abstracts: poster presentations

CELL BIO

An ASCB | EMBO Meeting

Dec. 3-7, 2022 • Washington, DC

Sunday, December 4, 2022

Science Education: Primarily Undergraduate 1

B1/P1001

A Case Study on Genomic Imprinting Facilitates Learning of an Abstract Topic

L. K. Goudsouzian[1], S. M. Lo[2]; [1]Biology, DeSales University, Center Valley, PA, [2]University of California at San Diego, La Jolla, CA.

Genomic imprinting is a form of non-Mendelian inheritance. Students in molecular biology and genetics college classrooms can struggle to understand genomic imprinting, in part because both high school and college instruction in genetics has traditionally prioritized lessons on Mendelian patterns of gene inheritance. We hypothesized that the use of a case study would help students better learn the topic of genomic imprinting. We wrote an interrupted case study which focused on genomic imprinting. The case study consists of three short popular news articles which relate to the topic of genomic imprinting. Each article is followed by a set of 2-4 questions. Students read each article and discuss its associated questions in small groups, then with the entire class, before moving on to the next article. We deployed the case study in an intermediate-level molecular biology course at a small, liberal-arts university. We assessed student learning and attitudes towards the case study (total of 50 pre/post matched pairs). In four true-false assessment questions, our results showed that students' performance on after the case study was significantly higher than their performance before the case study (McNemar's test for dichotomous paired nominal data, pre vs. post case study, p ranging from < 0.05 to < 0.0001 with odds ratios ranging from 5.39 to 17.48). Students also self-reported increased knowledge on concepts related to genomic imprinting (two items on a four-point Likert-like scale, pre vs. post case study, Wilcoxon rank-sum test for non-parametric data, $p < 0.0001$ for both items, Cohen's d effect size = 2.22 and 2.81). Finally, students were likely to agree that the case study was beneficial to their learning and was an enjoyable classroom activity (five items, post only, at least 3.28 ± 0.70 on a four-point Likert-like scale). We conclude that the case study is an effective way to instruct students about this challenging topic.

B2/P1002

MIRIC (Mentoring the Integration of Research Into the Classroom) Network affinity groups produce diverse resources for CURE development

M. J. Wolyniak[1], L. F. Barton[2], K. K. Resendes[3], J. N. Fitz Gerald[4], N. A. Najor[5], E. J. Sandquist[6]; [1]Hampden-Sydney College, Hampden-Sydney, VA, [2]Austin College, Sherman, TX, [3]Westminster College, New Wilmington, PA, [4]Rhodes College, Memphis, TN, [5]University of Detroit Mercy, Detroit, MI, [6]Weber State University, Ogden, UT.

An effective way to engage students in science education is through course-based undergraduate research experiences (CUREs) at all levels of the curriculum. While a proven high-impact pedagogical practice, CURE implementation can be a daunting task. Long-term mentorship in a community of practice dedicated to CURE development is an outstanding way for current and future faculty to overcome barriers to creating CURE experiences tailored to their own unique classroom needs. The Mentoring the Integration of Research Into the Classroom (MIRIC) Network is an initiative of the Council

on Undergraduate Research (CUR) Biology Division that trains current and future instructors on how to effectively implement CUREs into their classes. MIRIC is a supportive community for novice and experienced CURE instructors seeking to develop and strengthen their work with authentic research experiences in the classroom. To this end, MIRIC invites its mentee and mentor participants to join discussions on topics including "starting from scratch", inclusive CURE design and implementation, CURE sustainability, and extending the CURE model to K-12 classes to hone their skills on a specific aspect of CURE development of particular interest to them. Here we highlight the work of our affinity group work to date and showcase our diverse projects and products which may be shared with or used by other potential CURE instructors. Survey data from MIRIC participants indicate the network has been successful in giving participants the skills and the support necessary to develop effective CUREs that address the specific needs of their classrooms. Collectively, this affinity group approach for group mentoring has been effective in helping MIRIC mentees to develop the skills and confidence necessary to implement an effective CURE.

B3/P1003

Creative activities promote student motivation and engagement in a large cell biology course

I. Bouhdana, M. Denis, G. J. Brouhard; McGill University, Montreal, QC, CANADA.

Student motivation and engagement are foundational to academic success but can be difficult to achieve in large enrolment cell biology courses. In social cognitive theories of motivation, students are motivated when they perceive value in an educational activity, and motivation can feed forward on three distinct dimensions of student engagement, namely behavioral, cognitive, and emotional engagement. This engagement, in turn, is critical to academic success. A key to stimulating student motivation is creativity. We wondered whether creative activities would have a positive impact on student motivation, engagement with course material, and academic performance in a 500-student undergraduate cell biology course at McGill University. Students were given assignments with the option to choose between four creative activities: (1) 3D printing a protein structure based on a PDB file; (2) baking cookies or other pastries shaped like a protein of interest; (3) creating a freeform visual art submission, such as a painting; or (4) making a meme about course material. Students who did not wish to submit creative activities could opt out by completing a self-graded quiz instead. We hypothesized that the creative group would report greater motivation and engagement that would feed forward into improved performance on course examinations. Twice during the semester, we collected quantitative and qualitative survey data from students designed to measure their levels of behavioral, cognitive, and emotional engagement; we then correlated this data with exam performance. Our results indicated that students who participated in creative activities perceived them as valuable and reported higher levels of behavioural and emotional engagement with course content relative to students who took self-graded quizzes. However, contrary to our hypothesis, students who took self-graded quizzes reported significantly higher levels of cognitive engagement with course material and outperformed other students on course examinations. Our results suggest that creative activities are motivational and engaging to cell biology students but must be designed to promote cognitive engagement in order to improve academic performance. Future implementation of creative activities should consider student motivations for engaging in these activities as the basis for their design and intentionally include a cognitive engagement component.

B4/P1004

Using molecular biology preprints to engage undergraduates in the scientific community and enhance their scientific literacy: evidence from a new curriculum on preprint peer review

G. McDowell[1], S. Fankhauser[2], M. Balgopal[3], **R. S. Lijek**[4]; [1]Lightoller, LLC, Chicago, IL, [2]Oxford College, Emory University, Oxford, GA, [3]Colorado State University, Fort Collins, CO, [4]Mount Holyoke College, South Hadley, MA

Science education focuses on how experiments are carried out and the knowledge generated by the resulting literature, but misses an opportunity to engage students in the critical validation process that translates one into the other - the peer review of scientific manuscripts. Peer review is integral to the scientific process and so we envision a paradigm shift that makes teaching peer review integral to science education (McDowell et al., MBOC, 2021). Explicit teaching about peer review unmasks part of STEM's "hidden curriculum": untaught knowledge that is required for success and which disproportionately hinders minoritized students. Few training opportunities exist in peer review and none are designed for or tested in undergraduates. With NSF support, we created and are evaluating a curriculum in which biology undergraduates learn about the importance and mechanisms of peer review, then write and publish their own reviews of manuscripts in prepublication ("preprints"). Preprints are widely available online for free throughout biology, and their use in molecular biology has exploded in recent years, making this curriculum broadly applicable. By having students authentically review works-in-progress created by practicing scientists, they meaningfully contribute to the scientific literature and community. Using COVID19 preprints also motivates students who might be otherwise disconnected by the pandemic to re-engage in a meaningful way. Our research asks: does this preprint peer review curriculum improve students' scientific literacy? Does it enhance students' sense of belonging in the scientific community? We tested these hypotheses using validated pre-post surveys (e.g. TOSLS, PITS) and thematic analysis of students' writing. Here, we present the first of three years of data collection at Mount Holyoke, a small liberal arts college for women and gender minorities. Ongoing work includes scale up of the curriculum at Colorado State and Emory Universities and dissemination of the resulting evidenced-based curriculum suitable for diverse settings (McDowell et al., Learned Publishing, 2022). Using this curriculum to enhance students' scientific literacy and sense of belonging in the scientific community has the potential to broaden participation and prepare students for success in the STEM workforce and create a scientifically-literate public.

B5/P1005

Fictional Case Study Effects Student Learning about Immune Cell Memory

K. M. Cooper; Molecular, Life, and Health Sciences, Loras College, Dubuque, IA.

In an immunology class there are many opportunities to connect basic cellular principles to diseases and medicine, making undergraduate students potentially more likely to care about and remember the concepts. However, finding medical case studies to teach basic immunological principles is challenging, as many are too complex for an introductory-level immunology class and more appropriate for graduate or medical students. A simple fictional case study was designed to actively engage students in discovering what hemolytic disease of the newborn is, how it can be prevented, and how the preventative treatment is based a general principle of immunological memory where naïve B cells do not activate if antibody is already present on the antigen they recognize. Students were given pretests, posttests, and surveys about their impressions of the case study and their learning. Data from two

different cohorts of students showed student interest and engagement, though the topic was still challenging for them to fully understand. Additionally, general ideas for writing approachable case studies related to the cell biology of the immune system are shared.

B6/P1006

Supporting Faculty in CURE Implementation: Resilience and Growth of the Genomics Education Partnership Community

A. L. Goodman[1], **M. S. Santisteban**[2], C. S. Silver Key[3], J. Siders[4], C. Reinke[5], W. Leung[6], S. Gehrke[7], L. K. Reed[8]; [1]Chemistry and Biochemistry, California Polytechnic State University, San Luis Obispo, CA, [2]University of North Carolina at Pembroke, Pembroke, NC, [3]North Carolina Central University, Durham, NC, [4]Ohio Northern University, Ada, OH, [5]Linfield University, McMinnville, OR, [6]Washington University in St. Louis, St. Louis, MO, [7]University of Washington, Seattle, WA, [8]The University of Alabama, Tuscaloosa, AL.

Early participation in undergraduate research promotes persistence in STEM and other positive student outcomes (Rodenbusch et al., 2017), yet Primarily Undergraduate Institutions (PUIs) often lack the resources required to provide broad access to research. The Genomics Education Partnership (GEP, https://thegep.org/) is a community of 261 faculty members from 215 institutions across the United States and its territories. The Partnership is dedicated to facilitating equity in undergraduate biology education by providing centralized resources to empower faculty to engage their students in experiential learning and Course-based Undergraduate Research Experiences (CUREs) in genomics and bioinformatics. In the past four years, our community has undergone substantial changes: a transition from centralized to distributed leadership, the development of focused growth efforts to recruit faculty from Minority Serving Institutions (MSIs) and Community Colleges (CCs), and a switch in modality from in-person to virtual New Member Training. To assess how these changes have impacted our community, we created surveys and interview protocols based on the study of other communities of practice in STEM education (Kezar and Gehrke, 2015), and used them to collect quantitative and qualitative data. **Outcomes:** Our community has grown from 118 to over 260 active members in the last two years. The rapid growth and transition to virtual communications have been augmented by the activities of regional nodes that hold in-person and online activities, such as student research symposiums, faculty meetings, and training for new members and teaching assistants. Preliminary analysis of the survey responses from 124 faculty (60% response rate at the time of survey) suggests that the GEP model serves a wide range of faculty — including those who were newly-trained and long-time members, those who seek innovative genomics curriculum, and those who focus on engaging students in genomics research projects. We are analyzing faculty implementation reports to characterize the patterns of CURE adoptions for faculty trained in different modalities and teaching at different types of institutions. **Broader Impacts:** Characterizing the development and functioning of a distributed faculty community supporting the transformation of STEM education practices can provide a model for other faculty communities focused on education reform. The GEP provides the opportunity for faculty and students at CCs, MSIs, and other PUIs to engage in scientific research and gain professional development. Supported by NSF IUSE-1915544 and NIH IPERT-R25GM130517.

B7/P1007

Case study exams decreased test anxiety in a general biology class

D. S. Alves[1], C. D. Kilgore[2]; [1]Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN, [2]Teaching and Learning Innovation, University of Tennessee, Knoxville, TN.

Case studies are stories often used as an educational message and the purpose of this activity goes beyond just telling a history to students, it inspires and engages students to process the information and reflect in depth about the subject. In the present work, students from an introductory biology course (Bio160 at the University of Tennessee) were exposed to a case study in an innovative quiz format. Bio160 is a large size introductory class for major and non-major students, in which students often report negative emotions associated with perceived difficulty. Based on student feedback, the most common negative emotion reported was anxiety that often manifested during tests, exams and quizzes. Anxiety can also affect student retention, and in science classes, it can lead undergraduate students to leave their major (England et al., 2017). The main goal of our project is to understand the impact of using a case study exam on student test anxiety. The case study used in the present work was related to cell cycle (mitosis and meiosis). Students' knowledge in cell cycle was evaluated by using a quiz format that was delivered during the presented story. In order to address the possibility of students having anxiety from the exposure of an unknown test format, students were introduced to similar case studies twice before the exam. Student feedback indicated that the case study test format motivated them to learn something new about chromosomal/genetics sex definition and that they were inspired to think outside the box regarding the subject tested during the quiz. Finally, the results indicated that students experienced a significant decrease on test anxiety when exposed to this innovative test format.
References: England, B. J., Brigati, J. R., and Schussler, E. E. (2017). Student anxiety in introductory biology classrooms: Perceptions about active learning and persistence in the major. PLoS ONE, 12(8), e0182506.

B8/P1008

What Do Biology Students Do When They Need Academic Help? An Examination of Student Help-Seeking Behaviors

S. N. Ewell[1], R. Dunk[1], A. Radocha[1], R. Youngblood[1], S. Ferdous[1], A. Chen[2], Y. Mehari[1], A. Peart[1], M. Seibenhener[1], C. J. Ballen[1]; [1]Auburn University, Auburn, AL, [2]University of California, Irvine, Irvine, CA.

Self-regulated learning behaviors have been identified as a key factor in facilitating STEM persistence (Park et al., 2019). Self-regulation is a predictor of student academic performance and encompasses a variety of skills including help-seeking behaviors (Karabenick & Dembo, 2011). Help-seeking is a self-regulated learning behavior that allows learners to acquire content knowledge and skills necessary for academic success (Micari & Calkins, 2021). However, due to various barriers, many students do not seek help when needed. Currently there is a lack of in-depth literature that holistically examines the academic help-seeking processes of undergraduate students enrolled in biology coursework. To characterize the help seeking processes of students enrolled in undergraduate biology courses, we distributed a voluntary survey across three lower-level biology courses and three upper-level biology courses during two semesters at two R1 universities that asked students to indicate their attitudes toward help-seeking and classroom factors that encourage student help-seeking (n=1,023). We found that across all courses, ~51.2% of students perceived that they needed help to understand course content. We then ran an EFA model which independently verified the existence of two factors in our

questionnaire data: the perceived benefits of help seeking, and avoidance of help seeking (Pajares et al., 2004). Quantitative analyses revealed several individual characteristics predicted students' perceptions of help-seeking. For example, mixed-model analyses indicated that STEM majors were more likely to seek help, and persons excluded because of their ethnicity or race (PEERs) were less likely to seek help. However, neither of these factors had an impact on the perceived benefits of help seeking, which varied based on the individual course. On average, students in some courses perceived benefits more than students in other courses in our study. Next, we coded open-ended responses using thematic analysis. We found that across all classes, students preferred to use informal sources of help (e.g., classmates). Finally, we found that the following instructor behaviors encouraged student help-seeking behavior: instructors who explicitly encouraged question asking, structured class time for questions, provided positive feedback to questions, and cultivated a positive classroom environment. Additionally, we found that large class sizes, perceived negative attitude of the instructor, and rapid coverage of course material all discouraged help-seeking. Together, these data allow for understanding of student help-seeking processes in large enrollment biology classes and provide intervention targets for students who may struggle with obtaining help when needed.

B9/P1009

Supporting the Social-Emotional Health of CAMP Program Students in STEM

S. W. Lee[1], S. Dingwall[2], **R. A. Cardullo**[2]; [1]University of California, Irvine, Irvine, CA, [2]University of California, Riverside, Riverside, CA.

Minoritized students find it challenging to be accepted in the science, technology, engineering, and mathematics (STEM) environm. Minoritized students are more likely to persist in STEM if they feel a sense of belonging. California Louis Stokes Alliance for Minority Participation (CAMP) is a program that aims to support and increase the number of minoritized STEM graduate students and professionals. CAMP exists at all nine undergraduate University of California (UC) campuses and at various California State Universities, California Community Colleges, independent colleges and universities, and national laboratories. CAMP support of students is multifaceted, including research opportunities, financial support, academic advising, career counseling, professional development workshops, graduate school admission and preparation, and social activities. A key component of the CAMP program is getting undergraduate students involved in faculty-mentored research. Trust in faculty research mentors is associated positively with increased motivation and career expectations in students. Across all nine undergraduate UC campuses, we investigated the social-emotional health (Co-Vitality survey) of CAMP students who were involved in research and its correlation with perceptions of trust in their faculty research mentor. Co-Vitality of CAMP students correlated moderately with trust in their faculty research mentor. There was no correlation between Co-Vitality of CAMP students and trust in their college. This study suggests that faculty research mentors represent a source of support for their students' social-emotional health.

B10/P1010

Socially Relevant University Biology Course for Non-majors

A. Campbell, A. McDonnell; Davidson College, Davidson College, NC

Although biology education researchers focus most of their attention on courses designed for biology majors, most university students are not science majors. These non-major students often must take one

science course in order to graduate. Courses required of non-majors often are referred to as general education (GenEd) requirements or institutional service courses. GenEd biology courses typically mirror the content of courses designed for majors but "softened" to reduce the stringency of the content. Rather than presenting non-majors with a survey of biology reminiscent of high school courses that pushed them away from science, we designed a topics course that connected socially relevant topics with biology. The course described here, *Biology For Life*, used two guiding principles. First, we chose topics inherently interesting to college students. Topics included weight regulation, artificial sweeteners, pandemics, vaccines, race and race-based medicine, sex determination, sleep, GMOs, marijuana, alcohol, and climate change. Second, we presented students with data taken from peer-reviewed publications so they could build analytical skills and quantitative literacy. We wanted students to develop skills and understanding that would serve them after college (for life) and not just enough to pass examinations. In addition, students learned about logical fallacies used in arguments and how to draw argument maps. These two tools reinforced the major lessons from each chapter and helped students improve their ability to distinguish valid from fallacious arguments. Formal and informal course evaluations underscored very strong student enthusiasm for the course. However, four students felt the course was overly political and biased. This and other criticisms have been addressed for the next iteration of the course. The course also included a laboratory component which focused on quantitative literacy and Microsoft Office proficiency to provide marketable skills students could use in their post-university careers. In its first iteration, the course was offered 100% online due to the pandemic though it will be taught in person whenever possible. The entire GenEd course will be published as a combined electronic textbook and lab manual to facilitate adoptions by others who teach online or in person.

B11/P1011

S-STEM Scholarship Program: Exploring the Effects of Psychosocial Factors on STEM Persistence at an HBCU.

C. Carter, M. Van Stry; Lane College, Jackson, TN.

Lane College, founded in 1882 by former slave Bishop Isaac Lane, has strong history of educating underserved, minority students through student-center approaches. The Lane College S-STEM program aims to increase the number of low-income students entering STEM fields by providing scholarships, co-curriculum and high impact program activities to support Lane College students majoring in biology, chemistry, computer science, mathematics, or physics. These activities include development and implementation of new first-year STEM courses, including CHE 110 Scientific Literacy and Critical Problem Solving and PHY 111 Galileo and the Church. We have successfully recruited 2 cohorts of S-STEM scholars who have participated in several project activities including bi-weekly cohort meetings, undergraduate research projects, community outreach, internships, mentoring, and professional development. For the research project, we are exploring the development of non-cognitive factors known to impact student persistence in STEM (Grit, Self-efficacy, Growth Mindset, etc.) as S-STEM scholars matriculate through the program. Our initial measures of Grit and Growth Mindset for cohort 1 do not show a significant difference between S-STEM scholars and the control group of academically talented Power of Potential scholars. However, we have experienced low student participation in surveys during the period of remote instruction in response to the COVID-19 pandemic. For cohort 2, we measured self-efficacy for students in cohort 2 using the self-efficacy formative questionnaire developed by Gaumer Erickson and colleagues. Our data for the five students cohort 2 who completed the survey show an average score of 80.8% for focus, 60.8% for steps, and overall 72.5%. These initial data suggest

that the students will benefit from targeted instruction aimed at developing growth mindset and self-efficacy.

B12/P1012

Using *C. elegans* to study novel RNA interference phenotypes to promote inquiry-based learning in an undergraduate Cell and Molecular Biology course

J. Sullivan-Brown, A. Durkan, C. Byrnes, E. Cooper, A. Hally, J. Sowa; West Chester University of Pennsylvania, West Chester, PA

Inquiry-based undergraduate laboratories are designed to introduce students to the excitement of making novel scientific discoveries and perform independent research. Our objectives were to provide undergraduates with opportunities to gain genuine experiences in contributing to fundamental scientific knowledge by characterizing novel RNAi phenotypes of relatively understudied genes in the worm *Caenorhabditis elegans*. In our efforts to create these experiences, we designed labs for an upper-level Cell and Molecular Biology course at a primarily undergraduate institution in which students characterized the RNAi phenotypes of the potential oncogene DCAF13 (DDB1 and CUL4 associated factor 13). We discovered that when *dcaf-13* is disrupted in *C. elegans*, the worms showed a significant effect to larval development as measured by determining overall length of worms at 48 hours of development. In addition, *dcaf-13*(RNAi) worms display either a failure or delay of reaching the L4 or adult stages. Our data also indicates that *dcaf-13*(RNAi) did not affect embryonic development in *C. elegans*; however, egg-laying was significantly decreased in *dcaf-13*(RNAi) worms suggesting a general role in fertility. We were able to publish this work in *microPublication Biology*, with student authors from the Cell and Molecular Biology course and continue to use this study as a platform for novel discoveries.

B13/P1013

Self-regulated learning skills are linked to academic success: is it all "wrapped" up in reflection?

A. Ratnayake, A. Bansal, N. Wong, T. Saseetharan, S. Prompiengchai, A. Jenne, J. Thiagavel, **A. Ashok**; University of Toronto-Scarborough, Toronto, ON, Canada

Metacognition is the process of accurately monitoring one's comprehension and study strategies to enable effective self-regulated learning (SRL). SRL promotes autonomy, and purpose, which are components of intrinsic motivation shown to drive academic achievement. Our goal was to use a student-partnered approach to investigate the value of reflective tools to hone metacognitive skill development in students. We hypothesized that by using tools that allow reflection and refinement of students' study strategies, we could promote SRL and academic success in a foundation biology course. Undergraduate students in a semester-long second-year biology course were provided with three reflection survey instruments at three critical time points. Each survey included prompts aimed to encourage students to devise a study plan, reflect on the effectiveness of their plan, and identify areas for improvement as they prepared for evaluations. Student responses were systematically analyzed using a qualitative data analysis approach and were correlated with their academic performance. Our study sought to investigate the role of metacognition, motivation, and behavior in student course performance by (i) identifying the most utilized self-regulated learning strategies in order to better support student learning in the future, (ii) investigating the role of reflection in enhancing metacognitive processing and academic performance, and (iii) understanding whether students created and/or

modified their study strategies as an outcome of self-regulation. Our results suggest that students were able to demonstrate metacognitive awareness, as they accurately reported on the effectiveness of the study strategies they employed throughout the semester and indicated a shift from passive to active reviewing techniques in future study planning. Students perceived the reflection instruments beneficial in identifying areas of improvement and developing long-term study habits. Students who found the instruments to be effective also demonstrated improved performance on course assessments, suggesting a strong correlation between metacognitive axes and academic performance. Finally, students reported using the resources provided (ie. tipsheets and videos) to create or modify study plans in preparation for the semester ahead; employing the resources in this manner was correlated with improved academic performance. We conclude that reflection plays a central role in promoting metacognitive development, which, through impacts on motivation and behavior, augments self-regulated learning and enhances academic performance. Our study supports the recommendation that systematic use of reflection tools can support student learning in foundation biology courses.

B14/P1014

Linking research in cell biology and genetics courses through a single research topic on ABCD1 overexpression

K. Kandl, J. Porterfield, L. Bowers, S. Fruehling, A. Walter, L. Listenberger; St Olaf College, Northfield, MN

Authentic mentored undergraduate research is known to improve student learning and engagement. Course-based undergraduate research experiences (CUREs) allow more students to participate in research early in their academic careers. However, a single semester of research embedded in a laboratory course may not be enough to support student engagement with a complex problem. We are expanding the CURE concept by linking research projects across two distinct yet interdependent courses required for the Biology major, Cell Biology and Intermediate Genetics. Each course enrolls 130-150 students per year and may be taken in either order. During the fall 2021 and spring 2022 iteration of our linked CURE model, students in Cell Biology and Intermediate Genetics courses studied the effect of overexpressing ABCD1, a peroxisomal long chain fatty acid transporter, on the expression of other genes in lipid metabolic pathways (Intermediate Genetics) and on cell growth, metabolism, and lipid storage (Cell Biology) in the freshwater ciliate, *Tetrahymena thermophila*. Results from the Laboratory Course Assessment Survey, show that students beginning in either course reported similar satisfaction in terms of collaboration, discovery and relevance, and iteration, suggesting that the linked CURE model is effective whether students' first introduction to the research topic is from a genetics or cell biology viewpoint. Students obtained meaningful results that they shared in a poster session, and these results are being used to drive additional independent research projects in our labs. As we implement this approach in all sections of Cell Biology and Intermediate Genetics, Biology majors will have a year-long research opportunity using multiple approaches to address a single question. We will present logistical considerations for implementation such as faculty buy-in and scheduling considerations for sharing of information between students in these courses. Importantly, this approach is adaptable to different questions and model organisms, providing a template for sustainable research opportunities.

B15/P1015

Promoting Student Engagement and Academic Success in Introductory Cell Biology by Utilizing a Concepts Enhancement Learning (C.E.L) Lounge

K. Baker, G. Weber, M. Gobbett; University of Indianapolis, Indianapolis, IN

In an effort to overcome student disengagement and underpreparedness, exacerbated by remote learning during the pandemic, a pilot program, the Concepts Enhancement Learning (C.E.L) Lounge, was implemented in Introductory Cell Biology. The goal of the C.E.L Lounge is to provide engaging activities that not only promote students' "hands-on, minds-on" learning but to develop their abilities to learn course content and adapt to traditional academic expectations. Students had a weekly opportunity to assess their understanding of course material by voluntarily attending the C.E.L. Lounge, completing the supplemental learning activity, and answering the associated questions. Activities included models, manipulatives, and images of cellular processes. Students were welcomed and encouraged to work together; as a result, students also engaged in peer instruction. Completion of C.E.L. Lounge activities was positively correlated with overall course grades. Furthermore, students' perceptions indicated that they felt the activities were not only engaging, but more importantly, increased their understanding of lecture material and helped them be better prepared for exams, thus promoting their overall academic success in the course.

B16/P1016

Design2Data CURE Biotechnology Students develop employable technician skills while Engaging in authentic research experiences

C. E. Rolle, K. Mennes, N. Campbell; Capital Community College, Hartford, CT

The Design2Data with β-Glucosidase CURE uses a crowdsourcing approach to engage undergraduate students in design-build-test experiments to functionally characterize the effects of single amino acid mutations. CUREs are a meaningful for students who would not otherwise engage in authentic research as a part of their biotechnician skills training. Students began the D2D CURE in a biotechnology course and continued as an independent research project. Target amino acids were identified in the protein structure using Foldit. Plasmids containing the mutants were generated using Kunkel mutagenesis. The recombinant proteins were expressed, purified, and their enzymatic activity characterized. None of the mutations impacted protein induction and expression. The enzymatic activity and thermostability of the mutants were evaluated. This data will contribute to the D2D database to address current predictive limitations of protein modeling software. Given that Capital Community College is a designated MSI and HSI, as well as over 70% female students, the addition of the D2D CURE to the Biotechnology AS Degree Program should contribute to the diversity of the STEM pipeline in the region.

Science Education: K-12

B17/P1017

A STEAM lesson plan diversifies middle school student conceptions of scientists

J. Gardiner[1], C. Meaders[2], S. Wynns[3], S. Nigam[4], J. Harris[5]; [1]Fox Chase Cancer Center, Philadelphia, PA, [2]Cell and Developmental Biology, University of San Diego, San Diego, CA, [3]Cabrillo National Monument, San Diego, CA, [4]University of California San Diego, San Diego, CA, [5]Xavier University, New Orleans, LA.

Role model interventions that are tied to place-based-learning and classroom curricula may be effective tools for promoting diversity in science, technology, engineering, and mathematics (STEM). To evaluate this premise, we developed a sixth-grade lesson plan that focused on teaching environmental conservation and highlighting diverse women in STEM. Our curricula used a three-touch educational model consisting of comic-based lesson plans, a local “field trip” to Cabrillo National Monument, trading cards featuring 19 diverse women scientists, and a conservation capstone poster presentation - all aligned to Next Generation Science Standards - to create a meaningful experiential and project-based curriculum. To evaluate the program, we used a mixed-methods, change over time model, including the Draw-a-Scientist test (DAST) to assess if student perceptions of scientists were altered from the curricula. Overall, thirty-three students completed the DAST before and after participation. We used inductive coding of student drawings and responses to questions about their drawings and coded for six types of scientist stereotypic features. We found that science stereotypes held by students decreased from an average of 3.03 (SD = 1.26) per pre-drawing to an average of 2.24 (SD =1.37) per post-drawing, and that this decrease was significant ($p = 0.008$). We conclude that by using innovative tools such as art and comics for STEM education/outreach that feature characters representing a diverse array of scientists with intersectional identities, educators can help shift student perceptions on who can be a scientist, potentially increasing diversity in STEM fields.

B18/P1018

Share Your Expertise and Enthusiasm with Teachers: Organize a Workshop for Teachers to Build Inexpensive Microscopes for Classrooms

B. Goldstein; University of North Carolina at Chapel Hill, Chapel Hill, NC.

To get low-cost microscopes in front of kids and teachers, I've been running microscope-building workshops for elementary school teachers. In this poster, I’ll share what I do so that others can duplicate and/or build upon what I’m doing. I use a modified version of Kenji Yoshino's microscope design, which he described in an Instructable entitled "$10 Smartphone to digital microscope conversion!" This is a simple and clever design for a microscope that takes advantage of ubiquitous smartphone cameras. The microscopes are mostly transparent, making their inner workings obvious to teachers and students. And all of the parts are readily available from hardware stores or online, so teachers or students can make more anytime if they wish. Based on feedback from teachers, I enlarged Kenji's design to accommodate iPads and other tablets, which are available in many elementary schools, and I've made other minor improvements. Teachers have reported back that groups of students interact with each other around each microscope as they look at the smartphone or tablet screen together — a big improvement over microscopes in which students might see only their own eyelashes, and in which only each student would know what they saw. For the workshops, I prepare enough materials for each teacher to build their own microscope to keep in their classroom. A workshop with about a dozen or

more teachers can equip a school with a complete set to move from classroom to classroom. Most of the workshops last 90 minutes, during which three things happen: (1) the teachers assemble the microscopes, (2) they develop expertise using the microscopes as they take pictures of things they brought with them, and they collect images to share (see image galleries at www.diymicroscopes.org), and (3) teachers brainstorm about how they'll use the microscopes to best match their curriculum needs and students' interests. My current work includes bringing workshops to high poverty school districts in North Carolina and to statewide teachers conferences, and working collaboratively to assess outcomes. At this poster there will be some of the microscopes for anyone to try, and I'll share a protocol that you can use to get started running your own workshops with teachers at local schools. And I'll be happy to talk with people about collaborating or helping you get started running workshops on your own.

B19/P1019

Engaging high school students in summer research with cell biology and environmental science connections

E. L. Behnfeldt, S. Fromherz; Biology, Saginaw Valley State University, Univ Ctr, MI.

Summer research for high school students can foster interest in science and STEM careers. However, the pre-requisite background needed for cell biology research, and distractions of summer may challenge sustained student interest. As part of a broader program at Saginaw Valley State University, Michigan, a regional public primarily undergraduate university serving a high proportion of students from rural and underserved backgrounds, we developed and, in the summer of 2021, implemented research projects for four area high school students in a mentored team setting. The team consisted of two high school teachers, two undergraduates and an SVSU professor team leader. Projects broadly centered on plastic waste, a topic area likely of high relevance in students' daily lives. We bridged from environmental science to cell biology with experiments on the impacts of microplastics on the phagocytic pathway in a model unicellular ciliate, *Tetrahymena pyriformis*. Over the course of 6 weeks, students collected and studied local aquatic microbiota, helped develop and carry out authentic research projects in *Tetrahymena*, and prepared and presented research posters for a wider community audience. We used microscopy workstations equipped with large screen monitors and video cameras, as well as dyed polystyrene beads, to readily capture and quantitate data, and to easily share real-time microscopic images with the larger group. Initially we used pulse-chase experiments to introduce methods before helping students to develop and carry out authentic and feasible projects. For example, one student quantified vacuole size as a function of microplastic size. Qualitatively, we observed significant learning gains by the students in research skills such as experimental design and analysis, microscopy, math, teamwork, and communication. Furthermore, the group experience benefited mentor team members in multiple ways. We will describe the structure and benefits of the mentored team structure, microscopy workstations, exercises to support student growth and confidence in cell biology research, and representative projects and data. Our results suggest that summer research projects connecting cell biology with the environment, carried out in a small group setting with diverse mentors, can provide positive learning experiences for high school students and may foster careers in STEM.

B20/P1020

Gaining STEAM!: Illuminating Research Through Art

J. Gardiner[1], K. Tran[2], K. Montgomery[3]; [1]Fox Chase Cancer Center, Philadelphia, PA, [2]University of Pennsylvania, Philadelphia, PA, [3]University of California San Francisco, San Francisco, CA.

Public access and exposure to scientific research is limited due to both restrictive paywalls and technical language written for specialists. While effectively communicating discoveries to non-specialists is imperative, it is no trivial task. Thus, **developing alternative approaches to disseminate discoveries to non-specialist audiences is essential to ensure broader populations can actively engage in scientific discourse.** The combination of art and storytelling in comics break down complex concepts while simultaneously providing an engaging medium. When applied to science, this makes the subject more approachable, memorable, and enjoyable. **To this end, we developed** a community-based science communication (SciComm) project, "*Gaining STEAM!"* which addresses two hurdles within science engagement and communication: 1) providing SciComm training for scientists to engage with non-experts and 2) facilitate discourse between scientists and the community about scientific research. **This was accomplished through**: developing workshops for scientists on effective science communication through storytelling and comics, collaborations with local artists to create comic books based on the scientist's research, and a public comics showcase displayed through posters. Surveys distributed at the *Gaining STEAM!* showcase revealed that 70% of non-UW-Madison affiliates were unaware of university sponsored research. Strikingly, 55% of UW-Madison affiliates were also unaware of research projects conducted at their university. **100% of individuals surveyed** indicated that the use of comics improved their comprehension of the scientist's research. **Collectively, these findings support the use of comics as a tool for improved science communication and public engagement with STEM.**

B21/P1021

A SEPA partnership to enhance middle school STEM education

R. Sloboda[1], M. Tine[2], V. May[3], A. Skinner[4], L. Foster-Johnson[5], M. Fenzel[6], K. Price[6]; [1]Biological Sciences, Dartmouth College, Hanover, NH, [2]Sociology, Dartmouth College, Hanover, NH, [3]Thayer School of Engineering, Hanover, NH, [4]Guarini School of Graduate and Advanced Studies, Hanover, NH, [5]Geisel School of Medicine, Hanover, NH, [6]Montshire Museum of Science, Norwich, VT.

Providing young students with a solid understanding of science and the scientific process through their exposure to scientific thought and analysis is crucial for their life-long science comprehension and its relevance to the world around them. Loss of interest in learning STEM and perhaps having a future career in STEM has been shown to begin in the middle school years and is much more acute in low-income rural communities where there are few resources, such as equipment, teacher support, and collegial interaction. Our SEPA project involves Dartmouth faculty, graduate students, and science education experts from the Montshire Museum of Science working with middle school science teachers to co-develop hands-on, NGSS-aligned, science units that 6th-8th grade teachers in low-income rural communities can use to enhance the STEM engagement and learning of their students. At this point in our five-year project, we have worked with 6th and 7th grade teachers at four under-resourced schools in VT and NH. Our poster provides links to the curricular units we have developed, which are freely available to any teacher interested in using them. Initial assessments of the impact of these units on those involved indicate the project has had positive effects on several groups. Teachers reported enhanced self-confidence when teaching science, more confidence in the application of NGSS to their

classes, and more confidence in the inclusion of engineering design in developing hands on, instructional activities for their students. Similarly, the graduate students enhanced their science outreach skills and improved their self-efficacy. However, after engagement with the units we developed, the impact on the STEM attitudes of students in our 6th and 7th grade target classes was mixed. Students who expressed an interest in science at the start of the school year sustained a positive attitude towards STEM, but STEM attitudes remained low for those students who initially expressed less interest in science. Our results thus far suggest that interventions in middle school may be ineffective, because by the time students have reached middle school their level of interest in science as a field of study and as a career path may have already been fixed. Thus, efforts to impact positively the level of interest middle school students have for STEM needs to begin earlier, e. g. when they are in the elementary grades. Our work is supported by a Science Education Partnership Award (SEPA) from NIGMS of the NIH.

Science Education: Graduate and Medical School

B22/P1022

Evidence that Video Didactic Preparation can Augment Problem Based Learning in Medical Education

K. L. Hamilton[1], P. Horneffer[2], T. P. Stein[3], G. S. Goldberg[3]; [1]Georgetown University, Washington, DC, [2]All American Institute of Medical Sciences, St Elizabeth, JAMAICA, [3]Rowan University, Stratford, NJ.

Problem based learning (PBL) utilizes a self-directed strategy. Group participation is needed for this process to succeed. Students without a background in biology or medicine can feel overwhelmed by the complexity of the subject matter and unable to participate in the group learning process. We incorporated curated educational videos in the PBL curriculum to help address this situation. First year medical students participated in this study in the form of a typical PBL session. They were then assessed on basic and clinical science knowledge and their learning experience. Student basic science and clinical knowledge were similar between the experimental and control groups. However, the experimental group scored 13% higher in their learning experience, and 400% higher in their perception of feeling prepared and participating in the group PBL experience with $p<0.05$ by t-test ($n=13$). Results from this study indicate that videos can be utilized to enhance the PBL process.

B23/P1023

Communicating scientific concepts through art

B. E. Mierzwa[1,2]; [1]Ludwig Institute for Cancer Research, San Diego, CA, [2]Department of Cellular and Molecular Medicine, University of California, San Diego, CA.

Creativity is an integral part of both science and art, and using it to combine these two seemingly different disciplines creates unique and effective ways to communicate scientific information, both inside and outside the scientific community. Along my classical academic career path, I have been exploring the visual aspect of science communication using hand-drawn illustrations, science fashion, and interactive media. My drawings aim to convey complex biological concepts using both abstract imagery and real scientific data. Each illustration requires breaking down the essence of scientific findings and translating them into aesthetic visuals using metaphors that describe complex biological processes in intuitive ways. This form of visualization provides a powerful tool to facilitate communication between researchers from diverse fields, spark fascination and curiosity for science, as well as inspire our next generation of scientists. Here, I will share my journey towards combining science

and art, highlight the impact of artistic practices on my own research and communication, and discuss avenues for scientists to explore this emerging field.

B24/P1024

Evaluating the feasibility, utility, and impact of engaging in mentorship assessment to improve doctoral mentoring relationships

H. N. Fedesco, **E. L. Dolan**, E. Kraner; Biochemistry & Molecular Biology, University of Georgia, Athens, GA.

A doctoral student's relationship with their research advisor is thought to be the most influential factor in the quality and success of their training experience, but these relationships are not always positive. We hypothesize that doctoral mentoring relationships can be enhanced by improving student-advisor communication. Communication scholars have identified a set of behaviors used in close relationships, called relational maintenance strategies, which help to sustain or increase relational quality. Examples include being open and positive about the relationship, which can enhance relationship satisfaction, commitment, and trust. Furthermore, research on mentoring shows that, when relationship quality is high, mentees are more likely to persist and succeed. With this in mind, we sought to create a mentoring assessment structure that would foster openness and positivity in student-advisor relationships. We developed a survey based on established measures of mentoring support, administered the survey to student-advisor dyads, and provided a report to each dyad along with tailored feedback and talking points. We studied the experiences of students and advisors as they completed the process to assess the feasibility, utility, and impact of engaging in mentorship assessment. First, we recruited 12 life science doctoral student-advisor pairs, who completed the survey and received a report. Then we interviewed a subset of them to understand their experiences and gather feedback for improvement. We revised and repeated the survey and reporting process with new pairs and conducted additional interviews, including interviewing 11 international students from 7 countries to gain insight into cultural issues that may influence whether and how students engage in mentorship assessment. We then conducted qualitative content analysis of the interviews to describe student and advisor experiences with and outcomes from the mentorship assessment. Students and advisors reported that they were able to send relational maintenance messages. International students also had positive reactions, indicating comfort with the process. Students and advisors both noted relationship improvements such as gaining new insights, improving communication, and more openly discussing needs. Students noted positive outcomes such as greater accountability, a more manageable workload, and improved clarity about expectations. Advisors reported that they felt validated about their mentoring efforts. Students and advisors reported only a few neutral/negative outcomes, largely related to the time and effort spent completing the survey.

B25/P1025

Investigating the Relationship Between Metacognitive Awareness and Metacognitive Accuracy in Introductory Biology Students

J. Wollesen, M. Aranda; SOUTHERN ILLINOIS UNIVERSITY EDWARDSVILLE, Edwardsville, IL

Introductory biology courses are often difficult for students because they require students to have metacognitive awareness, which is the ability to monitor one's learning. Perceptions of learning are used to predict performance on tasks, which demonstrates metacognitive accuracy. Higher awareness is

thought to correlate with higher accuracy; however, previous studies examining this relationship have been inconclusive. We hypothesized that that these inconsistent findings are due to additional variables, such as mindset and locus of control. It is crucial to understand the relationships between these variables because they can influence student success by helping students develop self-regulatory skills. Three research questions guide this study: 1. To what extent does metacognitive awareness correlate with metacognitive accuracy and exam scores? 2. To what extent do mindset or locus of control affect the relationship between metacognitive accuracy and exam scores? 3. To what extent do measures of awareness, mindset, locus of control, or accuracy change after students take exam 1? To measure these variables, students completed a survey before and after their first exam. Accuracy was measured comparing predicted to earned exam scores. We did not find the expected correlations between metacognitive awareness and metacognitive accuracy or exam scores. We did, however, find correlations to be present between metacognitive awareness, mindset, and locus of control. No significant difference was observed in responses collected before and after the first exam. Further analyses of these data will examine possible differences in relationships among variables between different groups of students, such as high, mid, and low performers. We also plan to examine patterns of study strategy use among students and compare these to measured levels of metacognitive awareness and accuracy. This study sought to further investigate the relationship between metacognitive awareness and metacognitive accuracy by examining two additional student characteristics: mindset and locus of control. These components, along with metacognitive skills such as awareness and accuracy, can impact student success. We believe this information could inform teaching practices by allowing instructors to identify and assist students in developing self-regulatory skills.

B26/P1026

Lessons learned from teaching science virtually to medical and graduate students during the COVID-19 pandemic

S. B. Lovitch[1], A. Jambhekar[2]; [1]Brigham and Women's Hospital, Boston, MA, [2]Harvard Medical School, Boston, MA

At the onset of the COVID-19 pandemic, science educators at all levels were asked to rapidly pivot from in-person to virtual teaching and adapt their curricular materials to a virtual format, often with little to no experience or guidance. While improving conditions have since allowed for resumption of in-person instruction, many institutions have elected to retain elements of virtual or hybrid instruction. Therefore, review of the lessons learned from the virtual education experience of 2020-21 is warranted, in order to identify effective tools and approaches, and to recognize potential pitfalls and consider strategies to avoid them. We reviewed our experience teaching virtually in three courses with distinct learner populations and educational goals, based on analysis of anonymized student evaluation data and comments as well as personal experience. The courses analyzed are *Foundations of Medicine*, a course for first-year medical and dental students covering basic science content; *Developing and Communicating Scientific Investigations*, a course for first-year PhD students in Systems, Synthetic, and Quantitative Biology; and *Pathology of Human Disease*, a course for second- and third-year PhD students in the Leder Program in Human Biology and Translational Medicine (a translational science-focused track open to students in various PhD programs). Several virtual education strategies were particularly effective and well-received by the students, including use of polling and interactive annotation; use of the chat function in Zoom as a non-disruptive venue for asking questions and providing mutual support; and use of breakout groups where students could easily share electronic

materials, including those generated during the course of the discussion. The most frequently encountered pitfalls were inability to complete sessions within their allotted time; to monitor multiple small-group discussions simultaneously; to practice and evaluate oral presentation skills; and to identify struggling students due to lack of non-verbal cues, exacerbated by students' reluctance to activate video. Ultimately, virtual education proved to be an effective, but intrinsically limited, venue for conveying scientific content to medical and graduate students, and could be significantly improved by applying the lessons learned from the COVID experience.

Imaging Technologies, Single Molecule Imaging, and Super-resolution

B28/P1027

Label-free morphological and biochemical characterization of pancreatic secretory cells

V. Loconte[1], A. Deshmukh[2], J. Cuala[2], B. Vanslembrouck[1], K. Chang[2], K. White[2]; [1]Dept of Anatomy, University of California, San Francisco, San Francisco, CA, [2]University of Southern California, Los Angeles, CA.

The dysfunction of α and β cells in the islet of Langerhans can lead to different types of diabetes1. The maintenance of the cell well-being is usually accompanied by rearrangements in the cellular ultrastructure, which can alter intracellular connectivity and cell functional activity2. High-resolution techniques such as electron and fluorescent microscopy have been able to distinguish single components in the islets; however, both methods require extensive cell manipulation to reconstruct the whole three-dimensional (3D) cellular architecture3,4. To address this challenge, we used a high-throughput label-free approach, which allows us to recognize α and β cells from mouse islets based on their subcellular features using soft X-ray tomography (SXT)5. SXT captured the 3D ultrastructural reorganization of a single cell without staining and slicing, preserving high spatial resolution (50 nm) and short time of collection (<10 min)6. For both mouse α and β cells, we mapped cell, vesicle, and nuclear volume, as well as vesicle diameter, and vesicle X-ray absorption, and determined univocal features that can be used to identify cell types without the need of additional labels. We identified significant larger cell volume and vesicle diameter for β cells as compared to α cells, whereas we observed higher vesicle molecular density for α cells. All together, these features provided hallmarks that can be used to distinguish among the two cell types in the mouse islets and across different species. Finally, we were able to define structural heterogeneity in both α and β cells, showing different cell subpopulations. Collectively, our method can be applied to systems that require low levels of manipulation and high resolution, and allows to map and quantitatively describe cellular phenotypes and their impact in healthy and diseased conditions. References: [1]Meier, J.J. and Bonadonna, R.C., 2013. Diabetes care, 36(Supplement_2), pp.S113-S119. [2]Loconte, V., *et al.*, 2022. Structure, 30(4), pp.510-521. [3]Valm, A.M., *et al.* 2017. Nature, 546(7656), pp.162-167. [4] Müller, A., *et al.* 2021. Journal of Cell Biology, 220(2). [5]Larabell, C.A. and Nugent, K.A., 2010. Current opinion in structural biology, 20(5), pp.623-631. [6]Ekman, A.A., *et al.* 2017. Biology of the Cell, 109(1), pp.24-38.

B29/P1028

Laboratory Cryo Soft X-ray Tomography for 3D Imaging of Whole Cells

K. Fahy[1], P. Sheridan[1], F. O'Reilly[2], **T. McEnroe**[1], W. Fyans[1]; [1]SiriusXT, Dublin, IRELAND, [2]UCD Dublin, Dublin, IRELAND.

Analysis of three-dimensional biological cell samples is critical for understanding the mechanisms of viral disease and for the development of novel therapeutics. Soft X-ray microscopy (SXM) is the unique technology that can image whole intact cells in 3D under normal and pathological conditions without labelling or fixation, at high throughput and spatial resolution [1-4]. The main challenge of SXM is that the photonic illumination required for imaging has heretofore only been available at synchrotron labs [5] and access is limited. SiriusXT has developed a lab-scale SXM for fast and inexpensive three-dimensional imaging of whole cells that can be readily performed in a laboratory. The capabilities of this compact imaging device, combined with complementary light and electron microscopy approaches, is currently being demonstrated through a series of virology use cases to generate new scientific knowledge on the viral life cycle and host cell response to viral infection [6]. Our studies will allow demonstration of the benefits of the lab-based system relative to both synchrotron based SXM as well as other imaging modalities. However, the most comprehensive view of the complex structure of cells is unlikely to come from a single microscope. We will facilitate correlation of SXM with light and electron microscopy by integration of a fluorescence microscope, dual modality of sample presenting scheme, including EM grids, and an automatic data analysis pipeline. In this paper, we will present tomography data from whole single cells, and discuss the sample preparation and data analysis workflows employed by the microscope.

Figure 1: Slice through a reconstructed huh-7.5 cell cryo preserved and imaged in the laboratory soft x-ray microscope. Whole cell imaging with natural contrast allows the immediate identification of many cellular organelles.

References [1] M Harkiolaki et al., Emerging Topics in Life Science **2** (2018), p. 18. doi: 10.1042/ETLS20170086

[2] J Guo and CA Larabell, Current Opinion in Structural Biology **58** (2019), p. 324. doi: 10.1016/J.SBI.2019.06.012[3] G Schneider et al., Nature Methods **7** (2010), p. 985. doi: 10.1038/NMETH.1533[4] FJ Chichón et al., Journal of Structural Biology **177** (2012), p. 202. doi:10.1016/J.JSB.2011.12.001[5] A Ekman et al., Synchrotron Light Sources and Free-Electron Lasers Springer, Cham. (2019) https://doi.org/10.1007/978-3-319-04507-8_43-3[6] K Fahy et al., JPhys Photonics **3** (2021), doi: https://doi.org/10.1088/2515-7647/abfc5a

B30/P1029

Development of POLArIS, a versatile probe for multi-color/multi-target orientation imaging in living cells

K. Sato[1], N. Nakai-Kadowaki[1], A. Sugizaki[1], K. Chiba[2], K. Saito[1], M. Kawagishi[1], Y. Tomabechi[3], S. B. Mehta[4,5], H. Ishii[4,6], N. Sakai[3,7], H. Ka[1], M. Shirouzu[3], T. Tani[4,8], S. Terada[1]; [1]Tokyo Medical and Dental University, Tokyo, JAPAN, [2]Ochanomizu University, Tokyo, JAPAN, [3]RIKEN Center for Biosystems Dynamics Research, Yokohama, JAPAN, [4]Marine Biological Laboratory, Woods Hole, MA, [5](present address) Chan Zuckerberg Biohub, San Francisco, CA, [6](present address) National Institute for Physiological Sciences (NIPS), National Institutes of Natural Sciences, Okazaki, JAPAN, [7](present address) RIKEN SPring-8 Center, Sayo-gun, JAPAN, [8]National Institute of Advanced Industrial Science and Technology (AIST), Ikeda, JAPAN.

Monitoring the orientation of biological molecules in living cells is a key approach to understanding the mechanisms of the emergence/dissolution of molecular architectures. Fluorescence polarization microscopy (FPM) can visualize the dipole orientation of fluorescent molecules and has been used for analyzing the architectural dynamics of biomolecules. To monitor the orientation of molecules of interest using FPM, they need to be labeled with fluorophores in a sterically constrained manner so that the fluorophores do not freely rotate. Recently, we reported a versatile probe for such labeling using fluorescent proteins, POLArIS (Probe for Orientation and Localization Assessment, recognizing specific Intracellular Structures of interest) (PNAS 2021). Original POLArIS is a recombinant binder affimer rigidly connected to a green fluorescent protein mutant and can target specific biomolecules of interest by combining with evolutionaly molecular engineering. We have later developed nanobody-based POLArIS, which is compatible with animal immune libraries, with color variations of cyan/green/yellow/red fluorescent proteins, enabling multi-color orientation imaging for multiple targets (BBRC 2021). As an initial test case of POLArIS, we developed POLArIS^{act}, which specifically binds to F-actin in living cells. Localization analysis using fluorescently labeled phalloidin in fixed cells and co-expression with other genetically encoded F-actin probes indicated that POLArIS^{act} is an excellent F-actin probe. FPM analysis confirmed that POLArIS^{act} can monitor the orientation of F-actin both *in vitro* and in living cells. We microinjected the mRNA of POLArIS^{act} into starfish oocytes and observed the actin dynamics during early developmental processes using FPM. We found that actin filaments radially extend from centrosomes during mitosis in early embryos. Detailed microscopic observation revealed that the distribution of the radially extended actin filaments is very similar to that of astral microtubules. We named this actin-based structure FLARE (FLuffy And Radial actin-aster associated with mitosis in Embryo).

B31/P1030

Paired-objectives photon enhancement (POPE) Microscopy

M. Tingey[1], S. Junod[1], B. Zhang[1], A. Ruba[2], W. Yang[1]; [1]Biology, Temple University, Philadelphia, PA, [2]School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA.

The first two authors contributed equally to this work.

Fluorescence microscopy techniques are widely employed for the visualization, localization, and observation of structures and dynamics in biological samples. In order to obtain optimal image resolution, collecting the maximum number of photons from each fluorophore is essential. Historically, researchers have addressed this need by increasing the excitation energy, engineering higher numerical aperture (NA) objectives, improving the quantum yield of fluorophores, and/or escalating the quantum efficiency of detectors. While these approaches have led to great advances in the field of microscopy, they cannot proceed linearly in a limitless manner. We noticed that more than half of photons are lost as fluorophores emit in a direction other than the objective of either an upright or inverted microscope. To collect these lost photons, we have developed a new system called paired-objectives photon enhancement (POPE) microscopy to increase photon capture of a fluorophore by nearly two-fold. This system captures these lost photons by aligning an 8f optical system and mirror directly opposite the lower objective of an inverted microscope, thereby reflecting more emitted fluorescence back through the lower objective and into the detector. Through the additive effect of the initial fluorescence and the reflected fluorescence combining, the number of photons captured nearly doubles. This improved photon budget allows a microscopy imaging system to have better optical resolution, higher sensitivity, and/or faster detection speed. This new technique has been demonstrated in confocal light microscopy,

epifluorescence microscopy, and super-resolution light microscopy; and can, in theory, be applied in a modular fashion to any and all other fluorescence microscopy methods.

B32/P1031

Multiplexed spatiotemporal tracking of RNA dynamics in live mammalian cells using fluorescence lifetime imaging microscopy

N. Sarfraz, E. Braselmann; Chemistry, Georgetown University, Washington, DC.

RNA localization, biomolecular association, and dynamics are all linked and closely influence RNA function.[1] For quantitative insight into these processes, it is essential to visualize RNAs live and track responses to cellular perturbations.[2] In this study, we established FLIM for live RNA tracking. Our genetically encoded RNA FLIM sensor builds upon the Riboglow platform.[3,4] Riboglow consists of a small molecule fluorescent probe that binds a genetically encoded RNA tag and changes fluorescence upon binding.[3,4] This prompted us to hypothesize that fluorescence changes in the probe could lead to significant changes in fluorescence lifetime (τ). We assessed the τ of the probe in live cells in the presence vs. absence of tagged RNA. As hypothesized, we observed differences in τ that lead to substantial fluorescence contrast between cells with vs. without RNA. The cellular contrast for FLIM measurements was robust and superior in direct comparison with fluorescence intensity-based contrasting.[4] We have demonstrated tagging of coding and non-coding RNAs in mammalian cells. Importantly, the RNA tags in FLIM-Riboglow may be varied to yield unique fluorescence lifetimes in the same laser channel. We successfully identified two different RNAs, enabling quantitative assessment of spatiotemporal RNA dynamics live.[4] Recently, we have expanded the platform to include another tag, producing a significantly different lifetime thus expanding our tracking capabilities to three RNAs for multiplexed RNA detection. Current efforts are underway to expand multi-parameter RNA tracking capabilities and establish physiologically relevant RNA tracking in diverse mammalian model systems.

B33/P1032

A novel sub-precision detection method (MCS-DETECT) identifies shape complexity of mitochondria-ER contacts (MERCs) in 3D STED super-resolution microscopy

B. Cardoen[1], K. Vandevoorde[2], G. Gao[2], P. Alan[2], W. Liu[2], A. Vogl[2], G. Hamarneh[1], I. R. Nabi[2,3]; [1]School of Computing Science, Simon Fraser University, Burnaby, BC, CANADA, [2]Life Sciences Institute, Department of Cellular and Physiological Sciences, University Of British Columbia, Vancouver, BC, CANADA, [3]School of Biomedical Engineering, University Of British Columbia, Vancouver, BC, CANADA.

With the inter-organelle distance of mitochondria-ER contacts (MERCs) below the resolution of 3D fluorescence and super-resolution microscopy, identification and morphological analysis of MERCs is restricted. We illustrate how a novel Membrane Contact Site detection algorithm (MCS-DETECT) is able to accurately reconstruct sub-precision MERCs from 3D STED super-resolution microscopy and describe their morphological diversity. Our approach reconstructs the sub-precision MERC interface using a windowed Spearman correlation of the 2nd intensity differential, making the approach robust against inherent fluctuations in fluorescence markers across channels and datasets. To enable quantitative analysis, we compute shape features of the produced contacts, as well as a confidence map to report on reliability of contact detection. We validate MCS-DETECT by a parallel electron microscopy (EM) study of elongated ribosome- studded MERCs (riboMERCs), present in HT-1080 but not COS-7 cells. MCS-DETECT reconstructs large, tubular riboMERCs selectively in HT-1080 cells and identifies morphological

differences between riboMERCs and large contacts induced by expression of an ER-mitochondria linker in COS-7 cells. MCS-DETECT registers decreased large riboMERCs in Gp78 knockout HT-1080 cells, and increased riboMERCs, that retain the elongated, tubular morphology, upon overexpression in COS-7 cells of wild-type Gp78 but not a Ring finger mutant Gp78 lacking ubiquitin ligase activity. Gp78-dependent riboMERCs present complex tubular shapes that intercalate between and contact multiple mitochondria. MCS-DETECT applied to whole cell 3D super-resolution microscopy therefore shows that Gp78 ubiquitin ligase activity regulates the formation of novel tubular shaped riboMERCs.

B34/P1033

MINFLUX facilitates the analysis of macromolecular complexes via light microscopy

C. A. Wurm, M. Velasco, K. Bahlmann; Abberior Instruments America, Jupiter, FL.

Cells assemble macromolecular complexes into scaffoldings that serve as substrates for catalytic processes. Although dimension of these scaffolds varies, their investigation is often hampered by the available resolution of the applied (light) microscopes and/or the molecular specificity of the available labeling technology.
MINFLUX nanoscopy is capable of resolving structures as small as 1 - 2 nm [1]. For this, single fluorophores are localized by reading out their fluorescence signal at pre-defined positions in its vicinity. 3D MINFLUX nanoscopy allows researchers to investigate the arrangement of molecules in biological samples with a localization precision < 3 nm in all three dimensions. By combining MINFLUX nanoscopy with conventional confocal microscopy, structures of interest can be identified using reference marker proteins whereupon the protein of interest is analyzed at a molecular level. Colocalization studies can take advantage of the resolution in the nanometer range using two-color MINFLUX imaging.
At the basis of a current research project, we will exemplify the possibilities of the current MINFLUX nanoscopy and how MINFLUX can be applied to overcome the limitations of alternative technologies.
References [1] Schmidt et al. (2021) MINFLUX nanometer-scale 3D imaging and microsecond-range tracking on a common fluorescence microscope. Nat Commun. 2021 Mar 5;12(1):1478

B35/P1034

Molecular architecture of clathrin plaques and pits defined by super-resolution network analysis (SuperResNET)

I. Khater[1], **T. Wong**[2], C. Hallgrimsson[1], S. Moparthi[3], S. Vassilopoulos[4], G. Hamarneh[1], I. Nabi[2]; [1]Simon Fraser University, Vancouver, BC, CANADA, [2]University of British Columbia, Vancouver, BC, CANADA, [3]Sorbonne Universite, Paris, FRANCE, [4]Sorbonne Université, Paris, FRANCE.

SuperResNET is an integrated software that performs a set of processing operations to quantify and visualize 3D point clouds generated by single-molecule localization microscopy (SMLM) data. SuperResNET is equipped with computational modules to correct for multiple-blinking of single fluorophores, filter-out noisy events, segment clusters of localizations, extract various cluster features, and apply machine learning-based grouping to identify the biological clusters. SuperResNET was previously applied to caveolin-1 labeled cells, identifying the structure of caveolae and three distinct non-caveolar scaffolds. Here, application of SuperResNET to clathrin labeled HeLa cells identifies three groups: Class I: larger plaques; Class II: intermediate sized hollow pits and vesicles; and Class III: smaller oligomers. Cos7 cells present reduced number of plaques and treatment with the clathrin endocytosis inhibitor pitstop decreases plaque number in HeLa cells. Consistently, large clathrin plaques are not

observed by platinum replica electron microscopy in pitstop-treated cells. A second grouping of Class II pits based on shape features distinguishes larger, spherical and more hollow structures from smaller, elongated and more compact structures that accumulate upon pitstop treatment. Association of clathrin pits with plaques together with loss of plaques upon pitstop inhibition of clathrin endocytosis supports clathrin plaques as a reservoir for clathrin-dependent endocytosis. SuperResNET 2D and 3D visualization tools help detail the structural transition of clathrin budding from flat lattices into curved pits representing a powerful tool for analysis of the molecular architecture of subcellular, macromolecular organelles and structures.

B36/P1035

High resolution imaging reveals microclustering and large-scale reorganization of Fcγ receptors during Rituximab-mediated phagocytosis of B lymphoma cells

S. Jo[1], **B. L. Scott**[2], N. P. D. Nurmalasari[2], B. R. Fischer[1], Y. M. Loyd[2], R. B. Anderson, III[2], A. D. Hoppe[1]; [1]Chemistry and Biochemistry, South Dakota State University, Brookings, SD, [2]Nanoscience and Biomedical Engineering, South Dakota Mines, Rapid City, SD.

Antibody dependent cellular phagocytosis (ADCP) is an important effector mechanism by which professional phagocytes remove infected or immunologically aberrant cells during adaptive immune responses or treatment with therapeutic antibodies. ADCP has largely been studied using small and rigid particles such as silica beads that display immobile antigens. However, infected host and malignant cells are large and most transmembrane protein antigens on their surface are mobile or transiently confined by their actin cytoskeleton. Here, we studied ADCP of B lymphoma cells by macrophages using high resolution imaging to visualize the clustering of Rituximab (anti-CD20) with respect to the macrophage membrane and recruitment of Spleen Tyrosine Kinase (SYK). We found that Fcγ receptors (FcγR) on the macrophage surface initially form microclusters, recruit SYK, and undergo large-scale reorganizations at the phagocytic synapse prior to and during engulfment of the target cell. Using CRISPR-Cas9, we created SYK knockout macrophages that were unable to complete phagocytosis. Instead, Rituximab accumulated in large patches at the base of forming phagosomes that were subsequently internalized by trogocytosis and the phagocytic cup receded. This result is consistent with SYK kinase activity being necessary to trigger redistribution of Rituximab-FcγR during engulfment thereby preventing antigenic modulation of the target. To manipulate antigen mobility, we presented IgG on supported lipid bilayers (SLB) with different lipid compositions to generate either fluid or immobile bilayers. SLB-coated beads with mobile bilayers produced more total tyrosine- and SYK- phosphorylation during internalization than immobile bilayers indicating that microcluster rearrangement enhances FcγR signal amplification. Finally, using CRISPR knockouts and drug inhibition, we found that FcγR microcluster reorganization during frustrated phagocytosis requires the WAVE2 complex and Arp2/3 in addition to SYK. Taken together, we conclude that FcγR microclustering and antigen mobility promotes FcγR-SYK signaling and the reorganization of these microclusters is important for minimizing antigen modulation and promoting successful ADCP.

B37/P1036

De novo spatial proteomic profiling of immune synapses using a machine learning-guided microscope

Y. Liu, **W. Chong**, C. Huang, Y. Lin, Y. Chen, C. Cheung, H. Chang, C. Chung, C. Chang, J. Liao; Syncell Inc., Taipei, TAIWAN.

The spatial proteome of the immune synapse (IS) between a target cell and a lymphocyte provides valuable insights for mechanistic studies in immuno-oncology and therapeutic applications. Unfortunately, the low abundance of IS and the lack of an absolute IS marker for IS enrichment before mass spectrometric (MS) analysis create barriers for an efficient IS proteomic profiling. Here, we made use of Microscoop^{TM}, an integrated platform combining microscopic imaging, machine learning-based algorithm and photochemical labeling, to achieve spatial specific enrichment of IS proteins. Raji B cells, which served as the antigen-presenting cells (APCs), was incubated with Jurkat T cells to induce IS formation. Multiple immunofluorescence images of CD3, a common IS marker, were first applied to train an image progressing algorithm with convolution neural network-based deep learning. Microscoop then automatically performed multiple rounds of fluorescence imaging, deep learning-driven pattern generation, and photochemical labeling to biotin-tag sufficient number of immune-synapses. Subsequent protein enrichment by streptavidin pull-down and MS analysis allowed identification of IS specific proteins. Using Microscoop, we successfully labeled and isolated proteins from spatially reorganized interfaces between T cells and APCs. Following proteomic analysis, several hundreds of proteins were identified, including the proteins, such as the tyrosine protein kinase LCK, that are known to be specifically associated with T-cell receptor (TCR) and involved in TCR signaling. More interestingly, we identified several novel proteins at the ISs, including proteins that are implicated in phosphatidylinositol signaling. Our data showcases the Microscoop technology as an efficient and innovative approach to unravel spatial proteome of complex biological targets. We also revealed novel factors that potentially regulate immune response at the APC-T cell interfaces, thus shedding light on immune checkpoint signaling and tumor immunotherapy.

B38/P1037

Validating AI pipelines for single cell image data

A. Asmar, Z. Benson, M. Halter, A. Plant; NIST, Gaithersburg, MD.

Measurements of dynamic changes in single cells over time provides insight into the coordinated fluctuations in gene expression within individual cells in a population. Time lapse imaging is the primary method for obtaining single cell dynamics, and we have developed an imaging workflow to capture large-scale, ultra-fast time-lapse imaging of cells. A total of more than 12 x 10^6 induced pluripotent stem cells (iPSC) can be imaged approximately every 3 minutes in phase contrast over many hours using continuous motion microscopy. Corresponding fluorescence images are acquired at 30min intervals, and together these data provide a training set for UNet inferencing that allows segmentation and tracking of unlabeled parent and daughter cells in iPSC colonies. Because our interest is to monitor and analyze intracellular single cell dynamics over a large number of cells, and because we are interested in probing these dynamics under different culture conditions as populations evolve in time, we are particularly interested in verifying the accuracy of our algorithmic workflows. The large size of the datasets precludes establishing ground truth with manual methods. We investigated the application of a reporter cell line to automatically validate our lineage tracking workflow. Using a multi-color “Rainbow” reporter line, where a mixed population of cells are differentially tagged using single or multiple constitutive

fluorescent proteins, we can establish ground truth for lineage tracking. Each cell/lineage can be assigned a unique RGB value and then compared with the output from the tracking of unlabeled cells. This workflow provides large-scale data acquisition, algorithm development and implementation, and system validation that allows us to segment and track millions of cells over short intervals and long times. This approach allows us to accurately determine division times within the population, and to quantify intracellular fluorescent biomarker fluctuations over multiple generations.

B39/P1038

REPORTING OF MICROSCOPY METADATA - HOW TO IMPROVE QUALITY AND REPRODUCIBILITY IN BIOIMAGING

M. Hammer[1], A. Rigano[2], J. Ryan[3], D. Grunwald[1], J. Lacoste[4], R. Nitschke[5], B. Schroth-Diez[6], C. Brown[3], **C. Strambio-De-Castillia**[2]; [1]RNA Therapeutic Institute, University of Massachusetts Medical School, Worcester, MA, [2]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, [3]Advanced BioImaging Facility - Department of Physiology, McGill University, Montreal, QC, CANADA, [4]MIA Cellavie, Montreal, QC, CANADA, [5]Life Imaging Center, University of Freiburg, Freiburg, GERMANY, [6]Light Microscopy Facility, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, GERMANY.

Biology increasingly relies on microscopy techniques that push the boundaries of spatial and temporal resolution to dramatically improve fundamental understanding of life and advance therapeutic solutions. Drivers of innovation such as new fluorescence techniques and detection systems have led to the routine use of complex microscopy assays, optical components, mechanical parts, and imaging conditions. As a result, microscopy has evolved from a simple instrument that provided qualitative results to a quantitative measurement tool with a variety of possible measures such as distances, times, or intensities.

Rigorous science requires that all relevant information (i.e., metadata) about an experiment be reported using the FAIR (Findable, Accessible Interoperable, and Reusable) principles. However, it is often difficult for microscope users to decide what information about their microscope is essential, and users often feel overwhelmed by the amount of technical information required to meet FAIR imaging standards. International bioimaging organizations such as BioImaging North America (BINA), Canada Bioimaging, the Royal Microscopy Society, German Bioimaging, Euro-Bioimaging, France Bioimaging, and Global BioImaging have recently joined forces with individual imaging scientists, microscope manufacturers, and standards organizations to form QUAREP-LiMi (www.quarep.org), support biomedical researchers, and provide a forum for building broad consensus on quality control, reporting, and reproducibility in microscopy.

Here we present the recently released tiered system of microscopy metadata specifications originally developed by the NIH-funded 4D Nucleome (4DN) Initiative and BINA to extend the Open Microscopy Environment (OME) data model (i.e., the basis for the popular Bio-Formats image data exchange format). Because this 4DN-BINA-OME (NBO) metadata model correlates experimental design complexity with reporting depth on microscope hardware, acquisition settings, and calibration control, it has found favor in the international imaging community and is currently being further developed to incorporate instrument manufacturer input as part of QUAREP-LiMi.

To extend the impact of the NBO-QUAREP specifications, the talk will introduce the Micro-Meta App and MethodsJ2 software tools developed to enable biomedical researchers to properly report microscopy metadata and improve the quality and reproducibility of their imaging experiments regardless of their

imaging experience. Micro-Meta app enables researchers to capture detailed metadata about microscope hardware and acquisition settings. MethodsJ2 is a Fiji/ImageJ plugin that collects image metadata from image files and the Micro-Meta App to generate methods text for publication.

B40/P1039

Aberration-corrected STED Microscopy with MATRIX Detection for Neuroscience Research

M. Velasco[1], K. Bahlmann[1], M. Meschkat[2], J. Matthias[2], C. Wurm[2]; [1]Abberior Instruments America, Bethesda, MD, [2]Abberior Instruments, Goettingen, Germany

Stimulated Emission Depletion (STED) microscopy is a well-established super-resolution imaging technique that enables the visualization of nanoscale structures in biological specimens. Among the super-resolution modalities, STED microscopy stands out due to its compatibility with challenging specimens such as biological tissue, a prevalent specimen type in neuroscience despite its complexity. In tissue samples, acquiring STED images with high signal-to-noise is challenging for two reasons. First, improving resolution results in a reduction of the volume of the effective point-spread-function, which leads to dimmer images. Since the STED effect is applied in the focal plane, the in-focus signal decreases, while the background signal from the rest of the sample has the same strength as in conventional confocal microscopy. This translates to a decrease in the signal-to-background ratio, especially in thick tissue samples where the out-of-focus background contribution is high. Second, thick tissue samples typically have non-constant refractive index maps that lead to optical aberrations that compromise the quality of the STED doughnut. This is especially true for the three-dimensional (3D) STED doughnut, and the outcome is a depletion profile that switches off fluorescence entirely rather than just confining it to a sub-diffraction-limited sized spot. This effect worsens deeper in tissue as optical aberrations accumulate, and it imposes a practical limit on the imaging depth of conventional STED microscopy in tissue. We present two approaches that work in-tandem to improve STED imaging in tissue. The first approach is MATRIX detection, which harnesses the power of an array-based detector to discriminate in-focus signal from out-of-focus background without the losses experienced with pinhole detection. MATRIX detection can thus significantly increase optical sectioning and signal-to-background ratios. MATRIX detection can also be combined with adaptive optics, which (in our implementation) relies on a deformable mirror to introduce phase distortions that negate those induced by the specimen. In this way, optical aberrations can be corrected and super-resolution can be maintained even when imaging deep in tissue. The unprecedented combination of MATRIX detection and adaptive optics is powerful and allows for deep-tissue super-resolution imaging experiments where nanoscale structures can be visualized with more native context. We will present the intuitive and user-friendly implementation of MATRIX detection and adaptive optics on a state-of-the-art Abberior Facility Line STED microscope, and we will demonstrate how aberration-corrected STED microscopy with MATRIX detection can be applied in a range of neurobiological specimens.

B41/P1040

Characterization of *C. elegans* autofluorescence using spectral scanning and Fluorescence Lifetime Imaging Microscopy (FLIM)

E. Cameron, **H. Hulsey-Vincent**, L. Dahlberg, D. Galati; Western Washington University, Bellingham, WA

C. elegans produce a disruptive amount of autofluorescence during imaging. The greatest signal comes from granules in the gut, and a lesser amount from the cuticle. This autofluorescence has been

characterized at low spatial resolution using spectral scanning and at high spatial resolution using band pass filters. However, it has not been characterized at high resolution spectrally and spatially. We performed high resolution spectral scans of the gut granules at 405 nm, 488 nm, 561 nm, and 647 nm excitations. This revealed distinct spectral populations, with the most prominent at 405 and 488 nm.
In addition to measuring spectral differences, we used Phasor-Fluorescence Lifetime Imaging Microscopy (FLIM) to characterize differences in photon emission lifetime. The cuticle and gut granules were characterized at 440 nm and 488 nm excitations. The cuticle had a single photon decay population at both 440 and 488 nm excitation, while the gut granules had three distinct photon decay populations at both 440 nm and 488 nm excitation.
Cuticle autofluorescence hinders detection of fluorescent proteins at low levels. Using Phasor-FLIM, the auto fluorescent cuticle and GFP tagged proteins exhibit distinct lifetime populations. We demonstrate that by using Phasor-FLIM, the dim fluorescent signals which would typically be lost to noise caused by autofluorescence of the cuticle or gut granules can be separated and quantified. Further studies involving C. elegans may use Phasor-FLIM masking to distinguish between autofluorescence background and true fluorescence of commonly used fluorescent proteins and dyes.

B42/P1041

Multi-scale Unet: Incorporating machine learning to improve automated cell segmentation in time-lapse images to facilitate linage tracking of primary human hematopoietic progenitors

K. M. Ta[1], E. Thompson[1], S. Boobalan[2], **E. McEwen**[3], C. Monterosso[3], J. Duncan[1], D. Krause[1], V. Scanlon[3]; [1]Yale University, New Haven, CT, [2]University of Connecticut, Storrs, CT, [3]UConn Health, Farmington, CT

Multi-parameter single cell imaging analyses permit novel and robust dissection of the temporal molecular mechanisms governing stem and progenitor cell fate. Given our ability to acquire high throughput image acquisitions, downstream analyses become the bottleneck of projects. An accurate automated analysis is necessary for high throughput imaging. Previously established cell imaging analysis algorithms heavily rely on manual curation of both cell segmentation and linking cell tracks on a per cell basis, or require heavy feature engineering and parameter tuning to achieve reasonable results, rendering these tools very time and labor intensive.(Magnusson et al., 2015; Qin et al., 2020; Zargari et al., 2021) Our goal is to improve the automation of cell segmentation in brightfield (automatically identify individual cells and their membrane boundaries) by taking advantage of the recent successes in data driven deep learning tools. To achieve this goal, we compared several automated cell segmentation algorithms (Baxter, Unet, DeepSea) as well as our novel Multiscale Unet algorithm on their ability to identify individual cells over multiple time frames and then ran the resultant segmentation files through the automated tracking component of the Baxter algorithm. We utilized our time-lapse image data set and ground truth tracking of primary human Megakaryocytic-Erythroid Progenitor cells (CD34+Lin-CD135-cD45RACD110+CD38midCD41-) (Scanlon et al., 2022) to assess the automated performance of each algorithm. Specifically, we compared the resultant cell counts in a consistent field of view at given time points, the positions of the centroids of the segmented cells, and compare lineage trees representing the heritage of each progeny. The Multiscale Unet segmentation algorithm gave the closest automated results compared to the ground truth dataset. These results deliver a major step forward in automated image analyses of high throughput image sets, and facilitate accurate, robust, and timely results regarding the temporal molecular mechanisms that lead to cellular behavioral phenotypic changes, such as lineage commitment of progenitor cells. Results from these types of analyses could lead to improved understanding of steady state hematopoiesis as well as pathogenic changes in

progenitor cells leading to cytopenias or cancer and may illuminate novel druggable targets that instruct progenitor cell fate.

B43/P1042

Green genetically encoded voltage indicators for rapid and prolonged two photon voltage recording *in vivo*

M. A. Land[1], E. N. Zaabout[1], X. Lu[2], S. Yang[2], Z. Liu[2], X. Dong[1], A. J. McDonald[1], Y. Gou[1], V. Villette[3], S. Lai[1], M. M. Pang[4], S. Su[4,5], C. Cai[6], E. Froudarakis[1,7], N. Zhou[1], S. S. Patel[1], C. L. Smith[1], P. Bizouard[3], J. Bradley[3], T. R. Clandinin[4], A. Giovannucci[6,8,9], A. S. Tolias[2,1], J. Reimer[1], S. Dieudonné[3], F. St-Pierre[2,1]; [1]Baylor College of Medicine, HOUSTON, TX, [2]Rice University, HOUSTON, TX, [3]PSL Research University, Paris, France, [4]Stanford University, Stanford, CA, [5]Columbia University, New York, NY, [6]University of North Carolina at Chapel Hill, Chapel Hill, NC, [7]Foundation for Research and Technology Hellas, Heraklion, Greece, [8]North Carolina State University, Chapel Hill, NC, [9]UNC Neuroscience Center, Chapel Hill, NC

Monitoring neural activity on the millisecond time scale in genetically defined cells is a goal of neuroscience, but remains challenging to perform *in vivo*. A limitation of current techniques is not being able to quantitatively monitor neuronal electrical (voltage) dynamics with single-cell or subcellular resolution from large and genetically defined populations of neurons. Genetically Encoded Voltage Indicators (GEVIs) are a promising tool to bridge this gap. GEVIs are fluorescence-emitting protein sensors that report membrane potential (voltage) dynamics as changes in brightness. However, current GEVIs suffer from low sensitivity to voltage changes and are relatively dim, limiting our ability to image smaller transients and voltage dynamics in deeper layers of the cortex. GEVIs also suffer from poor photostability severely limiting their ability to be used for behaviorally relevant time scale. These issues are further exaggerated when trying to image under two-photon microscopy, a method of choice for deep-tissue imaging. Here, we report novel GEVIs optimized for long-term *in vivo* imaging under two-photon illumination. Our sensor design is based on the published ASAP family of sensors in which a voltage-sensitive domain is coupled to an extracellular circularly permuted GFP. Using high-throughput multi-parametric screening under two-photon illumination we tested mutations at amino acid positions that are structurally and evolutionary important in ASAP. Using a holistic metric we identified top sensors with improvements in sensitivity, brightness and/or photostability for further combinatorial screening. From these combination screens we identified a champion sensor called Jellyfish-derived Electricity-reporting Designer Indicator for 2-Photon microscopy (JEDI-2P). JEDI-2P is brighter, has improved photostability, greater sensitivity and faster kinetics than other leading sensors. JEDI-2P has been shown to report light-evoked responses in axonal termini of *Drosophila* interneurons. Using both resonant-scanning and ULoVE random-access microscopy, we show that JEDI-2P can also detect voltage dynamics of individual cortical neurons in awake behaving mice for more than 30 min. Additionally, JEDI-2P can be used to robustly detect spikes at depths exceeding 400 µm in pairs of neurons. Further screening has identified a new generation of sensors with improved response amplitude and kinetics compared with JEDI-2P. These new sensors have been cloned into a variety of vectors and are available for *in vivo* beta testing. We anticipate that development of our advanced GEVI toolbox will enable more robust high-throughput and real-time neural recording.

B44/P1043

Scalable 3D printed inserts for increased stability of hepatic intravital microscopy

M. Cornelius; National Institute of Health, Bethesda, MD

Confocal-based intravital microscopy (IVM) of the liver allows direct visualization of dynamic processes to evaluate hepatic function and dysfunction. Specifically, qualitative, and quantitative parameters can be extracted with high spatial and temporal precision. However, preserving tissue physiological conditions while achieving stability on the microscope stage remains challenging. This is magnified in murine models for non-alcoholic fatty liver disease (NAFLD), and Type II diabetes (T2D) where increased weight and hepatic lipid content negatively impact stability. We developed novel intravital microscopy inserts to address these limitations, allowing improved tissue access and enhanced stabilization. The production process of this prototype platform is based on 3D printing of the insert in a single-step process, which includes scalable sizes to accommodate small to large-size mice. The feasibility of production and the advantages of this customizable and versatile design are shown and discussed in this paper. Our design represents a significant step forward in facilitating intravital imaging of metabolic dynamics in physiology, NAFLD, and T2D, using IVM in living mice

Keywords. 3D printing, hepatic lobule, hepatocyte, intravital microscopy, mouse liver, glucose uptake

B45/P1044

Isotropic, One-step 12-fold modified Expansion Microscopy (mExM)

R. X. Norman[1], J. Loi[2], M. Saotome[3], E. E. Recchia[1], Q. Rosemarie[2], S. L. Lesko[4], S. Patel[2], N. Sherer[4], B. Sugden[2], M. E. Burkard[1], A. Suzuki[2]; [1]Medicine, University of Wisconsin-Madison, Madison, WI, [2]McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, WI, [3]School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND, [4]Institute of Molecular Virology, University of Wisconsin-Madison, Madison, WI.

Macromolecular complexes are integral to cellular functions and many of them are smaller than the resolution limit of fluorescence microscopy. Super-resolution methods including STED, SIM, and STORM improve the resolution limit but are insufficient to resolve many of these structures and require specialized equipment, dyes, and mathematical processing. Expansion microscopy is an alternative super-resolution method, where the sample is embedded in polymers, and it expands upon the addition of water. The resolution of ExM can be improved by the expansion factor as it is independent of the microscope optics. In this study, we used N,N-Dimethylacrylamide (DMAA), and Sodium acrylate as monomers and polymerized the hydrogel without requiring any specialized equipment. We also designed a reusable imaging chamber and mounting media to reduce the drift of the hydrogel and photobleaching respectively during imaging. As isotropic expansion is critical to prevent sample distortion and has not been addressed in previous studies, we determined the isotropy of both the hydrogel and the embedded sample. We found that the hydrogel stably expanded ~12-folds isotropically in three dimensions. To validate the 3D isotropic expansion of specimens, we measured nuclear sizes and volumes in multiple cell lines including human organoids. We chose the interphase nuclei since it is the largest organelle and has unique features such as nuclear membranes and a conserved 3D architecture. We found that our optimized method achieves 12-fold isotropic expansion of nuclei in various monolayer cells and organoids. Next, to validate the preservability of nuclear structures after expansion, we determined the 3D chromatin conformation using the Pore-C method. We found that the 3D organization of chromatin is not disrupted upon expansion suggesting that our 12x mExM method

preserves the chromatin at the microscopic and molecular levels. To examine the structural preservation and resolution, we imaged microtubules (~25 nm width) and centrioles (~250 nm) using mExM and confirmed that these structures are well preserved after 12-fold expansion. The thickness of microtubules using mExM was ~29 nm compared to ~257 nm pre-mExM. In further validations, we found that our method was able to preserve RNA transcripts at the HIV-1 genome integration site in a cell. Additionally, our method could visualize single Epstein-Barr Virus (EBV) particles in a lytic host cell. The EBV envelope diameter measurement was comparable between mExM and electron micrograph, showing the strong potential of this method in studying nanometer-scale structures. Since this method uses commonly available reagents and equipment, we think that it could also serve as an economical super-resolution method for a wider scientific audience.

B46/P1045

Deformable Phase Plate: a New Technology for Plug-and-Play Adaptive Optics

S. M. Weber, P. Rajaeipour; Phaseform GmbH, Freiburg i.B., Germany

Adaptive optics (AO) comprises a set of powerful opto-electromechanical techniques that enable dynamic correction of optical imaging errors. Despite their proven advantages for various microscopy techniques in life sciences, especially for deep tissue imaging, the widespread use of AO is still hindered by extreme complexity and integration costs. These disadvantages are mostly related to the main elements of AO systems, typically deformable mirrors (DM), and the wavefront metrology methods. The integration of such hardware into microscopes usually requires significant modifications or, in most cases, the development of new setups taking into account the AO design constraints. The Deformable Phase Plate (DPP) described in our poster presentation is a refractive counterpart of DMs, able to compensate for high-order aberrations, enabling higher contrast, sharper images and tighter focal spots for multiphoton microscopy. Its transmissive working principle combined with sensorless aberration measurement techniques enables plug-and-play AO systems. Like a lens in a conventional cage, DPP can be inserted into the beam path of microscopes to provide dynamic correction of system- and sample-induced aberrations. In our contribution, we will demonstrate the use of DPP to compensate for sample-induced aberrations in the illumination path of a two-photon microscope for focusing deep into biological tissue. By dynamically measuring and correcting aberrations using DPP, we show that it is possible to significantly improve the quality of the images obtained while simplifying sample preparation. AO is progressing daily to become accessible to a wider range of users, and our DPP technology, commercialized by the spin-off Phaseform from Freiburg in Germany, has the potential to play a signifcant role in this development.

B47/P1046

Chemigenetic fluorescent biosensors for imaging and screening signaling protein activity

L. W. Miller; University of Illinois-Chicago, Chicago, IL

Genetically encoded, Förster resonance energy transfer (FRET) biosensors enable live-cell optical imaging of signaling molecules. Small conformational changes often limit the dynamic range of biosensors that combine fluorescent proteins (FPs) and sensing domains into a single polypeptide. To address this, we developed FRET and lanthanide-based FRET (LRET) biosensors with two key features that enhance sensitivity and dynamic range. For one, alpha helical linker domains separate FRET partners and ensure a large conformational change and FRET increase when sensing domains located

near each terminus interact. We have demonstrated that linkers increase dynamic range in a length-dependent manner. Another key feature that enhances sensitivity is the incorporation of luminescent Tb(III) complexes as FRET donors. The long excited-state lifetimes (~ms) of Tb(III) and Tb(III)-sensitized FP emission signals favor time-resolved luminescence (TRL) detection strategies that nearly eliminate non-specific fluorescence background. TRL plate readers and microscopes use pulsed light to excite specimens and detect long-lived signals after a brief delay (~µs) that allows short-lived (~ns) fluorescence to decay. LRET biosensors enable medium (96-well) and high (384-well) throughput TRL assays of protein-protein interaction inhibition in cell lysates or intact cells. This presentation elaborates the technology and presents data on cellular imaging and screening with sensors that measure Rac1 GTPase and PP1 phosphatase activities.

B48/P1047

Exploring cellular context with plasma-focused ion beam scanning electron microscopy

G. Perumal, R. Vernon, R. Chalmers, A. Rigort, J. Mitchels; Thermo Fisher Scientific, Hillsboro, OR.

Focused ion beam and scanning electron microscopes (FIB/SEM)can be used to obtain three-dimensional volumetric data from biologicalspecimens. The unique combination of an imaging and precision manipulation toolallows sequentially removing material with the ion beam and imaging the milledblock faces by scanning with the electron beam, an approach known as FIB/SEMtomography. This technique can be applied to both resin-embedded samples andcellular specimens which have been preserved by cryo-fixation. Forcryo-imaging, the FIB/SEM instrument needs to be equipped with a cryo-system toenable imaging of samples that have been cryo-immobilized (vitrified) by eitherhigh-pressure freezing (HPF) or plunge-freezing methods. Here we demonstrate how biological samples can be imagedusing multi-ion plasma FIB (PFIB) technology. We exploit the fast materialremoval capabilities of the plasma beam for the newly developed spin millmethod, which is suitable for processing and imaging large sample areas onresin-embedded samples. We show results for spin milling on a mouse organotypicslice, where we exposed a large area (horizontal field width 638µm) of thesample surface and subsequentially imaged three different areas of interestrevealing structural details. In addition, we also demonstrate new results forcontextual imaging of cryo-samples, which we mill with different ion beamsoriginating from the plasma source. The cryo-samples were milled using eithernitrogen or argon ions and 3D data was acquired by collecting 20 nm slice datawith the secondary electron detector. By volume imaging of frozen mouse braintissue, Chlamydomonas and CHO cells, fine structural details such as synapses,subcellular compartments and organelles can be visualized without the need to treatthe sample with fixatives or stains. The PFIB technique offers new possibilities for theexploration of biological samples and enables both large volume milling ofcells and tissues and precise preparation of samples for electroncryo-microscopy.

B49/P1048

FIB-SEM, a resolutive 3D large volume method to assess flagellar ultrastructural organization

E. Penard, S. Bonnefoy, M. Majrouh, N. Cayet, T. Blisnick, P. Bastin, A. Mallet; Institut Pasteur, Paris, France

Transmission Electron Microscopy (TEM) is the imaging method of choice to visualize intracellular structures but it is limited to the observation of 500 nm thick slice for electron tomography approaches. Many organelles such as cilia and flagella have a larger size which measure about 20 µm. To follow along

the whole flagellum, we need another 3D high-resolution approach to observe structural details like microtubules, involved in intracellular organization.The Focus Ion Beam-Scanning Electron Microscopy (FIB-SEM) is a 3D electron microscopy method allowing the obtention of a volume in x-y-z at isotropic resolution of 10 nm. This microscope is composed of an ionic beam which precisely mill our sample, and an electronic beam to observe the surface of our sample. Here, we study *Trypanosoma brucei,* and more particularly its flagellum with the axonemal microtubules and extra-axonemal structures. The *T.brucei* genome contains several TTLLs enzyme (Tubulin Tyrosine ligase like) involved in the tail addition of glutamate residues to tubulin α or β. We focalized on TTLL9 enzyme, involved in distinct glutamylation enzymatic activities and structural as well as functionality issues. Using next FIB-SEM, this provided a high-resolution isotropic 3D view of structural axonemal and flagellar defects along the whole flagellum of *ttll9-/-* knockout cells. Notably, it allows to visualize frequent disorganization of the axoneme or distribution of excessive Paraflagellar Rod structure (PFR) as well as mis-orientation of the central pair microtubules all along the flagellum. Our results clearly demonstrate and confirm the importance of FIB-SEM to address the topology of structural defects previously detected by TEM and prime importance to 3D image analysis. The FIB-SEM is a long technique requiring high expertise. In this poster, data acquired from isolated cells demonstrate that FIB-SEM can be used to answer important biological questions that can be addressed to various types of samples, microorganisms, infected cells or tissues.

B50/P1049

Autonomous Cell Feature Selection by Machine Learning

V. Tiryaki[1], **V. Ayres**[2], I. Ahmed[3], D. Shreiber[4]; [1]Siirt University, Siirt, Turkey, [2]Michigan State University, East Lansing, MI, [3]CUNY Medgar Evers, New York, NY, [4]Rutgers, The State University of New Jersey, Piscataway, NJ

Automated cell segmentation is key for rapid and accurate investigation of cell responses. As instrumentation resolving power increases, clear delineation of newly revealed cellular features at the submicron through nanoscale becomes important. Reliance on the manual investigation of myriad small features retards investigation; however, use of deep learning methods has great potential to reveal small cell features both at high accuracy and high speed, which may lead to new discoveries in the near term. Adaptation of deep learning methods for cellular investigations has received intense interest in the last decade because of the success of these methods in image classification, segmentation, and other problems involving recognition. Until recently, while cell segmentation could be completed with machine learning techniques, the performance was limited, and the selection of an optimum feature extraction method could be time-consuming. The main problem is determining the feature extraction method, which can vary depending on the classification problem. Deep learning methods can resolve this uncertainty because they allow automatic feature learning. In this study, semantic cell segmentation systems were investigated by implementing fully convolutional neural networks called U-nets for the segmentation of astrocytes cultured on poly-L-lysine-functionalized planar glass. As its first focus, the performances of major types of U-nets for automatic cell segmentation in AFM images were investigated. This is the first investigative study that we are aware of that uses U-net models for cell segmentation of AFM images. AFM height, deflection, and friction images were used as inputs separately and together, and the segmentation performances were investigated on five-fold cross-validation data. When transfer-learning methods were applied, statistically significant segmentation performance improvements were observed. Segmentation optimization for AFM versus electron

microscopy (EM) images was next investigated. The publicly-available dataset ISBI 2012 provided as an EM segmentation challenge was used and alternative current and classical methods were investigated. The best EM segmentation performance was obtained when Cellpose transfer learning [2] was applied while U-VGG19 outperformed for AFM images. The higher performance of U-VGG19 on the AFM data shows that transfer learning on the deep networks originally trained with big data can outperform transfer learning on the deep networks that are originally trained with a more similar but smaller datasets. [1] Tiryaki VM, et al. Cytometry: Part A, 2022, 101(6): 507-520. (DOI: 10.1002/cyto.a.24533)[2] Stringer C, et al. Nat Methods, 2021, 18:100-106. (DOI: 10.1038/s41592-020-01018x)

B51/P1050

Label-free imaging of adipocyte differentiation process and lipid-droplet analysis using low-coherent holotomography

H.-J. Kim; Tomocube, Inc., Daejeon, Korea, Republic of

Visualization and time-lapse tracking of adipocytes and their lipid droplets (LDs) in the process of differentiation are challenging due to the long-term differentiation period. The traditional fat staining method, such as oil red-O staining, had a disadvantage in that it was impossible to observe the state of living. Using fluorescence dye (BODIPY or Nile Red), living cells can be observed but it is difficult to track them for a long period due to the persistence problem of fluorescent dyes. Holotomography (HT) has emerged as a useful tool for imaging live specimens without additional pre-treatment such as fixation. Here, we applied a recently developed low-coherent holotomography imaging system (HT-X1) which provides label-free 3D visualization of live cells. Since LDs in HT images have a high refractive index (RI), they can be clearly distinguished from cells. We performed differentiation of 3T3-L1 fibroblast into adipocyte in the HT-X1 system and acquired a time-lapse movie of the differentiation process for 14 days. The high RI sphere structure was verified LD by fluorescent staining. We also observed the redifferentiation process from primary dedifferentiated adipocyte into mature adipocyte for 42 days. Images were acquired every 3 days in the same region, and the continuously increasing LD contents were quantitatively measured. The volume, projected area, and dry mass of LDs were increased according to the adipocyte maturation. Through the results, we suggest that low-coherent holotomography has an exclusive capability for phenotypic and quantitative studies of adipogenesis and LD accumulation in various cell types.

B52/P1051

Quantitative phase imaging from brightfield: a new gold standard for cell confluence and counting

T. M. Joska, H. Fried, K. Chapman, A. Blanchard; Thrive Bioscience, Beverly, MA

Live cell imaging is a cornerstone in cell biology but manual evaluation of cell health by confluence, doubling rates, and cell counts remain labor intensive and subject to human error. Automated imaging approaches often only provide qualitative data, and frequently require toxic dyes that can confound results and harm or kill the cells in question. Thrive Bioscience, Inc. provides automated live-cell quantitative phase imaging from brightfield images (QPIB) across wells and plates within minutes offering greater reproducibility than manual techniques. The Thrive CellAssist system is a tightly integrated hardware and software solution for acquiring, analyzing, storing, and visualizing live cell images. Expandable from a single bench-top unit to a global network of automated, environmentally controlled imagers, it elevates imaging of live cells in culture from an occasional, manual process with

sporadic documentation, to a reproducible, routine, automated process that provides unparalleled insight into your cells' behavior. The Thrive CellEval software uses QPIB over every pixel to render stitched 3D images that outline cells as a means to count and derive confluence measures, track cell motility and growth rate, and much more. Confluence and cell counting measurements are validated over time with precise segmentation and optical volume measures. Further uses are automating scratch gap/migration assay by 1) automated identification of the scratch gap region, 2) the selection of regions devoid of the plowing effect, and 3) tracking of growth into the gap region over time. **At Thrive Bioscience**, *we image differently*. For more information on the CellAssist and CellEval software, visit our booth to explore the power of z-stacks and learn how brightfield images deliver superior laboratory workflows to improve experimental outcomes.

B53/P1052

Pipeline development for correlative cryofluorescence microscopy and electron tomography of isolated plasma membranes.

W. Sun, D. Michalak, K. Sochacki, N. Mizuno, J. E. Hinshaw, J. Taraska; National Institutes of Health, Bethesda, MD

The proteins associated with the plasma membrane of mammalian cells control motility, adhesion, cargo import and export, and signaling. Visualizing their structures in the heterogenous molecular environment of the cell is key to understanding their cellular functions in health and disease. Cryo-electron tomography (cryoET) is a powerful tool for identifying and reconstructing *in situ* protein structures at sub-nanometer resolution. Despite its strengths, the efficacy of cryoET is limited by sample thickness (requires <200 nm) and has few tools for identifying proteins of interest. Thus, methods for sample thinning and protein labeling are necessary for a broad implementation of the technique. Specifically, the standard ion-milling techniques used for sample thinning are not well-suited for observing proteins at the plasma membrane. Here, we optimize a cryo-correlative light microscopy and electron tomography pipeline for the study of plasma membrane-associated organelles and protein complexes. This pipeline employs a method called “unroofing” to isolate plasma membranes immediately before freezing. By modifying the unroofing procedure we can produce ultra-thin samples of both the basal and apical plasma membrane from different types of cells. Protein probes designed for correlative microscopy are being developed and optimized to allow us to identify specific proteins of interest in cryoET images. This pipeline is being benchmarked with sub-tomogram averaging of previously solved structures. The goal of this work is to enable efficient high-resolution visual proteomics of the plasma membrane of eukaryotic cells.

B54/P1053

Visualizing cytosolic and mitochondrial ATP changes upon metabolic inhibitions *in vitro* via a novel multi-organelle ATP biosensor

D. White, III, Q. Yang; Louisiana State University Health Sciences Center - New Orleans, New Orleans, LA

Adenosine 5’ triphosphate (ATP) is the main fuel resource utilized by all living cells. 90% of ATP is produced via mitochondrial oxidative phosphorylation (OXPHOS), with the remaining portions produced by glycolysis in the cytosol. Our lab designed a genetically-encoded fluorescent ATP indicator, called smacATPi (simultaneous mitochondrial and cytosolic ATP indicator), that allows for real-time visualization of cytosolic and mitochondrial ATP *in-vitro*. smacATPi combines previously described

individual cytosolic and mitochondrial ATP indicators, encoded for chimeric proteins of GFP- and mApple-F_0F_1-ATP synthase ϵ subunit (*B. subtilis*), with the latter containing a mitochondrial targeting sequence. Bioenergetics measured using a Seahorse bioanalyzer showed no metabolic interferences in cultured HEK293T cells transfected with smacATPi. To investigate the utility of smacATPi, mitochondrial and cytosolic ATP signals were investigated post-treatment of metabolic effectors. 2-deoxyglucose (2-DG) (25µM), a glycolysis inhibitor, triggered a 30% rapid decline in cytosolic ATP production and a 15% decrease in mitochondrial ATP. Oligomycin (5µM), a complex V inhibitor, induced a 10% decrease in mitochondrial ATP production and a 10% increase in cytosolic ATP. Within the inner mitochondrial membrane (IMM), the ADP/ATP carrier (AAC) protein plays a vital role in the appropriate movement of ADP and ATP between the cytosol and mitochondria. Although AAC has been extensively studied, its effects on ATP production have not yet been evaluated - especially in the setting of hypoxia. Atractyloside (ATR) is an AAC inhibitor, blocking ATP and ADP movement across the IMM. After ATR (100µM) treatment, both mitochondrial and cytoplasmic ATP signals were respectively decreased by 15% and 10%, indicating that inhibition of AAC-mediated ADP import would reduce both cytosolic and mitochondrial ATP content. In hypoxia, mitochondrial ATP was increased, and cytosolic ATP was decreased. ATR (100µM) treatment 2 hours post-initiation of hypoxia triggered an increase in cytosolic ATP compared to hypoxia alone, along with a downward trend of mitochondrial ATP. These results, for the first time, demonstrate real-time cytosolic and mitochondrial ATP changes upon metabolic inhibitions, suggesting complex and rapid ATP regulation between cytosolic and mitochondrial compartments under normoxic and hypoxic conditions.

Actin and Actin Associated Proteins 1

B56/P1054

Unraveling the role of the Arp2/3 complex in the NHEJ pathway of DNA repair

J. Salat, D. Mullins; University of California, San Francisco, San Francisco, CA

Actin has been extensively studied in the cytoplasm for its structural functions such as cell morphology, movement, and adhesion. On the other hand, research on the functions of actin in the nucleus has only begun to emerge. Previous work in our lab has shown nuclear actin filaments form in response to DNA damage in mammalian cells. The development of a nucleus-specific actin probe was crucial in visualizing actin filaments in the nucleus and determining that actin assembly is required for the DNA damage response. Actin regulators are involved in controlling when and where actin is assembled. The Actin Related Protein 2/3 (Arp2/3) complex is an actin regulator that nucleates an actin filament off an existing filament to form a branch. In the cytoplasm, the Arp2/3 complex nucleates actin branches that generate force to move membranes and is important for many cellular processes such as cell movement and phagocytosis. Recent research has found that the Arp2/3 complex is present in the nucleus where it is required for efficient DNA repair in the homology-directed repair pathway. Due to the Arp2/3 complex involvement in DNA repair, we tested whether it can bind to DNA and our fluorescence anisotropy data show the Arp2/3 complex binds to single-strand DNA with a K_d of 98 nM and double-strand DNA with a K_d of 382 nM. We also found that Arp2/3 nucleation activity is not disrupted by DNA binding and shown that in CH12 cells, a mouse B-cell model, the Arp2/3 complex is required for efficient cell proliferation and non-homologous end joining (NHEJ) during immunoglobulin class switch recombination.

B57/P1055

Nuclear actin polymerization promotes DNA damage response

A. Varlet; Cornell University, Ithaca, NY

The DNA damage response (DDR) is a key process to maintain genetic stability and integrity. Recent findings support that nuclear actin assists DDR by serving as a scaffold for protein complexes or as an ATPase. However, it is not clear whether nuclear actin filaments (F-actin) are required for these processes. To identify the role of nuclear F-actin in DDR, we generated mouse and human cell lines stably expressing GFP tagged actin targeted to the nucleus using a nuclear localization sequence (GFP-NLS-actin), using either wild-type (WT) actin or a non-polymerizable actin R62D mutant. Double strand breaks were induced by treating cells with phleomycin for 3 hours and DDR was measured by staining for γH2Ax. Expression of NLS-actin R62D resulted in significantly reduced levels of γH2Ax in response to phleomycin treatment compared to NLS-actin WT and NLS-GFP controls. To exclude the possibility of a delay in γH2Ax phosphorylation in response to inhibition of nuclear actin polymerization, we treated cells with phleomycin for various durations up to 24 hours. Cells expressing mutant actin showed lower levels of γH2Ax that persisted for 24 hours, in comparison to both NLS-actin WT and NLS-GFP. Lastly, to assess whether nuclear F-actin regulates γH2Ax phosphorylation in response to various types of DNA lesions, we induced either replication stress with camptothecin and hydroxyurea, or single strand DNA breaks with 4-nitroqulinoline N-oxide. Expression of NLS-actin R62D caused a significant reduction of γH2AX in response to either drug treatments compared to NLS-actin WT control cells. Altogether, these results suggest that impairing nuclear actin polymerization inhibits γH2Ax phosphorylation in response to various types of DNA damage. Nonetheless, the molecular mechanism by which nuclear F-actin modulates DNA damage or DDR remains to be clarified. Insights into how nuclear F-actin regulates DDR will broaden our understanding of fundamental aspects of nuclear actin in cell and molecular biology and reveal new potential therapeutic avenues for the treatment of multiples defective DDR-associated diseases such as cancer.

B58/P1056

Shifts in Nucleocytoplasmic Compartmentalization of the Actin-Binding Protein CapG Analyzed With FRAP

M. Fernandez, M. Sinha, M. Renz; Stanford, Palo Alto, CA

The Gelsolin-related actin-binding protein CapG is the only member of its family that distributes diffusively in the entire cell, i.e., cytoplasm and cell nucleus. The function of CapG in the cell nucleus is unknown. In previous work, we showed a difference in CapG's nucleocytoplasmic shuttling in cancer and normal cells. Here, we set out to further characterize determinants of CapG's nucleocytoplasmic compartmentalization.

We are measuring changes in CapG-GFP nuclear import kinetics in one and the same cell by using Fluorescence Recovery after Photobleaching (FRAP) repeatedly in the same cell. Metabolic depletion using Na-azide and 2-de-oxyglucose decreases the CapG nuclear import indicating an energy-dependent nuclear transport process. The decrease, however, is not irreversible and can be rescued by washing the cells and replacing complete media. The addition of epidermal growth factor (EGF) increases nuclear CapG import in 40% of the measured cells. It is a fast, non-genomic effect, taking effect after only 8 min, further increasing after 30 min, and plateauing at about 50 min. At the same time, the addition of EGF does not shift the steady-state distribution of CapG as shown by a constant nucleocytoplasmic

fluorescence intensity ratio before and after the addition of EGF. In cells that have been serum-starved for 20-hrs and arrested in G0, the addition of EGF triggers an increase in CapG nucleocytoplasmic shuttling in up to 80% of the analyzed cells. Based on our preliminary data, mutating the potential phosphorylation site S337 does not abrogate the EGF-triggered increase in CapG transport. We continue to examine critical phosphorylation sites using repeated FRAP measurements in one and the same cell. We established repeat FRAP experiments in one and the same cell that does not result in apparent cell toxicity but can resolve increase and decrease in CapG nucleocytoplasmic shuttling with EGF and metabolic depletion, respectively. We will continue to use this assay to further describe determinants of CapG intracellular compartmentalization.

B59/P1057

Cdc42ep3-bound septin filaments promote actin filament assembly.

M. Tomasso, S. Padrick, P. Mehetre, E. Brinckman, R. Ravi, J. Byrnett; Drexel University College of Medicine, Philadelphia, PA

Septins are filament-forming proteins that are involved in numerous cellular processes. Septins colocalize with actin, microtubule and membrane structures. The Cdc42 effector protein Cdc42EP3 (also known as BORG2) regulates septin localization to cellular actin structures, but it is unknown how Cdc42EP3 controls septin-actin association. Using biochemical analysis with purified components, we show that Cdc42EP3 binds to both septins and actin in a Cdc42-regulated fashion. Importantly, septin-bound Cdc42EP3 accelerates actin filament polymerization. Septin filaments composed of SEPT2, SEPT6 and SEPT7 directly interact with actin filaments, although this occurs less efficiently in pre-formed filaments. Thus, Cdc42EP3 is not needed to recruit actin filaments to septin filaments at equilibrium. Instead, Cdc42EP3 recruits actin monomers to septin filaments, promoting localized actin filament formation and septin-actin structure assembly.

B60/P1058

Coordinated septin-actomyosin assembly in the stable bleb cortex

A. M. Paguntalan, P. W. Oakes, J. R. Beach, A. Caillier; Loyola University Chicago, Chicago, IL

The cell cortex is an active contractile network that provides the basis for many essential cellular processes, including morphogenesis, migration, communication, and mechanosensing. Cortical networks are comprised of two key cytoskeletal components: filamentous actin (F-actin) and non-muscle myosin IIA (NMIIA), while additional actin-binding proteins regulate filament turnover, stabilization, bundling, and crosslinking. Mechanical properties, especially contractility, of the cortex depend on both the organization of F-actin and NMIIA-dependent force production. Cortical mechanics are known to modulate cell-matrix adhesion, membrane dynamics, ion channel activity, and more. Thus, understanding the dynamic assembly of cortical networks is of fundamental importance. Septin-family GTP-binding proteins have been proposed as molecular scaffolds for cytoskeletal organization, such as the septin collar that mediates assembly of the contractile actomyosin ring during cytokinesis. At sites of mechanical strain, septins can assemble into higher order structures and bind actin filaments via NMIIA and other actin-binding proteins to reinforce structural stability. Yet, the precise mechanisms by which the mammalian septin cytoskeleton contributes to cortical architecture and stability remain unclear. Because examination of cortical networks is often obscured by high network density and internal cytoskeletal structures, a simplified model of the cortex, amenable to molecular imaging with high

spatiotemporal resolution, is required. Using a specialized micropillar confinement system, we mechanically compress mammalian fibroblasts and induce a transition from mesenchymal to stable bleb-based migration, characterized by the down regulation of stress fibers and focal adhesions and the formation of a single elongated leader bleb. By expressing fluorescently tagged cytoskeletal constructs (e.g. actin, NMIIA, septins, or Rho GTPases), we track the localization and coincidence kinetics of these proteins in our stable bleb system via high resolution live-cell microscopy. We confirm that, as previously described, NMIIA-driven contractility at the proximal base of the bleb generates a cortical density gradient via retrograde advection, which enables stable bleb-based migration. Due to the diversity of cortical architecture and lack of internal structures, this confinement-based inducible-bleb system provides a minimalist, yet relevant, model of the contractile cortex. Further studies will reciprocally investigate the necessity and sufficiency of septins and actomyosin during the assembly and remodeling of these contractile cortical networks, thereby providing insights into biophysical properties of cortices found in every cell in the body.

B61/P1059

Septin-actin crosstalk at the bud neck in*S. cerevisiae*

J. Magliozzi, S. D'Amaddio, B. Goode; Brandeis University, Waltham, MA

Septins and actin make up two separate cytoskeletal polymer systems in eukaryotic cells. However, mounting evidence from diverse species indicates that septins play important roles in regulating actin cytoskeleton spatial organization and dynamics. The goal of our work is to define the mechanistic basis for septin-actin crosstalk, using budding yeast as a model. In *S. cerevisiae*, septins polymerize into a higher order networks found at the bud neck, and recruit and scaffold > 50 other proteins to control polarized cell growth and cytokinesis. Using super-resolution microscopy, we recently discovered that yeast septins are organized into 8-10 evenly-spaced vertical bars, or 'pillars', which align with actin cables to facilitate transport of secretory vesicles to the bud tip (Garabedian et al., 2020). In addition, we showed that septin pillars are decorated by the formin Bnr1 and the F-BAR protein Hof1, which directly inhibits Bnr1, directly binds to F-actin, and links septins and F-actin in vitro. Here, we begin to investigate how three other septin-associated proteins located at the bud neck potentially influence actin cable organization and function: IQGAP (Iqg1), type-II myosin (Myo1), and the F-BAR protein Syp1 (homolog of FCHO1/2). Importantly, most of what is known about the functions of these three proteins is restricted to cytokinesis, whereas here we are investigating their potential roles much earlier in the cell cycle, during bud development. Using auxin inducible degron (AID) tags, we show that acutely depleting cells of either Iqg1 or Myo1 leads to severe defects in actin cable organization and secretory vesicle transport. Further, we show that Syp1, similar to Hof1, directly binds to and bundles F-actin, and physically links septins and actin in vitro. However, in contrast to Hof1, Syp1 enhances (rather than inhibits) Bnr1-mediated actin nucleation. Overall, these results demonstrate that yeast septin pillars recruit multiple proteins that directly bind F-actin and/or formins, each making genetically unique contributions to controlling actin cable organization and dynamics.

B62/P1060

Mathematical modeling and biochemical analysis support partially ordered CaM-MLCK binding

M. J. S. MacEwen[1], D. Rusnac[1], H. Ermias[1], J. P. Dexter[2], Y. Sancak[1]; [1]Pharmacology, University of Washington, Seattle, WA, [2]Data Science Initiative and Department of Human Evolutionary Biology, Harvard University, Cambridge, MA.

Smooth muscle contraction depends on the activation of myosin light-chain kinase (MLCK) by calcium ions (Ca^{2+}) and calmodulin (CaM). Despite extensive biochemical analysis of this system, aspects of the mechanism of activation remain controversial, and competing theoretical models have been proposed for the binding of Ca^{2+} and CaM to MLCK. Though many of these models make assumptions about binding parameters, there is little literature that tests the models biochemically. Nevertheless, the proposed Ca^{2+}-CaM-MLCK binding models are analytically solvable for an equilibrium steady state and give rise to distinct predictions that hold regardless of the numerical values assigned to parameters. These predictions form the basis of a recently proposed, multi-part experimental strategy for model discrimination. Our team implemented this strategy by measuring Ca^{2+}-CaM-MLCK binding using an *in vitro*, plate reader-based FRET system. This system quantifies the binding of either wild-type or mutant CaM with defective EF-hand domains to a FRET reporter that uses a fragment of MLCK protein as a proxy for the full protein. We also performed Octet analysis (Bio-Layer Interferometry) as a second method to detect binding between FR and CaM and to corroborate the principal findings of our plate reader-based FRET assays. We observed binding between MLCK and either wild-type CaM or CaM with an N-terminus EF-hand mutation; we observed zero binding between MLCK and CaM with a C-terminus EF-hand mutation. Interpretation of our binding data in light of the mathematical models suggests a partially ordered mechanism for the binding of CaM to MLCK. In the literature of systems biology, it is rare for competing mathematical models to be assessed using biochemical experimentation, much less for such experimentation to decisively favor one model above the others. We believe that our unconventional and powerful strategy brings a valuable fresh perspective to the field. Our work may also prove useful in a translational or pharmaceutical context, as MLCK and its activation by CaM have been linked to the pathogenesis of human disease.

B63/P1061

ROCK activation contributes to Arp2/3 complex-deficient macrophage phenotypes

R. Manickam, **J. Rotty**; Uniformed Services University, Bethesda, MD.

In recent years there has been significant interest in understanding the interplay between different populations of actin filaments, and the factors that organize them. One prominent example is the mutually antagonistic relationship between non-muscle myosin II and Arp2/3 complex. One aspect of this relationship is likely due to the well-established antagonism between Rho (which activates myosin II) and Rac (which activates Arp2/3 complex). However, there is likely still more mechanism to uncover regarding the interplay between these important cytoskeletal regulators. Previous findings revealed that genetic deletion of the Arp2/3 complex (*Arpc2-/-*) in macrophages generates phenotypes consistent with hyperactive myosin II. *Arpc2-/-* macrophages are significantly smaller than wildtype (WT), protrude in part via blebbistatin-sensitive surface blebs, and use myosin II to migrate faster than their WT counterparts. New data suggests that ROCK activation drives these *Arpc2-/-* phenotypes. ROCK-dependent phosphorylation of cofilin and myosin light chain are enhanced in KO macrophages. Furthermore, inhibition of ROCK or myosin II causes *Arpc2-/-* macrophages to spread and become less

elongated, thereby returning to a size and shape similar to WT cells. These data together suggest that significant elements of the *Arpc2-/-* macrophage phenotype may actually be due to ROCK/myosin II hyperactivation. Phenotypes consistent with enhanced cortical contractility led to the hypothesis that Piezo mechanosensitive ion channels may be activated in the *Arpc2-/-* macrophages. Several groups have demonstrated that Piezo activation induces ROCK activity. Treatment of WT macrophages with the Piezo agonist Yoda1 was sufficient to rapidly disrupt lamellipodia and contract cells, replicating features of the *Arpc2-/-* phenotype. This suggests that enhanced Piezo-ROCK positive feedback overwhelms the Arp2/3 complex's antagonism toward ROCK. Future experiments will be focused on determining whether acute Arp2/3 complex regulation influences Piezo activity and myosin II's ability to access the actin cortex. These experiments have the potential to reveal a mechanism whereby a precise Arp2/3 and ROCK balance dynamically alters cell morphology and behavior.

B64/P1062

An unconventional myosin-based mechanism of vesicle transport in *Toxoplasma gondii*

J. A. Kellermeier, T. E. Sladewski, Jr, **A. T. Heaslip**; Molecular and Cell Biology, University of Connecticut, Storrs, CT.

In many eukaryotes, vesicle transport is driven by molecular motor proteins that bind and transport cargo along F-actin or microtubule tracks. In the apicomplexan parasite *Toxoplasma gondii (T. gondii)*, F-actin (Act1) and Myosin F (MyoF) transport numerous vesicular cargos, including secretory vesicles. However, it is unclear how MyoF and Act1 facilitate vesicle transport because their cellular organization and biochemical properties are poorly understood. To gain mechanistic insight into how Act1 and MyoF function to transport cargo, we recombinantly purified tag-free Act1 from SF9 cells, and used a real-time microscopy-based actin growth assay to show that Act1 can form long (>60 μm) F-actin filaments *in vitro* with a 10-fold higher critical concentration than mammalian actin. We then characterized the motile properties of MyoF on its native track. Using single-molecule imaging approaches, we find that MyoF is not processive as a single motor. However, teams of MyoF can move continuously towards the plus-end of F-actin, where they become retained. Surprisingly, the tail domain of MyoF also binds directly to F-actin bundles, raising the intriguing possibility that MyoF does not associate directly with vesicular cargo but instead functions as an actin organizer in the cell. To explore this hypothesis, the localization of MyoF-EmGFP was assessed using live cell microscopy. This revealed no vesicle enrichment; instead, MyoF had a dynamic filamentous organization throughout the cytosol. To determine if MyoF plays a role in actin organization, actin was visualized in live parasites using an Actin Chromobody (ActinCB) tagged with EmGFP upon MyoF knockdown. In control parasites, we observed highly dynamic actin filaments or bundles that underwent continual rearrangement. In contrast, MyoF knockdown resulted in the formation of large actin bundles adjacent to the Golgi which had drastically reduced dynamics. Collectively, these results demonstrate that MyoF influences vesicle transport by controlling the dynamics and organization of the actin cytoskeleton in *T. gondii*.

B65/P1063

Using MVID-Causing Mutations to Investigate MYO5B Motor Function

D. M. Bowman[1,2], M. J. Tyska[1,2], J. R. Goldenring[1,2,3]; [1]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, [2]Epithelial Biology Center, Vanderbilt University Medical Center, Nashville, TN, [3]Section of Surgical Sciences, Vanderbilt University Medical Center, Nashville, TN.

Myosin Vb (MYO5B) is a non-conventional myosin motor that has been implicated in plasma-membrane recycling and apicobasal polarity, primarily in the intestinal epithelium and liver. Mutations in MYO5B lead to the autosomal recessive disorder microvillus inclusion disease (MVID) that causes life-threatening diarrhea in neonates due to the mis-trafficking of apical transporters and proteins. MVID-causing MYO5B mutations are truncations or frameshifts that can lead to loss of MYO5B and point mutations in specific domains of the myosin motor. It is unknown how individual point mutations impact the function of the MYO5B motor. Understanding how different MVID-causing mutations in MYO5B disrupt the function of the motor functionality is critical to learning how MYO5B operates within the apical recycling and delivery pathways. I hypothesized that point mutations in the MYO5B motor head lead to defects in motor functionality by impacting the ability to bind actin, translocate, or hydrolyze ATP. To address my hypothesis, I used a live-cell assay to examine the functionality of the MYO5B motor and mutants independently of the cargo domain. I created a truncated MYO5B (amino acids 1-1015) motor construct with a C-terminal triple citrine tag and constructs containing select patient MYO5B motor mutations. The MYO5B motor domain constructs were co-expressed with mCherry-espin in the protrusion forming cell line, LLC-PK-CL4. The localization of the MYO5B-motor at the tips of microvilli indicated a functional motor, while a lack of MYO5B-motor at the tips of microvilli indicated a dysfunctional motor. A tip to cytoplasm ratio was used to quantify this change in distribution. In accordance with previous literature, the wild-type MYO5B motor localized to the tips of microvilli with a ratio of 4.087, while the P660L mutation localized to the bases of microvilli and had a tip ratio of 0.1000, providing more evidence that this mutation is a rigor mutation. Patient mutations I408F and R824C did not accumulate at the tips of microvilli with a tip ratio of 0.1380 and 0.4963, respectively. Surprisingly some MVID-causing MYO5B mutations did not fully impair MYO5B motor function. G519R had a tip ratio of 1.870, and D492G had a tip ratio of 1.660. Further investigation needs to be done to determine the effect these mutations have on the overall protein function. Studying the subtle differences in the phenotype of patient mutations could lead to a better understanding of the disease pathology and MYO5B function.

B66/P1064

Bending forces and nucleotide state jointly regulate F-actin structure

M. J. Reynolds, C. Hachicho, A. G. Carl, R. Gong, G. M. Alushin; Laboratory of Structural Biophysics and Mechanobiology, The Rockefeller University, New York, NY.

ATP hydrolysis-coupled actin polymerization is a fundamental mechanism of cellular force generation. In turn, force and actin filament (F-actin) nucleotide state regulate actin dynamics by tuning F-actin's engagement of actin binding proteins (ABPs) through unknown mechanisms. Here, we show that actin nucleotide state modulates F-actin structural transitions evoked by bending forces. Cryo-electron microscopy structures of ADP- and ADP-P_i-F-actin with sufficient resolution to visualize bound solvent reveal inter-subunit interfaces bridged by water molecules that could mediate lattice flexibility. Despite

extensive ordered solvent differences in the nucleotide cleft, these structures feature nearly identical lattices and essentially indistinguishable protein backbone conformations unlikely to be discriminable by ABPs. We next introduce a machine-learning enabled pipeline for reconstructing bent filaments, allowing us to visualize both continuous structural variability and side-chain level detail. Bent F-actin structures reveal major rearrangements at inter-subunit interfaces characterized by striking alterations of helical twist and deformations of individual protomers, transitions which are distinct in ADP- and ADP-P_i-F-actin. This suggests phosphate rigidifies actin subunits to alter F-actin's bending structural landscape. We therefore propose actin nucleotide state can serve as a co-regulator of F-actin mechanical regulation, as bending forces evoke nucleotide-state dependent conformational transitions that are likely detectable by ABPs.

B67/P1065

Phactr4 Coordinates with Arp2/3 Complex to Drive Membrane Protrusion in Multiple Macrophage Contexts

R. Manickam, J. D. Rotty; Biochemistry, USUHS, Bethesda, MD.

There are several human diseases, termed "actinopathies" which result from genetic variants of actin cytoskeleton proteins, leading to clinical immunodeficiencies. Of these, several actinopathies are linked, directly or indirectly, to dysregulated Arp2/3 complex function. While the function of Arp2/3 in the structural actin cytoskeleton has been well characterized, the potential mechanisms underlying heightened inflammatory phenotypes and immune dysfunction related to Arp2/3 deficiency remain less well understood. Unpublished data from our lab uncovered a novel potential Arp2/3 interacting protein called Phactr4, a relatively understudied protein which binds protein phosphatase 1 (PP1) as well as monomeric actin. Previous findings have shown that genetic deletion of the Arp2/3 complex (*Arpc2-/-)* in macrophages leads to phenotypic changes including increased serine/threonine phosphorylation of several proteins, including: phospho-cofilin, phospho-myosin light chain, p-ERK, p-P38, p-P65 and others. This points to a link between phosphoregulation and the Arp2/3 complex. We hypothesize that Phactr4 provides specificity to PP1 in certain dynamic contexts by coordinating with the Arp2/3 complex. Subpopulations of Phactr4 co-localize with Arp2/3 at the leading edge, and this population increases after induced cell spreading. Interestingly, *Arpc2-/-* macrophages display differential phagocytic ability, where the CR3 pathway is much less capable at internalizing iC3b-opsonized beads, while the defect in internalization of IgG-opsonized beads is less severe. Our preliminary work suggests that Phactr4 is preferentially localized to CR3-driven phagocytic cups, and is largely absent from FcR-driven phagocytic cups. These data together suggest that Phactr4 and Arp2/3 may be coordinating to drive membrane protrusion during certain dynamic cellular events, such as cell spreading, directed migration, and complement mediated phagocytosis. Future experiments will focus on characterizing Phactr4 deficient macrophages and whether Arp2/3 complex activity recruits Phactr4 to the leading edge. Understanding the Arp2/3-dependent interactions involved with the less well characterized Phactr4-PP1 complex will help us better understand the mechanisms underlying migration and phagocytosis in an immunological context.

B68/P1066

Determining the role of Plastin-3 in osteoblast mineralization and mechanosensation

S. M. Chin, S. Jansen; Washington University in St. Louis, Saint Louis, MO.

Plastin-3 (PLS3) is a calcium-sensitive actin-bundling protein that has recently been linked to the development of childhood-onset osteoporosis. The physiological role of PLS3 in bone health is unclear and, by extension, it also remains an open question how disruption of PLS3 affects cellular functions and ultimately contributes to early-onset osteoporosis. Here, we investigated the role of PLS3 in focal adhesions, which are large molecular complexes that link the actin cytoskeleton to the ECM and contribute to cellular processes such as mechanosensation and cell adhesion. Our results demonstrate that endogenous PLS3 shows a distinct localization in nascent vs. mature focal adhesions. In nascent focal adhesions, PLS3 shows a diffuse localization, however, in mature focal adhesions, PLS3 closely colocalizes with the actin stress fiber in the tail end of the focal adhesion. Interestingly, characterization of PLS3 pathogenic mutations defective in calcium-sensitivity and/or bundling activity reveals altered localizations within focal adhesions, suggesting that both calcium-regulation and bundling contribute to the function of PLS3 in focal adhesions. To gain more insight into the role of PLS3 in focal adhesions, we tested how control and PLS3 knockdown osteoblast cells sense/respond to changes in substrate stiffness using polyacrylamide hydrogels coated in Matrigel. While control cells plated on substrates of different stiffnesses exhibit increases in cell size in response to increasing substrate stiffness, PLS3 knockdown cells exhibit similar sizes regardless of substrate stiffness. Characterization of the actin cytoskeleton in control cells demonstrates changes to the actin cytoskeleton on stiffer substrates consistent with increased cell tension, including formation of actin stress fibers and larger focal adhesions, while PLS3 knockdown cells display similar cytoskeletal morphologies regardless of substrate stiffness. Taken together, these findings suggest that PLS3 knockdown osteoblasts are impaired in either sensing changes to the stiffness of their environment or transducing those cues to the actin cytoskeleton, which may contribute to the development of osteoporosis in patients carrying mutations in the PLS3 gene.

B69/P1067

An interphase actin wave promotes mitochondrial content mixing to maintain cellular homeostasis

S. M. Coscia[1], A. S. Moore[2], E. L. F. Holzbaur[1]; [1]University of Pennsylvania, Philadelphia, PA, [2]Howard Hughes Medical Institute, Ashburn, VA.

Cellular health is dependent on mitochondrial homeostasis and the proper regulation of mitochondrial dynamics. We discovered a mechanism regulating mitochondrial dynamics via a cycling wave of actin polymerization/depolymerization. In metaphase the force of actin polymerization propels mitochondria in a comet-tail mechanism, resulting in equivalent partitioning of mother cell mitochondria between daughters. Actin cycling persists in interphase, however during this cell cycle stage F-actin assembly leads to mitochondrial fission, as we observed fragmentation dependent on the essential fission mediator DRP1. We hypothesized that cycling produces force which is resisted by tethering of mitochondria to microtubules, leading to mitochondrial tubulation and thus fission. In support, upon chemical microtubule depolymerization, actin wave-associated mitochondria ceased to fragment and instead displayed enhanced motility as revealed by tracking and displacement index analysis. Next, we focused on the machinery driving cycling, which is blocked by inhibitors of the kinase CDK1. We probed for the involvement of putative CDK1-regulated F-actin nucleators, including the formin FMNL1. Depletion of FMNL1 blocked actin cycling and expression of a non-phosphorylatable mutant at the CDK1

site had a dominant negative effect. We next probed the interphase function of this wave. We noted a loss of mitochondrial membrane potential, as measured by TMRE uptake, in cells depleted of FMNL1. Moreover, Seahorse analysis indicated that FMNL1-depleted cells consumed oxygen at a slower rate, leading to lower ATP levels. While these data indicate that interphase cycling is required to maintain mitochondrial health, we found that the actin wave continues to propagate following CCCP-induced mitochondrial depolarization, indicative that altered mitochondrial health does not feed-back to inhibit cycling. Finally, we asked if the interphase wave promotes mitochondrial content mixing, required to maintain mitochondrial function. In support, inhibition of cycling blunted the spread of mitochondrially-targeted photoactivatable GFP and mitochondrial tracking revealed that mitochondria fragmented by the wave often re-fused with distinct neighbors. Thus, we propose that interphase actin cycling maintains mitochondrial health by enhancing content mixing to promote inter-organelle complementation.

B70/P1068

Delphilin FH2 Linker Subdomain Prolines affect Profilin-Actin Polymerization

B. Christian, M. Quinlan; Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA.

Formins make up a large family of actin-binding protein that nucleate actin filaments and promote filament elongation. The formin, Delphilin, is uniquely expressed in Purkinje cells, a highly branched neuron important for learning. Its interaction with Glutamate Receptor Delta 2 (GluRδ2) at the post-synaptic density has been documented, but otherwise Delphilin's biological role remains unknown. We previously biochemically characterized Delphilin as an actin nucleator which preferentially polymerizes non-muscle actin over muscle actin isoforms. Close examination of Delphilin's actin-binding FH2 domain shows that the Linker subdomain has a high proline-content compared to other formin family Linker subdomains: 5 prolines of 19 total amino acids in human Delphilin. Most Linkers are on average 20 residues long and thought to be disordered. The proline residues in the Delphilin-Linker are proximally placed such that there is a high probability of a polyproline II helix in this Linker, making it more rigid than most Linkers. I am investigating the contribution of this Linker subdomain to Delphilin's actin nucleating activity. I purified a construct of the C-terminal half of human Delphilin (hDelFF), including the FH1 and FH2 domains (Delphilin has no tail). I also created a version in which the prolines in the Linker are mutated to glycines (hDelFF-LM). With these, I performed bulk elongation assays with pyrene-actin. Both the hDelFF and hDelFF-LM constructs completely inhibit actin filament elongation at saturating concentrations, with a K_d of about 10 nM. Interestingly, I observe a difference when profilin is added. With profilin, hDelFF-mediated elongation is not fully inhibited at saturation. In contrast, the hDelFF-LM slows elongation more effectively than the wild-type hDelFF construct. Thus, the Linker subdomain is important for actin polymerization when using profilin-actin. We are working to determine the mechanism and whether the observed property is specific to Delphilin or present among formins. We are also looking into how the FH1 or profilin-actin may change a formin's FH2 gating properties. Gating is thought to be a characteristic solely attributed to the FH2 domain, but literature suggests that the addition of profilin may alter the gating limit in several formins. I am investigating this phenomenon in Delphilin and other highly characterized formins.

B71/P1069

β-actin protein is essential for retinal physiology

P. Vedula[1], M. E. Fina[2], B. A. Bell[3], S. S. Nikonov[2], A. S. Kashina[1], D. W. Dong[1]; [1]Biomedical Sciences, University of Pennsylvania, Philadelphia, PA, [2]Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [3]F.M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Actins are abundant and ubiquitously expressed proteins that are essential components of the cytoskeleton in both muscle and non-muscle cells. Higher vertebrates express two non-muscle actins - β- and γ- cytoplasmic actins. These two proteins differ by only 4 conservative substitutions in their N-terminus, that have been completely preserved over 320 million years of evolution. The functional differences between these nearly identical, and highly conserved proteins have been a mystery. Here, we addressed the role of these N-terminal substitutions using a gene-edited mouse model expressing γ-actin both natively and from the nearly intact β-actin gene. These mice completely lack β-actin protein but retain the nucleotide-level elements of both cytoplasmic actin genes. Thus, any phenotypes in these mice are caused by the replacement of β-actin-specific N-terminal amino acids with those of γ-actin, making this model uniquely suited to address the biological role of these amino acid differences *in vivo*. Strikingly, while these mice undergo normal embryogenesis, the absence of β-actin leads to physiological defects in the retina, including reduced light sensitivity in photoreceptors and progressive retinal degeneration, accompanied by disorganization of actin structures in the retina layers. Importantly, the phototransduction G-protein is mislocalized in the photoreceptors. Furthermore, these mice show prominent defects in the microvilli, actin-rich cytoplasmic protrusions, of the retinal pigment epithelium and the Müller glial cells . Our results demonstrate a crucial function for the highly conserved amino acid differences between β- and γ-actin in the proper structural organization and physiology of the mammalian retina.

B72/P1070

Vinculin organizes actomyosin and maintains epithelial cell-cell junctions at tricellular tight and adherens junctions

J. N. Iseler, L. van den Goor, A. L. Miller; Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI.

Epithelial cells form barriers that coat and protect organs and organisms from their environments. Epithelial tissue function requires both adherens junctions to adhere cells together and tight junctions to create a selectively permeable barrier between neighboring cells. Junctional integrity must be maintained when epithelia are challenged by mechanical stress, such as food passing through the intestines or the bladder expanding. Vinculin, a mechanosensitive protein, strengthens adherens junctions in response to mechanical stress through strengthening connections to an actomyosin (F-actin and Myosin II) array. We hypothesize that Vinculin reinforces tricellular tight and adherens junctions in response to increased tension by maintaining proper actomyosin organization. To test this idea, we challenged the junctions of the gastrula stage *Xenopus laevis* epithelium using two different techniques: addition of extracellular ATP and a tissue stretcher. First, we aimed to characterize how Vinculin and F-actin are reorganized at adherens junctions after either tension-increasing treatment by quantifying changes in fluorescence intensity of mNeon-Vinculin and LifeAct-RFP (an F-actin probe). After ATP treatment, we found that Vinculin intensity is enriched at tricellular junctions and reorganizes from

distinct “spots” into elongated “spokes” along the junction. Our preliminary data shows similar trends for Vinculin localization after using the tissue stretch device. In both stretched and ATP-treated tissues, medial apical and junctional F-actin intensity is increased under high tension. Next, we aimed to investigate whether Vinculin supports tricellular junction integrity. To test this, we performed immunofluorescence imaging of the tricellular tight junction protein Angulin-1 and actomyosin after Vinculin knockdown. Preliminary experiments show that Vinculin knockdown decreases Angulin-1 intensity at tricellular junctions and disrupts actomyosin organization at cell vertices, suggesting that Vinculin plays a role in maintaining tricellular tight junctions. Taken together, these data suggest that mechanosensitive recruitment of Vinculin to tricellular adherens junctions under increased tension is essential for maintaining junctional integrity and proper actomyosin organization.

B73/P1071

Fast actin disassembly and mechanosensitivity of the actin crosslinking protein fimbrin during clathrin-mediated endocytosis

S. Mousavi[1,2], X. Li[1,2], M. Lacy[3,2], J. Berro[1,2,4]; [1]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, [2]Nanobiology Institute, Yale University, West Haven, CT, [3]Yale School of Medicine, New Haven, CT, [4]Department of Cell Biology, Yale University School of Medicine, New Haven, CT.

The actin cytoskeleton is central to force production in numerous cellular processes in eukaryotic cells. During clathrin-mediated endocytosis (CME), actin dynamic is required when membrane tension or turgor pressure is large. Previous experimental work from our lab showed that several endocytic proteins, including actin and actin interacting proteins, turn over several times during the formation of a vesicle during CME in yeast. Their dwell-time distributions, which indicate how long individual molecules stay at endocytic structures, have a peak of around 1 s, and the distribution for the filament crosslinking protein fimbrin contains a second peak of around 0.5 s. To better understand the nature of these dwell-time distributions, we developed a stochastic model for the dynamics of actin and its binding partners. Our model demonstrates that very fast actin filament disassembly is necessary to reproduce experimental dwell-time distributions. Our model also predicts that actin-binding proteins rapidly decorate growing filaments and filaments are fully decorated. Last, our model predicts that fimbrin detachment from actin endocytic structures is mechanosensitive to explain the extra peak observed in the dwell-time distribution for the actin crosslinker fimbrin.

B74/P1072

Adaptive Role for Actin Cross-linkers in Force Generation at Sites of Clathrin-mediated Endocytosis in Yeast

J. Hill, D. Drubin; University of California, Berkeley, Berkeley, CA.

In budding yeast, clathrin-mediated endocytosis (CME) requires the synergistic activities of actin polymerization and actin-binding proteins such as crosslinkers to generate the forces required for invagination of the plasma membrane against high turgor pressure. Despite their essential role in actin force generation, few studies have addressed actin crosslinker function and mechanism in live cells. Endocytic internalization is impeded when the main yeast actin filament crosslinker, the fimbrin-related protein Sac6, is absent, or when membrane tension is raised by elevating turgor pressure. Here we combine live cell imaging studies of Sac6 and mathematical modeling to gain new insights into the role

of actin crosslinkers in actin force generation. By quantitatively measuring the maximum number of Sac6 molecules at sites of CME in cells with elevated turgor pressure, we show that sites with more crosslinkers are more effective at internalization under high load. Additionally, we used an experimentally constrained, agent-based mathematical model to recapitulate the result that endocytic networks with more double-bound crosslinkers internalize the plasma membrane against increased turgor pressure more effectively. In simulations, endocytic networks with more double-bound crosslinkers have less actin on average than in networks with fewer double-bound crosslinkers, consistent with more filaments experiencing a stall force as they grow against the membrane. These networks also have a higher fraction of growing plus ends oriented towards the plasma membrane where addition of new actin monomers contributes to force generation and internalization of the vesicle. Our results provide a richer understanding of the crucial role played by actin filament crosslinkers during actin network force generation, highlighting the contribution of these proteins to the adaptive response of the actin cytoskeleton to increased load in the context of clathrin-mediated endocytosis.

Higher-Order Actin-Based Structures

B75/P1073

Cdc42-Dependent Perinuclear Stress Fibers are a Functionally Distinct Network With Septins, Myo18Aalpha and MRCK

M. Utgaard, S. Chandrasekar, P. Oakes, J. Beach; Cell and Molecular Physiology, Loyola University, Chicago, IL.

Stress fibers are actomyosin bundles that form throughout many cell types. Numerous types of stress fibers have been reported with distinct compositions, assembly mechanisms, and functions. Ventral stress fibers are located on the ventral cortex and are anchored to the substrate on both ends by focal adhesions. A distinct sub-population of ventral stress fibers are the perinuclear stress fibers (PSF). Relative to other stress fibers throughout the cell, these PSFs have been less explored and their function remains ambiguous. Consistent with published studies, in addition to actin and myosin 2, we find that the perinuclear network contains the Cdc42 effector kinase MRCKα, the motor-dead myosin 18Aα, and septins, a fourth component of the cytoskeleton involved in numerous cellular processes. Interestingly, our super-resolution imaging reveals that while septins often align with the actomyosin stress fibers, they also appear to form distinct, adjacent structures. In contrast, septins are near perfectly coincident with myosin 18Aα and MRCKα, which were previously known to complex with one another. Considering Cdc42 is a known upstream modulator of both MRCK and septins, we explored Cdc42 contributions to PSFs. We observe that wild-type EGFP-tagged Cdc42 localizes to PSFs, but this localization is dramatically enhanced in a constitutively-active Cdc42 mutant. Constitutively-active Cdc42 expression also enhanced septin recruitment to PSFs, and inhibition of Cdc42 with a small molecule (ZCL278) caused septins to dissociate from PSFs and relocalize to the peripheral cortex. Fluorescent recovery after photobleaching (FRAP) of fibroblasts expressing EGFP-sept2 demonstrated that septin on PSFs has a slower turnover than septin that is localized at the cortex, consistent with these being two distinct septin structures. Finally, we find that PSFs that are decorated with septins/MRCKα/myosin18Aα do not exert significant traction stresses relative to other stress fibers in the cell. Our testable working model is that this septin/MRCKα/myosin18Aα network is inhibiting actomyosin force generation to support nuclear mechanics.

B76/P1074

Tunable autoinhibition of plastins/fimbrins defines their unique role in the actin cytoskeleton organization

C. Schwebach[1], E. Kudryashova[1], R. Agrawal[2], W. Zheng[3], E. Egelman[3], **D. Kudryashov**[1]; [1]Ohio State University, Columbus, OH, [2]Cornell University, Ithaca, NY, [3]University of Virginia, Charlottesville, VA.

Plastins (a.k.a. fimbrins) are conserved actin-bundling proteins contributing to the organization of lamellipodia, microvilli, and the contractile ring. Accordingly, plastins participate in various cellular events such as motility, endocytosis, and cytokinesis by organizing actin in both aligned bundles (parallel or antiparallel) and branched networks. We hypothesized that this unique versatility of plastins stems from a tunable inhibitory association between their actin-binding domains (ABD1/2). We demonstrate here that while ABD2 in isolation can bind actin with a low nanomolar Kd, it is potently inhibited by an equally strong interaction with ABD1, resulting in a rather weak bundling. Uncoupling the ABDs via phosphorylation of a Serine residue at their interface (or a mutation mimicking thereof) strongly potentiates actin cross-linking and dramatically affects the actin cytoskeleton in transfected cells. We suggest that the strong and tunable allosteric inhibition between the domains allows plastins to modulate the cross-linking strength, contributing to the remodeling of actin assemblies of different morphologies and defining the unique place of plastins in actin organization.

B77/P1075

Human Epithelial Cell Polarity is Dependent on ARHGAP18, an ERM Specific RhoA GAP, to Regulate Microvilli and Apical Actomyosin Networks.

A. T. Lombardo, C. A. R. Mitchell, R. Zaman, D. J. McDermitt, A. Bretscher; Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Vertebrate tissue assembly relies on the mechanical and biochemical polarity of cells, with each cell having regions of distinct morphologies and protein concentrations. Creation of polarized cellular regions is defined in part by the broad gradients of small GTP-binding proteins which regulate actomyosin cytoskeletal networks. These networks link to the plasma membrane to establish the cell structures that define each differentiated cell. Here, we show ARHGAP18 binds to, and is activated by, the actin-membrane-linker ezrin, to regulate RhoA specifically at the apical membrane of epithelial cells. We utilize human cells totally lacking the expression of all three ERMs (Ezrin Radixin Moesin) their activating kinases LOK and SLK or ARHGAP18 to define a RhoA negative feedback loop regulating polarity determination through actomyosin organization. Using spinning disc confocal imaging, scanning electron microscopy and Super-Resolution Stochastic Optical Reconstruction Microscopy (STORM) we track individual microvilli and visualize changes in single actin filaments to define a local regulation of RhoA on a microdomain scale. Additionally, we utilize fluorescent biosensors to visualize the localized activation of RhoA and downstream reorganization non-muscle myosin-II from within the terminal web to aberrant activation inside microvilli when ARHGAP18 is lost. Collectively this work seeks to address a decades old question of how the ERM family of proteins negatively regulate the RhoA signaling pathway in humans. Our findings offer insight into how cells simultaneously regulate both whole cell scale polarity signals and the maintenance of specific cytoskeletal structures.

B78/P1076

An adhesion-based mechanism stabilizes apical microvilli at the margins of transporting epithelial cells

C. S. Cencer[1], J. B. Silverman[1], S. Mao[1], L. M. Meenderink[2], E. S. Krystofiak[1], B. A. Millis[1], M. J. Tyska[1]; [1]Vanderbilt University, Nashville, TN, [2]Vanderbilt University Medical Center, Nashville, TN.

Transporting epithelia of the kidney and small intestine utilize actin-supported cell surface protrusions, known as microvilli, to expand surface area available for solute transport. Microvilli found on the surface of these epithelia constitute a well-organized “brush border” made up of thousands of protrusions connected via a tip-localized intermicrovillar adhesion complex (IMAC) composed of cadherins CDHR2 and CDHR5. Previous work in our laboratory showed that microvilli of early, differentiating cells are unstable and exhibit a cycle of growth and collapse, while mature cells contain robust and stable clusters of microvilli. Whether the IMAC contributes to nascent microvilli accumulation and stabilization during cell differentiation remains unclear. Here we show that, at time points early in differentiation, epithelial cells present two general populations of microvilli: (1) a marginal population at the edges of cells, characterized by high protrusion density, and (2) a medial population characterized by much lower protrusion density. Strikingly, marginal microvilli extend across cell-cell junctions to physically contact microvilli on neighboring cells, using transjunctional CDHR2 and CDHR5 adhesion complexes. Additionally, Fluorescence recovery after photobleaching and microvilli tracking experiments revealed that transjunctional adhesion complexes between marginal microvilli are more stable than those bridging medial clusters of microvilli. Tracking analysis on live kidney proximal tubule CL4 cells showed that marginal microvilli are not motile, suggesting that transjunctional adhesion serves as an anchoring point for nascent microvilli. Given the stabilizing nature of transjunctional adhesion complexes, we predicted that cell-cell junctions may be influenced by apical CDHR2/CDHR5 transjunctional contacts. Indeed, in a CDHR2 KO mouse model and in CDHR2 KO CL4 cells, endogenous signal of tight junction protein ZO-1 is abnormal. As a result, KO cells exhibit increased dynamics within a monolayer, leading to stretched and disorganized cell phenotype. Overall, these findings suggest a new, adhesion-based mechanism for the stabilization of microvilli and support of cell-cell junctions, changing our understanding of how transporting epithelial cells utilize cell-cell contacts to create optimal tissue structure.

B79/P1077

Defining mechanisms of microvilli biogenesis using direct visualization

I. Gaeta[1], L. Meenderink[2], M. Postema[1], C. Cencer[1], M. Tyska[1]; [1]Vanderbilt University, Nashville, TN, [2]Veterans Affairs Tennessee Valley Health Care System, Nashville, TN.

Microvilli are actin-bundle-supported surface protrusions that play essential roles in diverse epithelial functions, from nutrient absorption to mechanosensation. Despite the importance of microvilli for proper epithelial cell function, the mechanism by which cells build microvilli and the proteins involved in this process are not well understood. Classic electron micrographs indicate the presence of an electron dense protein complex at the distal tips of microvilli, which has been long hypothesized to coordinate protrusion growth due to its proximity to the fast-growing ends of actin filaments. Indeed, time-lapse data revealed that specific factors, including epidermal growth factor pathway substrate 8 (EPS8) and insulin-receptor tyrosine kinase substrate (IRTKS) (also known as BAIAP2L1), appear in diffraction-limited puncta at the cell surface and mark future sites of microvillus growth. New core actin bundles elongate from these puncta in parallel with the arrival of ezrin and subsequent plasma membrane encapsulation.

In addition to de novo growth, we also observed that new microvilli emerge from pre-existing protrusions. Moreover, we found that nascent microvilli can also undergo collapse, resulting in disassembly of the core actin bundle itself. To further develop our understanding of microvilli biogenesis, we used a biotin proximity labeling approach to probe for new molecules involved in microvillus growth, using EPS8 as bait. Mass spectrometry of biotinylated hits identified a previously uncharacterized proximal protein, KIAA1671. In silico domain analysis indicates this protein is a large (~200 kDa), primarily disordered protein that contains few recognized domains. We find that KIAA1671 localizes to the base of the brush border in native intestinal tissue and polarized epithelial cell culture models, and to actin positive structures in non-polarized cell types. Furthermore, we find that KIAA1671 co-accumulates with EPS8 at sites of microvillus growth, suggesting that KIAA1671 is involved in the growth of microvilli. Thus, using biotin proximity labeling in conjunction with live cell imaging, we have identified a novel factor that targets to the apical surface of epithelial cells and may be involved in microvilli morphogenesis. These studies are the first to offer a temporally resolved microvillus growth mechanism and highlight factors that participate in this process; they also provide important insights on the growth of apical specializations that will likely apply to diverse epithelial contexts.

B80/P1078

A novel population of short actin filaments at stereocilia tips contribute to a tip-down widening mechanism

X. Liao[1], **B. Perrin**[1], C. Tung[1], J. F. Krey[2], P. G. Barr-Gillespie[3]; [1]Indiana University - Purdue University Indianapolis, Indianapolis, IN, [2]Oregon Hearing Research Center, Oregon Health and Science University, Portland, OR, [3]Oregon Health and Science University, Portland, OR.

Stereocilia are actin-based protrusions on auditory sensory cells that convert mechanical energy into ion influx upon deflection by sound waves. Stereocilia size is crucial for this mechanotransduction function. The length and width of a stereocilium is dictated by its core of bundled, parallel actin filaments (F-actin). The core filaments are oriented with their fast-growing barbed ends towards stereocilia tips and their slower-growing pointed ends oriented towards bases. F-actin in the stereocilia core is highly stable, but actin at stereocilia tips turns over more rapidly. Here, we provide evidence that the dynamic actin at tips includes short actin filaments (tip filaments) that are separate from the core filaments and suggest that these tip filaments contribute to stereocilia growth. We identified tip filaments by probing permeabilized postnatal mouse cochlear tissue with purified, exogenous His-tropomodulin1 (His-TMOD1). Tropomodulins are well-characterized proteins that bind pointed ends of actin filaments, but not barbed ends. His-TMOD1 labeled the tips of stereocilia in all rows before postnatal day 6 (P6), with labeling declining until P9. Since actin in the stereocilia core have only their barbed ends at stereocilia tips, we propose that the pointed ends detected by the His-TMOD1 probe are short actin filaments that are not part of the core. Tip filament levels are highest when stereocilia are widening, suggesting they may contribute to this aspect of stereocilia growth. Correspondingly, transient overexpression of EGFP-actin changed the distribution of pointed ends so they were more evident in the stereocilia shaft, which is consistent with actin filaments adding to the core in a tip-down fashion. In addition, live-cell imaging revealed that overexpressed EGFP-actin initially localized to stereocilia tips, but then extended down the stereocilia shaft over time. Super-resolution imaging of fixed samples showed that the newly expressed EGFP-actin signal surrounded the stable, preexisting F-actin core. Together, these data suggest a tip-down widening mechanism where tip filaments may initiate actin polymerization and create new parallel F-actin at the periphery of the stereocilia core.

B81/P1079

Comparative analysis of bacterial actin-based motility mechanisms

M. Bacher, D. Serwas, M. D. Welch; MCB, University of California, Berkeley, Berkeley, CA.

Bacteria in the genus *Rickettsia* are arthropod-associated obligate intracellular organisms that have evolved to utilize host cell components for their survival. After invading host cells and gaining access to the cytoplasm, many *Rickettsia* species polymerize host actin filaments on their surface and undergo actin-based motility. *Rickettsia* express two different bacterial proteins that drive motility. RickA is well conserved between species and promotes actin polymerization by activating the host Arp2/3 complex. Sca2 is more divergent between species, suggesting species-specific differences in its biochemical mechanisms of action. For the disease-causing spotted fever group species *R. parkeri*, actin-based motility occurs in temporally distinct phases, with "early" (<1h post infection) motility driven by RickA, and "late" (>8h post infection) motility driven by Sca2. The ancestral and non-pathogenic species *R. bellii* expresses orthologs of RickA and Sca2, but it remains unclear how these two factors contribute to the mechanism and timing of actin-based motility in this species. To examine the biochemical activities of *R. bellii* RickA and Sca2, we purified glutathione S-transferase (GST) fusion proteins. We found that GST-RickA and GST-Sca2 conjugated to fluorescent beads promote actin polymerization in *X. laevis* egg extract, suggesting a direct role in bacterial-induced actin assembly. We next examined the timing of *R. bellii* actin-based motility in infected A549 human lung epithelial cells. *R. bellii* only initiated actin-based motility at times >4h post infection, while *R. parkeri* initiated motility within 1h post infection. Moreover, actin tails formed by *R. bellii* were thinner in width and more curved in shape. To further compare the ultrastructure of *Rickettsia* actin tails, we have initiated cryo-electron tomography studies. Our initial observation of actin tails formed by *R. parkeri* revealed that individual actin filaments emanate from the bacterial pole and are organized into parallel unbranched arrays. Together, these data suggest that RickA and Sca2 orthologs from diverse *Rickettsia* species function to polymerize actin, yet there are species-specific differences that result in variations in the timing and mechanism of actin-based motility. Further comparative analyses will shed light on the evolution of motility mechanisms and their role in pathogenicity.

B82/P1080

Arp2/3 complex-nucleated, light-dependent filopodia in amoeboid algae perform bidirectional cyclosis

P. Avasthi, **C. D. MacQuarrie**; Arcadia Science, Berkeley, CA.

Chlorarachniophytes are a unique class of marine algae. Many species in this group perform amoeboid motility and can form long extensions reaching up to 1 mm in length and 2 µm in diameter. These extensions appear to serve multiple functions including predation, motility, and possibly photosynthesis. Through live-cell DIC imaging, we observed rapid, bidirectional cytoplasmic transport occurring in these extensions, reminiscent of cytoplasmic streaming in fungi and plant cells. Here we explore the contributions of the actin cytoskeleton to this unique structure. *Amorphochlora amoebiformis* and *Bigelowiella longifila* cells were treated with cytoskeletal drugs or fluorescent dyes and observed using confocal microscopy. We observed multiple components being trafficked through these extensions including mitochondria, chloroplasts, DNA, and tubulin. Furthermore, the formation of these extensions appears to be actin dependent. Treatment with actin polymerization inhibitors Latrunculin B or

Cytochalasin D resulted in stubby extensions, while treatment with CK-666, which inhibits the branched-actin nucleating Arp2/3 complex, prevented the formation of these extensions entirely. Surprisingly, these extensions are light-dependent, suggesting a novel link between chloroplast activity and actin nucleation in algae.

B83/P1081

Liquid-like assembly of VASP catalyzes actin polymerization and bundling

K. Graham[1], A. Chandrasekaran[2], L. Wang[3], A. Ladak[1], E. Lafer[3], P. Rangamani[2], J. Stachowiak[1]; [1]The University of Texas At Austin, Austin, TX, [2]University of California San Diego, La Jolla, CA, [3]University of Texas Health Science Center at San Antonio, San Antonio, TX.

The actin cytoskeleton underlies many critical cellular processes including morphogenesis, trafficking, and motility. The organization of actin filaments into higher order networks or bundles is often required to sustain the forces involved in many of the mechanical functions of the cytoskeleton. Actin filament bundling is regulated by a family of actin-binding proteins. Recent work has revealed that several proteins comprising this cytoskeletal network have been found to undergo liquid-liquid phase separation. How might liquid-like phases contribute to filament organization and bundling? Here, we show that the processive actin polymerase and bundling protein, VASP, forms liquid-like droplets under physiological conditions. These VASP droplets catalyze actin polymerization and bundling through a mechanism that depends on the relative material properties of the filaments and the droplets. Specifically, as actin polymerizes within VASP droplets, elongating filaments partition to the perimeter of the droplet to minimize filament curvature, forming an actin-rich ring within the droplet. The rigidity of this ring is balanced by the droplet's surface tension, as predicted by a continuum-scale computational model. However, as actin polymerizes and the ring grows thicker, its rigidity increases and eventually overcomes the surface tension of the droplet, deforming into a linear bundle. The resulting bundles contain long, parallel actin filaments that grow from their tips, reminiscent of filopodia. Once the parallel arrangement of filaments is created within a VASP droplet, it propagates through the addition of new actin monomers to achieve a length that is many times greater than the initial droplet. Significantly, the fluid nature of the droplets is critical for bundling, as more solid droplets resist deformation by preventing the rearrangement of filaments that is necessary to form bundles. These results reveal a novel mechanism of filament bundling that may be relevant to the assembly of cellular architectures such as filopodia, stress fibers, and focal adhesions.

B84/P1082

Physical and kinetic determinants of shape changes in liquid droplets of processive actin polymerase, VASP containing actin filaments

A. Chandrasekaran[1], K. Graham[2], L. Wang[3], A. Ladak[2], E. Lafer[3], J. Stachowiak[2], P. Rangamani[1]; [1]Mechanical and Aerospace Engineering, University of California, San Diego, San Diego, CA, [2]Department of Biomedical Engineering, University of Texas at Austin, Austin, TX, [3]Department of Biochemistry and Structural Biology, University of Texas Health Science Center at San Antonio, San Atonio, TX.

Actin, one of the most abundant cytoskeleton proteins in cells, aided by accessory proteins, forms elaborate networks of a wide array of shapes tailored specifically for cellular functions such as cell growth, adhesion, and locomotion. Growing evidence suggests that phase separation of essential proteins, including the actin-binding proteins, promotes spatial and dynamic control of cell chemical

responses. However, the role of phase separation in mechanochemical responses of actin networks is poorly understood. Here, we use computational modeling to study liquid droplets of the processive actin polymerase Vasodilator stimulated phosphoprotein (VASP). Actin selectively partitions inside the VASP droplets to form actin filaments resulting in an actin shell confined in VASP droplets. Using a continuum modeling, we show that as actin accumulates, VASP droplets deform from circular to elliptical droplets. Additionally, we predict that the droplet deformation happens above a critical actin ring thickness. These modeling predictions are consistent with experimental observations. The shape changes of the droplets are driven by the competition between the actin bending energy and the droplet surface energy. Finally, we explore the kinetic aspects of such transitions using agent-based modeling in CytoSim and find the conditions that favor the formation of an actin shell and ring inside a droplet with high surface energy. We identify filament elongation rate and crosslinker unbinding rate as crucial parameters that dictate the final shape of the actin network. In summary, we have developed a framework to understand how the chemical evolution of actin filaments affects the mechanical properties of a liquid droplet-actin system.

B85/P1083

Molecular condensation regulates actin nucleation during immune signalings

Y. Miao; Nanyang Technological University, Singapore, SINGAPORE.

Rapid actin cytoskeleton remodeling on demand is essential to coordinate cellular processes during eukaryotic cell immune signaling. Host cells respond acutely by increasing actin polymerization and stabilization during host-pathogen communication. For example, upon recognizing pathogenic microbe, plants trigger rapid actin polymerization via condensing, thereby activating the actin nucleation factor via diverse mechanisms on the cell surface. Here, I will present a central mechanism by which membrane-associated formin condensation remodels actin cytoskeleton dynamically, in time and space, during early plant immune signaling. In addition, plant type I-formin is plasma membrane-integrated nucleator behavior like a mammalian integrin protein, which senses extracellular biophysical and chemical cues. We will also present the mechano-regulation of formin condensation and activity during cell immune signaling by modulating plant surface scaffolding structures, cell wall (CW)-plasma membrane (PM)- and actin cytoskeleton (AC) continuum.

B86/P1084

Ribonucleoprotein condensates regulate actin network polarization in the filamentous fungus Ashbya gossypii

Z. Geisterfer, A. Gladfelter; Biology Department, University of North Carolina At Chapel Hill, Chapel Hill, NC.

The dynamic restructuring and the precise positioning of polarized cytoskeletal networks, such as the actin cytoskeleton, is essential for the development of diverse cell morphologies, cell motility, cell signaling, and a host of other processes. Despite nearly 100 years of active research and a deep inventory of actin-regulatory proteins, how a cell positions these molecules at the right place and time remains poorly understood. Recent insights have implicated a role for biomolecular condensates in the spatial organization of the actin cytoskeleton in neurons, T-cells, and yeast. Though the mechanisms by which biomolecular condensates contribute to the regulation of the actin cytoskeleton are poorly understood, biomolecular condensation represents a potentially powerful mechanism by which cellular

material may concentrated or focused. Early studies in the Gladfelter lab have shown a necessary role for the RNA-binding protein, Whi3, in promoting lateral branching events in the genetically tractable model system, A. gossypii. Notably, Whi3 phase-separates at existing and incipient branch sites with mRNA transcripts coding for the formin, Bni1, and master-scaffolder, Spa2. Importantly, preliminary studies also show Whi3-coated beads are sufficient to generate and maintain polarized actin networks in A. gossypii cell-free extracts, showing Whi3 ribonucleoprotein (RNP) granules are sufficient to trigger actin assembly without other spatial landmarks. In addition, Whi3-bound mRNA transcripts are subject to translational repression in bulk cell-free extract studies, suggesting that Whi3 RNP granules regulate actin network polarization through focused protein synthesis of actin machinery and scaffolders. It remains unknown if and how Whi3 condensates control translation, and how the activity of these compartments are then coordinated. Similar membrane-less regulatory "hubs" are likely conserved in higher eukaryotes and may act in signal transduction in the post-synapse in neurons, or in homeostasis in large syncytia such as muscle cells or the placenta.

B87/P1085

Substrate stiffness regulates tunneling nanotube formation and function

S. Lin[1], A. P. Liu[1,2,3,4]; [1]Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, [2]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, [3]Cellular and Molecular Biology Program, University of Michigan, Ann Arbor, MI, [4]Department of Biophysics, University of Michigan, Ann Arbor, MI.

Cells constantly sense and respond to mechanical stimuli from their microenvironment. One of the most studied mechanical factors in the complex microenvironment is substrate stiffness. Although substrate stiffness is known to be a critical physical factor mediating cell dynamics and function, how it regulates actin-based protrusions is incompletely understood. As long-distance cell-cell communication conduits, tunneling nanotubes (TNTs) are actin-based membranous protrusions that hover over the substrate and extend over tens of micrometers. Although the molecular mechanisms of TNT formation have been intensively studied, it remains an open question concerning the mechanical d mechanisms underlying TNT formation. Here, by generating a series of silicon substrates covering the range of matrix stiffness in physiological tissues, we demonstrate that substrate stiffness plays a critical role in TNT formation and function. Cells grown on softer substrates formed not only more TNTs but also longer TNTs with faster growth speed. Since traction force and cell mechanical properties such as membrane tension are known to be modulated by substrate stiffness, it is plausible that substrate stiffness regulates TNT via these biomechanical factors. Interestingly, we found that increase in TNT formation on softer substrate does not lead to increase in vesicle transfer, a functional criterion of TNT. We found that while efficiency of vesicle transfer is positively correlated with cell-cell connection by TNT, it is negatively correlated with TNT length, suggesting that the length of TNT may be a critical factor when considering TNT functionality. Altogether, our findings reveal that substrate stiffness not only regulates the biogenesis and dynamics of TNT, but also impact the functionality of these structures. As tissue stiffness changes in numerous disease conditions, our study sheds light on how substrate stiffness could be linked to direct cell-cell communication during disease progression.

B88/P1086

Anillin and myosin synergistically contract actin networks

A. Beber, R. Podhájecký, P. Šliková, Z. Lánský, M. Braun; Institute of Biotechnology CAS, Prague, CZECH REPUBLIC.

Actin-based contractility underlies the dynamics of the cell cortex and cytokinesis. Myosin is the canonical molecular motor propelling this contractility. Additionally, anillin, an actin filament crosslinker, present at the cortex and the cytokinetic contractile ring, likewise generates contractile forces. While anillin binds both actin and myosin, it is unclear if the proteins cooperate. Here we show in a minimal reconstituted system that anillin and myosin synergistically control the contraction of actin filament bundles, two-dimensional networks and rings. This can be explained by our observation that only in the presence of both proteins single actin filaments robustly contract, while myosin or anillin alone, did not alter the geometry of single actin filaments. Based on our simulations we hypothesize that this process depends on the direct interaction between these two proteins. Our results suggest anillin promotes myosin-dependent rearrangements within actin networks

B89/P1087

Cortical tension drug screen links mitotic spindle integrity to RhoA pathway

D. Wang[1], **P. Gunning**[2], Y. Wang[3], X. Di[1], F. Wang[4], A. Wanninayaka[2], D. Jin[1], E. Hardeman[2]; [1]University of Technology Sydney, Sydney, AUSTRALIA, [2]University New South Wales, Sydney, AUSTRALIA, [3]University of Sydney, Sydney, AUSTRALIA, [4]Beihang University, Beijing, CHINA.

Mechanical force generation plays an essential role in many cellular functions including mitosis. Actomyosin contractile forces mediate the separation of daughter cells in mitosis and are implicated in mitotic spindle integrity via cortical tension. To identify potential novel regulators of mitotic cortical tension we conducted a small molecule screen of compounds previously identified from a high chemical diversity library of compounds that impact the organization of the actin cytoskeleton. In addition to screening 150 compounds that produce diverse reorganization of the actin cytoskeleton we also tested 32 compounds that are known to impact progression through mitosis. We initially measured the levels of actin and myosin in the mitotic cortex as a first pass followed by direct measurement of cortical tension to identify proteins implicated in this process. We show inhibitors of Rho kinase and compounds that target the actin-associated protein tropomyosin 3.1 (Tpm3.1) are the most potent inhibitors of cortical actomyosin enrichment and tension. Tpm3.1 is essential for non-muscle myosin 2A (NM2A)-mediated cortical tension independent of the cortical levels of actin and NM2A. The dependence of cortical tension on Tpm3.1 is isoform specific since knockdown of Tpm4.2 has no impact on cortical tension. We further demonstrate a critical role for cortical tension in Tpm3.1-driven rescue of mitotic spindle integrity induced by anti-cancer microtubule depolymerizing agents. Induction of microtubule depolymerization acts via GEF-H1 release and downstream RhoA activation to increase cortical actomyosin and Tpm3.1. Inhibition of GEF-H1 and other steps in the RhoA pathway synergize with microtubule depolymerizers to produce multipolar mitotic spindles. Overall, this suggests that microtubule dynamics regulate actomyosin cortical function via RhoA to ensure integrity of the mitotic spindle. Central to this mechanism is the dependence of NM2A on Tpm3.1 to produce the functional engagement of actin filaments responsible for cortical tension.

B90/P1088

Determining the role of the mammalian formin, formin homology 2 domain-containing 3 (FHOD3), in cardiomyocytes

D. Valencia[1], M. Quinlan[1], A. Nakano[2]; [1]Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA, [2]Molecular, Cell, and Developmental Biology, University of California, Los Angeles, Los Angeles, CA.

The highly organized structure in striated muscle cells, known as the sarcomere, is responsible for contraction. A mammalian formin known as Formin HOmology Domain containing 3 (FHOD3) has been shown to be important for sarcomere development and maintenance in cardiomyocytes. However, the specific mechanisms of sarcomere formation and maintenance remain unclear. The objective of this research is to better understand which biochemical activities of FHOD3 are necessary or sufficient for sarcomere formation and maintenance in cardiomyocytes. Here, using bulk actin assembly assays and seeded elongation assays, we found that FHOD3 is a moderately strong nucleator and weak elongator *in vitro*. Further, we successfully performed a proof-of-principle rescue experiment of wild-type FHOD3 in cardiomyocytes through an siRNA knockdown, followed by adenoviral infection to allow for exogenous expression of wild-type FHOD3. This rescue experiment paves the way for future experiments using function-separating mutants of FHOD3 to understand whether nucleation strength or elongation ability are more important for proper sarcomere formation in cardiomyocytes. This work will help us better understand the mechanisms by which many formins build specific structures *in vivo*.

B91/P1089

Control of actin cable length by decelerated growth and network geometry

S. G. McInally, J. Kondev, B. L. Goode; Brandeis University, Waltham, MA

The sizes of many subcellular structures are coordinated with cell size to ensure that these structures meet the functional demands of the cell. In eukaryotic cells, these subcellular structures are often membrane-bound organelles, whose volume is the physiologically important aspect of their size. Scaling organelle volume with cell volume can be explained by limiting pool mechanisms, wherein a constant concentration of molecular building blocks enables subcellular structures to increase in size proportionally with cell volume. However, limiting pool mechanisms cannot explain how the size of linear subcellular structures, such as cytoskeletal filaments, scale with the linear dimensions of the cell. Recently, we discovered that the length of actin cables in budding yeast (used for intracellular transport) precisely match the length of the cell in which they are assembled. Using mathematical modeling and quantitative imaging of actin cable growth dynamics, we found that as the actin cables grow longer, their extension rates slow (or decelerate), enabling cable length to match cell length. Importantly, this deceleration behavior is cell-length dependent, allowing cables in longer cells to grow faster, and therefore reach a longer length before growth stops at the back of the cell. In addition, we have unexpectedly found that cable length is specified by cable shape. Our imaging analysis reveals that cables progressively taper as they extend from the bud neck into the mother cell, and further, this tapering scales with cell length. Integrating observations made for tapering actin networks in other systems, we have developed a novel mathematical model for cable length control that recapitulates our quantitative experimental observations. Unlike other models of size control, this model does not require length-dependent rates of assembly or disassembly. Instead, feedback control over the length of the cable is an emergent property due to the cross-linked and bundled architecture of the actin filaments

within the cable. This work reveals a new strategy that cells use to coordinate the size of their internal parts with their linear dimensions. Similar design principles may control the size and scaling of other subcellular structures whose physiologically important dimension is their length.

B92/P1090

Actin wave mediates cell shape-dependent actin accumulation for protrusive activity

K. Yagami, K. Baba, S. Misu, H. Katsuno-Kambe, K. Okano, Y. Sakumura, Y. Hosokawa, N. Inagaki; Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan

Actin filaments (F-actins) accumulate at cell protrusions, for example, leading edge in migrating cells, microvilli in epithelial cells, invadopodia in cancer cells, and dendritic spine in neurons. However, how F-actins accumulate at cell protrusion is unclear. Previously, our group reported the mechanism of actin wave propagation along neuronal axons. Actin waves propagate by polymerization of F-actins which are anchored to the extracellular adhesive substrate through the clutch molecule Shootin1 and the cell adhesion molecule L1-CAM. G-actins dissociating from the rear of the filaments move toward the polymerizing ends by diffusion, because G-actin concentration is locally higher at the rear due to disassembly and is lower at the front due to polymerization. Through this mechanism, actin waves transport actin and actin-associated proteins along axons. The purpose of this study is to examine a possibility that F-actins accumulate at cell protrusions via actin waves.First, we prepared a glass substrate with triangle cell adhesion area. U-251 glioma cells cultured on the substrate formed triangular shape. In the triangular cells, F-actins accumulated at the three corners. Higher accumulation was observed when the corner angle is smaller. This result suggests that F-actins sense the cell shape and spontaneously accumulate at cell protrusions. Second, we observed F-actins that migrate in the direction of polymerization as actin waves at ventral and lateral sides of glioma cells using total internal reflection fluorescence microscopy. The ventrally-generated actin waves reached the cell edge and moved laterally. Shootin1b co-migrated with the actin waves. Shootin1b-KO delayed the speed of ventral and lateral actin waves and disturbed F-actin accumulation at the corners of the triangle. In addition, mathematical models demonstrated that the mechanism of actin waves can explain the F-actin accumulation at cell protrusions. Furthermore, Shootin1b-KO delayed cell polarity formation and migration of glioma cells. These data suggest that the cell shape-dependent actin accumulation via actin waves plays a key role in cellular protrusive activity.

B93/P1091

3D architecture of phagocytic podosomes examined using iPALM

J. C. Herron[1], **S. Hu**[1], T. Watanabe[1], A. T. Nogueira[1], B. Liu[1], M. E. Kern[1], J. Aaron[2], A. Taylor[2], M. Pablo[1], T.-L. Chew[2], T. C. Elston[2], K. M. Hahn[1]; [1]University of North Carolina At Chapel Hill, Chapel Hill, NC, [2]Howard Hughes Medical Institute Janelia Research Campus, Ashburn, VA

Actin-based structures known as podosomes play important roles in migration, phagocytosis, and other cellular processes. Using interferometric photoactivated localization microscopy (iPALM), we were able to examine the actin structure of phagocytic podosomes with 15-20 nm resolution. These studies were supplemented by structured illumination microscopy (SIM) used to examine the distribution of actin-associated molecules, including paxillin, talin, myosin II, alpha-actinin, and cortactin. We used a frustrated phagocytosis model, in which macrophages attempt to engulf circular patterns of IgG antibodies attached to coverslips. They form a phagocytic apparatus, but are ‘frustrated’ because they

are unable to fully engulf the IgG. This generates arrays of podosomes with regular, well-defined geometry, enabling us to apply a semi-automatic pipeline based on persistent homology to identify and measure features across podosome populations. This revealed that podosomes have an hourglass shape, with an actin knob protruding into the plasma membrane, and two actin networks extending from the hourglass. Associated proteins were concentrated at the base and in some cases along the sides of the podosomes. The morphology of phagocytic podosomes will be important as further studies reveal how the arrangement of signaling molecules controls function, and how podosomes sense and respond to mechanical forces.

B94/P1092

Reconstitution of the transition from a lamellipodia- to filopodia-like actin network with purified proteins

C. Suarez, J. D. Winkelman, A. J. Harker, P. M. McCall, A. N. Morganthaler, M. L. Gardel, D. R. Kovar; University of Chicago, Chicago, IL

How cells simultaneously assemble and maintain functionally diverse actin filament networks with distinct architectures and dynamics within a common cytoplasm is an outstanding question. Filopodia are finger-like membrane protrusions composed of long, parallel actin filaments bundled by fascin, which sense extracellular chemical and mechanical signals to help steer motile cells. Two processive actin filament barbed end elongation factors, formin and Ena/VASP, localize to filopodia tips and drive their elongation. One hypothesis is that filopodia are generated from the lamellipodia, which consists of densely packed, branched actin filaments that are nucleated by Arp2/3 complex and kept short by capping protein. Are different actin filament elongation factors necessary and sufficient to facilitate the emergence of filopodia with diverse characteristics from a highly dense network of short-branched capped filaments?We combined bead motility and micropatterning biomimetic assays, with multi-color Total Internal Reflection Fluorescence microscopy imaging, to successfully reconstitute the formation of filopodia-like networks (FLN) from densely-branched lamelipodia-like networks (LLN) with eight purified proteins (actin, profilin, Arp2/3 complex, Wasp pWA, fascin, capping protein, VASP and formin mDia2). Although saturating capping protein concentrations inhibit FLN assembly, the inclusion of mDia2 or VASP differentially rescues the transition of LLNs to FLNs. Formin mDia2-generated FLNs are relatively long and lack capping protein, whereas VASP-generated FLNs are comparatively short and contain capping protein. Our biomimetic reconstitution systems reveal that formin or VASP are necessary and sufficient to induce the transition from a LLN to a FLN, and establish robust in vitro platforms to investigate FLN assembly mechanisms.

Motors: Dyneins

B96/P1093

Using cryo-EM to visualize snapshots of dynein's activation pathway

A. A. Kendrick, A. E. Leschziner, S. L. Reck-Peterson; Cellular and Molecular Medicine, University of California, San Diego, San Diego, CA.

Regulation of cytoplasmic dynein-1 (dynein) is critical for diverse functions of eukaryotic cells, including cell division and long-range intracellular transport. Both dynein and Lis1, an essential dynein regulator, are mutated in patients with neurodevelopmental diseases and are conserved from fungi to mammals.

Dynein activity is controlled by an autoinhibited state called “Phi”, in which its two motor domains interact in a way that prevents motility. Lis1 is important for promoting the formation of active dynein complexes and it has been proposed to do so by stabilizing a dynein conformation that is not autoinhibited. Recently, we solved a structure of dynein bound to Lis1 in which two Lis1 dimers are inserted between two dynein motor domains; this “Chi” conformation appears to capture an early step in the dynein activation pathway. What other steps are involved in the full activation of dynein remains unknown. Here, our goal was to use Cryo-EM to visualize as many of these steps as possible to determine the structural changes underlying dynein activation by Lis1. We did so by purposely introducing heterogeneity during our Cryo-EM sample preparation; including ATP in our samples allowed dynein to go through its mechanochemical cycle. Using this approach, we captured eight distinct dynein and dynein-Lis1 structures from the same sample. These snapshots reveal novel conformations of dynein-Lis1 that we propose represent additional intermediate states in the dynein activation pathway. We will present a new model for how Lis1 relieves dynein autoinhibition and promotes conformations that are compatible with motility.

B97/P1094

Microtubule binding-induced allostery promotes Pac1/LIS1 dissociation from dynein prior to cargo transport

C. Beauchamp-Perez[1], N. Flint[1], L. G. Lammers[1], Y. Wang[2], P. Chai[2], K. Zhang[2], **S. M. Markus**[1]; [1]Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO, [2]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.

The lissencephaly-1 gene (Lis1, or Pac1 in budding yeast) is a highly conserved regulator of the retrograde microtubule motor dynein. Lis1 functions in part by stabilizing an open/partially active conformational state of dynein, and by enabling dynein localization to the plus ends of dynamic microtubules. Although dynein-Lis1 binding is required for dynein cargo transport functions, dissociation of this complex prior to initiation of motility appears to be equally important, as preventing as much leads to defects in dynein function. We sought to understand whether and how dynein-Lis1 binding affinity is modulated. To this end, we engineered dynein mutants that lock the motor in a constitutively microtubule-bound (MT-B) or microtubule-unbound (MT-U) conformational state. Whereas the MT-B mutant exhibits low affinity for Pac1 in cells, we find that the MT-U mutant binds with high affinity to Pac1, and as a consequence remains almost irreversibly bound to microtubule plus ends in cells. Using a combination of in vivo and in vitro approaches, we find that a monomeric motor domain fragment is sufficient to exhibit these opposing affinities for Lis1, and that this phenomenon is conserved with human proteins. A cryoEM structure of the human MT-B mutant reveals changes at the Lis1-binding surface that may account for the differential binding affinity of dynein for Lis1 when it is either bound or unbound to microtubules. Our data reveal that microtubule binding-induced conformational changes in dynein coordinate its dissociation from Lis1/Pac1, which is required for dynein activity.

B98/P1095

Lis1 bridges dynein to dynactin’s p150 arm to regulate complex assembly and motility

C. Lau, K. Singh, A. Carter; MRC-LMB, Cambridge, UNITED KINGDOM.

The dynein/dynactin/adaptor motor complex is a tightly regulated molecular machine that transports cargo along microtubules. One of its main regulators is Lis1, mutations of which cause the

neurodevelopmental disease lissencephaly. Lis1 is known to bind to the dynein motor domain and is important for the initiation of transport. Key questions are how Lis1 stimulates dynein's interaction with dynactin and whether it remains attached to the resulting complex during transport. Here we determine the cryo-EM structure of Lis1 bound to a dynein/dynactin/adaptor complex on microtubules. We use JIP3, the cargo adaptor for lysosomes, showing for the first time that this family of adaptors activates dynein. Unexpectedly we find Lis1 bridges dynein's motor to dynactin's p150 arm, a critical domain for processive dynein/dynactin movement. Our structure is the first observation of the p150 arm in its fully open and active conformation, showing its interaction with dynein's intermediate chain as well as Lis1. Our work explains how Lis1 directly aids dynein/dynactin complex formation and provides a mechanism for it to remain attached during cargo transport.

B99/P1096

A dynein-Lis1 structure captures an intermediate state in dynein's activation pathway

E. P. Karasmanis[1], J. M. Reimer[1], A. A. Kendrick[1], J. A. Rodriguez[1], J. B. Truong[1], A. E. Leschziner[1], S. L. Reck-Peterson[1,2]; [1]University of California San Diego, San Diego, CA, [2]Howard Hughes Medical Institute, Chevy Chase, MD.

Cytoplasmic dynein-1 is the major motor responsible for intracellular transport of cargo towards microtubule minus ends. Dynein motors are autoinhibited in a "Phi" conformation and undergo a series of conformational changes to form active complexes, which consist of two dynein dimers, the dynactin complex and activating adaptor(s). We and others have recently shown that Lis1 is required to form these active dynein complexes. Here, using cryo-electron microscopy, we solve a high-resolution structure of two Lis1 dimers inserted in-between a dynein motor dimer. Our structure reveals two new contact sites between dynein and Lis1 and is suggestive of the mechanism by which Lis1 promotes the relief of dynein autoinhibition. Using structure-guided mutagenesis, we disrupted each of these sites and showed that they are required for Lis1's regulation of dynein in vivo in *S. cerevisiae*. In vitro reconstitution of human dynein complexes in the presence of wild type or mutated Lis1, suggests that these contact sites are also important for Lis1's role in forming activated human dynein-dynactin-activating adaptor complexes. We propose that our structure represents an intermediate in dynein's activation pathway.

B100/P1097

Structure of dynein-dynactin on microtubules shows tandem adaptor binding

S. Chaaban, A. P. Carter; MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

Cytoplasmic dynein is a microtubule motor that is activated by its cofactor dynactin and a coiled-coil cargo adaptor. Up to two dynein dimers can be recruited per dynactin, and interactions between them affect their combined motile behaviour. Different coiled-coil adaptors are linked to different cargos, and some share motifs known to contact sites on dynein and dynactin. There is currently limited structural information on how the resulting complex interacts with microtubules and how adaptors are recruited. Here, we develop a cryo-EM processing pipeline to solve the high-resolution structure of dynein-dynactin and the adaptor BICDR1 bound to microtubules. This reveals the asymmetric interactions between neighbouring dynein motor domains and how they relate to motile behaviour. We find unexpectedly that two adaptors occupy the complex. Both adaptors make similar interactions with the

dyneins but diverge in their contacts with each other and dynactin. Our structure has implications for the stability and stoichiometry of motor recruitment by cargos.

B101/P1098

Evolutionarily conserved roles of the dynein intermediate chain and Ndel1 in assembly and activation of dynein

K. Okada[1], **B. R. Iyer**[2], L. G. Lammers[2], P. Gutierrez[1], W. Li[1], S. M. Markus[2], R. J. McKenney[1]; [1]Biochemistry and Molecular Biology, University of California Davis, Davis, CA, [2]Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO.

In the absence of cargo, cytoplasmic dynein assumes an autoinhibited conformation and must be activated for its transport function through the regulated assembly of a dynein-dynactin-adapter (DDA) protein super complex. The interaction between dynein and dynactin was first ascribed to a small portion of the N-terminus of the dynein intermediate chain (IC) and a fragment of the coiled-coil domain of the dynactin subunit p150^{Glued}. This intrinsically disordered N-terminal segment of the IC (ICN) also directly interacts with the dynein regulators Nde1 and Ndel1, which compete with p150^{Glued} for binding to ICN. However, high-resolution cryo-EM structures of DDA complexes did not reveal the relevant domains of ICN and p150^{Glued}, raising questions about the importance of this interaction. Using a combination of *in vitro* and *in vivo* analyses we assessed the importance and evolutionary conservation of the ICN-p150, ICN-Ndel1, and Ndel1-Lis1 interactions in the assembly of DDA complexes. Deletion of ICN abolishes dynein's interaction with dynactin at plus ends in budding yeast, and abrogates the assembly of active DDA complexes *in vivo* and *in vitro*. In both yeast and mammalian systems, we find that the ICN plays an evolutionarily conserved role in binding Ndel1 (Ndl1 in yeast), which recruits LIS1 (Pac1 in yeast) to the dynein complex. Interestingly, we find that Lis1/Pac1 cannot simultaneously bind to Ndel1/Ndl1 and the dynein motor domain, indicating that Lis1 must dissociate from Ndel1 prior to binding dynein. Whereas addition of either Ndel1 or p150^{Glued} disrupt DDA complex assembly *in vitro*, neither affected preassembled DDA complexes, nor bound to processively moving motor complexes, indicating that the ICN may preferentially bind p150^{Glued} in the context of an active DDA complex. With the help of AlphaFold2 structure predictions, we delineate residue level interaction surfaces between the human and yeast ICN-Lis1-Ndel1 tripartite complex. Our study reveals previously unknown regulatory steps in the dynein activation pathway and provides a more complete model for how both Lis1/Ndel1 and dynactin/cargo-adapters integrate to regulate dynein motor activity.

B102/P1099

Using multi-scale computational approach to investigate Dynein's motility

L. Li; University of Texas El Paso, El Paso, TX.

Dynein is a molecular motor for cargo transportation and force generation in cells. Dysfunction of dynein is associated with many diseases, such as ciliopathies, lissencephaly and other neurodegeneration disorders. Understanding the functions of dynein is crucial for developing new treatments of such diseases. Electrostatic interactions play important roles in proteins including dyneins. A lot of efforts have been made to study the electrostatic interactions in biological systems. However, it is extremely challenging to accurately calculate the electrostatic interactions in large biological systems such as dynein. I will introduce a novel multi-scale simulation approach which is used to study dynein's motion along microtubules. The electrostatic binding funnel around microtubule is observed, which

drags the dynein to the binding pocket. The electrostatic forces on dynein residues form a torsion which reorients the dynein when it is in an un-native orientation. Furthermore, the electrostatic component of the binding energy of dynein and microtubule strongly affects the velocity and run length of the dynein. These results reveal the mechanisms of dynein's motility and functions along microtubule, which shed light on treatments of molecular motor related diseases.

B103/P1100

The Kash5 protein involved in meiotic chromosomal movements is a novel dynein activating adaptor

R. Agrawal[1], J. P. Gillies[1], J. L. Zang[1], J. Zhang[2], S. R. Garott[3], H. Shibuya*[2], J. Nandakumar*[1], M. DeSantis*[1]; [1]MCDB, University of Michigan, Ann Arbor, MI, [2]University of Gothenburg, Gothenburg, SWEDEN, [3]Biological Chemistry, University of Michigan, Ann Arbor, MI.

Dynein is the primary retrograde molecular motor that harnesses ATP hydrolysis to move cargo on microtubules in multiple biological contexts. Dynein meets a unique challenge in meiosis by moving chromosomes tethered to the nuclear envelope to facilitate homolog pairing essential for gametogenesis. The nuclear envelope (NE) is intact during prophase I, implying that dynein is separated from its cargo by two lipid bilayers (the inner nuclear membrane [INM] and outer nuclear membrane [ONM]) and the perinuclear space between them. Despite being a process essential to meiosis progression and fertility, how dynein is activated to drive these movements across the NE remains unknown. Dynein tethers to chromosomes via the highly conserved linker of nucleoskeleton and cytoskeleton (LINC) complex at the NE. The LINC complex consists of a SUN protein (SUN1) and a KASH protein (KASH5) that span the INM and ONM, respectively and bind each other in the perinuclear space. While SUN1 tethers the chromosomes to INM, KASH5 binds dynein completing the attachment between dynein and the chromosomal cargo. Binding of an activating adaptor is required to make dynein processive, however, the identity of the activating adaptor required for dynein to move meiotic chromosomes is unknown. We show that meiosis-specific nuclear envelope protein KASH5 is a dynein activating adaptor. Using GST-pull downs, co-immunoprecipitation, isothermal calorimetry, and SEC-MALS we show that KASH5 directly binds dynein using a mechanism conserved among activating adaptors. Using single-molecule total internal reflection fluorescence (TIRF) microscopy, we demonstrate that purified KASH5 converts dynein into a processive motor that walks processively on microtubule tracks. We generated a homology model to map the dynein-binding surface of KASH5, identifying mutations that abrogate dynein binding in vitro and disrupt recruitment of the dynein machinery to the nuclear envelope in cultured cells and mouse spermatocytes in vivo. Together, this work identifies the first transmembrane activating adaptor of dynein and provides detailed molecular insights into how dynein is activated to power chromosome movements in meiosis to pair homologs.

B104/P1101

Ninein expression and involvement in phagocytosis in macrophages

S. Omer, N. Gandhi, R. Harrison; University of Toronto, Scarborough, ON, CANADA.

Phagocytosis of pathogens and foreign particles is a central process of the innate immune system. After target internalization by macrophages, the resultant phagosomes undergo an intricate maturation process that overlaps with its movement toward the cell center, ultimately resulting in degradation of the engulfed particle. Our work and others have shown that the minus-end-directed dynein microtubule motor is recruited to late phagosomes and is critical for phagosome maturation. It remains unclear

whether dynein activity and dynein adaptors are involved in earlier steps of particle internalization. Here we show that the microtubule-nucleator ninein that is known to localize primarily to the centrosome, is recruited to phagocytic cups co-localizes with F-actin and EEA1, early endosomal markers in the macrophage-like RAW 264.7 cell line. Similar accumulations at the phagocytic cups were also observed for dynein motor and its cofactor, dynein intermediate chain as early as 5 min after encountering IgG-opsonized particles. Ninein knockdown experiments showed defective macrophage spreading and reduced internalization of IgG- opsonized particles. We examined the potential mechanism of defective phagocytosis and found that ninein depletion impaired microtubules nucleation, reduced the radial microtubule array and resulted in defective dynein localization. These findings suggest that ninein participates in the early events of Fcγ-mediated phagocytosis, potentially by regulating MT organization and/or facilitating dynein-mediated early phagocytic force generation. We also discovered that macrophages express three ninein isoforms. Interestingly, western blotting of isolated latex-bead phagosomes revealed the presence of dynein and dynactin together with an additional smaller ninein isoform, suggesting distinctive regulation of ninein and dynein cellular functions within macrophages.

B105/P1102

Phagosomal pH regulates motor protein kinetics in macrophages

S. Patel, H. S. Shamroukh, K. C. Kondapalli, **S. K. Tripathy**; Department of Natural Sciences, University of Michigan - Dearborn, Dearborn, MI.

Macrophages are professional phagocytes involved in elimination of microbial pathogens and antigen presentation to T-cells of the immune system. Macrophages internalize microbes trapping them within a vacuole known as the phagosome. Directed transport of the phagosome on the microtubules, towards the minus ends, for fusion with lysosomes is crucial for pathogen degradation. Motion of the phagosome on the microtubules is bidirectional, with a tug-of-war between dynein and kinesin motor proteins, directing the phagosome towards and away from the lysosome respectively. The factors regulating this phagosome transport are, however, not completely clear. In a recently published study, we showed that the sodium proton exchanger, NHE9, regulates luminal pH of the phagosome to impact phagosome transport. Specifically, we demonstrated that an increase in luminal pH leads to significant decrease in the processive movements and run length without effecting the velocity. Our objective here, was to expand on these observations and characterize the impact of luminal pH on the kinetics of the motor proteins under load. To this end, we engineered stable overexpression of NHE9 in the well-established macrophage cell line, RAW264.7. We determined that phagosomes in control cells were consistently more acidic at all the evaluated time points post-ingestion of gram-negative bacteria (Escherichia coli) or gram-positive bacteria (Staphylococcus aureus), relative to phagosomes in NHE9 overexpressing macrophages. Next, we conducted optical trapping experiments at single-molecule resolution on 800nm carboxylate-coated polystyrene beads, phagocytosed by RAW 264.7 cells. We observed that under load, the average binding time of dynein was significantly higher than kinesin for the acidic phagosomes of control cells. Interestingly, the binding times of these motor proteins were similar for the relatively alkaline phagosomes in NHE9 overexpressing cells. Based on these observations, we hypothesize that the reduction in run length is a direct consequence of the decreased dynein binding time. It is plausible that the increase in diffusive motion of the relatively alkaline phagosomes, we noted previously, is due to an unresolved tug-of-war between the opposite motors. These findings have significant implications for our understanding of macrophage mediated immune response.

B106/P1103

Elucidating mitochondrial transport regulation using synthetic cargo in living cells

C. Gladkova, R. D. Vale; HHMI Janelia Research Campus, Ashburn, VA.

Mitochondrial microtubule-based transport shapes the cellular mitochondrial network alongside cycles of fusion, fission and mitochondrial turnover. Defects in these processes that collectively govern mitochondrial dynamics contribute to neurodegeneration. Anterograde transport supports energetically demanding peripheral processes such as cell motility and synaptic activity, while retrograde transport favours mitochondrial turnover in the cell body. Anterograde kinesin-1 and retrograde dynein/dynactin motor complexes both interact with mitochondria via a conserved two-component Miro/TRAK adaptor complex to facilitate bidirectional mitochondrial motility. Because of the close coupling of mitochondrial transport with other facets of mitochondrial dynamics, it has been challenging to isolate specific cues that regulate mitochondrial transport directly. To probe the direct molecular determinants of mitochondrial transport and directionality in cells, we have developed an orthogonal protein scaffold-based cargo assay. We recapitulate the Miro/TRAK mitochondrial adaptor complex by displaying the mitochondrial transport adaptor Miro on the surface of a self-assembled 60-subunit icosahedral particle in cells [1], and co-expressing a cytosolic form of TRAK. Through detailed molecular dissection, live-cell imaging and biochemistry we define elements that regulate the balance between the two opposing motors. We also reveal how these regulators may tune mitochondrial transport in response to environmental changes. Our tool enables us to bridge molecular-level understanding of mitochondrial transport from *in vitro* work and the complex regulation of mitochondrial transport in cells.

[1] Hsia Y et al, 2016, Design of a hyperstable 60-subunit protein icosahedron *Nature*, **535**, 136-9

B107/P1104

Structural and mechanistic insights into dynein-based mRNA sorting during *Drosophila* development

M. A. McClintock[1], K. Singh[2], S. Chilaeva[1], A. P. Carter[2], S. L. Bullock[1]; [1]Cell Biology, MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM, [2]Structural Studies, MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

Subcellular targeting of mRNA by microtubule-based motors is a widespread mechanism for spatial control of protein function. Much of our understanding of this process comes from studies of *Drosophila* oogenesis and embryogenesis, which rely on asymmetric protein expression for axis determination and polarized cell functions. In these systems, sequence-divergent RNA localization signals are detected by the non-canonical RNA-binding protein Egalitarian (Egl) and direct the activation of cytoplasmic dynein via association with the coiled-coil adaptor Bicaudal-D (BicD). How divergent localization signals are recognized by Egl is unclear. Furthermore, it is not understood how dynein motor activity is adapted by localizing mRNAs during oogenesis to produce different localization patterns of axis determinants. Using cryo-electron microscopy, we have determined the structures of Egl-BicD complexes bound to RNA localization signals from transcripts that adopt different subcellular localizations. We describe the modular architecture of the RNA-binding pocket of Egl, which is formed by novel double-stranded RNA-binding motifs and a previously annotated exonuclease domain. This modularity confers flexibility to the complex and enables accommodation of RNA localization signals that are structurally related yet not identical. Additionally, by combining single-molecule reconstitutions with *Drosophila* genetics, we show that engagement of Egl with the localizing transcript *gurken* (*grk*) promotes the adaptation of dynein behavior by the hnRNP Squid (Sqd). Our data suggest that Sqd promotes the multimerization of both *grk*

mRNA and active dynein motor complexes, leading to specific targeting of the mRNA to the dorso-anterior region of the oocyte. Collectively, our data provide the first structural insights into recognition of RNA localization signals by a microtubule-bound motor and reveal control of mRNA copy number as an effective strategy for modulating the localization of RNA-motor complexes.

B108/P1105

New adaptor proteins of dynein-dynactin motor complex

T.-Y. Yeh[1], A. Vemu[1,2], B. Clarkson[1], J. Bennett[1], K. Meurer[1], S. A. Ketcham[1], T. A. Schroer[1]; [1]Johns Hopkins University, Baltimore, MD, [2]National Institute of Neurological Disorders and Stroke, Bethesda, MD

Cytoplasmic dynein is well known for transporting intracellular cargoes along microtubules. However, how it is targeted to its many cargoes is not fully defined and other roles have not been completely explored. Here we show the diversity of cytoplasmic dynein functions in endomembrane organization using the small molecule dynein inhibitor, dynarrestin. We find that dynein stabilizes endocytic recycling tubules on microtubules, and that it serves as a "brake" to slow kinesin-mediated movement of post-Golgi carriers.Dynactin is an essential component of the dynein motor complex. The complex is stabilized by "activating adaptor" proteins, which tether dynein to dynactin and various cargoes. High-resolution cryo-EM studies have shown that many adaptors directly contact the dynactin DCTN5 (p25) component at distinct sites. Using DCTN5 knockdown followed by mutant rescue we identified two functional motifs: a basic loop and the C-terminal alpha helix. Lysine residues K74 or K78 in the basic loop are required for binding to adaptors (BICD1/2 and Hook1/FIP3, respectively) that mediate dynein-based membrane transport. The C-terminal alpha-helix, which is essential for viability in mice, is required for normal regulation of actin dynamics and primary ciliogenesis. This motif is required for association with a different set of adaptors (Hook2, Hook3, girdin, daple). Furthermore, we used a bioinformatics approach to identify three novel adaptor candidates. These proteins can bind to dynein and dynactin directly, and their binding relies on DCTN5. DCTN5 loop K74 and K78 residues and C-terminus differently contribute to these adaptor candidates interaction with dynein/dynactin.

B109/P1106

Investigating the mechanism of the dynein regulatory protein, Ndel1

S. R. Garrott, A. Siva, J. P. Gillies, R. C. El Jbeily, M. E. DeSantis; University of Michigan, Ann Arbor, MI

Dynein is a microtubule minus-end directed motor protein that functions in cell division, intracellular cargo trafficking, and neuronal migration. The paralogs Ndel1 and Nde1 are involved in all of dynein's processes and are implicated in numerous neurodevelopmental diseases, but the mechanisms and direct effects of Ndel1/Nde1 in dynein regulation are unclear. The discovery of dynein's conformational equilibrium, whereby dynein shifts from an autoinhibited conformation to an active conformation, has been instrumental to uncovering the mechanisms of other dynein regulatory proteins, but Ndel1/Nde1 have not been studied in this context. In this study, we investigated Ndel1's mechanisms of dynein regulation, using dynein's conformational equilibrium as a new framework to understand Ndel1. We confirmed Ndel1-dynein binding sites and determined affinities of these sites using quantitative pull-down assays; we also used this method to determine how Ndel1 influences dynein binding to other key regulatory proteins. We next used single molecule TIRF microscopy to determine how Ndel1 affects motility of the active dynein-dynactin-cargo adaptor complex. We also used TIRF microscopy to probe

how Ndel1 and Lis1 function together in regulating dynein motility. Finally, we used negative stain electron microscopy to examine Ndel1's effects on dynein conformation. The major finding of this study is that, in contrast to dynactin, cargo adaptors, and Lis1, which promote dynein activity, Ndel1 acts as a scaffold that inhibits activation of dynein motility and modulates other regulatory proteins' ability to bind dynein. The results of this study provide a comprehensive view of Ndel1's function in dynein regulation, placing its function into the larger dynein regulatory network.

B110/P1107

Spatial Regulation of Dynein Forces on Microtubule Asters in Frog Eggs

T. Mitchison, C. Field; Harvard Medical School, Boston, MA

Centrosomes are positioned by dynein pulling on astral microtubules. In small cells, where microtubules reach the cortex, this force is exerted mainly by cortical dynein. In large Xenopus eggs, where microtubules do not reach the cortex for much of the cell cycle, dynein is thought to pull on astral microtubules from sites scattered throughout the cytoplasm, but spatial regulation of dynein in large cells is poorly understood. After anaphase of 1st mitosis, centrosomes move away from the midplane of the egg, which is defined by microtubule bundles coated with Chromosome Passenger Complex (CPC). Previous models hypothesized that dynein forces on centrosomes are directed outwards from the midplane by differences in aster radius or surface area between the sides facing the midplane vs. the outside. We hypothesized that the AURKB activity of the CPC might locally inactivate dynein at the midplane, causing a force asymmetry that directs centrosome movement. We are testing this hypothesis in egg extracts using polarized sperm asters, which recruit CPC to a crescent on the chromatin-proximal side. Using an improved method for freezing egg extract, we analyzed aster growth and movement with high reproducibility. Polarized asters moved in response to dynein-dependent forces with the CPC-positive side usually trailing, consistent with negative regulation of dynein by the CPC. Beads coated with the dynein adapter HOOK2 recruit dynein and model organelles. Co-recruitment of CPC to HOOK2 beads inhibited their transport by dynein. This inhibition required AURKB activity, consistent with inhibition of dynein by AURKB. Preliminary biochemistry suggested that AURKB did not inhibit binding of dynein or dynactin subunits to HOOK2. Instead, active CPC recruited KIF20A, a plus end-directed motor that may oppose dynein motility. This finding suggests a role for KIF20A in regulating the balance of motor-dependent forces on centrosomes, in addition to its known role in recruiting CPC to midplane microtubules.

B111/P1108

Exploring dynein regulation in directed cell migration

J. L. Zang, A.-M. Zheng, D. Gibson, M. DeSantis; University of Michigan, Ann Arbor, MI

Directional cell migration requires the dramatic reorganization and coordination of all cytoskeletal networks in the cell. Although cell migration is largely an actin driven process, the microtubule network also plays a vital role in mesenchymal migration. A polarized microtubule network provides necessary tracks for the continuous traffic of polarization proteins towards the leading edge, which is important for directional persistence during migration. This microtubule orientation is established by cytoplasmic dynein-1 (dynein), the main minus-end directed microtubule motor in the cell. Upon a migratory stimulus, dynein produces traction forces on the microtubule network to move the centrosome and golgi apparatus to be anterior to the nucleus in the direction of migration. While it is known that dynein

enriches at the cortex of a migrating cell to induce the centrosome repositioning, the molecular mechanisms of how dynein localizes to the leading edge cortex or is activated during migration are currently unknown. Using proximity-dependent biotinylation coupled with mass spectroscopy on two known dynein regulators (Lis1 and Ndel1), biochemistry, and cell biology, I have identified novel machinery governing dynein regulation at the cell cortex. Here I show the necessity of this machinery for the dynein dependent reorientation of the centrosome and the maintenance of direction during cell migration. I hypothesize that upon migration, specific proteins localized to the cortical leading edge recruit Lis1 and Ndel1, which then locally activate a small pool of dynein for the proper polarization of the microtubule network. This work provides mechanistic insight into the regulation of dynein's activity in mesenchymal cell migration.

B112/P1109

Impact of a Dynactin p62 (DCTN4) Variant on Lung Epithelial Functions

E. Mattson, S. Ma, T. Schroer; Johns Hopkins University, Baltimore, MD

A variant of the dynactin component p62(DCTN4 rs35772018; hereafter DCTN4-Y270C) has been correlated with earlier onset of chronic Pseudomonas infection in CF patients, and worsened outcomes in ARDS, two conditions which lead to chronic inflammation and wounding of the lung epithelium. Response of the lung epithelium to infection and damage involves its role as an innate immune tissue, its barrier function, and its ability to migrate and undergo wound healing. I will describe what we have found with regards to the role of DCTN4-Y270C in all three of these functions. Our lab has shown that cells expressing DCTN4-Y270C migrate more slowly than WT in scratch-wound assays. We also see altered focal adhesions, defective integrin cycling, and aberrant actin dynamics. This suggests that DCTN4-Y270C impacts dynein-dependent transport of integrin and possibly other cargoes. To gain insight into the molecular basis of the subcellular trafficking and cell migration defects, we used an unbiased proximity-dependent biotinylation (BioID) screen. Several promising differentially interacting candidates were identified in the BioID datasets, including RUFY1, a Rab4/14 binding protein that has been shown to regulate integrin trafficking, and Ephexin-4, which has been implicated in actin dynamics and microtubule mediated focal adhesion disassembly. The bronchial epithelium is an innate immune tissue, and RNA sequencing of human bronchial epithelial (HBE) cells expressing Y270C revealed an altered immune profile with downregulation of several pathways involved in innate immune response. Alteration of these pathways and downregulation of critical anti-microbial peptides likely play a role in impaired antimicrobial function of DCTN4-Y270C.Finally, although we have observed normal tight junction formation in polarized DCTN4-Y270C HBEs, we observe alterations in adherens junction proteins with downregulation of E-cadherin and upregulation of N-cadherin. This, along with upregulation of several other EMT related genes suggests a partial EMT phenotype in the Y270C HBE. The functional consequences of this still need to be explored. In the context of the DCTN4-Y270C mutation, cystic fibrosis and ARDS can be viewed as a sensitizing background that enhances this mutation's negative impact on lung disease. Knowledge of the mechanisms by which DCTN4-Y270C exerts its effects will not only open up the opportunity for development of therapies to combat its detrimental effects, but will also allow us to predict if those with DCTN4-Y270C will be more susceptible to certain environmental insults that they should be advised to avoid such as smoking and exposure to infection (SARS-CoV2).

B113/P1110

Investigating the Molecular Evolution of Dynein and the Dynactin Complex

S. Bohling, H. Goodson; University of Notre Dame, Notre Dame, IN

The last eukaryotic common ancestor, LECA, is the progenitor of all eukaryotic organisms. We can learn about LECA through the study of early diverging organisms, and understanding the cell biology of LECA can provide insight into the cell biology of all eukaryotes. Red algae diverged from the lineage leading to green plants over 1 billion years ago. The cytoskeleton is essential for key cellular processes including cell division, intracellular transport, and cell motility. Therefore, learning more about the cytoskeleton of red algae could provide insight into the biology of LECA and thus into the function of cytoskeletal proteins in all eukaryotic cells. My work uses bioinformatic and computational biology approaches to identify the molecular motor cytoplasmic dynein and its accessory dynactin complex in both red algae and their phagotrophic sister phylum *Rhodelphis*. This work is interesting because until recently, plants (including red algae) were thought to completely lack cytoplasmic dynein/dynactin, and it was often assumed that these proteins were limited to the clade containing animals and fungi. Our work shows that dynein is found in *Rhodelphis* and several clades of red algae; consistent with this observation, several components of dynactin complex are found in these organisms as well. This observation shows that cytoplasmic dynein existed in at least the common ancestor of plants and animals/fungi. Interestingly, some red algae (Atlantic *Porphyra umbilicalis* and its Pacific relatives) seem to be in the process of losing their dynein proteins, as the dynein motor is present, but divergent, and dynactin complex proteins are either difficult to recognize or absent. Future studies will look at the relationship between dynein and dynactin complex proteins in red algae and other divergent organisms to gain a better understanding of the cell biology of LECA.

Motors: Kinesins in Mitosis and Spindle Regulation

B114/P1111

MKLP1 mediates RNA granule assembly and protein translation in the midbody

S. Park, R. Dahn, A. R. Skop; University of Wisconsin-Madison, Madison, WI.

MKLP1 is a spindle-midzone kinesin motor protein that functions in midzone/midbody microtubule assembly, and is necessary for the completion of cytokinesis. Abscission results in two daughter cells, and one midbody remnant (MBR)--a RNA-enriched extracellular vesicle suspected to play a role in mediating cell fate. Recent work from the Prekeris and Simpson labs have shown that midbody remnants (MBRs) can increase cell proliferation indexes when placed on naïve HeLa cells (Peterman, 2019; Rai, 2021), suggesting that information being transferred to cells. To determine how the midbody/MBR assembly might be mediated by MKLP1 and known midbody RBPs, we sought to take a cell biological and genetic approach. Using RNAScope™ Poly A mRNA probes, we discovered that RNAs are abundant in the midbody matrix, a region devoid of tubulin antibody. This accumulated mRNAs mass at the midbody is wrapped by MKLP1, and is lost in MKLP1 siRNA-treated cells. The RBPs, Arc and ESCRTIII, are not necessary for RNA accumulation at the midbody matrix, as Poly A probes reveals a normal accumulation of RNA. Next, using Click-It chemistry, we utilized homopropargylglycine (HPG), a Methionine analogue, to visualize active protein synthesis in the central matrix of midbody, which was co-incident with the Poly A RNAscope pattern, and also sensitive to MKLP1 knockdown, suggesting that either MKLP1 directly plays a role in translation event, or that decreased translation is due to lack of the

assembly of midbody-enriched RNA. Lastly, we discovered that Arc and ESCRTIII are necessary for mediating the levels of protein translation in the midbody. Active protein synthesis does not require the following midbody RBPs: Annexin A11, ATXN2L, Stau1, and TIS11b. In sum, we suggest that MKLP1 mediates the assembly and translation of RNAs in the midbody/MBRs, and that both Arc and ESCRT-III appear to modulate the levels of translation in the midbody. How the midbody RNP assembles and behaves as a novel translationally active extracellular vesicle (MBR stage) in cell communication is a focus of our lab.

B115/P1112

Using a Comprehensive Approach to Study the Mechanisms of Eg5 and Microtubule

W. Guo[1], J. E. Sanchez[2], L. Li[1]; [1]The University of Texas at El Paso, EL PASO, TX, [2]The University of Texas at El Paso, El Paso, TX.

Kinesins are microtubule-based motor proteins that play important roles ranging from intracellular transport to cell division. Human Kinesin-5 (Eg5) is essential for mitotic spindle assembly during cell division. By combining molecular dynamics (MD) simulations with other multi-scale computational approaches, we systematically studied the interaction between Eg5 and the microtubule. We find the electrostatic feature on the motor domains of Eg5 provides attractive interactions with the microtubule. Additionally, the folding and binding energy analysis reveals that the Eg5 motor domain performs its functions best when in a weak acidic environment. Molecular dynamics analyses of hydrogen bonds and salt bridges demonstrate that, on the binding interfaces of Eg5 and the tubulin heterodimer, salt bridges play the most significant role in holding the complex. The salt bridge residues on the binding interface of Eg5 are mostly positive, while salt bridge residues on the binding interface of tubulin heterodimer are mostly negative. In contrast, the interface between α and β-tubulins is dominated by hydrogen bonds rather than salt bridges. Compared to the Eg5/α-tubulin interface, the Eg5/β-tubulin interface has a greater number of salt bridges and higher occupancy for salt bridges. This asymmetric salt bridge distribution may play a significant role in Eg5's directionality. The residues involved in hydrogen bonds and salt bridges are identified in this work and may be helpful for anticancer drug design.

B116/P1113

Charge Changes in the Neck-linker of KIF18A Alter its Microtubule Preference, Cause Spindle Positioning Defects, and Impact Mitotic Function

K. A. Queen[1], A. Cario[1], S. E. Vandal[2], A. F. Thompson[1], C. L. Berger[1], J. Stumpff[1]; [1]University of Vermont, Burlington, VT, [2]Dartmouth Geisel School of Medicine, Hanover, NH.

Eukaryotic cells express many kinesins (45 in mammals) to support diverse cellular processes. Despite the unique functions these motors serve, kinesins share a conserved enzymatic region consisting of a motor domain and neck linker. The neck linker of kinesins undergoes conformational changes to ensure productive unidirectional stepping along microtubules. Our work focuses on the kinesin-8 KIF18A, which has an extended neck linker that not only serves to promote unidirectional stepping but also allows for obstacle navigation and subsequent accumulation to the plus-ends of kinetochore microtubules. Given the critical functions of the neck linker, we were interested in understanding how modifications within this structural region affect the canonical mitotic functions of KIF18A. KIF18A is a mammalian mitotic kinesin that plays important roles in chromosome alignment, kinetochore-microtubule attachment, and mitotic progression. These functions are mediated by the accumulation of KIF18A at the plus-ends of

kinetochore microtubules where it suppresses microtubule dynamics. KIF18A has three identified sites of modification within its neck linker, which occur at K356, S357, and K360, suggesting this may be an important regulatory region. Using an inducible cell line approach, we found that a phospho-mimetic S357D mutation alters the localization of KIF18A within the spindle, moving the motor off stable kinetochore microtubules and onto dynamic peripheral microtubules. The S357D mutant also exhibits altered localization in monopolar and interphase cells. Changes in localization are accompanied by defects in the ability of KIF18A S357D to promote mitotic progression, support chromosome alignment, and maintain spindle position. We investigated if this altered localization and mitotic defects are due to changes in single molecule motility or tubulin density but found no changes compared to wild-type KIF18A. However, removing the kinetochore microtubule specific microtubule associated protein HURP, allowed for re-localization of KIF18A S357D to k-fiber plus ends. This re-localization resembles the effects of removing obstacles from kinetochore microtubules in the presence of a shortened neck-linker KIF18A mutant. Taken together, this work indicates that KIF18A S357D may mimic a shortened neck-linker state, essentially excluding KIF18A S357D from kinetochore microtubules, resulting in alterations in the localization and the function of the motor.

B117/P1114

The β-tubulin tail differentially regulates kinesin-5 motors

E. C. Thomas, J. K. Moore; University of Colorado - Anschutz Medical Campus, Aurora, CO.

The tubulin code hypothesis predicts that tubulin proteins create programs for regulating the activities of kinesin motors, but we still lack an understanding of how molecular differences in microtubule networks can regulate kinesin function. The carboxy-terminal tails of tubulins are highly divergent and could provide an adjustable platform to control kinesin function. In this work, we identify through a genetic screen two budding yeast kinesin-5 motors, Cin8 and Kip1, that differentially interact with the β-tubulin tail. In wild-type cells, interactions with the β-tubulin tail recruit Cin8 to the spindle and establish a sequential recruitment of Kip1, which is otherwise insensitive to the β-tubulin tail. We determined that a negatively charged patch of acidic amino acids in the β-tubulin tail mediates the interaction with Cin8 and promotes its motility toward the plus-end of microtubules. Two positively charged regions within the Cin8 motor domain coordinate these charge-charge interactions with the negative β-tubulin tail. These regions are divergent in Kip1, providing a basis for its differential interaction with the β-tubulin tail. Finally, we demonstrate that interactions between Cin8 and the β-tubulin tail are necessary to promote stable, controlled spindle elongation during anaphase. The β-tubulin tail is highly divergent across species and is targeted for a variety of posttranslational modifications; it may therefore represent a mechanism for tuning spindle elongation across species and cell types. Interestingly, the positively charged regions of Cin8 are present in mammalian kinesin-5, suggesting that the mechanism may be conserved. Altogether this work proposes a molecular mechanism for how interactions between tubulin tails and kinesin motors can increase kinesin motility to promote spindle function.

Microtubules in Disease

B119/P1115

Tau Phosphorylation Impedes Cohesive Envelope Formation

V. Siahaan[1], E. Žatecká[2], A. Karhanová[1], M. Braun[1], Z. Lansky[1]; [1]Czech Academy of Sciences, Institute of Biotechnology, Prague, CZECH REPUBLIC, [2]Department of Cell Biology, Faculty of Sciences, Charles University, Prague, CZECH REPUBLIC.

In neurodegenerative diseases, collectively termed tauopathies, malfunction of the protein tau and its detachment from axonal microtubules are correlated with axonal degeneration and often are associated with abnormal phosphorylation of tau. Tau proteins, being intrinsically disordered, individually diffuse on microtubules and upon cooperative formation of cohesive envelopes, alter the spacing of the tubulin dimers constituting the microtubules[1]. Thereby, tau envelopes differentially regulate the accessibility of the microtubule lattice for other proteins, including molecular motors and microtubule severing enzymes[2]. Phosphorylation of tau reduces the affinity of tau for microtubules, however, the impact on envelope formation is not known. Pin1 is a peptidyl-prolyl cis-trans isomerase that catalyzes the isomerization of phosphorylated Serine/Threonine-Proline motifs in tau, restoring the affinity of phosphorylated tau for microtubules. Here, using in vitro reconstitution, we show that (a) phosphorylation impedes the formation of tau envelopes, while de-phosphorylation of tau enhances envelope formation and (b) Pin1 promotes envelope formation of phosphorylated tau. Our results demonstrate a mechanism by which Pin1 promotes the functioning of tau after phosphorylation, suggesting how Pin1 may decrease deleterious consequences of tau phosphorylation and hyperphosphorylation in neurodegenerative disease.

1. Siahaan, V. *et al.* Microtubule lattice spacing governs cohesive envelope formation of tau family proteins. *Nat Chem Biol* (in press).
2. Siahaan, V. *et al.* Kinetically distinct phases of tau on microtubules regulate kinesin motors and severing enzymes. *Nat Cell Biol* **21,** 1086-1092 (2019).

B120/P1116

Trisomy 21 increases microtubules and disrupts centriolar satellite distribution

B. L. McCurdy, C. E. Jewett, A. J. Stemm-Wolf, C. G. Pearson; University of Colorado Anschutz Medical Campus, Aurora, CO.

Trisomy 21, the source of Down syndrome, causes a 0.5-fold protein increase of the chromosome 21-resident gene Pericentrin (PCNT) and reduces primary cilia formation and signaling. Here, we investigate how PCNT and chromosome 21 genetic imbalances disrupt primary cilia. Using isogenic RPE-1 cells engineered with increased chromosome 21 dosage, we find PCNT protein accumulates around the centrosome as a cluster of enlarged cytoplasmic puncta that localize along microtubules (MTs) and at MT ends. These cytoplasmic PCNT puncta impact the density, stability, and localization of the MT trafficking network required for primary cilia formation. The PCNT puncta appear to sequester cargo peripheral to centrosomes in what we call ‘pericentrosomal crowding’. The centriolar satellite proteins PCM1, CEP131, and CEP290, important for ciliogenesis and signaling, accumulate at enlarged PCNT puncta in trisomy 21 cells. Reducing PCNT when chromosome 21 ploidy is elevated is sufficient to decrease PCNT puncta and pericentrosomal crowding, reestablish a normal density of MTs around the centrosome, and restore ciliogenesis to wild-type levels. A transient reduction in MTs also decreases

pericentrosomal crowding and partially rescues ciliogenesis in trisomy 21 cells, indicating that increased PCNT leads to defects in the MT network deleterious to normal centriolar satellite distribution. Trisomy 21 cilia frequencies eventually increase to wild-type levels after sustained duration in starvation media. This suggests that MT-dependent trafficking events required for ciliogenesis are initially waylaid but resolved with time. Importantly, initial steps of ciliogenesis require removal of the centriolar capping protein CP110 from the mother centriole. We find that depletion of CP110 protein levels in elevated chromosome 21 ploidy rescues primary ciliogenesis to normal levels. This suggests that bypassing MT-based trafficking events when chromosome 21 dosage is elevated rescues primary ciliogenesis and reinforces our conclusion that chromosome 21 aneuploidy disrupts MT-dependent intracellular trafficking required for primary cilia formation.

B121/P1117

Dystrophin and Ensconsin Cooperate to Maintain Nuclear Spacing in Myofibers by Regulating Distinct Aspects of Microtubule Organization

J. R. Padilla, A. Burgess, G. Aleck, L. Ferreira, E. S. Folker; Biology, Boston College, Chestnut Hill, MA.

Nuclei are evenly spaced in myofibers and disruptions in this spacing are a hallmark of poorly functioning muscles. Whereas some muscle disorders such as Emery-Dreifuss muscular dystrophy, which is linked to mutations in genes encoding nuclear envelope proteins, can be tied directly to the spacing of nuclei, Duchenne Muscular Dystrophy (DMD), caused by a mutation in *dystrophin* is more difficult to connect to nuclear spacing. Nevertheless, mispositioned nuclei are evident in DMD patients. To determine whether *dystrophin* regulates nuclear spacing independent of myofiber regeneration, we depleted Dystrophin from *Drosophila* muscles at specific developmental stages. Depletion of Dystrophin during active nuclear movement did not affect the nuclear spacing, however, depletion of Dystrophin after the completion of nuclear movement resulted in a loss of nuclear spacing indicating that Dystrophin is necessary to maintain nuclear spacing in differentiated myofibers. Intriguingly, nuclear spacing was not affected by the depletion of any other component of the dystroglycan complex indicating that Dystrophin regulates nuclear spacing by a novel mechanism. Because Dystrophin can bind microtubules, we screened microtubule associated proteins for functional interactions with *dystrophin* that regulate nuclear spacing*. Ensconsin/MAP7* was the only gene identified to functionally interact with *dystrophin.* Animals carrying a mutation in both genes had significantly more disrupted nuclear spacing than animals with a mutation in either gene alone. To identify the underlying mechanism of this functional interaction, we examined microtubule organization. Disruption of Ensconsin or Dystrophin expression reduced the number of nucleus-associated microtubules. Additionally, the disruption of Ensconsin expression reduced the length of microtubules whereas the disruption of Dystrophin increased the looping of microtubules and resulted in microtubules that grew back toward the nucleus. Simultaneous disruption of both Ensconsin expression and Dystrophin expression caused muscles to have shorter microtubules with more dramatic looping and a reduced number of nucleus-associated microtubules. Taken together, these data suggest that *dystrophin* and *ensconsin* cooperate to maintain nuclear spacing in myofibers via their regulation of distinct aspects of microtubule organization.

B122/P1118

A Specific Isoform of the Nesprin Msp300 Organizes the Perinuclear Microtubule Organizing Center in Fat Body Cells

G. M. Morton[1], Y. Zheng[2], C. Zheng[1], T. L. Megraw[1]; [1]Biomedical Sciences, Florida State University, Tallahassee, FL, [2]Xiamen University, Xiamen, CHINA.

The goal of this project was to determine how the Nesprin Msp300 controls assembly of the microtubule-organizing center (MTOC) on the nuclear surface in Drosophila fat body cells. Msp300 appears to form the foundation for this MTOC on the nuclear surface and is required to recruit the microtubule minus-end stabilizer Patronin and the microtubule polymerase Msps to form a functional MTOC. Remarkably, the microtubule nucleator γ-tubulin is not required at the fat body perinuclear MTOC. Msp300 is a component of the linker of nucleoskeleton and cytoskeleton (LINC) complex, which spans the nuclear envelope and provides a physical connection between the cytoplasm and the interior of the nucleus. Comprising the LINC complex are the KASH-domain and SUN-domain proteins, which are present on the outer and inner nuclear membrane, respectively. Surprisingly, while Msp300 is required for MTOC formation, the sole SUN protein in Drosophila is not required, indicating that Msp300 associates with the nuclear surface in a non-conventional manner in fat body cells. The Msp300 gene is complex, encoding at least eleven isoforms. Using a combination of mutant and RNAi isoform depletion approaches, we have identified a set of three similar isoforms that are specifically necessary for MTOC formation. The consequences of disrupting the fat body non-centrosomal MTOC is microtubule disruption, nuclear mispositioning, and impaired retrograde endocytic trafficking, and we show that the Msp300 isoforms we identified are necessary for some and perhaps all of these functions. In contrast, the loss of other Msp300 isoforms does not significantly impair ncMTOC function. Overall, our results reveal a novel function of a unique set of Msp300 isoforms in the fat body to organize the perinuclear MTOC.

B123/P1119

The tail anchored membrane protein SLMAP3 orchestrates muscle development by rearrangement of the microtubule organizing center (MTOC)

A. P. Dias, T. Rehmani, M. Salih, B. Tuana; Cellular and Molecular Medicine, University of Ottawa - Faculty of Medicine, Ottawa, ON, CANADA.

We have previously shown that SLMAP3 is a component of the centrosomal MTOC, to where it is directed by its N-terminal sequences including the FHA domain (J Cell Sci (2004) 117 (11): 2271-2281). SLMAP3 also localizes in the nuclear envelope (NE), where it is anchored by its C-terminal transmembrane domain. Muscle development is regulated by cytoskeleton remodelling, which includes the reorganization of MTOC components from the centrosome to the NE. Such a switch is important for myoblast fusion and proper nuclear positioning for normal myotube and muscle formation. Defects in these processes are associated with muscle dysfunction. To investigate the role of SLMAP3 in vivo, we generated SLMAP3 knockout (KO) mice using the Cre-Lox system, which exhibit late embryonic/perinatal lethality. RNA-seq analysis of SLMAP3 KO embryos indicated enrichment of biological processes involved in muscle development. Histological analysis of quadriceps indicated ~40% reduction of fiber length in SLMAP3 KO embryos compared to wild type (WT). Deletion of SLMAP3 in C2C12 mouse myoblast by CRISPR/Cas9 impacted myotube formation with ~98% reduction in fusion index and 7.6-fold less myosin heavy chain protein expression after 4 days in differentiation medium. Analysis of SLMAP3 interactors

by Immunoprecipitation-Mass Spectrometry (IP-MS) indicated its association with MTOC proteins, including akap9, pde4dip, pcm1 and pericentrin. Pericentrin staining indicated defects in the MTOC rearrangement in both SLMAP3 KO C2C12 cells and SLMAP3 null embryonic skeletal muscle. During differentiation, approximately 50% of the myogenin+ SLMAP3 KO C2C12 cells displayed NE-MTOC compared to 97% of myogenin+ WT cells. Still, the majority of the NE-MTOC in the KO cells exhibited weak and less defined pericentrin distribution, similar to that observed in skeletal muscle of SLMAP3 null embryos. Also, the mean distance between the nuclei in SLMAP3 null embryonic skeletal fibers was about half of the distance observed in WT myofibers, indicating impairment of nuclei positioning and defective NE-MTOC distribution. Our results indicate a novel role for SLMAP3 in myogenesis, via mechanisms involving MTOC dynamics orchestrated through its unique structural elements. (Supported by CIHR).

B124/P1120

Regulation of microtubule acetylation during the DNA damage response promotes efficient repair

S. S. Wallis, S. A. Godinho; Cancer Cell and Molecular Biology, Barts Cancer Institute, London, UNITED KINGDOM.

The microtubule cytoskeleton forms an integral part of eukaryotic cells. Over the past decade, there has been growing evidence that microtubules also play an important role in the repair of double strand breaks (DSBs) during the DNA damage response (DDR) by increasing DSB mobility. Recent work suggested that microtubule post-translational modifications (PTMs) are involved in the DDR, with acetylated tubulin levels increasing in response to DNA damage. This is particularly interesting as microtubule acetylation has been associated with increased kinesin-1 activity, which is known to affect DSB repair efficiency. However, how microtubule PTMs regulate the DDR and whether this can be exploited to enhance cancer treatment remains largely unknown. In this work, we show that microtubule acetylation, but not other PTMs tested, increases in a biphasic manner during the DNA damage response: an early peak in response to DNA damage and late peak correlated with DNA damage repair. Cells with defective DDR display a significantly lower late peak of microtubule acetylation, suggesting that efficient repair is important for increased tubulin acetylation at later time points. Furthermore, we show that depletion of alpha-tubulin acetyltransferase 1 (αTAT1), the enzyme responsible for microtubule acetylation, dramatically impairs the DDR, while overexpression of αTAT1 increases repair speed. Furthermore, using live-cell tracking of DNA damage foci, we observe a significant decrease in DSB mobility in cells depleted of αTAT1. Taken together, these results suggest that DSB mobility is regulated by tubulin acetylation and important for DNA repair. We propose a model by which the DDR is fine-tuned by microtubule acetylation to promote enhanced kinesin-1 activity to ensure efficient and timely repair. Finally, we demonstrate how changing levels of microtubule acetylation can be exploited to enhance the response to a commonly used chemotherapeutic, opening new avenues to treat cancer.

B125/P1121

The ubiquitous Microtubule Associated Protein 4 (MAP4) controls organelle distribution by regulating the activity of the kinesin motor

I. Nabti[1], B. J. N. Reddy[2], R. Rezgui[1], W. Wang[2], S. P. Gross[2], **G. T. Shubeita**[1]; [1]New York University Abu Dhabi, Abu Dhabi, UNITED ARAB EMIRATES, [2]University of California Irvine, Irvine, CA.

Regulation of organelle transport by molecular motors along the cytoskeletal microtubules is central to maintaining cellular functions. We show that the ubiquitously-expressed tau-related protein, MAP4, can bias the bidirectional transport of organelles towards the microtubule minus-ends. This is concurrent with MAP4 phosphorylation, mediated by the kinase GSK3β. We demonstrate that MAP4 achieves this by tethering the cargo to the microtubules, allowing it to impair the force generation of the plus-end motor kinesin-1. Consistent with this, MAP4 physically interacts with the motor complex via dynein and dynactin, and associates with cargoes through its projection domain when phosphorylated. Its phosphorylation coincides with perinuclear accumulation of organelles, a phenotype that is rescued by abolishing the cargo-microtubule MAP4 tether, or by pharmacological inhibition of dynein, confirming the ability of kinesin to inch along, albeit inefficiently, in the presence of phosphorylated MAP4. These findings have broad biological significance because of the ubiquity of MAP4 and the involvement of GSK3β in multiple diseases, more specifically in cancer, where MAP4-dependent redistribution of organelles may be prevalent in cancer cells, as we demonstrate here for mitochondria in the lung carcinoma epithelial cells.

B126/P1122

Tubulin Posttranslational Modification by Taurine is Tissue Selective, is increased in some Tumors, and Occurs in Single Molecule Patterns

A. White, **D. Sackett**; National Institute of Child Health and Human Development, NIH, Bethesda, MD.

Posttranslational modification (PTM) of tubulin encompasses a number of chemical changes to alpha and beta tubulin. These occur mostly on the disordered, acidic C-terminal tails (CTT) of 10-20 residues of alpha and beta that extend out from the surface of the microtubule (MT). An interesting PTM series entails the removal of the C-terminal tyrosine from alphas that have been incorporated into the MT, and its replacement when the tubulin is released as free dimer. We recently reported that taurine occurs as a C-terminal adduct to the de-Tyr CTT, blocking re-Tyr. Taurine is the most abundant free amino acid in the body and has long been thought to never be incorporated into any proteins. We used mass spectrometry to demonstrate taurine adducts to the C-terminus of de-Tyr alpha tubulin in chicken red blood cell tubulin, but now know that it is more widespread. We developed an antibody to alpha-CTT-taurine which shows that taurine-tubulin is abundant in rodent brain, skeletal muscle, and heart but absent or nearly so in some other tissues. Similarly, some but not all cell lines derived from these tissues of rodents and humans show abundant taurine-tubulin. Tissue microarrays of normal and tumor samples shows increased taurine-tubulin in some tumors (such as some sarcomas) but not in others (such as breast cancers). Examination of the distribution of taurine-tubulin in cultured cells by super-resolution STED microscopy shows a remarkable pattern of taurine modification on the MT. Taurine adducts occur as single molecules of alpha tubulin-taurine separated by regular spacings with no taurine modification. Thus, taurine incorporation at the C-terminus of de-Tyr alpha tubulin is the only known incorporation of taurine into any protein, and a possible modulator of the evolutionarily conserved

cyclic Tyr / de-Tyr modifications of tubulin. It shows a unique tissue selectivity and pattern of MT labeling, and is increased in some tumors.

B127/P1123

Non-Centrosomal Microtubule Organising Centres Controlled by Light in Pluripotent Cells

J. Greaney[1], H. Janovjak[2], J. Zenker[1]; [1]Australian Regenerative Medicine Institute, Monash University, Melbourne, AUSTRALIA, [2]Flinders Health and Medical Research Institute, Flinders University, Adelaide, AUSTRALIA.

The arrangement of microtubule organising centres (MTOCs) in a cell is a key mediator of cellular identity by controlling the architecture of the microtubule network and its associated organelles and biomolecules. Commitment of stem cells to a particular cell lineage is characterised, in part, by the transition from non-centrosomal MTOCs (ncMTOCs) to centrosomes. However, the real-time regulation of MTOC switching during pluripotency exit remains elusive.
We set out with the objective to design an optogenetically-controlled version of calmodulin-regulated spectrin-associated protein 3 (CAMSAP3), which drives the formation of the interphase bridge, the characteristic ncMTOC in the preimplantation mouse embryo. Opto-CAMSAP3 localised and functioned as normal in dark conditions and, upon light-illumination, dissociated from microtubules, rendering it non-functional with sub-cellular and sub-second spatiotemporal precision in the preimplantation mouse embryo and in human induced pluripotent stem cells (hiPSCs).
Unexpectedly, this led to the discovery of an array of perinuclear CAMSAP3 deposition in the preimplantation mouse embryo responsible for mediating the interphase perinuclear microtubule architecture. Known from mammalian muscle cells and *Drosophila melanogaster* embryos, perinuclear ncMTOC activity determines nuclear organisation and signalling. Thus, lacking centrosomes, the cells of the preimplantation embryo present an extraordinary plasticity in the usage of various ncMTOCs.
Our novel Opto-CAMSAP3 tool enables targeted disruption of CAMSAP3-dependent ncMTOC activity, while leaving other avenues of MTOC activity unaffected. The identification of the real-time changes in microtubule organisation underlying pluripotency and cell lineage specification *in vivo* and *in vitro* may open the door for new ways to regulate microtubule organisation and cell behavior non-invasively for applications in regenerative and reproductive research.

B128/P1124

Mediation of cisplatin resistance through cis and trans effects on microtubule dynamics

K. Mahalingan[1], S. Horibata[2], R. Patel[3], Y. Fan[3], J. Hotz[3], K. MacRenaris[2], D. Meerzaman[4], M. Gottesman[3], A. Roll-mecak[1]; [1]NINDS, Bethesda, MD, [2]MSU, East Lansing, MI, [3]NCI, Bethesda, MD, [4]NINDSNCI, Bethesda, MD

More than half a century after its discovery, cisplatin remains one of the most widely used anti-cancer drugs in the world. Despite this, cisplatin resistance is a major problem in cancer therapy. In ovarian cancer, cisplatin resistance accounts for more than 60% of all mortality. Thus, there is an urgent need to understand the mechanisms of cisplatin action. Although cisplatin cytotoxicity has long been associated with DNA damage, our RNA seq analysis of cisplatin-resistant ovarian cancer cells show many changes in microtubule cytoskeleton genes that correlate with drug resistance. Using *in-vitro* assays we show that clinical concentrations of cisplatin directly affect microtubule dynamics by increasing the frequency of microtubule depolymerization events. Cisplatin resistant cells counteract this destabilization through

changes in expressed tubulin isotypes and by over-expressing tubulin polymerization promoting protein (TPPP3). By reconstituting for the first-time microtubule dynamics with tubulin purified from cisplatin sensitive, resistance and re-sensitized cells we find that changes in tubulin isotypes in resistant cells directly stabilize microtubules, and that TPPP3 synergizes with the tubulin isotype repertoire for maximal microtubule stabilization to counteract the destabilization effects of cisplatin. TPPP3 depletion restores partial cisplatin sensitivity. Consistent with this, we find that patients with low TPPP3 expression have markedly improved therapeutic outcome. The demonstration of the direct effects of cisplatin on microtubule dynamics and that microtubule dynamics changes contribute to cisplatin resistance have direct bearing on cisplatin associated neuropathies and ototoxicity as well as cisplatin and paclitaxel combination chemotherapy, as the two drugs have opposing effects on microtubule dynamics. Our study also demonstrates that tubulin isotype repertoire changes, long associated with drug-resistance in tumors, have direct effects on microtubule dynamics and that they evolve synergistically with changes in the proteome associated with microtubules.

B129/P1125

Microtubule Acetylation Promotes Eribulin-induced Cell Death by Accelerating ER-Mitochondria Ca^{2+} Transfer

S. Song, J. Jeong, S. Keum, Y. Hwang, M. Ji, M. Lee, Y. Hwang, J.-H. Choi, S. Rhee; Chung-ang University, Seoul, Korea, Republic of

Eribulin is a newly developed anticancer drug that has been developed for patients who have not responded to first- or second-line anticancer therapy. However, after a series of treatments, most breast cancer cells developed resistance to eribulin. The goal of this study is to identify the signaling pathway induced by eribulin to provide clues for overcoming eribulin resistance. In breast cancer cells, eribulin treatment reduced microtubule (MT) dynamics while increasing its acetylation around the nucleus, but not in eribulin-resistant cells. The eribulin sensitivity to the breast cancer cells was reduced by silencing of α-tubulin N-acetyltransferase 1 (*ATAT*1), a major MT acetyltransferase. In addition, combining eribulin with tubacin, a MT acetylation restored MT dynamics and increased the cell death in eribulin-resistant cells. Mechanistically, eribulin-induced microtubule acetylation around the nucleus confers cells with the ability to promote mitochondrial-endoplasmic reticulum (ER) clustering, which accelerates Ca^{2+} transfer from ER to mitochondria, eventually leading to cell death. Taken together, our findings suggest that MT acetylation is a potential target for treatment of eribulin-resistance breast cancer cells.

B130/P1126

Determining the Role of Acetylated Microtubules in Double-Strand Break Repair and Mobility

J. F. Cátala Torres, A. Deb Roy, T. Inoue; Johns Hopkins University Sch Med, Baltimore, MD

The DNA damage response (DDR) protects us against constant genotoxic insults by coordinating the detection and repair of DNA damage. DNA double-strand breaks (DSBs) are one of the most dangerous forms of DNA damage, due to their propensity to cause mutations that may lead to disease. Although the molecular mechanisms behind DSB repair have been extensively studied, only recently has chromatin mobility come into focus. Upon the onset of DNA damage, microtubules (MTs) interact with nuclear envelope proteins to promote the mobility of DSBs and aid in their repair. Despite the central role of MTs in damaged chromatin mobility, it is not known how they are regulated in response to DNA damage. MT post-translational modifications (PTMs) present a quick and reversible way through which

the DDR can regulate DSB mobility. We and others have observed that MT acetylation increases following the induction of DNA damage. This PTM is evolutionarily conserved across eukaryotes and is exclusively mediated by alpha-tubulin N-acetyltransferase 1 (αTAT1). We have observed that mouse embryonic fibroblasts (MEFs) with an αTAT1 knock-out (KO) contain high basal levels of DSBs. These observations led us to hypothesize that the DDR drives MT acetylation to promote the movement and repair of DSBs. We utilized MEF cells from αTAT1 KO mice to study defects in the DDR in an MT acetylation-free background. We expressed fluorescently labeled 53BP1—a DDR protein—in these cells and observed the repair and mobility of DSBs using live cell microscopy. Our data show that upon treatment with a radiomimetic drug to induce DNA damage, cells lacking αTAT1 are more prone to accumulate DSBs. Wild-type cells repaired most DSBs by 3h, while αTAT1 KO cells did not fix the damage in that timeframe. In addition, we observed that DSBs in cells lacking αTAT1 move less, as determined by their displacement within a 10 min period. These results suggest an important role for microtubule acetylation in the DDR. Given that cells lacking αTAT1 showed defects in both DSB repair and mobility, it is possible that microtubule acetylation promotes DSB mobility to assist in repair. We will use synthetic biology tools developed in our lab to elucidate the mechanism behind this new role of acetylated microtubules in DNA damage repair and gain insight into the development of mutation-driven diseases such as cancer.

B131/P1127

Do microtubule depolymerizers induce inflammation in quiescent and non-quiescent cells?

L. J. Horin, T. J. Mitchison; Harvard University, Boston, MA

Microtubule (MT) destabilizing drugs are used in the clinic as chemotherapeutics for both liquid and solid tumors such as leukemia, melanoma, and non-small cell lung cancer. Although they are widely thought to act by targeting rapidly dividing cells, these therapies can also cause toxicity in tissues composed of quiescent and post-mitotic cells such as neuronal and vascular cells, suggesting that disruption of MT integrity can induce a cell stress response in non-dividing cells. In the literature there are conflicting reports of whether MT drugs causing inflammatory responses, and, as such, further work to clarify the link between MTs and cellular stress response is warranted, especially in different cellular contexts. To examine whether treatment with MT destabilizers causes inflammatory responses, we used several methods to detect markers of inflammation in non-quiescent and quiescent cells. First, we used pathway analysis of multiple published transcriptomics datasets and found that in cardiac tissue and quiescent RPE1s, several genes were upregulated that correlate with NFkB activation in response to short-term treatment with CA4 and other MT depolymerizers. Next, we measured relative NFkB activation in non-quiescent A549 Dual reporter cells in response to a 16 hr treatment with combretastatin A4 (CA4) and TNFα. There was a slight 1.4-fold increase in NFkB, though this was less than the 4.5-fold and 2.0-fold induction by treatment with 1 ng/ml and 0.1 ng/ml TNFα. This suggests there is not a significant effect of CA4 on inflammation in non-quiescent cells. Furthermore, co-treatment of CA4 and TNFα did not amplify NFkB expression, suggesting there is no synergistic effect between the two pathways. Finally, we used immunofluorescent labeling of p65 and phospho-NFkB to measure activation and translocation of NFkB in quiescent RPE1s and serum-starved human pulmonary microvascular endothelial cells (HPMECs). In both groups, treatment with 1 uM CA4 for 2 hours slightly decreased the intensity of these labels compared to DMSO, but there was no difference in nuclear translocation of NFkB into the nucleus. Overall, these data suggest that CA4 does not upregulate inflammatory pathways to a significant degree in quiescent and non-quiescent cell culture models.

Future work will use live cell fluorescence assays to sensitively clarify the relationship between MT depolymerization and inflammation.

Microtubule Networks in Mitosis

B132/P1128

Investigating the role of γ-TuRC minus-end capping in microtubule regulation *in vitro* and in cell division

A. Y. Berman, M. Wieczorek, T. M. Kapoor; The Rockefeller University, New York, NY.

The γ-Tubulin Ring Complex (γ-TuRC) plays an important role in centrosomal and non-centrosomal microtubule formation and organization during mitosis. The γ-TuRC is an ~2.3 MD assembly consisting of γ-tubulin, GCP2-6, MZT1 and 2, and actin that together form a cone-shaped structure. Fourteen γ-tubulins are arranged in a ring at the top of the complex, poised to interact with α,β-tubulins. Microtubule regulation by the γ-TuRC is central to mitosis when microtubule dynamics are increased and microtubule formation and organization are regulated to assemble a bipolar spindle. The γ-TuRC performs at least two functions: it can serve as a template for microtubule nucleation, and it can bind to either nucleated or pre-formed microtubules as a minus-end cap. While recent advances in the structural and biochemical analysis of the γ-TuRC have increased our understanding of γ-TuRC-dependent microtubule nucleation, its microtubule capping activity remains poorly characterized. Furthermore, the role of the γ-TuRC's capping activity in a cellular context remains unclear. To address this, we utilized biochemical reconstitutions and cellular assays to characterize the γ-TuRC's capping activity. Single filament assays showed that γ-TuRC capping of dynamic microtubules, which suppressed growth and shrinkage at minus-ends, had lifetimes of ~1 minute. In contrast, γ-TuRCs remained associated with the minus-ends of newly nucleated microtubules for several minutes. γ-TuRCs reconstituted with nucleotide-binding deficient γ-tubulin (γ-tubulin$^{\Delta GTP}$) formed ring-shaped complexes that did not nucleate microtubules, but capped dynamic microtubule minus-ends at lifetimes similar to wild-type complexes. In dividing cells, knockdown of γ-tubulin and add-back of γ-tubulin$^{\Delta GTP}$ resulted in mitotic spindle defects. However, microtubule regrowth assays revealed that while knockdown of γ-tubulin suppressed non-centrosomal microtubule formation, add-back of γ-tubulin$^{\Delta GTP}$ rescued this process. Our results suggest that γ-TuRC minus-end capping, a nucleotide-binding-independent activity, is required for non-centrosomal microtubule formation during cell division.

B133/P1129

The mitotic spindle protein CKAP2 regulates microtubule dynamics and ensures faithful chromosome segregation.

L. Paim, T. S. McAlear, A. Lopez-Jauregui, S. Bechstedt; Anatomy and Cell Biology, McGill University, Montreal, QC, CANADA.

During mitosis, cells rely on the concerted action of microtubule-associated proteins (MAPs) and motor proteins to assemble the mitotic spindle that orchestrates chromosome segregation. Cytoskeleton-Associated Protein 2 (CKAP2) is predominantly expressed during mitosis and previous work from our lab has shown that it potently increases microtubule nucleation and stability *in vitro*; however, its roles during mitosis in cells are poorly understood. Therefore, to study the function of CKAP2 in cells, we performed CRISPR-Cas9 genome editing to generate knock-out (KO) clones using human fibrosarcoma

cell line HT-1080. Immunofluorescence experiments of wild-type (WT) cells demonstrated that CKAP2 is barely detectable during interphase, however, with the onset of mitosis, CKAP2 localises primarily at the centrosomes and spindle microtubules, subsequently shifting its localisation to the chromatin during telophase. As expected, CKAP2 expression was lost in all three KO clones and significant spindle abnormalities, such as multipolar spindles, chromosome misalignments and lagging chromosomes at anaphase were more frequently observed in KO cells as compared to WT cells. In addition, multiple nuclear abnormalities were detected in KO cells more frequently than in WT cells, with cells displaying multinucleated/fragmented nuclei and abnormal donut-shaped nuclei. Consistent with a role of CKAP2 on chromosomal stability, chromosome spreads and FACS analyses revealed that CKAP2 KO cells were highly aneuploid, and two out of three KO clones displayed a near-tetraploid genome. To investigate the mechanism whereby the loss of CKAP2 impacts chromosomal stability, we performed live cell imaging experiments of cells expressing a fluorescent EB3 marker to directly assess microtubule dynamics, and found that microtubule growth rates were significantly reduced in KO cells, both during mitosis and interphase, as compared to WT cells. Our findings demonstrate the powerful role of CKAP2 as a microtubule nucleation factor in cells and provide a mechanistic explanation for the severe spindle phenotypes and oncogenic potential of CKAP2 misregulation.

B134/P1130

Midzone Microtubule Dynamics in Anaphase cells

C. Fermino Do Rosario[1], Y. Zhang[1], A. Shorey[1], J. Stadnicki[1], J. Ross[2], P. Wadsworth[1]; [1]UMass Amherst, Amherst, MA, [2]Syracuse University, Syracuse, NY.

During anaphase, antiparallel overlapping midzone microtubules elongate, contributing to chromosome segregation and specification of the location of contractile ring formation. Midzone microtubules are dynamics in early but not in late anaphase; however, the kinetics and mechanism of stabilization are incompletely understood.Quantification of midzone microtubules dynamics following photoactivation of LLCPk1 cells expressing PA-GFP tubulin shows that immediately after anaphase onset, a single highly dynamic population of microtubules is present (avg t ½ fast 17 sec). As anaphase progresses, both dynamic and more stable population of microtubules coexist, with a gradual increase in the dissipation half-time for the slower population (avg t ½ slow 85 - 183 sec early to later anaphase). By midcytokinesis, however, only static microtubules (no dissipation of fluorescence) are detected. We tested the contributions of furrowing and of midzone associated proteins to midzone microtubule dynamic behavior. Blocking furrow ingression using either C3 or latrunculin treatment resulted in two spatially distinct microtubule populations in telophase cells: peripheral microtubules that were highly dynamic (avg t ½ 25 sec) and central microtubules with fast and slow populations (avg t ½ fast 9 sec and t ½ slow 127 sec) which did not transition to a static array. Depletion of either PRC1 or Kif4a, which did not block furrow ingression in most cells, also prevented formation of a static array in telophase cells, although microtubules were partially stabilized with turnover similar to mid anaphase cells. In Kif4a depleted or C3 treated telophase cells, the length of PRC1 decorated microtubule overlaps were nearly 5X longer than the short highly compacted zones in control telophase cells. In summary, these results demonstrate that dynamic turnover and sliding midzone microtubules is gradually reduced as anaphase progresses and microtubules are further stabilized during cytokinesis. These data reveal the importance of the PRC1/Kif4a module and midzone microtubule compaction in generating a static midzone.

B135/P1131

Augmin prevents merotelic attachments by promoting proper arrangement of kinetochore and bridging fibers

V. Štimac, I. Koprivec, I. Tolić; Rudjer Boskovic Institute, Zagreb, CROATIA.

The mitotic spindle functions as a molecular micromachine that evenly distributes chromosomes into two daughter cells during cell division. Spindle microtubules in human cells are nucleated at centrosomes, at kinetochores, and on the lateral surface of pre-existing microtubules by the augmin complex. However, it is unknown how the augmin-mediated nucleation affects distinct microtubule bundles and thereby mitotic fidelity. Here we show, by using superresolution microscopy of human spindles along with depletion of the augmin complex subunits HAUS6 or HAUS8, that midplane-crossing microtubules are no longer organized into a bridging fiber that connects sister k-fibers when augmin is depleted, suggesting that augmin is responsible for the highly organized architecture of the mitotic spindle. Augmin depletion leads to a three-fold increase in segregation errors when the checkpoint is weakened. A significant number of lagging chromosomes appeared in the inner part of the mitotic spindle close to the main spindle axis, where the midplane-crossing microtubule disorganization is most prominent and proper bridging fibers are often absent. In augmin depleted cells, backtracking of lagging kinetochores until metaphase revealed their larger tilt and shorter interkinetochore distance, suggesting that lack of augmin leads to compromised microtubule arrangement facilitating the formation of merotelic attachments. Merotely was both visible in metaphase cells as well as persistent in anaphase following augmin depletion, indicating that augmin is necessary to both prevent and resolve the segregation errors through an interplay of bridging and k-fibers. The impairment of microtubule arrangement following augmin depletion is reflected in the 25% reduction of k-fibers and 65% reduction of bridging fibers, which are often completely missing. The absence of bridging fibers is characterized by a reduction of the interkinetochore distance. Altogether, our results demonstrate a critical role of augmin in the formation of a properly structured mitotic spindle, suggesting a model where augmin promotes mitotic fidelity and tension on kinetochores by generating highly organized mechanical entities consisting of two sister kinetochore fibers connected by a bridging fiber.

B136/P1132

Model mitotic spindle and chromosome as coupled oscillators

J. Zhu, C. Edelmaier, B. Heck, E. Bartle, P. Maddox, E. Nazockdast; University of North Carolina, Chapel Hill, NC.

Mitotic chromosomes of cells throughout phylogeny oscillate during prometaphase and metaphase. Chromosome oscillation can be used as a mechanical read-out of the location, direction, and magnitude of the forces within the spindle. Understanding these force generation mechanisms will further provide insights into how the same forces result in chromosome alignment during metaphase and segregation during anaphase. Several factors are believed to contribute to the oscillation of chromosomes: microtubule poleward flux, centromere stretch, the dynamic instability of microtubules, polar ejection force, and motor proteins. However, the relative contributions of these factors remain poorly understood. **We tested how mechanical couplings at three interfaces: the microtubule and the kinetochore, the kinetochore and the chromosome, and between chromosomes in tandem, contribute to chromosome oscillations.** To investigate the detailed structure of these interfaces, we developed a single molecule live imaging technique - Halo-FSM - founded on the principle of conventional

fluorescent speckle microscopy. Using this technique, we quantified the dynamics of individual microtubules at the kinetochore, with adequate resolution to discern evidence of the asynchrony of dynamics instability between microtubules at a given kinetochore. We also pinpointed the position of the microtubule tip with ~20 nm accuracy in living mitotic cells. Our preliminary data not only invoke the possibility that more than one population of kinetochore-microtubule exists within a given k-fiber but also suggests that microtubule ends penetrate deeper into the kinetochore than previously appreciated. Further, while classic laser ablation experiments in grasshopper spermatocytes assured that kinetochore pairs are mechanically independent, our results with mammalian Ptk1 cells suggest that this is not true in all organisms. In summary, we present evidence for a novel structural principle of the mitotic spindle as a coupled oscillator system.

B137/P1133

Kinetochore centering and oscillations are regulated by overlap-length dependent forces

P. Risteski, J. Martinčić, M. Jagrić, I. M. Tolić; Ruder Boskovic Institute, Zagreb, CROATIA.

Pre-anaphase kinetochore oscillations at the spindle midplane occur in the majority of the cell types and result from a tug-of-war between sister kinetochore fibers (k-fibers). Recently, we have shown that k-fibers are mechanically coupled to overlap bundles, i.e. bridging fibers, which slide apart and drive microtubule poleward flux of sister k-fibers. However, how overlap bundles are involved in the regulation of kinetochore centering and de-centering events that result in kinetochore oscillatory motions at the spindle equator has not been explored. By developing a new speckle microscopy assay we found that longer k-fiber fluxes at a higher rate than the shorter one, moving the kinetochores towards the center. This differential k-fiber flux arises due to asymmetry in the size of overlap regions between k-fibers and bridging fibers. Kinetochore centering efficiency is better when overlaps are shorter and thus k-fiber flux is slower than the bridging fiber flux. In addition to k-fiber flux being length-dependent in metaphase, we found that kinetochore congression velocity in prometaphase is reversely proportional to the flux of the k-fiber associated with proximal pole, which directly supports the idea that microtubule poleward flux drives kinetochore centering in both phases. Interestingly, k-fiber flux rates correlate with the kinetochore oscillation velocities and overlap lengths across a panel of siRNA treatments, implying that the velocity of kinetochore movements is a result of forces transmitted from the overlaps. We measured polymerization at the plus ends and depolymerization at the minus ends of both growing and shrinking sister k-fibers and found these events to be correlated. This correlation was also observed when polymerization and depolymerization events were increased in spindles with increased overlaps due to Kif18A depletion. Moreover, we found that plus ends of growing k-fibers largely polymerize whilst those of shrinking k-fibers exhibit pausing or slowly polymerizing events, thereby suggesting that shrinking k-fibers de-center kinetochores via depolymerization at their minus ends. Thus, we propose that overlaps promote kinetochore centering via length-dependent sliding forces and de-centering via flux-driven regulation of microtubule polymerization and depolymerization events on growing and shrinking k-fibers.

B138/P1134

Three-dimensional reconstruction of mitotic spindles in the late *C. elegans* embryo

C. Okafornta, M. Köckert, G. Fabig, T. Müller-Reichert; Experimental Center, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, 01307 Dresden, GERMANY.

The hallmark of mitosis in eukaryotic cells is the efficiency of the bipolar spindle apparatus to align and segregate the chromosomes. While there are many microtubules (MTs) that make up the spindle network, they can be classified by the interaction of their plus ends with target sites. For instance, kinetochore MTs (KMTs) associate with the chromosomes to connect them with the centrosomes. In the one-cell *C. elegans* embryo, KMTs make up about 2.8% of all MTs and achieve an indirect kinetochore-to-centrosome connection by anchoring into the spindle network (Redemann et al., 2017, *Nat. Commun*. 8: doi.org/10.1038/ncomms15288). Here, we use three-dimensional (3D) electron tomography to analyze the length distribution of KMTs in the spindles of late *C. elegans* embryos (Lindow et al., 2021, *J. Microsc.* 284: 25-44). We analyzed mitotic spindles in 16- and 32-cell embryos with the identity of each of the embryonic cells since the development in *C. elegans* is well annotated (Sulston et al., 1983, *Dev. Biol.* 100: 64-119). We show that the connection of KMTs to the centrosomes in spindles of later embryonic cells, especially from the 16-cell to the later stages of the *C. elegans* embryo, favors a switch to a direct connection. We discovered further that, although the total number of the MTs in a relatively small cell decreased by an order of magnitude, the total number of the KMTs remained approximately constant and was thus similar to the number of KMTs as previously observed in the one-cell embryo. Our aim is to analyze this switch in the kinetochore-to-centrosome connection in spindles of different *C. elegans* cell lineages.

B139/P1135

Differential regulation of astral microtubules in a shared cytoplasm

A. Bera, M. L. Gupta; Iowa State University, Ames, IA.

Microtubules (MTs) are essential, intrinsically dynamic cytoskeletal filaments, polymerized from heterodimeric tubulin. Thousands of MTs can work collaboratively to accomplish complex functions like cell division/migration and intracellular cargo transport, with a high degree of fidelity to ensure cell viability and genome stability. Failure in spatiotemporal coordination of MTs can penalize processes like mitosis/meiosis, oogenesis/oocyte maturation and embryo/neurodevelopment. But the mechanisms coordinating the actions of many dynamic MTs to engineer complex cellular machines, or how subsets of MTs faithfully cooperate within the overall network, remain largely unknown. Such mechanisms are difficult to discern in complex cells, containing thousands of MTs. Using *Saccharomyces cerevisiae*, a simple eukaryotic model with easily trackable astral MTs, we uncovered a novel phenomenon controlling MT number inside the daughter cell, or bud, during mitosis. In anaphase cells with mispositioned spindles, the entry/exit of MTs into the bud is regulated by the net MT occupancy status of the bud. This phenomenon is independent of bud neck size, MT length, spindle's distance from the bud neck or even total number of spindles inside the cell. Utilizing live-cell epifluorescence imaging, we have uncovered that highly conserved bud neck components and the plus-end directed, MT-depolymerizing motor, kinesin-8/Kip3, potentially regulate MT entry/exit into the bud. Therefore, we propose a preliminary model wherein controlling MT number inside the bud maximizes the efficiency of cell's spindle positioning mechanism. Elucidating these mechanisms represents a significant advance in

understanding how the actions of many dynamic MTs is coordinated to achieve diverse and complex cellular processes.

B140/P1136

Regulation of spindle pole architecture by the microtubule focusing machineries

G. Guilloux, R. Gibeaux; CNRS - Institut of Genetics and Development of Rennes, Rennes, FRANCE.

The spindle is a bipolar structure, composed of microtubules (MTs), which can adapt its size and architecture among cell types and organisms to ensure mitosis faithfulness. However, how specific spindle morphologies are established is poorly understood. To address this question, we use *X. laevis* and *X. tropicalis* egg extracts. Indeed, *X. tropicalis* spindles are not only shorter than their *X. laevis* counterparts, but they are also architecturally different, notably at their poles. Using these two systems, we aim to understand the mechanisms that control the variability of spindle pole architecture. To overcome the fact that motors involved in pole formation, such as XCTK2 and Eg5, are also involved in organizing the spindle mid-zone, we reduced the complexity of the spindle by studying MT focusing in asters that mimic spindle pole organization. We first compared different types of MT asters (triggered by centrosomes, DMSO or Ran mutant) between species. While the three aster types exhibited size difference between the two species, only those triggered by DMSO presented an increased MT density at the aster center for *X. tropicalis* as observed at spindle poles. By mixing *X. tropicalis* and *X. laevis* extracts and assembling such asters, we found that common focusing mechanisms act in these two species. Measurements of MT growth speed showed no difference between species, which is thus an unlikely mechanism to explain the observed differences. Adapting expansion microscopy to analyze asters, we found aster centers not to be a simple focal point as expected from low-resolution observations, but rather a meshwork. This suggested crosslinking of MTs and a likely important role of Eg5 and/or XCTK2 for pole organization. Surprisingly, while inhibition of Dynein only mildly defocused asters, inhibition of Eg5 and XCTK2 led to MT focusing around a spherical structure and to asters focusing as spirals, respectively. This suggests a complementary action of these three motors, to allow for MT minus-end clustering and MT arrangement stabilization through anti-parallel and parallel interactions.

B141/P1137

Asymmetric requirement for α-tubulin over β-tubulin

L. C. Wethekam, J. K. Moore; Department of Cell and Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO.

Most eukaryotes express families of genes for the α- and β-tubulin monomers that comprise the tubulin heterodimer. How cells regulate the balance of α- and β-tubulin to maintain stoichiometry for heterodimers, and the consequences of monomer imbalance, are not understood. In this study, we use the budding yeast *S. cerevisiae* to investigate the role of gene copy number in maintaining levels of α- and β-tubulin proteins and how shifting the expression of α- or β-tubulin genes impacts the supply of tubulin proteins and microtubule function. We find that α-tubulin gene copy number is uniquely important for maintaining an excess α-tubulin protein compared to β-tubulin protein and prevents the accumulation of toxic levels of β-tubulin. Decreasing β-tubulin gene copy number or increasing α-tubulin expression has minor effects on microtubule function and cell proliferation. In contrast, increased expression of β-tubulin relative to α-tubulin is toxic to cells, leading to the formation of noncanonical

tubulin-containing polymers, unstable mitotic spindles, and disrupted cell proliferation. We use a structure-guided mutagenesis approach to show that β-tubulin toxicity arises from the catalytically inactive GTP-binding pocket that is normally sandwiched between α- and β-tubulin in the heterodimer, and the recruitment of XMAP215/Stu2 to noncanonical polymers formed by super-stoichiometric β-tubulin. The latter suggests a previously unappreciated binding mode between β-tubulin and XMAP215/Stu2. Based on these results, we propose a novel model for the relationship between α- and β-tubulin in which α-tubulin must be maintained in excess to promote GTP hydrolysis and to prevent the accumulation of non-catalytic assemblies of β-tubulin.

B142/P1138

Rotation of microtubule asters under confinement

A. Sami[1], D. B. Stein[2], G. Kabacaoglu[3], J. S. Oakey[4], T. Sulerud[1], M. J. Shelley[2], J. C. Gatlin[1]; [1]Molecular Biology, University of Wyoming, Laramie, WY, [2]Center for Computational Biology, Flatiron Institute, New York, NY, [3]Mechanical Engineering, Bilkent University, Ankara, TURKEY, [4]Chemical Engineering, University of Wyoming, Laramie, WY.

During interphase of the cell cycle, the microtubule (MT) cytoskeleton forms a radial array referred to as the MT aster. The MTs that compose this structure (astral MTs) serve a broad range of functions within the cell including structural mechanics, cell motility, cell division, intracellular transport, and spatial organization. Several of these critical functions require that the aster position itself in the cell such that its center is close to that of the cell's. However, the mechanisms by which this positioning occurs, and that of the nucleus and mitotic spindle indirectly, are poorly understood. To better understand the forces generated by MTs during aster positioning, MT asters were assembled in hydrogel micro-enclosures (artificial cells) containing discrete volumes of cell-free extracts from *Xenopus laevis* eggs. Growing asters readily find the center of their enclosures, and once centered, asters spontaneously begin to rotate. For small cells this rotation is unidirectional and persistent, while for larger cells it is oscillatory with rotation periodically changing direction. In yet larger cells, rotation of any sort is not observed. We find that a minimal *in silico* model of astral MTs interacting through the cytoplasm and impinging upon the cell periphery explains these observations. For example, the model shows rotational switching results from the collective interplay between multiple bending modes of peripherally pinned MTs. A coarse-grain model of the collective dynamics of MT ensembles shows that the allowable bending modes are controlled by MT density; increased density can suppress the higher-order bending modes that drive switching. Both full simulations and experiments with increasing density of MTs confirm this prediction. This suggests that the MT aster can be conceptualized as a device assembly whose operating states arise from the interactions of its internal collective modes. Additionally, these studies demonstrate that the architecture of the MT aster allows pushing forces to be exerted over longer than expected distances, suggesting that MT interactions with cell boundaries must be tightly regulated in real cells.

B143/P1139

TPX2 dependent regulation of spindle architecture

G. Sepulveda[1], X. Zhou[1,2], L. Slevin[1,3], C. Brownlee[1,4], R. Heald[1]; [1]University of California, Berkeley, Berkeley, CA, [2]AbbVie, South San Francisco, CA, [3]Swedish Maternal and Fetal Specialty Center, Seattle, WA, [4]Stony Brook University, Stony Brook, NY

In all eukaryotes, the bipolar, microtubule-based spindle functions to segregate chromosomes to daughter cells during cell division, yet spindle size and microtubule organization vary dramatically across different species and cell types. In particular, meiotic spindles frequently lack astral microtubules at their poles, while mitotic spindles of many animal species possess long astral arrays that are most striking in the early embryonic divisions of a number of marine invertebrates. One candidate factor for modulating astral microtubule density and length is the microtubule-associated protein TPX2, which localizes to spindle poles and possesses microtubule nucleation activity regulated by the nuclear localization signal (NLS)-binding protein importin alpha. TPX2 also interacts with the mitotic kinase Aurora A through its N-terminus, and the kinesin-5 (Eg5) motor through its C-terminus. Although conserved among animals, TPX2 differs dramatically in primary sequence, with an intrinsically disordered region (IDR) in the amino terminal half of the protein capable of liquid-liquid phase separation. Through phylogenetic sequence comparison, we have identified TPX2 homologs in sea urchin (*S. purpuratus*) and sea squirt (*C. intestinalis*) that contain a significantly longer IDR. We hypothesize that this extended domain enhances co-condensation with tubulin, thereby increasing microtubule nucleation and aster size. Further, we have identified a conserved NLS motif located within this disordered region, KKLK in *X. laevis* TPX2, that when mutated to AALA results in significantly higher microtubule nucleation activity during spindle assembly. We are now expressing and purifying wild-type and NLS mutant TPX2 proteins from a variety of different species and testing their effects on spindle architecture and aster formation by adding them to *X. laevis* egg extracts, and determining the role of Aurora A and Eg5 in aster formation by treatment with specific inhibitors. Overall, these experiments will provide new insight into how TPX2 acts to modulate spindle architecture across species and during embryonic development.

B144/P1140

Spindle dynamics and orientation depends in force generators configuration

V. Gomez Herrera[1], M. Anjur-Dietrich[2], D. Needleman[3], R. Farhadifar[4], M. Shelley[1]; [1]New York University, New York, NY, [2]Harvard, Cambridge, MA, [3]New York University, Cambridge, MA, [4]Flatiron Institute, New York, NY

During cell division, the mitotic spindle forms inside cells and segregates chromosomes. The spindle's position sets the division plane, which is essential for proper growth and development. Force mechanisms regulating the position of the spindle are not yet understood. Here, we develop a coarse-grained model of spindles in cells, which accounts for microtubule dynamics, pulling forces from cortically bounded motor proteins, and fluid drag. We show that the spindle's resistance to rotation is largely driven by pulling forces from the motor proteins rather than the drag imposed by the cytoplasm. We also show that the arrangement of motor proteins affects the spindle's resistance to rotation- for configurations where multiple motors stack at the same region, the spindle's resistance to rotation significantly reduces. Our findings are consistent with measurements in human tissue culture cells, where the spindle resistance to the rotation has been quantified.

B145/P1141

In vivo interrogation of a nanoscale cytoskeleton-associated condensate using super-resolution microscopy

K. Morelli[1], S. M. Meier[2], M. Willis[1], A. Zhao[1], Y. Barral[2], J. Vogel[1]; [1]McGill Department of Biology, Montreal, QC, CANADA, [2]ETH Zürich Department of Biology, Zürich, SWITZERLAND.

Eukaryotic cells coordinate interactions between actin filaments and microtubules to serve various important cellular functions. One such function is positioning and alignment of the mitotic spindle during asymmetric cell division in budding yeast. To position the spindle, astral microtubules which are nucleated at the spindle pole bodies (SPBs, yeast centrosomes) and project into the cytoplasm interact with the polarized actin network which originates at the tip of the bud through the plus-end tracking (+TIP) Kar9. Recent work demonstrates that Kar9 and two other +TIPs, the EB1 homolog Bim1 and the CLIP170 homolog Bik1, form a liquid-like condensate which tracks the plus ends of growing and shrinking astral microtubules. However, directly observing predicted liquid-like behavior in living cells is challenging because Kar9 condensates have diameters around 200 nanometers or less in vivo. Therefore, we use lattice structured illumination microscopy super-resolution to study behaviors which emerge from material properties and determine condensate functions in vivo. Using time series with >15 frames per second, we develop a method to annotate dynamic microtubule behavior according to dynamic instability parameters using Kar9 as a plus end marker. Strikingly, shorter metaphase microtubules appear to grow and shrink along the contour of longer microtubules from the same SPB. These astral microtubules undergo rapid angular motions of astral microtubules relative to the SPB which most likely result from actin-bound myosin V pulling on Kar9 at their tips. Remarkably parallel microtubules remain linked during these motions. We conclude that microtubules are bundled by a mechanism that persists during phases of microtubule dynamic instability and rapid motion through the cytoplasm.

Ciliary/Flagellar motility

B147/P1142

Intracellular connections between basal bodies promote the coordinated behavior of *Tetrahymena* motile cilia

A. Soh[1], L. Woodhams[2], A. Junker[1], C. Enloe[3], B. Noren[3], A. Harned[4], C. Westlake[5], K. Narayan[4], J. Oakey[3], P. Bayly[2], C. Pearson[1]; [1]University of Colorado Anschutz medical campus, Aurora, CO, [2]Washington University in St. Louis, St. Louis, MO, [3]University of Wyoming, Laramie, WY, [4]National Cancer Institute, Bethesda, MD, [5]National Cancer institute, Frederick, MD.

The generation of efficient fluid flow is crucial for organismal development and homeostasis, sexual reproduction, and cell motility. Multiciliated cells possess fields of motile cilia that beat with metachronal synchrony to propel fluid. Cilia-driven fluid flow produces extracellular hydrodynamic forces that synchronizes ciliary beating. However, hydrodynamic coupling between neighboring cilia is not the sole mechanism for ciliary synchrony. Cilia are nucleated by basal bodies (BBs) that link to each other and to the cell's cortex via BB-associated appendages. The intracellular BB and cortical network is hypothesized to couple cilia and transmit cilia coordination cues. The extent of intracellular ciliary connections and the nature of these stimuli remain unclear. How BB connections influence the dynamics

of individual cilia has not been established. Using FIB-SEM imaging in *Tetrahymena thermophila*, we show that cilia are both longitudinally and laterally coupled by the underlying BB and cortical cytoskeletal network. To visualize the behavior of individual cilia in live, immobilized *Tetrahymena* cells, we developed Delivered Iron Particle Ubiety Live Light- (DIPULL) microscopy. Quantitative analysis of ciliary dynamics reveals that BB connections influence ciliary waveforms, but not ciliary length or beat frequency. Loss of BB connections in the *disA-1* mutant causes shorter and slower ciliary power strokes. Computer modeling analysis predicts that short and slow power stroke trajectories lead to reduced ciliary force output. Consistent with this, cells with disconnected BBs exhibit slower cilia-driven fluid flow. Disruption to BB connections also causes inconsistent power strokes between adjacently positioned cilia, leading to elevated frequencies of ciliary tangles and slower cell motility. In summary, BB connections promote effective ciliary power strokes and synchronize ciliary beating for efficient fluid flow and cell motility.

B148/P1143

***In situ* cryo-electron tomography reveals the asymmetric architecture of mammalian sperm axonemes**

Z. Chen[1], G. A. Greenan[1], M. Shiozaki[2], Y. Liu[1], W. M. Skinner[3], X. Zhao[2], S. Zhao[2], R. Yan[2], Z. Yu[2], P. V. Lishko[3], D. A. Agard[1], R. D. Vale[2]; [1]University of California, San Francisco, San Francisco, CA, [2]Janelia Research Campus, Ashburn, VA, [3]University of California, Berkeley, Berkeley, CA.

In their quest for optimal efficiency, sperm have evolved exquisite engineering solutions to deliver their genetic information to oocytes. The flagella of mammalian sperm display non-planar, asymmetric beating, in contrast to the planar, symmetric beating of flagella from sea urchin sperm and unicellular organisms. The molecular basis of this difference is unclear. Here, we perform *in situ* cryo-electron tomography of mouse and human sperm, providing the highest-resolution structural information of the axonemes to date (up to 6.0 Å). Our subtomogram averages reveal mammalian sperm-specific protein complexes within the microtubules and regulatory complexes of dyneins. The structures and interaction network of these mammalian sperm-specific complexes suggest their potential roles in enhancing the mechanical strength of the axonemes and regulating dynein-based axonemal bending. Intriguingly, we find that each of the nine outer microtubule doublets is decorated with a distinct combination of sperm-specific complexes. We propose that this asymmetric distribution of proteins differentially regulates the bending and sliding of each microtubule doublet and may underlie the asymmetric beating of mammalian sperm flagella. How these complexities and asymmetries arise during mammalian sperm development will be the next key question.

B149/P1144

Hydrodynamic mechanism of formation of metachronal waves in arrays of cilia

B. Chakrabarti[1], S. Fürthauer[2], M. Shelley[3]; [1]Center for Computational Biology, Simons Foundation Flatiron Institute, New York, NY, [2]Institute of Applied Physics, TU Wien, Vienna, AUSTRIA, [3]Center for Computational Biology, Simons Foundation Flatiron Institute, New york, NY.

Motile cilia are slender, hair-like cellular appendages that spontaneously oscillate under the action of internal molecular motors and are typically found in dense arrays. These active filaments coordinate their beating to generate metachronal waves that drive long-range fluid transport and locomotion. Understanding the emergence and properties of such metachronal waves in ciliary arrays is a multiscale problem central to developmental biology, transport phenomena, and nonequilibrium physics, with

potential biomedical applications. Until now, our understanding of their collective behavior largely comes from the study of minimal models that coarse grain the relevant biophysics and the hydrodynamics of slender structures. Here we build on a detailed biophysical model to elucidate the emergence of metachronal waves on millimeter scales from nanometer-scale motor activity inside individual cilia. Our study of a one-dimensional lattice of cilia in the presence of hydrodynamic and steric interactions reveals how metachronal waves are formed and maintained. We find that, in homogeneous beds of cilia, these interactions lead to multiple attracting states, all of which are characterized by a conserved integer charge. This allows us to design initial conditions that lead to predictable emergent states. Finally, and very importantly, we show that, in nonuniform ciliary tissues, boundaries and inhomogeneities provide a robust route to metachronal waves. Due to the novelty of our modeling and computation, we have unraveled the spatiotemporal self-organization of nanometric motor proteins in coordinating collective dynamics spanning millimeters: bridging length scales over six orders of magnitude.

B150/P1145

FAP47, HYDIN and CPC1 in the central pair apparatus of *Chlamydomonas* flagella

Y. Tani[1], H. Yanagisawa[1], T. Yagi[2], M. Kikkawa[1]; [1]Graduate School of Medicine, University of Tokyo, Tokyo, JAPAN, [2]Department of Life Sciences, Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, Hiroshima, JAPAN.

Motile cilia have a so-called “9+2” structure, which consists of nine doublet microtubules and central pair apparatus. The central pair apparatus has two microtubules surrounded by several projections, which are thought to interact mechanically with radial spokes and control the flagellar beating. Recently, the components of the central pair apparatus of motile cilia have been identified by proteomic and genomic analysis. Still, the mechanism of how the central pair contributes to the flagellar motility has much to be revealed. Here, we focused on high molecular weight components of the central apparatus, FAP47, HYDIN, and CPC1, and studied the swimming phenotypes and 3D localizations of these components using *Chlamydomonas* mutants. First, the swimming speed of FAP47-deficient cells is equal to the wild type’s speed, and the HYDIN-deficient mutant shows slightly slower swimming than the wild type. *fap47 x cpc1* double mutant cells swim much slower than cpc1 single mutant cells, indicating that the lack of FAP47 makes the motility defect of *cpc1* worse. GFP-tagged FAP47 rescued this motility defect. Among these three proteins, FAP47 is less studied, so we focused on FAP47. The FAP47-deficient mutants swam straighter than the wild type, which indicates the regulation of the flagella is disordered. Second, we suspect that this malfunctioning of flagella is caused by structural defects of the central pair apparatus, so we also checked the structural contribution of FAP47 in the central pair apparatus. FAP47 localizes the entire length of axonemes in indirect immunofluorescence microscopy and cryo-electron tomography revealed that the FAP47-deficient strain partially lacks the C1-C2 bridge structure. In conclusion, the lack of FAP47 destabilizes the central pair apparatus and affects the regulation of flagellar beating.

B151/P1146

Basal body polyglutamylation is modulated by differential ciliary forces

E. Collet, C. Pearson; UNIVERSITY OF CO ANSCHUTZ MEDICAL CAMPUS, Aurora, CO.

Motile cilia are evolutionarily conserved structures that produce hydrodynamic forces through highly coordinated beating. These hydrodynamic forces are critical for generating fluid flow in multiciliated epithelial cells and in the motility of multiciliated single-celled organisms. Ciliary beating is accomplished through dynein-driven sliding of the microtubule doublets which run the length of the cilium, resulting in a prominent, asymmetric beat stroke. These microtubule doublets are nucleated and anchored to the cell cortex by basal bodies (BBs), which resist and stabilize cilia against these asymmetric forces. While several molecular components and microtubule post-translational modifications have been identified as BB-stabilizing factors, the mechanisms by which BBs are stabilized against differential ciliary forces remain largely unexplored. Through modulation of the ciliary forces experienced by the ciliate Tetrahymena thermophila, we find that the levels and distribution of microtubule polyglutamylation at the BB actively respond to differential ciliary forces. Increasing or decreasing ciliary forces results in a corresponding increase or decrease in BB polyglutamylation. Additionally, we show BBs exhibit a distinctly variable, yet asymmetric, pattern of polyglutamylation localization at average ciliary forces. When subjected to increased ciliary forces, this asymmetry is promptly lost. Furthermore, BBs experiencing reduced ciliary forces exhibit an asymmetric polyglutamylation pattern distinct from that of control BBs. We propose that an upregulation of BB polyglutamylation is required to protect against elevated ciliary forces, and that these forces result in a saturation of polyglutamylation which masks the asymmetry exhibited at lower force levels.

B152/P1147

Generation of a mouse IFT25GFP knock-in mouse model

W. Li, Y. Yap, A. Kearney, C. Kelly, Z. Zhang; Wayne State University, Detroit, MI

Intraflagellar transport 25 (IFT25) is a component of the IFT-B complex. In mice, even though this IFT component is not required for cilia formation in the somatic cells, it is essential for sperm formation. However, the intracellular localization of this protein in male germ cells is not known given no antibodies are available for histologic studies. To examine localization of the protein in male germ cells and further investigate the mechanism of IFT in sperm formation, particularly to look into the dynamic trafficking of the protein, we generated a mouse IFT25/GFP knock in mouse model using the CRISPR/cas9 system, with the mouse IFT25 protein fused with a GFP tag in the C-terminus. Three independent lines were analyzed. Western blotting using both anti-IFT25 and anti-GFP antibodies showed that the IFT25/GFP fusion protein has the same tissue distribution as the endogenous IFT25 protein, with the highest level in the testis. Examination of localization of the IFT25/GFP in isolated germ cells revealed that the fusion protein was present in the cytoplasm of spermatocytes and round spermatids and a strong signal was present in the developing sperm flagellar. Diffusion analysis of IFT25 within the manchette and developing flagellar revealed the presence of both mobile and immobile fractions. Preliminary fluorescence recovery after photobleaching (FRAP) data revealed 40% of the IFT25 were mobile with a diffusion rate of 0.5 $\mu m^2/s$ while the remaining 60% of the IFT25 were immobile and did not recover after photobleaching. On going analyses will assess varying IFT25 mobility throughout the developing sperm.

B153/P1148

Rapid changes in ciliary waveform and beat frequency produce jumping behaviors in the predatory marine ciliate *Strobilidium*

D. Galati, M. B. Olson, T. Ridgway, B. Morales, M. G. Campbell, W. Zwolinski; Western Washington University, Bellingham, WA

Marine ciliates link microbially derived organic carbon to crustacean zooplankton at the lowest trophic levels. To succeed at these lower trophic levels, marine ciliates use diverse motility behaviors to capture prey (microbes) and escape predators (zooplankton). These motility behaviors include persistent unidirectional swimming, helical trajectories, anchored "bobbing", seemingly random "meandering", and bursts of rapid acceleration known as "jumping." One of the most well-studied jumping marine ciliates is *Strobilidium*, which accelerates from rest to speeds greater than 50 mm/sec in less than one second. Although the ecological importance and swim speeds associated with *Strobilidium* jumping have been characterized, the ciliary processes the enable jumping are not clear. To overcome this, we investigated confined single *Strobilidium* cells with high-speed oblique illumination microscopy. We found that *Strobilidium* jumping is initiated by a near instantaneous change in ciliary waveform and beat frequency that occurs over several milliseconds. The change in waveform and beat frequency propagates around a ring of fused oral cilia before slowly returning to the pre-jump status. The pre- and post-jump ciliary states are nearly identical, and there is no observable change in cell shape prior to jumping. Collectively, these results suggest that a ciliary state-switching mechanism initiates at or near the fused oral cilia to allow *Strobilidium* to alternate between two different velocity states.

Ciliopathies

B154/P1149

Characterization of a new ciliopathy-related disorder caused by mutations in WDR44 that affect RAB11-dependent ciliogenesis initiation

S. Shakya[1], A. Accogli[2], T. Yang[3], C. Insinna[1], S. Park[3], C. Westlake[1]; [1]Center for Cancer Research, NCI Frederick, Laboratory of Cellular and Developmental Signaling, Frederick, MD, [2]IRCCS Istituto Giannina Gaslini, Genova, ITALY, [3]Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul, KOREA, REPUBLIC OF.

Primary cilia are antenna-like structures required for developmental signaling pathways such as Hedgehog (Hh). These organelles are derived from the mother centriole (MC) through a complex process called ciliogenesis which is initiated by preciliary vesicle (PCV) trafficking and docking to the MC. Notably, ciliopathy disorders have been linked to defects in PCV-MC docking, but not PCV trafficking. RAB11 is required for PCV transport and interaction with WDR44 through its NH_2-terminal RAB11 binding domain (RBD) prevents ciliogenesis initiation by a poorly understood mechanism. Here we describe a new X-linked ciliopathy-related disorder caused by mutations in the RAB11 effector WDR44. We identified seven missense and one nonsense WDR44 variants in the COOH-terminal WD40 repeat domain from male patients that display ciliopathy-related features including musculoskeletal abnormalities, craniofacial dysmorphism, and kidney disease. Fibroblasts isolated from severely affected patients have aberrant ciliogenesis initiation and reduced Hh signaling implicating dysfunctional cilia as causative in disease. Zebrafish embryos expressing human WDR44 variants show ciliopathy-related phenotypes that correlate with the severity of the disease spectrum observed in these patients.

Moreover, the expression of WDR44 variants in human cells and zebrafish embryos reduces ciliation. Remarkably, missense variants associated with more severe disease show strongly reduced expression, which was unexpected based on the WDR44 function in blocking PCV trafficking. We discovered that the gain-of-function for these proteasome-sensitive WDR44 variants is associated with enhanced vesicular localization resulting from higher affinity binding to RAB11. Molecular structure predictions and coimmunoprecipitation studies support a direct interaction between the WDR44 RBD and the WD40 repeat domains, and strikingly the WDR44 patient missense variants disrupt these interdomain interactions. Together, our work demonstrates that WDR44 interdomain associations are important for regulating RAB11 effector binding, and the mutations observed in our patients alter these interactions resulting in impaired ciliogenesis initiation causing a ciliopathy-related disorder.

B155/P1150

Bardet Biedl Syndrome Alters the Ciliary Localization of Appetite Signaling Proteins in the Paraventricular Nucleus

G. Canales[1], J. Reiter[1,2], C. Vaisse[3]; [1]Biochemistry and Biophysics, Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA, [2]Chan Zuckerberg Biohub, San Francisco, CA, [3]Department of Medicine, Diabetes Center, University of California, San Francisco, San Francisco, CA.

Obesity is a hallmark feature of Bardet-Biedl syndrome (BBS), a ciliopathy caused by loss of function of the ciliary GPCR trafficking complex called the BBSome. A ciliary GPCR in the paraventricular nucleus (PVN) of the hypothalamus, called Melanocortin 4 Receptor (MC4R), is the most common gene mutated in monogenic obesity. *Mc4r*$^{-/-}$ mice develop hyperphagia and obesity, revealing the role of MC4R in food intake and body weight regulation. Disrupting primary cilia in the mouse adult CNS is sufficient to cause hyperphagic obesity and neuronal primary cilia function can be affected by diet and Leptin levels. MC4R co-localizes and works with Adenylyl Cyclase (ADCY) at PVN primary cilia to control feeding in vivo. I hypothesize that ciliopathies cause obesity by disrupting ciliary MC4R signaling in the PVN. Using mouse ciliopathy models of BBS and an endogenously tagged MC4R-GFP, I will show that localization of ADCY3 to PVN cilia is significantly reduced, while MC4R localization is increased. Our findings hint that central regulation of appetite signaling in BBS may be perturbed by a loss of Adenylyl Cyclase in the PVN, a model that we are testing using conditional genetic approaches. We are also investigating how the ciliary trafficking of ADCY3 is perturbed in some neuronal cilia but not others. This work has important implications for how we treat obesity in BBS patients.

B156/P1151

Loss of the Membrane-Binding Protein ciBAR1 in Mice Leads to Ciliary Defects

E. Kim[1], M. Hoque[1], J. Chen[1], F. Li[2], K. Takemaru[2]; [1]Molecular and Cellular Biology Graduate Program, Stony Brook University, Stony Brook, NY, [2]Department of Pharmacological Sciences, Stony Brook University, Stony Brook, NY.

Cilia—ubiquitous, microtubule-based organelles that protrude from the apical cell surface—play a variety of roles, including mechano- and chemosensation, cell signaling, and fluid dynamics. Dysfunction of cilia is associated with pleiotropic diseases, known as ciliopathies. The current body of literature suggests that the unique composition and morphology of the ciliary membrane are crucial for maintaining the function and homeostasis of cilia. However, the molecular players and mechanisms underlying the formation and maintenance of the ciliary membrane remain poorly understood. Previous

work by our lab and other groups demonstrated that the evolutionarily conserved coiled-coil protein Chibby1 (Cby1) localizes to the ciliary base and plays key roles in ciliogenesis in various model organisms. More recently, our lab reported that the membrane-binding proteins, Cby1-interacting BAR domain-containing 1 and 2 (ciBAR1 and 2; formerly known as FAM92A and B), physically interact with Cby1 to promote ciliogenesis. Bin/Amphiphysin/Rvs (BAR) domain-containing proteins are known to dimerize and form crescent-shaped structures that generate or preferentially bind to curved membranes to facilitate a plethora of cellular processes, such as endocytosis and vesicular fusion and fission. Coexpression of ciBAR and Cby1 induces membrane tubule-like structures in mammalian cultured cells. However, the *in vivo* functions of ciBAR1 and 2 are currently unknown. To elucidate the role of ciBAR1 in mammalian development, we have generated a ciBAR1$^{-/-}$ mouse model. We found that about 20% of ciBAR1$^{-/-}$ mice show embryonic lethality, and the rest appear runted but survive into adulthood with no gross morphological abnormalities. Consistent with the important role of ciBAR1 in ciliogenesis, ciBAR1$^{-/-}$ mouse embryonic fibroblasts (MEFs) exhibited a robust decrease in the number of primary cilia. In MEFs, ciBAR2 is not expressed, and Cby1 is detectable at the ciliary base in the absence of ciBAR1. Furthermore, we found that, like Cby1$^{-/-}$ mice, ciBAR1$^{-/-}$ mice develop cystic lesions in the pancreas most likely due to dysfunctional primary cilia. Taken together, our findings indicate that ciBAR1 plays a critical role in ciliogenesis, at least in certain ciliated cell types, in mice.

B157/P1152

Characterization of the Disease-Causing Mechanism of KIF3B Mutations from Ciliopathy Patients

S. Rogers, J. Reid, R. Dasari, C. Goben, J. M. Adams, **M. F. Engelke**; School of Biological Sciences, Illinois State University, Normal, IL.

The heterotrimeric kinesin-2 motor KIF3A/KIF3B/KAP drives intracellular transport, which is essential for ciliogenesis and ciliary function. Two point mutations in the KIF3B subunit (E250Q and L523P) have recently been linked to human disease (Cogne et al., 2020, Am. J. Hum. Genet.). Heterozygous carriers of these mutations display retinitis pigmentosa and, in some cases, other classical ciliopathy phenotypes such as postaxial polydactyly, as well as hepatic fibrosis and heart malformation (E250Q) and kidney dysfunction (L523P). However, the molecular mechanism leading to disease is currently unknown. Here we use *Kif3b*-knockout 3T3 cells, which cannot make cilia, to characterize these mutations. We find that re-expression of wildtype KIF3B restored ciliogenesis in 79.4% of transfected cells. In contrast, expression of the mutants resulted in virtually no cilia: E250Q rescued ciliogenesis in 0% and L523P in 0.4% of transfected cells. Next, we functionally characterized motor complexes containing the disease-causing mutations. Fluorescent-tagging revealed that the L523P mutant localized diffusely throughout the cytoplasm, indistinguishable from wildtype KIF3B, while the E250Q mutant decorated microtubules. Further characterization of the E250Q mutation, located in the nucleotide-binding switch II element of the motor domain, revealed it to be a novel rigor mutation that causes motor complexes to tightly bind to but not move along microtubules. The L523P mutation falls within the coiled-coil stalk domain of the motor. Bioinformatic analysis suggests that this mutation causes a break in the alpha-helical structure of this domain, suggesting that this mutation might interfere with the formation of the trimeric KIF3A/KIF3B/KAP complex. To our surprise, protein interaction assays revealed that motor complex assembly is unaffected. Instead, induced activation of the L523P mutant by truncation of the tail domain did not result in the expected peripheral accumulation of the mutant motor and thus indicates a defect in processive motility along microtubules. In summary, this work elucidates the molecular effects of disease-causing mutations in the KIF3B subunit on the kinesin-2 holoenzyme. Future work will establish

how the impaired function of kinesin-2 leads to ciliary disease. This work is funded by the NIGMS grant 1R15 GM137248-01A1 to MFE.

B158/P1153

Screening of a small molecule library for chemicals that enhance motile ciliogenesis using zebrafish and mice

G. Chandrasekaran[1], H. J. Ryu[2], G. B. Lee[3], H. Lu[4], S. Roy[4], M. A. Bae[5], H. Hyun[1], J. H. Ahn[3], E. Y. Choi[2], S. Y. Choi[1]; [1]Biomedical Sciences, Chonnam National University Medical School, Hwasun 58128, KOREA, REPUBLIC OF, [2]Biomedical Sciences, University of Ulsan College of Medicine, Seoul 05505, KOREA, REPUBLIC OF, [3]Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, KOREA, REPUBLIC OF, [4]Institute of Molecular and Cell Biology, Proteos, 61 Biopolis Drive 138673, SINGAPORE, [5]Bio & Drug Discovery Division, Korea Research Institute of Chemical Technology, Daejeon 34114, KOREA, REPUBLIC OF.

Cilia are classified into primary (non-motile) cilia and motile cilia. Ciliopathies are a group of genetic disorders caused by dysfunctional cilia. Motile cilia play a vital role in clearing mucus and foreign matter along the trachea and bronchi, cerebrospinal fluid circulation, ova movement from the ovary, and sperm motility. Defects in the motile cilia result in hydrocephalus, chronic respiratory infections, and infertility. Restoring motile cilia with respect to structure and function is critical to treating motile ciliopathies. However, there has been little, if any, known empirical research and effective therapeutic modalities for motile ciliopathies to date. To develop small molecules that can increase motile cilia's number, length, or function, a high throughput small molecule library screen was performed using *Tg(foxj1a:eGFP)* zebrafish larvae. Foxj1 transcription factor is a master regulator of motile ciliogenesis. Eight hit compounds (HC) were identified by this screening. The HCs were further validated with anti-acetylated α-tubulin immunostaining on WT zebrafish and primary mouse tracheal epithelial cells (mTECs). Drug metabolism and pharmacokinetics (DMPK) studies also identified HC-2 as druggable. In fact, at the molecular level, HC-2 increased the expression of several essential genes involved in motile ciliogenesis. Moreover, HC-2 effectively rescued the ependymal motile cilia in a zebrafish genetic model with reduced motile cilia. Most importantly, the administration of HC-2 to chronic obstructive pulmonary disease (COPD) mice model restored the otherwise lost/reduced tracheal motile cilia and aided in reducing airway resistance. Finally, using a novel hydroxyapatite-dye bead-based affinity purification assay, the GEMC1/MCIDAS-DP1 protein complex was preliminarily identified as a potential molecular target of HC-2, warranting further investigation. Given the above, our findings show promising pharmacological properties of HC-2 and support the idea of using it as a direct pharmacological intervention against defective motile cilia to ameliorate motile ciliopathy.

B159/P1154

mRNA therapy restores cilia function in a *ccdc40* zebrafish mutant model for Primary Ciliary Dyskinesia

M. Rasteiro, S. Lopes; Nova Medical School, Lisbon, Portugal

Primary ciliary dyskinesia (PCD) is an autosomal recessive disease characterized by neonatal respiratory distress, bronchiectasis, infertility, and *situs inversus*, all of these outcomes of defective cilia function. Mutations in more than 50 genes encoding cilia components have been confirmed to cause PCD. *ccdc40* is one of these genes that encodes a protein involved in the assembly of the internal dynein arms, the

nexin-dynein regulatory complexes, and the radial spokes, thus playing a crucial role in the correct assembly of the cilia ultrastructure and as a result impacting in the mucociliary clearance. The *CCDC40* gene has been described as causing a PCD variant resulting in more severe symptoms and a worse prognosis regarding respiratory complications than other PCD genes. Mucociliary clearance is essential to keep the lungs free of pathogenic agents. In PCD patients this mechanism is deficient or absent resulting in chronic respiratory infections. Up to the moment, there is no curative therapy available. PCD patients can only address their symptoms with antibiotics and physical therapy. Our animal model is a zebrafish with a mutation in the *ccdc40* gene presenting *situs inversus* and lack of cilia motility in the olfactory pit resulting from cilia ultrastructure disorganization observed by TEM. Zebrafish present a wide variety of ciliated organs, among them multiciliated epithelial tissues like the one present in the human respiratory system. When we administer a transient mRNA therapy (*CCDC40* human mRNA) coupled to a lipidic nanoparticle carrier into these zebrafish embryos, we are able to restore ciliary beat frequency and function in the olfactory pit to values indistinguishable from wild-type siblings. Our current results reinforce the human-zebrafish homology and show us that the transient gene therapy using this lipidic nanoparticle technology is effective in delivering the human *CCDC40* mRNA to the cells resulting in a strong rescue ability of the olfactory pit cilia movement. We believe that our findings are paving the way for future treatment for PCD patients.

B160/P1155

Neuronal primary cilia regulate postnatal pyramidal cell positioning to the deep and superficial sublayers in the mouse cortex

J. Yang, L. Qiu, K. Bicja, S. Mirhosseiniardakani, X. Chen; university of new hampshire, Durham, NH

It is well-recognized that primary cilia regulate embryonic neurodevelopment, but little is known about their roles during postnatal neurodevelopment. The hippocampal striatum pyramidal (SP) is subdivided into deep and superficial sublayers and gradually condensed into a compact lamina in the first postnatal two weeks, whereas simultaneously the volume of the neocortex greatly expands. It is elusive how pyramidal neurons position to two cortical sublayers postnatally. Here, we show that the axonemes of primary cilia in the deep and superficial sublayers of hippocampal SP point in the opposite directions, while neuronal cilia in neocortical sublayers display the same orientation. Neuronal primary cilia in the CA1 SP undergo marked changes in morphology and orientation from postnatal day 5 (P5) to P14, concurrent with cell positioning to two sublayers and with neural maturation. Surprisingly, the centrioles of late-born neurons migrate excessively to cluster at SP bottom before cilia protrusion and a reverse movement back to the main plate. Similarly, this “pull-back” movement of centriole/cilia is also identified on late-born cortical pyramidal neurons. We further reveal that ablation of neuronal cilia selectively in the mouse forebrain leads to significantly megalencephaly and increased superficial neurons positioned in the deep sublayer of the neocortex during the early postnatal stage. Together, this study provides the first evidence that primary cilia regulate pyramidal neuronal positioning in the mouse cortex and late-born pyramidal neurons undergo a reverse movement for cell positioning.

B161/P1156

Regulation of postnatal cortical development by neuronal primary cilia

K. Bicja, J. Yang, L. Qiu, X. Chen; University of New Hampshire, Durham, NH

Neuronal primary cilia (NPC) are small, centriole-based surface projections known to regulate embryonic neurodevelopment by multiple signaling pathways. However, whether NPC modulate postnatal brain development is not clear. The first objective of my research was to examine cilia orientation in the hippocampal dentate gyrus using transgenic mice overexpressing Arl13b, a protein regulating ciliogenesis, and overexpressing Centrin2, a protein marking the centriole base of cilia (Arl13b+ mice). Arl13b+ mice display elongated cilia in all regions of the hippocampus. In the postnatal dentate gyrus, the cilia showed a loosely opposite orientation, and they were generally perpendicular to the lamina of the granule cell layer. This result is consistent with our previous research about the opposite cilia orientation in the hippocampal CA1 region, which supports that directionality of NPC displays oppositely in compact laminated structure in the forebrain. The second objective was to determine cortical differences among control mice, Arl13b+ mice, and forebrain-specific cilia knockout mice (Ift88 flox/flox Emx1-Cre) via the tools of micro-CT and immunostaining. Interestingly, Ift88-Emx1 KO brains were slightly larger than their littermate brains, while Arl13b+ brains were slightly smaller than their control brains. Immunostaining was performed using anti-SATB2 and anti-CTIP2 antibodies, with SATB2 labeling the superficial sublayer and CTIP2 labeling the deep sublayer in the neocortex. Compared to controls, KO brains showed a higher SATB2 expression level in layer V, with a less clear border between layer V and IV. However, the cell density of controls and KO brains in layer V did not show significant differences. These preliminary results suggest that NPC play a critical role in regulating postnatal cortical development.

B162/P1157

The role of the retinal pigment epithelium in ciliopathy-mediated retinal degenerations

D. Reichert[1,2], C. Lewallen[1], R. Sharma[1], D. Ortolan[1], H. May-Simera[2], K. Bharti[1]; [1]NIH, Bethesda, MD, [2]JGU, Mainz, Germany

Primary cilia are microtubule-based antenna-like structures protruding from the cell surface of almost all cell types in the human body. Thought to be an evolutionary remnant, it was only recently discovered that primary cilia are cellular organelles critical for a wide range of physiological functions and developmental pathways. Mutations in ciliary proteins, that cause defects in cilia maintenance and function, are associated with a multitude of severe diseases, collectively termed ciliopathies. Retinal degeneration is the most common phenotype of syndromic and non-syndromic ciliopathies, subsequently leading to vision loss. The role of primary cilia in photoreceptors of the retina has largely been defined, however the function of primary cilia in other ocular tissues remains unknown. Of particular interest is the retinal pigment epithelium (RPE), a highly polarized monolayer in the back of the eye, essential for visual function. There is increasing evidence that the primary cilium and ciliary proteins are important in the development and function of the RPE. To discover the role of cilia in the RPE and how cilia defects affect RPE physiology in ciliopathy-mediated retinal degenerations, we generated induced pluripotent stem cell (iPSC) lines from seven ciliopathy patients with mutations in different cilia associated genes. These include Bardet-Biedl syndrome, Joubert syndrome, Leber's congenital amaurosis, Usher syndrome and PRPF31-mediated retinitis pigmentosa. All iPSC lines were successfully differentiated into mature iPSC-RPE. Flow cytometry showed that more than 95% of

differentiated cells expressed TYRP1 in all samples. RPE maturity was assessed by staining for RPE markers RPE65, Ezrin and Collagen IV. All lines formed a monolayer with transepithelial resistance of several hundred $\Omega*cm^2$. These newly generated lines will be compared to matched-control iPSC-RPE to understand the RPE disease phenotype. The generated lines will shed light on how the primary cilium impacts RPE differentiation, maturation, and function. This study provides the first human *in vitro* RPE disease models for a broad variety of ciliopathies, which can help develop treatment strategies for retinal degeneration in ciliopathy patients.

B163/P1158

Cep162 deficiency delays primary cilium formation through CP110 persistence at the mother centriole and causes retinal degeneration in humans

N. Nuzhat[1], J. Willer[1], H. Truong[1], E. D. Baere[2], H. J. Bolz[3], J. Pearring[1]; [1]University of Michigan, Ann Arbor, MI, [2]University of Ghent, Ghent, Belgium, [3]Senckenberg Center for Human Genetics, Frankfurt, Germany

In general, human mutations present in core ciliary components can involve virtually any organ, with frequent affection of the retina since the light-sensitive photoreceptor outer segment is an adapted primary cilium. Additionally, the process of ciliogenesis requires hundreds of proteins many of which also function outside of the cilium, such as playing key roles during cell cycle. In this study, we identified a homozygous *CEP162* frameshift variant, c.1935dupA (p.(E646R*5)), in two unrelated patients diagnosed with non-syndromic retinal dystrophy. CEP162 is a centrosome and microtubule-associated protein required for proper transition zone assembly during ciliogenesis and has been linked to neuronal development. Determining how the ciliary protein, CEP162, contributes to other cellular processes will help us to better understand the diversity of human ciliopathy phenotypes.
Using genetic, cell-based, and *in vivo* modeling, we found that even though mRNA levels were reduced, the truncated CEP162-E646R*5 protein was expressed and localized to the mitotic spindle during mitosis and directly bound to taxol stabilized microtubules. However, truncated CEP162 was absent from the basal body during ciliogenesis causing a delay in ciliation of patient derived primary fibroblasts. In these patient fibroblasts, we find ciliation is stalled due to the persistence of CP110 at the mother centriole; however, they ultimately extend abnormally long cilia that are missing key transition zone components. In *CEP162* knockdown cells, the truncated CEP162-E646R*5 protein was unable to restore the loss of cilia indicating it is unable to function at the cilium. CEP162 is expressed in human retina and we show that wild-type CEP162, but not truncated CEP162-E646R*5, specifically localizes to the distal end of centrioles in mouse photoreceptor cilia. Despite lack of CEP162-E646R*5 localization at the cilium, we find that both wild-type and truncated CEP162 were able to rescue photoreceptor cell loss induced by shRNA mediated knockdown of CEP162 in the developing mouse retina. Together, our data suggests that truncated CEP162-E646R*5 retains microtubule binding and association to the mitotic spindle that allows it to function during neuroretina development. However, it behaves like a loss of function mutation at the cilium leading to defects in recruitment of some transition zone proteins and ultimately leading to non-syndromic retinal dystrophy in patients.

Cytokinesis 1

B165/P1159

Mis-segregated DNA blocks an abscission promoting phosphatase pathway to halt cytokinesis

T. C. Panagiotou[1], A. Wilde[1,2]; [1]Molecular Genetics, University of Toronto, Toronto, ON, CANADA, [2]Biochemistry, University of Toronto, Toronto, ON, CANADA.

Cytokinesis is the final stage of cell division where the duplicated genome is equally segregated into two new daughter cells. Successful completion of cytokinesis is required for genome fidelity. Failure to correctly segregate chromosomes during cytokinesis leaves them trapped within the final intercellular bridge (ICB) that connects daughter cells[1]. If abscission - the physical separation of daughter cells - proceeds, chromosomal breakage can occur that leads to tumorigenesis[2]. To ameliorate this, cells activate a cell cycle checkpoint that inhibits the endosomal sorting complex required for transport (ESCRT) membrane-fissioning machinery within the ICB. This checkpoint is orchestrated by the kinase Aurora B that directly phosphorylates the ESCRT Chmp4C to stall abscission[3,4]. Differentially phosphorylated and localized pools of Chmp4C exist within the ICB, but it is only the unphosphorylated Chmp4C that localizes to the abscission site to promote scission[5]. Yet, once segregation errors have been rectified and cells commit to abscission, Aurora B activity persists in the ICB[6]. The molecular mechanism of how this checkpoint is overcome remains unclear. We report that the checkpoint is countered through a DNA-sensitive PP2A-B56ε phosphatase pathway. PP2A-B56ε is targeted to the ICB where it colocalizes with Chmp4C. The PP2A-B56ε phosphatase interacts with Chmp4C *in vitro* and *in vivo* during cytokinesis where it is required to properly localize Chmp4C to the abscission site. Depletion of B56ε delays the abscission process and results in an accumulation of hyper-phosphorylated, inactive Chmp4C in the ICB. Most strikingly, in cells treated with the segregation error inducing drug ICRF-193[7], PP2A-B56ε is absent from ICBs that harbor trapped chromosomes. Based on these findings, we propose that the PP2A-B56ε phosphatase effects a DNA-sensitive, abscission-promoting pathway by dephosphorylating Chmp4C to overcome the abscission checkpoint and complete cytokinesis.

References: [1] *Annu Rev Cell Dev Biol* **28**, 29-58 (2012). [2] *Science* **368** (2020). [3] *Science* **345**, 332-6 (2014). [4] *Science* **336**, 220-5 (2012). [5] *Open Biol* **6** (2016). [6] *J Biol Chem* **294**, 18639-49 (2019). [7] *J Cell Biol* **126**, 1341-51 (1994).

B166/P1160

The requirement for anillin in cytokinesis changes with ploidy

A. Piekny, K. Larocque, S. Koh, M. C. Husser, C. Brancheriau; Biology, Concordia University, Montreal, QC, CANADA.

Cytokinesis is required to physically separate the daughter cells toward the end of mitosis. This process occurs due to the ingression of an actomyosin ring, which must be properly positioned to avoid aneuploidy. Recent studies from our lab suggest that reciprocal gradients of active Ran and importins coordinate ring position with the segregating chromosomes. Proteins with nuclear localization signals (NLSs) can bind to importins, which are released by active Ran. The RanGEF RCC1 is bound to histones and generates active Ran near chromatin, while RanGAP downregulates Ran in the cytosol. We found that the ring protein anillin, requires importin-binding for its cortical recruitment and function in HeLa cells. This led to a model where cells with higher ploidy rely on a differential gradient of importins in the equatorial plane to control anillin function for ring positioning. In support of this model, we found that

anillin's requirement correlated with ploidy among different cultured human cells. To determine if ploidy influences anillin requirement through importins, we used CRISPR-Cas9 to integrate mNeonGreen into the anillin locus in HCT116 (colorectal carcinoma) cells, which have near-diploid modal chromosomal numbers. Only 15% of anillin was required to support cytokinesis in these cells, which displayed either failed ingression or failed abscission. After increasing ploidy, 50% of anillin was required for cytokinesis and all cells failed ingression or had oscillation phenotypes. In addition, anillin was cortically localized in HCT116 cells prior to mitotic exit, with broad localization at the equatorial cortex, while it was only localized to the equatorial cortex in cells with high ploidy. To determine if anillin's requirement for cytokinesis depends on importin-binding, we used a bicistronic plasmid to co-express RNAi-resistant NLS-mutant anillin and anillin shRNAs to knockdown endogenous protein. In HCT116 cells expressing this plasmid, NLS-mutant anillin failed to localize to the cortex prior to mitotic exit, but still localized to the equatorial cortex and cytokinesis was successful, while in cells with high ploidy, NLS-mutant anillin recruitment was delayed and localized with a narrower breadth, and cytokinesis failed. Our findings suggest that ploidy is one of the parameters controlling anillin's requirement in cells, which is largely through importin-binding.

B167/P1161

Diverse mechanisms regulate contractile ring assembly for cytokinesis in the two-cell *Caenorhabditis elegans* embryo

I. Ozugergin, M. Husser, A. Piekny; Concordia University, Montreal, QC, CANADA.

Cytokinesis occurs at the end of mitosis to physically separate a cell into two. This process occurs through the ingression of an actomyosin ring that cleaves the cell. Although the core cytokinesis machinery is conserved among metazoans, it is regulated by multiple pathways that appear to vary among cell types and organisms. Since few studies have compared cytokinesis between two cell types in the same model system, we performed the first in-depth characterization of cytokinesis in the two-cell *C. elegans* embryo. The embryo at this stage is ideal for comparative studies, as the larger AB cell is fated to be somatic tissue and the smaller P_1 cell is fated to be germline. We found that these cells have different cytokinesis kinetics supported by distinct myosin levels and organization, and that P_1 cells have a notable delay in ring assembly. Through perturbation of RhoA or polarity regulators and the generation of tetraploid strains, we found that ring assembly is influenced by multiple factors. We observed a negative correlation between size and assembly in P_1 cells, which also have lower levels of myosin. Since size could affect chromatin-associated cues that control actomyosin organization, we tested the role of the Ran pathway in these cells. Prior studies from our group found that active Ran forms an inverse gradient with importins that control the cortical recruitment of the ring scaffold, anillin, in HeLa cells. We found that while active Ran regulates ring assembly in AB and P_1 cells, it functions through ANI-1/anillin in AB cells, but not in P_1 cells. We propose that Ran signaling and low myosin levels delay ring assembly in P_1 cells to coordinate the timing of ring closure with their somatic neighbors. Our findings prompted us to characterize cytokinetic differences in mammalian cells. By generating multiple endogenously tagged cell lines to follow cytokinesis, we observe differences in the breadth and accumulation of cytokinesis proteins, and in the duration and symmetry of ring closure. We are determining how these differences arise, as well as their relevance for controlling different aspects of ring closure. Taken together, our findings show that it is imperative to utilize a wider range of cell types - both cultured and *in vivo* - to reveal the cytokinetic diversity across life forms and the mechanisms controlling this diversity.

B168/P1162

Endogenous tagging of anillin, Ect2 and RhoA reveals cytokinetic diversity in mammalian cells

M. C. Husser, I. Ozugergin, V. J. J. Martin, A. Piekny; Concordia University, Montréal, QC, CANADA.

Cytokinesis physically separates a cell into two daughter cells at the end of mitosis. This process occurs via the ingression of a RhoA-dependent actomyosin ring that assembles in the equatorial plane between the segregating chromosomes. The GEF (guanine nucleotide exchange factor) Ect2 generates active RhoA at the equatorial cortex, where it binds to effectors to generate actomyosin filaments. Another RhoA effector, anillin, acts as a scaffold to connect actomyosin with the plasma membrane for ring positioning. Different pathways regulate ring assembly and positioning in mammalian cells, but most studies were done using overexpressed transgenes in HeLa cells, and the contribution of these pathways in different cell types is not known. First, we used CRISPR/Cas9 to endogenously tag key cytokinesis regulators RhoA, Ect2 and anillin with mNeonGreen in HeLa cells, and characterized their spatiotemporal localization during cytokinesis in live cells. Then, to compare cytokinesis among cell types, we tagged endogenous anillin with mNeonGreen in cell lines that are not typically used to study cytokinesis: HEK293 (human embryonic kidney), HCT116 (colorectal cancer), HepG2 (hepatocellular carcinoma) and MDCK (Madin-Darby canine kidney) cells. Comparing anillin localization as well as the timing and symmetry of ring closure revealed diversity in how these cell types undergo cytokinesis. We found that: 1) anillin was cortically localized prior to mitotic exit in cells that had lower ploidy and/or were larger; 2) cells with broader anillin localization had faster ring closure, supporting a role for breadth in the alignment or removal of filaments for efficient ingression; and 3) cells capable of adopting apicobasal polarity had more extreme asymmetric ring closure, supporting a role for this machinery in controlling ingression. Combining the use of endogenous tags with quantitative measurements helped us capture cytokinetic diversity across a broader range of mammalian cell types and generate new knowledge of the mechanistic differences regulating cytokinesis in different cell types. The tools and protocols for the endogenous tagging of RhoA, Ect2, anillin and other proteins in human cells are available to the community.

B169/P1163

Aurora A kinase promotes clearing of contractile ring components from the cell poles in human cells

F. Wolff[1], E. Zanin[2]; [1]Ludwig-Maximilians-Universität München, Munich, GERMANY, [2]Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, GERMANY.

In the last step of cell division, called cytokinesis, the genetic material as well as other cellular content gets distributed from the mother cell among the two daughter cells. To ensure correct distribution, it is of major importance that cytokinesis is precisely controlled in time and space. During cytokinesis, the GTPase RhoA is active at the cell equator in a narrow zone, where RhoA induces assembly of the contractile ring underneath the plasma membrane. The formation and positioning of the contractile ring is directed by stimulatory signals from the spindle midzone, and inhibitory signals associated with the astral microtubules. Whereas the stimulating signals from the midzone are well characterized, the molecular nature of the aster-based inhibitory signals is unclear. We have recently shown that the kinase Aurora A, which localizes to the centrosomal asters, clears contractile ring components from the poles in *C. elegans*. Here we analyzed, whether Aurora A function in polar clearing is conserved in human cells. To address this, we inhibited Aurora A kinase in HCT116 and HeLa cells, and monitored the

localization of the active RhoA-binding protein anillin during anaphase. We found that after Aurora A inhibition anillin levels are increased at the cell poles and the equatorial anillin zone is wider. Together this suggests that the inhibitory role of Aurora A kinase in clearing contractile ring components from the poles and restricting them to a narrow equatorial zone is conserved from worms to humans. Currently, we are following a candidate-based approach to identify Aurora A phosphorylation targets during cytokinesis in human cells.

B170/P1164

The nuclear localization of Ect2 is required for abscission

N. Pham, J. Del Corpo, M. Husser, A. Piekny; Biology, Concordia University, Montreal, QC, CANADA.

Cytokinesis is the physical separation of a cell into two daughters by the ingression of an actomyosin ring. This process is tightly controlled to prevent aneuploidy. In anaphase, the centralspindlin complex recruits and activates the guanine nucleotide exchange factor (GEF) Ect2 at the equatorial cortex to generate active RhoA for ring assembly. After the ring ingresses in telophase, it transitions to form a midbody that is required for abscission. Ect2 localizes to the midbody, but then leaves and accumulates in the daughter nuclei, while centralspindlin remains at the midbody. Here, we show that the localization of Ect2 to the nuclei is required for abscission. A prior study showed that the over-expression of an N-terminal Ect2 fragment that localizes to the central spindle and competes with endogenous Ect2 causes cells to fail abscission. Another study showed that after Ect2 leaves the midbody, centralspindlin forms a complex with Fip3, which mediates secondary ingression and abscission. Thus, the levels of Ect2 at the midbody are important for late cytokinesis, but it is not clear why since the role of RhoA at this stage is not understood. One hypothesis is that Ect2 removal causes a decrease in active RhoA for cortical remodelling and abscission. Another not mutually exclusive hypothesis is that the removal of Ect2 frees Cyk4 to form complexes with Fip3 and/or proteins required for secondary ingression and abscission. To visualize changes in RhoA during cytokinesis, we used CRISPR-Cas9 editing to endogenously tag RhoA with mNeonGreen in HeLa cells. RhoA is enriched in the furrow as expected and remains visible at the midbody until around the same time that Ect2 localizes to the nuclei. To show that the localization of Ect2 is required for its function, we mutated the nuclear localization signal (NLS) in the S-loop domain to abolish its nuclear localization. Indeed, in the absence of endogenous Ect2, NLS mutant Ect2 fails to localize to the daughter nuclei and persists at the midbody causing cytokinesis failure. The timing of this failure is consistent with when RhoA levels decrease at the midbody. To further characterize this phenotype, we are measuring changes in the levels of endogenous RhoA at the midbody with mutant Ect2, and will monitor changes in the localization of Fip3 as well as other midbody proteins required for abscission.

B171/P1165

Intrinsic and extrinsic inhibition of cortical flow underlies symmetry-breaking of unilateral cytokinesis

K. Sugioka, C. Hsu, G. Sangha, W. Fan; Department of Zoology/Life Sciences Institute, University of British Columbia, Vancouver, BC, CANADA.

The contractile ring is supramolecular machinery critical for physical partitioning of the dividing cell. Eccentric closure of the contractile ring, called unilateral cytokinesis, often orients along the body axis to maintain tissue integrity. Despite the identification of unilateral cytokinesis regulators, the initial symmetry-breaking mechanism remains elusive. Here, we report that intrinsic and extrinsic inhibition of

the cortical flow breaks cytokinesis asymmetry in *Caenorhabditis elegans*. By coupling high-resolution 4D imaging and *in vitro* cell contact reconstitution assay, we found that unilateral cytokinesis of the zygote P_0 and the two-cell stage AB are regulated by the actin-dependent and adhesion-dependent mechanisms, respectively. In P_0 and AB, intracellular compression and intercellular adhesion locally inhibit the furrow-directed cortical flow and cleavage furrow ingression, respectively, resulting in the eccentric ring closure. Our study demonstrates that the local mechanical suppression of the ring-dependent cell cortex pulling is a common symmetry-breaking cue underlying the different modes of unilateral cytokinesis.

B172/P1166

Investigating the role of importin-β during cytokinesis

C. Brancheriau, K. Larocque, S. Pokharel, A. Piekny; Biology, Concordia University, Montréal, QC, CANADA.

Cytokinesis is the separation of a cell into two daughter cells at the end of mitosis. It occursdue to the assembly and ingression of a contractile ring controlled by multiple pathways. Thisprocess must be tightly controlled to prevent changes in cell fate or aneuploidy, which cancause disease. We found that a chromatin-sensing pathway positions the ring between thesegregating chromosomes. The GEF RCC1, which is bound to histones, generates Ran-GTPnear chromatin, while RanGAP inactivates Ran in the cytosol, creating a Ran-GTP gradient.Typically, importins bind to proteins with nuclear localization signals (NLS) and Ran-GTPreleases them. We found that importins bind to the NLS of anillin, a ring protein, and isrequired for its recruitment to the division plane where it functions to position the ring inHeLa cells. Anillin's requirement for cytokinesis varies widely, and we hypothesize that thismechanism may play a stronger role in cells where Ran-GTP levels are higher due toparameters such as fate, ploidy, cell size, or shape. In support of this model, we found thatincreasing ploidy in HCT 116 cells, which have lower requirements for anillin compared toHeLa cells, caused changes in anillin's localization and requirement. We are now obtainingevidence to show that importins and their control of anillin function changes in response toploidy. This includes using the Rango4 FRET probe generated by Dr. Kalab, which contains theimportin-β binding domain from snurportin sandwiched between a CFP-YFP pair, and FREToccurs when importin-β cannot bind due to high Ran-GTP. With this probe, we will showwhere importin-β is free to bind to NLS-proteins during anaphase, and how the levels of freeimportin-β change with ploidy. We are also endogenously tagging the KPNB1 locus withmNeonGreen in HeLa and HCT 116 cells to determine how the localization of endogenousimportin-β compares after increasing ploidy in HCT 116 cells. In addition, we are building anoptogenetic tool to control the activity of importin-β during cytokinesis by fusing it to a shortpeptide, CRY2, which is capable of rapid and efficient homo-oligomerization upon blue lightexposure. By activating this tool in specific regions of the cell, we can induce spatiotemporalchanges in importin-β and determine its requirement for cytokinesis.

B173/P1167

The number of cytokinesis nodes in mitotic fission yeast scales with cell size.

W. Sayyad, T. Pollard; Yale University, New Haven, CT.

Cytokinesis nodes are assemblies of stoichiometric ratios of proteins associated with the plasma membrane, which serve as precursors for the contractile ring during cytokinesis by fission yeast. The total number of nodes is uncertain, because of the limitations of the methods used previously. Here we

used the ~140 nm resolution of Airyscan super-resolution microscopy to measure the fluorescence intensity of small, unitary cytokinesis nodes marked with Blt1-mEGFP in live fission yeast cells early in mitosis. The ratio of the total Blt1-mEGFP fluorescence in the broad band of cytokinesis nodes to the average fluorescence of a unitary node gives about 190 unitary cytokinesis nodes in wild type fission yeast cells early in mitosis. Most, but not all of these nodes condense into a contractile ring. The number of cytokinesis nodes scales with cell size in four strains tested, although large diameter *rga4Δ* mutant cells form somewhat fewer cytokinesis nodes than expected from the overall trend. The Pom1 kinase restricts cytokinesis nodes from the ends of cells, but the surface density of Pom1 on the plasma membrane around the equators of cells is similar with a wide range of node numbers, so Pom1 does not control cytokinesis node number. However, varying concentrations of either kinase Pom1 or kinase Cdr2 with the *nmt1* promoter showed that the numbers of cytokinesis nodes increase above a baseline of about 190 with the total cellular concentration of either kinase.

B174/P1168

Speed oscillations in cytokinetic ring ingression suggest interplay between mechanical and biochemical feedback

M. E. Werner[1], D. Ray[2], C. Breen[3], F. Jug[4], A. Maddox[1]; [1]Biology, University of North Carolina, Chapel Hill, NC, [2]University of North Carolina, Chapel Hill, NC, [3]University of Wisconsin, Madison, WI, [4]Human Technopole, Milan, ITALY.

The cytokinetic ring is a transient contractile structure that provides the mechanical force to separate two nascent daughter cells. It assembles in the division plane in response to local activation of the small GTPase RhoA via the local recruitment and reorganization of cortical actomyosin and associated regulators and crosslinkers. Recent theoretical work predicted that complex contractile dynamics could result from the interaction between cortical mechanics and the biochemical feedback loops known to underlie oscillations of RhoA activity and the abundance of its downstream cytoskeletal targets. To explore how cytokinetic ring organization and composition contribute to ring ingression, we used a combination of high resolution four-dimensional fluorescent microscopy combined with computer vision based analysis to follow cytokinetic ring ingression with unprecedented temporal resolution. We observed that ring ingression kinetics are spatially and temporally non-uniform. Specifically, ingression follows an oscillatory pattern of acceleration and deceleration in ingression speed rather than a constant speed as previously suggested. Quantification of the amplitude and frequency of ingression speed oscillations revealed a period of 20 seconds, which corresponds to that of RhoA activity pulses we measured in the cleavage plane during early anaphase using a RhoA activity biosensor. 20-second oscillations co-exist with longer and shorter periods, with slower oscillations becoming more prevalent as furrowing progresses. Faster- ingressing segments exhibited speed oscillations with both higher amplitude, lower frequency, and higher correlation of oscillation parameters with neighboring segments when compared to slower ingressing parts of the same ring. Both speed oscillation frequency and amplitude are sensitive to perturbations of RhoA or myosin activity. Taken together, our results support the hypothesis that contractility within local, semi-autonomous units dictates the overall dynamics of the cytokinetic rings. Our findings also provide experimental evidence for the proposed influence of material properties and force generation on the biochemical feedback regulation of non-muscle actomyosin contractility.

B175/P1169

Distortion of meiotic midbody retains translated proteins in mouse oocyte

G. Jung[1], S. Park[2], A. Skop[2], K. Schindler[1]; [1]Department of Genetics, Rutgers University, Piscataway, NJ, [2]Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI.

Oocyte meiosis leads to the formation of a healthy egg that supports the foundation of new life. Cytokinesis of meiosis I is critical for egg quality because it is necessary for physical separation of progeny cells and for preventing polyploidy. Moreover, cytokinesis leads to the formation of the midbody (MB), a transient, membrane-less organelle with translationally active ribonucleoprotein (RNP) granule qualities. Mitotic cells release MBs via symmetric abscission but can then uptake MBs through phagocytosis. MB uptake correlates with cell fate determination, stemness, and tumorigenicity, consistent with MBs being RNP granules. However, mammalian oocytes undergo asymmetric divisions during meiosis, suggestive of asymmetric cytokinesis and abscission. Whether oocytes have MBs and, if so, what function they have is not known. Because MBs can influence somatic cells, we hypothesized that inheriting a meiotic MB is critical to sustain pre-implantation embryonic development and to acquire totipotency after fertilization. To address these gaps, we first identified MB structures in mouse oocytes based on the localization of key structural proteins MKLP2 (arms), PRC1 (core), and MKLP1 (ring) described in mitotic cells. We found a unique distortion to the midzone spindle and MB ring not observed in mitosis. To determine the cause of the ring distortion, we tested the hypothesis that microtubules exert uneven forces during cytokinesis. By high-resolution live-cell imaging, we found that the abundance of microtubules on the egg side is greater than on the polar body, indicating microtubule asymmetry drives the distortion. We next asked if meiotic MBs are also RNP granules. By conducting fluorescence *in situ* hybridization and immunocytochemistry, we found that polyadenylated RNAs and ribosomal proteins are enriched at the MB ring, in line with the localized translation we detected in this region. Finally, we asked if the distortion-derived cap acts as a barrier that prevents nascent proteins from leaving the egg and escaping into the polar body. We evaluated translation signal localization after disrupting the cap with nocodazole. Unlike control eggs which retained translation in the egg, eggs with no cap showed translation signal leakage into the polar body. To expand on the potential role of MBs in ensuring developmental competence, we are parthenogenetically activating eggs with and without cap disruption and comparing the development efficiency to the blastocyst stage. Together, these data show asymmetries at the subcellular level distort the MB in mouse oocytes and highlight the potential role the distortion has in guaranteeing egg quality and developmental competence.

B176/P1170

Building the Cytokinetic Contractile Ring in an Early Embryo: Composition and Organization of Initiating Myosin II Clusters

G. Reyes[1,2], Q. W. McKim[3,2], H. Y. Herzon[3,2], K. Herrera[3,2], N. T. Lo[3,2], C. B. Shuster[1,2], J. H. Henson[3,2]; [1]Biology, New Mexico State University, Las Cruces, NM, [2]Friday Harbor Labs, University of Washington, Friday Harbor, WA, [3]Biology, Dickinson College, Carlisle, PA.

Significant questions remain regarding the assembly and structural progression of the cytokinetic contractile ring (CR) in animal cells. We have investigated the nanoarchitecture and dynamics of the CR in early sea urchin embryos using super resolution light microscopy (3D-SIM, STED and iPALM) and platinum replica TEM. The sea urchin experimental system offers the distinct advantage of being able to examine the CR in both first division whole embryos and isolated cortices. To date our studies have

indicated that the CR initiates as a broad stripe of clusters containing myosin II, actin, septin and anillin, which then congress over time into a narrower, more linearized and contractile array within the cleavage furrow. Our present work indicates that MKLP1, the kinesin-like microtubule motor component of the Rho-activating Centralspindlin complex, colocalizes with these initiating clusters and the nascent CR in whole embryos and isolated cortices, although this colocalization diminishes with CR maturation. Conversely, the actin cross-linker alpha-actinin colocalizes with late stage CRs but not with the initiating clusters. This is consistent with our previous results showing that these clusters assemble in the absence of actin filaments, and using para-aminoblebbistatin (PAB) - a more soluble and photostable derivative of blebbistatin - we have now demonstrated that clusters can also assemble in the presence of myosin II ATPase inhibition. Embryos treated with PAB undergo nuclear divisions and can exhibit anaphase B-like elongation and early telophase shallow furrowing but become binucleate and eventually progress through the 2-4 cell nuclear division. Embryos retain cytokinesis competence in the presence of PAB as shown by the cell division displayed by embryos washed out of the drug or exposed to photo-inactivation. Collectively these results highlight the importance of the initiating myosin II clusters in setting up the organization of the embryo's CR, in the targeting of Rho-activation via Centralspindlin, and the absence of a requirement for actin filaments or myosin II ATPase function in CR assembly.

B177/P1171

Inhibition of polymeric SUMO-2/3 chain signals induces the formation of chromatin bridges

X. Zhang[1], T. Chen[1], R. Zhang[2], A. Noor[1]; [1]Biology, SUNY Buffalo State College, Buffalo, NY, [2]Troy High School, Troy, MI.

Polymeric SUMO-2/3 chain modification of various protein targets acts as the signals for recruiting the SUMO-targeted ubiquitin E3 ligases, including RNF4 and RNF111, leading to their ubiquitination and proteasomal degradation in mammalian cells. However, the functional significance of poly-SUMO-2/3 chain modification is not well understood. To address this question, we specifically blocked the poly-SUMO-2/3 chain signals by overexpressing a GFP-tagged RNF4 N-terminal fragment containing four tandem SUMO-interacting motifs (GFP-SIMs) with a high-affinity binding to poly-SUMO-2/3 chain signals. We found that the GFP-SIMs proteins are largely associated with poly-SUMO-2/3 chain modified proteins at PML nuclear bodies in human HeLa cells, especially after a treatment with arsenic trioxide known to stimulate poly-SUMO-2/3 chain modification of PML proteins. In addition, the transient expression of GFP-SIMs induced the formation of chromatin bridges and caused an accumulation of poly-SUMO-2/3 chain modified proteins at the midbody in cells with chromatin bridges. Consistent with the defect triggered by blocking the poly-SUMO-2/3 chain signals, global inhibition of SUMOylation by treating cells with ML-792, a specific inhibitor of the SUMO-activating E1 enzyme, increases the DNA damage foci during interphase and the chromatin bridges during mitosis and cytokinesis. Therefore, poly-SUMO-2/3 chain signals are critical for ensuring accurate chromosome segregation and maintaining genome stability.

B178/P1172

Structure-function analysis of RGA-3/4, a cytokinetic Rho GAP

S. Kim, W. M. Bement; University of Wisconsin, Madison, WI.

Traditionally, cytokinesis has been viewed as essentially linear, with Rho being activated in the beginning of the process to drive contractile ring formation, and then inactivated once cytokinesis is complete.

However, propagating waves of Rho activation and inactivation and complementary waves of F-actin assembly and disassembly - referred to as cortical excitability - are focused at the equatorial cortex during cytokinesis in amphibian and echinoderm embryos, indicating that Rho is undergoing continual flux through the GTPase cycle. Cytokinetic cortical excitability is proposed to result from Ect2-dependent Rho activation coupled with delayed F-actin-dependent Rho inactivation. RGA-3/4, a GTPase activating protein (GAP) for RhoA, is an excellent candidate as a link between F-actin and Rho inactivation during cytokinetic cortical excitability. RGA-3/4 is required for cytokinesis and gets recruited to the equatorial cortex prior to the onset of furrowing. It colocalizes with F-actin in the cytokinetic apparatus and depends on F-actin for its cortical localization. In spite of its importance in cytokinesis and implication in cytokinetic cortical excitability, the mechanism to how RGA-3/4 interacts with F-actin and how it is being regulated is completely unknown. To further our knowledge about RGA-3/4, we have performed structure and function analysis. RGA-3/4, when expressed alone in *Xenopus* oocytes, localizes to and reorganizes the cortical actin network into interlocking cables. This activity is independent of its GAP activity, but requires the amino terminal portion of RGA-3/4 as well as the last 20 aas of the GAP domain. When co-expressed with Ect2 in *Xenopus* oocytes, RGA-3/4 induces cortical excitability. In contrast to actin reorganization, this activity requires GAP activity as well as the portions of the protein needed for actin reorganization. Further, induction of cortical excitability also requires a minimum of ~120 aas of the region immediately downstream of the GAP domain (aa234-350). To better understand how actin reorganization and cortical excitability are induced by RGA-3/4, I am in the process of studying RGA-3/4-F-actin interaction in vitro.

B179/P1173

Early zygotic gene product Dunk interacts with anillin to regulate Myosin II during *Drosophila* cleavage

J. Chen, B. He; Biological Sciences, Dartmouth College, Hanover, NH.

Drosophila cellularization is a special form of cytokinesis that partition the peripherally localized syncytial nuclei into individual cells. Similar to typical animal cytokinesis, an early event in cellularization is the recruitment of non-muscle myosin II (“myosin”) to the cleavage furrows, where myosin forms an interconnected basal array before reorganizing into individual basal cytokinetic rings. The initial recruitment and organization of basal myosin are regulated by *dunk*, a cellularization-specific gene, but the mechanism is unclear. In a genome-wide yeast two-hybrid screen, we identified anillin (Scraps in *Drosophila*), a conserved scaffolding protein in cytokinesis, as the primary binding partner of Dunk. Anillin has been previously shown to regulate the assembly and function of the basal cytokinetic rings during mid- to late cellularization, but its role and regulation in early cellularization are unexplored. We show that anillin colocalizes with myosin as early as at the onset of cellularization, when anillin and myosin localize together in discrete puncta at the apical cortex and co-move towards the newly formed furrow, facilitating the formation of basal myosin array. Loss of anillin results in myosin localization defects at the basal array that closely resembles the myosin phenotype in *dunk* mutant embryos. Furthermore, we show that anillin colocalizes with Dunk at the cleavage furrows during the early cellularization. Loss of Dunk does not prevent the recruitment of anillin to the cleavage furrows but affects the spatial distribution of anillin. Finally, we present evidence that Dunk functionally interacts with anillin during cellularization in regulating the integrity of the myosin array. Based on these results, we propose that Dunk regulates myosin recruitment and organization during early cellularization by binding and regulating anillin. Our work demonstrates a previously unappreciated role for anillin in regulating cortical myosin dynamics in early cellularization. The interaction between a cellularization-

specific protein and the conserved cytokinetic machinery may shed light on how the latter can be adapted in evolution to achieve specific, non-canonical forms of cytokinesis.

B180/P1174

A checkpoint system enforces the staging of secretory events in cytokinesis

E. L. Weiss, J. L. Brace, T. A. Volpe; Molecular Biosciences, Northwestern University, Evanston, IL.

Checkpoint pathways create dependency relationships in temporally staged events of cell division. We have defined such a system in budding yeast cytokinesis, where cytokinetic actomyosin ring contraction, synthesis of an extracellular septum, and abscission are quickly followed by a sharp burst of hydrolytic enzyme secretion that destroys the septum to allow separation of mother and daughter cells. While these enzymes are produced in early cytokinesis, septum destruction never begins before abscission is complete. Notably, we find that secretion of septum destruction enzymes is blocked when cytokinesis is impaired. This strong delay of late cytokinetic secretion requires Fir1, an intrinsically disordered protein that contains numerous short conserved protein intearaction motifs. In the absence of Fir1 treatments that perturb early septation and actomyosin ring function cause complete and lethal failure of cytokinesis, a far more severe phenotype. Fir1, localizes to the cytokinesis site and is quickly proteolyzed upon septum completion, but is profoundly stabilized when septation is aberrant. Fir1 appears to block separation enzyme secretion in part by inhibiting the NDR/LATS kinase Cbk1, a key component of a "hippo" signaling pathway that induces mother-daughter separation. We find that disruption of cytokinesis triggers activation of a MAPK pathway that likely potentiates Fir1-dependent secretion block. Intriguingly, we also find that only daughter cells are capable of secreting post-abscission septum destruction enzymes in mother-daughter pairs that uniformly express the proteins. Thus, these cells protect the phased process of cytokinesis with exacting spatial regulation and checkpoint-mediated temporal staging.

B181/P1175

The impact of polarized adhesion on the efficiency of epithelial cytokinesis

M. Gonçalves[1,2], C. Ramos[1], M. Osswald[1], C. Lopes[1], C. Sunkel[3,2], M. Eurico[1]; [1]Epithelial Polarity and Cell Division Lab, IBMC/i3S - Instituto De Investigação E Inovação Em Saúde, Porto, PORTUGAL, [2]Doctoral Program in Molecular and Cell Biology (MCBiology), ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Porto, PORTUGAL, [3]Cell Division and Genomic Stability Lab, IBMC/i3S - Instituto De Investigação E Inovação Em Saúde, Porto, PORTUGAL.

Epithelial tissues are a critical barrier in multicellular organisms. Its proper function relies on the distribution of adhesion complexes along an apical-basal axis, which ensures epithelial integrity. During development and throughout adult life, epithelial tissues undergo mitotic proliferation, which demands coordination of adhesion with the dramatic shape and cytoskeleton changes that are necessary to allow daughter cell separation. However, it is still unclear how the adhesion complexes impact the robustness of cell division in this polarized and multicellular environment. To tackle this question, we performed an *in vivo* RNAi screen in the *Drosophila* follicular epithelium to determine which cell adhesion molecules impact epithelial cytokinesis. One promising hit was the extracellular matrix receptor Dystroglycan (Dg), which we found to promote cytokinesis robustness. Live imaging uncovered the spatial redistribution of Dg-GFP, which is enriched in the basal side of ingressing cleavage furrow since the beginning of ring constriction. This was also observed for Dystrophin (Dys), the intercellular linker by which Dg interacts

with the actin cytoskeleton. We are now characterizing how the Dg-Dys complex ensures epithelial cytokinesis robustness. Current data shows that the Dg-Dys complex promotes the constriction of the actomyosin ring and also facilitates abscission. Altogether, these results pave the way to understand how cytokinesis is coupled with adhesion in order to ensure the correct genetic ploidy of epithelial cells during growth and development.

B182/P1176

Myosin XI motors participate in a cortical cytoskeletal assembly to orient plant cytokinesis

C. Huang[1], F. Peng[2], Y. Lee[1], B. Liu[1]; [1]University of California, Davis, Davis, CA, [2]University of Pennsylvania, Philadelphia, PA.

Plant cells are immobile, surrounded by the cell wall such that anisotropic growth is brought about by the production of new cells in formative orientations. Plant growth and form are dependent on oriented cell divisions that employ the cortical microtubule array, preprophase band (PPB), to predict cell plate placement. It has been elusive how this transient cytoskeletal array imprints the spatial information at the cell cortex for later stages of mitotic cell division. We discovered in *Arabidopsis thaliana* that the Myosin XI motor MYA1 localized to the cortical division site (CDS), previously occupied by the PPB. Furthermore, the CDS-localized kinesin motor, POK1, assembles with MYA1 to form into a ring of macromolecular assemblies that guide cell plate deposition. This finding uncovered the mechanism of how the two cytoskeletal networks interact to regulate division plane orientation during vegetative growth in flowering plants.

B183/P1177

Cdc42 GTPase activating proteins Rga4 and Rga6 coordinate septum synthesis and membrane trafficking at the division plane during cytokinesis

B. F. Campbell[1], B. S. Hercyk[2], A. R. Williams[2], E. San Miguel[2], H. G. Young[2], M. E. Das[1]; [1]Biology, Boston College, Chestnut Hill, MA, [2]Biochemistry & Cellular and Molecular Biology, The University of Tennessee - Knoxville, Knoxville, TN.

Fission yeast cytokinesis is driven by simultaneous septum synthesis, membrane furrowing, and actomyosin ring constriction. The septum consists of a primary septum flanked by secondary septa. First, delivery of the glucan synthase Bgs1 and membrane vesicles initiate primary septum synthesis and furrowing. Next, the glucan synthase Bgs4 is delivered for secondary septum formation. It is unclear how septum synthesis is coordinated with membrane furrowing. The Rho GTPase Cdc42 promotes delivery of Bgs1 but not Bgs4. We find that after primary septum initiation, Cdc42 inactivators Rga4 and Rga6 localize to the division site. In rga4Δrga6Δ mutants Cdc42 activity is enhanced during late cytokinesis and cells take longer to separate. Electron micrographs of the division site in these mutants exhibit malformed septum with excess primary septum and irregular membrane structures. These mutants have a larger division plane with enhanced Bgs1 delivery but fail to enhance accumulation of Bgs4 and several exocytic proteins. Additionally, these mutants show endocytic defects at the division site. This suggests that Cdc42 regulates primary septum formation and only certain membrane trafficking events. As cytokinesis progresses, Rga4 and Rga6 localize to the division site to decrease Cdc42 activity to allow coupling of Cdc42-independent membrane trafficking events with septum formation for proper septum morphology.

B184/P1178

Spatiotemporal dynamics of cell plate development during plant cytokinesis

R. Sinclair[1], M. Wang[1], Z. Jawaid[1], S. Wong[1], D. Cox[1], J. Heddleston[2], E. Wait[2], J. Aaron[2], T. Wilkop[1], **G. Drakakaki**[1]; [1]University of California Davis, Davis, CA, [2]Janelia Research Campus, Ashburn, VA

Cytokinesis in plants is fundamentally different from that in animals and fungi. In plant cells, a cell plate forms through the fusion of cytokinetic vesicles and ultimately develops into the new cell wall, partitioning the cytoplasm of the dividing cell. The formation of the cell plate entails multiple stages that involve highly orchestrated vesicle accumulation, fusion and membrane maturation, which occur concurrently with the timely deposition of polysaccharides such as callose, cellulose and cross-linking glycans. The polysaccharide callose is thought to play a vital role in stabilizing the cell plate during its expansion, however many fundamental questions remain unanswered. Overcoming genetic lethality, we used the pharmacological probe endosidin 7 to specifically inhibit callose deposition during cytokinesis. Using 4D imaging, employing lattice light sheet microscopy, we interrogated the temporal dynamics at the cell plate in the presence and absence of callose. We approximated cell plate sub-structures with testable shapes and adopted the Helfrich-free energy model for membranes, including a stabilizing and spreading force, to understand the transition from a vesicular network to a fenestrated sheet and mature cell plate. Quantitative analysis along with biophysical modeling enabled the dissection of stage transition at the cell plate and identified key transition points at which different components such as callose are critical for cell plate development. Further isolation of cytokinetic vesicles followed by multi-platform analysis identified cargoes targeted to cell plate. In total, the combination of the described approaches provided insights cell plate formation during plant cell division.

Centrosome Assembly and Functions

B185/P1179

Mammalian Spermatocytes have the Capacity to Segregate Chromosomes without Centriole Duplication and Centrosome Separation

M. W. Skinner[1,2], C. Simington[1], P. López-Jiménez[3], K. Baran[1], J. Xu[1], J. Page[3], R. Gómez[3], A. Holland[4], P. Jordan[2,1]; [1]Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, [2]Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences, Bethesda, MD, [3]Autonomous University of Madrid, Madrid, SPAIN, [4]Johns Hopkins University School of Medicine, Baltimore, MD.

Centrosomes are the canonical microtubule organizing centers (MTOCs) of most mammalian cells, including spermatocytes. Centrosomes are comprised of a centriole pair within a structurally ordered and dynamic pericentriolar matrix. To segregate homologs during meiosis I and sister chromatids during meiosis II, centrioles undergo two rounds of duplication and centrosomes mature and separate twice. The first centriole duplication event occurs during leptonema at the same time meiotic double-strand breaks are formed. Centrosomes mature during latter stages of meiotic prophase, then separate at diakinesis to opposite sides of the nucleus. When the nuclear envelope breaks down, separated centrosomes serve as bipolar nucleation sites for the microtubules responsible for segregation of homologous chromosomes during meiosis I. At interkinesis, centrioles are duplicated, and centrosomes are separated again to facilitate segregation of sister chromatids during meiosis II. Using mouse mutant models and chemical inhibition, we have blocked centriole duplication and centrosome separation

during spermatogenesis and discovered that spermatocytes adopt a backup mechanism for chromosome segregation. Strikingly, this mechanism is unique from the acentriolar MTOCs that form bipolar spindles in oocytes, which require canonical centrosome components, including gamma-tubulin, pericentrin, and CEP192. From our in-depth analysis, we have discovered microtubule-associated proteins involved in the acentrosomal microtubule organization that are capable of mediating chromosome segregation during spermatogenesis.

B186/P1180

Identifying New Regulators of Centriole Biogenesis

C. Peneda, M. Bettencourt-Dias; Instituto Gulbenkian De Ciência, Oeiras, Lisbon, PORTUGAL.

Centriole biogenesis is a highly regulated process and has been widely studied. Several key proteins and molecular mechanisms involved in centriole number and length control have been identified. However, how these processes are regulated is still not fully understood. Therefore, the aim of our project was to identify new regulators of centriole biogenesis. In our work, we used light microscopy, super resolution and electron microscopy techniques and were able to identify LZTS2 as a new regulator of centriole elongation. Our results will thus help to unravel new regulatory pathways governing centriole elongation.

B187/P1181

E3 ubiquitin ligase, FBXW7 targets centriole duplication protein, STIL for degradation

U. Anand, B. Badarudeen, A. Muliyil, M. Ebrahim, A. Asokan, R. Khurup, T. K. Manna; IISER TVM, Thiruvanathapuram, INDIA.

Centrosomes are the main microtubule organizing centers in animal cells. During the cell cycle, centrosomes duplicate only once during S phase to ensure that at mitotic onset, a cell carries two centrosomes, that are required for serving as poles of the mitotic spindle. Initiation of new centriole biogenesis involves recruitment of a ring-like oligomeric structure consisting of CEP152 and its associated proteins onto the proximal end of the mother centriole, which follows recruitment and activation of polo-like kinase 4 (Plk4). Plk4 kinase seeds the new centriole by phosphorylating pro-centriole protein STIL (SCL/TAL1 interrupting locus) and facilitating formation of the cartwheel-like template by recruiting SAS-6. Elevated expression of several of these factors, notably STIL, SAS-6 and Plk4 induces centriole over-duplication and multipolar defects. Mechanisms how the levels of centriole duplication factors are maintained are poorly understood. Here we find that E3-ubiquitin ligase. SCF-FBXW7 with its substrate targeting subunit FBXW7 regulates level of STIL in cultured human cells. siRNA-mediated depletion of FBXW7 increased the level of STIL, more prominently during the G1/S phase, the time when new centriole formation begins. FBXW7 over-expression results in down-regulation of STIL level and further, it is rescued, when the substrate-binding WD40 of FBXW7 was deleted. Concordant with these findings, we also found that localization STIL at the centrioles is reduced, when the ligase is overexpressed. Biochemical results showed that FBXW7 interacts with and ubiquitinates STIL via its WD40 domain. Pharmacological inhibition of PLK4, which phosphorylates STIL, suppressed FBXW7-mediated STIL degradation suggesting its role in this process. Inhibition of Plk4 kinase activity also compromised the binding between FBXW7 and STIL, suggesting a role of the kinase in mediating STIL-FBXW7 interaction. We also found that STAN domain of STIL, which binds to SAS-6 to form centriole

cartwheel, is involved in FBXW7-mediated degradation and interaction with FBXW7. Results suggest that STIL STAN domain degradation by SCF-FBXW7 could be involved in controlling new centriole assembly.

B188/P1182

MPS1-mediated potentiation of Aurora A activity causes spindle orientation defects in the *Drosophila* follicular epithelium

N. Leça[1,2], M. Osswald[3], C. E. Sunkel[1,2], E. Morais-de-Sá[3], C. Conde[1]; [1]Cell Division and Genomic Stability group, i3S - Institute for Research and Innovation in Health, Porto, PORTUGAL, [2]ICBAS - Institute of Biomedical Sciences Abel Salazar, Universidade do Porto, Porto, PORTUGAL, [3]Epithelial Polarity and Cell Division group, i3S - Institute for Research and Innovation in Health, Porto, PORTUGAL.

Proper orientation of the mitotic spindle during cell division is critical for determination of the progeny fate and for accurate tissue architecture. Here, we show that overexpression of the master mitotic checkpoint kinase MPS1 in *Drosophila* follicular epithelium cells leads to massive defects in spindle orientation and to the formation of a multilayered tissue. Live cell imaging and immunofluorescence analysis of *Drosophila* S2 cells revealed that increased activity of centrosome-tethered MPS1 is sufficient to induce pronounced rotation of the mitotic spindle through mis-regulation of the canonical Mud-Dynein/Dynactin orientation machinery. Correct distribution of Mud-Dynein/Dynactin at the cell cortex is established by tightly-regulated phosphorylation of Pins by Aurora A. We found that centrosomal activity of Aurora A is significantly incremented following MPS1 overexpression and *in vitro* kinase assays revealed that MPS1 activity directly potentiates Aurora A T-loop auto-phosphorylation. Notably, expression of constitutively active Aurora A or of a phosphomimetic version of Pins recapitulates the abnormal spindle rotation phenotype. Importantly, depletion or chemical inhibition of Aurora A efficiently prevent the rotation of the mitotic spindle in cells overexpressing MPS1. Furthermore, follicular epithelium multilayering caused by MPS1 overexpression is significantly reduced in *Drosophila* heterozygous mutants of Aurora A. We propose that, when overexpressed, a cytosolic pool of MPS1 is able to reach the centrosomes and potentiate Aurora A auto-activation. This likely causes unbalanced hyperphosphorylation of Pins, thus precluding correct localization of Mud-Dynein/Dynactin at the cell cortex and consequently resulting in the ectopic cortical accumulation of astral microtubule pulling forces that drive spindle rotation. Our results unravel a novel and unanticipated cross-talk between the mitotic checkpoint and the spindle orientation signaling pathways.

B189/P1183

Investigating Centrosome Inactivation during Cellular Differentiation

R. K. Ng, J. Magescas, J. L. Feldman; Biology Department, Stanford University, Stanford, CA.

Centrosomes are membrane-less organelles composed of a pair of microtubule-based centrioles surrounded by proteins known collectively as the pericentriolar material (PCM). In animal cells, centrosomes act as microtubule-organizing centers (MTOCs) by nucleating and localizing microtubules to form the mitotic spindle. Centrosomes become “activated” as MTOCs through the mitotic kinase-dependent accumulation of PCM proteins around centrioles. In telophase, centrosomes become “inactivated” through the loss of MTOC activity by the disassembly of PCM proteins and microtubules through phosphatase activity. Centrosome inactivation is a feature of all cycling animal cells, a hallmark of cellular differentiation, and centrosome hyperactivity has been associated with many types of cancers. However, the mechanisms underlying centrosome inactivation and the function of centrosome

inactivation during cellular differentiation is unknown. To understand the normal function of centrosome inactivation during differentiation, we are using two approaches to activate centrosomes in *C. elegans* differentiated cells and are assessing the associated cellular and tissue-level phenotypes. First, we inhibited PP2A, a phosphatase implicated in centrosome inactivation, specifically in embryonic intestinal cells which resulted in aneuploidy, mitotic arrest, and centrosome hyperactivity. In addition, intestinal cells no longer polarized, preventing proper intestinal connectivity with the rest of the digestive tract and resulting in larval lethality. As a second approach, we developed a nanobody-based targeting strategy to drive the active mitotic kinase PLK-1 to the centrosome. Expression of this CAP-Trap (Constitutively-Active PLK-1-Trap) in embryonic intestinal cells reactivated interphase centrosomes as demonstrated by the presence of growing microtubule plus-ends emanating from the centrosome, and we are currently assessing the impact of this reactivation. Expression of CAP-Trap in differentiated phasmid neurons caused defects in neuronal morphology and the perdurance of centriole proteins. Together, these data indicate that the regulation of PP2A and PLK-1 activity are involved in centrosome inactivation in differentiating intestinal cells and neurons *in vivo*, and future studies will determine if centrosome inactivation is required for proper intestinal and neuronal differentiation and function.

B190/P1184

Centrosomal localization of Plk4 during centriole duplication and amplification

C. Sullenberger, P. Avazpour, D. Luvsanjav, D. Kong, J. Loncarek; National Cancer Institute at Frederick, Frederick, MD.

Background: Dividing somatic cells restrict the number of centrosomes to two per cell, which is critically important for the formation of bipolar mitotic spindles and accurate chromosome segregation, in addition to other centrosome-related cellular events. Centrosome number relies on a precise duplication pattern of two resident mother centrioles, which duplicate only once at the beginning of S phase by each initiating only one new centriole (procentriole) in their vicinity. Procentrioles initiate when polo-like kinase 4 (Plk4) binds to and phosphorylates STIL, which, in turn promotes its association with SAS-6, and the formation of a Plk4/STIL/SAS-6 complex, as a basis for procentriole assembly. **Aim:** To we dissect the dynamics and spatial organization of centrosomal Plk4 during procentriole initiation during centriole duplication and amplification with improved resolution. **Methods:** We used human cultured cells, some inducibly expressing Plk4, and examined Plk4 centrosomal dynamics by employing super resolution microscopy approaches that allow spatial resolution of at least 25 nm. Additionally, biochemical methods were used to investigate cytosolic levels of Plk4, and a specific Plk4 inhibitor centrinone was used to modulate Plk4 activity. **Results:** Super resolution imaging revealed a detailed Plk4 pattern in G1 and early S phase cells, which further details a previously observed ring-to dot Plk4 centrosomal pattern during procentriole initiation. Our findings raise the question of whether Plk4 focusing proceeds or follows procentriole initiation. In addition, we show that overaccumulation of active and inactive Plk4 on centrosomes follows different centrosomal dynamics and we speculate possible reasons for this observation. Our study provides new insights into the behavior of elusive Plk4, which is critically important for centrosome homeostasis and human health.

B191/P1185

Characterization of Orb2-dependent microcephaly in *Drosophila*

T. Hailstock, D. A. Lerit; Cell Biology, Emory University, Atlanta, GA.

Neural stem cells (NSCs) maintain a balance between self-renewal versus neurogenesis by asymmetric cell division, where dysregulation of this homeostatic state leads to neurodevelopmental disorders, such as microcephaly. NSC centrosomes play an important role in the regulation of this process by aligning the mitotic spindle along an invariant apical-basal axis to generate a self-renewing stem cell and a daughter cell. We recently identified the RNA-binding protein *Drosophila* Orb2 as a putative regulator of centrosome-enriched mRNAs. As an ortholog of human cytoplasmic polyadenylation element binding (CPEB) proteins, its implications in mRNA localization and translational control suggest a role for Orb2 in NSC spindle organization and neuronal specification. Preliminary work in our lab reveals Orb2 subcellular localization to the NSC cytoplasm, a broader cellular localization within the optic lobe region of larval brains at the neuroepithelial ridge and axons, and colocalization with glia-specific markers such as anti-Repo. These findings support the hypothesized role of Orb2 in supporting asymmetric centrosome maturation in NSCs while apparent enrichment in glia cells suggests expression outside of NSCs. Due to an apparent developmental delay, we defined a protocol to systematically compare brain volumes in age-matched *orb2* null animals relative to controls. These studies indicated loss of *orb2* results in microcephaly, suggesting an underlying requirement for Orb2 in regulating brain size. However, the specific contribution and mechanism of Orb2 in regulating neurodevelopment remains unknown. To examine our central hypothesis that glia cell dysfunction contributes to *orb2*-dependent microcephaly, current work to deplete *orb2* via RNAi in defined cell types including glia cells and/or neuroepithelial precursors is underway. Future directions include determination of developmental stage-specific requirements for Orb2. Advanced characterization of reproductive and behavioral variance observed in *orb2* mutants is also an exciting area of interest. Together with the utility of our colorimetric age-matched regime in *Drosophila*, resulting findings will provide insight into the neurodevelopmental basis of microcephaly.

B192/P1186

Architectural basis for cylindrical self-assembly governing Plk4-mediated centriole duplication in humans

J. Ahn, L. Zhang, H. Ravishankar, K. Kirsch, Y. Zeng, J. Park, **K. Lee**; Cancer Innovation Laboratory, National Cancer Institute, National Institutes of Health, BETHESDA, MD.

As the main microtubule-organizing center in animal cells, the centrosome is critically required for normal cell division and proliferation. How the pericentriolar material (PCM) surrounding the barrel-shaped centriole is organized to form a functional centrosome remains elusive. We found that two long coiled-coil proteins, Cep63 and Cep152, interact with each other to form a 2:2 heterotetrameric complex that can self-assemble into a nanoscale cylindrical architecture *in vitro*, and whose organizational dimension and arrangement pattern closely mirror those of its respective endogenous proteins around the centriole. Additionally, we performed size-exclusion chromatography, sedimentation equilibrium ultracentrifugation, and interferometric scattering mass spectrometry and showed that the heterotetrameric building block generates octameric and hexadecameric complexes in a concentration-dependent manner, suggesting that the cylindrical self-assembly is formed through stepwise processes. By using MINFLUX nanoscopy, which offers low-nanometer-scale localization

precision in a three-dimensional space, we further showed that mutants defective in forming the Cep63•Cep152 heterotetramer exhibited crippled pericentriolar Cep152 organization, consequently failing to promote polo-like kinase 4 (Plk4)'s dynamic relocalization from around the centriole to the future procentriole assembly site as well as Plk4-mediated centriole duplication. Remarkably, the entire self-assembly process could be driven by two short, uncharacterized regions (which we named "self-assembly modules") in Cep63 and Cep152 capable of cophase-separating and generating cylindrical self-assemblies *in vitro*. Fluorescence recovery after photobleaching revealed that the self-assembled architecture is highly dynamic, undergoing internal rearrangement within the assembly while exchanging its components with those in the surroundings. Dynamic turnover of pericentriolar Cep63 and Cep152 has also been observed in human centrosomes. Taken together, given the evolutionarily conserved organization of PCM, this work could serve as a paradigm for investigating the structure and function of centrosomal scaffolds in other organisms, while offering a new direction for probing organizational defects in PCM-related human diseases.

B193/P1187

The *Caenorhabditis elegans* Centrosome is Surrounded by a Membrane Reticulum, the Centriculum, that Affects Centrosome Size and Function

S. Drey[1], R. Maheshwari[1], M. Rahman[1], M. Onyundo[1], O. Cohen-Fix[1], G. Fabig[2], T. Müller-Reichert[2]; [1]National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH, Bethesda, MD, [2]Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Dresden, GERMANY.

Centrosomes serve as the primary microtubule organizing centers during animal cell division. At the core of each centrosome is a pair of centrioles that recruits the pericentriolar material (PCM), which anchors and nucleates microtubules. Despite being considered membraneless organelles, the centrosomes of many cell types, including human cells, are surrounded by endoplasmic reticulum-derived membranes of unknown structure and function. We sought to characterize the centrosome-associated membrane and its possible regulation of centrosomes and microtubules. Using volume electron microscopy, we show that the *C. elegans* centrosome is surrounded by a membrane reticulum that we call the centriculum, for centrosome-associated membrane reticulum. Our current investigation reveals that microtubules collide with the centriculum, indicating the centriculum may serve as a microtubule "filter" by limiting the number of microtubules that fully elongate. Furthermore, we see that the centriculum fuses with the nuclear envelope as mitosis progresses, pointing toward a possible role in anchoring the centrosomes to the nuclear envelope and transducing the pulling forces of microtubules to the nuclear membranes to promote nuclear envelope breakdown. In addition to its possible functions in centrosome anchoring and force transduction, the centriculum may also affect centrosome size. The centriculum can be enlarged by disrupting ER membrane junction formation or increasing microtubule stability. These conditions result in expansion of the PCM and increased microtubules in the vicinity of the centrosome, suggesting that the centriculum affects centrosome structure and function. In converse, the centriculum collapses around the centrosome and condenses the PCM after induction of microtubule catastrophe. In conclusion, we show that centrosomes are surrounded by a reticular network of ER-derived membrane which may play roles in centrosome anchoring to the nuclear envelope, force transduction of the mitotic spindle to the nuclear membranes, and regulation of centrosome size and function.

B194/P1188

Pericentrin-Like-Protein is a Kinesin-1 InteractorThat Drives Centriole Motility.

M. R. Hannaford, R. Liu, N. Billington, C. J. Fagerstrom, B. J. Galletta, J. R. Sellers, N. M. Rusan, N. M. Rusan; NHLBI, NIH, Bethesda, MD.

Centrosomes are one of the primary organizers of the microtubule (MT) cytoskeleton within cells. They comprise a pair of centrioles which act as a platform for a matrix of proteins termed the pericentriolar material (PCM). PCM is required for the nucleation and anchoring of MTs. Through MT organization, centrosomes are important for the mitotic spindle, cilia and flagella. To fulfill these varied functions, centrosomes must achieve proper positioning within the cell. Therefore, centrosomes must be motile. Typically, centrosomes are thought to position themselves via motor proteins pushing or pulling on the MTs anchored at the centrosome. However, in some cases, centrioles are motile without the presence of PCM or anchored microtubules, we refer to these as inactive centrioles. In this work we investigated how inactive centrioles are able to move through interphase cells. We reveal that these centrioles are cargo, being transported along the interphase microtubule array. We show that Kinesin-1 localizes to the centriole and is important for motility via an interaction between the Kinesin cargo binding tail and Pericentrin Like Protein (PLP); a coiled-coil rich protein which localizes to the outer centriole. Reverse yeast-2-hybrid screening allowed us to identify specific mutations which block the interaction between Kinesin-1 and PLP, live cell imaging then demonstrated that these mutations block interphase centriole motility. Reconstitution of this protein complex *in vitro* revealed that interaction with PLP is dependent upon the relief of Kinesin-1 autoinhibition. In this work we propose the first detailed mechanism of how centrioles can move independently of their role as an MTOC. We will further discuss our recent in vitro and in vivo efforts to dissect the mechanism of Kinesin-1 activation by kinesin activators, which promote PLP binding and efficient centriole motility.

B195/P1189

A complex pattern of ZYG-1-mediated phosphorylation of SAS-5 controls centriole assembly

P. Sankaralingam[1], S. Wang[2], K. F. O'Connell[1]; [1]National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, [2]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, MD.

Centrioles are microtubule-based cylindrical structures which act as a platform to concentrate microtubule nucleating machinery, thus forming the centrosome, the cell's major Microtubule-Organizing Center (MTOC). In dividing cells, centriole duplication is a tightly linked to the cell cycle and happens once every S phase. Mis-regulation of the process is linked several pathological conditions such as cancer and microcephaly. In *C. elegans*, centrioles are built by the sequential addition of several proteins - SAS-7, SPD-2, ZYG-1, SAS-5 & SAS-6 and SAS-4. While, the homologs and the general theme of assembly are highly conserved, the molecular mechanism of centriole biogenesis is not completely understood. In particular, how the kinase activity of ZYG-1 regulates the entire process is unknown. We addressed this question using *in vitro* refolded recombinant centriolar proteins. We found that ZYG-1 physically interacts with and preferentially phosphorylates SAS-5 *in vitro*. Using mass spectrometry, we identified 14 Ser/Thr residues that were phosphorylated *in vitro*. Intriguingly, many of these residues are conserved among nematodes. Mutational scanning of endogenous *sas*-5 using CRISPR/Cas9 genome editing revealed that S10 and S331/338/340 are indispensable for proper SAS-5 function *in vivo*. Replacing S10 or S331/338/340 with alanine or glutamate led to a fully penetrant loss of function

phenotype. The mutants could be conditionally rescued using a RNAi-resistant *sas*-5 transgene. Upon knocking down the transgene, SAS-5$^{S10A/S10E}$ embryos exhibited monopolar spindles during the early embryonic divisions. Conversely and unexpectedly, we found that SAS-5$^{S331/338/340A\ (3A)}$ embryos exhibit multipolar spindles. Consistent with an overactive centriole assembly pathway in this mutant, we found that the level of SAS-5^{3A} protein was significantly elevated. In contrast, SAS-5$^{S10A/S10E}$ levels were unaffected. Using S10-phospho specific antibodies, we found that SAS-5 is phosphorylated at centrioles *in vivo* and that the cell-cycle dependent pattern of phosphorylated SAS-5 differs significantly from the behavior of bulk centriole-associated SAS-5. Overall, our results suggest that ZYG-1 both positively and negatively regulates SAS-5 through phosphorylation to stringently control centriole biogenesis.

B196/P1190

Mechanism of centrosomal protein recognition and regulation by the ubiquitin ligase TRIM37

A. Bellaart[1,2], **A. Brambila**[1,2], F. Meitinger[1,2,3,4], K. Oegema[1,2,3], M. Ohta[1,2,3,4], A. Desai[1,2,3]; [1]Department of Cell and Developmental Biology, School of Biological Sciences, University of California San Diego, La Jolla, CA, [2]Ludwig Institute for Cancer Research, La Jolla, CA, [3]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, [4]Okinawa Institute of Science and Technology, Onna, JAPAN.

Centrosomes, small cellular organelles comprised of a centriolar core that is decorated with appendages and surrounded by a pericentriolar protein matrix that nucleates microtubules, are composed of more than 100 interacting components. How centrosomal protein assemblies are confined to the vicinity of centrioles and prevented from forming in the cytoplasm is not well understood. TRIM37 is a tripartite motif (TRIM) family ubiquitin ligase that serves as a guardian of centrosomal singularity, preventing the formation of ectopic centrosomal protein assemblies. When TRIM37 is absent, one of the ectopic assemblies that forms is a highly organized condensate containing the centrosomal protein centrobin. Centrobin-scaffolded condensates frequently nucleate microtubules in mitosis, leading to defects in chromosome segregation that may underlie abnormalities observed in the TRIM37 loss-of-function human disorder mulibrey nanism. These observations raise the question of how TRIM37 recognizes its centrosomal substrates to prevent their interaction/overaccumulation and how it simultaneously prevents the formation of ectopic assemblies while permitting the formation of normal assemblies at centrioles. Unique among TRIM family ligases, TRIM37 has a TRAF domain predicted to mediate protein-protein interactions. To understand how TRIM37 identifies its centrosomal substrates, we are investigating how it regulates two centrosomal proteins, centrobin and CEP192. In *in vitro* interaction assays following co-expression in FreeStyle 293-F cells, we found that TRIM37 associates with both centrobin and CEP192 and that binding is reduced by mutation of the TRAF domain. By testing a series of centrobin truncation mutants, we identified a 200 amino acid region of centrobin that mediates its interaction with TRIM37. Alphafold modeling identified short motifs within this region that are predicted to mediate TRAF domain binding. A truncation removing one motif partially reduced binding while a truncation removing all 3 motifs abrogated binding. Similar efforts are being pursued to understand the TRIM37-CEP192 interaction. Efforts to identify TRIM37 ubiquitination sites within centrobin and CEP192 and to employ in vivo replacements to examine the function of the TRIM37 TRAF domain and of specific interaction/ubiquitination regions of centrobin and CEP192 are also ongoing.

B197/P1191

Evolution of cell populations with different levels of centriole amplification

M. Dias Louro[1], C. Bank[1,2,3], M. Bettencourt-Dias[1]; [1]Instituto Gulbenkian De Ciência, Oeiras, PORTUGAL, [2]Institut für Ökologie und Evolution, Universität Bern, Bern, SWITZERLAND, [3]Swiss Institute of Bioinformatics, Lausanne, SWITZERLAND.

Understanding how cellular features evolve is a fundamental, but understudied problem, in biology. In the context of somatic cell evolution, many of these features show atypical levels of phenotypic variation, which have been associated with the development of human diseases such as cancer. One such feature is the abundance of subcellular structures, such as centrioles.
Whereas centriole number is usually constant in healthy proliferating cells, it is highly heterogeneous in cancer cells, with a tendency for abnormally high numbers, a condition known as centriole amplification. Centriole amplification is associated with hallmarks of cancer such aneuploidy and increased invasion, but its role in cancer initiation remains contentious. It has been proposed that low numbers of extra centrioles can trigger tumorigenesis whereas high numbers are excessively deleterious and suppress tumor formation. However, the relationship between the number of extra centrioles and/or the relative frequency of cells with abnormal numbers and the evolutionary dynamics of cells in the process of cancer evolution is poorly understood.
Here, we performed experimental evolution of human cell populations with different degrees of centriole amplification. Our goal was to quantify how centriole number and fitness changed over time depending on the initial degree of centriole amplification. Using a breast cancer model carrying a doxycycline-inducible Plk4 overexpression system, a key regulator of centriole biogenesis, we obtained ancestral populations with different levels of centriole amplification, which we evolved for two months in the presence of doxycycline. We observed that doxycycline-treated cell populations suffered a relative fitness penalty, proliferation deficit, and cell cycle disruption, proportional to the dose of doxycycline. Eventually, the relative fitness and population size of doxycycline-treated populations converged to those of control populations. We observed that cells with extra centrioles were progressively lost during experimental evolution, in a manner which depended on the concentration of doxycycline. Centriole number decrease was correlated with the upward trend in relative fitness and population size, indicating that doxycycline-treated populations adapted due to negative selection against cells with extra centrioles. Surprisingly, Plk4 mRNA levels were elevated both in the ancestral and evolved populations, suggesting that selection acted downstream of Plk4 overexpression. Our results showed that extra centrioles could not be maintained during experimental evolution despite constant Plk4 overexpression, suggesting that additional mechanisms may be required to sustain centriole amplification in live tumors.

B198/P1192

The Unkempt RNA-binding, satellite protein promotes PLK4 induced centriole over-duplication

A. Martinez, A. J. Stemm-Wolf, R. M. Sheridan, C. G. Pearson; University of Colorado Sch Med, Aurora, CO.

Centriole duplication drives the assembly of the centrosome, the microtubule organizing center (MTOC). PLK4 is a master regulator of centriole duplication that controls early events in centriole assembly, centrosome assembly, and centriolar satellite integrity. Maintenance of the number of procentrioles formed is necessary for ensuring only one centrosome is assembled during S phase. Overduplication of

centrioles can lead to centrosome amplification (CA) multipolar mitosis, chromosomal instability (CIN), and aneuploidy. PLK4 overexpression promotes centriole over-duplication by dysregulating assembly factors such as centriole structural proteins, satellites, and the PCM. How PLK4 dysregulates centrosome assembly factors to promote centriole overduplication remains unclear. We find that Unkempt (UNK), an RNA-binding zinc finger protein, is upregulated when PLK4 is artificially overexpressed in RPE-1 cells, suggesting that UNK modulates PLK4 induced centriole overduplication. We show that UNK localizes to the centrosome and to centriolar satellites. Using PLK4 overexpression in RPE-1 cells, UNK knockdown attenuates centriole over-duplication, suggesting that UNK promotes PLK4 induced centriole over-duplication. In addition, early centriolar assembly proteins, CEP192, CEP152, PLK4, and SAS6 are not effectively recruited during centriole assembly. Moreover, we show that UNK promotes localization of centriolar satellite scaffold proteins, PCM1 and CEP131, required for trafficking centrosomal proteins and centriole over-duplication. In summary, these data suggest that UNK promotes the localization of early centriole assembly proteins needed for PLK4 induced centriole over-duplication.

B199/P1193

Pericentrin is Essential for Differentiation in Rhabdomyosarcoma Cells

T. Allsage, T. Alahdadi, G. Bertram, Z. Gromley, **A. Gromley**; Lincoln Memorial University, Harrogate, TN.

One of the most common pediatric soft tissue cancers is rhabdomyosarcoma, comprising nearly 5% of all cancers in children. This cancer arises due to a defective myoblast differentiation process (myogenesis) that is thought to be caused by a disruption in the normal functions of the centrosome and primary cilia. Our previous studies have shown that the centrosome protein centriolin may play an important role in the normal function of the primary cilia and myogenesis, as disruption of the centriolin gene leads to cell cycle arrest, cell death, and impaired differentiation in cultured rhabdomyosarcoma cells. This suggests that specific components of the centrosome and the primary cilia could play crucial roles in the differentiation process of these cells. It is known that centriolin is localized to the maternal centriole within the centrosome, but many other proteins also contribute to the structure and function of this organelle. To determine if the effects we have seen previously are specific to centriolin or if other centrosome proteins have a similar role in myogenic differentiation, the CRISPR/Cas9 gene editing system was used in cultured human rhabdomyosarcoma cells to eliminate pericentrin, a centrosome protein that is located within the pericentriolar material surrounding the centriole. Upon disruption of the pericentrin gene using CRISPR/Cas9, cellular differentiation of rhabdomyosarcoma cells was affected, mirroring the phenotypes seen with centriolin loss. However, cell viability appeared to be unchanged. This is in stark contrast to the cell death that is characteristic of centriolin CRISPR treated cells. Our findings provide evidence that multiple proteins of the centrosome, each residing in distinct locations, are required for normal differentiation in rhabdomyosarcoma and, further, disruption of specific centrosome components can lead to programmed cell death. We propose that manipulation of individual centrosome components may be an effective means of tempering the growth of rhabdomyosarcoma and may be a viable approach for the treatment of this disease. Future studies will focus on identifying any additional centrosome proteins with similar properties.

B200/P1194

RNA binding proteins coordinate the post-transcriptional regulation of centrosomal *Plp* mRNA

J. Fang, D. Lerit; Emory University School of Medicine, Atlanta, GA.

The deregulation of centrosomes is associated with developmental disorders, including microcephaly, ciliopathy, and cancer. As major microtubule-organizing centers, centrosomes are composed of a pair of centrioles surrounded by pericentriolar material (PCM), a matrix of proteins required for microtubule nucleation. Also localizing to centrosomes are a small number of mRNAs. However, how and why RNAs localize to centrosomes remain critical unanswered questions. Conserved among the RNAs localizing to centrosomes is *pericentrin (PCNT)*-like protein (*Plp*). *Drosophila* PLP is a key component of the centrosome required for PCM scaffolding and microtubule organization. The disruption of *Plp* in *Drosophila* results in embryonic lethality, while the deregulation of *PCNT* in humans is associated with MOPD II and ciliary defects associated with Trisomy 21. To investigate mechanisms of *Plp* mRNA localization to centrosomes, we examined the contributions of two RNA-binding proteins during *Drosophila* embryogenesis. Our recent work shows oo18 RNA binding protein (Orb) interacts with *Plp* mRNA, contributes to *Plp* mRNA polyadenylation, and promotes PLP protein expression. Moreover, overexpression of full-length PLP can recover the PCM disorganization, cell division defects, and embryonic lethality caused by *orb* depletion, demonstrating *Plp* is an important downstream target of Orb. However, Orb is dispensable for robust *Plp* mRNA localization to centrosomes. To understand how *Plp* mRNA localizes to centrosomes, we investigated another multifunctional RNA binding protein, FMRP, the product of the fragile X mental retardation 1 gene (*Fmr1*). Our preliminary data indicate FMRP interacts with *Plp* mRNA and promotes its localization to centrosomes. These data support the idea that different RNA binding proteins coordinate centrosomal mRNA localization and translation. In addition, we observed the *Plp* coding region is required and sufficient for mRNA localization, and we will present our efforts to uncover the minimal localization element. Consistent with a model requiring active polysome transport, we found that treatment with translation inhibitors impairs *Plp* mRNA localization. This work lends mechanistic insights into RNA localization to centrosomes, which may be involved in the etiology of human diseases.

B201/P1195

***Spd-2* gene duplication reveals cell type-specific assembly of pericentriolar material**

R. S. O'Neill[1], F. C. Welsh[2], A. Sodeinde[3], C. J. Fagerstrom[1], B. J. Galletta[1], N. M. Rusan[1]; [1]NHLBI, NIH, Bethesda, MD, [2]University of Washington, Seattle, WA, [3]Yale, New Haven, CT.

The centrosome is the major microtubule organizing center (MTOC) of the cell, ensuring proper spindle formation during cell division by recruiting pericentriolar material (PCM) proteins which in turn nucleate microtubules. In the most general sense, centrosomes are assumed to function similarly across cell types during division; however, a more nuanced view is emerging that PCM proteins and MTOC activity are differentially regulated across cell types, leading to functionally important diversity among different types of dividing cells. To gain insight into cell type-specific regulation of PCM proteins, we investigated a gene duplication of the PCM gene *Spd-2* using an evolutionary cell biological approach, reasoning that gene duplication could give rise to centrosome gene duplicates with cell type-specific functions. In *D. melanogaster*, *Spd-2* is a single copy gene, is ubiquitously expressed and functions by organizing PCM in both brain neuroblasts and spermatocytes. In contrast, *D. willistoni* has both *Spd-2A* (ancestral) and *Spd-2B* (derived). To explore expression and function we generated *D. melanogaster* animals with GFP-

tagged *Spd-2A* and *Spd-2B* transgenes, including their *D. willistoni* native regulatory elements. Similar to Spd-2, Spd-2A organizes PCM in neuroblasts, but was insufficient for MTOC function in meiotic spermatocytes. In contrast, Spd-2B was only expressed in spermatogenesis, where it organized PCM during meiosis. Interestingly, driving expression of Spd2-B in the brain rescued neuroblast MTOC, but driving Spd-2A in spermatocytes still failed to rescue meiotic MTOC, indicating an evolutionary change in *Spd-2A* affecting meiotic function. Finally, we used chimeric transgenes to map these evolutionary changes to a ~120 amino acid C-terminal tail domain of Spd-2A which was sufficient to prevent proper MTOC organization in meiosis. Together, these results indicate that somatic and germline cells have different requirements for PCM, and that Spd-2 is differentially regulated at the C-terminal tail to satisfy these requirements. Further, our evolutionary cell biological study points to a general model of Spd-2 function where the C-terminal tail mediates a priming step that precedes PCM recruitment. Our current work aims to use additional chimeras, point mutants, and transgenes to gain further insight into this putative Spd-2 tail priming mechanism.

B202/P1196

Site-Specific Phosphorylation of ZYG-1 Regulates ZYG-1 Stability and Centrosome Number

M. Song, J. Medley, J. DiPanni, N. Yim, B. Shaffou, B. Sebou, E. Cramer, C. Wu, M. Kabara; Oakland university, Rochester, MI

Spindle bipolarity is critical for genomic integrity. As centrosome number often dictates mitotic bipolarity, tight control of centrosome number is vital for faithful cell divisions. The kinase Plk4/ZYG-1 plays a conserved role as a master regulator of centrosome assembly. In *Drosophila* and mammalian cells, Plk4 stability is known to be regulated by autophosphorylation-mediated proteasomal destruction. In *C. elegans*, molecular genetic studies have shown that ZYG-1/Plk4 activity is regulated by another kinase, Casein Kinase II (CK2), and phosphatases (PP1, PP2A-B55/SUR-6), while autophosphorylation-mediated regulation of ZYG-1 is yet to be tested in *C. elegans*. Here, we investigated how protein kinase CK2 regulates ZYG-1 stability in *C. elegans*. Given that CK2 kinase activity negatively regulates centrosome duplication by controlling centrosomal ZYG-1 levels, we hypothesized CK2 directly phosphorylates ZYG-1, and CK2-dependent phosphorylation of ZYG-1 triggers proteasomal destruction of ZYG-1, contributing to proper levels of ZYG-1. First, we show that CK2 directly phosphorylates kinase-dead ZYG-1 *in vitro,* and ZYG-1 physically interacts with CK2 in *C. elegans* embryonic protein lysates. Using *in silico* tools, we identified several ZYG-1 serine residues conforming to consensus CK2 target residues and focused on four serine sites within the ZYG-1-Linker-1 domain critical for centrosomal ZYG-1 loading and the ZYG-1-SAS-6 binding. To test the functional impact of site-specific phosphorylation of ZYG-1 *in vivo,* we used CRISPR editing and generated the *C. elegans* strains carrying mutations at the four serine residues replaced with alanine (S-to-A: Non-Phosphorylatable; NP) or aspartic acid (S-to-D: Phospho-Mimetic; PM). Our data illustrate the NP-ZYG-1 mutations result in elevated levels of both cellular and centrosomal ZYG-1, restoring bipolar spindles and embryonic viability to hypomorphic *zyg-1* mutants, suggesting that the NP-ZYG-1 mutation stabilized ZYG-1. As expected for overexpression, extra centrosomes are often observed in the NP-ZYG-1 mutant embryos. By contrast, the PM-ZYG-1 mutations lead to reduced ZYG-1 levels and aggravate *zyg-1* mutant phenotypes. Finally, we show inhibiting 26S proteasome blocks degradation of the unstable PM-ZYG-1 form, whereas the stable NP-ZYG-1 form becomes partially resistant to proteasomal degradation. Collectively, our data support a model where site-specific phosphorylation of ZYG-1 by CK2 regulates ZYG-1 stability via proteasomal degradation. Therefore, CK2-dependent phosphorylation of ZYG-1 provides an additional mechanism to fine-tune

ZYG-1 levels during cell cycle progression, leading to one and only one centrosome duplication during the early *C. elegans* cell division.

B203/P1197

The regulation of centriole number by the microtubule-remodeling factor *ssna*-1

J. A. Pfister[1], L. Agostini[2], N. Mizuno[2], K. F. O'Connell[1]; [1]National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, [2]National Heart, Lung, and Blood Institute, Bethesda, MD

The centrosome functions as the primary microtubule-organizing center (MTOC) of the cell and plays important roles in ciliogenesis and assembly of the mitotic spindle. It is composed of an orthogonally oriented pair of barrel-shaped structures known as centrioles surrounded by a dense proteinaceous matrix called the pericentriolar material (PCM). In mitotic cells, centrioles duplicate once per cell cycle in a process that is highly regulated to insure proper centriole number. Improper duplication, resulting in either too few or too many centrioles, disrupts spindle structure and cilia function. Indeed, abnormal centriole number has been linked to cancers, primary microcephaly, and a variety of ciliopathies. Work in *C. elegans* has identified a core group of conserved proteins essential for centriole biogenesis; among these is the master regulatory kinase ZYG-1, a homolog of Plk4, and SAS-6 a key component of the central scaffold. Here we report our molecular and genetical analysis of the *C. elegans* protein SSNA-1, whose human ortholog has been shown to function in neuronal branching and microtubule remodeling. In vitro studies demonstrate that worm SSNA-1 can bind and branch microtubules like its vertebrate counterpart. Using CRISPR-mediated genome editing, we have deleted the *ssna-1* gene and find that this results in a significant decline of embryonic viability and the formation of multipolar spindles. While our analysis indicates that the loss of SSNA-1 results in centriole overduplication, we find that *ssna-1* and *zyg-1* genetically interact in complex manner suggesting that SSNA-1 plays both positive and negative roles in centriole assembly. In particular, we find that loss of SSNA-1 alters centriolar composition such that centrioles have less ZYG-1 and more SAS-6. We have localized SSNA-1 in the embryo and find that during the first cell cycle SSNA-1 is restricted to centrioles; however, during the subsequent divisions, SSNA-1 also localizes to novel satellite-like structures that surround the PCM. Finally, our *in vitro* protein analysis has identified residues that are necessary for function and reveals that high-order oligomerization is essential for SSNA-1 function.

B204/P1198

In Vivo Interactome Profiling of Interferon Regulatory Factor 6 (IRF6) by TurboID-based Proximity Labeling

S. Huang, T. C. Cox[1]; University of Missouri-Kansas City, Kansas City, MO

Mutations in *IRF6* are arguably the most common cause of cleft lip/palate. IRF6 is a member of the interferon regulatory transcription factor (IRF) family which regulate the cell's response during viral infection. However, IRF6 is unique among the family in that it also has a critical developmental role, specifically in embryonic epithelia. Previous data indicate that IRF6 regulates epithelial cell proliferation/differentiation and cell-cell adhesion, although the regulatory networks in which IRF6 functions, and the specific proteins with which it interacts, remain largely unknown. It is notable, though, that IRF6 is predominantly located in the cytosol, with its nuclear import tightly regulated. Proximity-dependent *in vivo* biotin labelling coupled with mass spectrometry provides a sensitive tool by which to identify protein-protein interactions. To better understand IRF6 function within the cell, we

performed IRF6 proteomic profiling based on the promiscuous biotinylase enzyme, TurboID. This analysis identified several known proteins that have been directly implicated in cleft lip/palate, supporting the notion of a distinct cytoplasmic function for this transcription factor. Notably, our analyses also identified many vesicle transport proteins (endocytic adaptor proteins/recycling proteins), suggesting that IRF6 could directly regulate the trafficking of adherens junction proteins by interacting with components of intracellular vesicles. Other labeled proteins of note include several centrosomal proteins such as PCM1 and CEP170, which may support a mechanism whereby IRF6 also impacts cell cycle progression. Further experiments indicated that IRF6 could co-localize with PCM1, and centrosomal enrichment of IRF6 was significantly enhanced by phosphomimetic mutations of key serine residues in its C-terminus. Specific IRF6 interactors are currently being verified by mammalian two-hybrid and co-immunoprecipitation experiments. Taken together, these data will shed light on our understanding of the regulatory mechanisms of IRF6 controlling craniofacial development.

Spindle Assembly 1

B205/P1199

miR-31 regulates cytoskeletal dynamics to impact formation of the mitotic spindle

J. L. Song, C. Remsburg, K. Konrad; Biological Sciences, University of Delaware, Newark, DE.

miR-31 is a highly conserved microRNA that is known to play a role in cell proliferation, migration, and differentiation. Using the sea urchin as a model organism, we observed that miR-31 inhibitor injected embryos are developmentally delayed throughout cleavage stages and have an increased lethality rate at blastula stage. We identified miR-31 to localize at the midzone of the mitotic spindle and perinuclearly in divided blastomeres. miR-31 inhibitor injected embryos display longer astral microtubules and increased interpolar and kinetochore microtubules, as well as increased filamentous actin compared to the control. To understand the mechanism of these cytoskeletal defects, we identified that miR-31 suppresses several actin remodeling transcripts, including *Rab35*, *Gelsolin* and *Fascin*. Interestingly, *Rab35*, *Gelsolin* and *Fascin* transcripts co-localize with miR-31. The 3'UTRs of *Gelsolin* and *Fascin* are sufficient for localization to the mitotic spindle. miR-31 inhibition results in an increase in Fascin protein at the mitotic spindle midzone and perinuclearly in blastomeres in interphase. Our research, as well as previous studies, indicate proper actin dynamics are critical for formation of the mitotic spindle. These findings lead to our hypothesis that miR-31 regulates local translation of transcripts that mediate actin to impact formation of the mitotic spindle. Since proper mitotic spindle formation is a prerequisite for faithful segregation of chromosomes, identifying how miRNAs mediate spindle formation and mitosis is critical for the fundamental understanding of early cell division, birth defects, and predisposition to cancer.

B206/P1200

Differential regulation of sliding microtubule bundles in metaphase and anaphase

S. Forth, E. Tai, A. Alfieri; Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY.

The proper organization of the mitotic spindle is required for successful chromosome segregation during cell division. This highly dynamic network consists of microtubules that are organized, bundled, and transported by motor and non-motor proteins that generate precisely balanced 'active' pushing and 'passive' frictional forces to give the spindle its mechanical robustness. How these mesoscale forces are

produced and regulated by ensembles of mitotic proteins has been unclear. We are addressing this knowledge gap by directly measuring force production across reconstituted micron-scale microtubule bundles and simultaneously observing by single molecule fluorescence microscopy the localization of proteins that build these networks. Here we demonstrate that the essential microtubule crosslinking protein PRC1 performs distinct mechanical tasks in metaphase and anaphase to regulate motor protein activity. In metaphase, when CDK/cyclin-B activity is high, PRC1 is phosphorylated at two threonine residues near its microtubule-binding domain, while in anaphase these marks are removed. Surprisingly, we find that a phosphomimetic PRC1 construct organizes smaller bundles containing fewer filaments than the wildtype protein. In addition, this metaphase PRC1 analog produces significantly less mechanical resistance against motor-driven microtubule sliding than the anaphase analog. These changes are biochemically regulated by phosphorylation of microtubule-adjacent residues within the PRC1 protein, and help explain the functional differences observed in cells between bridging fibers that connect sister kinetochore fibers in metaphase and the central spindle in anaphase that bridges the two separating spindle halves.

B207/P1201

F-actin is essential for the fidelity of bipolar spindle formation in mouse oocytes

E. J. Soto-Moreno, A. Balboula; University of Missouri, Columbia, MO.

The spindle provides the machinery for accurate chromosome segregation. In contrast to somatic mitotic cells, in which centriole-containing centrosomes regulate bipolar spindle formation, mouse oocytes lack centrioles and, therefore, bipolar spindle assembly depends on self-organization of numerous acentriolar microtubule organizing centers (MTOCs) into two poles. Accordingly, following nuclear envelope breakdown, MTOCs undergo two critical steps to assemble a bipolar spindle: 1) MTOC clustering (MTOC-MTOC aggregation) and 2) MTOC sorting (*i.e.,* MTOC distribution towards the poles). The traditional view is that, in mammalian oocytes, microtubules (MTs) and their associated proteins are the only cytoskeletal components responsible for regulating spindle bipolarity. In mammalian oocytes, F-actin localizes to the MT spindle. Interestingly, we found that F-actin also colocalizes and surrounds MTOCs in metaphase I (Met I) oocytes. The biological significance of F-actin localization to the spindle/MTOCs for bipolar spindle assembly is unknown. We found that perturbing global or cytoplasmic, but not cortical, F-actin resulted in MTOC clustering and sorting defects, and decreased bipolar spindle formation at Met I. We investigated the role of spindle/MTOC-localized F-actin in regulating MTOC organization using two different approaches. First, we found that F-actin localization to the spindle depends on unconventional myosin X (MyoX, that links F-actin to MTs by having both MT and F-actin binding domains). siRNA-mediated depletion of MyoX or expressing a dominant negative MyoX mutant (mutation at the MT binding domain) abolished F-actin localization to the spindle, phenocopied MTOC clustering and sorting defects in F-actin-perturbed oocytes and increased the incidence of aneuploidy. Such phenotypes were rescued by forcing F-actin localization to the spindle in MyoX-depleted oocytes using MAP4-UtrCH. Second, we optimized a novel photoswitchable optojasp approach that can manipulate actin dynamics at a µm precision to perturb F-actin selectively at the spindle/MTOCs, following its exposure to 405 nm laser pulses. Perturbing spindle-localized F-actin in oocytes using optojasp approach resulted in clustering and sorting defects compared to laser-exposed untreated or optojasp-treated controls. Importantly, exposing one spindle pole to laser pulses in optojasp- treated Met I oocytes (with already clustered and sorted MTOCs) resulted in MTOC clustering and sorting defects compared to those at the non-laser exposed spindle pole. Taken together, these

findings reveal an unexpected function of F-actin in bipolar spindle assembly in mammalian meiosis, a process that is notoriously prone to mistakes.

B208/P1202

Spindle scaling in cellular differentiation

T. Kletter[1], S. Reusch[1], A. Halavatyi[2], A. Biswas[3], M. Pleitner[4], O. Muñoz[5], B. Neumann[2], V. Zaburdaev[5], S. Reber[1]; [1]IRI Life Sciences, Humboldt-University of Berlin, Berlin, GERMANY, [2]ALMF, European Molecular Biology Laboratory, Heidelberg, GERMANY, [3]Max-Planck-Institute for the Science of Light, Erlangen, GERMANY, [4]University of Applied Sciences, Berlin, GERMANY, [5]Friedrich–Alexander University Erlangen-Nürnberg, Erlangen, GERMANY.

During early metazoan embryogenesis, the mitotic spindle scales with decreasing cell size across successive reductive divisions. However, it is unclear how cells cope with the challenge of organelle scaling when they are differentiating into the various embryonic tissues. The developing brain is notoriously sensitive to mutations in spindle or centrosome-associated genes and here, spindle defects may cause neurodevelopmental disorders. Using adaptive feedback microscopy, we investigated spindle scaling in murine embryonic stem cells driven towards neural progenitor fates. Over the course of differentiation, cells and spindles underwent concerted changes in shape and size. However, from an early timepoint, differentiating cells showed unexpectedly diverging spindle scaling compared to the non-differentiating cells. Spindle mass, and thus spindle scaling, is governed by regulation of microtubule nucleation and dynamics, processes that are highly intertwined with the biophysical properties of the cytoplasm at large. These properties are known to change when cells transition between differentiation states. Using quantitative phase imaging, we found that indeed the differentiating population had altered mitotic cell mass densities. Thus far, with this work we propose that, ultimately, spindle size scales with cellular dry mass rather than with the cell's spatial dimensions.

B209/P1203

Intracellular enrichment of lipid droplets concentrates tubulin to promote microtubule assembly and chromosome segregation errors

G.-Y. Chen, M. Chung, M. A. Lampson; University of Pennsylvania, Philadelphia, PA

Cancer progression is highly associated with chromosome instability arising from mitotic errors, but the underlying mechanisms cannot be entirely explained by mutations in oncogenes or tumor suppressor genes. In addition to genetic defects, physical constraints at the cell and tissue levels can cause mitotic errors. Hepatic cells provide a natural system to study the impacts of intracellular physical constraints on mitosis because they frequently enrich lipid droplets during cancer progression. We show that droplet enrichment increases chromosome segregation errors independent of spindle assembly defects, indicating impaired regulation of kinetochore-microtubule attachments. Furthermore, we find that droplet enrichment increases microtubule polymer by increasing total tubulin concentration. We propose that droplets exclude cytoplasmic volume and promote crowding to increase microtubule polymerization and kinetochore-microtubule attachment errors. Thus, physical constraints generate unstable chromosomes by disturbing cytoskeletal dynamics as a mechanism leading to the genomic variability characteristic of cancer cells.

B210/P1204

ZYG-8 regulates outward sorting forces to achieve proper force balance in*C. elegans*acentrosomal spindles

E. R. Czajkowski, N. Divekar, S. Wignall; Northwestern University, Evanston, IL.

Although centrosomes help organize spindles in most cell types, oocytes lack these structures; we are leveraging *C. elegans* to understand how acentrosomal oocyte spindles form and are stabilized. Using this system, we previously demonstrated that microtubule minus ends are sorted outwards during spindle assembly, where they form multiple poles that coalesce until bipolarity is achieved. Kinesin-12/KLP-18 is the primary outward sorting motor, while kinesin-5/BMK-1 provides a redundant outward force and dynein generates an inward force. However, how these motors are regulated to achieve proper force balance is not known. Now, we have gained insight into this question through studies of ZYG-8, a conserved kinase with a doublecortin-like microtubule binding domain. To study the functions of ZYG-8, we applied the auxin-inducible degron system; this approach enabled both long-term depletion, to investigate a role for ZYG-8 in spindle assembly, and acute removal from pre-formed spindles, to assess a role in maintaining spindle stability. Following long-term ZYG-8 depletion, bipolar spindles formed but were significantly longer, had disrupted pole organization, and were often bent; we observed similar phenotypes upon short-term depletion from pre-formed spindles. These phenotypes suggested that there was excessive outward force on the spindle in the absence of ZYG-8. Notably, when we co-depleted KLP-18 with ZYG-8, we also found evidence for excess outward force. KLP-18 depletion alone results in a failure to sort minus ends outwards during spindle assembly, resulting in a monopolar spindle with all minus ends at the central pole. However, when KLP-18 and ZYG-8 were both depleted prior to spindle assembly, minus ends were not only found at the central pole, but were also located at the periphery of the array. Moreover, when we removed ZYG-8 from pre-formed monopolar spindles, we observed the formation of structures with multiple poles, suggesting that minus ends were being sorted outwards, fragmenting the central pole. These phenotypes suggested that ZYG-8 may normally suppress an activity capable of exerting outward force. Given that KLP-18 was depleted in the monopolar spindle assay, we hypothesized that ZYG-8 may be regulating BMK-1, the redundant outward sorting motor. To test this hypothesis, we acutely removed ZYG-8 from monopolar spindles in a *bmk-1* mutant strain; under these conditions monopolar spindles remained intact and minus ends were not sorted outwards. This result suggests that ZYG-8 functions to dampen the activity of BMK-1, and that this regulation is essential to achieve and maintain proper force balance in the acentrosomal oocyte spindle.

B211/P1205

Regulation of Twist in the Human Anaphase Spindle

L. Neahring, S. Dumont; University of California San Francisco, San Francisco, CA

At each cell division, nanometer-scale motors, crosslinkers, and microtubules give rise to the micron-scale spindle. Although many mitotic motors have been shown to walk in helical paths around microtubules in vitro, the human spindle exhibits only slight global twist. This raises the question of how molecular torques are balanced to build a nearly achiral spindle. Here, we define the mechanisms that generate and resist twist in the anaphase spindle. We find that left-handed twist in RPE1 and MCF10A cells increases at anaphase, and that it is dramatically enhanced by inhibition of NuMA or dynein. We show that KIF4A, a kinesin that regulates microtubule dynamics and sliding in the anaphase midzone, is

required for this left-handed twist. We also uncover a role for the cell cortex, as disrupting actin filaments reduces anaphase twist. Our results suggest a model in which the left-handed torques generated by mitotic kinesins are increased at anaphase by the relocalization of KIF4A and counteracted by NuMA/dynein, either via active right-handed torque generation or by NuMA/dynein-dependent crosslinking that increases the spindle's torsional rigidity. Together, our work sheds light on the spatiotemporal regulation of spindle twist, a recently recognized phenomenon that may have implications for spindle mechanics and function.

B212/P1206

Measuring and modeling the dynamics of mitotic error correction

G. Ha[1], P. Dieterle[1], H. Shen[2], A. Amir[1], D. Needleman[1]; [1]Harvard University, Cambridge, MA, [2]Reverie Labs, Cambridge, MA

The mammalian mitotic spindle normally segregates an equal number of chromosomes to daughter cells with high fidelity. Over the course of spindle assembly, many initially erroneous attachments between kinetochores and microtubules are fixed through a process called error correction. Despite the importance of chromosome segregation errors in cancer and other diseases, we lack methods to characterize the dynamics of error correction and how it can go wrong. We have developed a novel experimental method and analysis framework to quantify chromosome segregation error correction in human tissue culture cells with live cell confocal imaging of spindle assembly, timed premature anaphase, and automated counting of kinetochores after cell division. Our results are consistent with an exponential decrease of errors over time, and an asymmetric initial state of erroneous attachments with high intercellular variability. We have developed a coarse-grained model which can quantitatively explain both the measured error correction dynamics and the distribution of anaphase onset times under different molecular perturbations. Our experimental and analysis frameworks allow us to quantitatively determine the dynamics of error correction for the first time. In order to develop a biophysical model of error correction, we will follow up on these results by characterizing how the regulation of microtubule stability and tension-dependent attachments determines error correction dynamics.

B213/P1207

Morphological growth dynamics, active microtubule mechanics, and mechanical plasticity of the vertebrate meiotic spindle

T. Fukuyama[1], L. Yan[2], M. Tanaka[3], M. Yamaoka[3], K. Saito[3], S.-C. Ti[4], C.-C. Liao[5], K.-C. Hsia[5], Y. T. Maeda[2], **Y. Shimamoto**[3]; [1]National Institute of Natural Sciences, Aichi, Japan, [2]Kyushu University, Fukuoka, Japan, [3]National Institute of Genetics, Shizuoka, Japan, [4]The University of Hong Kong, Hong Kong, Hong Kong, [5]Academia Sinica, Taipei, Taiwan

The spindle is a self-organized subcellular structure built from microtubules and microtubule-associated proteins. The spindle's bipolar shape is essential for the equal partitioning of replicated chromosomes between the two daughter cells. However, multipolar spindles can stochastically arise in a subset of a cell population, leading to chromosome segregation errors. How microtubules occasionally favor non-bipolar self-organization is not known. Here, using a combination of large-field fluorescence imaging, quantitative shape analysis, machine learning-based phenotype sorting, and Xenopus egg cytoplasmic extracts, we find that the microtubule structures growing into bipolar and multipolar shapes exhibit

distinctly different self-organization dynamics. Bipolar spindles are built through a predominant bipolar growth while little exploring other shape morphologies. Multipolar spindles are built while exhibiting a large shape fluctuation, which starts at an early self-organization phase and shows a characteristic temporal pattern. Our high-resolution imaging identifies a local defect in the premature, early microtubule network that can be the source of the multipolar shape growth. We also find that the spindles exhibit substantial shape stability upon maturation but can undergo phenotypic switching in response to a transient force perturbation applied using microneedles. Together with molecular perturbation assays, we discuss the biophysical mechanism of how varied spindle shapes emerge and are stabilized within the dynamic cytoplasm.

B214/P1208

The chromokinesin KLP-19 regulates the microtubule overlap in the central spindle of the C. elegans embryo during mitosis, independently of the microtubule bundling protein SPD-1

S. Redemann, M. Magaj, V. Zimyanin; University of Virginia, School of Medicine, Charlottesville, VA.

The faithful segregation of chromosomes during mitosis is essential for the health and survival of any eukaryotic organisms. Any failure in this process has detrimental consequences such as developmental abnormalities and cancer. During mitosis microtubules assemble into a bipolar spindle that segregates the sister chromatids in anaphase. Recent publications have highlighted the importance of the spindle midzone, the region between the chromosomes, for the process of chromosome segregation. The detailed mechanisms of midzone assembly and function during chromosome segregation has however remained elusive. A number of publications have led to a model of midzone formation and function in mammalian cells that relies on a combination of microtubule organization by bundling of antiparallel microtubules by PRC-1 (*C.elegans* spd-1), regulation of microtubule growth dynamics by KIF4a (*C.elegans* klp-19) and motor driven microtubule sliding by a combination of motors. Combining a detailed 3D structural analysis by electron tomography with state-of-the art light microscopy we have found that the spindle midzone in *C. elegans* is composed of different microtubule subclasses that likely differ in their dynamics. Thus, suggesting a possible differential regulation of microtubule growth during anaphase. Using Fluorescence recovery after photobleaching and second harmonic generation imaging we found that while microtubules within the midzone move pole-wards, the length of the antiparallel microtubule overlap zone in the spindle midzone is constant throughout anaphase, and independent of cortical pulling forces as well as the presence of the microtubule bundling protein SPD-1. Further investigations of the microtubule bundling protein SPD-1 and the chromo-kinesin KLP-19 in *C. elegans*suggest that in contrast to previously reported in mammalian cells, KLP-19 functions independently of SPD-1. Our data suggests that KLP-19 plays an active role in regulating the number of microtubules within the midzone as well as the size of the antiparallel overlap region throughout mitosis.

B215/P1209

Laser Ablation and Fluid Flows Show a Single Force Mechanism Governs Spindle Positioning

H. Wu[1], G. Kabacaoğlu[2], E. Nazockdast[3], H. Chang[4], M. J. Shelley[2,5], D. J. Needleman[1,2]; [1]Harvard University, Cambridge, MA, [2]Flatiron Institute, Simons Foundation, New York, NY, [3]The University of North Carolina, Chapel Hill, NC, [4]Academia Sinica, Taipei, TAIWAN, [5]New York University, New York, NY.

Few techniques are available for elucidating the nature of forces that drive subcellular behaviors. Here we develop two complementary ones: 1) femtosecond stereotactic laser ablation (FESLA), which rapidly creates complex cuts of subcellular structures, thereby allowing precise dissection of when, where, and in what direction forces are generated; and 2) assessment of subcellular fluid flows, by comparing direct flow measurements, using microinjected fluorescent nanodiamonds, to large-scale fluid-structure simulations of different force transduction models. We apply these new techniques to study spindle/centrosome positioning in *Caenorhabditis elegans* early embryos, and probe the contributions of three postulated force mechanisms: *i)* microtubule pushing, *ii)* cytoplasmic pulling, and *iii)* cortical pulling upon centrosomal microtubules. Based on our results, we construct a biophysically-based model to explain the centrosome dynamics. Taken together, we demonstrate that cortical pulling forces provide a general explanation for many behaviors mediated by centrosomes, including pronuclear migration/centration and rotation, metaphase spindle positioning, asymmetric spindle elongation and spindle oscillations. In sum, this work establishes new methodologies for disentangling the forces responsible for cell biological phenomena.

B216/P1210

Mammalian kinetochore-fibers maintain their own lengths, but require poles for spindle coordination

M. Richter; UCSF, San Francisco, CA

At each cell division, nanometer-scale components self-organize to build a micron-scale spindle. In mammalian spindles, microtubule bundles called kinetochore-fibers attach to chromosomes at one end and focus into spindle poles at the other. Despite evidence suggesting poles set length and other spindle properties, their role remains poorly understood. In fact, many species do not have spindle poles. Here, we probe the role of the pole in giving rise to the mammalian spindle's length, dynamics, and function, inhibiting dynein to generate spindles whose kinetochore-fibers do not focus into poles. We find that unfocused kinetochore-fibers have a mean length indistinguishable from control, but a broader length distribution, and reduced length coordination intracellularly and between sisters. Further, we show that unfocused kinetochore-fibers, like control, can grow back if shortened by laser ablation, albeit more slowly, and thus actively recover their length. We find that while control kinetochore-fibers robustly recover their length by increasing plus-end polymerization and suppressing minus-end depolymerization, these dynamics are severely reduced in unfocused ones, slowing length recovery. Finally, we show that spindles with unfocused kinetochore-fibers fail to correctly segregate chromosomes. Thus, while mammalian kinetochore-fibers locally maintain and recover their own lengths without spindle poles, poles globally coordinate them across space and time.

B217/P1211

Myosin 10 supports mitotic spindle bipolarity by promoting PCM integrity and supernumerary centrosome clustering

Y. Yim[1], A. Pedrosa[1], X. Wu[1], R. Cheney[2], J. Hammer[1]; [1]National Institutes of Health, Bethesda, MD, [2]University of North Carolina, Chapel Hill, NC.

Myosin 10 (Myo10) is a member of the MyTH4/FERM domain family of unconventional, actin-based motor proteins. Previous studies have shown that Myo10 supports cell adhesion during interphase via its integrin-binding FERM domain and spindle dynamics during mitosis via its microtubule-binding MyTH4 domain. Here we characterized Myo10's contribution to mitosis using Myo10 knockout HeLa cells and MEFs isolated from a Myo10 knockout mouse. Most notably, both of these knockout cells exhibit a pronounced increase in the frequency of multipolar spindles. Staining of unsynchronized metaphase cells showed that the primary driver of spindle multipolarity in knockout MEFs and knockout HeLa cells lacking supernumerary centrosomes is PCM fragmentation, which creates y-tubulin-positive, centriole-negative microtubule asters that serve as additional spindle poles. For HeLa cells possessing supernumerary centrosomes, Myo10 depletion further accentuates spindle multipolarity by impairing centrosome clustering. These results indicate, therefore, that Myo10 supports spindle bipolarity by maintaining PCM integrity in both normal and cancer cells, and by promoting supernumerary centrosome clustering in cancer cells. Finally, complementation experiments show that Myo10's FERM domain-dependent interaction with integrin is essential for both the clustering of supernumerary centrosomes and the maintenance of spindle pole integrity, and that its MyTH4 domain-dependent interaction with microtubules is primarily required for the maintenance of pole integrity.

B218/P1212

PLK-1 is required for establishing and maintaining acentrosomal spindle organization in *C. elegans* oocyte meiosis

J. Narula, S. Wignall; Northwestern University, Evanston, IL

Sexual reproduction relies on meiosis, a specialized cell division program that produces haploid gametes. Female gametes (oocytes) in most organisms lack centrosomes, yet the mechanisms by which acentrosomal spindles are formed and stabilized are poorly understood. Previous work has detailed steps in the spindle assembly pathway in *C. elegans* oocytes and has characterized a layered ring-shaped protein complex ("ring complex") that encircles each chromosome and is required both for spindle assembly and chromosome congression. However, key questions remain regarding how these components work together to ensure the creation of a bipolar spindle, and how these processes are regulated.

Polo-like kinases (PLKs) are critical regulators of chromosome segregation and bipolar spindle formation in mitosis across species. In *C. elegans*, polo-like kinase 1 (PLK-1) plays an essential role in centrosome maturation during mitosis and is required for nuclear envelope breakdown in oocytes. However, whether this kinase performs other functions during meiosis is not known. Here we report that PLK-1 dynamically localizes to several subcellular locations in oocytes including the spindle poles, kinetochore, ring complex, and chromatin, and that the chromosomal passenger complex (CPC) and BUB-1 are required for PLK-1 to target to the ring complex. To investigate the role of PLK-1 further, we utilized auxin-induced degradation (AID) to deplete PLK-1 from oocytes either prior to spindle assembly or after bipolar spindle formation. Depletion of PLK-1 prior to spindle assembly resulted in a failure of nuclear

envelope breakdown, consistent with previous findings. Additionally, we found organizational defects when depleting PLK-1 from fully formed bipolar spindles, indicating that PLK-1 also plays a role in maintaining the spindle after bipolarity has been established. Lastly, we observed the formation of ectopic microtubule asters in these depletion conditions, suggesting a unique role for PLK-1 during acentrosomal meiosis. Taken together, our work identifies PLK-1 as a key player in *C. elegans* meiosis, thus incorporating this protein into a larger model for acentrosomal spindle assembly and chromosome dynamics.

B219/P1213

Segregation of the univalent X chromosome in the two striped planthopper *Acanalonia bivittata*

A. Borseth, K. Felt, E. Stowe, L. Paliulis; Bucknell University, Lewisburg, PA

Correct segregation in meiosis I depends on homologous chromosomes pairing to form bivalents. To achieve a successful reduction in ploidy, bivalents align on the metaphase plate and then homologues segregate during anaphase I. While bivalent formation is generally required for correct segregation in meiosis I, many organisms pose an exception to this requirement. Our objective was to study on such exception, the two striped plant hopper *Acanalonia bivittata* (Hemiptera, Auchenorrhyncha), which has a univalent X chromosome. *A. bivittata* were collected and their identification was confirmed through DNA barcoding. Chromosome number was found to be consistent with previously published karyotypes for the species. To observe the behavior of unpaired chromosomes in male primary spermatocytes, live-cell and confocal imaging of stained cells was conducted. The univalent X chromosomes were found to be characterized by independent and delayed segregation that occurred after autosomal segregation during early anaphase I. Delayed segregation of the univalent chromosome was associated with the loss of microtubule connections to one spindle pole, leaving the chromosome aligned on the metaphase plate, often in a position outside the main spindle body. This study characterized the behavior of an unpaired chromosome in an alteration to the traditional meiotic program. This work has translational implications in organisms in which a chromosomal pairing partner is lost, such as the diminution and potential loss of a Y chromosome in mammals.

B220/P1214

Mitotic Spindle Remodelling in Response to Severe Metabolic Stress

J. Ng[1], G. Charras[1], S. Banerjee[2]; [1]University College London-41600960, London, United Kingdom, [2]Carnegie Mellon University, Pittsburgh, PA

In response to severe metabolic stress, some organisms enter a reversible state known as suspended animation (SA), in which all observable activities, such as growth and motility, come to a halt. Remarkably, following SA, the organisms can resume their life cycle, unharmed. Upon SA entry, how individual cells switch off their activities and preserve memory of their pre-SA asymmetries are little understood. Here we seek to investigate how cell division, one of the most dynamic cellular processes, is perturbed during SA. We treated mitotic HeLa cells with metabolic inhibitors and paid particular attention to the mitotic spindle. We observed that microtubules (MT) in different parts of the mitotic spindle exhibit differential changes in length under severe metabolic stress, suggesting that their dynamics are spatially controlled by distinct energy-dependent processes. After release from the metabolic stress, most cells were able to resume division. To understand the mechanistic nature of the spindle remodelling, we developed a mathematical model to determine how changes in the mechanical

properties and forces within the spindle during energy depletion may contribute to the observed behaviour. The results suggest that apart from a decrease in motor activities, changes in spindle stiffness, MT polymerization and MT flux are all required to explain spindle remodelling. The model also predicts the lengthening of the overlap regions of the half-spindles and a reduction of tension in the spindle. Overall, they show that the spindle can be viewed as a composite material made up of components with different sensitivities to energy depletion. The size of the spindle is predominantly controlled by the opposing forces generated by these components. These findings raise new questions about what energy-dependent processes are connected to the physical properties of the spindle and how the remodelling preserves the memory for division.

Cancer Therapy - Inhibitor Activity 1

B222/P1215

The Antitumor Activity ofcannabis sativa/CBD in prostate cancer pc3 cells

L. R. Motadi; University of Johannesburg APK, Johannesburg, SOUTH AFRICA.

Prostate cancer is the second most frequently occurring carcinoma in males worldwide and one of the leading causes of death in men around the world. Recent studies estimate that over 1.4 million males are diagnosed with prostate cancer on an annual basis, with approximately 375 000 succumbing to the disease annually. With current treatments continuing to show severe side effects, there is a need for new treatments. In this study we looked at the effect of cannabis sativa extract, cannabidiol and cisplatin on prostate cancer cells, PC3. Methods In addressing the above questions, we employed the MTT assay to measure the antiproliferative effect on PC3 cells following treatment with varying concentrations of Cannabis sativa extract, cisplatin and cannabidiol. xCELLigence was also used to confirm the IC50 activity in which cells were grown in a 16 well plate coated with gold and monitor cell attachment. Caspase 3/7 activity was also measured using 96 wellplate following treatment. Western-blot and qRT-PCR was also used to measure the gene expression of tumour suppressor genes, p53, Bax and Bcl2. Animal studies were employed to measure the growth of PC3-mouse derived cancer to evaluate the effect of compounds in vivo. Results From the treatment with varying concentrations of Cannabis sativa extract, cannabidiol and cisplatin, we have observed that the three compounds induced antiproliferation of PC3 cancer cell lines through the activation of caspase 3/7 activity. We also observed induction of apoptosis in these cells following silencing of retinoblastoma binding protein 6 (RBBP6), with upregulation of p53 and bax mRNA expression, and a reduction in Bcl2 gene expression. The growth of tumours in the mouse models were reduced following treatment with cisplatin and cannabidiol.

B223/P1216

Analysis of off-target effects on cardiac muscle by drugs used to treat multiple myeloma and breast cancer

J. Wang; Cell and Developmental Biology, SUNY Upstate Medical University, syracuse, NY.

We have previously demonstrated that myofibril assembly occurs in three steps (premyofibrils to nascent myofibrils to mature myofibrils) representing increased complexity of protein arrangements (Rhee et al., 1994; White et al., 2018). Our discovery that inhibitors of the Ubiquitin Proteasome System (UPS) prevent nascent myofibrils from progressing to mature myofibrils in cultured embryonic quail

muscle supports the three-step model, and indicates some proteins in nascent myofibrils in skeletal muscles must be proteolyzed to permit the maturation of mature myofibrils. These UPS inhibitors have no effects on the formation of premyofibrils and nascent myofibrils, and no effects on existing mature myofibrils. Similar UPS inhibitors treatments on cardiomyocytes isolated from embryonic chick embryos, and on hiPSC-derived cardiomyocytes also led to the halt in nascent myofibrils progressing to mature myofibrils. Our experiments using UPS drugs on skeletal and cardiac muscled cells suggest possible explanations for the off-target cardiac and skeletal muscle effects of UPS inhibitors that are also used on multiple myeloma patients. We suggest that analyses of cultured embryonic heart cells and human cultured muscle cells will provide a preclinical assay for testing of novel cancer drugs with improved outcomes for patients., an important "…goal of the emerging field of cardio-oncology…" (Narezkina and Nasim, 2019). As a test of our suggestion, we have used a new UPS drug suggested for cancer patients that is a ligase inhibitor. This compound did inhibit myofibrillogenesis in embryonic quail skeletal muscle cells. However, it had no effect on myofibrillogenesis in embryonic chick heart cells at the same concentration. In support of our suggested assay, the ligase that the new UPS inhibitor binds to is not present in cardiac muscle cells, but is present in skeletal muscle cells. Use of doxorubicin to treat breast cancer patients has a serious effect on the hearts, and skeletal muscles. Our experiments indicates that this compound inhibits the assembly of myofibrils, and in contrast to UPS inhibitors lack of effects on mature myofibrils, doxorubicin induces the disassembly of mature myofibrils. The idea that doxorubicin interferes with the assembly and/or maintenance of mature myofibrils may explain its cardiotoxic effects on breast cancer patients.

B224/P1217

Podoplanin Expression and Ex Vivo Lectin Effects on OSCC Cells from Clinical Trial Subjects

T. J. Hellmig[1], E. J. Brace[1], S. Parikh[1], S. Basu[1], A. J. Shienbaum[2], D. I. Suster[3], E. M. Capitle[3], E. Kalyoussef[3], D. Roden[3], S. Baredes[3], M. Fatahzadeh[4], G. S. Goldberg[1]; [1]Rowan University, Stratford, NJ, [2]Keystone Medical Laboratories, Throop, PA, [3]Rutgers New Jersey Medical School, Newark, NJ, [4]Rutgers School of Dental Medicine, Newark, NJ.

Oral cancer kills over 13,000 people in the USA and over 145,000 people worldwide each year. Current treatments include surgical procedures that can leave patients with permanent sequalae and decrease their quality of life. Novel therapies are needed to treat oral cancer. Maackia amurensis seed lectin (MASL) can target the podoplanin (PDPN) receptor which is expressed on OSCC cells that cause over 90% of oral cancers. These findings prompted the investigation of MASL as a novel agent in an ongoing clinical trial (NCT04188665) aimed at oral cancer. Here, we examined PDPN expression and the effects of MASL on OSCC cells derived from patients enrolled in this trial. IHC detected robust levels of PDPN expression on OSCC cells in oral lesions from these patients. Western blot analysis confirmed PDPN expression by these cells adapted to culture. Submicromolar (770 nM) MASL concentrations decreased the motility and viability of these OSCC cells by over 70% and 10%, respectively. Moreover, higher (2880 nM) MASL concentrations completely inhibited the motility of these cells, and decreased their viability by over 50%. These results support the consideration of MASL as a potential OSCC anticancer agent.

B225/P1218

Antiproliferative Activity of Male and Female *Maclura pomifera* Extracts in ER-positive Breast Cancer Cell Lines

M. Rumpa, C. Maier; Biology, Texas Woman's University, Denton, TX.

Breast cancer is one of the most diagnosed cancers in women around the world. Natural products of plant origin have been recognized as prominent sources of anti-cancer drugs. *M. pomifera* is a dioecious (separate male and female plants) tree native to the south-central USA, reported to have anti-inflammatory, antinociceptive, and antiproliferative properties. Native Americans have used plant parts for cancer treatment, but few cancer research studies have focused on *M. pomifera*, limiting our understanding of its medicinal properties. The goal of this research is to identify capsaicin-like compounds in *M. pomifera* plant extracts and determine their antiproliferative activities in the ER-positive breast cancer MCF-7 and T47D cell lines. Capsaicin, a bioactive phytochemical abundantly present in chili peppers, is known as a potent transient receptor potential vanilloid type 1 (TRPV1) calcium-permeable ion *channel* agonist. Other plants synthesizing capsaicin-like compounds could provide new therapeutic agents for cancer treatments. To identify capsaicin-like phytochemicals, stem and leaves of male and female *M. pomifera* were sequentially extracted with hexane, ethyl acetate, ethanol, and methanol. *M. pomifera* contains capsaicin-like compounds based on a colorimetric assay, TLC, and UPLC-ESIMS/MS. Antiproliferative activities evaluated using MTS assays revealed that *M. pomifera* plant extracts possess antiproliferative properties. Both male and female *M. pomifera* plant extracts inhibited growth of MCF-7 and T47D cells. Male extract at 500 µg/ml concentration inhibited both MCF-7 and T47D cells by 80% compared to female extract (45%). Blocking of TRPV1 with capsazepine significantly increased cell viability suggesting that *M. pomifera* phytochemicals works through TRPV1 in inducing cell death. We hypothesize that *M. pomifera* capsaicin-like compounds trigger apoptosis through the mitochondrial apoptotic pathway due to intracellular calcium overload *via* TRPV1. To find out whether plant extracts increase the calcium concentration in the cell, cells were pretreated with the calcium chelator BAPTA-AM and then treated with plant extracts. Chelation of calcium ions significantly increased cell viability, revealing that *M. pomifera* phytochemicals activate TRPV1 thus overloading cells with calcium ions and triggering cell death. Future work will focus on chemically characterizing capsaicin-like phytochemicals in *M. pomifera* extracts and elucidating their mechanism of action in inducing apoptosis in cancer cell lines *via* the TRPV1 ion channel. The findings could lead to the discovery of natural products for the development of new cancer treatment drugs.

B226/P1219

MiR92b-targeted miRNA inhibitor-containing Gold Liposomal Formulation Reduces Cell Growth in Glioblastoma Cells

A. O. Sanchez Alvarez[1], N. Grafals-Ruiz[1], F. Valiyeva[1], R. Rabelo[2], P. E. Vivas-Mejía[1,3]; [1]Comprehensive Cancer Center UPR, San Juan, PR, [2]University of Puerto Rico Rio Piedras Campus, San Juan, PR, [3]Department of Biochemistry, University of Puerto Rico Medical Science Campus, San Juan, PR.

MicroRNA (miRNA) dysregulation plays a central role in the initiation, progression, and drug resistance of most malignancies. MiRNA-based therapies are designed to target upregulated or downregulated miRNAs with oligonucleotide miRNA inhibitors (OMIs) or oligonucleotide miRNA mimics (OMMs), respectively. Although OMIs and OMMs are promising therapies for cancer treatment, their significance has been hindered as these molecules are quickly degraded when systemically administered. MicroRNA-

92b (miR92b) is aberrantly abundant in glioblastoma (GBM), the most aggressive of the primary brain tumors. Brain-targeted drug delivery systems have the extra challenge of overcoming the blood-brain barrier (BBB). Apolipoprotein E (ApoE) is under study for its role as a lipid transport protein in the brain, making it suitable for brain targeting. The internalization of nanocarriers is increased with ApoE peptide functionalization compared to empty liposomal formulation in U87 cells. Our lab recently published successful systemic delivery of miR92b-OMIs containing gold-liposomal formulation into the brain of a GBM syngeneic mouse model. However, this formulation was prepared with 15 nm gold nanoparticles ($AuNPs_{15}$), and very low OMI conjugation and high AuNP agglomeration were observed. Therefore, optimization and standardization of this procedure are necessary to obtain a formulation with potential clinical use. The herein project furtherly explores the role of miR92b in the U87 human GBM cell line while addressing the above-described challenges. We used AuNPs of different sizes and observed that 5 nm AuNPs ($AuNPs_5$) increased the AuNP-OMI conjugation, reduced the AUNP aggregation, reduced the size of the nanocarrier system (liposomes), and increased the homogeneity of the liposomal formulation. These traits also result in enhanced cost efficiency due to less waste of raw material. The liposomal nanocarrier system using $AuNPs_5$ results in a 22% size reduction from the previously reported value with a 50% increase in homogeneity. Using $AuNPs_5$ gold-liposomal formulation the previously observed tendency was intensified, achieving better internalization compared to empty liposomes and $AuNPs_5$ gold-liposomal formulation without ApoE. In vitro administration of miR92b-OMI to U87 cells resulted in decreased proliferation and a reduction in the ability to migrate. Quantitative PCR confirmed complete inhibition of miR92b after transfection. A caspase 3 assay suggests apoptosis after miR92b inhibition. These findings help elucidate the role of miR92b in GBM for future therapy designs.

B227/P1220

Anti-Cancer Ruthenium Complex KP1019 Causes Ribosomal Biogenesis Stress in Yeast

J. S. Blackman, P. K. Hanson, S. L. Gary; Biology, Furman University, Greenville, SC.

Platinum-based drugs (cisplatin, carboplatin etc.) are used to treat many cancers, but limitations include harsh side effects and evolution of drug resistance. The ruthenium based complex indazole *trans*-[tetrachlorobis(1*H*-indazole) ruthenate(III)], also known as KP1019, may overcome these limitations and appeared promising in an early clinical trial. Prior studies demonstrated KP1019's ability to cause DNA damage and alter the cell cycle, however the drug's impact on cells is not fully understood. More recently, the ribosome has been implicated as a potential target of KP1019 in both cancer cells and the model organism *Saccharomyces cerevisiae*. For example, in a previous proteomic analysis in yeast, KP1019 increased expression of translation and ribosome related genes. To understand KP1019's impact(s) on ribosomes, here we examine KP1019 sensitivity of yeast lacking genes that encode ribosomal structural proteins or proteins involved in ribosome biogenesis. In the presence of KP1019 these deletion strains appeared drug resistant, displaying a higher growth rate than wildtype yeast. To determine whether the altered gene expression and mutant growth kinetics might be due to KP1019's effect on ribosomal biogenesis, we used fluorescence microscopy to monitor the localization of GFP-tagged large ribosomal protein Rpl7a. We discovered that KP1019 treatment causes a dose-dependent increase in the proportion of cells with a single focus of Rpl7a-GFP, as opposed to a diffuse cytoplasmic distribution. Additionally, we used Sik1-RFP and Elo3-BFP proteins to visualize the nucleolus and endoplasmic reticulum respectively to refine the drug-induced localization of Rpl7a-GFP localization as being nucleolar, a result that suggests KP1019 does indeed interfere with ribosomal biogenesis.

Identification of this process as a target of KP1019 opens new opportunities for rational design of combination drug therapies.

B228/P1221

Chromatin remodeling in sodium valproate-treated U251MG glioblastoma cells as assessed by image analysis

C. B. M. Oliveira, M. A. Rocha, M. L. S. Mello; Department of Cell Biology, University of Campinas - UNICAMP, Campinas SP, BRAZIL.

Sodium valproate (VPA), a drug widely prescribed as an anticonvulsant, is a well-known class I histone deacetylase inhibitor (HDACi), a DNA demethylation inductor and a promising antitumor agent. Cells of glioblastoma, a very aggressive tumor of the central nervous system, have been reported to contain increased levels of DNA methylation in several of their gene promoters. When cultivated in the presence of VPA, glioblastoma cells not only undergo histone H4 hyperacetylation but also exhibit decrease in their DNA methylation profile. In this work, we investigated whether these VPA-induced epigenetic changes are accompanied by chromatin remodeling characteristics in Feulgen-stained U251MG glioblastoma cells that could be revealed using image analysis. Such an analysis was performed in an Axiophot 2 microscope equipped with AxioCam HRc video camera and Kontron KS-400-3 software (Carl Zeiss, Oberkochen/Munich, Germany). U251MG cells cultivated in the presence of 1 mM and 10 mM VPA for 4 and 24 h and of 5 µM 5-aza-CdR for 28 h (a control of DNA demethylation) were used. Increase in the acetylation levels of histone H3 was demonstrated with the VPA treatments but not with the 5-aza-CdR assay, whereas decrease in the global levels of 5-methylcytosine followed treatments with both drugs. A decreased variability in the degree of chromatin packing per nucleus and in nuclear entropy, defined in terms of number of bits needed to store densitometric values per nuclear image, in the cells treated with the longer VPA treatment especially at the 10 mM concentration, but not with the 5-aza-CdR assay, revealed chromatin decondensation. These results suggest that the chromatin remodeling induced by VPA in U251MG cells, that may favor gene expression, is more involved with histone acetylation than with DNA demethylation.

Financial support: FAPESP (grant no. 2015/10356-2), CAPES, CNPq (grants no. 421299/2018-5, 304797/2019-7)

B229/P1222

Assessment of NK Cell-based Immunotherapies Using an Optimized Multiplexed Assay and Advanced Flow Cytometry

J. Lovchik, M. Carter; Sartorius Bioanalytical Instruments, Inc., Albuquerque, NM

NK cells are potent cytotoxic effector cells that offer great promise for clinical utilization in treating cancers without the adverse side-effects associated with T cell-based cytotherapies. Hence, there is currently a focus on the development of NK cell-based cancer immunotherapies, including chimeric antigen receptor-engineered NK (CAR-NK) cells and tumor-specific monoclonal, bi-specific and tri-specific NK cell engaging antibodies (mAbs, BIKES, and TRIKES) that can induce NK cell-mediated antibody dependent cellular cytotoxicity (ADCC). In this study, we utilized a Burkitt lymphoma cell model to examine the effect of cytokine activation on NK cell-mediated killing, and the potency of different anti-hCD20 mAbs in NK cell-mediated ADCC across multiple donors. Assessing tumor cell killing is critical in evaluating the efficacy of new NK cell-based immunotherapies. However, full characterization

requires concurrent analysis of multiple factors, including expression of activation markers and cytokine production which involve performing multiple assays requiring a high number of effector cells. For this reason, we utilized a rapid, high throughput, multiplexed, flow cytometry assay that not only measures target cell killing, but also determines expression of NK cell activation markers and cytokine production using 10 µL of sample in either 96 or 384-well plate format. The results showed that direct tumor cell killing was induced upon cytokine activation and corresponded to increased production of several cytokines, plus expression of CD25 and CD69 activation markers. Tumor cell killing was also induced upon addition of certain anti-CD20 mAbs to unactivated NK cells, although the level of ADCC depended on which mAb was used. Corresponding increases in Granzyme B, CD178 (Fas Ligand), IFNγ, TNF, and RANTES secretion were also observed. In general, higher levels of ADCC and effector protein secretion were seen using a non-fucosylated mAb. However, differences were observed in both the degree of ADCC and cytokine expression between donors that corresponded to differences in their CD16 genotype. Overall, use of this multiplexed assay revealed tumor cell killing by NK cells was effectively enhanced by cytokine activation or addition of anti-tumor mAbs, and simultaneously provided characterization of the NK cells in relation to tumor target killing.

B230/P1223

A Novel PSMA Targeting Small Molecule Drug Conjugate Can Successfully Enter PSMA-Positive Prostate Cancer Cells Through Receptor Mediated Endocytosis

B. McAllister[1], L. Caromile[1], C. Berkman[2]; [1]UConn Health, Farmington, CT, [2]Washington State University, Pullman, WA

A common roadblock in cancer research is drug delivery. The primary tumor microenvironment can prevent a drug from reaching its target, which can cause off-target effects if not tumor specific. Prostate specific membrane antigen (PSMA) is a transmembrane protein with high therapeutic potential due to its specific overexpression in prostate cancer. Targeting PSMA for drug delivery is already being implemented; however, many designs struggle with efficacy and durability. This study aims to investigate a PSMA targeting small molecule drug conjugate's (SMDC) ability to target PSMA-positive prostate cancer cells, with the goal of improving delivery, uptake, and efficiency of treatment moieties such as radioligand therapy. The SMDC, 5FAM-X-FPO-42 (5FAM) contains a specific, irreversible PSMA targeting region and is conjugated to a 488 fluorophore. To demonstrate that PSMA SMDC was specific, we treated C42B PSMA-positive and C42B$^{PSMA\text{-}KO}$ cells with the SMDC and found that 5FAM specifically interacted with cell surface PSMA of the C42B PSMA-positive cells, not the C42B$^{PSMA\text{-}KO}$, and was subsequently internalized into the cell. It is known that PSMA is internalized into the cell by receptor mediated endocytosis of clathrin coated pits. To further characterize the SMDC's trafficking through the cell, we found that PSMA and 5FAM both colocalized in the endosome (EEA1) and lysosome (LAMP1) but only low levels of PSMA was visible in the endoplasmic reticulum (PDI) and Golgi (RCAS1). The SMDC was not detected hinting that the 5FAM might have been cleaved off the linker by low pH. Cell fractionation and a western blot verified that PSMA was in the membrane/organelle fraction of C42B PSMA-positive cells. These results provide evidence that the PSMA targeting SMDC is effectively and exclusively targeting PSMA-positive cells *in vitro*. To further investigate the trafficking of the PSMA SMDC, this study aims to follow the movement of PSMA undergoing endocytosis while interacting with the SMDC to investigate if PSMA is recycling back to the cell membrane. PSMA recycling is essential for treatment of PSMA positive prostate cancer as it continuously provides a therapeutic target. Therefore,

this research will provide a better understanding of the trafficking of the SMDC, its interaction with PSMA during receptor mediated endocytosis and its potential clinical utility.

B231/P1224

Differential Radiation Responses in alt-NHEJ Pathway in Human Neuroblastoma Cells and Human Glioblastoma Cells

Q. Randle, G. Regisford, P. Saganti, H. Wang; Biology, Prairie View A&M University - Prairie View, TX, Prairie View, TX.

Radiation DNA double-strand breaks are repaired by homologous recombination(HR) and non-homologous end-joining (NHEJ). Poly(ADP-ribose) polymerase I (PARP-1) is involved in the alternative NHEJ pathway (alt-NHEJ). To improve radiotherapy efficacy in human malignant glioblastoma tumors and to decrease toxicity in human neurons, we investigated the radiation response in human neuroblastoma cells (SH-SY5Y) and human malignant glioblastoma cells (U251 MG) and the regulation of poly(ADP-ribose)polymerase I (PARP-1), Ataxia telangiectasia-mutated (ATM) and Nicotinamide adenine dinucleotide (NAD) on radiation sensitivity, DNA damage, and repair, apoptosis, and autophagy. Cell survival fractions of radiation dose were determined by clonogenic assays in U251 MG and SH-SY5Y cells, pretreated with a triple-component complex consisting of PARP inhibitor, ATM inhibitor, and NAD supplements. Necrosis and apoptotic cells were detected by a combination of propidium iodide and Hoechst 333342staining and analyzed using Flow Cytometry and confocal microscopy. DNA damage was detected by immunostaining gamma-H2AX and 53BP1 and DNA damage foci were observed under confocal microscopy and quantified by Image J. Radiation-induced autophagy was detected by fluorescent Live Cell Imaging of GFP-LC3 MediatedAutophagy under confocal microscopy. Our results demonstrated that the triple-component complex significantly increased the radiation sensitivity of U251 MG, but notSH-SY5Y cells. The synergistic interaction of the triple complex inhibitors on DNA damage and repair is under investigation. Our study suggests that modulation of the alt-NHEJ pathway may enhance radiotherapy efficacy in human malignant glioblastoma tumors.Differential Radiation Responses in alt-NHEJ Pathway in Human NeuroblastomaCells and Human Glioblastoma Cell

Cancer Therapy-3D Culture Models

B232/P1225

AI-enabled Novel Workflow to Evaluate Cell Therapy Using 3D Spheroid Models

Z. Tong, A. Lim, A. Katiyar, T. Baranowski, J. Baade, O. Sirenko; Molecular Devices, LLC, San Jose, CA.

T-cell therapies are designed to help our immune system fight cancer cells, including CAR T cells, tumor infiltrating lymphocytes (TIL), and other genetically modified T cells. In recent years, the field of cell therapy has started to expand, among which, the launch of the first CAR T-cell therapies to treat blood cancer in 2017 was a critical milestone in this field. Despite its boom, the discovery of novel immunotherapies that specifically enhance T-cell response against cancer cells remains a challenge task, especially when trying to find robust in vitro models to evaluate these immunotherapies throughout their development. In the past, the models have been limited to the use of suspension cells and 2D cell monolayers, which lack the key features of 3D extra-cellular microenvironment. In recent studies, 3D advanced tumor models, such as multicellular spheroids, are believed to bring a closer resemblance to the in vivo clinical scenario because it has more complex cell-cell interactions, optional extracellular

matrix proteins, and a layered structure. In addition, these models have the added requirements for immune cells to penetrate the layered structure during killing. Moreover, the information associated with how the phenotype of the 3D model evolves and how the penetration of T cell into the 3D model can be measured has not been fully explored and exploited to evaluate the cell therapy.
To address some of the obstacles mentioned above, we used multi-cellular spheroids cultivated from Hela cell line in 96-well round bottom plate as our 3D model. In this study, we stained and activated the human peripheral blood mononuclear (PBMC) cells with PMA/ionomycin for 6 hrs, treated the spheroids with stimulated PBMC for 3 days, and imaged the whole plate with a high-content confocal imager every 2hrs. For the evolution of the phenotypic change of the spheroids analysis, a deep learning-based segmentation model was generated to mask the whole spheroid and the edge of the spheroid. The resulting masks were then used to classify the spheroids using machine learning, where the clear distinct phenotypic changes of the spheroids were observed compared to controls, allowing for live monitoring of the T cell efficacy. To analyze how T cells penetrated inside the spheroids, we calculated the coordinates of the penetrated T cells and the distance of cell movement from the nearest edge of the spheroid mask. By collecting the statistics of the distances and the time, we measured the speed of the T cell penetration into spheroids. Overall, our results show that the 3D spheroid models and the high-content analysis workflow may potentially be used as a metric to evaluate the efficacy of cell therapy in vitro.

B233/P1226

The Identification and Characterization of FOLFOX Resistance in Colorectal Cancer Patient Derived Organoids

J. Morris; Cellular and Molecular Medicine, University of Arizona, Tucson, AZ.

Resistance to chemotherapy drugs is a well-documented issue inhibiting the treatment of a wide variety of cancers. Colorectal cancer (CRC) is the third most common neoplasm worldwide and has the third highest mortality rate. However, the longtime gold-standard FOLFOX chemotherapy regimen, consisting of 5-FU and Oxaliplatin, results in only 70% survival rates in Stage 3 CRC patients, worse in Stage 4. More recent targeted therapies have marginally improved on this number, but the fact remains that CRC represents a highly heterogeneous neoplasm and resistance to standard chemotherapeutic regimens poses a significant barrier in treating this devastating disease. There is an immediate need to better understand how this resistance arises. We hypothesize that a small number of cells with high phenotypic plasticity (HPP) are able to remain dormant during the initial chemotherapy treatment then reenter the cell cycle to cause recurrence and metastasis months or even years after treatment has concluded. Patient-derived organoids (PDOs) are a recently developed *ex vivo* model that allows for the study and extensive characterization of such HPP cell populations that may be found in patient tumors. We are developing and expanding various PDO populations to examine them genotypically and phenotypically on the single-cell level through RNA sequencing and high throughput imaging, respectively. By identifying these HPP cell populations, we then discover drugs that prevent the development of drug-resistant subpopulations. In the long term, we use our tumor phenotype profile to develop models that predict the responsiveness of tumors, including their drug-resistant HPP subpopulations, to well-documented and clinically used oncology drugs, streamlining treatment and improving survival outcomes. Thus far we have characterized the phenotypes of five different tumor PDO lines as well as a polyp control. We observe that each tumor has a vastly different morphological and phenotypic profile

and as such are susceptible to different drugs. However, some drugs appear to target the stem cell compartment and thus slow or stop the growth of all lines examined so far.

B234/P1227

Evaluating drug response in 3D triple negative breast cancer organoids with high content imaging and analysis

O. Sirenko; Molecular Devices, San Jose, CA.

Triple negative breast cancer is a clinically aggressive subtype, with high rates of metastasis, recurrence, and drug resistance and few clinically approved small molecule targeted therapies. There is a critical need to develop methods for efficient testing drug efficacy in patient-derived tumor samples to discover new therapeutic targets. Additionally, most potential oncology drugs fail the drug development pipeline despite having promising data for their efficacy *in vitro*, further reflecting the need for research models that more accurately model tumor biology. Two-dimensional (2D) cell culture remains the primary method of drug screening, despite being considered less physiologically relevant than three-dimensional (3D) culture. Challenges with 3D culturing methods, such as increased complexity and technical issues in performing and analyzing experiments, have prevented its widespread adoption as a primary screening method during drug studies. However, these challenges can be addressed by using automated high content imaging and analysis systems. In the present study, we describe automation of imaging and cell culture methods which enables scaling up complex 3D cell-based assays and compound screening. In addition, we show advanced analysis approaches and descriptors that allow scientists to gain more information about complex cellular systems, disease phenotypes, and compound effects. Using these methods, we measured responses to drug treatment in 3D mammospheres, including cytotoxicity, metabolism, and altered cell morphology. Tumoroids were formed from primary cells isolated from a patient-derived tumor explant, TU-BcX-4IC, that represents metaplastic breast cancer with a triple-negative breast cancer subtype and treated with 165 compounds of approved cancer drugs at multiple concentrations. We characterize multiple quantitative descriptors for tumor phenotypes and compound effects including characterization of tumoroid size, integrity, cell morphology and viability. In addition, the Cell Painting method was used for 3D tumoroids for evaluation of phenotypic effects. Principal component analysis was used to identify hits and cluster them based on similarity of phenotypes. Eight compounds were detected that demonstrated effects at low concentrations (10 nM), including romidepsin, trametinib, bortezomib, carfilzomib, panobinostat, that will be further investigated as potential drug candidates. 33 additional compounds were identified as effective at higher concentrations. In this study, we demonstrate methods for increased throughput and automation in 3D cancer assays that are suitable for compound screening using patient-derived samples.

B235/P1228

Genomic heterogeneity in pancreatic cancer organoids and its stability with culture

O. Usman[1], L. Zhang[2], G. Xie[1], H. M. Kocher[3], C. Hwang[4], Y. Wang[1], X. Mallory[2], **J. Irianto**[1]; [1]Department of Biomedical Sciences, Florida State University, Tallahassee, FL, [2]Department of Computer Science, Florida State University, Tallahassee, FL, [3]Barts Cancer Insitute, London, UNITED KINGDOM, [4]University of California Davis, Davis, CA.

The establishment of patient-derived pancreatic cancer organoid culture in recent years creates an exciting opportunity for researchers to perform a wide range of *in vitro* studies on a model that closely

recapitulates the tumor. Among the outstanding questions in pancreatic cancer biology are the causes and consequences of genomic heterogeneity observed in the disease. However, to use pancreatic cancer organoids as a model to study genomic variations, we need to first understand the degree of genomic heterogeneity and its stability within organoids. Here, we used single-cell whole-genome sequencing to investigate the genomic heterogeneity of two independent pancreatic cancer organoids, as well as their genomic stability with extended culture. Clonal populations with similar copy number profiles were observed within the organoids, and the proportion of these clones was shifted with extended culture, suggesting the growth advantage of some clones. However, sub-clonal genomic heterogeneity was also observed within each clonal population, indicating the genomic instability of the pancreatic cancer cells themselves. Furthermore, our transcriptomic analysis also revealed a positive correlation between copy number alterations and gene expression regulation, suggesting the functionality of these copy number alterations.

B236/P1229

Novel patient derived colorectal cancer organoid platform for automated high throughput drug discovery applications.

A. Lim[1], Z. Tong[1], E. Fraser[2], V. Durban[2], P. Macha[1], O. Buddhikot[1], T. Baranowski[1], j. Baade[1], A. Katiyar[1], H. Amijee[2], O. Sirenko[1]; [1]Molecular Devices, San Jose, CA, [2]Cellesce Biotechnology, Cardiff, UNITED KINGDOM.

There is a critical need to develop methods for efficient testing of drug efficacy using patient-centric models, to discover new therapeutic targets. Most potential oncology drugs fail at the later stages of the drug development pipeline and in clinical trials, despite having promising data for their efficacy in vitro. This high failure rate is partly due to insufficient predictive models being used to screen drug candidates in the early stages of drug discovery.

3D cell models, specifically patient-derived organoids (PDOs), offer a promising solution to this problem. Cells grown in 3D can better mimic cell-cell interactions and the tissue microenvironment, including cancer stem cell niches. Studies show that patients and their derived organoids respond similarly to drugs, suggesting the therapeutic value of using PDOs to improve therapeutic outcomes. However, challenges commonly associated with using these organoids, such as assay reproducibility, ability to scale up, and cost have limited their widespread adoption as a primary screening method in drug discovery.

To address some of the hurdles associated with the use of PDOs in large scale screens, a semi-automated bioprocess has been developed for the controlled production of standardized PDOs at scale. They are of a uniform size, high viability and are produced in repeatable batches in an assay-ready format.

In this study, patient-derived colorectal cancer organoids were seeded in 384 or 96-well microtiter plates manually, or with an automated cell bioprinter and dispenser. These PDOs were treated with selected anti-cancer compounds at various concentrations. Compound effects were monitored over time using transmitted light imaging. For the analysis of organoid growth and development, a deep learning-based image segmentation model was developed to automate the segmentation of the organoids. Using this approach, we tracked the effects of the compounds on colorectal organoid size, morphology, texture, and additional morphological and phenotypic readouts. A viability assay was carried out using live/dead cell dyes and the PDOs were imaged in 3D on a high-content confocal imager. Out of the tested panel of known anti-cancer drugs, we found that PDOs treated with

romidepsin and trametinib showed the most significant reduction in size, with a greater number of dead cells compared to the other compounds and controls. Overall, our results show the potential for the utility of PDOs in both precision medicine and high throughput drug discovery applications, when using automation with high-content imaging.

B237/P1230

Probing tumor spheroid hypoxia using red/near-infrared ratiometric O_2-sensitive nanoparticles

A. C. Debruyne[1], I. A. Okkelman[1], S. M. Borisov[2], R. I. Dmitriev[1]; [1]Human Structure and Repair, University of Ghent, Gent, BELGIUM, [2]Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Graz, AUSTRIA.

The role of the tumor microenvironment is paramount in supporting cell survival, promoting local invasion and metastatic dissemination. While the limited nutrient availability and hypoxia are known to be important contributors to the 'niche' environment and lead to metabolic cell heterogeneity, their quantitative non-destructive analysis is rather difficult. For instance, analysis of real-time oxygenation in such 3D cell cultures as spheroids and organoids, is rarely performed. To address this, we produced cell-penetrating O_2-sensitive nanoparticle probes, which can enable measurements of spheroid oxygenation and consumption rate on a conventional fluorescence microscope. We used near-infrared O_2-sensitive (PtTPTBPF) metalloporphyrin and red aza-BODIPY reference dyes to increase the light penetration depth and used well-known biocompatible polymer nanoparticles (Eudragit RL100 and PMMA-AA) to achieve efficient cell staining. The resulting probe, termed 'MMIR' (multi-modal infrared) enables measuring oxygenation gradients via fluorescence emission ratiometric measurements or the phosphorescence lifetime imaging microscopy (PLIM).To test the brightness, photostability, phototoxicity, and reproducibility of MMIR, we evaluated different reference:sensing dye (1:1, 1:0,5 and 0,5:0,5) ratio and the presence of nanoparticle charge groups. We examined multiple cell lines including HCT116, PANC-1, dental pulp stem cells, HUVEC, MCF-7, and SKOV-3. Preliminary results showed cell line-dependent staining and lower aggregation in case of negatively charged probe. No significant toxicity was found and after optimizations of the staining protocol, nanoparticle probes were used for staining 3D spheroid cultures and subsequent analysis of O_2 gradients. Surprisingly, the human colon cancer HCT116 spheroids displayed an "inverted" oxygenation gradient, with elevated oxygenation at the spheroid core, compared to the cells located at the periphery. This can be explained by the presence of necrotic core and the large size of spheroids (~700 µm), which cannot consume O_2. In addition, using the MMIR probe we studied how the growth medium composition can influence the oxygenation gradients. Collectively, presented ratiometric O_2-sensing nanoparticle probes provide means for multi-parameter quantitative oxygenation measurements and characterization of 3D cultures. Funding: supported by the Special Research Fund (BOF) grant of Ghent University (BOF/STA/202009/003).

B238/P1231

Investigating the effects of confined cell migration on chromatin organization

J. McAllister[1], C.-R. Hsia[2], O. Hasan[1], J. Judd[1], S. Lee[1], R. Agrawal[1], C.-Y. Chang[1], P. Soloway[1], J. Lammerding[1]; [1]Cornell University, Ithaca, NY, [2]National Institutes of Health, Bethesda, MD

Cancer cells must migrate through small interstitial spaces during metastasis. These invasion events require extensive deformation of the cell body and nucleus, which can result in changes in chromatin organization. Previous studies have shown that 2D migration can induce heterochromatin formation in

cells, but this effect has not yet been investigated in 3D environments. In my research, we use polydimethylsiloxane (PDMS) microfluidic devices that mimic 3D interstitial spaces to study the effect of confined migration on chromatin organization. We demonstrate that confined 3D migration induces significantly more heterochromatin formation in human fibrosarcoma cells than unconfined 3D migration, and that histone-modifying enzyme HDAC3 is involved in this process. We have also shown that confined migration leads to changes in chromatin accessibility, which may alter gene expression to confer cells migratory advantages during confined migration. Whereas previous studies found that mechanical compression can induce heterochromatin formation via nuclear translocation of HDAC3, we found that HDAC3-dependent increases in heterochromatin during confined migrations are due to an activating change in its phosphorylation status. Previous studies have also shown that mechanical stimuli (stretch) can activate mechanosensitive ion channels and lead to changes in chromatin organization. Thus, we hypothesize that confined migration activates mechanosensitive Ca^{2+} channels, which contribute to heterochromatin formation. Indeed, preliminary experiments suggest that inhibition of stretch-sensitive Ca^{2+} channels and Ca^{2+} chelation reduces heterochromatin formation during confined migration in fibrosarcoma cells. These findings are an important first step to understanding how confined migration influences chromatin modifications and may provide insights into future therapies for invasive cancers.

B239/P1232

Exploring the role of fibroblast-shed syndecan-1 ecto domain molecules in the promotion of mammary tumor formation

R. Bellin, G. Aucello, A. Maher, C. McCutcheon, M. Slanetz, J. Kelly, B. Lawton; College of the Holy Cross, Worcester, MA

It has been shown that chemical cross-talk between ductal epithelial cells and stromal fibroblasts in mammary gland tissue can play a role in promoting the development of breast cancer, specifically the formation of ductal carcinomas. Studies have suggested that the ecto domain of syndecan-1 (sdc-1), a transmembrane heparan sulfate proteoglycan, is one possible molecular basis for this cross-talk as it can serve as driver of epithelial cell division by acting as a growth factor co-receptor. In addition, clinical studies have linked the aberrant expression of sdc-1 in tumor proximal fibroblasts to poor breast cancer prognosis. In our current study, using a combination of conventional 2D tissue culture approaches and a 3D mammary duct-on-a-chip model system, we are testing the hypothesis that sdc-1 ecto domain molecules shed from stromal fibroblasts can serve as co-receptors for epithelial cell FGF receptors, thereby stabilizing the growth factor-receptor complex and driving elevated levels of epithelial cell division. In 2D dish-based studies, we have demonstrated that NIH-3T3 fibroblast cells actively express and shed sdc-1 ectodomain molecules when grown in culture, an expression pattern that not typical of most fibroblasts present in tissues. When epithelial cells were co-cultured with NIH-3T3 cells using a 2D Boyden chamber system, FGF-driven mammary epithelial cell proliferation was significantly increased in comparison to epithelial cells grown in the absence of fibroblasts. To expand the physiological relevance of these findings, we have adapted an existing 3D mammary duct-on-a-chip model system to allow the inclusion of stromal fibroblasts and used CRISPR/Cas9 to generate a paired fibroblast line unable of expressing sdc-1. We are using a combination of qualitative ductal atypia formation assays and quantitative in-chip proliferation assays to test if our 2D culture findings can be replicated in a culture system with a more tissue-relevant 3D structure.

B240/P1233

Simple 3-D *in-vitro* Solid Tumor Gliospheres Advance the Study of Cellular Interactions Between CAR T-cells and Solid Brain Tumor Cells

I. I. Torres-Vazquez, E. Rosa-Molinar; The University of Kansas, Lawrence, KS

Chimeric antigen receptor (CAR T)-cells are developed by genetically modifying patients' T cells to express an artificial T cell receptor designed to be specific against the patient's tumor associated antigen, and they are used in CAR T-cell therapy to treat non-solid and solid brain tumors. Despite CAR T-cell therapy's effectiveness against non-solid brain tumors, Car T-cells' penetration and efficacy in treating solid brain tumors such as glioblastoma multiforme (GBM) are significantly limited.
To overcome this barrier, we developed a simple 3-D *in-vitro* solid tumor gliospheres to study the cellular interactions between CAR T-cells and solid brain tumor cells. We generated 3-D gliospheres from low-passage, serum-free cell lines derived from GBM patient tumors, GBM-39 (MC PDX NR, GMB 39); as a control we generated 3-D gliospheres using a commercially available cell line, LN-18 (ATCC, CRL-2610). Multi-modal widefield fluorescence imaging combined with whole-mount immunostaining for glial, neuronal, glioma stem cell markers, as well as EGFR permitted us to determine the cellular phenotype present as well as the presence of EGFR within GBM-39 and LN-18 gliospheres. To study the cellular interactions between the cellular phenotypes, present in the GBM-39 and LN-18 gliospheres and Car T-cells, both GBM-39 and LN-18 gliospheres were exposed to Jurkat epidermal growth factor receptor (EGFR) CAR T-cells. We imaged the GBM-39 and LN-18 gliospheres exposed to EGFR CAR T-cells at 24, 48, and 72 hours. In GMB-39 gliospheres, the effects of EGFR CAR T-cells were observable five (5) days post exposure. In contrast, in LN-18 gliospheres, EGFR CAR T-cells effects were observable 24 hours post exposure.
In the GBM 39 type, EGFR CAR T-cells were not found throughout the gliospheres; rather they were restricted to the outer layer. In the LN-18 gliospheres, EGFR CAR T- cells rapidly penetrated and freely distributed themselves throughout the gliospheres. In addition, LN-18 gliospheres were reduced in size. These differences suggest something is limiting EGFR CAR T-cells' penetration of GBM-39 gliospheres which is the focus of ongoing research effort.
Research Supported by: KUCC/NCI GR16221

Bioengineering, Microenvironment and Metastasis

B241/P1234

Mechanism and Targeting of Adaptive Resistance to Anchorage Independent Cell Death in Cancer

M. Monavarian[1], E. F. Page[1], A. Kumari[1], R. Rajkarnikar[1], L. Quintero-Macias[1], S. Sathoo[2], L. Lanov[3], F. Massicano[3], E. A. Worthey[3], M. K. Jolly[2], E. V. Broude[4], A. Singh[5], K. Mythreye[1]; [1]Division of Molecular Cellular Pathology, Department of Pathology, O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham Heersink School of Medicine, Birmingham, AL, [2]Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore, INDIA, [3]Center for Computational Genomics and Data Sciences, University of Alabama at Birmingham, Birmingham, AL, [4]Department of Drug Discovery and Biomedical Sciences, University of South Carolina College of Pharmacy, Columbia, SC, [5]Electrical and Computer Engineering, University of Delaware, Newark, DE.

Intraperitoneal metastasis in cancer such as ovarian (OC) is characterized by accumulation of malignant ascites containing tumor cells that evade cell death in suspension contributing to poor patient outcomes

including distant metastasis and enhanced chemoresistance. We find that development of resistance to cell death under anchorage independence or anoikis resistance can occur in an oncogene agnostic but transcriptional reprogramming manner. Adapted cells are significantly more invasive and chemo resistant in vitro and promote distant metastasis to the lungs in vivo. Bulk transcriptomics and kinome analysis reveals strong MYC dependent repression programs and increased JAK STAT activities, with resistant cells characterized by hybrid epithelial/mesenchymal phenotypes and reduced apoptosis. In testing models for development of resistance we find that difference in proliferation rates, does not explain the outcomes in the longitudinal data. Thus using a modified Luria-Delbrück's "fluctuation analysis' we find a transiently primed stress-tolerant state where single cells reversibly switch back to being stress sensitive after being tolerant for several generations. This transient memory as estimated from the extent of clone-to-clone fluctuations in survival under stress is consistent with experimental loss of the tolerant state. Notably, inhibition of transcriptional reprogramming globally early in the process using specific CDK8/19 inhibitors abrogates development of resistance and concomitantly suppresses metastasis in vivo. These and additional characteristics of resistant cells will be presented.

B242/P1235

microRNA-18a regulates the metastatic properties of oral squamous cell carcinoma cells via HIF-1α expression

S. Kim[1], J. Oh[2], J. Choi[1]; [1]Oral Pathology, Gangneung-Wonju National University, Gangneung-si, KOREA, REPUBLIC OF, [2]Oral and Maxillofacial Surgery, Gangneung-Wonju National University, Gangneung-si, KOREA, REPUBLIC OF.

Rapid metastasis of oral squamous cell carcinoma (OSCC) is associated with a poor prognosis and a high mortality rate. However, the molecular mechanisms underlying OSCC metastasis have not been fully elucidated. Although deregulated expression of microRNA (miRNA) has a crucial role in malignant cancer progression, the biological function of miRNA in OSCC progression remains unclear. This study aimed to investigate the function of miRNA-18a in OSCC metastatic regulation via hypoxia-inducible factor 1α (HIF-1α). miRNA-18a expressions in patients with OSCC (n = 39) and in OSCC cell lines (e.g., YD-10B and HSC-2 cells) were analyzed using quantitative real-time polymerase chain reaction. HIF-1α protein expressions in OSCC cells treated with miRNA-18a mimics or combined with cobalt chloride were analyzed using western blotting. The miRNA-18a expression-dependent proliferation and invasion abilities of OSCC cells were analyzed using MTT assay, EdU assay, and a Transwell® insert system. miRNA-18a expression was significantly lower in OSCC tissue than in the adjacent normal tissue. In OSCC cell lines, HIF-1α expression was significantly decreased by miRNA-18a mimic treatment. Furthermore, the migration and invasion abilities of OSCC cells were significantly decreased by miRNA-18a mimics and significantly increased by the overexpression of HIF-1α under hypoxic conditions relative to those abilities in cells treated only with miRNA-18a mimics. miRNA-18a negatively affects HIF-1α expression and inhibits the metastasis of OSCC, thereby suggesting its potential as a therapeutic target for antimetastatic strategies in OSCC.

B243/P1236

Excess cholesterol synthesis driven by upregulation of lamin B receptor causes nuclear envelope fragility in metastatic melanoma

M. A. Baird[1], A. Cartagena-Rivera[2], C. Jewett[3], M. Pirooznia[1], R. Fischer[1], C. Waterman[1]; [1]National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, [2]National Institute of Biomedical Imaging and Bioengineering (NIBIB), NIH, Bethesda, MD, [3]Johns Hopkins University, Baltimore, MD.

Malignant melanoma (MM) is characterized by its mutational heterogeneity and aggressive metastatic spread. During metastasis, MM cells migrate through diverse microenvironments, including regions of dense tissue confinement to reach the vasculature. Microenvironmental confinement of tumor cells causes nuclear deformation, resulting in loss of nuclear envelope (NE) integrity and DNA damage, improper repair of which leads to genomic aberrations and genetic heterogeneity in the population. We hypothesize during metastatic progression, expression of NE genes is altered, increasing nuclear deformability and fragility, thus leading to increased genetic heterogeneity within the population. To test this hypothesis, we used a PDMS confinement device to assay for NE fragility in a range of metastatic and benign cancer cell types, revealing that metastatic cells displayed increased susceptibility to NE rupture. We then performed bioinformatic analysis of RNA-seq data sets from patient samples of MM and benign nevi, revealing potential NE proteins upregulated in metastatic disease. A targeted siRNA-based screen of upregulated NE genes showed knockdown of lamin B receptor (LBR) strongly reduced NE fragility in MM cells, and ectopic overexpression of LBR was sufficient to increase NE fragility in benign melanocytes. Similarly, atomic force microscopy showed knockdown of LBR in MM cells caused decreased nuclear deformability, while overexpression of LBR in benign melanocytes was sufficient to increase nuclear deformability. siRNA rescue experiments utilizing protein domain truncations and human disease-related point mutations in LBR demonstrated that the cholesterol synthase activity of LBR was required for increased NE fragility and deformability, independent of LBR's additional roles tethering heterochromatin and lamin B to the NE. Furthermore, reduction of LBR decreased cellular cholesterol and caused altered cholesterol distribution in the NE, potentially creating local membrane defects for NE rupture. These results show that upregulation of LBR in MM plays dual roles in reducing nuclear deformability and increasing NE rupture through alterations in cholesterol distribution in the NE. Utilizing whole genome sequencing, mouse tumor models and in vitro tumor spheroids, current work focuses on LBRs potential role in generating chromothripsis and genetic diversity in MM tumors.

B244/P1237

A link between size and phenotype in tetraploid cancer cells

M. Bloomfield, T. Chau, S. Huth, D. McCausland, A. McCaffrey, K. Fluet, D. Cimini; Virginia Tech, Blacksburg, VA.

Changes in DNA content can affect cell and nuclear size, and both tetraploidy and cell/nuclear size alterations have been linked to tumorigenesis. To investigate a potential link between tetraploidy and cell/nuclear size in tumorigenesis, we experimentally induced whole genome doubling (WGD) in diploid (2N) breast and colorectal cancer cells and generated single-cell tetraploid (4N) clones. We found that cell and nuclear volume do not always scale with DNA content after WGD and identified 4N clones that were classified as small and large based on their cell and nuclear size. While cell and nuclear volume doubled in the large 4N clones, they only increased by ~50% in the small 4N clones compared to 2N

counterparts. Both the small and large 4N clones maintained near-4N genomes, confirming that the size variations are not caused by a difference in chromosome number. Analysis of tumor histology images from The Cancer Genome Atlas (TCGA) also revealed that nuclear size of cancer cells increased in WGD+ compared to WGD- tumors. Interestingly, cancer cell nuclear area varied among WGD+ tumors, consistent with our small and large 4N clones, indicating that size variations also occur following WGD *in vivo*. Further characterization of our 4N clones showed that the small and large 4N clones displayed distinct mitotic phenotypes and tumor-like behavior. The large 4N clones had a higher incidence of unaligned chromosomes, multipolar spindles, and anaphase lagging chromosomes compared to the small 4N clones and the 2N cells. Moreover, we found that the small 4N clones outperformed the large 4N clones and 2N parental cells in several phenotypic assays, including cell migration and soft agar colony formation. These findings suggest that the small 4N clones may have increased tumorigenic potential relative to the large 4N clones. We are currently testing the hypotheses that cancer cell nuclear area is predictive of clinical outcome in WGD+ tumors, and that the mechanism(s) restricting cell and nuclear size scaling after WGD are responsible for the phenotypic differences in the 4N cells. Overall, our findings suggest that cell/nuclear size could be an important factor for the prognosis of WGD+ tumors.

B245/P1238

CanCel TatToo: receptor-mediated ablation of cancer cells and beyond

V. Purde[1], R. Shakya[1], E. Kudryashova[1], T. Cripe[2], **D. Kudryashov**[1]; [1]Ohio State University, COLUMBUS, OH, [2]Nationwide Childrens' Hospital, COLUMBUS, OH.

The application of protein toxins for therapeutic cell ablation is limited by their severe side effects due to high on- and off-target toxicity. We propose that the selectivity of targeting can be enhanced by intein-based toxin reconstitution from two dysfunctional fragments delivered to the cytoplasm via selective independent pathways. We designed a robust splitting algorithm and achieved reliable cytoplasmic reconstitution of functional Diphtheria toxin from engineered intein-flanked fragments upon receptor-mediated delivery of one of them to the cells expressing the remaining part. Retargeting the delivery machinery towards different receptors overexpressed in cancer cells enables selective ablation of specific sub-populations in mixed cell cultures. In a mouse model, the transmembrane delivery of a split-toxin construct potently inhibits the growth of subcutaneous xenograft tumors expressing the split-counterpart. Interestingly, delivery of a split part to the cells infected by a virus encoding the second part, clears the virus, allowing the cells to survive even high MOI doses of the virus in cell culture. Receptor-mediated delivery of engineered split-proteins provides a platform for precise therapeutic and experimental ablation of tumors or desired cell populations while also expanding the applicability of the intein-based protein trans-splicing for various applications.

B246/P1239

Role of Aging Associated Vascular Senescence on Ovarian Cancer Burden

A. Kumari[1], M. Monavarian[1], L. Macias[1], B. Horst[1], R. Arend[2], K. Mythreye[1]; [1]Department of Pathology and O'Neal Comprehensive Cancer Center, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL, [2]Department of Obstetrics and Gynecology-Gynecologic Oncology, University of Alabama at Birmingham, Birmingham, AL.

Ovarian cancer is the most lethal gynecological cancer, typically diagnosed at advanced stages post-menopausally. The ovaries, which are a primary site of tumor growth for all ovarian cancers, are subjected to rapid changes in the vasculature upon the onset of menopause. In a corollary manner, the vasculature is a primary therapeutic target for the management of advanced ovarian cancers indicating the significance of tumor angiogenesis in diseases progression and metastasis. Given the association between aging endothelial senescence and angiogenesis, we hypothesized that pre-existing senescent endothelial cells in aged and/or menopausal ovaries could contribute to ovarian cancer growth and invasion in the ovaries. To first distinguish the effects of follicular changes associated with the onset of menopause versus the effect of age we used 4-vinylcyclohexene diepoxide (VCD) for inducing menopause in young mice and a physiologically aged model. Menopausal changes of the ovary in both models were confirmed by follicle count and hormonal profile. Intrabursal implantation and assessment of tumor growth kinetics of mouse ovarian cancer cells (MOVCs) (ID8p53-/-) into the bursa of the ovaries from both these models revealed that physiologically aged mice had high tumour growth kinetics as compared to young (6 week old) mice. However, tumour burden in VCD induced mice was not significantly altered as compared to age-matched control mice suggesting that age-associated changes in ovaries contribute to tumor growth. We next assessed whether ovarian endothelial cells contribute to tumour growth, invasion into the ovary, by implanting MOVCs mixed with primary mouse ovarian endothelial cells (MOECs) intrabursally, and comparing to MOVCs alone. We find that mice implanted with MOVCs and MOECs together had significantly higher tumour burden as compared to MOVCs alone. Examining MOECs from aged mice indicate strong senescence features as evident by β-galactosidase staining, γH2Ax immunofluorescences, p16, TRF-1 (Telomeric repeat factor-1) and PAI-1 (Plasminogen activator inhibitor-1) levels. We find that condition media from senescent MOECs from older mice promoted invasion of cancer cells *in vitro*. These findings suggest a potential direct contribution of the aged and senescent vasculature to tumour growth and invasion into the ovary. These findings and ongoing studies will be presented.

B247/P1240

Engineering antibody-based fragments to HPV oncoproteins

T. Smalley, A. Nicolaci, S. Pilon-Thomas, J. Binning; Moffitt Cancer Center, Tampa, FL.

BACKGROUND: Antibodies and their derivatives have revolutionized therapies and research and continue to expand science's biological toolkit. Specifically, the single domain antibody fragment (nanobody) has been a focus of researchers due to its advantageous characteristics such as small size, affinity, specificity, and solubility in the cytosol. In line with these advancements, we developed nanobodies specific to the early oncoprotein E6 found in the Human papillomavirus (HPV) genome. HPV infections are the most common sexually transmitted disease and are linked to ~ 4.5% of the total cancer incidence worldwide; however, there are no targeted therapies for HPV-related diseases and some HPV pathology remains elusive. We hypothesize that the nanobody's engagement of the viral E6

oncoproteins will suppress tumor growth and induce apoptosis in HPV(+) tumor cells, while having no off-target effects in non-infected cells. To test this hypothesis, we will use a DNA-based delivery system to induce intracellular expression of E6 nanobody antagonists in tumors.METHODS: Nanobodies against HPV E6 were selected using a yeast surface display platform. To determine the inhibitory effects of the nanobodies on the HPV(+) cell line CaSki, a colony formation assay (ability to form colonies) and Annexin V-APC/PI assay (apoptosis) were utilized. Further, we derivatized our nanobodies into nanobody-PROTACs. To assess the potential of our PROTACs to promote the proteasomal degradation of E6, a E6-GFP probe was transfected into HEK293T cells alone and in combination with the PROTAC. Levels of GFP were monitored using flow cytometry and microscopy.RESULTS: The colony formation assay showed the significant inhibitory effects of the A2 (n=6, $p < 0.05$) and C11 (n=6, $p < 0.05$) nanobodies. Further analysis of the inhibitory nanobodies showed 2-fold increases in late apoptosis, illustrating neutralizing candidates to move *in vivo*. Nanobody-PROTACs were also shown to promote E6 degradation. Expression of E6-GFP decreased when co-expressed with the nanobody-PROTAC (n=3, $p <0.05$), suggesting the bifunctional nanobody-PROTAC is recruiting E6 to a functional E3 ligase and marking it for proteasomal degradation.CONCLUSION: The summation of these current screens has allowed us to determine the most promising nanobody candidates for inhibition of endogenous E6 in HPV(+) cells. In addition, the results from our preliminary nanobody-PROTAC assay warrants further exploration of this orthogonal approach. Further studies involving the HPV(-) cervical cancer cell line C33A will be used to determine any off target or cytotoxic effects.

B248/P1241

Haploinsufficient tumor suppressor PRP4K is negatively regulated during epithelial-to-mesenchymal transition

L. E. Clarke, A. Cook, S. Mathavarajah, A. Bera, J. Salsman, **E. Habib**, C. V. Iderstine, M. Bydoun, S. M. Lewis, G. Dellaire; Dalhousie University, Halifax, NS, Canada

The pre-mRNA processing factor 4 kinase (PRP4K, also known as PRPF4B) is an essential gene in mammals. However, PRP4K is considered a haploinsufficient tumor suppressor, as partial loss of PRP4K expression in breast and ovarian cancer is associated with aggressive tumour phenotypes including taxane chemotherapy resistance, increased cell migration and invasion *in vitro*, and cancer metastasis in mice. Increased cell migration and invasion is associated with epithelial-to-mesenchymal transition (EMT) during cancer development. Therefore, we examined how partial depletion of PRP4K expression in the mammary epithelial cell lines HMLE and MCF10A affected cell migration the relationship of PRP4K protein expression to the process of EMT. Reduction of PRP4K expression by small hairpin RNA (shRNA) in these epithelial cell lines induced partial EMT marked by the upregulation of mesenchymal markers, such as fibronectin and Zeb1. In addition, depletion of PRP4K reduced or had no effect on 2D migration in the scratch assay but resulted in greater invasive potential in 3D transwell assays. In comparison, depletion of PRP4K in mesenchymal triple-negative breast cancer cells (MDA-MB-231) resulted in both enhanced 2D migration and 3D invasion, with 3D invasion correlated with higher fibronectin levels in both MDA-MB-231 and MCF10A cells. Surprisingly, these cellular changes occurred without changes in E-cadherin as would be expected for induction of full EMT. Induction of complete EMT in MCF10A cells, by treatment with WNT-5a and TGF-β1, or depletion of eukaryotic translation initiation factor 3e (eIF3e) by shRNA, resulted in significantly reduced PRP4K expression. Mechanistically, induction of EMT by WNT-5a/TGF-β1 reduced PRP4K transcript levels, whereas eIF3e depletion led to reduced PRP4K translation. Finally, reduced PRP4K levels after eIF3e depletion correlated with increased YAP activity

and nuclear localization, both of which are reversed by overexpression of exogenous PRP4K. Thus, PRP4K is a haploinsufficient tumor suppressor negatively regulated by EMT, that when depleted in normal mammary cells can increase cell invasion without inducing full EMT.

B249/P1242

Localization of phosphofructokinase-1 to the plasma membrane through a novel interaction with the sodium/hydrogen exchanger NHE1

H. L. Hansen, B. A. Webb; West Virginia University, Morgantown, WV

Dysregulation of glycolysis and increased cell migration are hallmarks of cancer. Cancer cells are polarized, suggesting that glycolytic enzymes may be spatially regulated. We found that the first rate limiting enzyme of glycolysis phosphofructokinase-1 (PFK1) is enriched to the lamellipodia of migrating human and rat breast cancer cells. We hypothesize PFK1 is recruited to lamellipodia to locally generate ATP for cell migration; however the mechanism of recruitment is unknown. Glycolytic enzymes, including PFK1, pyruvate kinase M2, and lactate dehydrogenase A, have been shown to localize to the plasma membrane through interactions with transmembrane ion transport proteins, suggesting the formation of a glycolytic metabolon. We previously identified a novel interaction between the liver isoform of PFK1 (PFKL) and the ubiquitously expressed sodium/hydrogen exchanger NHE1, which is essential for pH homeostasis and directed cell migration and asked if this interaction could localize PFKL to lamellipodia. First, we asked if PFKL and NHE1 colocalize by immunofluorescent labelling. We found that PFKL and NHE1 colocalize in membrane ruffles of CCL39 Chinese hamster ovary fibroblasts but not NHE1-deficient CCL39 cells. To determine the affinity and identify the regions of interaction we generated GST-tagged chimeras of regions of the NHE1 cytoplasmic tail. Using sedimentation binding assays, we found that PFKL binds to a 70 amino acid region (637-707) of NHE1 with high affinity (695±162nM). Next, we asked if the NHE1-PFK1 interaction was isoform specific and determined that PFKL and the muscle isoform PFKM bind to this region; however, the platelet isoform PFKP does not. Next, we asked if the NHE1-PFKL interaction was evolutionarily conserved and found that mouse PFKL and zebrafish PFKLA and PFKLB were able to bind. To identify the residues of PFKL that bind to NHE1, we compared surface residues conserved between the isoforms and species. We have identified 21 potential amino acids on PFKL that participate in this interaction. Future studies will generate binding incompetent mutants of PFKL to determine if the interaction with NHE1 is required to locally produce ATP. Identifying the mechanism of PFKL recruitment to the plasma membrane will provide insight into the spatiotemporal dynamics of glucose metabolism and organization of glycolytic enzymes in cells.

B250/P1243

Single-cell spatial molecular profiling of an *in vitro* 4T1 model of tumorigenesis

G. Abbott[1], S. Forward[1], M. Fahlberg[1], Y.-C. Lin[1], S. Anmalsetty[2], D. A. Wink[2], S. J. Lockett[2], W. F. Heinz[2], S. J. Kwok[1]; [1]LASE Innovation, Woburn, MA, [2]National Institute of Health, Frederick, MD

Probing the spatial context and dynamic behaviors of individual cancer cells is critical for understanding tumorigenesis, metastasis and treatment resistance. However, single-cell spatial information that is obtained from microscopy cannot easily be connected to molecular profiling methods such as flow cytometry and single cell sequencing. Here, we develop an approach using novel laser particles (LPs) to optically barcode individual cells and connect imaging to proteomic characterization with flow cytometry. LPs are micron-sized, semiconductor-based probes that emit narrowband laser light (<0.3

nm) and have been developed for massively multiplexed cell tracking. We employ an *in vitro* 4T1 murine breast cancer model of tumorigenesis, in which cells are grown in a restricted exchange environment chamber to form a 2D "disk" that recapitulates oxygen and nutrient gradients in a tumor. The disk is tagged with the LPs and imaged to record the spatial location of each barcode. The disk is then dissociated and stained for surface and intracellular markers and measured on a flow cytometer that reads out both fluorescence and LP barcodes. The barcodes read out during imaging and cytometry are matched and the data are combined for each barcoded cell. A key challenge was ensuring LPs found on cells during imaging were retained through the cell processing steps and the cytometry acquisition. We developed and optimized methods for tagging the disk using a biotin-streptavdin binding. We found that cells need to have 3LPs to be uniquely identifiable. To maximize the tagging and recovery of cells we tested several different fixation and dissociation methods for the cell disk. We found that fixation with PFA and dissociation with Accutase provided the most stable recovery and results of tagged cells. Overall, we were able to tag ~30% of the cells in a 2D disk (~1,000 cells) with 3+ LPs and measure them using flow cytometry. We are validating our method by comparing to previously published fluorescence microscopy data, reproducing spatial gradients of surface and intracellular markers (PMID: 33859337). Our results establish LPs as a novel method for cellular barcoding and connecting single-cell information from different platforms, from imaging to cytometry, cytometry to imaging, and imaging/cytometry to sequencing. Supported in part by NCI Contract: 75N91019D00024

B251/P1244

Nickel Sulfide Nanoparticles Conjugated with Graphene and their Use as Photothermal Therapeutic Agents in Breast and Ovarian Cancer Models

G. Ray[1], G. Neelgund[1], H. Wang[2], G. Regisford[3]; [1]Chemistry, Prairie View A&M University, Prairie View, TX, [2]Physics, Prairie View A&M University, Prairie View, TX, [3]Biology, Prairie View A&M University, Prairie View, TX.

Nickel sulfide (NiS) nanoparticles are important transitional metal chalcogenides and are explored for their possible applications. NiS nanoparticles have high absorption in the near-infrared (NIR) region, which is a basic need for application in the photothermal therapy (PTT) of cancer. PTT relies on the NIR absorption ability of the photothermal agent employed. The NIR absorption of NiS nanoparticles is improved by its conjugation with graphene oxide (GO) and graphene (GR) nanosheets. The objective of this study was to design and synthesize robust near-infrared (NIR) active photothermal agents comprised of nickel sulfide nanoparticles with graphene oxide and graphene nanosheets for the advancement of PTT. Two NIR responsive nanomaterials *viz.,* GO-NiS and GR-NiS were prepared by hydrothermal method. Before deposition of NiS nanoparticles, GO nanosheets were prepared by modified Hummers and Offeman method, and thus produced GO nanosheets that were modified to GR nanosheets through conjugation with ethylene diamine. The successful formation of GO-NiS and GR-NiS in the nano regime was characterized using UV-vis spectroscopy, ATR-FTIR spectroscopy, powder XRD, and thermogravimetric analysis (TGA). The response of GO-NiS and GR-NiS towards the absorption of NIR radiations was estimated by the illumination of aqueous dispersions of GO-NiS and GR-NiS to 980 nm laser. We investigated the biological effect of GO-Nis and GR-Nis nanoparticle breast cancer cells (MCF7) and ovarian cancer cells (UWB1.289) combined with 980 nm laser irradiation. Cell toxicity was determined using MTT assay and Trypan blue staining. Necrosis and apoptotic cells were detected by a combination of propidium iodide and Hoechst 333342 staining and analyzed using Flow Cytometry and confocal microscopy. DNA damage was detected by immunostaining gamma-H2AX and 53BP1 and DNA

damage foci were observed under confocal microscopy and quantified by Image J. Our results demonstrated that laser radiation induces cell death in both cancer cells and GO-Nis and GR-Nis in a dose- and concentration-dependent manner. In conclusion, the GO-NiS and GR-NiS have demonstrated a significant photothermal effect under irradiation to NIR laser. The use of nickel sulfide nanoparticles with graphene and graphene oxide sheets for treatment in killing cancer cells will allow for a more cost-efficient treatment with no harmful side effects.

B252/P1245

Prostate Specific Membrane Antigen (PSMA) in Prostate Cancer CellsParticipates in Prostate Cancer Bone Metastasis

R. Sohan, L. Caromile; University of Connecticut Health Center, Farmington, CT

Metastasis to bone affects 80% of advanced prostate cancer (PCa) patients. Once bone metastasis forms, the 5-year survival rate drops to <60%. Prostate-specific membrane antigen (PSMA) is found on the surface of prostate epithelial cells and is a prognostic biomarker of PCa progression both in the primary and metastatic bone tumors. However, PSMA's interaction within the metastatic bone microenvironment via the "vicious cycle" of bone metastasis is not well understood. Therefore, this study aims to elucidate if PSMA's status within the immortalized human PC cells C42B can affect bone formation in the permissive human osteoblast cell line (hFOB 1.19) through the recreation of the bone microenvironment. To ensure that PCa cell lines can form bone-like mineral, we treated the C42B cell lines with osteogenic growth media containing 50 µg/mL L-ascorbic acid and 10 mM β-glycerophosphate for 0-10 days. Alizarin Red staining (ARS) verified that PSMA positive C42B could form calcium based mineral deposits and alter known bone biomarkers transcript levels (DKK1, RANKL, VIM, BMP2, ALPL). Additionally, the bone biomarker transcript BMP2 was upregulated in C42B$^{PSMA\ KO}$ but not in the PSMA positive C42B which is associated with increased PCa reoccurrence in this cell type. Overall, knocking out PSMA of our C42B PCa cell line resulted in a less aggressive phenotype as measured by decreased ability to mineralize and a change in the profile of known bone biomarkers. Future directions include trans-well experiments to mimic the PCa bone microenvironment's vicious cycle and exosome isolation.

B253/P1246

A Copper Sulphate Nanoparticle Complex Sensitizes Radiation and PARP Inhibitors in Human Cancer Cells

G. C. Regisford[1], H. Wang[2], G. Neelgund[2], F. Guice[1], D. Ruiz[3], P. Saganti[2]; [1]Biology, Prairie View A&M University, Prairie View, TX, [2]Chemistry and Physics, Prairie View A&M University, Prairie View, TX, [3]Genetics and Genomics, Texas A&M University, Prairie View, TX.

To improve therapeutic efficacy and decreased toxicity in the nanosized mediated drug delivery model, we examined the effects of a copper sulfate nanoparticle coupled with heparin and folic acid (HP-CuS-FA), combined with radiation and a PARP inhibitor on the proliferation of Human cervical carcinoma (HeLa) cells and normal human skin fibroblast cells (AG1522). An MTT assay was used to assess the toxicity of the chemically synthesized and fully characterized HP-CuS-FA nanoparticles on HeLa and AG1522 cells. Clonogenic assays were used to determine the radio-sensitizing effects of HP-CuS-FA nanoparticles. Necrosis and apoptotic cells were detected by a combination of propidium iodide and Hoechst 333342 staining and were analyzed using Flow Cytometry and confocal microscopy. DNA damage was detected by immunostaining gamma-H2AX and 53BP1 and DNA damage foci were

observed under confocal microscopy and quantified by Image J. Our results indicated that HP-CUS-FA nanoparticles induced significantly more cell death in Hela cells than in AG1522 cells. Treatment of HeLa and AG1522 cells with HP-CuS-FA resulted in apoptosis, necrosis, and the generation of reactive oxygen species in mitochondria. Treatment of HeLa and AG1522 cells with HP-CUS-FA nanoparticles and a PARP inhibitor (ABT888) led to an increase in cytotoxicity, DNA damage, and apoptosis. While colony formation was reduced with increasing radiation dose, the pretreatment of cells with HP-CUS-FA NP and PARP inhibitor (ABT888) significantly reduced the colony formation following radiation exposure. Our data show that the combination treatment of CuS-FA nanoparticles and a PARP inhibitor enhances the efficacy of radiation therapy by increasing the reactive oxygen species, inducing apoptosis and DNA damage. This study suggests that HP-CuS-FA nanoparticles and a PARP inhibitor have a synergistic effect on cancer cell death and are potential nano radio-sensitizing agents for the treatment of cancer.

Immunomodulation and the Tumor Microenvironment

B254/P1247

Anti-tumor macrophages increase intracellular iron in cancer cells and inhibit proliferation.

D. Greiner, S. Varady, M. Roh-Johnson; University of Utah, Salt Lake City, UT.

Different immune landscapes can promote or inhibit tumor progression. Interactions between tumors and immune cells alter how tumors maintain and use vital metabolites such as iron. In mammals, iron is a primary cofactor in many cellular activities, including proliferation. Tumors require sufficiently high levels of iron for sustained proliferation; however, too much iron can be toxic to cells, thus cells have developed mechanisms to tightly regulate their iron levels. Macrophages are a key immune cell type in solid tumors with differing cell fates that can either promote or inhibit tumor growth. Macrophages also regulate environmental iron: Anti-tumor macrophages are thought to act as an iron sink, sequestering iron from cancer cells and preventing cancer cell growth, while pro-tumor macrophages are thought to act as an iron source for cancer cells, promoting cancer cell growth. These hypotheses are primarily generated from measuring iron influx and efflux in macrophages, independently of how these processes affect cancer cell iron levels. Contrary to this model, our data suggest that anti-tumor macrophages cause elevated levels of available iron in cancer cells. Furthermore, we found that cancer cells exhibit increased levels of an iron import protein and reduced levels of an iron export protein in the presence of anti-tumor macrophages. These results suggest that anti-tumor macrophages cause cancer cells to have aberrantly high levels of iron, potentially due to dysregulation of iron homeostasis. Thus, we will test the hypothesis that anti-tumor macrophages inhibit cancer cell growth by increasing intracellular levels of available iron to drive ferroptosis, iron-mediated-cell death. This work may suggest the potential for the use of ferroptosis agonists in conjunction with potential anti-cancer treatments that aim to reprogram the molecular immune landscape.

B255/P1248

Macrophages Promote Tumor Cell Extravasation across an Endothelial Barrier through Thin Membranous Connections

A. Genna, C. Duran, D. Entenberg, J. Condeelis, **D. Cox**; Albert Einstein College Med-Jack & Pearl Resnick Campus, New York, NY

Macrophages are important players involved in the progression of breast cancer, including in seeding the metastatic niche. However, the mechanism by which macrophages promote tumor cell extravasation at metastatic sites is not clear. Examining time-lapse intravital-imaging (IVI) data sets of breast cancer cell extravasation in the lung, we detected the presence of Thin Membranous Connections (TMCs) between macrophages in lung parenchyma and tumor cells in the vasculature. These TMCs resembled similar structures formed between macrophages and tumor cells called tunneling nanotubes that we previously demonstrated to be important in tumor cell invasion *in vitro* and *in vivo* (Hanna 2019). We used an *in vitro* assay to mimic macrophage-driven tumor cell extravasation, in which an endothelial monolayer and a matrigel-coated filter separated tumor cells and macrophages from each other. We observed TMCs formed between macrophages and tumor cells through the endothelial layer on top of the porous filter. Furthermore, tumor cells are more frequently found to associate with pores that contain macrophage protrusions. Macrophage conditioned media was insufficient to stimulate tumor cell extravasation *in vitro*, consistent with a requirement for contact between macrophages and tumor cells. To determine if TMCs were important for tumor cell extravasation we used macrophages with reduced levels of endogenous M-Sec (TNFAIP2), which causes a defect in tunneling nanotube formation. As predicted, these macrophages showed reduced macrophage-tumor cell TMCs. There was also a concomitant reduction in tumor cell extravasation *in vitro* with M-Sec deficient macrophages compared to control macrophages, using either a human or murine breast cancer cell line. To determine the role of macrophage-tumor cell TMCs *in vivo* we generated an M-Sec deficient mouse. In a small study using an *in vivo* model of experimental metastasis we detected a significant reduction in the number of metastatic lesions in M-Sec deficient mice compared to wild type mice. There was no difference in the size of the metastases, consistent with a defect specific to tumor cell extravasation and not metastatic outgrowth. Overall, our data indicate that macrophage TMCs play an important role in promoting the extravasation of circulating tumor cells in the lung.

B256/P1249

CAR-Macrophages reprogram neighboring macrophages for efficient cancer cell phagocytosis

T. Waddell, Q. Xue, M. Roh-Johnson; University of Utah, Salt Lake City, UT.

Macrophages in the tumor microenvironment are influenced to function as pro-tumor immune cells, facilitating blood vessel growth, cancer cell proliferation, immune inhibition, and cancer cell migration. The interactions between these tumor-promoting macrophages and cancer cells lead to the recruitment and differentiation of other tissue-resident macrophages to also function as tumor-promoting macrophages. To disrupt the pro-tumor environment, we are testing whether chimeric antigen receptor-expressing macrophages (CAR-Macrophages) can convert tumor-promoting macrophages into an anti-tumor fate and facilitate cancer cell killing. We developed synthetic and inducible CAR-Macrophages using the FRB/FKBP/rapamycin system. Macrophages expressing FRB as an antigen receptor were cultured with melanoma cells expressing FKBP. Upon adding rapamycin, we visualized a significant increase in CAR-Macrophage-mediated phagocytosis compared to non-rapamycin treated

control. We then added naïve macrophages to these cultures and found that the naïve macrophages were induced to become anti-tumor-like and efficiently phagocytosed cancer cells. Bolstered by our findings using a synthetic CAR-Macrophage system, we then sought to develop a CAR-Macrophage that targets an endogenous tumor antigen. We designed CAR-Macrophages targeting chondroitin sulfate proteoglycan 4 (CSPG4), a large glycoprotein expressed on the plasma membrane and specific to and enriched in cancer cells. We cultured anti-CSPG4-CAR-Macrophages with melanoma cells and observed a significant increase in phagocytosis mediated by anti-CSPG4-CAR-Macrophages compared to non-specific control CAR-Macrophages. Current work focuses on using primary human cells and in vivo mouse models to further test whether tissue resident-macrophages are converted to anti-tumor-like macrophages and whether efficient melanoma cell phagocytosis is sustained. These results point to a powerful mechanism where CAR-Macrophages not only participate in tumor cell killing but facilitate a general anti-tumor state in endogenous macrophages.

B257/P1250

Intravital imaging of the myeloid immune cell landscape during tumor initiation and progression

T. D. Madsen, W. Wang, R. Weigert; NCI, CCR, LCMB, National Institutes of Health, Bethesda, MD.

How the immune cells transition from tumor-fighters to tumor-facilitators is still poorly understood. Early responders such as neutrophils, macrophages and other myeloid cells have been shown to undergo changes in gene expression and behavior, arguably being transformed in new cell types such as the poorly defined tumor-associated neutrophils (TANs) and macrophages (TAMs). Little is known about the sequence of events leading to this switch in the immune system, at what stage of cancer development it occurs, and what components in the tumor cells and surrounding microenvironment trigger these transitions. This knowledge would be highly valuable to design successful immunotherapies for cancer.To this end, we developed a mouse model in which we followed myeloid cell populations during the development of oral squamous cell carcinoma using state-of-the-art intravital multiphoton microscopy. Tumorigenesis was triggered by treatment with a carcinogen (4-Nitroquinoline 1-oxide) in the drinking water. The entire dorsal tongue epithelium was imaged at cellular resolution followed by focused imaging of developing tumors at sub-cellular resolution bi-weekly over a period of 24 weeks. By using transgenic mice that express fluorescent proteins specifically in myeloid cells, we recorded the history of their recruitment and infiltration before and during the development of the cancerous lesions from hyperplastic and dysplastic stages to invasive carcinoma. Moreover, the behavior and dynamics of the myeloid cells was correlated with the metabolic state of the tumor cells and the structure of the extracellular matrix.Preliminary results from multiple mice and tumors showed that myeloid infiltrations arose around week 3 from the administration of the carcinogen, epithelial hyperplasia around week 8, dysplasia at week 16 and carcinoma *in situ* around week 20-24. Interestingly, early lesions were often accompanied by peripheral recruitment of myeloid cells but not by their infiltration into the tumors. At more advanced stages, myeloid cells infiltrated inside the tumor, concomitantly with a reduction of both glycolytic and oxidative phosphorylation.
In conclusion, we have recorded the history of multiple tumors and their interactions with myeloid cells using our intravital imaging system on 4NQO-treated transgenic mice. This has shown a correlation between lesion size, metabolic state and myeloid cell infiltration and laid the groundwork for future in-depth characterization of the myeloid subtypes associated with each type of lesion.

B258/P1251

Analysis of early cellular and sub-cellular changes during tumor initiation and progression in live animals.

W. Wang[1], K. Lee[1], R. Coffey[2], A. Sorkin[3], R. Weigert[1]; [1]National Institutes of Health, Bethesda, MD, [2]Department of cell and developmental biology, Venderbilt University Medical Center, Nashville, TN, [3]Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA.

Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers with a 65 % 5-year survival rate. It accounts for more than 650,000 cases and 330,000 deaths worldwide annually and it is projected to increase by 30% in the next 10 years. Understanding the mechanisms that control initiation, progression, and spreading of the tumor to distal sites is of paramount importance to develop effective therapies. To this end, we have used a well-established carcinogen model in live mice that mimics the progression, histopathology, and molecular characteristic of the HNSCC observed in patients. Here, we show, for the first time, the visualization of the onset and progression of cancerous and pre-cancerous lesions through the various stages the same animal. We discovered that only 15% of the lesions fully develop into persistent in situ or invasive carcinomas, whereas the remaining lesions reach different stages (e.g. severe dysplasia and carcinoma) and then heal spontaneously. Using intravital subcellular microscopy (ISMic), we acquired information on the cellular and subcellular changes in the tumor cells and associated microenvironment (e.g., blood vessels, extracellular matrix, and immune cells). Specifically, we correlated the lesion progression with changes in the cell morphology, mitochondrial metabolism, immune cell response, autophagy, and EGFR signaling, thus providing a working hypothesis on the mechanism controlling tumor progression in vivo which can serve as a springboard to develop novel therapies.

B259/P1252

The Chemokine Receptor CCR1 is Essential for Microglia Stimulated Glioma Invasion

S. J. Coniglio, N. Zeren, S. Morgan, M. Uppiliappan; SIST, Kean University, Union, NJ.

Glioblastoma Multiforme (GBM) is the most aggressive form of adult brain tumor with a median survival time of twelve months. GBM is highly resistant to conventional therapy which includes surgical resection of the tumor, radiation treatment and chemotherapy. GBM cells are highly motile and invasive resulting in infiltrative tumors with poorly defined borders. GBM tumors are heavily infiltrated with microglia cells which are known to stimulate GBM cell invasion. Our laboratory has previously demonstrated that microglia strongly stimulates GBM invasion both in-vitro and in orthotopic animal models. This interaction was found to be dependent on CSF-1R which is expressed on all tumor infiltrating macrophages/microglia. Blockade of the CSF-1R using compounds such as pexidartinib (PLX3397) can inhibit microglia/macrophage-stimulated GBM invasion *in-vitro* and *in vivo*. A variety of chemokines are upregulated in the GBM tumor microenvironment and facilitate “cross-talk” between microglia and GBM cells eliciting a chemotactic response. We postulated that inhibition of CCL3 associated receptors such as C-C receptor 1 (CCR1) might also inhibit GBM invasion, thus, a CCR1 antagonist could prove efficacious for blockade of microglia-induced glioblastoma invasion in vitro. Many potent CCR1 antagonists have been described in the literature. We chose four of these compounds with two distinct structural cores, all with reported IC50’s of less than 200 nM for inhibition of CCR1 binding versus CCL3. We examined the ability of these antagonists to block microglia-stimulated glioblastoma invasion using an in-vitro coculture invasion assay. Using quantitative PCR arrays, we also show that expression of

chemokines and chemokine receptor genes is greatly altered in GBM conditioned media-treated microglia. Understanding the pattern of tumor-associated macrophage/microglia chemokine secretion in GBM may present additional targets for chemotherapeutic intervention and enhance immunotherapy.

B260/P1253

Optimizing a T cell suppression assay to test for the presence of myeloid derived suppressor cells (MDSC) in mouse models with soft tissue sarcomas

Z. Jamil[1,2]; [1]CUNY Brooklyn College, Brooklyn, NY, [2]John Hopkins University, Baltimore, MD

Optimizing a T cell suppression assay to test for the presence of myeloid derived suppressorcells (MDSC) in mouse models with soft tissue sarcomas

ABSTRACT

INTRODUCTION: In recent years, immunotherapy has emerged as a cancer treatment due toits potential to reinvigorate the immune system of patients for fighting cancer cells. However, insoft tissue sarcomas (STS), theTumor Immune Microenvironment largely consists ofimmunosuppressive cell types known as Myeloid Derived Suppressor Cells (MDSC). MDSCinhibits T cell activation and proliferation. Here, we optimize T cell proliferation conditions bystimulation beads via staining of T cells with a fluorescent tracking dye at differentconcentrations. Ultimately, utilizing this assay, we will assess the immunosuppressive function ofMDSC isolated from a STS mouse model by co-culturing them with spleen T cells.

METHODS: Wild-type mice had their spleens excised and processed to obtain splenocytes. Thenumber of live and dead cells present in the spleen were counted using a hemocytometer toestablish the stimulation beads to cell ratios of 0:1, 1:1, 1:2, and 2:1. The cells were observedunder the microscope to ensure bead to cell conjugation, cell viability, and media contamination.This was closely followed by the removal of beads and running the cells through flow cytometryto determine the optimal staining conditions and timeframes needed for T cell proliferation.

RESULTS: We revealed the 72 hour-mark to be the ideal timeframe for assessing T cellproliferation at the 0:1, 1:1, 1:2, and 2:1 beads to cells ratio. The staining conditions showcasedgood resolutions and peaks in flow cytometry for 1uL of dye in 10M cells and 5M cells and0.1uL of dye in 1M cells. Despite the resolution for 0.5 uL of dye in 5M cells and 0.5uL of dyein 1M cells being good, their peaks did not exhibit good distinction. For 0.1uL of dye in 5Mcells, the cells did not display a good resolution even though they seemed to be proliferating.

CONCLUSION: We determined that the 72 to 120 hours is the ideal condition for T cellactivation and proliferation in our system. In addition, our data suggests 1uL of dye for 10Mcells and 5M cells along with 0.1uL of dye for 1M cells to be the optimal staining conditions forflow cytometry studies. These parameters establish the ideal conditions for T cell activation andproliferation in our mouse model STS which will help us evaluate the immunosuppressivecapacity of MDSC in our in vitro assay and strengthen our understanding of immunotherapytreatment.

B261/P1254

Mre11 antagonizes nucleosome sequestration of cGAS to suppress tumorigenesis

M. Jo; University of North Carolina At Chapel Hill, Chapel Hill, NC

Oncogene-induced replication stress generates cytoplasmic chromatin fragments (CCF) that activate cGAS/STING-mediated innate immune signaling to inhibit tumor development. However, the mechanism of cGAS activation by CCF remains enigmatic, particularly given the constitutive inhibition of cGAS by high-affinity histone acidic patch (AP) binding. Here we show that the Mre11-Rad50-Nbn (MRN) complex is required for cGAS activation and p53-independent tumor suppression during Myc-induced mammary neoplasia. MRN binding to nucleosomes releases cGAS from sequestration, thereby enabling cGAS activation by cytosolic DNA. Mre11-dependent cGAS activation antagonizes mammary epithelial neoplasia by stimulating ZBP1/RIPK3/MLKL-mediated necroptosis. In human triple-negative breast cancer, ZBP1 downregulation correlates with increased genome instability, decreased immune infiltration, and poor patient prognosis. These findings establish Mre11 as essential for cGAS-dependent innate immune activation and necroptosis engagement during tumorigenesis.

B262/P1255

High Mobility Group Box 1 Protein Localization and its Effect on the Immune Response in the Lung Tumor Microenvironment

K. Jafri[1], N. Andre[2], **G. E. Simmons, Jr**[2]; [1]Cornell University, Ithaca, NY, [2]Cornell University College of Veterinary Medicine, Ithaca, NY

High mobility group box protein 1 (HMGB1), a pro-inflammatory damage-associated molecular pattern protein, drives inflammation in the lung tumor microenvironment. Furthermore, HMGB1 has been previously demonstrated to suppress functional anti-tumor immune responses in this context. Recently, we have identified HMGB1 as a protein that is functionally dependent on monounsaturated fatty acid (MUFA) availability. Utilizing a combination of molecular assays and 3-dimensional cultures, we now show that HMGB1 is sequestered intracellularly, rather than being secreted extracellularly in a MUFA-dependent fashion. We hypothesized that MUFAs promote an anti-tumor immune response via modulating HMGB1 release from cancer cells. Thus, by restricting extracellular HMGB1 levels, HMGB1-responsive proinflammatory/tumor signaling pathways (e.g., NF-κB) will be suppressed. Taken together, these observations suggest an approach to decrease maladaptive immune and inflammatory responses mediated via innate immune cells. Exploiting the MUFA/HMGB1 signaling axis may increase the efficacy of cancer therapies targeting lung malignancies.

Cancer Stem Cells

B263/P1256

Differential metabolic adaptations define responses of winner and loser oncogenic mutant cells in skin epidermis *in vivo*

A. Hemalatha[1], R. Perry[2], V. Greco[1]; [1]Genetics, Yale University - New Haven, CT, New Haven, CT, [2]Endocrinology, Yale University - New Haven, CT, New Haven, CT.

Skin epidermal stem cells detect, and correct aberrancies induced by oncogenic mutations. Different oncogenes invoke different mechanisms of epithelial tolerance: while wild-type cells outcompete β-

catenin-Gain-of-Function (βcatGOF) mutant cells, $Hras^{G12V}$ mutant cells outcompete wild-type cells. We asked how metabolic states change as wild-type stem cells interface with mutant cells, and how this ultimately drives different cell competition outcomes *in vivo*. Combining two powerful experimental modalities -1. optical redox imaging to visualize endogenous levels of the co-enzymes NAD(P)H/ FAD, adapted and evolved within our *in vivo* imaging platform to enable long term tracking and visualization of metabolic states of cells within the live animal, and 2.$^{13}C_6$- glucose tracer-assisted mass spectrometry to measure metabolic fluxes directly - we provide unprecedented resolution of the metabolic state at the interface of Mutant and Wild-type stem cells within the epidermis *in vivo*. By tracking endogenous redox ratio (NAD(P)H/FAD) with single cell resolution in the same mice over time, we show that wild-type epidermal stem cells maintain robust redox ratio despite their heterogeneous cell cycle states. We discover that both βcatGOF and $Hras^{G12V}$ models lead to a rapid drop in redox ratios. However, the "winner" cells in each model (wild-type cells in βcatGOF and mutant cells in $Hras^{G12V}$ model), rapidly recover their redox ratios, irrespective of the mutation induced. Glucose catabolic flux studies reveal that both βcatGOF and $Hras^{G12V}$ mutant epidermis upregulate flux through TCA cycle. Contrastingly, the "winner" mutation $Hras^{G12V}$ specifically upregulates pyruvate to lactate flux (glycolysis readout) while the "loser" βcatGOF mutation downregulates this step. Hence, we reveal the metabolic adaptations that define the hallmarks of winners and losers during cell competition *in vivo* and uncover the nodes of regulation unique to each cell fate. These novel findings are in contrast to the expectations of Warburg effect, a fundamental concept in cancer metabolism, wherein proliferative cells expressing oncogenic mutations are expected to upregulate glycolysis at the expense of downstream TCA cycle and mitochondrial oxidation. Instead, our study suggests that decoupling lactate levels (glycolysis) and mitochondrial oxidation could be a strategy used by "winners" in cell competition. This study also reveals a metabolic plasticity inherent to epidermal stem cells that affect cell competition outcome with profound implications for therapeutically eliminating oncogenic mutations from the skin epidermis.

B264/P1257

Role of HOX gene expression in differentiation of colon cancer stem cells

B. Osmond[1,2], C. O. B. Facey[2], L. M. Opdenaker[2], C. Zhang[1,2], B. M. Boman[1,2,3]; [1]Biological Sciences, University of Delaware, Newark, DE, [2]Cawley Center for Translational Cancer Research, Helen F Graham Cancer Center & Research Institute, Newark, DE, [3]Thomas Jefferson University, Philadelphia, PA.

HOX genes are a highly conserved subset of genes that encode transcription factors critical for stem cell (SC) function and embryonic development. Several HOX genes have also been implicated in a myriad of cancers, including colorectal cancer (CRC). However, how HOX genes play a role in SC regulation and how dysregulation of HOX genes contributes to cancer development is still not fully understood. Current research indicates that HOX expression is regulated by the WNT and retinoic acid (RA) signaling, which are signaling pathways that become dysregulated in cancer. *Goal*: To determine how regulation of HOX genes specifies differentiation of colonic SCs into specialized cell lineages in the colon and how dysregulation of HOX genes contributes to CRC development. *Hypotheses*: *i*) Each of the different cell types within the human colonic epithelium has a distinct HOX gene expression signature; *ii*) All-trans retinoic acid (ATRA) induced differentiation of CRC cells leads to changes in HOX gene expression that correlate with specific HOX expression signatures. *Results*: Immunostaining shows that specific HOX proteins (e.g., HOXA9 & HOXC9) are selectively expressed in ALDH-positive SCs and expression of these HOX genes is increased in CRC tissues compared to normal epithelium. Bioinformatics analysis predicts that these HOX genes, along with HOXA4, HOXA10, and HOXC8, are regulated by the RA signaling

pathway. To investigate the effect of RA signaling on HOX gene expression, we studied how ATRA influences CRC cells (HT29 & SW480). We found that ATRA treatment of CRC cells: 1) Decreased proliferation and increased cellular differentiation, particularly along the neuroendocrine cell (NEC) lineage; 2). Significantly changed HOX gene expression. For example, western blot and qPCR analyses showed that treatment of CRC cells with ATRA decreases expression of HOXA4, HOXA9 & HOXD10 and increases expression of HOXC8 & HOXC9. Nanostring mRNA profiling also shows that ATRA treatment of CRC cells increases HOXA3 & HOXA5 and decreases HOXA10, HOXB6 & HOXB9. By defining how these HOX gene expression changes in response to ATRA treatment in comparison to the HOX gene signatures of different colonic cell lineages will help us understand the role HOX genes play in SC differentiation along specific cellular lineages and how to target HOX gene in cancer treatment.

B265/P1258

p27 Increases Breast Cancer Stem Cell (CSC) Expansion by Recruiting STAT3 and CBP on Chromatin to Induce Angptl4 and Jag1 Expression

S. Razavipour[1], K. Jang[2], H. Yoon[2], M. Kim[2], M. Shin[2], D. Zhao[2], D. Van Booven[2], A. Bagheri[1], J. Slingerland[1]; [1]Georgetown University, Washington, DC, [2]University of Miami, Miami, FL.

p27 is a key regulator of the cell cycle. It plays a dual role during tumorigenesis. p27 acts as a tumor suppressor through its cell cycle inhibitory role, and as an oncogene to promote cancer invasion and metastasis when phosphorylated at T157 and T198 by PI3K activated kinases. Our lab demonstrated that p27 activates STAT3, a driver of Embryonic Stem Cell (ES) and Cancer Stem Cell (CSC) self-renewal, to induce Epithelial-Mesenchymal Transition (EMT). In mice, knock-in of the cell cycle defective p27 mutant (p27CK-) was previously shown to promote stem cell expansion in multiple tissues, suggesting p27 may regulate stem cells. Here, we showed that C-terminally phosphorylated p27 increases CSC properties, including tumor sphere formation and CSC markers by recruiting STAT3 and a histone acetyltransferase, CBP, on the promoter of the stem cell-driver genes to induce their expression. Our RNA-seq data in breast cancer cell lines with overexpression and knockdown of phosphomimetic p27CK-T157D/T198D (p27CK-DD) showed that p27-activated genes are associated with stem cell pathways. We have confirmed that overexpression of p27CK-DD increases tumor spheres, ALDH1 activity and expression of embryonic stem cell transcription factors (ES-TFs) to increase tumor initiating stem cells in vivo in limiting dilution assays. ChIP-seq of p27 and STAT3, analyzed together with RNA seq has identified that p27 cooperates with STAT3 to induce expression of genes governing CSC self-renewal. Our Chip-seq data revealed that 65% of STAT3 target genes are co-bound with p27 and p27 knockdown decreases STAT3 binding enrichment on p27/STAT3 co-target gene promoters. Combined p27/STAT3 ChIP-seq/ RNA-seq analysis in our cell lines revealed that p27 modulates STAT3 recruitment on several stem cell-driven genes including *MYC*, *SOX2*, *JAG1,* and *ANGPTL4* promoters to regulate their expression. These targets were validated by qPCR and ChIP-qPCR experiments. We also showed that p27 recruits CBP to p27/STAT3 co-target gene promoters and increases chromatin accessibility by increasing H3K27acetylation. Knocking down of *ANGPTL4,* a known regulator of stem cell properties and anoikis resistance, in p27CK-DD overexpressed cell lines further indicate that p27CK-DD-driven upregulation of sphere formation and ES-TF expression is mediated through Angptl4.Taken together, these data reveal a novel function for p27 as a transcriptional regulator of CSC-promoting genes. In breast cancer cell lines, p27 recruits STAT3/CBP to several stem cell-driver gene promoters to induce their expression and promote CSC expansion.

B266/P1259

Intestinal epithelial cell proliferation and gut growth regulated by steroid sex hormones

T. Kandelouei, M. R. Lewis, B. Edgar; Oncological Sciences, Huntsman Cancer Institute, Salt Lake City, UT.

Intestinal epithelial cell proliferation & gut growth regulated by steroid sex hormones Study objective: To establish a mechanistically detailed paradigm of how a sex-specific steroid regulates progenitor cells function in the intestinal tissue as a non-sex organ. **Methods:** We use Drosophila and mice as model systems. Drosophila mid-gut is similar to the mammalian intestine, has a simple structure, and powerful genetic tools exist for probing the molecular genetic mechanisms. The major steroid hormone in *Drosophila* is 20- hydroxyecdysone (20HE). We perform genetics tests, including RNA-Seq of whole mid-gut and FACS-sorted progenitor cells, metabolomics and lipidomics analysis, and Cut&Tag. These experiments help us identify the genes that are transcriptionally regulated by 20HE and how 20HE signaling affects other signaling pathways and physiological processes such as ISC metabolism, proliferation, lineage symmetry/asymmetry, and gut growth. We will also explore whether sex steroids regulate proliferation and gut epithelial homeostasis in mice to test if a *Drosophila* like system operates in mammals. We investigate the effects of sex steroid hormones, assaying effects on GI tract size, morphology, cell proliferation, and gene expression. **Results:** A recent discovery in *Drosophila* has shown that 20HE, produced by ovaries after mating, stimulates intestinal stem cell (ISC) proliferation and gut growth. We look through the detailed mechanisms underlying the 20HE contribution to ISC proliferation and gut growth. Our RNA-Seq data has shown the upregulation of genes involved in peptide and glutathione metabolic processes and proliferation in 20HE-Fed compared to control flies; we followed this with metabolomics and lipidomics analysis that we currently working on data sets. Our preliminary results in mice showed higher crypts and tissue thickness/ growth effect in E2 (estrogen) and E2/P4 (E2/progesterone) treated mice compared to the control group. We will look further into the detailed mechanisms that might be induced by steroids to regulate ISC division in mice. **Conclusion:** This work will provide lead data for a better understanding of how sex steroids regulate intestinal physiology & pathology. This work will advance our knowledge of how steroid signaling affects the sex-biased incidence of many human diseases, notably those involving altered stem cell behavior, such as cancer.

B267/P1260

Uncovering the role of microRNAs that target stem cell genes in the emergence of cancer stem cell heterogeneity during colorectal cancer development

M. Lausten[1,2], V. A. Stark[1,2], C. O. B. Facey[2], L. M. Opdenaker[2], B. M. Boman[1,2,3]; [1]Biological Sciences, University of Delaware, Newark, DE, [2]Cawley Center for Translational Cancer Research, Newark, DE, [3]Thomas Jefferson University, Philadelphia, PA.

Our goal is to identify microRNAs (miRNAs) that target genes that encode different proteins involved in stem cell (SC) signaling pathways involved in tumorigenesis of colorectal cancer (CRC). One reason behind the lack of efficacy in cancer treatment is due to the development of SC overpopulation. *Hypothesis*: specific miRNAs target different SC genes, which leads to the emergence of multiple cancer SC (CSC) sub-populations and tumor heterogeneity. Accordingly, we used flow cytometry to identify CSC sub-populations based on expression of SC markers including ALDH1, CD166, LGR5, and LRIG1 in the HT-29 CRC cell line. We quantified and isolated CSC sub-populations using FACS and measured the expression of miRNAs in each CSC sub-population using NanoString profiling. The level of miRNA upregulation in each CSC sub-population was then ranked based on differential expression of the

miRNAs between different CSC sub-populations. We further analyzed the LGR5 and ALDH SC sub-populations because they showed the largest difference between CSC sub-population sizes with the lowest size (<1%) of the co-positive (LGR5+/ALDH+) cell sub-population. LGR5+ and ALDH+ CSC subpopulations had the highest numbers of differentially expressed miRNAs. This is relevant because WNT signaling mainly occurs via LGR5+ CSC and retinoic acid (RA) signaling mainly occurs via ALDH+ CSCs. We report herein miRNAs that are differentially expressed between ALDH+/LGR5- compared to ALDH-/LGR5- subpopulation and LGR5+/ALDH- versus ALDH-/LGR5-. The mRNAs expressed in CSC subpopulations from Nanostring profiling were analyzed using bioinformatics for their predicted target mRNA binding by upregulated miRNAs. Our results show that: *1*) multiple CSC subpopulations exist in the HT-29 cell population; *2*) each CSC subpopulation has a unique miRNA signature. Notably, we identified miR-660-3p to be specifically upregulated in the LGR5+/ALDH- CSC subpopulation. Moreover, we found, using target prediction tools, that miR-660-3p is predicted to bind to the 3'UTR of *RXRA* mRNA. The binding of miR-660-3p to *RXRA* mRNA was validated utilizing luciferase assay in HT-29 cells. *RXRA* mRNA is significantly upregulated in the ALDH+/LGR5- CSC sub-population but not in the LGR5+/ALDH- CSC subpopulation. We previously reported that RXRA is selectively expressed in ALDH + CSC sub-populations. Taken together, these results indicate that expression of miR-660-3p, by inhibiting *RXRA* expression, decreases RA signaling in LGR5+ CSCs. It is also known RXRA binds with β-catenin suppressing protein expression and transcriptional activity. Thus, upregulated miR-660-3p targets a key gene in the RA signaling pathway (*RXRA*) which could contribute to the emergence of SC sub-populations during CRC development.

B268/P1261

Altered Keratinocyte Stem cell conditioning through Polycomb group proteins leads to accelerated skin tumorigenesis in animals prenatally exposed to arsenic

A. Chauhan[1,2], S. Gangopadhyay[1,2], V. Sharma[3], S. Singh[1,2], K. Koshta[1,2], V. Srivastava[1,2]; [1]Developmental Toxicological Division, CSIR Indian Institute of Toxicological Research, Lucknow, INDIA, [2]Academy of Scientific and Innovative Research(AcSIR), Ghaziabad, INDIA, [3]Department of Biotechnology, Faculty of Engineering and Technology, Manav Rachna International Institute of Research and Studies, Haryana, INDIA.

Exposure to arsenic has been linked with several chronic conditions, including lung, bladder, and skin malignancies. In endemic regions, prenatal exposure to arsenic is linked with worse health outcomes in children. In our animal experiments, we observed that gestational exposure to arsenic(0.04mg/kg) accelerated skin tumorigenesis, accompanied with amplified and activated keratinocyte stem cell (KSCs) pool in the skin of offspring. The function of these KSCs is unknown. In present study, female BALB/c mice were exposed to sodium arsenite (0.04mg/kg) in drinking water during gestation, with no further dose administration to the offspring. Primary arsenic conditioned KSCs isolated from2-day pups were evaluated for their differentiation, proliferation, and invasive potential and underlying mechanisms involved. Our study shows that only prenatal exposure to arsenic is enough to increase proliferative and differentiative capacity in KSCs as measured by increased PCNA, Involucrin, Krt1,Krt5 & CD34 expression in both *in vivo* and *in vitro* systems. Pulse chasing stem cells by BrdU incorporation in 2 day pups revealed increased stem cell pool in maternally exposed animals. *In vitro* BrdU uptake and scratch assay verified high division & migratory potential with high survival rate as observed by decreased bax/bcl-xL ratio and low TUNEL positive cells. Early life exposure to As contributed to the activation of MAPK proteins via IGF2R in KSCs as revealed by high phosphorylated levels of b-raf, MEK1, ERK1/2 & IGF2Rin

immunoblot assay. The results were further validated by inhibiting pERK1/2 by U0126. An increased ratio of activating H3K4me1 to deactivating H3K27me3 methyl marks on IGF2R promoter was confirmed by ChIP assay in As conditioned cells with reduced binding of PRC2 group silencer, EZH2 in the same region. The data suggested disrupted imprinting of IGF2R gene responsible for hyper-activating MAPK cascade in KSCs that further contributed to enhanced epidermal proliferation and tumorigenesis. Overall our study shows a positive association between maternal arsenic exposure at environment relevant doses and accelerated skin tumorigenesis. The arsenic conditioned KSCs possessed high differentiation and proliferative potential which was modulated by epigenetic memory modules of the PRC2 group. This contributed to a reduced tumor latency period and enhanced tumor invasiveness in exposed progeny.

B269/P1262

Anti-tumor response of retinoic acid agents based on the retinoid signaling pathway genotype of colorectal cancer cells

V. O. Hunsu, C. O. B. Facey, L. Opdenaker, B. M. Boman; Helen F Graham Cancer Center, Newark, DE.

There are no curative treatments for advanced colorectal (CRC) due to stem cell (SC) overpopulation that drives tumor growth and from resistance of cancer SCs (CSCs) to conventional treatments. An alternative strategy is to discover ways to therapeutically sensitize human CRC stem cells to differentiation-inducing effects of retinoic acid (RA) agents. Indeed, all-*trans*-retinoic acid (ATRA) is known to be a highly effective treatment for acute promyelocytic leukemia (APL) which is caused by translocation in the RA receptor alpha (*RARA*) gene. Results from our bioinformatics analysis on mutations and overexpression of RA signaling component genes in CRC showed that RA pathway genes are often altered in CRCs. Thus, we surmised that the response of retinoic acid (RA) agents may have effects on CRC cells depending on their pathway genotype. Our ***hypothesis*** is that the ability of RA agents to induce differentiation of CSCs depends on the RA pathway genotype expressed in CRC cells. Our ***approach*** was to test the ability of RA agents (ATRA, 13-*cis* retinoic acid, 9-*cis* retinoic acid) and RA metabolism blocking agents (Liarozole, Talarozole) to inhibit proliferation and induce differentiation of CRC cell lines that have different mutations in RA signaling components. Nanostring Profiling was also used to measure the effects of the agents on mRNA expression of CSC markers, indicators of cell differentiation, and RA receptors. Our ***results*** show that the RA pathway genotype affects the anti-response of RA agents on CRC cell lines. Indeed, both HCT116 and SW480 CRC cell lines, which have mutants in *RARA* and/or *RXRG* displayed resistance to ATRA. In contrast, HT29 CRC cells, which have wild- type RA receptors, are sensitive to ATRA. All three of these CRC cell lines showed similar dose responses to the CYP26A1 inhibitor Liarozole. This latter finding indicates that regardless of RA receptor mutations, inhibition of ATRA metabolism increases intracellular RA levels in a similar manner on all 3 cell lines. Nanostring mRNA profiling showed that ATRA-treated HT29 cells have increased expression of RARA, CYP26A1 (ATRA metabolizing enzyme) and KRT20 (differentiation marker); and a decreased expression of CSC markers LGR5 and ALDH1A1. This indicates that CRC cells with a normally-functioning RA signaling pathway can be induced to increase differentiation and to decrease CSC numbers. In ***summary***, we show that colonic SC differentiation can be induced by RA signaling but anti-tumor responses are attenuated by RA pathway mutations. These findings have vast clinical importance for designing differentiation-type therapies.

B270/P1263

Cancer stem cells as the novel origin of myeloid-derived suppressor cells

S. Afify, M. Seno; Department of Cancer Stem Cell Engineering, Faculty of Interdisciplinary Science and Engineering in Health Systems, Institute of Academic and Research, Okayama, Japan

BACKGROUND:Tumor microenvironment contains a variety of stromal cells, including myeloid-derivedsuppressor cells (MDSCs). MDSCs have been described as a non-lymphoid populationof immune suppressor cells enriched in cancer patients.AIM: In the current study we are aiming at uncovering the relationship between cancerstem cells and the generation of MDSCs.METHODS:Monocytes in peripheral blood collected from healthy donors were first reprogrammedinto pluripotent stem cells (PSCs). Then the human PSCs were converted into CSCs inthe presence of the conditioned medium derived from human liver cancer cell line. Thecharacteristics of CSCs were assessed for the potential of self-renewal, differentiation,and tumorigenicity together with the expression of genetic markers.RESULTS:The resultant cells are confirmed as CSC exhibiting sphere-forming potential, anddifferentiation potential in vitro and developing malignant tumors with micrometastasesin the liver of nod-scid mice. The primary cells derived from the malignant tumor,exhibited strong immunoreactivity to anti-human CD34 and anti-c-KIT antibodiessuggesting that they were enriched with hematopoietic progenitor cells. Markersassociated with MDSCs were found expressed in both of the converted cells and theprimary cells. According to these results, CSCs are implied to be capable ofdifferentiation into MDSCs that support the growth and survival of tumor cells. CONCLUSIONS: The results of this study will provide new insights into CSCs andtheir relationships with tumor microenvironments supporting chronic inflammationenriched with cytokines and growth factors, inducing regulatory T cells suppressingimmune responses, and maintaining CSCs themselves as the result.

B271/P1264

Restoring the balance between WNT and retinoic acid signaling to promote differentiation of cancer stem cells in treatment of colorectal cancer

C. O. B. Facey[1], V. O. Hunsu[1,2], B. T. Osmond[1,2], C. Zhang[1,2], L. M. Opdenaker[1], B. M. Boman[1,2,3]; [1]Helen F. Graham Cancer Center and Research Institute, Newark, DE, [2]University of Delaware, Newark, DE, [3]Thomas Jefferson University, Philadelphia, PA

Mutation in the *APC* gene causes dysregulated WNT signaling in 80-90% of CRC cases. Overpopulation of ALDH+ stem cells occur in a progressive manner in the development of CRC in patients with familial adenomatous polyposis (FAP). Given that ALDH is a key member of the retinoic acid signaling (RA) pathway, we hypothesize that human CRC develops due to an imbalance between dysregulated WNT and RA signaling. Herein, we aim to restore proper WNT and RA signaling and identify a common link or target that could promote differentiation of CSCs along the neuroendocrine cell (NEC) lineage. Accordingly, to determine the effects of restoring wt-*APC* in *APC*-mutant HT29 CRC cells, we conducted *in vitro* experiments to examine Wnt/β-catenin signaling, sensitivity to retinoids, cell proliferation, expression of stem cell markers, quantification of ALDH+ stem cells, and NEC maturation. We utilized Nanostring profiling, TCF reporter assay, western blot, and fluorescence activated cell sorting analyses to ascertain these effects. We found that induction of *wt-APC* expression decreased WNT/β-catenin signaling and reduced protein expression of WNT-target genes. Notably, inducing *wt-APC* decreased ATRA-induced expression of the WNT target gene *CYP26A1* (by 50%), which is predicted to, in turn,

increase RA signaling by lowering degradation of RA. Indeed, inducing w*t-APC* increased sensitivity of CRC cells to ATRA-induced apoptosis and inhibition of cell proliferation in a dose-dependent manner. Expression of ALDH1A1 decreased (3-fold) with ATRA treatment and inducing *wt-APC* extended the decrease by an additional 2-fold. Furthermore, upon inducing *wt-APC,* we found an increase in the protein expression of several NEC markers including CHGA, GLP2R, NSE (ENO2), and SSTR1, and an increased number of GLP2R+ NECs. Based on Nanostring profiling and bioinformatic analysis, we discovered a protein network and cell signaling mechanism that links the WNT and retinoic acid pathways via CYP26A1. Thus, by inducing *wt-APC* expression and exogenously activating RA signaling, we found a way to increase differentiation of SCs along the NEC (and other) lineage(s). Translating this strategy *in vivo* might lead to new, more effective treatments involving retinoids for CRC patients.

B272/P1265

Modulation of AXIN2 expression leads to changes in cell cycle, apoptosis, & the colon cancer stem cell population.

C. Zhang[1,2], C. O. B. Facey[1], L. M. Opdenaker[1], B. M. Boman[1,2]; [1]Cawley Center for Translational Cancer Research, Helen F. Graham Cancer Center & Research Institute, NEWARK, DE, [2]Department of Biological Sciences, University of Delaware, Newark, DE.

Colorectal cancer (CRC) is driven by stem cell (SC) overpopulation yet diagnosed via tissue disorganization. Etiology of CRC is complex and much effort has focused on studying SC overpopulation in tumor biology and tissue disorganization in cancer pathology, but regulatory mechanisms linking these neoplastic processes which occur in CRC need clarification. The initiating event in development of most CRCs is increased WNT-signaling caused by APC mutations. The negative regulator of WNT-signaling activity is the beta-catenin destruction complex, which is organized by AXIN1 & AXIN2 scaffold proteins. A growing body of scientific evidence shows that AXIN proteins are key to destruction complex function and CRC development. Our HYPOTHESIS is that aberrant expression of AXIN2 leads to changes in cell cycle, apoptosis, and cancer stem cells that promotes CRC growth and development. Accordingly, we created siRNA-mediated AXIN2 knockdown and lentiviral-based AXIN2 overexpressing CRC cell models (HT29 & SW480) to study the effect of modulating AXIN2 levels in vitro. Our RESULTS show that AXIN2 is involved in multiple regulatory mechanisms: 1) AXIN2 knockdown or overexpression led to decreased CRC cell proliferation; 2) Modulating AXIN2 led to changes in expression of WNT-target genes & SC markers ALDH & LGR5 3) ALDH+ SC population size increased in response to increasing AXIN2, and vice versa. 4) Cell cycle was delayed in both AXIN2 knockdown and overexpressing CRC cell lines. Moreover, our bioinformatics analyses of the effect of tankyrase inhibitors (TIs), which prevent AXIN degradation, in treatment of CRC indicate that efficacy of TIs against CRC cells depends on the APC genotype. CONCLUSION Taken together, our findings show that AXIN2 - a critical factor in WNT signaling - plays a significant role in the cancer cell phenotype including changes in the cell cycle, apoptosis, and CSC population. SIGNIFICANCE: Changes in AXIN2 levels appear to impact critical functions that contribute to SC overpopulation and tissue disorganization that drives tumor development. Thus, discovering ways to target AXIN2-based mechanisms has vast clinical importance.

B273/P1266

Effects of sulforaphane association to conventional therapy for treating triple-negative breast cancer

L. L. Coutinho[1], R. Cheng[2], L. Ridnour[2], M. S. Junqueira[1], R. Chammas[1], D. Wink[2], T. C. Tortelli[1], **M. Rangel**[1]; [1]University of Sao Paulo, Sao Paulo, Brazil, [2]National Cancer Institute, Frederick, MD

Most tumors are comprised of functionally heterogeneous cells, and only a limited, but highly dynamic subpopulation of cells known as cancer stem cells(CSCs) can self-renew and initiate tumors at a high frequency. The triple-negative subtype of breast cancer(TNBC) shows a significantly higher number of CSCs when compared to non-TNBC tumors. This characteristic contributes to the aggressive phenotype exhibited by TNBC and impacts the treatment of patients harboring these tumors since CSCs are involved in the acquisition of resistance to conventional antineoplastic therapies. The embryonic master regulator Cripto-1(CR-1) is known to be increased in TNBC, mainly in the CSCs population contributing to the maintenance of their stem-like phenotype. Also, studies have shown that the 'REDOX' molecules Nitric Oxide (NO) and one of the enzymes involved in its synthesis, Nitric Oxide Synthase 2(NOS2), are able to induce carcinogenesis through different processes, including the promotion of stem-like properties in TNBC cells. Here, we sought to sensitize TNBC cells to conventional chemotherapy by negatively modulating CR-1 and NOS2/NO using sulforaphane(SFN), a nutraceutical agent known to especially target the CSCs population. This natural isothiocyanate compound found in cruciferous vegetables has also been shown to interfere with both CR-1 and NO activity in pathological conditions, such as TNBC and *H. pylori* infection, respectively. To address our hypothesis that SFN would sensitize cells to chemotherapy, we used in this study four cell lines representing the distinct TNBC subtypes, MDA-MB-468, HCC70, MDA-MB-453, and MDA-MB-231. We tested their response to doxorubicin(DOX), and cisplatin(CIS), and found that all of them were sensitive to DOX, displaying a significant reduction in cell viability at very low doses. On the other hand, only the basal-like MDA-MB-468 and HCC70 cells responded to CIS at low doses. The luminal androgen receptor MDA-MB-453 and the mesenchymal MDA-MB-231 cells showed resistance to CIS treatment, with a subtle decrease in cell viability only at higher doses. However, when SFN was associated with CIS treatment, both cell lines, exhibited a significant reduction in cell viability accompanied by elevated early apoptosis activation and cell death. Additionally, we observed that MDA-MB-231 cells treated with CIS alone exhibited increased expression of the pluripotency genes *CR-1* and *Oct-4*, and the epithelial-to-mesenchymal transition marker *N-cadherin*, but the combination therapy CIS-SFN was able to revert this effect. Finally, our results revealed that SFN treatment alone was able to impair cytokine-induced *CR-1*, *NOS2,* and *COX2* overexpression in MDA-MB-231 cells and decrease DETANO(NO-donor)-induced HIF-1α stabilization in these cells.

Oncogenes and Tumour Suppressors - Protein Degradation and Cell Death

B274/P1267

Palmitoylation-driven PHF2 Ubiquitination Remodels Lipid Metabolism through SREBP1c Axis in Hepatocellular Carcinoma

D. Jeong[1], J. Seo[1,2], J. Fukuda[2], Y. Chun[1]; [1]Seoul National University College of Medicine, Seoul, KOREA, REPUBLIC OF, [2]Yokohama National University, Yokohama, JAPAN.

Oncogenic signals and malignant phenotypes are closely correlated with lipids and lipid-related enzymes. Palmitic acid (PA) is the most common saturated fatty acid in humans and is known to mediate

palmitoylation of target proteins. Although PA and palmitoylation affect many oncogenic proteins, its pathophysiological functions are not thoroughly understood. In this study, we presented that PA acts as a molecular checkpoint of lipid reprogramming and cancer progression in hepatocellular carcinoma (HCC). Mechanistically, a palmitoyl-transferase ZDHHC23 palmitoylates plant homeodomain finger protein 2 (PHF2) at cysteine 23, which subsequently mediates ubiquitin-dependent degradation of PHF2. Further experiments using a mutant containing a cysteine-to-alanine substitution showed that PA-induced lipid accumulation and cell proliferation is largely dependent on PHF2 palmitoylation. We also found that PHF2 functions as a tumor suppressor by acting as an E3 ubiquitin ligase of sterol regulatory element-binding protein 1c (SREBP1c), a master transcription factor of lipogenesis. PHF2 directly interacts with and destabilizes SREBP1c, thereby reducing SREBP1c-dependent proliferation and lipogenesis in HCC cells. Notably, SREBP1c increases free fatty acids in HCC cells, and the consequent PA induction triggers the PHF2/SREBP1c axis. Clinical dataset of HCC patients revealed that the higher ZDHHC23 and SREBP1c and the lower PHF2 expression are closely associated with poor prognosis of cancer. As a result, the circuit of PA/PHF2/SREBP1c modulates HCC progression and could be a potential marker in patients with HCC. Since PA seems to be central to this axis, we suggest that levels of dietary PA should be carefully monitored in patients with HCC.

B275/P1268

Fbxw7 triggers degradation of WDR7 to prevent mitotic slippage

S. Haenle-Kreidler, K. T. Richter, **I. Hoffmann**; Cell Cycle Control and Carcinogenesis, F045, DKFZ, Heidelberg, GERMANY.

During prolonged mitotic arrest induced by anti-microtubule drugs, cell fate decision is determined by two alternative pathways, one leading to cell death, the other inducing premature escape from mitosis by mitotic slippage. Cancer cells are able to evade mitotic cell death by performing mitotic slippage, which means that they exit from mitosis without completing a normal cell division. Therefore, mitotic slippage promotes survival and chemoresistance of cancer cells and represents a major problem in chemotherapy. FBWX7, a member of the F-box family of proteins and substrate-targeting subunit of the SCF (SKP1-CUL1-F-Box) E3 ubiquitin ligase complex promotes mitotic cell death and prevents mitotic slippage. Most of the FBXW7 substrates are widely studied protooncogenes including Cyclin E, c-Myc, Notch and c-Jun. FBXW7 functions as tumor suppressor and mutations in the FBXW7 gene have been observed in a variety of human cancers. We find that WDR5, a component of the mixed lineage leukemia (MLL) complex of Histone 3 Lysine 4 (H3K4) methyltransferases is a substrate of FBXW7. WDR5 binds to FBXW7 in vivo and in vitro and its ubiquitin-mediated proteasomal degradation is mediated by FBXW7. Furthermore, we find that WDR5 depletion counteracts FBXW7 loss-of-function by reducing mitotic slippage and polyploidization. Our data elucidate a new mechanism in mitotic cell fate regulation which might contribute to prevent chemotherapy resistance in patients after anti-microtubule drug treatment.

B276/P1269

Basal Gp78-dependent mitophagy promotes mitochondrial health and limits mitochondrial ROS

P. Alan[1], **K. Vandevoorde**[1], B. Joshi[1], B. Cardoen[2], G. Gao[1], Y. Mohammadzadeh[1], G. Hamarneh[2], I. R. Nabi,[1]; [1]University of British Columbia, VANCOUVER, BC, CANADA, [2]Simon Fraser University, Burnaby, BC, CANADA.

Mitochondria are major sources of cytotoxic reactive oxygen species (ROS) that contribute to cancer progression. Mitophagy, the selective elimination of mitochondria by autophagy, monitors and maintains mitochondrial health and integrity, eliminating ROS-producing mitochondria. However, mechanisms underlying mitophagic control of mitochondrial homeostasis under basal conditions remain poorly understood. Gp78 E3 ubiquitin ligase is an endoplasmic reticulum membrane protein that induces mitochondrial fission and mitophagy of depolarized mitochondria. Here, we report that CRISPR/Cas9 knockout of Gp78 in HT-1080 fibrosarcoma cells increased mitochondrial volume and rendered cells resistant to carbonyl cyanide m-chlorophenyl hydrazone (CCCP)-induced mitophagy. These effects were phenocopied by knockdown of the essential autophagy protein ATG5 in wild-type HT-1080 cells. Use of the mito-Keima mitophagy probe confirmed that Gp78 promoted both basal and damage-induced mitophagy. Application of a spot detection algorithm (SPECHT) to GFP-mRFP tandem fluorescent-tagged LC3 (tfLC3)-positive autophagosomes reported elevated autophagosomal maturation in wild-type HT-1080 cells relative to Gp78 knockout cells, predominantly in proximity to mitochondria. Mitophagy inhibition by either Gp78 knockout or ATG5 knockdown reduced mitochondrial potential and increased mitochondrial ROS. Live cell analysis of tfLC3 in HT-1080 cells showed the preferential association of autophagosomes with mitochondria of reduced potential. Basal Gp78-dependent mitophagic flux is therefore selectively associated with reduced potential mitochondria promoting maintenance of a healthy mitochondrial population and limiting ROS production.

B277/P1270

Pharmacological PIP4K2 inhibition disrupts mitochondrial homeostasis and autophagy in acute leukemia

K. Lima[1], D. A. Pereira-Martins[1], L. B. L. de Miranda[2], J. L. Coelho-Silva[3], R. C. Cavaglieri[1], J. J. Schuringa[4], E. M. Rego[1], J. A. Machado-Neto[2]; [1]Laboratory of Medical Investigation in Pathogenesis and Targeted Therapy in Onco-Immuno-Hematology, Faculdade de Medicina, University of São Paulo, São Paulo, BRAZIL, [2]Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, BRAZIL, [3]Department of Medical Imaging, Hematology, and Oncology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, BRAZIL, [4]Department of Experimental Hematology, Cancer Research Center Groningen, University Medical Center Groningen, University of Groningen, Groningen, NETHERLANDS.

Introduction: PIP4K2s comprise a family of lipid kinases responsible for the production of phosphoinositides with structural and signal transduction functions. PIP4K2A and PIP4K2C expressions have been identified as prognostic factors in acute myeloid leukemia (AML) patients. Ancestry-related polymorphisms in the PIP4K2A gene are associated with the risk of developing acute lymphoblastic leukemia (ALL). Recently, a selective pharmacological inhibitor of PIP4K2s was developed (THZ-P1-2). **Aims:** To evaluate the antineoplastic potential of THZ-P1-2 in AML and ALL cells. **Methods:** A panel containing 12 AML and 9 ALL cell lines was used. Cell viability was evaluated by MTT assay, apoptosis by annexin V/PI and flow cytometry (FC), mitochondrial membrane potential by JC-1 and FC, formation of

acidic vesicular organelles (AVO) by acridine orange and FC, cell cycle by propidium iodide and FC, and cell signaling by qPCR and Western blot. ANOVA and Bonferroni post-test were used. A p-value < 0.05 was considered significant. **Results:** In AML and ALL cells, THZ-P1-2 reduced cell viability in a concentration-dependent manner. THZ-P1-2 induced apoptosis, loss of mitochondrial membrane potential, and AVO formation (all p < 0.05). Cell cycle analysis indicated an increase in cells in subG1 and a decrease in cells in G2/M (all p < 0.05). Molecular analysis indicated that THZ-P1-2 has a paradoxical role in autophagy, at first the compound induced the initiating stages of autophagy (increased LC3II) and then inhibited the final stages of autophagy flux (reduction of p62/SQSTM1), which may trap the cell in a state of inertia. DNA damage (γH2AX) and apoptosis (PARP1 cleavage) markers were induced by THZ-P1-2. Gene expression profile indicated that processes such as intrinsic apoptotic signaling pathway, signal transduction in absence of ligand, autophagosome organization, macroautophagy, and response to starvation are involved in the mechanism of action of the compound (all FDR *q* < 0.05). **Conclusion:** The contribution of PIP4K2-related genes to acute leukemias has been revealed in recent years, ranging from prognostic markers to the association with genetic susceptibility. Our study provides insight into pharmacological PIP4Ks inhibition targeting mitochondrial homeostasis and autophagy shedding light on a new class of drugs for these diseases. Supported by FAPESP and CNPq.

B278/P1271

AD80 inhibits aurora kinases and induces mitotic catastrophe and autophagy in pancreatic cancer cells

K. Lima[1], L. B. L. de Miranda[1], A. D. B. Garnique[1], B. O. de Almeida[1], M. C. do Nascimento[2], G. M. Machado-Santelli[1], E. M. Rego[2], **J. A. Machado-Neto**[1]; [1]Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil, [2]Faculdade de Medicina, University of São Paulo, São Paulo, Brazil

Introduction: Pancreatic cancer is one of the most lethal human neoplasms and its therapeutic repertoire remains limited. Advances in the understanding of the molecular complexity involved in the biology of the disease have paved the way for new therapeutic opportunities. AD80 is a multikinase inhibitor that inhibits S6K as well as RET, RAF, and SRC and displays antineoplastic effects in hematological and solid tumors. **Aims:** To uncover cellular and molecular effects of AD80 in pancreatic cancer cells. **Methods:** MIA PaCa-2, PANC-1, and AsPC-1 pancreatic cancer cell lines were used. Cell viability was evaluated by MTT assay, clonogenicity by colony formation assay, DNA content analysis by propidium iodide and flow cytometry, morphology by immunofluorescence (IF), formation of acidic vesicular organelles (AVO) by acridine orange and fluorescence microscopy, and cell signaling by PCR array and Western blot. Spheroid was also used to access the effects of the drug in 3D models. ANOVA and Bonferroni post-test were used. A *p*-value < 0.05 was considered significant. **Results:** In pancreatic cancer cells, AD80 reduced cell viability in a concentration- and time-dependent manner (all $p < 0.05$). AD80 also strongly decreased clonogenicity (all $p < 0.05$). DNA content analysis indicated an increase in polyploidy cells (all $p < 0.05$), which was confirmed by IF. In the molecular scenario, AD80 reduced S6RP and histone H3 phosphorylation and induced γH2AX and PARP1 cleavage. AURKA phosphorylation and expression were markedly decreased by the drug. In PANC-1 cells, AD80 strongly induced autophagic flux (consumption of LC3B and SQSTM1/p62). A total of 32 out of 84 autophagy-related genes were modulated by AD80 and it was associated with vacuole organization, macroautophagy, response to starvation, cellular response to nitrogen levels, and cellular response to extracellular stimulus (all FDR *q* < 0.05). In 3D pancreatic cancer models, AD80 also effectively reduced cell viability (all $p < 0.05$). **Conclusion:** AD80 induces mitotic aberrations, DNA damage, autophagy, and apoptosis in pancreatic cancer cells. Our exploratory study establishes novel targets underlying the antineoplastic activity of the

drug and provides insights into the development of therapeutic strategies for this disease. Supported by FAPESP, CNPq, and CAPES.

B279/P1272

Doramectin induces apoptosis in B16F10 melanoma cells

M. Crotts, J. Jacobs, R. Baer, J. L. Cox; A T Still University, Kirksville, MO

Introduction: Metastatic melanoma is resistant to current pharmacologic regimens that act mainly through apoptotic mechanisms. This indicates novel therapies are needed, specifically those acting via alternative cell-death pathways. Doramectin has shown promising results inhibiting neuroblastoma growth via autophagy. Our research question was will doramectin induce autophagy in murine melanoma B16 cells as a means for cell-death.
Method: B16 viability and proliferation were assessed with MTT analysis. Cells were treated with doramectin (15 uM), both doramectin and an indicator of cell-death, as compared to untreated control cells (media). Likewise, MDC analysis was completed to observe autophagy. Flow cytometry and Western blot analyses were conducted to observe protein amplification with doramectin treatment to assess cell-death pathways. Mitotracker was used to analyze post treatment cellular morphology.
Results: MTT analysis of doramectin treated cells displayed a significant increase in cell death when compared to control. Furthermore, time-course analysis of doramectin treatment over 24 hours yielded significant differences in nuclear morphology with apoptotic indications such as shrunken nuclei and nuclei fragments. Flow cytometry analysis of doramectin treated cells showed apoptosis as a major mode of cell death with some necrosis. Western blot analysis showed increased levels of CHOP with doramectin treatment. Mitotracker yielded significant differences in mitochondrial morphology.
Conclusion: Doramectin does not induce autophagy in murine melanoma B16 cells *in vitro*. Rather, it induces cell-death via apoptosis. However, morphological changes and the presence on necrosis suggest an alternative mechanism which may include reactive oxidative species. Further work exploring novel mitochondrial targeted therapies are needed.

B280/P1273

Silibinin protects against staurosporine-induced apoptosis in malignant melanoma B16F10 cells

J. Jacobs, M. Crotts, R. Baer, J. L. Cox; A T Still University, Kirksville, MO

Background: Rare melanoma subtypes have few therapeutic options to treat advanced disease and current treatments show minimal anti-tumor effect. Patients minimally benefit from combined targeted therapy with immunotherapy and kinase pathway inhibitors. Silibinin from milk thistle is characterized as having pharmacological activity through anti-carcinogenic and anti-inflammatory effects. PKC inhibitor Staurosporine is pro-apoptotic and currently undergoing phase II clinical trials for treatment of rare melanoma subtypes. The current study provides a possible mechanism as to why combined therapy shows minimal results and contradicts previous studies describing the use of silibinin as a potential clinical combination therapy. Our data suggests silibinin protects against staurosporine- induced cell death in B16F10 melanoma cells. Individual treatment of either silibinin or staurosporine on B16F10 melanoma cells is pro-apoptotic in a dose- and time-dependent manner. MTT assays show increased cell death, NucBlue staining shows decreases in cell volume and nuclear size in addition to changes in nuclear morphology, and further studies indicated DNA fragmentation and decreased p-AKT and p-iKBa levels. However, pretreatment with silibinin prior to staurosporine treatment decreases cell death,

increases cell volume and nuclear size, and decreases apoptosis significantly. This indicates that silibinin protects cells from staurosporine-induced apoptotic cell death and should not be used in combined targeted therapy in patients with rare melanoma subtypes undergoing treatment with a staurosporine derived PKC inhibitor.

B281/P1274

CDK5 interacts with MST2 and modulates the Hippo pathway

M. Passi, S. Zahler; Ludwig-Maximilians-Universität München, Munich, Germany

The Hippo-YAP/TAZ pathway is a prevalent evolutionary conserved pathway from *Drosophila* to mammals and plays a key role in controlling organ size, cell growth, self-renewal, and tissue homeostasis. The Hippo pathway consists of a MST/LATS kinase module and a YAP/TAZ-TEAD transcription module, and regulates the transcriptional activity of YAP and TAZ, responding to a broad range of mechanical clues from shear stress to cell shape, cell-cell contact, extracellular matrix rigidity and stiffness. In general, the Hippo pathway kinases are considered tumour suppressors. Activation of YAP/TAZ is inhibited by phosphorylation caused by LATS1/2 and MST kinases, which results in cytoplasmic sequestration and degradation of YAP/TAZ. While unphosphorylated, YAP/TAZ translocate to the nucleus and bind to TEAD family transcription factors to induce gene expression. We have recently identified an interaction between CDK5, an unusual member of the family of cyclin-dependent kinases, and MST2 in a yeast-two-hybrid screen. Accordingly, in the current study, we aim to shed a light on the involvement of CDK5 in the Hippo pathway by employing. CDK5 knockdown and overexpression in Huh-7 cells. While CDK5 did not directly affect phosphorylation of MST2, phosphorylation of LATS1 (at Thr1079) was changed by knockdown of CDK5. Transcriptional YAP activity was also changed accordingly (measured by reporter gene assay). Since the kinase activity of CDK5 seems not to be crucial for the process (inhibition by roscovitine, a CDK5 inhibitor), CDK5 seems to act like a scaffold bringing the other proteins together and providing a platform for the Hippo pathway cascade.

Keywords: - CDK5, Hippo pathway, YAP/TAZ, MST1/2, LATS1/2, kinases, overexpression, knockdown.

B282/P1275

CYPRESS ESSENTIAL OIL LIMITS PROLIFERATION OF FIBROSARCOMA CELLS

D. Garcia, M. Catalfumo, D. Lobo, D. Lobo; Monmouth University, West Long Branch, NJ

CYPRESS ESSENTIAL OIL LIMITS PROLIFERATION OF FIBROSARCOMA CELLS

Dianelys Garcia, Michael Catalfumo, and Dorothy LoboBiology Department, Monmouth University, West Long Branch, NJ 07764

Cypress oil is an essential oil derived from evergreen coniferous trees native to Southern Europe and Western Asia. Cypress oil exerts anticancer properties due to their natural terpenes which induce apoptosis and cell cycle arrest, in turn limiting tumor growth and metastasis. The components of cypress essential oil include a total of 20 constituents which represent 98.1% of the oil. These include: α-pinene (48.6%), δ-3-carene (22.1%), limonene (4.6%) and α-terpinolene (4.5%) which are the main components comprising 79.8% of the oil. A cell line of HT-1080 (fibrosarcoma) cells were treated with cypress essential oil at different concentrations and the effects were determined. Proliferation was measured by direct cell counting using trypan blue dye exclusion and MTT assay. Proliferation of HT-1080 fibrosarcoma cells demonstrated a significant decrease with increased concentration of cypress essential oil. In addition, high concentrations of cypress essential oil also decreased MTT activity which

therefore confirmed a decrease in viability due to essential oil treatment. Western blot analysis will be used to ascertain if the decreased viability is a result of apoptosis by detection of PARP cleavage. The effects of cypress essential oil will also be tested on normal fibroblast cells to compare differences in signaling. The signaling of normal-contact inhibited cells treated with cypress will be compared to the cancerous cell line.

B283/P1276

Manuka Essential Oil Decreases Proliferation and Causes Apoptosis in HT-1080 Fibrosarcoma Cells

N. I. Bass, **D. Hutter Lobo**; Monmouth University, West Long Branch, NJ

Since research on the effects of essential oils on human cell lines is limited, the goal of this project was to treat cancer cells lines with manuka essential oil at different concentrations and ascertain the effects on cell proliferation on a variety of cell lines, including normal fibroblast (CUA-4) and fibrosarcoma (HT-1080) cells. Manuka oil is popular in many skincare products because of its antibacterial and anti-inflammatory properties that treat several skin conditions. However, manuka oil also contains an active ingredient that is commonly found in herbicides and is potentially toxic to human cells at certain concentrations. To study the effects of this essential oil on cultured cells, cell lines were grown on 24-well plates, and subconfluent cultures were treated with varying concentrations of manuka oil for 24 hrs. The effect of the oil on proliferation and viability was measured through direct cell counting using trypan blue dye exclusion and through the use of an MTT assay. As the concentration of oil increased, viability of all cell lines tested decreased. MTT assay results also reflected this trend, with a significant decrease in MTT activity seen in cells treated with 500 µg/ml manuka oil. To determine if the decreased cell numbers observed from manuka oil treatment is the result of apoptosis, PARP cleavage assays were performed in HT-1080 cells. HT-1080 cells were found to have significant levels of PARP cleavage after 4 hours of treatment with manuka essential oil, indicating that the cells were undergoing apoptosis.

Regulatory and Noncoding RNAs

B285/P1277

Effects of Osteoclasts on Transcriptional Regulation of Non-Coding RNAs in Osteoblasts Grown on Titanium with Nanotopography

R. L. B. Trevisan[1], E. P. Ferraz[2], J. A. R. Gordon[3], C. Tye[3], J. B. Lian[3], G. S. Stein[3], J. L. Stein[3], A. L. Rosa[1], **M. M. Beloti**[1]; [1]School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, BRAZIL, [2]School of Dentistry, University of São Paulo, São Paulo, BRAZIL, [3]Department of Biochemistry and Vermont Cancer Center, University of Vermont Larner College of Medicine, Burlington, VT.

The bone remodeling process is crucial for titanium (Ti) osseointegration and depends on the crosstalk between osteoclasts and osteoblasts, which may be affected by epigenetic mechanisms, including the action of long non-coding RNAs (lncRNAs) and microRNAs (miRNAs). Ti with nanotopography (Ti Nano), generated by H_2SO_4/H_2O_2 treatment, attenuates the negative effects of osteoclasts on osteoblasts. Thus, we hypothesized that osteoclasts affect transcriptional regulation of non-coding RNAs (ncRNAs) in osteoblasts grown on Ti Nano in a different way than on machined Ti (Ti Control). We aimed to investigate the effects of osteoclasts on the regulation of ncRNAs in osteoblasts grown on Ti Nano compared to Ti Control. Osteoblasts were cultured on Ti Nano or Ti Control, and osteoclasts were cultured in inserts in a co-culture model for 48 h; non-cocultured osteoblasts were used as control.

Using RNA-seq (DESeq2: FC>1.7; p<0.05), we identified 4328 modulated mRNAs and 252 modulated lncRNAs, plotted in a heatmap according to their expression patterns. Correlation analyses between lncRNAs and mRNAs as well as the cluster distribution were identified through K-Means and Elbow methods (RStudio), considering the Pearson correlation and coefficients 1.0 and -1.0. Many genes were regulated by osteoclasts, mainly in osteoblasts grown on Ti Nano. The real-time PCR (p≤0.05) showed that osteoclasts upregulated the gene expression of *H19*, *Snhg1*, *Neat1* and *Zfas1* and downregulated *Carmn*, *Tug1*, *Dnm3os* and*Kcnq1ot1* in osteoblasts cultured on Ti Nano. In osteoblasts grown on Ti Nano, positive correlations between the expression of *Kcnq1ot1*, a lncRNA that contributes to osteogenic differentiation by sponging miR-214, and the bone marker mRNAs *Alpl*, *Bglap*, *Bmp8a*, *Col1a1* and *Vim* in the absence of osteoclasts, and negative correlations in the presence of osteoclasts, were observed. Although osteoclasts downregulated *Kcnq1ot1* and upregulated miR-214 in osteoblasts grown on both Ti surfaces (p≤0.05), such effects were more pronounced on Ti Nano. Thus, we conclude that Ti Nano induces osteoblast resistance to the negative effects of miR-214, which is reversed by the presence of osteoclasts due to, at least in part, the modulation of osteogenic mRNAs through a *Kcnq1ot1*-miR-214 circuit. **Financial support:** FAPESP-Brazil (# 2017/12622-7, 2017/23888-8, 2018/17356-6 and 2019/09349-2) and NIH-USA (R01AR039588 and R01DE029311).

B286/P1278

Diagnostic potential of circulating miRNAs in metabolic syndrome

M. Khan[1], A. Dandare[1,2], A. Liaquat[3], M. Rafiq[1,3]; [1]COMSATS University Islamabad, Islamabad, PAKISTAN, [2]Usmanu Danfodiyo University Sokoto, Nigeria, Sokoto, NIGERIA, [3]Shifa Tameer-E-Millat University, Islamabad, PAKISTAN.

The global prevalence of metabolic syndrome (MS) is 20-25 %. Inadequate information about underlying pathophysiological factors of MS is a major challenge for designing treatment approaches that target the root of the disease, and the reasons for the increasing cases of the syndrome in the world. This study aimed to evaluate the potential role of miRNAs for novel treatment and diagnostic approaches to the MS. Dysregulation of miRNAs and their target genes was measured in MS patients and healthy controls. The serum level of insulin, C-reactive protein, and adiponectin were quantified using ELISA. Relative expression of miR-17-5p, miR-320a, miR-548c-3p, miR-579-3p, and miR-21-5p along with their target genes of JAK/STAT signaling pathway; *STAT3, JAK2, SOCS4, IL6R, AKT1, PIK3CB, PIAS1,* and *PTPN11* was measured using qPCR. We observed the existence of dyslipidemia, elevated level of serum insulin, and C-reactive protein (CRP) accompanied by decreased adiponectin in MS patients compared to the control group. miR-17-5p, miR-320a, miR-548c-3p, and miR-579-3p were significantly up-regulated, whereas *STAT3, JAK2, SOCS4,* and *IL6R* genes were down-regulated in the MS patients. Existence of positive correlation was observed between the up-regulated miRNAs and insulin or CRP and inversely correlated with adiponectin concentrations. Interestingly, the miRNAs exhibited the order of diagnostic accuracy as miR-320a > miR-579-3p >> miR-548c-3p > miR-17-5p. The involvement of miR-17-5p, miR-320a, and miR-548c-3p in the pathogenesis of MS via the regulation of the JAK/STA signaling pathway was suggested, thus could be promising candidates for both treatment and diagnostic approaches to the disease.

B287/P1279

SARS-CoV-2-derived microRNAs activate inflammasome and interferon pathways in human lung and blood immune cells

L. G. Bimerew[1], F. Meng[1], Z. Azam[1], N. Wong[1], T. Lee[2], S. Yip[1], C. Huang[1,3]; [1]Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Kowloon, HONG KONG, [2]Department of Biomedical Engineering, The Hong Kong Polytechnic University, Kowloon, HONG KONG, [3]Research Institute for Future Food, The Hong Kong Polytechnic University, Kowloon, HONG KONG.

The causative agent of COVID-19, SARS-CoV-2 controls the host immune function for its benefit through various mechanisms. The role of virus-derived microRNAs (v-miRNAs) in COVID-19 pathogenesis has recently been reported, while the mechanism behind this process is incompletely understood. We aimed to identify the v-miRNAs in SARS-CoV-2 infected cells and their role in host immune regulation. Small RNA-seq library was prepared on-site and sequenced using the Illumina platform on RNA samples isolated from SARS-CoV-2 infected Calu-3 human lung cells and peripheral blood mononuclear cells (PBMCs). A number of SARS-CoV-2 encoded v-miRNAs were identified using stringent bioinformatics approaches. V-miRNAs and control miRNA mimics were synthesized and transfected into Calu-3 and PBMCs. Next, RT-qPCR, flow cytometry, western blotting, ELISA, and single-cell RNA-seq (scRNA-seq) were performed to identify and validate the putative targets of v-mRNAs and their global response on immune cell populations. ScRNA-seq data is analyzed in the 'R' (version 4.1.2) environment using the 'iCellR' package and graphs and charts are prepared in GraphPad Prism. We identified the top three SARS-CoV-2-*N* encoded v-miRNAs (v-miRNA-Ns) and we further validated the expressions in COVID-19 patients' nasopharyngeal aspirate and *ex vivo* infected PBMCs. Further cellular pathway analysis showed that PBMCs transfected with v-miRNA-N-mimics express significantly higher IL-1β, Caspase 1, and NLRP3 protein levels, indicating their involvement in inflammasome activation. *N* encoded v-miRNAs induce COVID-19-like features demonstrated by our scRNA-seq platform in both overall and individual immune cell groups (in PBMCs), featured by upregulation of key COVID-19-related gene activity. Functional analysis of differentially expressed genes identified interferon signaling as the most enriched pathway along with apoptosis signaling genes in the v-miRNA-N groups. In line with previous findings, our analysis showed a similar trend of interferon signaling genes in the v-miRNA groups (e.g., *XAF1, IFI44L, IFI44, HERC6, STAT1, TRAF3, MX1 and ISG15*). We show here for the first time that SARS-CoV-2 *N* encoded v-miRNAs could modulate inflammatory and cell death processes by hijacking host gene regulations and downstream signaling networks.

B288/P1280

Unveiling the potential role of myopia-SNPs on non-coding RNA-mediated ceRNA network in human iPSC-derived retinal ganglion cells

N. Wong[1,2], Z. Jiang[1], R. Kwarteng[1], S. Luo[1], V. Choh[2,3], S. Yip[1,2], C. Huang[1,2]; [1]Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Kowloon, HONG KONG, [2]Centre for Eye and Vision Research Limited, Sha Tin, HONG KONG, [3]University of Waterloo, Waterloo, ON, CANADA.

Myopia is a multifactorial visual disorder characterized by axial elongation of the eyeball along with structural changes in the choroid, retina, and sclera. The specific etiology is still unclear but both genetics and environmental factors are considered to be contributors to myopia. Our study aimed to characterize the regulatory role of non-coding RNAs in retinal ganglion cells (RGCs), which may

potentially contribute to myopia. Human induced pluripotent stem cells (iPSCs) were differentiated into RGCs under specified culture conditions. The differentiated cells were collected on day 40 and subjected to RT-qPCR and flow cytometry analysis to check the cell identity. RNA-seq and small RNA-seq were performed to identify differentially expressed genes (DEGs) (| $\log_2$FoldChange | >2, adjusted *P*-values < 0.05), including differentially expressed-long non-coding RNA (DE-lncRNA) and microRNA (DE-miRNA). Moreover, bioinformatics analyses of potential competing endogenous RNA (ceRNA) interactions involved in RGC differentiation was completed. A significant upregulation of RGC marker genes (ATOH7, BRN3A, ISL1, SNCG) was detected by RT-qPCR, and flow cytometry analysis showed >60% $THY1^+$ cells, which represents an enriched population of RGC lineage. RNA-seq data revealed 7,753 DEGs in 40-day RGCs compared to iPSCs, while 2,944 of them are lncRNAs. 18,432 SNPs associated with myopia (myopia-SNPs) were identified from genome-wide association studies and mapped to the genomic location of DE-lncRNA. MAPT-AS1 and MAPT-IT1 were two upregulated DE-lncRNAs showing >10 myopia-SNPs located on their exons. By *in silico* analyses, putative binding regions of DE-miRNA with myopia-SNPs located on them were identified on MAPT-AS1 and MAPT-IT1. MiR-302a-5p and miR-6797-5p were found to be the top downregulated DE-miRNAs that have binding sites on MAPT-AS1 and MAPT-IT1, respectively. Further analyses revealed a list of mRNAs that are potentially targeted by miR-302a-5p and miR-6797-5p, which could subsequently construct ceRNA networks in RGCs. To conclude, putative lncRNA-miRNA-mRNA networks were identified during RGC differentiation and myopia-SNPs may alter such interactions by disrupting the miRNA-binding sites.

B289/P1281

Long non-coding RNA H19 regulates retinal gene expression during neural retina/photoreceptor differentiation of human mesenchymal stem cells

F. Meng[1], **Z. Jiang**[1], J. Sun[1], N. Wong[1,2], T. Krause[1,3], V. Choh[2,4], S. Yip[1,2], C. Huang[1,2]; [1]Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Kowloon, HONG KONG, [2]Centre for Eye and Vision Research Limited, Sha Tin, HONG KONG, [3]FH IMC University of Applied Sciences, Krems, AUSTRIA, [4]University of Waterloo, Waterloo, ON, CANADA.

Degenerative retinal diseases cause impairment of retinal cellular functions, resulting in premature cell death and ultimately direct vision damage. There is currently still no effective treatment for these degenerative retinal diseases. Therefore, understanding the molecular mechanisms in addition to further optimization for stem cell-based retina lineage differentiation may provide an improved cellular and regenerative therapy. The objective of this study is to investigate the regulatory role of long non-coding RNA H19 and its derived microRNA miR-675 in the differentiation of human mesenchymal stem cells (hMSCs) into neural retina/photoreceptor cells. Bone marrow-derived hMSCs were induced to undergo retinal differentiation by cytokine cocktail treatment (DKK1, IGF-1, Noggin, and bFGF). Total RNA was isolated from hMSCs cultured in the differentiation medium for 3, 7, 14 and 72 hours post H19-siRNA transfection. Expressions of retina-relevant marker genes NRL, OPN1MW, and RORB plus H19/miR-675-3p and -5p were determined by quantitative RT-qPCR. The mRNA targets and functional networks of H19/miR-675 were analyzed using bioinformatics tools with a focus on genes involved in retina development. Up-regulation of H19, OPN1MW, and RORB was detected in hMSCs after 3-day retinal differentiation, and peaked at day 14. After H19-siRNA transfection, the expression of H19 was inhibited 11.3-fold, and down-regulation of OPN1MW and RORB were shown on day 3 (11.9-fold and 2.7-fold) compared to control-siRNA-transfected hMSCs. The major H19 isoform H19_203, which contains the miR-675 region, was detected in hMSCs instead of H19_206. By *in silico* analysis, we

identified 49 potential miRNAs that targeted the retinal relevant genes. Among them, DKK3, NEUROD1/2, OPN1MW, OPN1SW, OPN4/5, OTX1, PAX6, RORA/B/C, and RXRG were the targets of miR-675-3p and -5p. In addition, H19 contains the binding sites of 13 of these miRNAs. This implies that H19 may act as the miRNA sponge during retinal differentiation. Gene Ontology enrichment analysis showed that these putative miRNA-lncRNA-mRNA interactions may be involved in the functional pathways of intraciliary transport and visual learning and behavior. Specifically, this newly identified H19-mediated miRNA-lncRNA-mRNA interactome may play an important role in neural retina/photoreceptor differentiation through targeting genes involved in retina development.

B290/P1282

Phase separation of Lysine Specific Demethylase-1 recruits TERRA on telomere to trigger R-loop mediated ALT pathway

M. Xu[1], D. Senanayaka[2], D. Chenoweth[3], N. Reiter[2], H. Zhang[1]; [1]Carnegie Mellon University, Pittsburgh, PA, [2]Marquette University, Milwaukee, WI, [3]University of Pennsylvania, Philadelphia, PA.

Histone methyl regulatory enzymes and chromatin-associated RNAs influence the dynamic organization of the genome and modulate gene expression. The lysine-specific demethylase 1A (LSD1 or KDM1A) catalyzes the removal of histone mono- and dimethyl functional groups (H3K4me1/2 or H3K9me1/2), resulting in repressive or activating transcriptional marks respectively. Here we report a new function of LSD1 through its phase separation with RNA in telomere maintenance via the alternative lengthening of the telomere (ALT) pathway that is based on homology-directed DNA synthesis. We find LSD1 localizes to ALT telomeres, and LSD1 deficiency leads to a loss in ALT signatures, such as telomere clustering and telomeric DNA synthesis. The presence of LSD1 on ALT telomeres requires the telomere repeat-containing RNA (TERRA), a lncRNA that promotes ALT pathway via R-loop formation. Interestingly, the presence of TERRA on ALT telomeres also requires LSD1. To determine the mechanism underlying the mutual dependence of LSD1 and TERRA on telomeres, we used a chemical dimerization approach to recruit LSD1 to telomeres and observed increased TERRA level and R-loop formation. In addition, LSD1 recruitment triggered local phase separation on telomeres. In vitro reconstitution shows that TERRA and LSD1 indeed undergo phase separation. The formation of LSD1 condensates depends on the structure of TERRA, with condensate formation preferentially occurring in a length-dependent manner that correlates with the number of UUAGGG hexanucleotide repeats. Our findings reveal a new pathway where LSD1 undergoes phase separation with TERRA to facilitate R-loop formation for telomere maintenance in ALT cancer cells.

B291/P1283

MicroRNA profiling in pseudoexfoliation syndrome using next-generation sequencing

A. Khera, S. Pandav, F. TT; Postgraduate Institute of Medical Education and Research, Chandigarh, India

Aim: XFS etiopathogenesis is linked to multiple genes and microRNAs. However, the precise etiopathogenesis is unknown. The goal of this study was to use NGS to profile miRNA expression in XFS. **Material & Methods:** During cataract surgery, anterior lens capsules (ALC) from XFS, XFG, and cataract (CAT) patients were obtained. ALC was used to extract RNA. Insilico analysis of significantly expressed genes and miRNAs was performed. qPCR was used to confirm the expression levels of 6 miRNAs, LOXL1 and elastin. **Results:** In all groups, insilico analysis revealed differential expression of genes and miRNAs. The Kegg pathway analysis showed various pathways involved in disease pathology. The expression of

LOXL1 and elastin, and the miRNAs involved in LOXL1 transcription revealed that miR-141 and miR-200 may be involved in disease pathogenesis. **Conclusion:** More research on the expression of miR-141 and miR-200 in large number of samples of XFS is required to come to the conclusion.

B292/P1284

Differential Gene Expression Analysis of the Anterior Cingulate Cortex Shows Highly Divergent Molecular Response Between Depressive and Resilient Groups in a Mouse Model of Major Depression

J. Cortés Silva[1,2,3], T. Peña-Centeno[1], L. Kaurani[1], J. Zhou[1], S. Burkhardt[1], A. Fischer[1,3]; [1]German Center for Neurodegenerative Diseases (DZNE), Göttingen, Germany, [2]International Max Planck Research School for Genome Science, Göttingen, Germany, [3]Universitätsmedizin Göttingen, Göttingen, Germany

Major Depression (MD) is one of the most common neuropsychiatric disorder with incidence numbers that currently exceed 300 million people suffering from this disease worldwide. Although the symptoms and treatment for MD follow well-defined guidelines, the mechanisms of action at the molecular level are still unknown. In the present study the Social Defeat (SD) paradigm was applied to mice to study the genetic basis of MD, and the molecular mechanisms that may mediate resilience to environmental stressors. The presentation of chronic SD over a period of 10 days allowed us to distinguish between those subjects who responded to the stress-induced procedure (depressive group), and those who did not show any effect (resilient group) at the behavioral level. The distinction of the groups was made considering the immobility times of the mice during the Forced Swim Test (FST) compared to a control group. Cognitive function, specifically the processes related to spatial, reference and working memory, were addressed using the Morris Water Maze (MWM) and the Y-Maze test when the SD and FST procedure was completed. After the identification of the groups and the cognitive tests, the Anterior Cingulate Cortex (ACC) of the subjects was dissected to perform TotalRNA-seq gene expression analysis. Animals: 21 wildtype B6J C57bl and 14 CD1 mice. TotalRNA-seq: Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus: Dual-indexed libraries. Results: RNA-seq gene expression analysis of the ACC confirms a clear distinction between the depressive (n=7), resilient (n=7) and control (n=7) groups, as it was seen in the behavioral tests. Gene ontology (GO) from the Differential Gene Expression (DGE) analysis, indicated an upregulation of the circadian process in the depressive group, a biological function that appears to be intensely affected in people suffering from MD. Along the same lines, a negative regulation of the glucocorticoid receptor signaling pathway, which has been shown to be reactive to stressful stimuli from the environment, is present. Biological function analysis shows a significant downregulation of the defense and immune response in the depressive group. All dysregulation observed in the depressive group after the DGE analysis, was absent in the resilient and control groups. Conclusions: the results will be discussed.

RNA Localization and Transport

B293/P1285

Novel Microscopy Tools Reveal Dynamic Sub-cellular Distributions of Circadian Clock Components in Filamentous Fungus

Z. Wang[1], B. M. Bartholomai[1], J. J. Loros[2], J. C. Dunlap[1]; [1]Molecular and System Biology, Geisel School of Medicine at Dartmouth, Hanover, NH, [2]Biochemistry & Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Circadian rhythms are endogenous daily oscillations driven by an intracellular molecular clock that helps organisms better coordinate their lives with the environment. Organisms from fungi to animals share a similar phosphorylation-driven transcription/translation negative feedback loop as the core clock mechanism, an oscillator composed of positive and negative elements. Research on the filamentous fungus model organism, *Neurospora crassa*, has provided answers to many fundamental clock-related questions. In *N. crassa*, the White Collar Complex (WCC), a heterodimeric transcription factor, serves as the positive element driving the transcription of *frequency* (*frq*). Frequency protein (FRQ), with Casein Kinase-1 (CK-1a) and other regulators, forms the negative element complex that inhibits the function of WCC and stops *frq* transcription. Molecular components of the circadian clock have been described over decades of genetic and molecular biological studies. However, little is known about their dynamics and regulation at the subcellular level. *Neurospora crassa* grows as a syncytium analogous to muscle cells, but the subcellular distribution of transcripts must facilitate precise temporal control throughout the syncytium. We applied single molecule RNA-FISH (smFISH) for analysis of mRNA spatial patterning in fixed cells. While *frq* mRNA cycles in abundance throughout the circadian day, we found surprising heterogeneity in the proportion of transcriptionally active nuclei. Moreover, *frq* transcripts are spatially clustered near to nuclei in a time-of-day dependent manner. The intrinsically disordered RNA-binding protein PRD-2, which binds both *frq* and *ck-1a* mRNAs and displays liquid-liquid phase separation, is crucial to the clustering. This spatiotemporal clustering may organize clock mRNAs to facilitate local translation and assembly of clock protein complexes, while the ability to undergo fission/fusion of PRD-2-mediated biomolecular condensates containing *frq* and *ck-1a* mRNAs may prevent local heterogeneities in the distribution of clock components. Recently, live-cell imaging has emerged as a useful tool in circadian research. We implemented novel microscopy tools for filamentous fungi along with strategies, including 4-color imaging and microfluidics compatible with multi-day growth and imaging, to facilitate live-cell imaging of low-abundance circadian proteins. Through 3-color live-cell imaging of WCC, FRQ and a nuclear marker in single cells, we have followed the circadian dynamics of their subcellular localization in high spatiotemporal resolution. We also optimized and are using FRAP and photoconvertible fluorescent proteins for investigating the nucleocytoplasmic transport mechanism of clock proteins.

B294/P1286

Transport of Myelin Basic Protein mRNA is Essential for Myelination

J. C. Nowacki[1], L. M. Meservey[2], A. Valenzuela[2], H. S. Richardson[1], H. Nguyen[2], J. Lin[2], H. Zeng[2], M. Fu[1]; [1]NINDS, NIH, Bethesda, MD, [2]Stanford, Stanford, CA.

mRNA transport is an evolutionarily conserved cellular phenomenon observed even in the simplest of organisms (yeasts). Oligodendrocytes, the myelin-producing cells of the central nervous system, have an

elaborate cytoskeletal architecture and can myelinate up to 50 axons per cell. Myelin basic protein (MBP) is one of the two most abundant proteins expressed by oligodendrocytes, and *in vitro* studies have established that the 3'UTR is necessary for *Mbp* mRNA localization outside the cell body. Our lab previously generated a mouse model lacking the *Mbp* 3'UTR to investigate whether transport and local translation is necessary for myelination *in vivo.* Preliminary qPCR data has indicated that *Mbp* coding-region mRNA is downregulated in 3'UTR KO mice. Here, we show that oligodendrocytes cultured from Mbp 3'UTR KO animals show *Mbp* mRNA transport defects, KO animals are hypomyelinated, and display tremors and gait defects, thus supporting the necessity of *Mbp* mRNA transport for myelination.

B295/P1287

Localization of mRNAs to Centrosomes Requires the Pericentriolar Material Scaffold

W. Tian, J. Fang, D. A. Lerit; Emory University, Atlanta, GA.

Pericentriolar material (PCM) is a proteinaceous matrix surrounding the centrioles that promotes centrosome microtubule-nucleating activity. We and others previously showed the pericentriolar components Centrosomin (Cnn) and Pericentrin-like protein (PLP) functionally cooperate to organize a PCM scaffold required for centrosome structure and microtubule organization. We recently showed that several centrosomal mRNAs are localized to the centrosome and contribute to centrosome function under regulation of RNA-binding proteins. However, whether the PCM is required to recruit or anchor mRNA to the centrosome remains incompletely understood. We hypothesize that PCM functions as a scaffold to anchor centrosomal mRNAs at centrosomes. To test our hypothesis, we used a classic *cnn* allele to disrupt PCM organization and investigated consequences to the localization of *centrocortin* (*cen*) and *plp* mRNAs. We used single molecule fluorescence in situ hybridization (smFISH) and Asterless-YFP (Asl-YFP) to label mRNAs and centrosomes, respectively, to visualize mRNA enrichments at the centrosome in syncytial *Drosophila* embryos. Given the significantly smaller volume of Asl-labeled centrioles as compared to centrosomes, we adapted our customizable Subcellular Distribution computational pipeline and compared mRNA distributions as quantified from either the centroid or surface of centrosomes to permit quantification using a variety of centrosome markers. Upon disrupting PCM organization, our preliminary data indicate that the enrichment of *cen* mRNAs at centrosomes and the number of mRNAs in granules are significantly decreased. These data demonstrate that proper PCM organization is needed for *cen* mRNA localization to centrosomes. In contrast, we observed no significant difference in *plp* mRNA localization to centrosomes in controls vs. *cnn* mutants, suggesting mechanisms of *cen* and *plp* mRNA localization are separable. We are presently increasing sample sizes to strengthen our quantification and studying additional mRNAs to investigate whether the PCM scaffold is more generally required for mRNA localization to the centrosome. Our work will help elucidate the mechanisms and biological significance of mRNA localization to centrosomes.

B296/P1288

Single Molecule Spatial Transcriptomics Demonstrates Spatial Heterogeneity of miRNA and Classifies beta Cells in Type 1 Diabetes

G. Chang[1], C. Seitz[1], F. Syed[2], C. Evans-Molina[2], J. Liu[1]; [1]Physics, Indiana University-Purdue University Indianapolis, Indianapolis, IN, [2]School of Medicine, Indiana University-Purdue University Indianapolis, Indianapolis, IN.

Recently emerged spatial transcriptomics approaches combine the RNA sequencing (RNA-Seq) with spatial localization to reveal the spatial heterogeneity of transcriptome in pancreatic islet. However, the interrogation of the transcriptomic expression in a single cell is missing, particularly the spatial distribution of each RNA molecule. Here we proposed studying the spatial distribution of the miRNA in single β cells obtained from human pancreatic tissues. A multi-dimensional quantitative model was established to describe the spatial distribution of individual RNAs as a library of “features”, which includes RNA expression, locations, clustering/dispersion, and reciprocal positions. In particular, the degree of RNA clustering/dispersion was described by the mathematical model of clusters, i.e. Ripley's H function. Features are first extracted from spatial distribution of miRNA 146 and 155 in β cells of human pancreatic tissues from non-diabetic and diabetic donors. Extracted features are then analyzed by statistical distribution modelling and supervised machine learning. Machine learning enables the classification of 3 groups of β cells (control, T1D, and AAb+) using spatial transcriptomic features with high accuracy (65%±3%). Furthermore, it offers quantitative evaluation of those distinctive features contributing to the classification and phenotyping. Expression levels of miRNA 146 and 155 in β cells are significantly different among pancreatic sections from non-diabetic donors and nPOD donors with T1D and AAb+. This differentiation further exhibits a spatial disparity, where there exists more nuclear miRNA 146/155 than cytoplasmic miRNA for β cells in the T1D and AAb+ samples. More interestingly, it is revealed that the distribution of miRNA is directly associated with β cell phenotype and T1D disease status. The Ripley's H function at 457 nm suggests a different degree of miRNA 155 dispersion of control and T1D. All evidence suggests the spatial heterogeneity of transcriptome of β cells in T1D, and this transcriptomic disparity has been leveraged to classify β cells into different pathological conditions. This work will not only disclose fundamental mechanisms that are associated with β cell survival in T1D; more practically, it could lead to important transcriptomic features of β cells that could have clinical relevance in stratifying the T1D phenotypes.

B297/P1289

Dynamic transcription start site usage and RNA binding protein complexing regulate *GJA1* mRNA translation to modulate gap junction formation.

E. E. Shrontz[1,2], M. J. Zeitz[1], J. González Velázquez[1], C. C. James[1,3], J. W. Smyth[1,2,4]; [1]Fralin Biomedical Research Institute, Roanoke, VA, [2]Virginia Tech Carilion School of Medicine, Ronaoke, VA, [3]Graduate Program in Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, VA, [4]Department of Biological Sciences, College of Science, Virginia Tech, Blacksburg, VA.

Gap junctions comprising connexin proteins enable direct electrical and metabolic intercellular communication and alterations in gap junction coupling underly several human pathologies including dementia, cancer progression, and cardiac arrhythmias. The most ubiquitously expressed connexin is connexin43 (Cx43, gene name *GJA1*). Investigation of Cx43 gap junction biology has historically focused on transcription, vesicular trafficking, and post translational modifications. The importance of

translational regulation, however, is highlighted by the recent discovery that Cx43 mRNA undergoes internal translation initiation to yield truncated protein isoforms, including GJA1-20k, which regulate gap junction formation. We previously reported that during TGF-β stimulation or stress, shortening of *GJA1* mRNA 5'UTRs occurs through alternate transcription start site (TSS) usage and limits GJA1-20k translation resulting in fewer gap junctions. The IMP family (also termed IGF2BPs) of RNA binding proteins (RBPs) regulate alternative and canonical translation initiation of several genes, which led us to investigate how they may impact *GJA1* mRNA. We hypothesize that alternative TSS usage modifies the inclusion of *cis* acting elements in the *GJA1*-5'UTR affecting RBP binding to regulate translation. Employing mouse and human *GJA1* knockout epithelial cells generated through CRISPR/Cas9, we introduced *Gja1* mRNA harboring various 5'UTR sequences to test what *cis*-elements confer specific localization and/or complexing with RBPs, including the IMPs. Using single molecule FISH to label *GJA1* mRNA, and antibodies targeting IMP1, IMP2, or IMP3 we find IMP1 complexes with *GJA1* mRNA predominantly and this complexing is reduced during TGF-β stimulation. Biochemical assays confirm *GJA1*/IMP1 binding by RNA-IP and synthetic reduction of IMP1 expression by RNA interference or CRISPR/Cas9 alters *GJA1* mRNA localization, reduces internal translation of GJA1-20k, and limits gap junction formation. We find comparable results during hypoxia in primary mouse cardiomyocytes and human induced pluripotent stem cell derived cardiomyocytes. Dynamic Gja1-5'UTR composition therefore represents a powerful mechanism connecting alterations in transcription to gene-specific translational regulation by RBPs such as IMP1, fine tuning intercellular communication during stress.

B298/P1290

Single molecule imaging reveals a conserved family of centrosomal mRNAs and a novel translation-dependent localization mechanism

A. Safieddine[1], E. Coleno[1], S. Salloum[1], A. Imbert[2], O. Kwon[3], R. Chouaib[1], M. Robert[1], X. Pichon[1], H. Le Hir[4], F. Mueller[5], T. Walter[2], **E. Bertrand**[1]; [1]IGH, CNRS, Montpellier, FRANCE, [2]MINES ParisTech, PSL-Research University, CBIO-Centre for Computational Biology, Paris, FRANCE, [3]Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Ecole Normale Supérieure, CNRS, Paris, FRANCE, [4]IGH, CNRS, Institut de Biologie de l'Ecole Normale Supérieure, FRANCE, [5]Imaging and Modeling Unit, Institut Pasteur, Paris, FRANCE.

Local translation allows for a spatial control of gene expression. Here, we performed RNA localization screen using high-throughput smFISH and discovered mRNAs locally translated at unexpected locations, including cytoplasmic protrusions, cell edges, endosomes, Golgi, the nuclear envelope and centrosomes. Surprisingly, mRNA localization frequently required ongoing translation, indicating widespread co-translational RNA targeting. We also discovered that several mRNAs accumulated in foci distinct from P-bodies, which served as specialized translation sites, i.e. translation factories. Most interestingly, we found a conserved family of mRNAs that localize to centrosomes in both human and drosophila cells. These mRNAs localize to centrosomes at different stages of the cell cycle and some also localize to cilia in quiescent cells. Drug treatments and reporter analyses revealed that mRNA localization required translation of the nascent protein. Moreover, using *ASPM* and *NUMA1* as models, single mRNA and polysome imaging revealed active movements of endogenous polysomes towards the centrosome at the onset of mitosis, when these mRNAs start localizing. These data identify a conserved family of centrosomal mRNAs, which localize by a novel mechanism involving active polysome transport mediated by nascent proteins.

B299/P1291

mRNA Location and Translation Rate Determine Nuclear Import Efficiency

A. N. Gasparski, K. Moissoglu, S. Meydan, N. R. Guydosh, S. Mili; National Institutes of Health, Bethesda, MD.

Numerous mRNAs are actively targeted to peripheral protrusions of mammalian migrating cells. However, the functional consequences of peripheral mRNA targeting are largely unexplored. Here, we investigate how peripheral localization of the *NET1* mRNA affects NET1 protein function. The NET1 protein exhibits dual localization. It functions in the cytosol as an activator of the RhoA GTPase, an important regulator of cell motility. NET1 is additionally imported into the nucleus through the action of nuclear localization signals (NLSs) found at the N-terminus. By specifically altering the location of the *NET1* mRNA between peripheral and perinuclear areas, we show that NET1 produced at peripheral locations associates less with importin β1 and persists to a higher extent in the cytoplasm. This mechanism requires an internal PH domain as well as rapid translation of the intervening sequence between the NLS and PH domains. By contrast, perinuclear mRNA location and slower translation rate favor NET1 association with importin β1 and nuclear targeting. Modulation of this mechanism profoundly impacts NET1 function in cell motility. Perinuclear *NET1* RNA localization causes reduced RhoA activity, impedes focal adhesion maturation and results in slower cell migration to an extent similar as that observed upon knockdown of NET1 expression. Overall, these results reveal that the location of protein synthesis and the rate of translation elongation can influence the ability of competing domains within a polypeptide to determine protein distribution and function.

B300/P1292

Genome-wide analysis of anterior-posterior mRNA localization in a giant single cell reveals a role for the microtubule cytoskeleton

A. Albright, W. Marshall; University of California, San Francisco, San Francisco, CA.

Compared to the development of pattern in multicellular tissues, far less is known about how individual cells can develop their own patterns and organization. Several studies in yeast and *Drosophila* embryos show a clear role of a polarized cytoskeletal system in the localization of specific mRNAs; however, these have largely relied on studies of individual genes and the genome-wide extent of this phenomenon remains unexplored. The giant ciliate *Stentor coeruleus* is a classical model that shows promise in investigating subcellular pattern formation owing to its large size, easy manipulability, and its ability to regenerate; all of which allow us to cut individual cells in half. To determine the dependence of mRNA localization on the microtubule cytoskeleton on a genome-wide scale, we knocked down beta-tubulin, a key component of the microtubule cytoskeleton, using RNAi and conducted single-cell RNA-sequencing and half-cell RNA-sequencing in *Stentor coeruleus* cells bisected along the anterior-posterior axis. Here, we show significant differential gene expression in many genes upon depletion of beta-tubulin in both the anterior and posterior regions of the cell when compared to the whole cell as well as to a negative control. Altogether, our results suggest that the microtubule cytoskeleton is critical for proper mRNA localization and expression of many genes along the anterior-posterior axis. Moving forward, our goal is to develop additional technologies in *Stentor coeruleus* and validate these data with an orthogonal method such as single-molecule fluorescence *in situ* hybridization (smFISH) or microinjection of fluorescent RNAs.

B301/P1293

Spatial Coordination of Cytosolic and Mitochondrial Translation

W. Li[1], E. Yun[1], C. Osman[2], R. H. Singer[1]; [1]Program in RNA Biology, Albert Einstein College of Medicine, Bronx, NY, [2]Faculty of Biology, Ludwig-Maximilians-Universität München, München, GERMANY.

Mitochondria produce the energy to fuel various biological activities. The mitochondrial DNA encodes a handful of genes that are essential for ATP production. A class of protein complexes, including the ATP synthase, have dual genetic origins-some of their subunits are encoded by the nuclear DNA while the other subunits are encoded by the mitochondrial DNA. It remains unclear how cells coordinate the nuclear and mitochondrial gene expressions to maintain the subunit stoichiometry. Here, I will present the novel single-molecule imaging methods to study the nuclear- and mitochondrial-encoded mRNAs. We found that the cytosolic and mitochondrial translation co-localize on opposite sides of the mitochondrial double membrane, thereby promoting the assembly of the ATP synthase. This study uncovers a new layer of regulation of the mito-nuclear crosstalk that is fundamental to organelle biogenesis.

This work is supported by American Heart Association Postdoctoral Fellowship #903024 (WL), and 1R35 GM136296-01 (RHS).

B302/P1294

Control of viral RNA translation via SARS-CoV-2 nucleocapsid protein condensation

C. Roden, A. Gladfelter; The University of North Carolina At Chapel Hill, Chapel Hill, NC.

Infections of SARS-CoV-2 virus have resulted in Covid-19, a global pandemic. The SARS-CoV-2 RNA genome encodes enzymatic machinery to initiate copying itself in the host cytosol and the structural proteins necessary to build new virions. How the virus controls the timing, localization, and specific levels of production of these varied proteins is poorly understood. The non-structural proteins are produced by Orf1ab and structural proteins are produced from sub-genomic RNAs (sgmRNAs) that must be individually processed. Notably, each individual structural protein encoding RNA and Orf1ab share the same 5'UTR sequence yet are not translated with equal timing or efficiency. Orf1ab is translated first followed by nucleocapsid (N-protein) then other structural proteins such Spike, Membrane, and Envelope which all are present in unequal abundance. This study focuses on understanding how translation is controlled through N-protein interactions with RNA sequence features. The SARS-CoV-2 N-protein is an intrinsically disordered RNA-binding protein that has been shown to form biomolecular condensates with viral RNA both cell free and in infected cells. Specific viral RNA sequence and structural features promote N-protein condensation. These include transcription regulatory sequences (TRS) which are recognized by the N-terminal (NTD) domain of N-protein in a structure dependent manner and long, double-stranded RNA stem loops recognized by C-terminal (CTD) domain. We report here that interaction between N-protein NTD and TRS-promotes translation of TRS-containing mRNAs present on 5'UTRs of viral RNAs. Blocking TRS-NTD interaction with mutations, small molecules, or a nanobody results in enhanced translational repression. N-protein is able to differentially regulate all TRS containing RNAs based on the degree the TRS is structured in each 5'UTR with different structures associated with each viral coding sequence. These data support a model by which SARS-CoV-2 N-protein regulates the translation efficiency of Orf1ab and sgmRNAs differentially via the strength of TRS-Nucleocapsid interactions present on the 5'UTRs of viral RNA. In this way, the virus can spatially and

temporally regulate translation for optimal replication. Disrupting N-protein condensation mediated translational regulation may provide a therapeutic target for coronavirus infections.

B303/P1295

Subcellular mRNA localization across tissue architecture and polarity type

D. Mason, S. Mili; National Cancer Institute, NIH, Bethesda, MD

Localization of mRNAs to distinct subcellular domains is a widely observed phenomenon that influences the function of the encoded proteins. In motile mesenchymal cells several mRNAs localize to the cell periphery to regulate protrusion formation and migration. This work has established a role for transcript localization in mesenchymal cells with a front-rear polarity, but comparatively little is known about the localization or function of these mRNAs in apical-basal polarized epithelia. To determine whether protrusion-localized mRNAs are localized in normal epithelia, we surveyed the localization of several endogenous mRNAs in various mouse epithelial tissues with different architectures (skin, tongue, intestine, and kidney). We observed that some, but not all, protrusion-localized mRNAs were preferentially polarized towards the basal cell membrane in all tissues tested. Interestingly, in multilayer epithelial tissues (i.e. skin and tongue) mRNA polarization was more prominent in the basal cell layer, relative to the suprabasal layers. This suggests that mRNA polarization is altered by a basal cell-specific polarization cue.To probe the underlying mechanism of basal mRNA polarization we tested whether we could recapitulate basal mRNA localization in a monolayer of MCF10A cells, a normal breast epithelial cell line. Consistent with our *in vivo* findings, basal mRNA enrichment occurs in *in vitro*monolayers. Intriguingly, however, we did not observe prominent basal mRNA localization in a multilayered organotypic skin culture model. To understand the mechanism of mRNA localization, we used a combination of anti-sense oligonucleotides and ectopically expressed mutants to identify regions in the 3’ untranslated region (3’UTR) of basal mRNAs that promote localization. Interestingly, the same sequence determinants are required for both basal localization in normal epithelia and for protrusion localization in mesenchymal cells. Overall, these results suggest that the same underlying molecular mechanism targets mRNAs to different subcellular locations, dependent on cell polarization (mesenchymal vs. epithelial) and tissue type. However, additional tissue-specific factors like topology may influence the execution of this mechanism. Our current efforts focus on establishing tissue-specific regulation and the physiologic function of polarized mRNAs in epithelial tissues.

B304/P1296

DEAD Box RNA Helicase 3-dependent RNA composition of Stress Granules

B. Cui, H. Ji, M. Aksenova, M. Wyatt, **M. Shtutman**; University of South Carolina, Columbia, SC

In response to stress, cytoplasmic RNA localizes to insoluble ribonucleoprotein granules, principally known as stress granules (SGs). DEAD-Box RNA helicase 3 (DDX3), a noted protein that plays a critical role in RNA metabolism, has recently been shown to modulate SG composition. But the role of DDX3 activity in SG dynamics is not well understood. We elucidate this question by determining the effects of DDX3 inhibition on the whole transcriptome of stress-induced SGs by using a small molecule inhibitor of DDX3, RK33, that targets DDX3's ATPase activity. We identified a differential modulation of DDX3-dependent sequestration of RNAs to SGs not only for coding but, more interestingly, for noncoding RNAs as well. Here we biochemically separated sodium arsenite-induced insoluble stress granules from soluble cytosolic fractions. We found specific classes of intronic and circular RNAs that are distinctly

targeted to SGs. Collectively, these results reveal that the enzymatic activity of DDX3 modulates the transcriptomic composition of SGs, and previously unexplored intronic RNA profiling in the SGs may contribute to understanding human diseases that associate with SG-dysfunction, such as viral infection and neurodegeneration.

B305/P1297

A Motor-Adaptor Complex Mediating Protrusion Localization of APC-Dependent mRNAs

K. Moissoglu, T. Wang, A. N. Gasparski, S. Mili; National Cancer Institute, NIH, Bethesda, MD

RNA localization to specific subcellular compartments is important for the spatiotemporal control of gene expression. We have previously identified a group of mRNAs that exhibit a characteristic peripheral distribution within protrusive regions of the cytoplasm. Work from our laboratory has established that this localization pathway is biologically relevant. Indeed, disrupting the localization of specific APC-dependent mRNAs, such as *RAB13* and *NET1*, results in inhibition of cell migration on 2D surfaces or invasion in 3D environments (Moissoglu et al, 2020; Chrisafis et al, 2020). This peripheral mRNA localization pattern depends on GA-rich sequences in the 3' untranslated region (UTR) of these transcripts and requires the function of the tumor suppressor protein APC as well as the presence of a stable, de-tyrosinated network of microtubules. We have now identified two additional elements, the kinesin KIF1C and the RNA binding protein CNBP (CCHC-type zinc finger Nucleic acid Binding protein), that operate in concert to mediate transport of APC-dependent mRNAs to peripheral locations. KIF1C associates with APC-dependent mRNAs and is required for their transport to the periphery and their ability to exhibit long and linear movements characteristic of active transport on microtubules. In these events, single KIF1C motors can be visualized moving together with individual mRNAs. Thus, KIF1C serves as a motor that actively transports APC-dependent mRNAs to peripheral protrusions (Pichon, Moissoglu et al, 2021). Using proteomic approaches, we have now further identified CNBP as an RNA-binding protein that participates in this transport mechanism. Specifically, CNBP associates directly with APC-dependent mRNAs and this interaction depends on the same GA-rich elements on the 3'UTR that are important for protrusion localization. CNBP additionally interacts with the KIF1C motor. Furthermore, CNBP is required for peripheral mRNA localization, and also importantly, for the interaction of APC-dependent mRNAs with KIF1C. We propose a model in which CNBP serves as an adaptor between the kinesin KIF1C and APC-dependent mRNAs in the transport pathway to their target peripheral locations.

Nucleocytoplasmic Transport

B307/P1298

Impaired Nucleocytoplasmic Transport in Neurological Diseases

B. Ding; Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, Shreveport, LA.

In eukaryotic cells, transcription and translation processes are physically separated by the nuclear envelope (NE). Newly transcribed mRNAs must be exported to the cytoplasm for protein synthesis, while some proteins require to be imported into the nucleus to fulfill their nuclear functions. This nucleocytoplasmic transport (NCT) across the NE is tightly regulated and is critical for maintaining cellular homeostasis. Its dysregulation leads to aging and many neurological diseases, including

amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Alzheimer's disease (AD). To decipher the pathophysiological mechanisms underlying NCT-impaired neurological diseases, the examination of NCT activities at the single cell level is critical. Recently, we reported the techniques for measuring the nuclear transport of both mRNA and protein cargos. *Fluorescent In Situ Hybridization* (FISH) coupled with oligo-dT probes were used to measure the distribution of Poly(A) RNAs (mRNA). A dual reporter (GFP-NES and RFP-NLS) was used to examine the protein nuclear transport. These approaches together with imaging analysis enable us to systematically quantify the NCT activities in cultured neurons. We modeled movement disorder dystonia using patient-derived motor neurons (MNs), which have been generated via direct conversion from patient fibroblasts and the differentiation of induced pluripotent stem cells (iPSCs). We first reported the disease-dependent cellular deficits of dystonia in patient-derived neurons, including deformed nucleus, mislocalized proteins, and impaired nucleocytoplasmic transport, providing another example of the impairment of NCT in neurological diseases. Our study also demonstrates the high value of patient-derived neurons in modeling neurological diseases.

B308/P1299

Hexokinase 2 translocates to the nucleus when glucose is depleted and does not regulate gene expression

M. A. Lesko[1], D. G. Chandrashekarappa[2], E. M. Jordahl[1], M. C. Schmidt[2], J. D. Durrant[1], A. F. O'Donnell[1]; [1]Biological Sciences, University of Pittsburgh, Pittsburgh, PA, [2]Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Glucose is a critical energy source for cells and serves as a building block for key biosynthetic processes. Hexokinases are central regulators of glucose metabolism, facilitating the first committed step in glycolysis: conversion of glucose to glucose-6-phosphate. Hexokinases are conserved across eukaryotes, and we use *S. cerevisiae* as a model to study hexokinase function. Yeast express three hexokinases with hexokinase 2 (Hxk2) as the predominant isoform in glucose-grown cells. In addition to phosphorylating glucose, Hxk2 has been proposed to regulate the glucose repression pathway by translocating into the nucleus to regulate transcription of metabolic genes with the transcriptional repressor Mig1. Nuclear shuttling of Hxk2 is thought to be regulated by phosphorylation, and this modification also controls the dimer to monomer transition for Hxk2, suggesting that these two states may be intimately linked. Using advanced confocal microscopy and automated image quantification approaches, we refine the model of Hxk2 nuclear shuttling, refuting the long-held view that Hxk2 translocates into the nucleus in high-glucose conditions. We instead present evidence that Hxk2 shuttles to the nucleus when glucose is depleted, a response also observed in mammalian hexokinases. We expand our understanding of cytosolic-nuclear shuttling for Hxk2 by identifying Tda1, a kinase known to control Hxk2 multimerization in response to glucose depletion, as key for controlling Hxk2 nuclear localization. We show that phosphorylation of Hxk2 at residue serine 15, once thought to be critical for regulating nuclear shuttling, is dispensable for Hxk2 localization changes, but does control monomer-dimer transition which is consistent with findings from the Kriegel lab. We further identify a single lysine in the N-terminal region of Hxk2 that is required for the regulated nuclear shuttling of Hxk2. Finally, we perform RNAseq analyses and demonstrate that Hxk2 plays almost no role in transcript regulation in cells either in replete glucose or in glucose starvation, with only three genes changing their expression profiles (Hxk1, Hxt1, and Suc2), likely a result of metabolic changes rather than direct transcription regulation. Taken together, our studies provide insight into the critical roles hexokinases play in regulating fundamental metabolic responses in cells.

B309/P1300

5-FU induces calpain mediated disruption of nuclear transport in cancer cells.

E. Stewart, K. Resendes; Westminster College, New Wilmington, PA.

Nuclear Pore Complexes (NPCs) mediate the travel of molecules into and out of the nucleus with help of nuclear transporters. The GTPase Ran assists these nuclear transporters in the binding/unbinding of their protein cargo. RanGTP exists primarily in the nucleus, with RanGDP in the cytoplasm, creating a strong gradient across the nuclear envelope. Disruption of this gradient can cause cell stress, which can then lead to apoptosis. 5-Fluorouracil (5-FU) is a widely used chemotherapy drug that we have previously demonstrated to disrupt the Ran gradient and nuclear transport by altering NPC permeability. These effects of 5-FU are Ca2+ dependent, but the exact mechanism is unknown. Calpains are calcium activated non-lysosomal cysteine proteases, which may be triggered by 5-FU. Ubiquitously expressed calpain 1 or calpain 2 are thought to, when activated by Ca2+, cleave certain nucleoporins, altering the permeability of the NPC by disrupting its structural integrity. Using western blot analysis, we have demonstrated that the cleaved active form of calpain 2, but not calpain 1, is present in HeLa cervical cancer cells in response to 5-FU. Additionally, use of the calpain inhibitor calpeptin significantly reduced the 5-FU induced increase in nuclear pore permeability. Specifically, addition of calpeptin reduced the number of 5-FU induced leaky HeLa cell nuclei from 25% to 14% and blocked the 5-FU Ca2+ mediated disruption of the Ran gradient. Our current studies seek to determine if 5-FU similarly induces activation of calpain 1 or 2 to disrupt nuclear transport in PANC1 cells, as pancreatic cancer is often treated with this chemotherapy. Together these studies will further our understanding of how 5-FU induces apoptosis, which in turn can help with the development of mechanisms to counteract the risk of 5-FU resistance encountered in cancer cells, in particular by building on the growing evidence that calcium supplementation may enhance the potency of 5-FU.

B310/P1301

The role of PCID2 and Centrin2 in nuclear export and centrosomal localization of BRCA1/BRCA2 in Hs578t breast cancer cells

L. Turturice, N. Polio, T. Gatesman, K. Resendes; Westminster College, New Wilmington, PA.

Centrin2 is a small calcium-binding protein ubiquitously expressed in eukaryotic cells that aids in centrosome stability and microtubule nucleation, as well as functioning in the TREX2 mRNA export complex. We have previously shown that Centrin2 and another member of the TREX2 complex, PCID2, both facilitate nuclear protein export in addition to mRNA export. PCID2, like Centrin2, also localizes to the centrosome during the process of duplication. Therefore, we propose that PCID2 and Centrin2 may play roles in regulating nuclear to centrosomal transport of centrosome duplication regulators. We found that siRNA knockdown of PCID2 led to an accumulation of the centrosomal regulators, BRCA1 and BRCA2 in the nuclei of Hs578T breast cancer cells. Co-localization studies with γ-Tubulin also indicated a loss of BRCA1 at the centrosome after PCID2 knockdown; however, the loss of PCID2 had no significant impact on the ability of BRCA2 to localize to centrosomes. These results indicate that PCID2 is involved in the nuclear export of both BRCA1 and BRCA2 but may only be important for the centrosomal localization of BRCA1. Current studies are exploring the possible impact of Centrin2 siRNA knockdown on the nuclear export and centrosomal localization of BRCA1 and BRCA2. Preliminary results suggest that a lack of Centrin2 also leads to nuclear accumulation of BRCA1 and BRCA2, with more replicates

underway. Additionally, studies of the centrosomal localization of both BRCA1 and BRCA2 following Centrin2 siRNA knockdown have been performed; however, the loss of Centrin2 disrupted localization of y-tubulin at the centrosome hampering our analyses and alternative markers are currently being explored. Together our findings will provide fundamental knowledge on the role of PCID2 and Centrin2 in nuclear transport and the centrosome cycle, leading to a better understanding of centrosomal amplification and tumorigenesis in a variety of cancer types.

B311/P1302

Nucleocytoplasmic transport is regulated by forces on the nucleus

K. L. Scott[1], P. Purkayastha[2], T. P. Lele[2,3,4], K. J. Roux[1,5]; [1]Enabling Technologies Group, Sanford Research, Sioux Falls, SD, [2]Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, TX, [3]Department of Biomedical Engineering, Texas A&M Universeity, College Station, TX, [4]Department of Translational Medical Sciences, Texas A&M University, Houston, TX, [5]Department of Pediatrics, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD.

The facilitated diffusion of molecules between the nucleus and cytoplasm through nuclear pore complex (NPC) channels that reside in the nuclear envelope (NE) is a key regulator of myriad cellular processes. Here, we used a light inducible transport reporter (LINuS) and a naturally occurring, inducible transcription factor (glucocorticoid receptor) to demonstrate that facilitated nucleocytoplasmic transport is significantly increased when force on the NE is decreased, either dramatically in the case of cell rounding or more selectively by disrupting mechanical tethers between the nucleus and the cytoskeleton. Loss of the A-type lamins that resist cytoskeletal mechanical forces leads to decreased transport, but that decrease in transport can be overcome by the disruption of those mechanical forces on the cell. Ran-GTPase, the driver of facilitated diffusion, leaves the nucleus at a higher rate in cells with reduced forces on the nucleus and results in a redistribution of Ran-GTPase to the cytoplasm. This redistribution can be rescued by overexpression of Ntf2, which is responsible for recycling Ran back to the nucleus. Disruption of forces on the nucleus led to an increase in the cytoplasmic levels of RNA, the export of which depends on facilitated transport. The passive diffusion of larger proteins through NPCs is also enhanced by profound relaxation of cellular forces. These results support the idea that nucleocytoplasmic transport is mechanosensitive, providing another mechanism by which cells can respond to their environment.

B312/P1303

Testing key physical parameters of an osmotic model for nuclear size control in fission yeast

Z. Tan[1,2], J. Lemière[1], F. Chang[1]; [1]University of California, San Francisco, San Francisco, CA, [2]Nankai University, Tianjin, China

In many cell types, the size of the nucleus scales with cell size so the nuclear-to-cell volume ratio (N/C ratio) is maintained as a constant. Recently our lab and others (Lemière et al., Deviri et al) proposed a physical “bag within a bag model” of two nested vesicles for nuclear size control based upon colloid osmotic pressure. In this model, nuclear volume is determined by a balance of osmotic pressures on the nuclear envelope and its membrane tension. In the fission yeast *S. pombe* the nucleus acts as an ideal osmometer whose size is determined by its osmotic environment, suggesting that nuclear membrane tension is negligible in these cells. Thus, key determinants of nuclear size are the numbers of macromolecules in the nucleoplasm and cytoplasm that produce colloid osmotic pressure. Here, we test

this osmotic model with experiments in which we vary key parameters in fission yeast. To alter relative colloid osmotic pressure, we will vastly overexpress mCherry-GST in the nucleus or cytoplasm. We predict that increasing mCherry protein content in the nucleus will increase the N/C ratio while overexpressing cytoplasmic mCherry may decrease the N/C ratio. To increase the mechanical effect of nuclear envelope on nuclear size, we plan to express human nuclear lamin constructs in fission yeast. We predict that increased nuclear envelope stiffness may decrease the N/C ratio and dampen nuclear swelling under hypoosmotic shock similar to what has been observed in Chondrocytes. These studies will provide critical quantitative experimental tests of this osmotic model for nuclear size.

B313/P1304

Mlv reverse transcription can occur in the nucleus

K. Salas-Briceno, S. R. Ross; University of Illinois, Chicago, IL

Reverse transcription plays an essential role in retroviral replication. It is thought to initiate from within the capsid and uses the reverse transcriptase enzyme to synthesize DNA from an RNA template. Since reverse transcription initiates within the capsid, uncoating and reverse transcription are probably mutually dependent. HIV can infect non-dividing cells, because there is active transport of the preintegration complex (PIC) across the nuclear membrane. There is still much debate regarding the timing and cellular location of reverse transcription for HIV, and whether it occurs solely in the cytoplasm or nucleus or both. Moreover, while it is known that Moloney Leukemia Virus (MLV) replication depends on cell division for replication in tissue culture cells, whether its reverse transcription is solely cytoplasmic has not been studied. In this study, we used NIH3T3 and primary mouse dendritic cells to determine where the different stages of reverse transcription occur and whether cell division is needed for nuclear entry. Our data strongly suggest that in both NIH3T3 cells and dendritic cells, the initial step of reverse transcription occurs in the cytoplasm and not in the nucleus. We also found that the MLV PIC requires cell division to enter the nucleus of NIH3T3 cells. However, we also detected MLV RNA/DNA hybrid intermediates in the nucleus of dividing NIH3T3 cells, suggesting that reverse transcription can continue after nuclear entry. In contrast to NIH3T3 cells, we found that MLV could infect non-dividing primary dendritic cells and that knockdown of a number of nuclear pore proteins dramatically reduced the appearance of integrated MLV DNA in these cells. These findings suggest that simple retroviruses, like HIV, also might have some mechanism to gain nuclear entry by traversing the nuclear pore complex in non-mitotic cells.

B314/P1305

The *Drosophila* EGFR ligand mSpitz is delivered to cytoplasmic capes at sites of non-canonical RNA export on the nuclear envelope *via* the endosomal system

F. Mattie[1], P. Kumar[2], M. Travor[3], K. Browder[4], **K. Shill**[1], C. Thomas[1]; [1]Pennsylvania State University, Unviersity Park, PA, [2]Government College for Women, Unviersity Park, India, [3]Pennsylvania State University, University Park, PA, [4]Genentech Inc., South San Francisco, CA

Nuclear-cytoplasmic communication is not limited to nuclear pores, with both proteins and RNA using alternative routes between these compartments. We previously characterized cytoplasmic capes (large invaginations of the nuclear envelope in *Drosophila*), that are enriched for the membrane-bound EGF receptor ligand mSpitz, endosome-related organelles and ubiquitylated proteins. Closely associated with capes are groups of perinuclear vesicles between the outer and inner nuclear membranes that resemble

those seen at sites of non-canonical ribonucleoprotein (RNP) export *via* budding. Here, we demonstrate that mSpitz delivery to cytoplasmic capes requires passage through the endosomal system. We also show that capes are indeed closely associated with sites of non-canonical RNP export as well as the *D*Frizzled2 receptor C terminal fragment (*D*Fz-2c), a core component of this export pathway. Timelapse microscopy of glands in intact larvae indicates that cytoplasmic capes are stable structures that persist for at least 90 minutes without conspicuous growth. We further show that capes appear with the growth of the salivary gland rather than at a specific developmental stage, suggesting that capes do not form in response to ecdysone signaling. We also demonstrate that the large F-actin binding protein β_H-spectrin that modulates endosomal trafficking, as well as spectrin tetramer formation are required for cape formation. Finally, we find that there is a slight elevation in the level of *D*Fz-2c at the nuclear envelope when β_H is knocked down. Cytoplasmic capes therefore represent a sub-specialization of the nuclear envelope where endosomal trafficking and RNP export are closely associated and may be functionally interdependent.

Nuclear Membrane Homeostasis

B315/P1306

PP2A-B55^{SUR-6} promotes nuclear envelope breakdown in *C. elegans* one-cell embryo

S. Kapoor, K. Adhikary, S. Kotak; Indian institute of Science (IISc) Bangalore, Karnataka, INDIA.

The nucleus constrains the cell's genetic material by forming a selective barrier to the entry of macromolecules from the cytoplasm. In animal cells, nuclear envelope breakdown (NEBD) allows the spindle microtubules to access and attach to the chromosomes within the nucleus at the onset of the M-phase. Proper chromosome-microtubule attachment ensures faithful segregation of genetic material into the two daughter cells. NEBD is regulated by the activity of critical kinases such as CDK-1, AIR-1 (Aurora A), and PLK-1. While these mitotic kinases are clearly crucial for NEBD, no phosphatase has yet been linked with NEBD at the mitotic entry. Here, we identified PP2A-B55^{SUR-6} as an essential regulatory subunit of PP2A phosphatase critical for timely NEBD in the one-cell *Caenorhabditis elegans* embryo. We found that in embryos that are depleted for PP2A-B55^{SUR-6}, nuclear membrane permeabilization is significantly delayed, and nuclear lamin and nucleoporins persist throughout mitosis. As a result, chromosomes' segregation is impaired. Notably, we found that the impact of PP2A-B55^{SUR-6} depletion on NEBD is not because of its effect on cell cycle progression or mislocalization of essential kinases such as PlK-1 or AIR-1. By performing a genetic epistasis experiment, we uncovered that nuclear lamin (LMN-1), but not nucleoporins, could be the target of PP2A-B55^{SUR-6}. Notably, genomically-tagged GFP- PP2A-B55^{SUR-6} localizes to the nucleus just before the onset of NEBD-suggesting PP2A-B55^{SUR-6} might directly regulate nuclear envelope breakdown. Overall, our findings implicate the PP2A phosphatase complex in a key process of NEBD in animal cells.

B316/P1307

Uncovering mechanisms by which barrier-to-autointegration factor controls dynamics of nuclear leakage and repair following nuclear envelope rupture

C. T. Halfmann[1], R. M. Sears[1,2], K. L. Scott[1], K. J. Roux[1,2]; [1]Sanford Research, Sioux Falls, SD, [2]Department of Pediatrics, Sanford School of Medicine, University of South Dakota, Vermillion, SD.

The nuclear envelope (NE) creates a critical barrier between the cytosol and nucleus during interphase that is key for cellular compartmentalization and protecting genomic DNA. However, the NE can rupture, a process that has been implicated in several NE-associated diseases and cancer. We and others previously reported that barrier-to-autointegration factor (BAF), a highly mobile DNA-binding protein, is required for repair of NE ruptures. Following NE rupture, BAF rushes into the nucleus, rapidly binds to nuclear DNA that is newly exposed to the cytosol, and recruits components to repair the NE, including LEM-domain proteins, A-type lamins, membranes, and ESCRT-III membrane repair machinery. To further reveal the mechanisms by which BAF aids in repairing NE ruptures, we expressed untagged BAF variants that modify its various interactions and/or localization in human fibroblasts depleted of endogenous BAF and analyzed the dynamics of NE rupture leakage and repair. We observed that BAF needs to be cytoplasmic and retain phosphoregulatable DNA binding in order to control rupture leakage and repair NE ruptures. Unexpectedly, loss of BAF's ability to recruit LEM-domain proteins or lamins via expression of a LEM-binding deficient BAF (L58R) or lamin-binding deficient BAF (A12T) did not substantially impact initial NE rupture leakage or repair efficiency, suggesting that recruitment of these proteins contribute little to the control of rupture leakage or the initial repair of NE ruptures. Inhibition of BAF dimerization prevented BAF enrichment at NE ruptures, exacerbated rupture leakage, and prevented efficient rupture repair. This was rescued by artificially tethering two BAF monomers together, suggesting that it is not just BAF's ability to bind but also crosslink DNA that leads to reduced leakage and efficient rupture repair. Our evidence also suggests that BAF creates a size-dependent diffusion barrier for proteins, with larger proteins being more significantly impeded by BAF's presence at the rupture. Surprisingly, the loss of A- or B-type lamins enables the repair of NE ruptures in BAF-depleted cells, suggesting that the nuclear lamina is inhibitory to rupture repair. Our ongoing studies seek to further understand the mechanisms by which BAF regulates rupture-induced nucleocytoplasmic exchange and the repair of NE ruptures, and mechanisms by which the nuclear lamina inhibits proper rupture repair in BAF-depleted cells.

B317/P1308

The dynamin-like protein Dnm1 mediates scission of the outer nuclear membrane in nuclear autophagy

P. J. Mannino, S. Chandra, D. J. Thaller, N. R. Ader, T. J. Melia, C. P. Lusk; Cell Biology, Yale University, New Haven, CT.

Autophagy is a catabolic process that encompasses the capture and delivery of cellular material into the lytic compartment for degradation. Autophagy can be leveraged to selectively capture and degrade damaged portions of organelles thereby maintaining homeostasis and preventing age-related decline in cellular function. How organelle membranes are remodeled in a manner that produces sub-compartments amenable to degradation by autophagy while also maintaining organelle identity and function is an open question. In comparison with other organelles (e.g., the endoplasmic reticulum and mitochondria) where many molecular details have been worked out, how the nuclear envelope (NE) is remodeled during nuclear autophagy (nucleophagy) remains ill-defined. We have been exploring nucleophagy by leveraging Atg39, a type-II integral membrane protein in budding yeast that is the only known NE cargo adaptor. We have demonstrated that Atg39 localizes to the outer nuclear membrane (ONM) likely by direct interactions between predicted amphipathic alpha helices in its lumenal domain and the lumenal leaflet of the inner nuclear membrane (INM). These sequence elements are also required for the coordinated remodeling of both the INM and ONM that captures integral INM proteins

and nuclear contents within lumenal vesicles upon Atg39 overexpression. To better understand the molecular players that execute nucleophagy, we are applying lattice light sheet microscopy (LLSM) with targeted genetic deletions to directly observe, and perturb, physiological Atg39-mediated nucleophagic events in living cells. Our data suggest that there are likely several genetically-dissectible steps in nucleophagy beginning with the condensation of Atg39 at the ONM, the capture of INM and nuclear cargo, the release of a NE fragment from the nucleus, and finally fusion with the vacuole. The entire process typically occurs within ~5 minutes. We homed in on dynamin-like protein 1 (Dnm1) as a potential factor that contributes to the cytosolic release of the NE fragment. Consistent with this hypothesis, Dnm1 colocalizes with Atg39 upon activation of nucleophagy. Analysis of *dnm1Δ* cells by LLSM revealed a stall in nucleophagy and retention of Atg39 at the nuclear periphery. Correlative light and electron microscopy at sites of Atg39 accumulation at the nuclear periphery in *dnm1Δ* cells revealed that Atg39 localized to lumenal vesicles derived from the INM that were surrounded by ONM. These data suggest that INM and ONM fission are genetically-dissectible and are likely temporally distinct events. Thus, we provide a quantitative and morphological timeline of nucleophagy and evidence that Dnm1 not only functions at mitochondria but also at the ONM.

B318/P1309

Nemp1 supports the nuclear envelope in the face of mechanical stresses to promote metazoan fertility

H. McNeill[1], A. Ganguly[1], B. Hakim[1], D. Hodzic[1], K. Ha[2], A. Pathak[1], H. Zmuda[1], Y. Tsatskis[3], A. Jurisicova[4]; [1]Washington University School of Medicine, St. Louis, MO, [2]Lunenfeld-Tanenbaum Research Institute, Toronto, ON, CANADA, [3]Sickkids, Toronto, ON, CANADA, [4]Luenfeld-Tannenbaum Research Institute, Toronto, ON, CANADA.

Nemp1 is an inner nuclear membrane protein we have shown is critical for fertility of worms, fish, flies and mice. Although Nemp1 KO mice are overtly normal, females are severely subfertile with a reduced ovarian reserve. We now show that Nemp1 mutant oocytes in mice are lost to death that occurs during early postnatal stages. Knockdown of Nemp in the *Drosophila* germline also leads to oocyte loss. Importantly knockdown of ATM and Chk2 rescue egg laying in *Drosophila*. These data suggest loss of Nemp activates a germline checkpoint. Preliminary data indicate that loss of Chk2 also rescues oocyte loss in mouse Nemp1KO ovaries. Loss of Nemp1 mutant oocytes occurs in the highly cross-linked ovarian cortex and loss of Nemp1 leads to a softer nuclear envelope (NE) in AFM studies. We hypothesized that the highly cross-linked ovarian cortex provided a mechanical stress that led to death of Nemp1KO oocytes. Collagen cross-linking is mediated by enzymes known as lysyl oxidases. We now show that loss of one genomic copy of LOX leads to a softer ovary. Remarkably, Nemp1KO, LOX+/- females have a partial restoration of fertility, supporting the proposal that Nemp1 acts to protect oocytes against mechanical stresses. Cultured cell experiments show that lethality associated with loss of Nemp1 is rescued when cells are plated on soft substrates. These data highlight the importance of Nemp1 and the nuclear envelope in protecting cells against mechanical stresses.

B319/P1310

The role of Prm3p in nuclear envelope expansion during yeast mating response

E. C. Morgan, V. Meleshkevich, O. Cohen-Fix; National Institutes of Health, Bethesda, MD

Despite the essential role the nucleus plays in numerous physiological processes, several fundamental features of its morphology and function remain unexplored. In particular, the mechanisms that determine nuclear size and shape are largely unknown. Altered nuclear size is observed in diseases such as cancer, and abnormal nuclear shape is often seen in aging cells. Nuclear morphology also changes in several developmental contexts. For example, hematopoietic stem cells possess a large oval-shaped nucleus that forms increasingly large invaginations during differentiation into a mature neutrophil, resulting in a large multi-lobed nucleus. Given this link between cell function and nuclear morphology, we sought to characterize the processes that regulate nuclear size and shape. The nucleus is surrounded by the nuclear envelope (NE), which is composed of two nuclear membranes that are continuous with the endoplasmic reticulum (ER). To study how the nucleus remodels its shape we use the budding yeast *Saccharomyces cerevisiae,* which displays altered nuclear morphology when exposed to mating pheromone. As part of the mating response, yeast cells arrest in G1 and their nuclei elongate. We determined that this takes place through an expansion of the NE without a proportional increase in nuclear volume. Since the yeast mating response is controlled largely by the transcription factor Ste12p, we conducted a high-throughput screen to determine which Ste12p-induced genes are necessary for the observed NE expansion. We found that absence of *PRM3*, which is highly induced upon pheromone exposure, resulted in significantly rounder nuclei during alpha factor treatment despite an otherwise robust transcriptional response. Thus, Prm3p appears to be a key regulator of NE expansion during yeast mating response. Prm3p is a 133aa protein that localizes to the nuclear envelope via a C-terminal anchor, and is conserved in budding yeasts. Prm3p may influence NE expansion in a variety of ways, from recruiting phospholipid synthesis-linked enzymes to influencing local membrane composition to permit expansion. We are currently generating a suite of *PRM3* partial deletion mutants with CRISPR-Cas9 to assess which portions of the protein are specifically necessary for NE expansion, and what mechanism of NE expansion Prm3p may facilitate.

B320/P1311

Cell type-specific response to rupture of the nuclear envelope

Y. Kamikawa[1], A. Saito[1], Z. Wu[1], K. Imaizumi[2]; [1]Hiroshima University, Hiroshima, Japan, [2]Hiroshima University, 広島市南区霞1, Japan

Recent works have highlighted that the nuclear envelope (NE) is damaged by various stress, such as mechanical stress under confined cellular migration, referred to as NE stress. NE stress leads deformation of the nucleus and even rupture of the NE. The molecular mechanisms that repair ruptured NE are extensively studied and crucial repair factors have been identified including a DNA binding protein BAF and its binding partner LEM domain proteins. We previously identified an endoplasmic reticulum resident trans-membrane transcription factor OASIS/CREB3L1 as a factor that responds to NE stress. The expression of OASIS is restricted to specific types of cells such as astrocytes and osteoblasts, suggesting cell-type specific response to the NE stress. To test this idea, the consequences of NE stress were compared in a variety of cancer cell lines using Boyden chamber assay, where cells migrate through constricted space of the porous membrane. We found that nuclear deformation as well as DNA damage induced by NE stress varies among these cell lines. Furthermore, live cell imaging revealed the

failure of repairing of ruptured NE in a certain type of cell line. Collectively, the response to NE stress and its underlaying molecular mechanisms are likely cell-type specific.

B321/P1312

Small, fat-filled lipid droplets are sufficiently rigid to indent a nucleus, dilute the lamina, and cause rupture

I. Ivanovska, M. Tobin, **D. Discher**; University Pennsylvania, Philadelphia, PA

The nucleus in many cell types is a stiff organelle, and yet fat-filled lipid droplets (FD's) in the cytoplasm are somtimes seen to indent and displace the nucleus. FD's are phase-separated liquids with a poorly understood interfacial tension γ that determines how FD's interact with other organelles. Here, micron-sized FD's remain spherical as they indent both the nucleus and peri-nuclear actomyosin, dilute Lamin-B1 locally independent of Lamin-A,C, and trigger rupture with locally persistent accumulation in the nucleus of cGAS, a cytosolic DNA sensor. FD-nucleus interactions initiate rapid mis-localization of the essential DNA repair factor KU80, and nuclear rupture associates with DNA damage and perturbed cell cycle. Similar results are evident in FD-laden cells after constricted 3D-migration, which is impeded by FD's. Spherical shapes of small FD's are consistent with a high γ that we measure for FD's mechanically isolated from fresh adipose tissue as ~40_mN/m - which is far higher than other liquid condensates, but typical of oils in water and sufficiently rigid to disrupt cell structures.

B322/P1313

Investigating the role of nuclear envelope permeability during budding yeast meiosis

M. Walsh, G. King, K. Chetlapalli, E. Ünal; University of California, Berkeley, Berkeley, CA

The nuclear envelope functions to separate nuclear material from cytoplasm, which is important for regulating cellular processes, separating transcription from translation, and protecting DNA. Studying natural changes to the nuclear envelope allows us to gain better insight as to how nucleocytoplasmic transport is established and maintained. In some types of cells, the nuclear envelope is disassembled during cell division, whereas other cells maintain the integrity and transport capabilities of the nuclear envelope throughout division. In the budding yeast *Saccharomyces cerevisiae*, mitotic divisions and the first meiotic division maintain nuclear integrity. However, during meiosis II, there is a transient change in the nuclear barrier which allows for nucleoplasmic proteins to diffuse throughout the cell. Notably, the nuclear envelope remains largely intact by electron microscopy (EM). This phenomenon also occurs in the fission yeast *Schizosaccharomyces pombe*, but its regulation and function remain enigmatic in both yeasts. This project aims to investigate the changes in nuclear envelope permeability in *S. cerevisiae* and determine the underlying regulatory mechanism(s).

B323/P1314

The role of Vps4 in ESCRT-III-mediated nuclear sealing

L. Chen, N. R. Ader, M. C. King, C. P. Lusk; Yale, New Haven, CT

Abstract Title: *The role of Vps4 in ESCRT-III-mediated nuclear sealing*Track: Cellular DynamicsAuthors: Linda Chen, Nicholas R. Ader, Megan C. King*, and C. Patrick Lusk**co-last authors
During metazoan cell division, the nuclear envelope (NE) disassembles upon mitotic entry before reassembling in mitotic telophase just prior to cytokinesis. Perturbation of this process is associated with

DNA damage and cell death. In human cells, the ATPase associated with diverse cellular activities (AAA+ ATPase) VPS4 has been implicated in the sealing of holes in the reassembling NE in concert with the endosomal sorting complexes required for transport-III (ESCRT-III) machinery. However, how VPS4 mediates this sealing in conjunction with the ESCRT-III machinery remains unclear. Specifically, it is an unresolved question whether VPS4 acts solely as a "disassemblase" for ESCRT-III filaments, or rather, if it is required for the maturation of the ESCRT-III complex early in the NE sealing process. Here, we use the sealing of the singular mitotic NE hole that occurs in the fission yeast *Schizosaccharomyces pombe* to investigate the precise role of Vps4 in regulating ESCRT-III activity at the NE. We find that endogenous expression of a Vps4 hypomorph is sufficient to drive the aberrant and constitutive localization of the first ESCRT protein to be recruited to the NE, Cmp7. Notably, this phenotype contrasts with our observations in cells lacking the downstream ESCRT-IIIs, Did4 and Vps24, where Cmp7 accumulates but is still removed at the following cell cycle. Surprisingly, cells expressing this Vps4 do not display a defect in nucleocytoplasmic compartmentalization, as measured by a fluorescent reporter of nuclear integrity. Overall, our work suggests that Vps4 plays a key role in ESCRT-III processive remodeling by allowing for the removal of Cmp7 and complete ESCRT-III filament assembly.

B324/P1315

CTDNEP1 inhibits interphase nuclear membrane rupture

A. I. Yashchenko, A. L. Gunn, E. M. Hatch; Fred Hutchinson Cancer Center, Seattle, WA

The nuclear envelope serves as a barrier between the DNA and the cytoplasm and transient loss of NE compartmentalization in interphase leads to increased genome instability, cell invasion, and metastasis. Several conditions contributing to nuclear membrane rupture, including nuclear compression and loss of lamin proteins, have been identified, but many factors regulating nuclear membrane stability and the molecular mechanisms of membrane repair remain unclear. To comprehensively identify proteins regulating nuclear membrane rupture and repair, we used a fixed-cell nuclear integrity reporter, based on the persistence of large cytoplasmic proteins in the nucleus after membrane repair, to develop a high-content siRNA screening pipeline. We performed a pilot screen and identified CTD nuclear envelope phosphatase 1 (CTDNEP1) as a top hit that increased nuclear membrane rupture upon depletion. CTDNEP1 localizes to the inner nuclear membrane, activates lipin 1, an enzyme catalyzing diacylglycerol (DAG) synthesis, and is critical for proper nuclear envelope assembly. We confirmed CTDNEP1 knockdown by qRT-PCR and observed an expected increase in ER area after siRNA transfection by immunofluorescence. We validated our screen result using two independent siRNAs against CTDNEP1 and observed a 2.4-fold increase in NE rupture frequency upon CTDNEP1 depletion and a modest increase in rupture duration by live-cell imaging with both siRNAs. To determine how CTDNEP1 depletion increases membrane rupture, we analyzed known rupture factors by immunofluorescence, including actin organization, nuclear lamina protein localization, and nuclear lamina organization. Qualitative analysis did not identify gross changes in any of these categories upon CTDNEP1 depletion, suggesting that CTDNEP1 acts in a new pathway, potentially directly on membrane mechanics. We are currently working to quantify our immunofluorescence assays and observe changes in membrane characteristics to better understand how CTDNEP1 regulates nuclear membrane stability.

B325/P1316

Biochemical analysis of lamin subunits suggests mutiple layers of regulation for lamin filament assembly

R. T. A. Pedersen, Y. Zheng; Carnegie Institution of Washington, Baltimore, MD

In metazoan cells, type V intermediate filaments proteins called lamins form a meshwork that underlies the nuclear envelope and supports the shape, organization, and function of the nucleus. During open mitosis, lamin filaments disassemble at mitotic entry and reassemble after anaphase. Disassembly of the lamin network has been shown to be triggered by phosphorylation of lamin subunits. Dephosphorylation of lamin subunits as the activity of mitotic kinases subsides is presumed to lead to reassembly of lamin filaments as the nucleus reforms, but the precise molecular pathway of lamin network reassembly remains obscure.
Here, we present analysis of endogenous lamin proteins in *Xenopus laevis* egg extracts that suggests that dephosphorylation alone cannot fully account of the regulated reassembly of lamin filaments following mitosis. Although metaphase-arrested extracts are characterized by high mitotic kinase activity and interphase-arrested extracts are characterized by high phosphatase activity, we find that the egg- and embryo-specific lamin isoform lamin-B3 is largely soluble in both types of extracts. The more ubiquitous B-type lamin, lamin-B1, is less soluble in both metaphase and interphase extracts, although this disparity between lamin-B3 and lamin-B1 is due at least in part to lamin-B1's larger membrane-bound population. The observation of soluble lamin protein in interphase extracts could be explained at least three ways: (1) lamin subunits are not dephosphorylated in these extracts, (2) dephosphorylation of lamin subunits is not alone sufficient to drive assembly, or (3) lamin assembly has a high critical concentration, so there is a large pool of soluble lamin subunits even after filament assembly. Analysis of the phosphorylation states of lamin-B1 and lamin-B3 demonstrates that both are hyperphosphorylated in interphase extracts, much as they are in metaphase extracts, suggesting that dephosphorylation of lamins at mitotic exit may be a regulated process rather than a widespread phenomenon. We propose that lamin assembly may be subject to multiple layers of molecular control, including both temporal regulation by the cell cycle state and spatial regulation by lamin subunit binding proteins.

Endosomes, Lysosomes, and Lysosome-related Organelles

B327/P1317

Golgi-derived vesicles facilitate the fission of lysosomal tubules by modulating lysosomal PI(3)P levels

M. Boutry[1], L. DiGiovanni[1], N. Demers[1], A. Fountain[2], S. Mamand[2], R. Botelho[2], P. K. Kim[1]; [1]Program in Cell Biology, The hospital for Sick Children, Toronto, ON, CANADA, [2]Toronto Metropolitan University, Toronto, ON, CANADA.

Lysosome reformation requires the recycling of lysosome membrane components from lysosomal organelles such as autolysosomes, endolysosomes and phagolysosomes. Membrane recycling involves the formation and fission of tubules emerging from lysosomal organelles. However, the mechanisms governing these processes in the different forms of lysosomal organelles are poorly understood. Further complicating the field is the potential differences in the recycling mechanism between lysosomal organelles. For instance, phosphatidylinositol-4-phosphate (PI(4)P) has been shown to promote the formation of tubules from phagolysosomes, whereas PI(4)P formed by PI4KIIIβ on autolysosomes has

been proposed to inhibit tubules formation. Using super-resolution live-cell imaging and manipulation of PI(4)P at lysosomal organelles, we investigated the role of PI(4)P in the formation of tubules from three different forms of lysosomal organelles. We find that PI(4)P is required to generate tubules from lysosomal organelles. Moreover, we show that PI4KIIIβ-dependent Golgi-derived vesicles are recruited to lysosomal organelles tubules fission sites where they actively contribute to the fission process by mediating a PI(3)P signaling. Our findings suggest a unifying mechanism for tubule formation and fission from lysosomal organelles, where PI(4)P promotes the formation of lysosomal tubules and PI4KIIIβ-mediated Golgi-derived vesicles regulate lysosomal PI(3)P to drive the fission process.

B328/P1318

Lysosomes contain an expansion compartment that mediates zinc transporter delivery to promote zinc homeostasis in*C.elegans*

A. Mendoza[1], N. Dietrich[2], C. Tan[3], D. Herrera[1], J. Kasiah[1], Z. Payne[1], D. Schneider[1], K. Kornfeld[1]; [1]Washington University, St. Louis, MO, [2]NIEHS, Epigenetics and Stem Cell Biology Laboratory, Durham, NC, [3]California Institute of Technology, Pasadena, CA.

Zinc is a transition metal that is essential for all life. Conserved zinc homeostatic mechanisms exist to respond when cytosolic levels are too low or too high. The Kornfeld lab uncovered high and low zinc homeostasis pathways in *C. elegans* that are involved in sensing cytosolic zinc levels and responding to return to homeostasis. Zinc dyshomeostasis leads to multiple human pathologies. Intestinal lysosomes are a site of zinc trafficking. The zinc exporter, CDF-2 stores zinc within lysosomes. We predicted that a mechanism exists to release zinc from lysosomes since there is one that stores it. To identify the zinc transporter that imports zinc we performed confocal fluorescence microscopy and showed that ZIPT-2.3 co-localizes with CDF-2. To determine ZIPT-2.3 function, we performed genetic and biochemical assays to show that ZIPT-2.3 imports zinc into the cytoplasm, and that the *zipt-2.3* gene is necessary for this function in zinc deficient conditions. Because CDF-2 and ZIPT-2.3 co-localize to gut granules, we tested their relationship by measuring their changes in expression in zinc replete, deficient, and excess conditions. Analysis showed that *cdf-2* and *zipt-2.3* mRNA and CDF-2 and ZIPT-2.3 protein expression is reciprocally regulated. Super resolution microscopy revealed that in addition to housing CDF-2 and ZIPT-2.3, lysosomes alter their morphology in response to available cytosolic zinc. Using line scans, we defined specific compartments and membrane boundaries across zinc conditions. Lysosomes contain acidified and expansion compartments. ZIPT-2.3 and CDF-2 populate membranes that surround the acidified compartment, while CDF-2 is localized to the expansion compartment. The acidified compartment is comprised of distinct regions named the LysoTracker and zinc regions. The expansion compartment is highly dynamic and deviates greatly in size. Our model predicts that in zinc deficient conditions, ZIPT-2.3 expression increases and CDF-2 expression decreases, promoting the net flow of zinc into the cytosol. In zinc excess conditions, CDF-2 expression increases, and ZIPT-2.3 expression decreases, facilitating net flow into the gut granule. The expansion compartment expands in zinc excess conditions but shrinks in zinc deficient and zinc replete conditions. Based on our observations we conclude that the purpose of the expansion compartment is to provide a structure for zinc trafficking while allowing the lysosome to participate in its canonical activities. Future studies will focus on how the expansion compartment is formed and if expansion compartment form in human lysosomes

B329/P1319

Fig4/PIKfyve/Vac14 complex loss of function results in altered PI4P subcellular distribution and calcium homeostasis

C. Kutchukian, V. A. Lam, M. Casas, A. J. Yarovaya, R. E. Dixon, E. J. Dickson; Physiology and Membrane Biology, University of California, Davis, Davis, CA.

At endosomal membranes, the phosphoinositide (PI) 5-phosphatase Fig4 and the PI 5-kinase PIKfyve function in a complex with the scaffold protein Vac14 to finely tune the synthesis and turnover of the low-abundant signaling lipid PI(3,5)P_2, essential for membrane trafficking and endocytosis. In humans, loss-of-function mutations in the genes encoding Fig4 and Vac14 cause severe neurological disorders including the amyotrophic lateral sclerosis, Charcot Marie Tooth disease and Yunis-Varón syndrome, highlighting a pivotal role for this complex in maintaining neuronal integrity. Multiple studies have reported that despite their divergent roles, Fig4, PIKfyve, or Vac14 loss-of-function result in similar decreases in PI(3,5)P_2, suggesting a common pathomechanism. An important unanswered question is whether Fig4/PIKfyve/Vac14 complex disruption impairs the cellular distribution of other PIP species. In the present study, we report that PI4P levels are significantly decreased at the trans-golgi network (TGN) in *FIG4*, *PIKFYVE* or *VAC14*-deficient HAP1 cells and in neurons treated with pharmacological inhibitors of class III PI 3-kinase and PIKfyve. We demonstrate that these changes are likely caused by reduced TGN levels of PI4P-generating enzymes PI4KIIα and PI4KIIIβ, as well as an enhanced oxysterol-binding protein (OSBP)-mediated PI4P transfer from the TGN to the endoplasmic reticulum at membrane contact sites (MCS). Functionally, decreases in TGN PI4P result in alterations in trafficking patterns from the TGN that influence plasma membrane voltage-gated calcium channels. We determined, using super-resolution microscopy and electrical field stimulation, that voltage-gated calcium channel distribution and activity is altered in neurons treated with class III PI 3-kinase and PIKfyve inhibitors. Upstream of changes in voltage-gated calcium channel dynamics is enhanced nuclear translocation of the transcription factor EB. Overall, our results suggest that the Fig4/PIKfyve/Vac14 complex tunes the molecular contents of ER-Golgi MCS to influence anterograde membrane trafficking and voltage-gated calcium channel distribution and activity. We propose that these deviant changes in lipid and calcium signaling pathways contribute to neurodegeneration in neurons with disrupted Fig4/PIKfyve/Vac14 complex function.

B330/P1320

The gastrosome: a new compartment in the phagocytic pathway

J. Zareba, E. Cattaneo, F. Peri; Department of Molecular Life Sciences, University of Zurich, Zurich, SWITZERLAND.

Phagocytosis is a central process for immunity and maintenance of tissue homeostasis. This way, professional phagocytes, such as tissue-resident macrophages, efficiently remove foreign particles and large apoptotic cells. While the engulfment of these targets is well understood, their subsequent digestion and recycling remain mostly unclear, even though these steps are essential to ensure proper phagocytosis. Recent work in zebrafish microglia and mammalian macrophages led to the discovery of the "gastrosome," a new compartment in the phagocytic pathway. Live imaging has shown that this unique vesicle fuses with mature phagosomes and functions as a collection compartment. Increased phagocytosis causes the dramatic enlargement of the gastrosome affecting cellular morphology and motility. To uncover the regulation and role of this newly discovered compartment we have taken chemical and reverse genetic approaches. This identified Npc1 and ABC transporters as essential in

phagocytosis and gastrosome biology. The development of new phagocytic markers in zebrafish has allowed real-time mapping of vesicular interactions over time providing new insights into the spatio-temporal coordination of phagocytosis, the role of the gastrosome, and how microglia react when these transporters are blocked.

B331/P1321

Toll-like receptor signaling remodels the lysosomal system in macrophages

N. Ilic, A. Logli, R. Botelho; Biology, Toronto Metropolitan University, Toronto, ON, CANADA.

Pattern recognition receptors (PRRs) play a central role in innate immunity by binding to conserved microbe-associated molecular patterns (MAMPs) displayed by microbes. PRR-MAMP binding leads to the downstream activation of several signalling pathways to prime the immune system for defence against these infectious threats. There are several families of PRRs that recognize specific types of MAMPs associated with specific classes of microbes. One such family of PRRs is the Toll-like receptors (TLRs), a group of transmembrane receptors found in the plasma membrane or the endosomes. Another family of PRRs are the NOD-like receptors (NLRs), a family of cytosolic receptors. Lipopolysaccharides (LPS) from gram-negative bacteria engage the plasma membrane TLR4 signalling. Previously, LPS-TLR4 signalling was shown to remodel the lysosomal system in macrophages and dendritic cells into an expanded, tubular network that appears to promote uptake of extracellular fluid and antigen presentation. These adaptations caused by TLR4 were found to be dependent on the mechanistic target of rapamycin (mTOR) pathway. However, there is little evidence of whether other TLRs and PRRs like NOD proteins also cause lysosome remodelling. Since lysosomes are critical in digesting microbes engulfed by phagocytosis, processing antigens, and antigen presentation, we aimed to analyze whether all other members of the TLR family (TLR1-9) and members of the NLR family (NOD1-2) caused lysosome remodelling in murine bone marrow-derived macrophages. We stimulated macrophages with cognate ligands of each TLR for 2 h and found that this caused lysosome tubulation and expansion, though to varying degrees. For example, Pam3CSK4, which stimulates TLR1/2, caused extensive tubulation, while HKLM, a TLR2 ligand, was more subdued. TLR ligands also had varying effects on lysosome expansion; while ODN1826, a TLR9 ligand, caused the largest expansion, stronger than LPS, there was no significant difference in total lysosome volume by ligands activating TLRs2-7. In contrast, agonists of NOD1 and/or NOD2 were relatively less potent in both tubulating and expanding total lysosome volume within 2 h of stimulation. To further investigate, we seek to identify if the mTOR pathway is required for the lysosome adaptations in activated macrophages and their impact on uptake of extracellular material and antigen presentation. Overall, we aim to determine the similarities and differences in the actions of endosomal and plasma membrane TLRs, which can potentially be helpful for future adjuvant research. Overall, our research provides a better understanding of PRR signalling and how lysosome remodelling may aid in immunity.

B332/P1322

Understanding the Significance of Unconventional Golgi Lysosome Interplay in Epidermis Homeostasis

S. MAHANTY[1], P. Bergam[2], D. Nair[3], V. Belapurkar[3], L. Eluvathingal[3], G. Raposo[2], S. Rao Gangi Setty[1]; [1]Department of Microbiology & Cell Biology, Indian Institute of Science, Bangalore, INDIA, [2]UMR 144, Structure and Membrane Compartments, Institut Curie, Paris, FRANCE, [3]Centre for Neuroscience, Indian Institute of Science, Bangalore, INDIA.

The outer skin layer epidermis provides a hydrophobic and antimicrobial barrier to the body and, is critical in maintaining skin health and hygiene. Keratinocytes are the major cellular constituent of epidermis which maintains skin barrier homeostasis through regulated differentiation. During differentiation, keratinocytes produce a class of lysosome-related organelles (LRO) called epidermal lamellar bodies (LBs) which critically regulate the skin barrier formation and homeostasis. In line, impairment in LBs formation is associated with life-threatening skin disorders. However, the molecular mechanism of LBs biogenesis/secretion remain unknown. Even though LBs share part of their cargoes with the conventional lysosomes and, their *trans* Golgi origin is speculated on the basis of electron microscopy (EM) observation, the role of neither of these organelles is elucidated so far in the context of LBs biogenesis. We revealed unique dispersed Golgi morphology and extensive dispersion of lysosomes in our *in vitro* model of differentiated keratinocytes. Remarkably, dispersed Golgi stacks make frequent contact with the lysosomes as observed by super-resolution live and steady-state experiments. Further observation by conventional EM and, immunogold labeling of ultrathin cryosections (immuno-EM) of differentiated keratinocytes show extensive vesicular Golgi morphology and its contact with the lysosomes. The Golgi lysosome contact is possibly tethered by peripheral Golgi protein GRASP65. In line, siRNA-mediated inhibition of GRASP65 led to the loss of Golgi-lysosome contact which in turn results in aberrant lysosome morphology and perinuclear accumulation of giant lysosome structures. Exploiting specific inhibition and gene expression approaches, we further reveal a possible direct role of functional Golgi and, the Golgi to lysosome contact respectively in the generation and maintenance of keratinocyte lysosomes. Taken together, our study highlights the modulation of Golgi morphology and, unravels its coordination in the generation of keratinocyte lysosomes which play a critical role in epidermis homeostasis. In my presentation, I will further emphasize how this interplay between Golgi and lysosomes possibly favors the biogenesis of LBs and its overall significance in keratinocyte physiology.

B333/P1323

Syntaxin 12 and COMMD3 are new factors that function with VPS33B in the biogenesis of platelet α-granules

A. L. Ambrosio, H. P. Febvre, S. M. Di Pietro; Colorado State University, Fort Collins, CO.

Blood circulating platelets are megakaryocyte-derived cells that mediate blood clotting and regulate angiogenesis, inflammation and malignancy. They accomplish these functions by releasing the content of their granules to circulation upon platelet activation. In particular, platelet α-granules are lysosome-related organelles that contain hundreds of proteins including growth and angiogenic factors and cytokines. Despite the fundamental role of α-granules, both in health and disease, their biogenesis is unclear. Mutations in only three proteins are known to cause α-granule defects and bleeding disorders in humans. Two such proteins, VPS16B and VPS33B, form a complex mediating transport of newly synthesized α-granule proteins through megakaryocyte endosomal compartments. It is unclear how the VPS16B/VPS33B complex accomplishes this function. Here we report VPS16B/VPS33B associates

physically with Stx12, a SNARE protein that mediates vesicle fusion at endosomes. Importantly, Stx12 deficient megakaryocytes display reduced α-granule numbers and overall levels of α-granule proteins, thus revealing Stx12 as new component of the α-granule biogenesis machinery. VPS16B/VPS33B also binds CCDC22, a component of the CCC complex working at endosome exit sites. CCDC22 competes with Stx12 for binding to VPS16B/VPS33B suggesting a possible hand-off mechanism. Moreover, the major CCC form expressed in megakaryocytes contains COMMD3, one of ten COMMD proteins. Deficiency of COMMD3/CCDC22 causes reduced α-granule numbers and overall levels of α-granule proteins, establishing the COMMD3/CCC complex as a new factor in α-granule biogenesis. Furthermore, P-Selectin traffics through the cell surface in a COMMD3-dependent manner and depletion of COMMD3 results in lysosomal degradation of P-Selectin and PF4. In conclusion, we identified two new players in α-granule biogenesis, Stx12 and COMMD3/CCC, making them candidates for platelet storage pool disorders of unknown molecular etiology. Mechanistically, our results suggest VPS16B/VPS33B coordinates the endosomal entry and exit of α-granule proteins by linking the fusogenic machinery with a ubiquitous endosomal retrieval complex that is repurposed in megakaryocytes to make α-granules.

B334/P1324

OCA7 defines pigmentation by regulating early stages of melanosome biogenesis

W. Beyers, A. Detry, S. Di Pietro; Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO.

Mutations in C10orf11 (OCA7) cause OculoCutaneous Albinism, a disorder that presents with hypopigmentation in skin, eyes and hair. The OCA7 pathophysiology is unknown and there is virtually no information on the OCA7 protein and its cellular function. Here, we discover that OCA7 localizes to the limiting membrane of melanosomes, the specialized pigment cell organelles where melanin is synthesized. OCA7 is recruited through interaction with a canonical effector binding surface of melanosome proteins Rab32 and Rab38. Using newly generated OCA7-KO MNT1 cells, we show OCA7 regulates overall melanin levels in a melanocyte autonomous manner by controlling melanosome maturation. Interestingly, we found OCA7 regulates PMEL processing, impacting fibrillation and the striations that define transition from melanosome Stage I to Stage II. Furthermore, the melanosome lumen of OCA7-KO cells displays lower pH than control cells. Together, our results reveal OCA7 regulates pigmentation through two well established determinants of melanosome biogenesis and function, PMEL processing and organelle pH.

B335/P1325

Effect of a Minor Phosphatidylinositol Phosphate on a Secretory Granule in Platelet and Megakaryocyte

C. Lu[1,2], A. Lee[1], L. Li[2], F. Pluthero[2], W. Kahr[2,1]; [1]Biochemistry, University of Toronto, Toronto, ON, CANADA, [2]Cell Biology, The Hospital for Sick Children, Toronto, ON, CANADA.

Platelets are blood cells that play a key role in hemostasis, inflammation and wound healing. They are derived from megakaryocytes (MKs) which mostly reside in bone marrow. Many aspects of platelet function are related to the release of proteins from α-granule, a lysosome-related-organelle which contain proteins such as fibrinogen and Von Willebrand factor (VWF). Neurobeachin-like 2 (NBEAL2) is required for the retention of cargo proteins by α-granules. NBEAL2 deficiency in MKs leads to the bleeding disorder Gray Platelet Syndrome (GPS), so called because patient platelets look pale in a blood

smear due to the lack of α-granules. The mechanism by which NBEAL2 recognizes α-granules to facilitate the maturation and stability of is unclear.Phosphatidylinositol phosphates (PIPs) comprise less than 1% of cellular lipids, yet they regulate fundamental biological processes including signalling, vesicle trafficking, and membrane dynamics. The specific intracellular distribution of PIPs is key to their role in regulating membrane trafficking and downstream signaling pathways. $PI(3,5)P_2$, a minor PIP localized to multivesicular bodies (MVBs) and lysosomes, plays a pivotal role in endosomal maturation. $PI(3,5)P_2$ can be only generated by the PIKfyve (phosphoinositide kinase, FYVE-type zinc finger containing) lipid kinase.NBEAL2 has a Pleckstrin homology (PH) domain, which is commonly found in PIP binding proteins. We hypothesize that NBEAL2 interacts with the membranes of α-granules via interaction with granule-associated PIPs. Our in-vitro data shows NBEAL2 and its PH-BEACH subdomain preferentially binds $PI(3,5)P_2$. One GPS-associated missense mutation abrogates $PI(3,5)P_2$ binding. We demonstrate that NBEAL2 interacts with PIKfyve complex using co-immunoprecipitation. Pharmaceutical inhibition of $PI(3,5)P_2$ synthesis in MKs causes a disassociation between NBEAL2 and α-granule membrane. We also observe a premature secretion of VWF from α-granules which recapitulates the phenotype of NBEAL2 deficient MKs. Our data suggests that the interaction of NBEAL2 and $PI(3,5)P_2$ is critical in α-granule maturation and MK cargo protein trafficking.

B336/P1326

In vitro reconstitution of calcium-dependent recruitment of the human ESCRT machinery in lysosomal membrane repair

S. Shukla, K. P. Larsen, C. Ou, K. Rose, J. H. Hurley; Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

The endosomal sorting complex required for transport (ESCRT) machinery is central to endolysosomal and plasma membrane repair. ESCRT recruitment to sites of damage occurs on a fast time scale, and Ca^{2+} has been proposed to play a key signaling role in the process. However, the upstream components needed to orchestrate the membrane repair process are poorly understood. With the goal of bridging this gap in understanding, we biochemically reconstituted the membrane repair machinery. Here, we show that the Ca^{2+}-binding regulatory protein ALG-2 binds directly to negatively charged membranes in a Ca^{2+}-dependent manner. Next, by monitoring the colocalization of ALIX with ALG-2 on negatively charged membranes, we attest to the upstream role of ALG-2 in bringing ALIX to the membrane. Furthermore, we show that ALIX recruitment to membrane orchestrates the downstream assembly of late-acting CHMP4B, CHMP3, and CHMP2A subunits along with the AAA+ ATPase VPS4B. Finally, we show that ALG-2 can also recruit the ESCRT-III machinery to the membrane via the canonical ESCRT-I/II pathway. Our reconstitution experiments delineate the minimal sets of components needed to assemble the entire membrane repair machinery and open a new avenue for the mechanistic understanding of endolysosomal membrane repair.

B337/P1327

Proinflammatory macrophages are better at clearing aggregated LDL and are more prone to foam cell formation

C. J. Ma, N. Steinfeld, F. R. Maxfield; Biochemistry, Weill Medical College/Cornell University, New York, NY.

Atherosclerosis is a chronic disease associated with long-term dyslipidemia and inflammation. The development of atherosclerotic plaque starts with foam cell formation, where macrophages infiltrate the intima of the aortic wall to digest modified low-density lipoproteins (LDLs). Pathology of atherosclerotic lesions showed that most of the modified LDLs are aggregated (agLDL) and retained in the aortic wall. Our lab has previously described the formation of an extracellular, acidic, hydrolytic compartment (lysosomal synapse), by which macrophages catabolize agLDL in a process termed digestive exophagy. In a recent study, we further demonstrated that macrophage-mediated digestive exophagy of agLDL is regulated by the Toll-like receptor 4 (TLR4) signaling pathway. Because TLR4 is a known regulator of inflammation, we hypothesized that macrophage interacting with agLDL would activate the macrophages into a pro-inflammatory state, and this activation would further enhance the catabolism of agLDL- leading to increased foam cell formation. To investigate whether digestive exophagy of agLDL results in an inflammatory response, we showed that agLDL treated-macrophages showed an increase in NFκB phosphorylation. In addition, we cultured macrophages expressing the inflammasome marker ASC-citrine with agLDL or acetylated LDL (AcLDL). We observed a gradual increase in the number of inflammasomes over a 24 h period in agLDL treated cells, whereas no changes were observed in AcLDL treated cells. Furthermore, lipopolysaccharide-primed and pro-inflammatory (M1) macrophages also demonstrated increased exocytosis of lysosomal contents to agLDL, an indication for increased digestive exophagy, when compared with non-activated (M0) or anti-inflammatory (M2) macrophages. Interestingly, M2 macrophages showed a decrease in lysosome exocytosis when compared to M0 macrophages. A similar trend is observed in their ability to form lysosomal synapses, which is measured by the degree of local actin polymerization around agLDL. Lastly, treating macrophages with MCC950, an NLRP3 inhibitor that blocks inflammasome formation and IL-1β release, significantly decreased exocytosis of lysosomal content. Overall, our data provide evidence that pro-inflammatory macrophages are the main contributors to the digestive exophagy of agLDL, and it is possible to modulate digestive exophagy by altering the inflammatory state of macrophages.

B338/P1328

Characterizing a novel mechanism that contributes to the development of atherosclerosis

N. Steinfeld, C. J. Ma, F. R. Maxfield; Weill Medical College/Cornell University, New York, NY.

The development of atherosclerosis is characterized by excessive cholesterol ester and lipid accumulation in macrophages, leading to foam cell formation. In atherosclerotic lesions, most lipoproteins are aggregates that are tightly crosslinked to the extracellular matrix, which makes digesting aggregated lipoproteins by endocytosis or phagocytosis difficult. Thus, it has been unclear how the cholesteryl esters in the core of aggregated extracellular LDL (agLDL) are hydrolyzed. Our laboratory's recent studies demonstrate a novel mechanism by which macrophages create tightly sealed compartments that surround portions of the aggregated LDL. These compartments are acidified by secretion of lysosomal enzymes, creating what we have termed a lysosomal synapse. Within the lysosomal synapse, cholesteryl esters are hydrolyzed by secreted lysosomal acid lipase leading to the

production of unesterified cholesterol outside the cell, in a process we have termed digestive exophagy (Reviewed in Maxfield et al., Traffic 2020). We believe that the transport of this cholesterol into macrophages is a primary driver of foam cell formation. However, many gaps remain in our understanding of the signaling and machinery involved in digestive exophagy, especially in the control of lysosome exocytosis.

We have data implicating the phospholipase Cγ1/2 (PLCγ1/2) and protein kinase Cα (PKCα) signaling axis in digestive exophagy based on both pharmacological treatment and genetic manipulation. Both inhibition of PLC using U73122 and siRNA knockdown of PLCγ1 or PLCγ2 results in a sharp reduction of lysosome secretion towards agLDL. PLC cleaves phospholipids into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). Soluble IP_3 then binds to the IP_3 receptor on a ligand-gated Ca^{2+} channel, leading to the release of Ca^{2+} into the cytoplasm. Chelation of Ca^{2+} using BAPTA-AM leads to a near total loss of lysosome exocytosis towards agLDL. Further downstream, conventional PKC isoforms are regulated by both DAG and Ca^{2+}. Treatment of macrophages with a DAG mimic and PKC activator, PMA, lead to a near 8-fold increase in lysosome exocytosis towards agLDL, while siRNA knockdown of PKCα reduced lysosome secretion upon contact with agLDL. We are in the process of further characterizing this mechanism and are pursuing both targeted and unbiased approaches to identifying novel regulators of this process.

B339/P1329

Rab7-positive late endosomes show rapid responses to lysosomal membrane permeabilization

R. Mulligan, B. Winckler; University of Virginia, Charlottesville, VA

Lysosomes are the main degradative organelles of the cell, responsible for macromolecular turnover and cellular signaling. Maintenance of proper lysosomal function is integral as lysosomal disruption compromises proteostasis, contributes to cellular dysfunction and threatens cellular death. Lysosomal membrane permeabilization (LMP) is one common form of lysosomal disruption that occurs in many pathologies, notably those impacting the central nervous system such as neurodegeneration and stroke. While homoeostatic cellular responses have been described to address LMP at the level of the lysosome, the role of endosomes in LMP have not been elucidated, despite their interconnectedness to lysosomal function. Using LLOMe as a pharmacologic inducer of lysosomal damage in normal rat kidney cells (NRK) and primary mouse embryonic fibroblasts (MEF), we have now found that the late endosomal small GTPase Rab7 is a rapid (<20mins) early responder to lysosomal damage states. Following lysosomal disruption, Rab7 accumulates on enlarged undamaged late endosomal compartments. Endosomal maturation from the trans-Golgi network through *early* late endosomes (Rab7+, cathepsin-B *lo*) is preserved, however maturation beyond this point is arrested. At the molecular level, Rab7 is hyper-activated and binds more GTP following LLOMe-induced LMP. To determine specific mechanisms by which Rab7 is activated and accumulates on late endosomes, we employed a diverse pharmacologic approach to specifically disrupt various aspects of lysosomal function. Indeed, though LMP cumulatively leads to loss of lysosomal degradative capacity and massive efflux of divalent cation stores including Ca^{2+}, it is specifically the loss of luminal lysosomal pH that contributes to Rab7 mediated changes in late endosomes. Together, these results suggest a homeostatic cascade to counteract pH collapse in LMP driven by Rab7 activation, which leads to arrest of endosomal maturation.

B340/P1330

Early Endosomes Act as Local Exocytosis Hubs to Repair Endothelial Membrane Damage

N. Raj[1], L. Greune[2], M. Kahms[3], K. Mildner[4], R. Franzkoch[5], O. E. Psathaki[5], T. Zobel[6], D. Zeuschner[4], J. Klingauf[3], V. Gerke[1]; [1]Institute of Medical Biochemistry (IMB), ZMBE, WWU, Muenster, GERMANY, [2]Institute of Infectiology, ZMBE, WWU, Muenster, GERMANY, [3]Institute of Medical Physics and Biophysics, WWU, Muenster, GERMANY, [4]Electron Microscopy Facility, Max Planck Institute for Molecular Biomedicine, Muenster, GERMANY, [5]Integrated Bioimaging Facility (iBiOs), Center of Cellular Nanoanalytics (CellNanO), University of Osnabrück, Osnabrueck, GERMANY, [6]Imaging Network, Cells in Motion Interfaculty Centre, WWU, Muenster, GERMANY.

The plasma membrane of a cell is subject to stresses such as mechanical forces resulting from shear stress and mechanical stretch, causing ruptures that must be repaired immediately to preserve membrane integrity and ensure cell survival. Defective plasma membrane repair can lead to eventual tissue damage and has been linked to numerous disease pathologies. Yet, the spatio-temporal membrane dynamics at the wound site and the source of membrane required for wound repair are poorly understood. Here, we show that early endosomes, previously known to function in the uptake of extracellular material and its endocytic transport, are involved in plasma membrane repair in human endothelial cells. Using live-cell imaging, STED, and correlative light and electron microscopy, we demonstrate that membrane injury triggers a previously unknown exocytosis of early endosomes that is induced by the Ca^{2+} entering through the wound. This novel early endosome exocytosis is spatially restricted to the vicinity of the wound site, occurs immediately upon wounding, and is mediated by the endosomal SNARE VAMP2, which is crucial for efficient membrane repair. Lysosomal exocytosis also occurs upon wounding but is not required to support membrane resealing in endothelial cells, as revealed by different membrane damage assays including two-photon live-cell laser ablation, glass bead rolling, and mechanical scratch-induced wounding. Thus, the specialized Ca^{2+}-triggered and localized exocytosis of early endosomes is an emergency-based process that supplies the membrane material needed for rapid wound closure in endothelial cells. This is essential to prevent wound-induced endothelial leakage and a resulting inflammatory reaction in a mechanically stressed environment.

B341/P1331

Does phagocytic receptor dynamics and signalling regulate phagocytic capacity, appetite, and recovery?

S. Soffiaturo[1], R. Botelho[2]; [1]Toronto Metropolitan University, Toronto, ON, CANADA, [2]Ryerson University, Toronto, ON, CANADA.

Within the immune system, there are specialized cells that carry out specific functions to fight infections and maintain health. Phagocytes such as macrophages are specialized cells that perform a critical process known as phagocytosis. Phagocytosis refers to the recognition, uptake, and degradation of particles by phagocytes. Initial stages of phagocytosis include the recognition of particles by receptors on the cell surface such as Fcγ receptors. After recognition, particles are engulfed within an internal compartment referred to as a phagosome. Through an array of signaling events, phagosomes mature and eventually fuse with lysosomes resulting in the degradation of the engulfed material. Although, macrophages uptake particles until they reach their limit and become “full”. This notion is referred to as phagocytic capacity. The limit to the number of particles that can be taken up by macrophages is dependent on size, shape, and composition. Currently, there is a large amount of knowledge

surrounding the role of receptors involved in Fcγ-mediated phagocytosis. Although, we do not know how receptors are involved in terms of phagocytic appetite and capacity, despite the extensive knowledge surrounding phagocytic capacity. Therefore, we hypothesized that depletion of Fcγ receptor levels abates appetite in cells at phagocytic capacity. To test this hypothesis, we chose to examine FcγRIII dynamics as cells reach capacity. We first performed immunofluorescence assays alongside streptavidin-biotin pulldown assays to examine FcγRIII surface levels as cells reach capacity following uptake of synthetic polystyrene beads in RAW 264.7 and primary macrophages. Our primary data suggests that surface levels of FcγRIII in RAW 264.7 and primary macrophages are not largely affected as cells reach capacity. In contrast, preliminary data suggest that total levels are altered potentially indicating a depletion in the cell's reservoir of receptors. Future experiments include examining if engagement of inhibitory signals like ITIM-based FcγRIIb helps reduce phagocytic appetite of macrophages at capacity. As particle recognition is a significant step within the process of phagocytosis, it is critical that we gain a better knowledge of it. Therefore, this work aims to provide insight into the role of phagocytic receptor dynamics and signaling in regulating phagocytic capacity, appetite, and recovery.

B342/P1332

Mu opioid receptor-mediated release of endolysosome iron increases levels of mitochondrial iron, reactive oxygen species, and cell death

P. W. Halcrow[1], N. Kumar[1], E. Hao[1], N. Khan[1], O. Meucci[2], J. D. Geiger[1]; [1]Biomedical Sciences, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND, [2]Physiology and Pharmacology, Drexel University School of Medicine, Philadelphia, PA.

Opioids are widely used analgesics and are prone to abuse due to their euphoric and dependency effects. Functioning through opioid receptors, opioids including morphine and DAMGO activate mu-opioid receptors (MOR), increase intracellular reactive oxygen species (ROS) levels, and induce cell death. Ferrous iron (Fe^{2+}) through Fenton-like chemistry can increase ROS levels and damage DNA and proteins. Endosomes and lysosomes (endolysosomes) are "master regulators of iron metabolism" central to iron trafficking as they contain readily-releasable Fe^{2+} stores and an acidic lumen linked to iron homeostasis. However, the opioid-induced mechanisms influencing endolysosome-iron homeostasis and downstream-signaling events inducing toxicity remain unknown. Here, using SH-SY5Y neuroblastoma cells we found that morphine and DAMGO de-acidified endolysosomes, decreased endolysosome Fe^{2+} levels, increased cytosol and mitochondria Fe^{2+} and ROS levels, depolarized mitochondrial membrane potential, and induced cell death; effects blocked by the nonselective MOR antagonist naloxone and the selective MOR antagonist β-funaltrexamine (β-FNA). Deferoxamine, an endolysosome-iron chelator, inhibited opioid agonist-induced increases in cytosolic and mitochondrial Fe^{2+} and ROS. Opioid-induced efflux of Fe^{2+} from endolysosomes and subsequent accumulation of Fe^{2+} in mitochondria were blocked by the endolysosome-resident two-pore channel inhibitor NED-19 and the mitochondrial permeability transition pore inhibitor TRO. Naloxone and β-FNA by themselves were able to retain Fe^{2+} in endolysosomes and decrease levels of cytosolic and mitochondrial Fe^{2+} and ROS. Opioid agonist-induced increases in cytosolic and mitochondrial Fe^{2+} and ROS as well as cell death appear downstream of endolysosome de-acidification and Fe^{2+} efflux. These findings provide new insight into the pharmacological actions of opioids and demonstrate clearly that the pool of iron in endolysosomes is sufficient to effect changes to other organelles and to affect key cellular functions including cell life and death.

B343/P1333

Huntingtin during axonal injury: Evidence for a retrogradely moving Huntingtin-Rab7-LAMP1-containing signaling endosome.

T. J. Krzystek, J. A. White II, R. Rathnayake, L. Thurston, H. Hoffmar-Glennon, Y. Li, **S. Gunawardena**; The State University of New York at Buffalo, Buffalo, NY

Huntingtin (HTT), the protein involved in Huntington's disease is a 350kDa protein of unknown function. Despite more than 350 binding partners identified for HTT across a wide range of cellular processes, including trafficking, the molecular mechanisms by which HTT and its binding partners function remain elusive. Here we provide evidence for a retrogradely moving HTT-Rab7 vesicular complex in Drosophila, mice and human neurons by using *in vivo* imaging in living axons coupled with a custom particle tracking analysis and pharmacological inhibitors. We identify that adaptors HIP1 and RILP aid the retrograde motility of LAMP-1 containing HTT-Rab7 late endosomes, but not autophagosomes. Reduction of Syntaxin17 and Chloroquine/BafilomycinA1-mediated pharmacological inhibition, but not reduction of ATG5, disrupted the *in vivo* motility of these vesicles. The retrogradely moving HTT-Rab7-LAMP1-containing late endosome can traffic long-distance signaling components such as the BMP-receptors TKV/WIT, neurotropic factor BDNF and axonal damage response components WND/DLK and JNK following axonal injury. Taken together, our observations unravel a previously unknown role for HTT in the retrograde movement of a Rab7-LAMP1-containing signaling late endosome, which has functional relevance during axonal injury.

B344/P1334

Insights into the Biological Role of the Lysosomal Trafficking Regulator Through Proteomic and Functional Analyses

J. Serra-Vinardell, M. B. Sandler, W. A. Gahl[,1], **P. Sharma**; NHGRI/NIH, Rockville, MD

Variants in the Lysosomal Trafficking Regulator (*LYST*) gene are associated with Chediak-Higashi Syndrome (CHS), a rare autosomal recessive disorder characterized by primary immunodeficiency and a high risk of progressive neurological problems. Enlarged lysosomes/ lysosome-related organelles (LRO) are the cellular hallmark of CHS. Though its name implies LYST in lysosomal trafficking, LYST deficient cells lack primary regulation of lysosome/LRO size and number. Therefore, there is a need to continue delineating the precise role of LYST. The daunting task of expressing large LYST protein (429 kDa) hampered researchers from manipulating LYST levels in the cell of interest. Here, we exploited the piggyBac transposon system to successfully express epitope-tagged LYST in different cell types, including neurons, to gain functional insight. We performed systematic proteomic analysis of affinity-purified samples by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and identified several LYST-interacting proteins. We further developed a co-immunoprecipitation strategy to identify endogenous LYST interacting proteins by LC-MS/MS in differentiated macrophages treated with toll-like receptor ligands. Together, our approaches led us to build an inventory of LYST interacting proteins across cell lines of distinct origins. We apply the LYST Proteomic map to functional analyses of candidate protein interactions and illuminate new mechanistic insights into how LYST controls cellular processes dysregulated in CHS.

B345/P1335

Intracellular vesicles in B lymphocyte activation

A. Music, S. Hämälistö, **P. Mattila**; University of Turku, Turku, Finland

There is a major lack in our knowledge of the cell biological features of lymphocyte function and activation, critical for understanding the mounting of the immune response both in health and disease. We have focused on characterizing the intracellular vesicle pool in B lymphocytes (B cells), particularly, upon cell activation and antigen processing, which are key steps towards antibody responses against specific antigens. Our research has identified high heterogeneity in the antigen-containing vesicles that direct freshly encountered and internalized antigens towards the compartments processing the antigen into peptide-MHCII complexes (Hernández-Pérez et al, Journal of Cell Science, 2019). Unlike in the general understanding of the early to late endosomal cascade maturation, we also found that vesicles with lysosomal features, such as low pH, capture both the internalized antigen as well as surface-derived MHCII right below the plasma membrane.
Here, we have continued our studies on unveiling the role of the lysosomal vesicle pool in B cells. Our results point towards several distinct vesicle pools, that differ, for instance, by their size, location, and the content of lysosomes associated proteins 1 and 2 (LAMP1 and LAMP2). Furthermore, inhibiting lysosomal function with cationic amphiphilic drugs (CADs) leads to marked changes in the B cell antigen receptor (BCR) -triggered activation of B cells. The cells where lysosomes are inhibited are not able to form normal immunological synapses and they show aberrant activation status of signaling proteins. In our work, we mainly utilize various high-resolution fluorescence microscopy techniques as well as electron microscopy.

B346/P1336

S100A4 regulates lysosomes

N. Dulyaninova, S. Sidoli, A. J. Haimowitz, M. J. Gamble, J. M. Backer, **A. R. Bresnick**; Albert Einstein College of Medicine, Bronx, NY

S100A4, a member of the S100 family of Ca^{2+}-binding proteins, is primarily expressed in fibroblasts and leukocytes. S100A4 is also expressed in cancer cells and is directly involved in tumor metastasis. Biochemical studies have shown that S100A4 binds with high affinity to the nonmuscle myosin-IIA heavy chain and mediates myosin-IIA filament depolymerization. Consistent with S100A4's *in vitro* biochemical activity, the loss of S100A4 in cells results in myosin-IIA filament overassembly, leading to a decrease in the migratory capacity of multiple cell types. We have previously shown that CSF-1-differentiated bone marrow-derived macrophages (BMMs) from S100A4$^{-/-}$ mice show defects in the function of podosomes; actin-rich protrusive structure that degrade the extracellular matrix (ECM). Podosomes in S100A4$^{-/-}$ BMMs are small and the BMMs show an 60% decrease in ECM degradation. To explore the mechanism for this defect, we measured lysosomal exocytosis, which contributes to matrix degradation. In response to A23187, a Ca^{+2} ionophore, S100A4$^{-/-}$ BMMs showed a 1.6-fold decrease in β-hexosaminidase release. Ionophore-stimulated retrograde movement of lysosomes towards the nucleus was also inhibited in S100A4$^{-/-}$ BMMs. To measure the fidelity of lysosomal protein sorting in the endocytic pathways and biosynthetic pathways, we measured CSF-1-stimulated degradation of the CSF-1 receptor, and A23187-stimulated trafficking of Lamp1. CSF-1 receptor internalization and degradation was comparable in wild type and S100A4$^{-/-}$ BMMs. Plasma membrane Lamp1 was low in unstimulated wild type BMMs and increased 2-fold in response to A23187. In contrast, plasma membrane Lamp1 was elevated in S100A4$^{-/-}$

BMMs and was unaffected by A23187. To examine lysosomes in more detail, we performed transmission electron microscopy on wild type and S100A4$^{-/-}$ BMMs. S100A4$^{-/-}$ BMMs showed a 5-fold increase in the number of autophagosomes, and a 3-fold increase in the number of autophagosomes containing lipid droplets. In addition, multilamellar bodies were greatly increased in S100A4$^{-/-}$ BMMs. Taken together, these data show an unexpected role for S100A4 in regulating lysosomal trafficking. Future experiments will use RNAseq and proteomic approaches to define the mechanisms for this surprising phenotype in S100A4$^{-/-}$ BMMs.

B347/P1337

Manipulation of the host cell endo-lysosomal system by the intracellular bacterial pathogen *Legionella pneumophila*

A. Steinbach, S. Mukherjee; University of California, San Francisco, San Francisco, CA

Legionella pneumophila (*L.p.*), an intracellular bacterial pathogen, manipulates eukaryotic host cell membrane trafficking to establish a replicative niche. While the bacterium resides within a phagosome-derived compartment throughout its lifecycle, this compartment, known as the *Legionella*-containing vacuole (LCV), resists trafficking to the lysosome. LCV formation and lysosomal fusion evasion is dependent on the secretion of bacterial proteins, termed effectors, into the host cell cytosol, as a secretion-null isogenic strain is degraded in the lysosome. As the mechanisms of lysosomal trafficking evasion by *L.p.* are poorly understood, the present study aims to address how the bacterium subverts normal endosome maturation at the LCV membrane to block fusion with the lysosome. While previous reports indicated that *L.p.* blocked endosome maturation of the LCV at early stages, application of time-lapse fluorescence imaging in the present study has revealed that both Rab5 and Rab7, the master regulators of early and late endosome identity, respectively, associate with the LCV. This result suggests that endosome maturation of the LCV progresses to a late endosome-like state, while still resisting trafficking to the lysosome. Unexpectedly, we have also found that a class of effector proteins are required for Rab5 association with the LCV, suggesting that Rab5 is actively recruited to the membrane during infection. To determine whether *L.p.* manipulates the function of endosomal regulatory proteins, mass spectrometry analysis was carried out on *L.p.* infected human cell lysates. This dataset and subsequent validation demonstrate that both Rab5 and Rab7 are mono-ubiquitinated during *L.p.* infection. Rab mono-ubiquitination is promoted by two bacterial effectors, SidC and SdcA, and appears to occur upon recruitment of the Rab to the LCV membrane. The mechanism and functional consequences of endosomal Rab mono-ubiquitination during infection, as well as the precise block point in endosome maturation at the LCV membrane, remain active areas of investigation. Taken together, these data suggest that *L.p.* employs a more subtle, multi-pronged approach to blocking trafficking of the LCV to the lysosome than previously proposed and represents progress in understanding of *L.p.*'s interaction with the endo-lysosomal system during infection.

B348/P1338

The Neurobiological Basis of Tourette Syndrome: A Behavioral Assessment of the *Drosophila* gene *Atg8a*

T. Hatfield; SIMMONS UNIVERISTY, Boston, MA

Tourette Syndrome (TS) is a complex, stigmatized, and misunderstood neurological condition primarily characterized by vocal and motor tics. The condition is highly heritable, with many implicated genes,

including *GABARAP.* This gene, whose expression negatively correlates with tic severity in some TS patients, is involved in autophagosome formation and $GABA_a$ receptor trafficking to the cell membrane. By overexpressing an RNAi via the GAL4/UAS system in *Drosophila melanogaster*, we aim to replicate *GABARAP's* decreased expression by knocking down the gene's ortholog, *Atg8a*, in the *Drosophila* nervous system. From these mutants, we aim to understand the origins of TS-like behavioral phenotypes produced in *Atg8a* mutants, and to gain insight into *GABARAP's* potential role in TS development.
Initial screenings of *Atg8a* mutants included over 800 larvae and 200 flies observed across 3 redundant RNAi lines for *Atg8a* of different sequences, reducing the probability of the observed phenotypes being caused by off-target effects. Using Tourette related behavioral traits, like the obsessive grooming and olfactory-learning ability, representative of compulsive motor tics and commonly co-morbid learning disabilities, we evaluate *Atg8a* mutants for Tourette-like phenotypes. In the learning assay, larval mutants of *Atg8a* demonstrate a significant decrease in learning ability across all *Atg8a* mutants compared to their UAS line counterparts, with their average change in LI being -72.42% ($p = 0.0043$). In adults, a similarly significant result is observed during the obsessive grooming assay. Across all RNAi knockdown mutants of *Atg8a*, there is a significant increase in grooming bout frequency and length, with their average percent changes from UAS line to mutants for all RNAi lines being 160.56% ($P = 0.00088$) and 127.50% ($P = 0.01273$) respectively.
These results demonstrate that knocking down *Atg8a* produces behaviors in *D. melanogaster* consistent with human TS patients. Although *GABARAP* plays a role in both autophagy and $GABA_a$ receptor trafficking, *Atg8a*, to our knowledge, has only been studied in the context of autophagy. This makes understanding *Atg8a's* potential GABAergic role as a *GABARAP* ortholog vital to understanding the origins of observed behavioral phenotypes. We intend to determine *Atg8a's* role as a *GABARAP* ortholog both to understand the etiology of these mutant phenotypes, and to shed light on the potential biological mechanisms driving this poorly understood human condition.

Vesicle Tethering, Docking and Fusion

B349/P1339

Role of exocyst phosphorylation in yeast: effect on exocyst structure and function

N. Buwa[1], D. Lepore[2], **L. Martínez-Núñez**[1], M. Feyder[1], B. Reneker[2], P. Opara-Nadi[1], B. Thomas[1], M. Munson[1]; [1]Biochemistry and Molecular Biotechnology, University of Massachusetts Chan Medical School, Worcester, MA, [2]University of Massachusetts Chan Medical School, Worcester, MA.

Membrane trafficking impacts a multitude of physiological phenomena including cell polarization, growth, signaling, and motility. Exocytosis requires vesicle fusion with the plasma membrane followed by cargo release outside the cell. SNARE-mediated vesicle fusion during exocytosis is regulated by the multi-subunit hetero-octameric tethering complex called exocyst. To carry out its functions, exocyst interacts with various players in the exocytic machinery, including SNAREs, the Sec1/Munc18 (SM) protein, small GTPases such as Rab and Rho, and the type V myosin in yeast. In order to control spatial and temporal specificity of fusion, the exocyst must be regulated. We propose that, in addition to the above binding interactions, exocyst is also regulated through phosphorylation of several of its subunits. Mapping of phosphorylation sites on the yeast exocyst cryoEM structure indicates that the majority of its phosphorylation sites are present in intrinsically disordered regions. Other phosphorylation sites are located near regions of inter-subunit interactions, or regions that bind non-exocyst partners. Ongoing mutational analyses in yeast aim to determine how phosphorylated residues affect cell growth, as well

as exocyst assembly, localization and function. We are also determining what structural changes are induced by phosphorylation. These studies will elucidate the role of subunit phosphorylation in exocyst assembly, activation, vesicle tethering and fusion.

B350/P1340

The Golgi complex serves as a platform for the DNA damage response pathways

K. Kuodyte[1,2], G. Galea[1], E. Lundberg[3], R. Pepperkok[1]; [1]Cell Biology & Biophysics, EMBL, Heidelberg, GERMANY, [2]Faculty of Biosciences, Heidelberg University, Heidelberg, GERMANY, [3]Science for Life Laboratory, KTH Royal Institute of Technology, Stockholm, SWEDEN.

DNA damage response (DDR) is a well characterized process, however, the majority of studies focus specifically on nuclear events, while cytoplasmic response to DNA damage remains largely unexplored. We have identified 15 DDR proteins that localize to the nucleus and to the Golgi complex. These proteins function in various distinct DNA repair mechanisms (e.g., HR, NHEJ, BER, MMR) and related signaling pathways. The dual-localizing DDR proteins were found to respond to specific DNA lesions through a shift in localization either from the Golgi to the nucleus or from the nucleus to the Golgi, based on their role in the specific DDR pathways.
We have identified several Golgins acting as anchors and regulators of these DDR proteins at the Golgi. More in-depth characterization of one of the dual-localized proteins RAD51C revealed that its Golgi localization is dependent on the golgin Giantin and the depletion of Giantin leads to aberrant DNA repair.Altogether, our data propose that the Golgi complex serves as a regulatory hub for various DNA repair mechanisms.

B351/P1341

A Functional Characterization of Golgi Tethers in*Saccharomyces cerevisiae*

A. Krahn, N. Johnson, B. Glick; Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

The Golgi apparatus lies at the heart of the cellular endomembrane system and is responsible for modifying and sorting proteins imported from the endoplasmic reticulum (ER). According to the cisternal maturation model, early Golgi cisternae arise from ER-derived membranes, mature biochemically into late Golgi cisternae through the retrograde vesicular recycling of resident Golgi proteins, and ultimately fragment into secretory carriers. The maturation of individual cisternae is evidenced by the arrival and departure of resident Golgi proteins. This process can be directly observed in the yeast *Saccharomyces cerevisiae*, which possesses non-stacked, optically resolvable cisternae. Although the components that drive cisternal maturation in *S. cerevisiae* have been largely identified, the vesicular recycling pathways at the Golgi and the specific molecular machineries that mediate each pathway are incompletely characterized. Recent work in our lab has identified some of these pathways and has measured the timing of arrival and departure for various membrane traffic components as a cisterna matures. Among these components are Golgi-associated tethers, which are thought to capture incoming vesicles and aid their fusion with cisternae. To identify which tethers act in each traffic pathway, we have developed an *in vivo* vesicle tethering assay. This assay involves ectopically localizing Golgi tethers and testing their abilities to capture transport vesicles containing different resident Golgi proteins. We have functionally characterized the coiled-coil golgins Imh1 and Sgm1 as well as the multi-subunit tether GARP. Our data demonstrate that Imh1 captures vesicles containing late Golgi proteins that recycle either within the Golgi or between the Golgi and the prevacuolar endosome (PVE). Sgm1

captures intra-Golgi vesicles during an intermediate stage of maturation, whereas GARP cooperates with Imh1 to capture intra-Golgi vesicles during a late stage of maturation. Neither Sgm1 nor GARP captures vesicles derived from the PVE. Our results reveal the specificities and interrelationships of these Golgi tethers.

B352/P1342

Kinetic Analysis of Vesicle Tethers that Act Late in Golgi Maturation

N. Johnson, A. H. Krahn, B. S. Glick; Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

The Golgi apparatus consists of disk-like membrane cisternae that modify and sort proteins and lipids. Golgi cisternae are transient structures that form *de novo* from the endoplasmic reticulum, then progressively mature by delivering early-acting resident proteins to younger cisternae while receiving late-acting resident proteins from older cisternae, and then finally dissolve into secretory carriers. This cisternal maturation process can be visualized directly in the yeast *Saccharomyces cerevisiae*, in which non-stacked Golgi cisternae appear as optically resolvable spots. During yeast cisternal maturation, many resident Golgi transmembrane proteins recycle within the organelle while some resident Golgi transmembrane proteins travel to prevacuolar endosome (PVE) compartments and back. Multiple membrane traffic pathways transport different classes of resident Golgi transmembrane proteins. 4D confocal microscopy reveals that each class of Golgi proteins has a characteristic kinetic signature, which reflects the timing of arrival and departure of the vesicles involved in the corresponding traffic pathway. A mechanistic understanding of Golgi maturation will require identifying the components that mediate each traffic pathway, including the Golgi-associated tethers that capture transport vesicles. We have focused here on identifying the traffic pathways that employ three late-acting Golgi tethers: the coiled-coil golgins Sgm1 and Imh1, and the multi-subunit tether GARP. Our kinetic and functional data paint the following picture. Sgm1 is recruited by the Rab GTPase Ypt6, and it operates in a traffic pathway that recycles Golgi proteins during an intermediate stage of maturation. This pathway leads to recruitment of the Arf-like GTPase Arl1, which in turn recruits Imh1. Then Imh1 operates in a traffic pathway that captures vesicles originating from the PVE. Imh1 also operates in a traffic pathway that recycles late-acting Golgi proteins. A component that recycles from the PVE apparently promotes the recruitment of GARP, which cooperates with Imh1 to capture vesicles carrying late-acting Golgi proteins. Thus, GARP has a previously unknown role in intra-Golgi recycling. These various tethers and traffic pathways have functional interconnections, and they form part of a molecular logic circuit that controls Golgi maturation.

B353/P1343

Identification of plasma membrane to trans-Golgi Network vesicle fusion machinery in Saccharomyces cerevisiae

J. Grissom, R. Chi; Biological Sciences, The University of North Carolina At Charlotte, Charlotte, NC.

Clathrin-mediated endocytosis (CME) is one of the most important cellular processes for the uptake of nutrients or other cargos at the cell surface. CME is a specialized form of receptor-mediated endocytosis involving clathrin and other endocytic proteins. During CME more than 60 proteins are recruited to plasma membrane (PM) sites for cargo capture, vesicle formation and internalization. Recent studies in budding yeast have shown that yeast have a minimal endomembrane system that is fundamentally

different than that of mammalian cells. In this new model, the trans-Golgi Network (TGN), acts as the primary acceptor of endocytic vesicles, sorting cargo for degradation. Given the vast amount of information obtained using the budding yeast endomembrane system, the field must now come to understand these pathways in the context of this new paradigm. Using a targeted genetic approach, we queried SNARE proteins that are known to localize and mediate vesicle fusion events within specific membranes of the yeast endomembrane system. While all yeast SNAREs have been annotated, interestingly none have been identified to mediate the fusion of plasma membrane-derived vesicles to the TGN. Using this targeted genetic approach screen, we have successfully identified SNARE proteins that mediate PM-TGN vesicle fusion.

B354/P1344

Syntaxin5's flexibility in SNARE pairing supports Golgi function

Z. Dsouza, I. Pokrovskaya, V. Lupashin; University of Arkansas for Medical Sciences, Little Rock, AR.

The Golgi - the central organelle in membrane trafficking, receives, modifies, and sorts secretory and endocytic cargo. Glycosylation, one of the major post-translational modifications are conducted within the Golgi where the majority of the glycosylation machinery resides. Golgi resident proteins are recycled from the late (trans) Golgi compartments to the early compartment (cis) by the trafficking machinery - including small GTPases, vesicular coats, tethers, and SNAREs, which orchestrates the events of vesicle formation, budding, and fusion. Mutations in the Golgi trafficking machinery are known to cause glycosylation defects at the cellular and organismal levels. The Conserved Oligomeric Golgi (COG) complex is the key modulator of vesicular trafficking and glycosylation at the Golgi. It functions upstream of the membrane fusion machinery and stabilizes the Golgi SNARE complex -STX5/GS28 (GOSR1)/GS15 (BET1L)/YKT6. We hypothesized that depletion of Golgi v-SNAREs would mirror defects observed in COG deficient cells. To test this, we created single and double knockouts (KO) of GS28 and GS15 in HEK293T cells and analyzed the resulting mutants using a comprehensive set of biochemical, mass-spectrometry (MS) and microscopy approaches. GS28 KO significantly affected GS15, but not the other two partners, STX5 and YKT6. V-SNARE deletion also did not disrupt Golgi morphology. While glycosylation and retrograde trafficking were unaffected in GS15 KO, GS28 KO showed mild glycosylation abnormalities and a delay in retrograde trafficking of SubAB toxin. Our analysis revealed that COG dysfunction is far more deleterious for Golgi function than double GS28/GS15 KO, indicating adaptation of vesicle fusion machinery in v-SNARE KO cells. Quantitative proteomic analysis of STX5-interacting SNAREs revealed an unexpected flexibility in Golgi SNARE pairing in human cells. We uncovered that two novel non-canonical Golgi SNARE complexes - STX5/SNAP29/VAMP7 and STX5/VTI1B/GS15/YKT6, were increased in GS28 KO cells. Moreover, SNAP29 was relocated to the Golgi area in GS28 KO cells in a STX5-dependent manner. Analysis of triple KO cells depleted for GS28/SNAP29/Vti1b revealed synthetic growth defects. This is the first study to show a remarkable plasticity in the mammalian intra-Golgi SNARE machinery, which is possibly controlled by the COG complex.

B355/P1345

Why are SNARE complexes rod-shaped?

D. An, J. Zeng, Z. A. McDargh, B. O'Shaughnessy; Columbia University, New York, NY.

SNARE proteins are the core components of the cellular machinery that fuses membranes for vital processes such as neurotransmitter or hormone release and intracellular trafficking. In common with

other fusion-catalyzing complexes such as those used by enveloped viruses for cell entry, the 4-helix SNARE complex is extended and rod-shaped, and several complexes fuse membranes cooperatively. Why has nature arrived at the same shape solution to the membrane fusion challenge in such diverse settings? Here we used ultra coarse-grained simulations to show the bulky rod shape is designed to generate entropic forces that drive membrane fusion. As the shape generating maximum excluded volume for a given number of protein residues, the rod is the optimal configuration for fusion-catalyzing entropic force.

The mechanism of SNARE-mediated fusion is controversial. We developed molecularly explicit simulations of synaptic vesicle-plasma membrane fusion on physiological msec timescales for the first time, which showed membrane fusion is driven by entropic forces among the rodlike SNARE complexes (SNAREpins) which pushed SNAREpins outwards, clearing the fusion site and pressing the membranes into an extended contact zone (ECZ). Entropic forces maintained SNAREpins at the outer ECZ edge, catalyzed a hemifusion stalk at the edge, and expanded the stalk into a hemifusion diaphragm (HD) large enough to allow tension to complete the fusion pathway by nucleating a simple pore in the HD. HD expansion was required for the final step since at physiological tensions rupture of the planar HD membrane is via a large diameter hydrophilic pore. The entropic forces originate in the bulky rodlike SNAREpin shape: with hypothetical membrane-spanning complexes lacking rodlike domains, weak entropic forces failed to fuse membranes.

This mechanism radically differs from a common view, that fusion is driven by the ~ 60 kT zippering energy of SNARE complexation. We find no support for this view as complexation occurs after microseconds (Kubelka et al., 2004; Xi et al., 2012), much faster than fusion.

Corroborating the predicted pathway, ECZ and HD intermediates were observed in reconstituted SNARE systems (Diao et al., 2012; Hernandez et al., 2012). Simulated fusion required SNARE complexation zippering forces of order ~ 20 pN or greater, similar to experimental values (Gao et al.,2012).

B356/P1346

Synaptotagmin 7 docks synaptic vesicles for Doc2α-triggered asynchronous neurotransmitter release

G. F. Kusick[1], Z. Wu[2], M. M. M. Berns[3], S. Raychaudhuri[1], K. Itoh[1], A. M. Walter[3], E. R. Chapman[2], S. Watanabe[1]; [1]Johns Hopkins University, School of Medicine, Baltimore, MD, [2]University of Wisconson-Madison, Madison, WI, [3]University of Copenhagen, Copenhagen, Denmark

The molecular basis of asynchronous neurotransmitter release remains enigmatic despite decades of intense study. Synaptotagmin 7 (syt7) and Doc2 have both been proposed as Ca^{2+} sensors that trigger this mode of exocytosis at synapses, but conflicting findings have led to controversy. Furthermore, how these proteins influence synaptic vesicle exocytosis is unknown: do they enhance fusion of synaptic vesicles that are already docked and ready to fuse, or do vesicle dynamics play a role? To address this, we measured glutamate release and activity-dependent ultrastructural changes in neurons lacking each of these proteins. Glutamate release was monitored using the optical reporter iGluSnFR in cultured hippocampal neurons and patch-clamp recordings of acute hippocampal slices from knockout mice, while docking of synaptic vesicles was assayed using time-resolved electron microscopy ('zap-and-freeze'). We found that at excitatory mouse hippocampal synapses, Doc2α is the major Ca^{2+} sensor for asynchronous release, while syt7 supports this process through activity-dependent docking of synaptic vesicles. In synapses lacking Doc2α, asynchronous release after single action potentials is strongly reduced, while deleting syt7 has no effect. However, in the absence of syt7, docked vesicles cannot recover on millisecond timescales. Consequently, both synchronous and asynchronous release depress

from the second pulse on during repetitive activity. By contrast, synapses lacking Doc2α have normal activity-dependent docking, but continue to exhibit decreased asynchronous release after multiple stimuli. Moreover, knocking out both of these Ca^{2+} sensors causes no further decrease in asynchronous release, consisting with them acting in series. These findings result in a new model whereby syt7 drives activity-dependent docking, thus 'feeding' synaptic vesicles to Doc2 for asynchronous release during ongoing transmission.

B357/P1347

A dynamic mechanically stabilized membrane reservoir mediates fast endocytosis

D. An[1], R. Su[1], P. J. Wen[2], C. Chan[2], L. Wu[2], B. O'Shaughnessy[1]; [1]Columbia University, New York, NY, [2]National Institute of Neurological Disorders and Stroke, Bethesda, MD.

We present experimental, data analytic and mathematical modeling results suggesting that repeated exocytic stimulation mechanically stabilizes an internal reservoir of plasma-membrane-attached vesicles that serves as a supply for fast endocytic internalization.

Exocytosis mediates neurotransmission, hormone secretion and other fundamental processes. Following fusion to the plasma membrane (PM), a vesicle releases cargo via a fusion pore and then either merges with the PM, remains in place or grows (Shin et al., 2020). Repeated stimulation delivers large amounts of membrane and other molecules to the PM, whose retrieval is achieved by endocytosis. A major pathway is classical clathrin-mediated endocytosis (CME), when membrane pinches inwards from the PM and is removed by membrane fission (Kaksonen & Roux, 2018). However, the timescale of CME is insufficient to restore PM homeostasis (area, tension) in the face of sufficiently fast repeated stimulation. Alternative ultrafast clathrin-independent modes of endocytosis have been identified, but their molecular origin is unknown.

Here we studied exocytosis of ~400-nm diameter dense-core vesicles in live, primary cultured bovine adrenal chromaffin cells stimulated by whole-cell calcium dialysis. Imaging multiple individual exocytosis events whose statistics were accumulated over 26 movies of 1-2 min each, we catalogued the relative frequency of vesicle-membrane merging, vesicle remaining in place, and vesicle growth events, as well as composite events (grow-merge, stay-merge, merge-stay). The pairwise distribution function revealed that ~ 20% of exocytosis events occurred at hotspots of repeated release.

Over 1-2 min, up to ~70 vesicles fused with the imaged portion of the PM, a huge addition of membrane given the high sensitivity of the tension of a membrane to its area. Part of the added membrane did not merge with the PM, but remained as a reservoir of PM-attached intracellular vesicles that reached steady state after ~ 20s. Cross pairwise distributions showed that membrane reservoirs lie close to hot spots.

To explore the reservoir mechanism and its significance, we developed a mathematical model which showed that repeated exocytosis at a hotspot lowers the local PM tension and the driving force for vesicle-PM merging, up to a threshold where a reservoir stabilizes. The reservoir then constitutes an adjustable supply of rapidly available vesicles for internalization, effectively short-circuiting the slow CME pathway. Thus, our results suggest repeated exocytic stimulation mechanically stabilizes internal reservoirs of PM-attached vesicles ready for fast endocytic internalization.

B358/P1348

BAR Domain Proteins and Actomyosin Regulate Fusion Pore Dynamics of Large Secretory Vesicles

T. Biton[1], N. Scher[1], E. Schejter[2], B. Shilo[2], O. Avinoam[1]; [1]Department of Biomolecular Sciences, Weizmann Inst Science, Rehovot, ISRAEL, [2]Department of Molecular Genetics, Weizmann Inst Science, Rehovot, ISRAEL.

Secretion by exocytosis occurs in every living cell, facilitating metabolism, signaling and trafficking. Large secretory vesicles (LSVs), up to 10 µm in diameter, are utilized in exocrine tissues to secrete a high volume of cargo. We recently showed that when LSVs fuse with the apical surface, unlike smaller vesicles such as synaptic or endocrine vesicles, they do not incorporate into or detach from the surface. Instead, LSVs stay connected to the surface through a dynamic fusion pore. While this occurs, the vesicular membrane is folded by an actomyosin meshwork that is recruited to the vesicle after fusion. In such a scenario, the vesicular and apical membranes remain physically and chemically distinct and membrane homeostasis is maintained. We hypothesized that fusion pore regulation plays an important role in this process. To understand how the fusion pore is regulated, I used live, super-resolution microscopy in the Drosophila larval salivary glands. I discovered that the fusion pore of LSVs displays a behavior consisting of three morphologic phases - expansion, stabilization, and constriction. I have shown that pharmacological and genetic perturbations of branched actin polymerization results in pores that fail to expand, leading to LSV detachment from the surface, or pores that fail to stabilize, resulting in a full collapse of the LSV into the surface and actomyosin independent content release. Additionally, inhibiting Myosin-II activity results in stalled vesicles that remain connected to the surface by a wide pore that does not constrict. To better understand the molecular mechanism regulating the fusion pore I performed a candidate-based screen. I identified three conserved Bin-Amphiphysin-Rvs homology (BAR) domain proteins that regulate the pore phases. I found that Missing in metastasis (MIM), is essential for both pore expansion and pore stabilization in a dose-dependent manner. Additionally, I was able to show that MIM localizes to the fusion site before fusion and during the secretory process and that the I-BAR domain of MIM is essential for MIMs localization and function. My experimental evidence suggests that the fusion pore of LSVs is regulated by a dedicated protein machinery involving BAR domain proteins and actin dynamics and that such machinery facilitates content release and membrane homeostasis in exocrine tissues.

B359/P1349

Multivesicular Endosome (MVE) Secretion Is Regulated By Temperature

A. Weisgerber[1], Z. Otruba[1], M. Nguyen[2], B. Bills[2], **M. Knowles**[2]; [1]Chemistry & Biochemistry, University of Denver, Denver, CO, [2]Molecular and Cellular Biophysics Program, University of Denver, Denver, CO.

Multivesicular endosomes fuse with the plasma membrane to secrete exosomes into the extracellular space. The regulation of this fusion event is not well understood, however, the recent design of CD63 based, pH sensitive, fluorescent probes, real time dynamics and kinetics of MVE fusion can be measured using TIRF microscopy. In this work, A549 cells, a model lung cancer cell line, expressed fluorescent fusion probes and were observed as the process of MVE membrane fusion occurred spontaneously and slowly in time. The temperature was varied from 23°C to 37°C and fusion happens more frequently at warmer temperatures. Interestingly, the temperature also affected the release kinetics of the fluorescent probes, as noted by the rate of fluorescence decay from the fusion site. Through fitting of

the release kinetics and diffusion-based simulations of exosomes leaving the fusion site, we determined that some exosomes are tethered to the cell surface and these tethers can dissociate slowly in time.

B360/P1350

Investigating Annexin and S100A11's role in membrane repair

N. Loew, K. Sonnemann, W. Bement; University of Wisconsin–Madison, Madison, WI.

Every cell is susceptible to stress- or contraction-induced membrane damage which, if left unrepaired, leads to uncontrolled cytoplasmic efflux, ion influx and ultimately cell death. To maintain homeostasis, cells employ a highly conserved membrane repair system characterized by rampant calcium-dependent vesicle-vesicle and vesicle-membrane fusion events around the wound site. Annexins are a family of calcium dependent lipid binding proteins that participate in membrane repair by regulating vesicle fusion events. While annexins are known to promote membrane repair, less is known about how the annexin-binding S100 family of proteins promote or otherwise regulate repair. The objective of this study is to investigate how annexin and S100A11 regulate membrane repair in frog oocytes. We expressed recombinant annexin and S100A11 proteins and injected the purified proteins into oocytes in order to visualize and quantify recruitment of each protein to the site of laser-induced damage. We found that both annexin and S100A11 were rapidly recruited to wounds and co-localized during the repair response. The S100A11 recruitment was calcium dependent; a S100A11 mutant unable to bind calcium was not recruited to wounds. Interestingly, annexin recruitment to wound sites was also diminished in the presence of the S100A11 mutant. Therefore, we conclude that annexin activity during membrane repair is regulated by S100A11. These results advance our understanding of membrane wound repair and identify S100 proteins as potential targets for therapeutic interventions aimed at promoting wound repair.

B361/P1351

Membrane remodeling properties of the Parkinson's disease protein LRRK2

X. Wang[1,2,3,4], Y. Wu[1,2,3,4], S. Cai[1,2,3,4], J. Ge[1], S. Lin[1,5], P. De Camilli[1,2,3,5,4]; [1]Yale University School of Medicine, New Haven, CT, [2]Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, [3]Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT, [4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, [5]Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven, CT.

Mutations in Leucine-rich repeat kinase 2 (LRRK2) are responsible for late-onset autosomal dominant Parkinson's disease (PD). LRRK2 has been implicated in a wide range of physiological processes including membrane repair in the endolysosomal system. Here, we report that purified LRRK2 directly binds acidic lipid bilayers in a cell-free system and can deform them into narrow tubules in a guanylnucleotide-dependent but ATP-independent way. Moreover, we found that LRRK2 shows preferential assembly on highly curved lipid nanotubes relative to spherical liposomes. We suggest that an interplay between the membrane remodeling and signaling properties of LRRK2 may be key to its physiological function. LRRK2, via its kinase activity, may achieve its signaling role at sites where membrane remodeling occurs.

B362/P1352

VPS33B and VPS16B, proteins deficient in ARC syndrome, form a pentameric bilobed complex

R. J. Y. Liu[1], Y. Al-Molieh[1], H. H. Y. Yao[1], R. S. Q. Geng[1], L. Li[2], F. G. Pluthero[2], S. Benlekbir[2], J. L. Rubinstein[2], W. H. A. Kahr[2]; [1]University of Toronto-47731885, Toronto, ON, CANADA, [2]The Hospital For Sick Children, Toronto, ON, CANADA.

Loss of function variants of *VPS33B* and *VIPAS39* (*VPS16B*) are causative for multisystemic arthrogryposis, renal dysfunction and cholestasis (ARC) syndrome, typified by early lethality. This indicates that VPS33B and VPS16B play essential cellular roles, and while these proteins have been shown to associate, little is known about the structure and function of the VPS33B/VPS16B complex. VPS33B is a member of the SEC1/MUNC18 (SM) protein family, and is thus thought to facilitate vesicular fusion like its homolog VPS33A, a component of the homotypic fusion and vacuole sorting (HOPS) complex. While it has been compared to the heterohexameric HOPS complex, the stoichiometry and arrangement of subunits in the VPS33B/VPS16B complex is not known. We show here that endogenous VPS33B and VPS16B from human cells form a large molecular weight complex of similar size to that of affinity-purified human VPS33B/VPS16B expressed in yeast. Circular dichroism confirmed VPS33B/VPS16B has a well-folded α-helical secondary structure. Size exclusion chromatography-multi angle light scattering, quantitative immunoblotting, small angle X-ray scattering and negative staining electron microscopy indicate the complex has a VPS33B:VPS16B ratio of 2:3, and a distinctive two-lobed shape. Examination of truncated and arthrogryposis, renal dysfunction and cholestasis (ARC) syndrome-causing variants of VPS33B and VPS16B expressed in yeast identified a VPS16B truncation that did not affect binding to VPS33B, as well as an ARC-associated VPS33B missense variant that disrupts complex formation. The demonstration that VPS33B and VPS16B form a unique bilobed complex containing only these proteins will facilitate future studies into their structure and function.

B363/P1353

Intersectin-1 condensates control replacement site vesicles

T. H. Ogunmowo[1], R. E. Pepper[1], A. Ho[1], S. Raychaudhuri[1], H. Wang[2], C. Hoffmann[2], I. Milosevic[3], D. Milovanovic[2], S. Watanabe[1]; [1]Cell Biology, Johns Hopkins University Sch Med, Baltimore, MD, [2]German Center for Neurodegenerative Diseases, Berlin, GERMANY, [3]University of Oxford, Oxford, UNITED KINGDOM.

Vesicles docked at release sites are used for synaptic transmission and quickly replaced by new vesicles. Replacement vesicles can rapidly toggle between undocked and docked states on a millisecond time scale during synaptic activity, and this dynamic transition controls the plastic nature of synaptic transmission. Thus, these vesicles must be held in the region close to the release sites. However, the precise location of replacement vesicles and how their transition to release sites is controlled remains mysterious. The objective of this study is to determine the mechanisms regulating replacement vesicles, and their physiological relevance. Here, we show that the multimodal scaffolding protein Intersectin-1 forms condensates to maintain vesicles near the active zone where release sites are located, and its interaction with Endophilin A1 mobilizes the vesicles to release sites. Specifically, heterologous expression of Intersectin-1 in HEK293 cells suggests that Intersectin-1 forms liquid-like condensates with Synapsin-1 and Synaptophysin-containing vesicles, indicating a potential role in vesicle sequestration by Intersectin-1. Intersectin-1 condensates can be dispersed by co-expression of Endophilin A1 or Ca^{2+} influx in HEK293 cells, suggesting that both Endophilin A1 and Ca2+ may free vesicles from Intersectin-1

condensates. Consistent with this notion, Intersectin-1 forms distinct spherical puncta just above release sites, between Rim1 and Synapin1 condensates, when mouse hippocampal synapses are observed by superresolution microscopy. These clusters are likely liquid molecular condensates since they are dispersed by an aliphatic alcohol which disrupts weak hydrophobic interactions and display diffusion-limited kinetics by internal FRAP. Our time-resolved ultrastructural analysis, zap-and-freeze electron microscopy, shows that vesicles within 30 nanometers of release sites are scarce when lacking Intersectin-1, suggesting that Intersectin-1 likely keeps vesicles in this region. After stimulation, new vesicles cannot dock immediately to replenish the release sites. Consequently, these synapses fail to facilitate synaptic transmission. Interestingly, in Endophilin A1 knockout neurons, the number of vesicles in replacement sites is normal, yet release sites are not rapidly replenished after stimulation. This defect is also observed in neurons expressing mutant Intersectin-1, which cannot interact with Endophilin A1. Taken together, these data suggest that Intersectin-1 localizes replacement vesicles near the active zone, and its interaction with Endophilin A1 promotes replenishment of release sites.

B364/P1354

investigations of annular gap junctions as delivery vehicles to cellular organelles

A. Cooper[1], S. Cetin-Ferra[1], B. S. Jones[1], A. O. Hinton, Jr.[2], S. A. Murray[1]; [1]Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, [2]Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN.

Gap junction channels facilitate the transfer of molecules between adjacent cells and regulate multiple cellular processes. These channels are composed of proteins, connexins. Connexin assembly into gap junction channels, and channel aggregation into gap junction plaques has been well characterized. However, the channel removal from the cell surface by a gap junction plaque internalization process that results in annular gap junction formation is less understood. The central dogma is that annular gap junctions are degraded however the fate of these annular gap junctions and their proteins is controversial. We hypothesize that annular gap junctions, in addition to being degraded, deliver their connexins to other cellular compartments. To characterize annular gap junction fate, we used SW-13 adrenal cells that express connexin 43 (Cx43), the most abundant connexin family member. Annular gap junction contact sites with lysosomes and mitochondria were characterized with transmission electron microscopy (TEM). Immunocytochemical colocalization with 3D computer-assisted reconstruction was used to analyze annular gap junction associations with lysosomal (LAMP), recycling (Rab 11, VPS35), and mitochondrial (TOM20) markers. With TEM, lysosomal annular gap junction membrane fusion was seen such that the lysosomal membrane and annular membranes appeared to be continuous with one another. There was no space intervening between annular gap junction and lysosomal membranes at these contact sites. In contrast, annular gap junction/mitochondrial membranes were never seen to be continuous with one another and at their contact sites there was an average 18.4 ± 0.7nm space (range 0-25nm). Even in the cases where no space was measured between annular gap junction and mitochondria membranes, the integrity of each organelle's membrane remained intact. With immunocytochemistry annular gap junctions colocalized with lysosomes. However, the percent colocalization of annular gap junctions with the recycling (Rab 11, VPS35), and with mitochondria markers was statistically greater than that for lysosomes/annular gap junctions. We suggest that while some annular gap junctions are degraded, others form close contacts that may allow connexin delivery to the plasma membrane via recycling endosomes, or to mitochondria for possible regulation of mitochondrial function. This work was supported by the UNCF/BMS EE Just Postgraduate Fellowship,

Burroughs Wellcome Fund CASI Award, Burroughs Welcome Fund Ad-hoc Award, NIH SRP Subaward to #5R25HL106365-12 from the NIH PRIDE Program, DK020593, Vanderbilt Diabetes and Research Training Center for DRTC Alzheimer's Disease Pilot & Feasibility Program and UNCF/BMS EE Just Faculty Fund Grant to A.H.J. and NSF grant #MC-2011577 to S.A.M.

B365/P1355

Composition and Structure of the VPS33B/VPS16B Complex

Y. AL-MOLIEH[1], R. J. Y. Liu[1], F. G. Pluthero[2], L. Li[2], S. Benlekbir[2], J. L. Rubinstein[2], W. H. A. Kahr[2]; [1]University of Toronto-47731885, Toronto, ON, Canada, [2]Hospital for Sick Children, Toronto, ON, Canada

Loss of VPS33B and/or VPS16B protein expression causes the multisystem neonatal lethal condition arthrogryposis, renal dysfunction, and cholestasis (ARC) syndrome, where symptoms include the failure of platelet precursor megakaryocytes to produce secretory α-granules. VPS33B is a member of the SEC1/MUNC18 (SM) protein family, and is proposed to facilitate vesicular fusion like its homolog VPS33A, a component of the homotypic fusion and vacuole sorting (HOPS) complex. We have determined that VPS33B and VPS16B form a large molecular weight complex in cells that contains only these proteins (unlike the heterohexameric HOPS complex). We have gained insights into the structure and composition of the VPS33B/VPS16B complex using a range of biochemical and biophysical techniques, including circular dichroism, size exclusion chromatography-multi angle light scattering (SEC-MALS), quantitative immunoblotting, small angle X-ray scattering and negative staining electron microscopy (EM). We have determined that the VPS33B/VPS16B complex contains 2 copies of VPS33B and 3 of VPS16B, and that it has a distinctive two-lobed structure. We investigated the relationship of components within the complex by adding a biotinylation signal to the C-terminus of VPS33B and binding avidin to the complex prior to EM analysis. This revealed that the VPS33B subunits occur in a *trans* conformation at either end of the complex, with VPS16B occupying the central region. Our findings represent the most detailed structural determination of the VPS33B/VPS16B complex to date.

B366/P1356

Plasma membrane disruption induce macropinocytosis and lysosome fusion after membrane repair

M. Konishi[1], Y. Egami[2], K. Kawai[3], N. Araki[3], **K. Miyake**[1]; [1]Int. Univ. Health & Welfare, Narita, Japan, [2]Kagawa Univ., Miki, Kagawa, Japan, [3]Kagawa, Miki, Kagawa, Japan

Plasma membrane is repaired within seconds after damage. It has been reported that lysosomes supply cell membranes to the damaged area for repair. In this study, how lysosomes fuse to the membrane was confirmed by live imaging. We also observed morphologically how contaminated cytoplasm is repaired over time after plasma membrane disruption. FITC-Lys-Dextran, 10 kD (Fdx) was injected into mice flexor digitorum brevis muscle, followed by electroporation. After a few days, fibroblasts, macrophages and muscle fibers with many sequestered vesicles were identified, and when these cells were subjected to membrane damage with a two-photon laser, active exocytosis of Fdx was observed from the fibroblasts and macropages. However, sequestered vesicles of muscle fibers were not secreted. We also observed Lgp120-GFP transfection-labelled lysosomes or LC3-GFP transfection-labelled autophagosomes in cultured cells such as BS-C-1 or muscle fibers, but exoxcytosis were not obserbed by membrane damage or ionomycin. Therefore, scratch loading was performed in HRP (10 mg/ml, D-PBS+) and fixed after recovery times of 15 s, 3 min, 30 min, 1 h, 6 h, 24 h, 36 h and 72 h after injury. HRPs were sequestered by the membrane 30-24 h after injury, with an increase in large lysosomal-like sequestered

vesicles surrounded by the sequestered membrane at 36 h and 72 h. Electron microscopic images of repaired cells at 30 min post-injury showed that they extended cell surface ruffling and intensely engulfed extracellular fluid and their own cytoplasm. Therefore, Rab35-GFP was transfected and LIVE imaging during damage repair using FM4-64 showed macropinocytosis. Furthermore, Fdx was introduced into the cells by scratch loading and changes over time were observed for 72 hours. As a result, as time progressed from 24 to 72 hours, more sequestered vesicles were observed in the cells. When cells with these sequestered vesicles were subjected to membrane repair again with two-photon laser, many of the sequestered vesicles fused to the nearby plasma membrane, which was not damaged, and the fluorescent material in them was found to be expelled by exocytosis. When these cells were transfected with Lgp120-GFP, many of the sequestered vesicles were considered to be secretory lysosomes. These findings indicate that macropinocytosis is induced by plasma membrane disruption and that there is a mechanism by which damaged cytoplasm is sequestered and expelled from the contaminated cytoplasm by exocytosis occurd by plasma membrane disruption.

B367/P1357

Dynamics of dysferlin and t-tubles during sarcolemma repair

T. Matsuda, **K. Miyake**; International University of Health and Welfare, Narita, Japan

5-30% of skeletal muscle fibers are constantly damaged by exercise and undergo membrane repair. We found that limb muscular dystrophies unable to produce dysferlin are unable to repair cell membranes, suggesting that dysferlin localises to intracellular vesicles of sarcoplasmic reticulum, muscle plasma membrane transverse tubule(t- tubule) or other origin and that sarcolemma repair is mediated by vesicles fusion. The detailed localisation of dysferlin and its kinetics during sarcolemma repair remain unclear. In this study, live imaging of dysferlin and t-tubules during sarcolemma repair was performed to morphologically analyse how the sarcolemma is repaired. Electroporation was used to introduce dysferlin-GFP and the t-tubule marker BIN1 (Amphiphysin-2)-GFP into the mouse flexor digitorum brevis muscle. 10-30 days later, multiphoton microscopy and Airyscan live imaging was performed. Using isolated mouse skeletal muscle, two-photon laser damage to sarcolemma visualised by FM reagents showed many vesicles forming longitudinal columns along t-tubules and many vesicles accumulating in the damaged area. Dysferlin was also localised in vesicles just below the plasma membrane and in the sarcoplasmic reticulum between myofibrils, but most of them were strongly localised in the t-tubules, as was BIN1. When the sarcolemma was injured, both dysferlin and BIN1 were observed to collect in the injured area. However, BIN1 was observed to collect inside the injured area earlier than dysferlin. This movement may be due to the fact that BIN1, which stabilises the t-tubules, first changes and prepares the t-tubule membrane for vesicle formation, from where dysferlin forms vesicles and transports them one after the other to the injured area. Thus, it was speculated that dysferlin localises predominantly in t-tubules. It is also possible that dysferlin and BIN1 both work to sarcolemma repair, but that their roles are different.

Neuronal Signaling and Metabolism

B369/P1358

Potential regulation of SYD-2 Liprin α; protein abundance by the Anaphase Promoting Complex (APC) and UNC-43 CaM Kinase II in C. elegans

A. Pressel, T. Prechtel, L. Campagnoli, K. Rush, D. Lester, J. R. Kowalski; Butler University, Indianapolis, IN.

Neuronal signaling makes up the basis of every thought, action, and movement; misregulation of neurological signaling contributes to neurological diseases. Specifically, a crucial balance of excitatory to inhibitory neuronal signaling (E:I balance) is controlled by synaptic proteins; yet details are incompletely understood. Prior studies used *Caenorhabditis elegans* as a model to study a conserved synaptic scaffolding protein, SYD-2 Liprin α, which acts in inhibitory GABA neurons to control signaling at the *C. elegans* neuromuscular junction (NMJ). However, regulation of SYD-2 Liprin α itself is incompletely understood. We tested whether two hypothesized regulators of SYD-2 Liprin α from mammalian systems, the anaphase-promoting complex (APC) ubiquitin ligase and the calcium-dependent UNC-43 CaM Kinase II (CaMKII), control SYD-2 Liprin α abundance in C. elegans GABA neurons. We previously showed the synaptic abundance of SYD-2 is regulated by the APC, which is present in GABA neurons to regulate NMJ inhibitory signaling. Like the APC, CaMKII negatively regulates Liprin α levels in mammalian cells, and *unc-43* loss-of-function *(lf)* C. elegans have increased muscle contraction similar to *apc(lf)* mutants, also consistent with negative regulation of SYD-2 Liprin α. Here, we provide preliminary evidence of SYD-2's direct regulation via APC ubiquitination, while its regulation by UNC-43 may happen locally, if at all. We found *C. elegans apc (lf)* mutants have decreased GABA release and more muscle contraction, and *syd-2(lf)* mutants have less muscle contraction that suppresses increased contraction of *apc(lf)* mutants. Imaging experiments demonstrated GFP::SYD-2, but not a GFP::SYD-2(**Δ**;;Dbox) mutant lacking APC recognition sites, accumulates at GABA neuron presynapses in *apc(lf)* mutants, consistent with negative regulation of SYD-2 by the APC. Double immunoprecipitation analyses support potential ubiquitination of GFP::SYD-2 Liprin α in *C. elegans*; tests to confirm APC dependence are underway. Initial Western blots of total protein lysates from *unc-43(lf)* mutants suggest little effect of *unc-43* on total GFP::SYD-2 levels; however, current experiments are testing local synaptic regulation. Future studies will continue both aspects of this project in efforts to understand SYD-2 regulation as a mediator of inhibitory signaling, a crucial component of neuronal balance.

B370/P1359

Investigation of cell autonomous and nonautonomous signaling of the G protein-coupled receptor, FSHR-1, in controlling neuromuscular structure and function in C. elegans

M. Buckley, A. Ritter, **M. McClain**, W. Jacob, D. Emch, J. Kowalski; Butler University, Indianapolis, IN.

Regulation of neuronal signaling balance is critical for nervous system function. At neuromuscular junctions (NMJs), this occurs via regulation of excitatory and/or inhibitory signaling events. The G protein-coupled receptor (GPCR), FSHR-1, is a key regulator of NMJ signaling, as well as germline differentiation, and stress responses. Prior research showed that *C. elegans* with *fshr-1* deletion exhibit decreased muscle excitation along with accumulation of fluorescently labeled synaptic vesicles in excitatory cholinergic motor neuron presynapses. We used paralysis, swimming assays, and quantitative imaging of the fluorescently-tagged synaptic vesicle protein SNB-1::GFP to confirm the NMJ defects of

fshr-1 loss-of-function *(lf)* mutants. Along with synaptic vesicle accumulation and reduced muscle contraction, *fshr-1(lf)* mutants exhibited build-up of the active zone proteins UNC-10/RIM and SYD-2/Liprinα in cholinergic motor neurons. However, where *fshr-1* acts to control NMJ function is unknown. Expression of *fshr-1* in all neurons, and in excitatory cholinergic or inhibitory GABAergic neurons alone, restored NMJ activity to these animals. Yet, the same *fshr-1* re-expression in cholinergic or GABAergic neurons failed to restore wild type synaptic vesicle localization. Endogenous *fshr-1* expression was detected in several head cells, along with its known intestinal expression, but not in body wall motor neurons, suggesting a complex site of action. Experiments in which re-expression of *fshr-1* under intestinal, neuronal, and glial promoters rescued the NMJ behaviors of *fshr-1* mutants further support non-motor neuron sites of action. Currently, we are imaging SNB-1::GFP to quantify the abundance and localization of synaptic vesicles in cholinergic NMJs of animals with *fshr-1* re-expressed under these non-motor neuron-specific promoters to determine if the rescue of NMJ function with these promoters correlates with expected changes in SNB-1::GFP accumulation. Future work will include use of tissue-specific deletions to identify where *fshr-1* is required for NMJ structure and function in diverse physiological conditions. Together these data should provide a more complete picture of the cell autonomous and non-autonomous mechanisms by which FSHR-1 controls NMJ function that may be relevant to mammalian FSHR, which has been implicated in neurological dysfunction.

B371/P1360

Investigation of FSHR-1 and its putative α;; and β;; glycopeptide ligands in the cell non-autonomous regulation of neuromuscular function

L. Bortey, W. Jacob, J. Kowalksi; Butler University, Indianapolis, IN.

Normal neuronal signaling regulates the balance of chemical messages to the postsynaptic cell. FSHR-1 is the sole *C. elegans* homolog to a family of glycopeptide hormone receptors involved in gonad function and required for normal neuromuscular activity. Previous studies show *fshr-1* mutants exhibit decreased muscle contraction and our preliminary data indicate these effects on muscle contraction can be caused through cell non-autonomous activity of FSHR-1 in distal tissues. However, the ligands that activate FSHR-1 signaling, as well as the mechanisms by which FSHR-1 promotes muscle excitation are unknown. Possible ligands for FSHR-1 may be the **α**;; glycopeptide FLR-2, the closest homolog to the human FSHR ligand, and *T23B12.8*, which encodes the **β**; glycopeptide homolog. We hypothesized FLR-2 and the **β**; glycopeptide work in a ligand-receptor relationship with FSHR-1 in *C. elegans* to control neuromuscular function. In swimming assays measuring neuromuscular function, *flr-2* **α**;; glycopeptide loss of function (lf) mutants showed the same deficits in motility as *fshr-1*(lf) mutants, demonstrating *flr-2* is required for neuromuscular signaling. The *T23B12.8* **β**; mutants also showed a decrease in motility, and swimming defects in **α**;;**β**; double mutants are non-additive, supporting a role for the two peptides in co-regulating muscle excitation. *fshr-1;flr-2* double mutants also showed similar decreases in motility as either of the fshr-1 and flr-2 mutants alone, suggesting *fshr-1* and *flr-2* **α**; glycopeptide work within the same pathway and supporting a model in which FLR-2 is an FSHR-1 ligand. Studies with *fshr-1*;**β**; mutants are planned. In addition to identifying FSHR-1 ligands, we are exploring the mechanisms by which FSHR-1 cell non-autonomously regulates signaling for muscle contraction. Current experiments are using RNAi interference to knock down a panel of secretory genes in worms overexpressing FSHR-1 in distal tissues (e.g., intestine, glia, head neurons) to test the hypothesis that FSHR-1 activation induces secretion of peptide or other factors required for muscle excitation. Together, our findings will contribute to understanding the role of a conserved GPCR, its glycopeptide ligands, and how its interaction with

multiple different pathways promotes neuronal signaling balance, which may provide insight important in approaching treatments for neurological diseases.

B372/P1361

Cannabinoid Receptor-Mediated Synaptic Signaling and Neural Plasticity in Central Olfactory Neurons

T. Heinbockel, Z.-J. Wang; Howard University College of Medicine, Washington, DC

Our studies aim to understand integrative and computational mechanisms that allow main olfactory bulb neurons to respond to afferent input and synaptic or feedback signals. The endocannabinoid (eCB) signaling system has been functionally implicated in many brain regions but our understanding of the role of cannabinoid receptor type 1 (CB1R) in olfactory processing remains limited. Endocannabinoids are known to mediate retrograde signaling at synapses in several brain regions through a form of short-term neural plasticity. Endocannabinoids are released from depolarized principal neurons and rapidly diffuse to presynaptic inhibitory interneurons to transiently reduce presynaptic firing and neurotransmitter (GABA) release (Depolarization-Induced Suppression of Inhibition, DSI). We study the function of the endocannabinoid system in regulating neural activity at synapses in the main olfactory bulb, the first central relay station in the brain for the processing of olfactory information coming from the nose. Our experimental approach uses electrophysiological recording techniques, specifically whole cell patch-clamp recordings. Previously, using anatomical approaches, we showed that CB1R is present in periglomerular processes of a GAD65-positive population of interneurons but not in mitral cells, key output neurons. We detected eCBs in the mouse main olfactory bulb as well as the expression of CB1R and other genes associated with the cannabinoid signaling system. Output neurons such as mitral cells and tufted cells in the olfactory bulb are computational elements in brain circuits that integrate incoming signals with membrane properties to generate behaviorally relevant synaptic output. Our data support the notion that retrograde signaling is present in neural circuits involving mitral and tufted cells. Mitral and tufted cells release endocannabinoids and, through retrograde signaling, inhibit presynaptic interneurons such as periglomerular cells, which controls the GABA release of these presynaptic neurons. This, in turn, allows mitral and tufted cells to temporarily regulate their synaptic input and relieve them from synaptic inhibition. Endocannabinoids function as retrograde messengers to regulate neural signaling and mediate plasticity at olfactory bulb synapses with potential effects on olfactory threshold and behavior. This publication resulted in part from research support to T.H. from NSF (IOS-1355034), NIH (P30AI117970), and Howard University College of Medicine.

B373/P1362

Synaptic deficits of fragile X syndrome in patient-derived isogenic induced neurons

C. MacKenzie, E. Rao, C. Arnold, J. Chrisman, J. Knopp, K. Hutson, C. Patzke; University of Notre Dame, Notre Dame, IN.

Fragile X syndrome (FXS) is one of the main forms of inherited intellectual disability and the most common monogenic cause of autism spectrum disorder. FXS is caused by loss of expression of the gene *FMR1*, most often via epigenetic silencing secondary to a pathogenic expansion of trinucleotide repeats in the 5' untranslated region of the gene. Excision of the repeat expansion can induce promoter demethylation and restore *FMR1* expression in patient-derived induced pluripotent stem cells (iPSCs). We used FXS patient-derived iPSCs, corrected the mutation by CRISPR/Cas9-mediated repeat deletion, and generated induced neurons (iNs) by overexpression of neurogenin-2. Lack of *FMR1* expression

results in irregular morphology, overabundance of synapses, synaptic vesicle pool dysregulation, and endocannabinoid signaling impairment in FXS iNs, compared to isogenic controls. Our findings highlight molecular deficits which may contribute to irregular brain development in FXS patients and might provide new avenues for future treatments of the condition.

B374/P1363

Multimeric and monomeric α-synuclein induce distinct effects on vesicle trafficking and mitochondria at synapses.

C. Roman-Vendrell[1], G. E. Fisher[2], M. Celikag[3], T. Bartels[3], J. R. Morgan[1]; [1]Marine Biological Laboratory, Woods Hole, MA, [2]University of Chicago, Chicago, IL, [3]UK Dementia Research Institute & University College London, London, UNITED KINGDOM.

Multimeric and monomeric α-synuclein induce distinct effects on vesicle trafficking and mitochondria at synapses.

Parkinson's disease and Dementia with Lewy Bodies are neurodegenerative diseases characterized by the abnormal aggregation of α-synuclein throughout neurons. Under physiological conditions, neurons express multimeric α-synuclein (60-100 kDa oligomers) and monomeric α-synuclein (14 kDa). Altering the balance between these molecular species leads to aggregation, synaptic dysfunction, and neurotoxicity. Moreover, accumulation of α-synuclein at synapses leads to synaptic deficits that precede or co-occur with other signs of neurodegeneration. However, the precise effects of distinct molecular species of α-synuclein at synapses, including synaptic vesicle trafficking and mitochondria morphology, remain unclear. Lamprey reticulospinal synapses provide an excellent model for these studies because we can acutely introduce precise amounts of different α-synuclein species directly to presynapses followed by detailed ultrastructural analysis using electron microscopy. Previously, we showed that excess brain-derived α-synuclein (mixed multimeric and monomeric), moderately impaired intracellular vesicle trafficking without effects on clathrin mediated endocytosis (CME). Currently, our goal is to determine which aspects of this phenotype are due to α-synuclein multimers or to monomers, by introducing them to synapses isolated in purified form. We found that excess purified multimeric α-synuclein partially phenocopies the effects of brain-derived α-synuclein, particularly, causing a decrease in the SV cluster, an increase in the size and number of cisternae/endosomes, without effects on plasma membrane or CME. In contrast, excess purified monomeric α-synuclein induced atypical fusion/fission events at the active zone and moderately impaired CME. We also analyzed mitochondria morphology and found that multimeric α-synuclein decreases the number of mitochondria at the synapse and that the remaining mitochondria are swollen and closer to the active zone. In contrast, monomeric α-synuclein decreased the number of mitochondria with no significant effects on their location or morphology. These data provide further evidence that the impacts of α-synuclein on synapses and mitochondria depend on which molecular species is accumulated. These results provide insight into the cellular mechanisms by which distinct α-synuclein contributes to synaptic pathology in neurodegenerative diseases.

B375/P1364

How does the DAF-7 TGF-beta signaling pathway affect the dense core vesicle protein IDA-1?

D. Zheng Fung, A. McGehee; Suffolk University, Boston, MA

Environmental factors influence behavioral responses in many organisms. We are interested in understanding the molecular changes in the nervous system that influence these changes. In *C. elegans*, overcrowding, scarce food supply, and high temperatures affect the DAF-7 TGF-beta signaling pathway. According to previous studies, mutation in the *daf-7* gene or other parts of the DAF-7 TGF-beta signaling pathway results in increased expression of the glutamate receptor GLR-1. Therefore, the DAF-7 TGF-beta signaling pathway regulates protein levels of GLR-1, and data from our lab suggests that IDA-1 might be involved. IDA-1 is a protein found on the surface of dense core vesicles (DCVs) and levels of IDA-1 are correlated with dense core vesicle signaling. In order to test whether a *daf-7* mutation affects IDA-1 levels we used quantitative fluorescence microscopy to measure GFP levels in reporter strains. An IDA-1-GFP protein fusion was imaged and measured in a *daf-7* mutant strain and a wildtype strain. Intensity measurements of IDA1-GFP fluorescence indicate that the abundance of IDA-1-GFP is significantly higher in the *daf-7* mutant strain compared to the wildtype strain. We found this same result in the ALA neuron and in cells around the vulva in *C. elegans.* Furthermore, in order to determine whether regulation was transcriptional or post-transcriptional, a reporter construct where *ida-1* promoter drives GFP was used (P*ida-1*::GFP). Fluorescence microscopy was employed to image and measure the GFP intensity in a *daf-7* mutant strain and a wildtype strain. Quantification of GFP fluorescence showed that there was no significant GFP difference between the *daf-7* and the wildtype strains. Together these results suggest that the DAF-7 TGF-beta signaling pathway is important in regulating levels of the dense core vesicle protein IDA-1, and that this regulation may be post-transcriptional.

B376/P1365

Investigating the relationship of the DAF-7/TGF- β signaling pathway, IDA-1 and GLR-1 through behavioral assays

F. Lampitiello, A. M. McGehee; Suffolk University, Boston, MA

Abstract

The nematode *C. elegans* responds to changes in its environment by regulating neurotransmission through several signal transduction pathways. These alterations regulate the transcription and levels of various proteins on the surface of neurons, which in turn influence behavior as well as adaptive strategies to this new environment. When environmental conditions are not favorable (such as a lack of food, extreme temperatures, or overcrowding), the DAF-7/TGF-β signaling pathway gets deactivated. It allows *C. elegans* to enter the Dauer stage by promoting the expression of genes associated with this stage. Previous research has shown that the DAF-7/TGF-β signaling pathway negatively controls the levels of the GLR-1 glutamate receptor expressed in the synapses of *C. elegans*. When a loss of function mutation is present in one of the components of the pathway GLR-1 levels increase. High levels of functional GLR-1 glutamate receptor are associated with an increased frequency of spontaneous reversals; this behavior was therefore used as an indicator for active GLR-1. How exactly the DAF-7/TGF-β pathway affects the levels of GLR-1 is unknown. We studied whether IDA-1 is involved in this regulation. IDA-1 is a transmembrane protein located on dense core vesicles (DCV) that contain neuropeptides. To investigate the role of IDA-1 spontaneous reversal assays coupled with locomotion assays were performed using the wild type strain, *daf-7* loss of function mutants, *ida-1* loss of function

mutants and *ida-1 daf-7* double mutants. The reversals assays highlighted a significant increase in spontaneous reversals in the *daf-7* mutants compared to the wild type, which is consistent with previous research. A difference, however, was not found between the *ida-1* single mutants and *ida-1 daf-7* double mutants. This outcome suggests that IDA-1 is required for the increase in reversals observed in the *daf-7* strain. To confirm that the change in reversals is due to a change in GLR-1 protein we imaged a GLR-1-GFP fusion protein in these same mutant strains. Similar to the behavioral results we saw increased GLR-1-GFP in the *daf-7* mutants as compared to wild type, but no difference in GLR-1-GFP levels between the *ida-1* and *ida-1 daf-7* double mutants. Together these results suggest that IDA-1 is required for regulation of GLR-1 levels through the DAF-7/TGF-β signaling pathway.

B377/P1366

Autism and schizophrenia-associated Shank3 mutants regulate the nuclear localization of the Par polarity complex

E. N. Ozsen, M. M. Voglewede, H. Zhang; Rutgers Robert Wood Johnson Medical School, Piscataway, NJ

The Shank family of scaffolding proteins are involved in neuronal development and synaptic plasticity. Mutations in Shank family members have been linked to neurodevelopmental disorders including autism spectrum disorders (ASD) and schizophrenia (SCZ). In particular, *Shank3* is one of the most common monogenic ASD genes and is associated with schizophrenia. However, the mechanisms underlying disrupted synapse formation and function in the Shank3 mutants remain unclear. Previous studies have shown Shank2 interacting with atypical protein kinase C (aPKC), a component of the partitioning-defective protein (Par) complex along with Par3 and Par6, which regulates tight junction formation in epithelial cells. However, it is still unclear whether Shank proteins display similar mechanistic properties in regulating synaptic function. We hypothesize that Shank3 regulates synaptic development through interactions with the Par3/Par6/aPKC complex, and that Shank3 mutants will show altered activities of the Par complex. To examine this hypothesis, we utilize two mutated forms of the *Shank3* gene, *Shank3* InsG3680 and *Shank3* R1117X, which are linked to ASD and SCZ, respectively. Overexpression of Par3, aPKC, and Shank3 in Rat2 cells reveals an increase in aPKC co-localization with the ASD and SCZ-associated Shank3 mutants as compared with wildtype Shank3. Furthermore, there is decreased co-localization between Par3 and aPKC in the Shank3 mutants, suggesting that Shank3 alters Par3-aPKC association. This co-localization of mutant Shank3 and aPKC is concentrated in the nuclei, with no changes in Par3 localization, suggesting that aPKC preferentially interacts with Shank3, leading to its dissociation from Par3 and redistribution into the nucleus. To further examine these findings, we are performing subcellular fractionations of Shank3 mutant mice brains to investigate the cellular localization of Par3, Shank3, and aPKC *in vivo*. Overall, this study can give insight into the interaction of Shank3 with the Par3/Par6/aPKC complex in autism spectrum disorder and schizophrenia, and how Shank3 mutations dysregulate proper synapse development and plasticity.

B378/P1367

Development of Expression and Purification Protocols for Characterizing Members of the Ly6 Protein

R. Sung, V. Vaganov, **Y. Hallman**, A. Michii, M. Felland; Carleton College, Northfield, MN.

The Ly6 proteins are a family of novel regulatory proteins conserved among multiple species that share significant structural homology with alpha neurotoxins commonly found in snake venoms, such as α-bungarotoxin. Previous work studying Ly6 function has relied on in vivo monitoring of endogenous

protein levels/behaviors; however, the ability to biochemically characterize and study Ly6 function using purified protein has been limited. Ly6 proteins share a conserved motif known as the Ly-6/uPAR (LU) domain, in which 10 cysteines form five disulphide bonds that maintain the highly flexible three-fingered structure of the LU domain. These predicted structural elements make the expression of Ly6 proteins in their soluble form challenging. In this study, optimal recombinant expression in E. coli and purification conditions for the ODR-2, a membrane-associated protein related to the Ly6 superfamily of GPI-linked signaling proteins found in C. elegans, were elucidated. ODR-2 has two predicted signal sequences, an N-terminal signal sequence for export to the plasma membrane and a C-terminal signal sequence for GPI attachment. We chose to test expression for constructs that were full length or had both signal sequences removed (to mimic the predicted sequence for the mature protein). We examined the impact of using a variety of solubility tags to support expression of soluble ODR-2; we found that use of N-terminal GST, MBP, and pelB leader sequence tags did not improve soluble expression while an N-terminal SUMO-tag did promote soluble expression in the supernatant. All constructs showed significant insoluble expression in the pellet. These conclusions were consistent across multiple expression strains, including BL21 DE3, Origami-2, Rosetta-gami, and SHuffle. Surprisingly, we found that optimal expression yield occurred at 30 or 35°C and a 3-hour inoculation time. We are in the process of scaling up expression and developing protocols for purification of both soluble and insoluble ODR-2.

B379/P1368

Epileptic activity in Drosophila melanogaster with a knockdown of Ube3a in different neuronal populations as a model of Angelman Syndrome

M. Moore, D. T. Taylor; Utah Valley University, Orem, UT

Angelman Syndrome is a rare inherited neurogenetic disease resulting in a neurodevelopmental disorder. This disorder stems from a mutation or deletion of the *Ube3a* (E3 ubiquitin ligase) gene leading to a loss of function of Ube3a. This loss of function interferes with the proteosome-mediated pathway. Some characteristics of this disease are developmental delay, recurring seizures, and severe intellectual disabilities. We studied seizure activity in male *Drosophila melanogaster* (fruit flies) with knockdowns of *Ube3a* in different subtypes of neurons. Epileptic activity was monitored in individual fruit flies using imaging chambers and mechanically induced seizures using a vortex assay. We hypothesized that there will be fewer seizures and a decrease in time to return to normal activity in crosses when valproate, an anticonvulsant, is administered. Mechanically induced seizure activity was recorded in three treatments groups which have *Ube3a* knocked down in glutamatergic, GABA-ergic, or all neurons. A positive control was also used: eas (easily shocked seizure phenotype). The seizure activity was tested in individual male *Drosophila* 3-5 days post-eclosion. They were tested 1-2 hours post-CO2 anesthesia, and transferred into their imaging chambers following the mechanical seizure induction via a vortex. Seizure activity was recorded for 4 minutes, providing enough time for the *Drosophila* to regain normal function post-seizure induction. Epileptic activity was analyzed and included total seizure duration, number of seizures, and total time to return to normal activity.

B380/P1369

Developing Behavioral Assays to Study Ly6 Protein Function in C. elegans

R. Sung, **I. Pozzi**, M. Shen, A. Dujsikova, K. Mali; Carleton College, Northfield, MN.

The Ly6 protein family is an understudied conserved family of proteins that is of interest due to their structural similarities to alpha neurotoxins. Alpha neurotoxins target nicotinic acetylcholine receptors; interestingly, Ly6 proteins such as lynx1 have also been shown to regulate nicotinic acetylcholine receptor function. In C. elegans, the Ly6 protein family consists of odr-2 and its nine homologs known as hot genes. Odr-2 has been implicated in functions relating to chemotaxis and pheromone sensing, while the function of the remaining hot genes remains unknown. Our long term goal is to determine the function of hot genes by creating knockout mutants, running behavioral assays, and comparing wild type behavior to our hot gene mutant behavior. We are in the process of generating and obtaining those hot gene mutants, and in preparation, we are establishing protocols for behavioral assays that may serve as methods for mutant characterization. be relevant to mutant characterization. We are using GFP transcriptional reporters for each hot gene to determine where those genes are expressed. We then use this expression pattern as a guide for identifying potential behaviors that may be impeded in a knockout mutant. Here, we focus on developing assays that could be beneficial for studying hot-3 function. Using a GFP transcriptional reporter for hot-3, we see hot-3 expression in the worm olfactory system and the vulval muscles. Based on these expression patterns, we hypothesized that an egg laying assay and a chemotaxis assay were most likely to reflect changes in behavior. To establish the validity of each assay, we compare N2 behavior to a mutant line with known defects in the observed activity. Chemotaxis assays with wild type (N2) worms as a negative control and odr-2 knockout mutants (CX2304) as a positive control for altered chemotaxis due to impaired Ly6 protein function show behavior consistent with previously reported findings. Egg laying assays with N2 worms as a negative control and egl-4 worms as a positive control for egg-laying deficiencies show behavior that aligns with previously reported results. We are in the process of using NeuroPAL to identify additional hot-3 expressing cells in order to identify additional behavioral assays. We anticipate this workflow will be useful for evaluating the function of other members of the Ly6 protein family in C. elegans.

Dynamics of Proteins and Organelles in Neurons

B381/P1370

Non-autonomous Signaling from Hypodermal cells Instructs the Formation of Neuronal Glycolytic Condensates *in vivo*

M. Singh, A. Wolfe, D. A. Colón-Ramos; Yale University, New Haven, CT.

Glycolysis, a glucose-consuming pathway required for cellular energy production, is well conserved and has been extensively characterized biochemically. Less is understood regarding how it is subcellularly organized, or coordinated across tissues *in vivo*. Previous work from our lab found that during transient hypoxia or neuronal stimulation, the rate-limiting glycolytic enzyme phosphofructokinase-1(PFK-1.1) dynamically re-localizes from the cytoplasm to condensates near synapses in *C. elegans* neurons. PFK-1.1 is also required at the synapse for the synaptic vesicle cycle(Jang, Nelson et al. 2016, Jang, Xuan et al. 2020). Here we demonstrate that PFK-1.1 condensate formation in neurons is regulated by a non-cell-autonomous signal derived from hypodermal(epidermal) cells. Via cell-specific rescues *in vivo*, we determine that non-autonomous disruption of glycolysis in hypodermal cells affects condensate

formation in neurons. Hypodermal cells in *C. elegans* are rich in metabolic genes and known to regulate metabolic processes(Clark, Meade et al. 2018, Kaletsky, Yao et al. 2018). Our data suggest that the state of glycolysis in the hypodermis signals, non-autonomously, to the neurons to regulate PFK-1.1 clustering, and metabolic state, during transient hypoxia, and reveal mechanisms that are involved in cross-tissue metabolic state communication and cell biological organization of glycolytic proteins.

B382/P1371

Metabolic compartmentalization supports synaptic function during hypoxia in *C. elegans*

S. Ravikumar[1], A. D. Wolfe[1], R. H. Goodman[2], D. A. Colón-Ramos[1]; [1]Department of Neuroscience, Yale University, New Haven, CT, [2]Vollum Institute, Oregon Health & Science University, Portland, OR.

The biochemical reactions required to generate energy for a cell have been well-characterized, yet less is known about how such metabolism is spatially organized and regulated. In specialized cells such as neurons, their unique polarization can result in subcellular changes in metabolic demand far from the cell body. In neurons of the nematode *C. elegans*, transient energy stress by hypoxia causes glycolytic enzymes to dynamically re-localize within the cytoplasm into liquid-like condensates at neuronal connections called synapses. This provides *in vivo* evidence for the existence of a metabolic complex and the intriguing possibility that these metabolic complexes can regulate local metabolic flux.
We identified a **G**lycerol-3-**P**hoshphate **D**e**H**ydrogenase (GPDH-2) required for synaptic vesicle recycling and localization of a glycolytic protein PFK-1.1 during hypoxia. To infer PFK-1.1 enzymatic activity in time and space, we used a fluorescent biosensor to measure concentration changes of its product, fructose 1,6-bisphosphate (FBP). Hypoxia causes a surge and subsequent drop in FBP concentration, presumably increasing overall rates of glycolysis. Interestingly, in *gpdh-2(-)* mutants FBP levels remain high. The reaction GPDH-2 catalyzes is coupled with the redox transfer of electrons from NADH, subsequently regenerating NAD+, an essential cofactor for many cellular reactions. We aim to explore the role of GPDH-2 and NADH/NAD+ ratios on glycolytic flux and synaptic function at single cell resolution. Finally, we observe that GPDH-2 forms accumulations preferentially in synaptic regions and future work will elucidate how it interacts with synaptic proteins and other cellular compartments in neurons.

B383/P1372

Energy Matters: Resetting synaptoenergetics is a new mechanistic target for the anti-bipolar actions of lithium

S. Li, G. Xiong, Z. Li, Z. Sheng; National Institutes of Health, Bethesda, MD.

Synaptic transmission imposes large energy demands that are met by local ATP synthesis, which is consumed to initiate action potentials and fuel synaptic vesicle refilling and recycling. Neurons face challenges in maintaining presynaptic bioenergetics or 'synaptoenergetics' during sustained synaptic activity (Li and Sheng, ***Nat Rev Neurosci*** 2022). Bipolar disorder (BD) is a manic-depressive illness characterized by unusual shifts in mood, energy, and concentration. Lithium is a widely used drug for treating BD patients with recurring manic and depressive episodes. However, its cellular target and mechanism of action for alleviating BD symptoms is not yet completely understood. A growing body of evidence suggests that brain bioenergetic failure and mitochondrial dysfunction are pathological hallmarks of BD. Our previous study demonstrated that mitochondria are recruited to presynapses through a crosstalk between AMPK signaling and syntaphilin (SNPH)-mediated mitochondrial anchoring; impaired mitochondrial maintenance fails to maintain sustained synaptic transmission (Li et al., ***Nat***

Metab 2020). This raises a fundamental question of whether impaired presynaptic energetics contributes to BD pathology. In this unpublished work, we address this question using *Snph* KO mice, which display defective presynaptic mitochondrial anchoring and synaptoenergetic deficits. Intriguingly, *Snph* KO mice recapitulate human mania-like behavioral traits, including hyperactive locomotion, anxiety- and depressive-like behaviors, and impaired sensorimotor gating, which are responsive to lithium. Treating *Snph* KO cortical neurons with lithium chloride restores synaptoenergetics by recruiting mitochondria through TRAK O-GlcNAcylation, thus rescuing synaptic efficacy and synaptic depression recovery after prolonged synaptic activity. We further establish human BD-linked iPSC-derived excitatory neuron models, which recapitulate synaptoenergetic deficits during sustained synaptic activity. More strikingly, human BD neurons show reduced SNPH expression. Altogether, using both human iPSC-derived BD neuronal models and *Snph* KO mice, we establish two emerging concepts: (1) defective synaptoenergetics is one risk factor for BD; and (2) restoring synaptoenergetics is a novel target underlying the anti-BD actions of lithium. (Supported by the Intramural Research Program of NINDS, NIH).

B384/P1373

Redox-dependent membrane trafficking of neurotransmitter transporters

S. Radhakrishnan[1], S. Amara[2]; [1]National Institute of Mental Health, Bethesda, MD, [2]National Institutes of Mental Health, Bethesda, MD.

Plasma membrane neurotransmitter transporters clear neurotransmitters from the extracellular space and play a key role in regulating neuronal signaling. The neuronal excitatory amino acid transporter 3, EAAT3, is essential for glutamate clearance and modulation of glutamatergic tone. However, EAAT3 also transports cysteine into the cell where it serves as a rate-limiting substrate for the synthesis of glutathione, a key neuronal antioxidant. EAAT3 mutations are associated with several neuropsychiatric disorders such as schizophrenia and obsessive-compulsive disorders that may be due to its role in glutamate or cysteine transport. The activity of plasma membrane transporters can be altered by changes in transport kinetics, expression levels and trafficking to and from the plasma membrane. Using radiolabeled neurotransmitter uptake assays and surface biotinylation assays in Neuro2A cells we showed that activation of protein kinase C (PKC) can increase cell surface localization of EAAT3, as previously reported in other cell lines. There is evidence that redox-triggered mechanisms can influence signaling through PKC by altering the catalytic properties or the subcellular compartmentalization of various PKC isoforms. This suggests a mechanism whereby EAAT3 trafficking could be modulated by cellular redox stress and PKC activation to increase cysteine import and stimulate glutathione biosynthesis. To study this, we examined whether oxidizing agents such as hydrogen peroxide can enhance PKC activation and increase EAAT3 plasma membrane localization. Using a genetically encoded PKC activity sensor, CKAR, we found that application of oxidizing agent, hydrogen peroxide increased PKC activation and enhanced surface expression of EAAT3 in both EAAT3-transfected Neuro2A cells and in primary cultures of mouse cortical neurons that express EAAT3 endogenously. Additional studies have addressed another potential mechanism for redox regulation of neurotransmitter transporters—the direct redox modification of cysteine residues. The dopamine transporter (DAT) contains highly conserved internal lysine and cysteine residues that could trigger a redox-dependent internalization mechanism where the electrophilic modification of a cytosolic cysteine facilitates a conformation change and ubiquitylation of an adjacent lysine residue. We have examined this possibility by assessing DAT trafficking with surface biotinylation, TIRF microscopy and dopamine uptake assays. Taken together

our observations suggest multiple mechanisms though which changes in cellular redox states can regulate the activity of neurotransmitter transport systems.

B385/P1374

Functional characterization of iPSC-derived astrocytes in monoculture

A. Bhattarai, 1st[1], E. Nickoloff-Bybel[2], R. Abedalthaqafi[3], K. Jordan-Sciutto[4]; [1]Life Sciences, Soka University of America, Aliso Viejo, CA, [2]Department of Pathology, University of Pennsylvania, Philadelphia, PA, [3]Department of Periodontics, University of Pennsylvania, Philadelphia, PA, [4]Department of Oral Medicine, University of Pennsylvania, Philadelphia, PA.

Astrocytes are important brain regulatory cells and are essential in supporting physiological processes in the healthy brain. A number of studies have shown astrocytic dysfunction plays a role in many neurological diseases. Much of what is known comes from animal models as obtaining primary human astrocytes is challenging, and beyond the reach of many labs. Recent studies suggest these cells may differ in their response to stressors compared to animal astrocytes. Therefore, there is a need for reliable humanized astrocyte models. Our lab is working towards optimizing a new experimental approach using induced pluripotent stem cell-derived (iPSC) astrocytes (iAst) to model human astrocytes *in vitro*. Preliminary studies suggest iAst in monoculture do not take up glutamate, a critical astrocyte function, to the same degree as when cultured with neurons, suggesting a less mature phenotype. Therefore, we investigated whether different culturing conditions and treatments could induce a more mature phenotype in monoculture. We cultured increasing numbers of iAst in the presence of different concentrations of neuron conditioned media (NCM), or db-cAMP, an agent used to mimic neuronal factors, for 7 days to understand if they changed their phenotype, as has been described for rodent astrocytes. We performed glutamate uptake assays to quantify astrocyte function and used immunofluorescence staining to look at changes in morphology. For glutamate assays, media was removed after 7 days and media containing 100uM glutamate was added. Supernatants were collected at 5, 60, 120, and 180 minute timepoints. Our results show that iAst at 20k-40k cells per well, but not 10k, show modest glutamate uptake ability. Our data also indicates treatment with 50% and 75% NCM, but not db-cAMP, may further increase glutamate uptake in iAst. We are now assessing whether this treatment also alters astrocyte morphology and activation, using the astrocyte specific markers S100b and GFAP. These changes could indicate a more mature or reactive astrocyte phenotype, which would alter the response of the astrocytes to disease relevant stimuli, such as inflammatory cytokines. These data are important as they suggest that while iAst in monoculture do not mimic *in vivo* astrocytes, the use of exogenous neuronal media and/or factors could be used to make them a better *in vitro* model system.

B386/P1375

Relationship between mitochondrial transport and neuronal architecture *in vivo*

E. Donovan, N. Bell, E. Sarkar, E. Barnhart; Biological Sciences, Columbia University, New York, NY.

In developing neurons, mitochondrial motility is critical for the growth and stabilization of axonal and dendritic arbors. Mitochondrial movement decreases as neurons mature, and it is unclear whether mitochondrial transport is required to maintain neuronal form and function in adults. To elucidate the physiological significance of mitochondrial transport in adult neurons, we measured and manipulated mitochondrial motility in adult horizontal system (HS) neurons in the *Drosophila* visual system. First, we

used *in vivo* confocal microscopy to measure mitochondrial exchange rates, the volume of mitochondria moving across the primary dendrite, in young (day 4) and old (day 28) flies. We found mitochondrial exchange rate decreases from ~1 μm^3 per min to ~0.25 μm^3 per min from young to old flies. Therefore, as flies age mitochondrial transport decreases, but even in old flies there remains significant mitochondrial transport. Next, we used *Drosophila* genetic tools to selectively perturb mitochondrial motility in adult HS neurons. We selectively up- or down-regulated expression of Milton, a conserved adapter protein essential for mitochondrial transport, in HS neurons. Overexpression had no significant effect on mitochondrial exchange rates in young flies, but mitochondrial exchange increased by 50% in older flies; RNAi knockdown eliminated mitochondrial exchange rates in both young and old flies. RNAi knockdown of Milton reduced mitochondrial localization in distal dendrites and increased mitochondrial localization in the soma. Strikingly, this decrease in mitochondrial localization in the distal dendrites was correlated with reduced neuron branching, and smaller dendrite arbors. Although RNAi knockdown of Milton neurons developed normally, since RNAi was restricted only to the adult, alterations in adult mitochondrial transport perturbed the morphology of the adult neuron. These results demonstrate that mitochondrial motility is necessary to maintain proper mitochondrial localization and dendrite integrity in adult neurons.

B387/P1376

Mitophagy receptor function in *Drosophila* neurons is mediated by *kenny*, homolog of mammalian optineurin

L. Randolph[1], Z. Haupt[2], **R. Insolera**[2]; [1]Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, [2]Ophthalmology, Visual and Anatomical Sciences, Wayne State University, Detroit, MI.

The maintenance of a healthy pool of mitochondria is essential to long-term neuronal health. Mitophagy acts as a critical mechanism to degrade damaged mitochondria in neurons, and disruption of this process is tightly associated with neurodegenerative disease. Previous work has shown that mammalian optineurin (OPTN), a gene mutated in familial forms of amyotrophic lateral sclerosis (ALS) and glaucoma, functions as a mitophagy receptor in cultured cells. However, OPTN's role in neuronal mitophagy *in vivo* and how its dysfunction perturbs neuronal physiology remains largely unknown. Here, we demonstrate that the *Drosophila* homolog of optineurin, *kenny (key)*, directly mediates the recruitment of the phagophore to mitochondria undergoing mitophagy. In larval motoneurons, endogenous kenny protein localizes to polyubiquitinated mitochondria. We find that kenny's domain structure mediates its function as a mitophagy receptor: its ubiquitin-associating (UBA) domain localizes the protein to polyubiquitinated mitochondria, and kenny's LC3-interacting region (LIR) domain enables recruitment of the phagophore. Loss of *kenny* in basal conditions, or in conditions of mitochondrial stress, stalls the progression of mitophagy and leads to the accumulation of polyubiquitinated mitochondria in neuronal cell bodies. Interestingly, despite putative autophagy receptor domain structure and tight localization to mitochondria undergoing mitophagy, we observe that the *Drosophila* homolog of mammalian p62, Ref(2)p, is not required for the recruitment of the phagophore in neuronal mitophagy. Physiologically, the absence of *kenny* causes age-dependent locomotor defects under basal conditions, and sensitizes animals to genetic and pharmacological mitochondrial stress manipulations. Overall, this work establishes *kenny* as an essential component of neuronal mitophagy in *Drosophila,* and sets the foundation to further understand the potential cellular and physiological consequences of disease mutations associated human OPTN in an *in vivo* system.

B388/P1377

Exceptionally long-lived mitochondrial proteins in mammalian brains

E. Bomba-Warczak, J. Savas; Northwestern University, Chicago, IL.

Mitochondria are critical for maintaining neuronal homeostasis and decreased mitochondria quality and impaired function is a point of convergence for many neurological disorders. Mitochondria are rejuvenated through several processes including mitogenesis, fission-fusion, and multiple quality-control mechanisms. Consistently, the average half-life of mitochondrial proteins in the brain is less than 3 weeks. Efficient surveillance and the continual renewal of mitochondrial components are thought to be essential for maintaining high-quality organelles. and impairments to these mechanisms are thought to lead to the progressive functional and structural degeneration of neurons. However, I recently discovered that a discrete subset of the mitochondrial proteome escapes the classical first-order degradation kinetics and persists for at least 4 months in the mouse brain. Using whole animal dynamic metabolic stable isotope with labeling and mass spectrometry (MS)-based proteomic analysis, I found that these mitochondrial long-lived proteins (mt-LLPs) are (1) present in tissues enriched with long-lived postmitotic cells, (2) are enriched within and near mitochondrial cristae invaginations, and (3) are spatially restricted within the same mitochondria. However, the mechanisms governing the persistence, maintenance, and potential contribution of mt-LLPs to mitochondrial function are currently unknown. We hypothesized that akin to other long-lived proteins, the exceptional longevity of mt-LLPs could play an essential role in the long-term stabilization of the mitochondrial cristae in long-lived, post-mitotic cells. Therefore, the ongoing research efforts are centered on the investigation of the spatio-temporal distribution of mt-LLP within mitochondrial networks using optical pulse-chase techniques and super-resolution microscopy in primary neurons. Further, we hypothesize that the ultrastructure of cristae invaginations physically separates mt-LLPs from degradation, which we examine using genetic manipulations targeting cristae integrity and QC-pathways. Together, investigating the localization of long-lived proteins and characterizing their relationship to cristae structure will increase our understanding of mitochondrial biology and of the role of mitochondrial dysfunction in neurons.

B389/P1378

CED-3 caspase and opposing kinesins direct mitochondrial confinement to govern Compartmentalized Cell Elimination

R. Sharmin[1], S. P. Carmona[2], G. Clark[1], K. Juanez[1], A. Elkhalil[1], M. W. Pellegrino[1], S. Shaham[2], P. Ghose[1]; [1]University of Texas at Arlington, Arlington, TX, [2]The Rockefeller University, New York, NY.

Morphologically complex cells, defined by their elaborate processes, such as neurons bearing axons and dendrites, are commonplace in the metazoan body. How morphologically complex cells are eliminated is poorly understood. Intracellular transport is especially important for morphologically complex cells yet its role in cell elimination is underexplored. We discovered a novel 'tripartite' killing program that dismantles the sex-specific CEM neurons and the morphologically complex tail-spike epithelial cell (TSC) during *C. elegans* embryonic development. This program, we term Compartmentalized Cell Elimination, or CCE, is distinguished by three cell regions dying in three different ways. Remarkably, the single process/dendrite of these cells shows two disparate elimination morphologies in two subcellular domains, morphologically similar to different types of developmental pruning, retraction and fragmentation, as well as injury-induced Wallerian degeneration and pathological neurodegeneration.

We present CCE as a powerful *in vivo* model for region-specific cell elimination. We report that mitochondrial retrograde transport, mediated by the Kinesin-1 homolog UNC-116, and soma sequestration of mitochondria are a pre-requisite for CCE. Our genetic evidence suggests that soma confinement of mitochondria is accomplished by the targeting of the anterograde motor UNC-104/Kinesin-3 by CED-3/Caspase. Our findings present a regulatory mechanism for the novel phenomenon of CCE, introduce a novel, *in vivo*, caspase target and provide insight into regulation of pruning/localized cell elimination as well as neurodegenerative diseases.

B390/P1379

Single particle tracking reveals the distinct motility patterns of different ER proteins and the overall morphology of ER in dendritic processes of mature neurons

L. Benedetti[1], C. J. Obara[1], P. De Camilli[2], T. A. Ryan[3], J. Lippincott-Schwartz[1]; [1]4D Cellular Physiology, HHMI Janelia Research Campus, Ashburn, VA, [2]Cell Biology and Neuroscience, Yale University School of Medicine, New Haven, CT, [3]Biochemistry, Weill Cornell Medicine, New York, NY.

Neurons are polarized cells whose function is to collect, integrate and relay electrical signals for communication with target cells. The development and maintenance of neurons is accompanied by a high degree of spatial and morphological specialization of intracellular membrane-bound organelles. Neuronal endoplasmic reticulum (ER), for example, extends from cell body to peripheral axon and dendritic elements over hundreds of microns. Since the size of ER tubules in neurons is extremely narrow, diffraction-limited, optical microscopy does not allow visualization of the intricate mesh of ER tubules. Consequently, little is known about the mobility, spatial distributions, and dynamic regulation of intrinsic ER proteins. To gain insight into these questions, we applied single particle tracking analysis combined with super-resolution imaging in dendrites from mature neurons. Summing up diffusion-trajectories of different ER proteins, we could visualize ER morphology across entire dendrites. We found the ER consisted of long, longitudinal tubules extending along dendritic processes. The tubules were interconnected by short perpendicular connections spaced ~1 µm apart, resembling the ladder-like organization of axonal ER in immature neurons reported recently by Voeltz and colleagues. ER proteins diffused throughout this system, but different proteins exhibited a unique motility pattern correlating with the protein's topology and ensemble of functional interactions.

B391/P1380

PDZD8 promotes autophagy at ER-Lysosome contact sites to regulate synaptic growth

R. Thakur[1], K. O'Connor-Giles[1,2]; [1]Neuroscience, Brown University, Providence, RI, [2]Carney Institute for Brain Science, Providence, RI.

Sites of apposition between organelles, referred to as membrane contact sites (MCSs), are hotspots for intracellular signaling, lipid metabolism, and organelle biogenesis/dynamics in eukaryotic cells. The endoplasmic reticulum (ER) forms an extensive and dynamic network of MCSs with almost all organelles. MCSs between the ER and endo-lysosomes are particularly abundant, suggesting important physiological roles.PDZD8 is an intrinsic ER transmembrane protein with a synaptotagmin-like mitochondrial lipid-binding protein (SMP) domain that has been reported to localize to ER-late endosome (LE)/lysosome and ER-mitochondria MCSs. PDZD8 is enriched in neurons. However, its role in the nervous system remains poorly understood. We identified Drosophila PDZD8 as a candidate regulator of synapse formation and function, and used CRISPR-Cas9 editing to generate null and

endogenously tagged alleles. We find that PDZD8 is expressed at synapses throughout the central nervous system and the larval neuromuscular junction (NMJ), where it localizes to ER-LE/lysosome MCSs. Activity-induced synaptic growth, neurotransmission, and locomotion are dysregulated in PDZD8 mutants, indicating key roles in nervous system development and function. Through genetic and in vivo cell biological studies, we have found that PDZD8 regulates synaptic growth via autophagy. Our analyses suggest that PDZD8 promotes autolysosome maturation to maintain autophagic flux in neurons. We further show that the C1-CC domain of PDZD8 functions to tether ER and LE/lysosomes membranes through lipid binding and is required to promote autophagy and synaptic growth. SMP-domain mediated lipid transfer activity at ER- LE/lysosome MCSs is also required to promote synaptic growth. Finally we show that autophagy negatively regulates the Wingless signaling pathway to promote PDZD8-mediated synaptic growth. Overall, we find that PDZD8-mediated ER-lysosome membrane interactions promote autophagy to regulate synaptic growth, revealing an important role for lipid transfer proteins in promoting communication in the brain.

B392/P1381

AP-1 recruits KIF13B to dendrite-selective vesicles at the trans-Golgi network

A. C. Montgomery, M. Bentley; Rensselaer Polytechnic Institute, Troy, NY

Neurons are the fundamental unit of the nervous system and mediate electrochemical signaling. To fulfill their signaling function, neurons are polarized and maintain two distinct compartments: axons that primarily send signals and dendrites that receive signals. Each compartment requires a specific complement of membrane proteins that is maintained by vesicle trafficking. At the Golgi, dendritically polarized membrane proteins are sorted into vesicles by the heterotetrameric adaptor protein complex AP-1. Nascent Golgi-derived vesicles undergo KIF13B-mediated transport and exclusively move in the somatodendritic region without entering the axon. Because cargo sorting and vesicle transport are necessarily sequential, they must be connected. To date, the molecular link between these trafficking events remains undefined. We performed live-cell microscopy of KIF13B in cultured hippocampal neurons to determine at what stage of the secretory pathway KIF13B is recruited to dendrite-selective vesicles. We found that neurons maintained a stable population of KIF13B at the Golgi. Immunocytochemistry showed that this population specifically localized to the trans-Golgi network and colocalized with AP-1. To test the hypothesis that AP-1 retains KIF13B at the Golgi, we used a novel assay to probe for protein-protein interactions in the native cytoplasmic environment of intact neurons. We found that AP-1 specifically bound KIF13B, but not other related members of the Kinesin-3 family. Specific disruption of the interaction between KIF13B and AP-1 perturbed dendrite-selective transport, indicating the requirement of this interaction for polarized trafficking. We propose the model that AP-1 connects the sorting of dendritically polarized membrane proteins into Golgi-derived vesicles to the recruitment of KIF13B to confer dendrite-selective transport. This is the first instance in which a mechanistic link between sorting and transport has been defined and it is likely that similar mechanisms participate in many other trafficking events, both in neurons and non-neuronal cells.

B393/P1382

Endocytosis in the axon initial segment maintains neuronal polarity

K. Eichel[1], V. Belapurkar[2], D. Perrais[2], K. Shen[1]; [1]Stanford University, Stanford, CA, [2]University of Bordeaux, Bordeaux, France

Neurons are highly polarized cells with distinct axonal and dendritic domains that send and receive signals, respectively. To achieve this polarization, neurons extensively use membrane trafficking mechanisms to compartmentalize a vast and diverse repertoire of proteins to each domain. The axon initial segment (AIS) is a critical boundary zone separating these domains and is known to function in neuronal polarity by sorting intracellular vesicles and slowing protein diffusion on the plasma membrane. However, it remains unclear how the AIS maintains stringent polarity over the decades-long lifetime of the neuron. We identified a conserved mechanism in which polarized membrane proteins are endocytosed in the AIS and subsequently degraded to maintain neuronal polarity. Using *C. elegans* to study AIS function in intact living animals, we find that dendritically and axonally polarized membrane proteins are endocytosed at the axon-dendrite boundary zone of the AIS. Endocytic removal prevents both the further diffusion of dendritic membrane proteins into the axon and the diffusion of axonal membrane proteins into the dendrite after their initial vesicular delivery to the axon. Engineering receptor interaction with the AIS master organizer, ankyrinG, antagonizes receptor endocytosis in the AIS and causes polarity deficits with subsequent morphological and behavioral defects. We then extend these finding to cultured rodent and induced human neurons. By measuring receptor endocytic rates in live rat hippocampal neurons, we find that the dendritically polarized transferrin receptor is endocytosed at the same rate in the AIS as in the dendrite. However, the post-endocytic targeting of receptors differs: receptors endocytosed in the AIS are targeted for degradation whereas those endocytosed in the dendrite are recycled back to the plasma membrane. We propose that robust receptor endocytosis in the AIS when coupled to degradation functions to clear axonal and dendritic membrane proteins from the AIS to maintain their compartmentalization. Our results reveal a conserved and essential endocytic clearance mechanism in the AIS that contributes to maintaining polarity over the long lifespan of the neuron. Through the study of one of the most polarized cell types, we have identified strategies by which cells achieve compartmentalization along a contiguous membrane.

B394/P1383

Plasticity of prefrontal projections mapped by axonal transport

E. L. Bearer[1], T. W. Uselman[1], C. S. Medina[1], R. E. Jacobs[2]; [1]University of New Mexico Sch Med, Albuquerque, NM, [2]Physiology, Keck School of Medicine, Los Angeles, CA.

The anterior cingulate area (ACA), located in the medial prefrontal cortex (mPFC), regulates flexibility and is implicated in decision-making. Loss of volume in mPFC of cocaine users correlates with decreased executive function and compulsive drug use. We previously reported that ACA projections are plastic, with destinations altered by genetic manipulations of monoamine systems. We now test whether a single experience of ethological threat during adulthood can influence ACA projection functional anatomy. Mice were exposed for 30 min to synthetic predator odor (2,3,5-Trimethyl-3-thiazoline, TMT). Three weeks later tracers were stereotactically injected into the ACA and their projections in the living mouse imaged, first with MEMRI (manganese-enhanced magnetic resonance imaging), and then after fixation, by optical microscopy. Mn(II), a contrast agent for MRI, enters active neurons through voltage-gated calcium channels, is packeged into transport vesicles and trafficked down axons by kinesin-based

fast axonal transport. MEMRI detects brain-wide projections in the living mouse at 100 μm resolution. MR images were captured before and at successive time points after ACA injection (0.5, 6, 24h). Analysis of MEMRI with voxel-wise SPM comparisons between time points revealed progression of Mn(II) distally. Application of our new *In Vivo* digital mouse brain atlas allowed automated quantification of Mn(II) accumulation, which detected dramatic differences in locations of projections between animals experiencing acute threat and those without. Regional segmentation of these projection maps showed decreases in anterior and basolateral amygdala, preoptic hypothalamus and superior central raphe, and increases in posterior and cortical amygdala, periaqueductal gray and dorsal raphe in threat-exposed versus no-threat groups. These changes best correlated with redirection of projections after NET disruption, which increases the amount of noradrenaline in the synaptic cleft similar to an effect of cocaine and may also mimic stress. No ACA volume loss was detected in non-threat exposed, NET-deleted mice by SPM of DTI ($p<0.005$ FDR). Thus a single experience of acute threat redirects ACA distal connections independent of volume loss, which may explain one aspect for how life-threatening experiences pose long lasting risks for anxiety and substance use disorders

B395/P1384

Coordinated Signaling of Laminin and BDNF Stimulates Local Translation and Point Contact Density in Neuronal Growth Cones

N. K. Kirkise, K. Welshhans; Biological Sciences, University of South Carolina, Columbia, SC.

Accurate neural wiring in the developing brain is mediated by growth cones, which are the pathfinding tips of growing axons that respond to environmental cues to find and connect with their synaptic partners. One mechanism initiated by these cues that regulates axon guidance is the local translation of mRNAs, such as *β-actin*, within growth cones. Although it is well-established that neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), stimulate local translation, the contribution of extracellular matrix (ECM) proteins, such as laminins, to this process is not well understood. Our lab has previously shown that local translation of *β-actin* mRNA occurs at point contacts, which are growth cone adhesions that link the ECM to the intracellular cytoskeleton. Thus, we hypothesized that laminin may stimulate local translation in axonal growth cones. Embryonic day 17 mouse cortical neurons were acutely stimulated with laminin 111 ± BDNF by bath application and used in (1) a puromycin assay to label all nascent protein synthesis and (2) a puromycin-proximity ligation assay (Puro-PLA) to label only newly translated β-actin. We found that brief stimulation with laminin 111 and BDNF together results in an increase in overall local protein synthesis and local translation of *β-actin* mRNA in growth cones. Stimulation with laminin 111 and BDNF together also results in an increase in axon length and point contact density. Interestingly, stimulation with either BDNF alone or laminin alone does not result in a significant increase in β-actin local translation, axon length, or point contact density, suggesting that coordinated signaling of these two factors is needed for axon development. However, there are many isoforms of laminin, each with differing temporal and spatial distributions. Thus, we repeated the puromycin assay using BDNF and either Laminin 111, 511, 211, or 221. Neurons cultured on Laminin 111 show increased local protein synthesis and axon length, whereas Laminin 221 results in a remarkable decrease in both local protein synthesis and axon length. Local translation regulates growth cone turning in response to external stimuli. Thus, these results suggest that laminin and BDNF have a synergistic effect on local translation and point contact density, thereby facilitating the movement and directionality of growth cones during axon pathfinding.

B396/P1385

Neuronal lysosomes intrinsically regulate site-specific mRNA translation

M. S. Fernandopulle, V. Ryan, F. Almsned, H. Yuan, A. Qi, S. Meydan, C. Drerup, M. Ward; NINDS, National Institutes of Health, Bethesda, MD.

Lysosomes are metabolic and signaling organelles whose dysfunction often leads to neurological disease. Long known as sites of nutrient sensing and recycling, lysosomes have recently been recognized as vehicles for mRNA transport in neurons, enabling translation of proteins far from the cell body. This phenomenon of "local translation" is critical for proper neuronal function; however, how cells regulate this process in space and time remains unclear. Using electron and live cell microscopy, we found that distal lysosomes in neurons were associated with both mRNAs and ribosomes, implicating lysosomes in jointly regulating RNA transport and translation. SunTag translation experiments revealed that lysosomes actively engaged in translation show decreased motility, suggesting a coordinated signaling mechanism for site-specific translation. To uncover the mechanistic basis of lysosome-associated translation, we developed a suite of functional proximity labeling approaches in iPSC-derived neurons that characterize translation on the lysosome surface. We catalog the landscape of mRNA binding to neuronal lysosomes, showing that these mRNAs are functionally diverse and predominantly localized to distal processes. We identify TRPML1, a lysosomal cation channel, as a major binding partner of lysosome-proximal ribosomes. TRPML1 is known to efflux Zn^{2+} in neurites, which halts lysosomal transport by direct motor protein inhibition. Activation of TRPML1 also dramatically stimulated lysosome-associated translation, mechanistically linking lysosome-mediated mRNA localization with site-specific translation. These results shed light on the neuronal function of TRPML1, in which recessive mutations cause the neurodegenerative disease mucolipidosis IV. More broadly, our work suggests a mechanism through which lysosomes disseminate and translate mRNAs in a locally regulated fashion.

B397/P1386

Locally Translated β-actin Associates with Both Adhesions and Filopodia in Growth Cones

K. Rygel, K. Welshhans; Department of Biological Sciences, University of South Carolina, Columbia, SC.

Local translation is the process by which specific mRNAs are localized and translated in subcellular regions. Numerous studies have demonstrated that local translation of *β-actin* mRNA in neuronal growth cones is required for appropriate axon guidance during nervous system development. However, where this newly translated β-actin is incorporated into the growth cone to functionally impact axon guidance has yet to be studied. The prevailing hypothesis in the field states that locally translated β-actin is important for filopodial dynamics since rapid extension and retraction of filopodia is necessary for growth cone turning, but this has not been directly tested. We recently demonstrated that *β-actin* mRNA is locally translated at adhesion sites, called point contacts, in growth cones. Point contacts are highly dynamic structures in motile growth cones that link the extracellular matrix to the intracellular actin cytoskeleton, and generate the force needed for growth cone motility. Based on our finding that *β-actin* mRNA is locally translated at point contacts, we hypothesized that nascent β-actin is needed for both filopodia and point contacts to regulate axon guidance. Using embryonic mouse cortical neurons, we performed fluorescent noncanonical amino acid tagging-proximity ligation assay (FUNCAT-PLA) to label newly translated β-actin and visualize where it is integrated within growth cones. We have found that nascent β-actin is localized in all regions of the growth cone including filopodia, lamellipodia, and the central domain. We also demonstrate that about 34% of newly translated β-actin colocalizes with

point contacts, suggesting that nascent β-actin may contribute to axon guidance, in part, by regulating adhesions. Using axon-specific β-actin siRNAs, we are investigating whether locally translated β-actin is required for both point contact and filopodial dynamics. Taken together, this work describes a novel role for nascent β-actin in growth cones and increases our understanding of the molecular mechanisms that regulate axon pathfinding, a process that is critical for nervous system development.

B398/P1387

Injury Signaling Kinases DLK and LZK Regulate Cell Death in Vincristine-treated Human Neurons

M. Nebiyou, J. Gomez-Deza, C. E. Le Pichon; NIH/NICHD, Bethesda, MD

Vincristine is a drug used to treat solid tumors in cancer patients and is known to cause chemotherapy induced peripheral neuropathy. We found that vincristine is toxic to iPSC-derived human neurons. Dual Leucine Zipper Kinase (DLK) and Leucine Zipper Kinase (LZK) are homologous, stress-sensing and injury-signaling proteins that can regulate neuron death. We tested whether DLK and/or LZK were necessary for vincristine-induced neuron death. We generated single and double CRISPR knockout lines for DLK and/or LZK and found that DLK or LZK single knockouts were partially protected from vincristine-induced toxicity while the DLK/LZK double knockouts were wholly protected compared to wild type. Blocking transcription in wild type neurons also prevented vincristine-induced toxicity. Using RNA seq, we confirmed that vincristine treatment induces a diverse transcriptional response. In each of the single knockout lines the transcriptional response to vincristine was blunted while in the double knockout line it was essentially abolished. We are currently collecting proteomic data to complement our transcriptomic data and examining which transcriptionally dependent pathways are necessary for toxicity.

B399/P1388

Temperature adaptation shapes the calcium response and morphology of a thermosensory neuron to drive temperature seeking behavior

J. R. McVay[1], J. A. Beagan[1], D. A. Colón-Ramos[1,2]; [1]Yale University, New Haven, CT, [2]Universidad de Puerto Rico, San Juan, PR

Sensory neurons across the evolutionary spectrum adapt their responses when chronically exposed to environmental stimuli, thereby altering behavior. AFD, the thermosensory neuron of the nematode *Caenorhabditis elegans,* represents an information-rich, genetically tractable model system with which we can study the molecular interface of sensation and behavior. *C. elegans* do not have an innate temperature at which they live, rather they form memories of preferred habitation temperatures and perform goal-directed behavior to return to this temperature, a behavior called thermotaxis. By quantifying single-neuron calcium activity and tracking the behavior of animals trained to have different habitation temperature preferences we find, consistent with previous studies, that AFD exhibits a limited 'perceptive range' of temperatures that it responds to which adapts on the same timescale as behavioral plasticity. Intriguingly we find that AFD's sensory tip, comprised of a primary cilium and a network of microvilli, exhibits structural remodeling which occurs on the same timescale as perceptive range adaptation, and as has been previously observed (Singhvi et al. 2016, Cohn et al. 2020). Similar to the mammalian retina, activity of a set of phosphodiesterases (PDEs) is required for proper adaptation of both the structural- and activity-based signatures of AFD's perceptive range.

B400/P1389

The conserved kinase NEKL-4/NEK10 orchestrates ciliary microtubule integrity and mitochondrial dynamics to modulate hyperglutamylation-induced neurodegeneration

K. M. Power, J. Shivas, C. Rongo, M. M. Barr; Rutgers University, Piscataway, NJ

Neurodegenerative diseases present a severe and significant medical concern that has historically proven difficult to study. The health of neurons depends in large part on mitochondrial localization and metabolic function. Microtubule-based transport is important for proper mitochondrial localization and function, and thus to neuron health. Consequently, proteins that affect microtubule stability play indirect yet important roles in the regulation of mitochondria function. A loss-of-function mutation of the deglutamylase CCP1 causes infantile-onset neurodegeneration and leads to excessive glutamylation of microtubules and defects in transport of mitochondria in neurons. CCP1 is evolutionarily conserved; its ortholog in *Caenorhabditis elegans*, *ccpp-1*, also functions in a subset of neurons where it affects microtubule stability of neuronal cilia. We performed a forward genetic screen to identify suppressors of *ccpp-1* age-related ciliary degradation. We identified a mutation in the NIMA-related kinase *nekl-4*, an ortholog of mammalian NEK10. NEK10 is associated with both cilia dysfunction and disrupted mitochondrial homeostasis in mammalian cells. We found that NEKL-4 did not play a role in regulating microtubule glutamylation and acted via an unknown mechanism. To uncover the NEKL-4 mechanism of action and to determine the role of NEKL-4 and CCPP-1 in mitochondrial dynamics, we generated CRISPR-tagged fluorescent wild type, constituently active, and kinase dead forms of NEKL-4. Wild-type and kinase-dead NEKL-4 did not localize to cilia, but was associated with mitochondria. In *ccpp-1* mutants, kinase-dead NEKL-4 translocated to cilia and suppressed ciliary degeneration. Time lapse microscopy showed that NEKL-4 and mitochondria are co-transported, which suggests that NEKL-4 may regulate mitochondria transport in the ciliated neurons. Additionally, our imaging showed that both *nekl-4* and *ccpp-1* affect mitochondrial morphology and localization in these neurons. We are currently testing the hypothesis that CCPP-1 and NEKL-4 affect mitochondrial function, which may impact cilia structure and function. Our work adds to a growing body of evidence suggesting a connection between cilia stability and mitochondrial function that, when perturbed, could instigate neuronal degeneration.

B401/P1390

Are organelle contacts the key to neuron and astrocyte resilience as well as their demise during neurodegeneration?

S. Rhoads, S. Hsu, V. Dong, T. Cohen, S. Cohen; University of North Carolina, Chapel Hill, NC

Organelle contacts are sites of close apposition between two or more membrane-bound organelles. They facilitate critical lipid, protein, and metabolite transfer for proper cell function. Recently, organelle contact dysregulation has been implicated in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). However, the extent of organelle contact dysfunction in ALS is not fully understood. Here, we use multispectral imaging, a fluorescence microscopy technique that can be used to simultaneously visualize six fluorescent structures (i.e., organelles) in live cells, to quantify the type and extent of organelle contacts. First, we investigated organelle contacts in live primary neurons and astrocytes at baseline. Our results showed that both cell types have distinct sets of organelle contacts that reflect cell-specific functions. Particularly, mitochondria content and contacts were more extensive in neurons likely reflecting their higher oxidative phosphorylation rates compared to astrocytes. Then, we examined the extent of organelle reorganization during acute pharmacological perturbations,

including oxidative stress and ER stress. Surprisingly, organelle contacts were not significantly altered. Our results could suggest that these cell types maintain specific organelle contacts because they are integral to proper cell function and survival. In future experiments, we will analyze if this resilience is maintained during ALS-related genetic perturbations and whether contact dysregulation is a prominent pathomechanism in ALS.

B402/P1391

Drosophila* motion vision neurons exhibit diverse ER calcium signals *in vivo

K. C. Delgado, E. L. Barnhart; Columbia University, New York, NY

Calcium gates synaptic vesicle release in neurons and is therefore a crucial regulator of neurotransmission. Neuron activation drives calcium influx across voltage gated calcium channels, but it is unclear how calcium handling by the endoplasmic reticulum (ER) contributes to cytosolic calcium concentrations and therefore neurotransmission *in vivo*. In this work we used *in vivo* two photon calcium imaging to measure stimulus-evoked calcium signals in the cytosol and ER in *Drosophila* neurons. In the cytosol, the sign of the calcium response is always the same — calcium concentrations always increase in response to preferred visual stimuli — regardless of cell type or compartment (i.e. axons versus dendrites). Response kinetics, on the other hand, vary across cell types: whereas some neurons response to visual stimuli quickly and transiently, others response more slowly and in a sustained fashion. In the ER, both the sign and timing of stimulus-evoked calcium signals differed across cell types and compartments. For example, in the axon terminals of second-order interneurons Mi1, the ER took up calcium during neuron activation but released calcium in the dendrites of the same cell. In Tm1 neurons, the ER took up calcium immediately after neuron activation in both axons and dendrites. Additionally, the addition of SERCA inhibitor thapsigargin to the brains of flies greatly decreased ER calcium signaling and altered cytosolic calcium signals, indicating that ER calcium dynamics during neuron activation can alter cytosolic calcium signals. The *Drosophila* visual system is an ideal model for investigating how ER calcium signals tune neuronal response kinetics, and how those responses shape cell and circuit function *in vivo*.

B403/P1392

Investigating pleiotropic roles of PINK1 and Parkin in neuronal homeostasis

A. M. Lamper; Columbia University, New York City, NY

PINK1 and Parkin are well established initiators of mitophagy, but can also modulate the activity of several mitochondrial fission, fusion, and motility factors. In Drosophila, PINK1 and Parkin induce the degradation of the major mitochondrial fusion factor, Marf, and a mitochondrial motility factor, Miro. Degradation of these proteins is hypothesized to arrest and isolate mitochondria, thereby facilitating mitophagy. However, it is unclear how PINK1 and Parkin regulate mitochondrial morphology and localization in vivo. Using confocal microscopy to image adult Drosophila neurons in vivo, we measured increased mitophagy levels in flies overexpressing either PINK1 or Parkin. These flies also exhibited extreme fragmentation of the mitochondrial network, consistent with PINK1/Parkin-dependent degradation of Marf and subsequent downregulation of fusion. Additionally, PINK1 or Parkin overexpression increased the amount of mitochondrial mass in the somata of these neurons, indicating modulation of mitochondrial trafficking or biogenesis. Interestingly, the average mitolysosome volume significantly increased in neurons expressing RNAi against Parkin, suggesting that mitolysosome size may

vary by mitochondrial degradation mechanism. These results support the idea of pleiotropic roles for PINK1 and Parkin in mitochondrial homeostasis, and suggest that defects in mitophagy may not be sufficient to fully describe the neurodegeneration seen in Parkinson's disease.

B404/P1393

Illuminating organelle dynamics during neuronal differentiation

M. C. Zanellati[1], R. Basundra[2], V. Dong[1], V. Williams[1], S. Hsu[1], M. Deshmukh[2], S. Cohen[1]; [1]UNC-Sarah Cohen Lab, Chapel Hill, NC, [2]UNC-Mohanish Deshmukh Lab, Chapel Hill, NC

Organelles undergo dramatic changes in shape, position, dynamics, and interactions with other organelles (together termed morphodynamics) to fine tune the metabolic state of a cell.
Until recently, scientists lacked the tools to study organelle organization in living cells at a systems level. We have developed a method for multispectral imaging of up to seven organelles simultaneously in live cells. We used this method to study organelle dynamics along the differentiation of induced pluripotent stem cells (iPSCs) into cortical neurons (iNeurons). We transfected iPSCs and iNeurons with organelle markers using custom piggyBac vectors 24 hours prior to imaging and collected multispectral images at five timepoints throughout neuronal differentiation, maturation, and *in vitro* aging: iPSCs, and iNeurons at day 7, day 14, day 21, and day 30. At each timepoint, >30 Z-stacks and >30 timelapse images were collected, from at least three biological replicates. Raw images were then subjected to linear unmixing and run through a Cell Profiler image analysis pipeline for segmentation and analysis of approximately 400 morpho-metrics including organelle area, size, shape, and number; number and area of the contacts (2 to 6-way). We found that the cell body (soma) contracts during differentiation from an iPSC to an iNeuron. Interestingly, some organelles scale with the soma, while others do not. We also observed changes in organelle morphology. For example, tubulation of mitochondria increased as iPSCs differentiated into cortical neurons and decreased again when the neurons aged. These data are consistent with increased oxidative phosphorylation as cells differentiate. We noted that organelle communication networks dramatically rewire throughout iNeuron differentiation. At early stages of differentiation, we observed rearrangement of mitochondria-organelle contacts. Mitochondria-ER and mitochondria-ER-Golgi contacts were high in iPSCs, and significantly decreased at early stages of neuronal differentiation. These contacts are consistent with glycolytic metabolism in stem cells being lost as they differentiate to iNeurons. Moreover, we found an increase in higher order contacts (4- and 5-way contacts) when neurons became mature, compared to young neurons and undifferentiated iPSCs. Finally, these high-order contacts were subsequently lost with *in vitro* aging. Our results suggest that extensive rewiring of organelle contacts is necessary to support metabolic changes throughout differentiation, and that the resulting organelle signature is required to sustain neurological functions.

B405/P1394

Interrogating how local axonal microtubule dynamics impact motor activity and synaptogenesis

J. Aiken, E. L. F. Holzbaur; University of Pennsylvania, Philadelphia, PA

Neurons rely on sustained, long-range trafficking of components to maintain the complex synaptic networks that encode the human experience. With a single neuron capable of forming thousands of distinct synapses, spatially precise delivery of the necessary synaptic components is paramount. Here, we explore how local regulation of microtubules, the track upon which neuronal cargos are transported, and long-range microtubule motors, the proteins responsible for cargo movement, influence synapse

distribution and delivery of essential synaptic cargos. We previously demonstrated that synaptic vesicle precursors (SVPs) are retained at presynaptic regions in rat primary neurons. These *en passant* presynaptic zones are enriched for dynamic microtubule ends, which promote detachment of the neuronal kinesin-3 motor KIF1A. Using live-imaging of human neurons derived from induced pluripotent stem cells (iPSCs), we reveal that even in the absence of robust synaptic connections, "protosynaptic" sites are hotspots for dynamic microtubules rich in GTP-tubulin, with a 5-fold increase in microtubule growth events observed in regions containing stable SVPs compared to non-synaptic regions. In the human i3Neuron system, SVPs demonstrate increased pause frequency at the protosynapses in both the anterograde and retrograde directions. Further, we have developed a novel heterologous synapse protocol utilizing microfluidic chamber technology to isolate human i3Neuron axons and the addition of neuroligin-expressing HEK cells to rapidly induce robust presynapse formation. We find that neuroligin-1 (NL1) expression in non-neuronal cells is sufficient to instigate presynapse formation in human i3Neuron axons within an hour of NL1+ cell addition. This method provides unique spatial and temporal control over presynapse formation, making exclusive presynaptic genetic and pharmacological manipulation possible and providing a convenient time scale upon which to observe/probe axonal synaptogenesis. Using this system, we find that axonal nocodazole treatment leads to a significant decrease in Synapsin I/II coverage over NL1+ HEK cells. Interestingly, while less Synapsin by volume is detected, the average intensity of accumulated Synapsin is not altered by nocodazole treatment. We propose a model where local microtubule modulation and cargo-specific motor behavior directs axonal traffic to help direct synaptogenesis and guide synaptic cargo delivery.

B406/P1395

Investigating competing constraints on dendrite architecture in Drosophila motion vision neurons

J. I. Kalai, E. J. Donovan, G. Napoleone, E. L. Barnhart; Columbia University, New York, NY

Form and function are inextricably linked in elaborately branched neurons. In particular, theoretical work suggests that specific parent-daughter power rules, which describe the relative widths of parent and daughter dendrites at branch points, are important for neuronal signal processing and stability. In theory, scaling according to Rall's Law, in which total dendrite cross-sectional area narrows across branch points, facilitates the efficient propagation of electrical signals in dendritic arbors. In contrast, our previous work suggests that scaling according to Da Vinci's Rule for Trees, in which cross-sectional area is conserved across branch points, facilitates intracellular transport and the reliable distribution of mitochondria throughout the neuron (Donovan and Agrawal et. al., 2022). To investigate how neurons balance these competing constraints on dendritic architecture, we measured mitochondrial motility and localization patterns in different types of *Drosophila* lobula plate tangential cells (LPTCs). Using in vivo confocal microscopy, we measured significant mitochondrial motility in several types of LPTCs, including CH, VS, and V1 neurons. In addition, by reconstructing mitochondria in ssTEM images of the Drosophila brain, we found that mitochondria are densely distributed throughout these same cell types. Finally, our preliminary measurements of dendrite architecture suggest that different types of LPTCs obey different forms of parent-daughter scaling rules, including Rall's Law and Da Vinci's Rule for Trees. In future studies, we will exploit this morphological diversity, along with in vivo measurements of mitochondrial dynamics and dendritic signal processing, to better understand how systems-level maintenance and intracellular homeostasis give rise to different dendritic architectures in neurons.

B407/P1396

Visual Input Synergizes With the Wnt/B-Catenin Pathway to Promote Proliferation in the Zebrafish Optic Tectum

J. Yue[1], O. L. Hagen[1], Y. Kim[1], E. Kushkowski[1], C. Roberts[1], H. Rouse[2], M. Varga[3], S. W. Wilson[2], K. L. Cerveny[1]; [1]Reed College, Portland, OR, [2]University College London, London, United Kingdom, [3]Eotvos Lorand University, Budapest, Hungary

Specialized stem cell niches arrayed throughout the zebrafish body support life-long growth. In the visual system, coordinated growth of the retina and optic tectum (OT) promotes accurate retinotopic mapping as new neurons are added throughout life. Studies of adult fishes have provided evidence that survival, proliferation, and differentiation of cells in the OT are correlated with retinal innervation, raising the possibility that retinal axons provide crucial information to control stem and progenitor cell behaviors in the optic tectum. To explore how retinal innervation influences OT growth in larval zebrafish, we examined brains lacking innervation from (i) both eyes as a result of the lakritz mutation and (ii) one eye as a result of surgical eye removal, and then compared them with wild-type larvae with two eyes. We observed elevated cell death in non-innervated and denervated OT lobes. In addition, we found fewer sox2+ stem cells and a smaller proportion of proliferating progenitors in lakritz and denervated OT lobes. To test the extent to which light-evoked retinal activity contributes to proliferation and survival in the optic tectum, we reared fish from 1-9 dpf in the dark and then asked how OT cells in innervated and non-innervated tecta behave. We found that innervation was still able to promote survival and proliferation, albeit to a lesser extent, when fish were reared in the dark. These data, combined with preliminary RNA-Sequencing data, led us to investigate whether the Wnt/B-catenin pathway was acting as an innervation-dependent trophic factor. Expression of wnt3a and Wnt/B-catenin target genes, lef1 and axin2, appears to be innervation dependent as in situ hybridization and RT-qPCR show decreased levels of these genes in non-innervated and denervated tectal lobes. Further supporting an innervation-dependent role for Wnt activation, we found that lakritz mutants treated with a Wnt-pathway agonist, 6-bromoindirubin-3-oxim (BIO), exhibited increased levels of OT cell proliferation that was indistinguishable from wild-type OT proliferation. Together our data provide new insight into how sensory organs and the brain regions they innervate coordinate their growth and development.

B408/P1397

Graduate Student

D. Fortson; Columbia University - New York, NY, New York, NY

Mitochondria are not discrete organelles. On the contrary, mitochondrial fission and fusion facilitate mixing of mitochondrial proteins throughout a dynamic, interconnected mitochondrial network. Defects in fission and fusion are associated with a host of neurodegenerative disorders. However, it is unclear how specific perturbations affect the rate and spatial pattern of mitochondrial mixing in neurons in vivo. In this work, we used a mitochondrially-targeted photoconvertible fluorescent protein, Mito-Dendra2, to measure mitochondrial mixing rates in wild-type Drosophila neurons in vivo. We photoconverted Mito-Dendra2 from green to red fluorescence in selected regions of Horizontal System (HS) neurons (e.g. in the primary or distal dendrites) and measured the rate of recovery of green fluorescence over time. Fluorescence recovery occurs more rapidly in primary dendrites than in the distal dendrite: three hours after photoconversion, recovery was 54+/-6% in primary dendrite and 39+/-4% in the distal dendrites. In addition, whereas more than 50% of mitochondria in the primary dendrite had mixed

mitochondrial content (i.e. both green and red fluorescence) three hours post photoconversion, only ~25% of mitochondria in the distal dendrites were both green and red. Altogether, these results suggest that the rate of mitochondrial mixing is higher in primary dendrite than in the distal dendrites. In future studies, we will investigate how specific perturbations of fission and fusion affect the spatiotemporal pattern of mitochondrial mixing, as well as mitochondrial health and neuronal function in vivo.

Lipids and Membrane Microdomains

B410/P1398

Rafting in a RUSH: membrane microdomains in secretory trafficking

I. Castello Serrano, R. Ippolito, K. R. Levental, I. Levental; Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA.

The determinants of sub-cellular distribution are not known for most membrane proteins. Although a variety of specific motifs responsible for cargo integration into coat/adapter mediated sorting schemes have been identified, many membrane proteins have no known motifs. A potential parallel mechanism for organizing membrane protein traffic is sorting by membrane microdomains known as lipid rafts. Such domains are small, dynamic assemblies of preferentially interacting lipids and proteins that have been widely implicated in signaling at the plasma membrane (PM) but are likely also present in various endo-membranes. Our lab has recently defined the structural determinants of preferential protein partitioning into these ordered membrane domains and shown that raft affinity is required for PM localization of a subset of membrane proteins. These observations suggested that sorting and trafficking of membrane proteins can be directed by their affinity for a particular membrane environment. To directly assess the role of membrane microdomains in the secretory pathway, we have taken advantage of a robust tool for synchronized protein traffic, known as RUSH (Retention Using Selective Hooks). Here, tagged proteins are retained in specific organelles by a resident “hook”, where they can be quickly released upon introduction of biotin, allowing direct and quantitative analysis of trafficking rates and destinations by fluorescence microscopy. We applied this system to a library of transmembrane domain (TMD) constructs to evaluate the role of raft affinity in secretory traffic, and the machinery involved therein. We find that while TMD-encoded raft affinity is fully sufficient for PM sorting, it is not sufficient for rapid exit from the endoplasmic reticulum (ER), which requires a specific cytosolic sorting motif. However, we find that Golgi exit rates are highly raft-dependent, with raft preferring proteins exiting ~2.5-fold faster than mutants with perturbed raft affinity. By super-resolution microscopy, we directly observe segregation of proteins in the Golgi apparatus based on their affinity for ordered membrane rafts. We rationalize these observations with a kinetic model of trafficking through the secretory pathway that implicates phase separation of organellar membranes in the kinetics of inter-organellar transport. These observations highlight a central role for lipid rafts in sorting in the secretory pathway and establish the core machinery for raft-mediated cellular trafficking from Golgi. The proposed kinetic model provides quantitative insights into the dynamics of TMD protein migration from ER to their final post-Golgi destination.

B411/P1399

ORP9 controls the abundance of accessible cholesterol in cell membranes by inhibiting OSBP at ER-TGN contacts

T. Naito[1], H. Yang[1], D. Koh[1], Y. Saheki[1,2]; [1]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, SINGAPORE, [2]Department of Molecular Physiology, Faculty of Life Sciences, Kumamoto University, Kumamoto, JAPAN.

Sterol is an essential structural component of cell membranes in eukaryotes. The dominant form of sterol in mammals is cholesterol, which is particularly enriched in the plasma membrane (PM), where it plays a critical role in cell signaling. The majority of cholesterol is transported inside the cell via vesicular transport. However, growing evidence suggests that non-vesicular lipid transport facilitated by lipid transfer proteins maintains cellular cholesterol distribution by transporting a biochemically distinct pool of cholesterol, known as accessible cholesterol, between various organelles and cell membranes. Previous studies from our group and others showed that evolutionarily conserved lipid transfer proteins, including GRAMD1s (GRAMD1a/1b/1c) and oxysterol binding protein (OSBP), contribute to this process. GRAMD1s transport accessible cholesterol from the PM to the endoplasmic reticulum (ER) when the levels of accessible PM cholesterol are elevated, while OSBP transports such cholesterol from the ER to the trans-Golgi network (TGN) via phosphatidylinositol 4-phosphate (PI4P)-driven countertransport. However, how the activities of these proteins are coordinated by the cell remains unclear. Here, we performed a mini-screen, using a novel cholesterol biosensor derived from the cholesterol sensing domain of GRAMD1s (GRAM-H), and identified ORP9, a member of OSBP-related proteins (ORPs), as a critical regulator of OSBP function. We generated HeLa cells lacking ORP9 and GRAMD1s and found that these quadruple knock-out cells showed massive accumulation of accessible cholesterol in the PM and the TGN membrane. Strikingly, inhibition of OSBP function in these cells was sufficient to remove accessible cholesterol from these membranes. Mechanistically, ORP9 extracts PI4P from the TGN and inhibits OSBP-mediated cholesterol transport at ER-TGN contacts. In the absence of ORP9, PI4P accumulates in the TGN, causing hyperactivation of OSBP-mediated cholesterol transport to the TGN as well as to the post-TGN membranes, including the PM. Furthermore, GRAMD1 aberrantly populates at ER-TGN contacts in cells lacking ORP9, suggesting its additional role in extracting cholesterol from the TGN. Our results show that ORP9 controls PI4P levels in the TGN, thereby coordinating the activities of OSBP and GRAMD1s at ER-TGN contacts to maintain cellular cholesterol distribution.

B412/P1400

Cholesterol-enriched domains contribute to myoblast migration by controlling focal adhesions

J. Vanderroost[1], N. Avalosse[1], D. Mohammed[2], P. Van der Smissen[1], D. Hoffmann[1], D. Alsteens[2], D. Tyteca[1]; [1]UCLouvain, Brussels, BELGIUM, [2]UCLouvain, Louvain-la-Neuve, BELGIUM.

Myoblast migration is crucial for myogenesis and muscle regeneration and requires membrane deformation, calcium ion exchanges and cytoskeleton rearrangements, a.o. However, the spatiotemporal control of myoblast migration remains elusive. This study tests whether and how plasma membrane (PM) cholesterol (chol) and/or sphingolipids can perform this role using mouse C2C12 myoblasts. Multiple lipid labeling and high-resolution confocal microscopy evidenced two types of lipid domains at the outer PM leaflet of resting myoblasts: sphingomyelin/chol/ganglioside GM1 (SM/chol/GM1)- and chol-enriched domains. Both domains showed a lower membrane stiffness than the bulk membrane as evidenced by atomic force microscopy. Super-resolution confocal microscopy

showed that upon migration, chol and SM polarized at the front whereas GM1 polarized at the rear. Spontaneous migration (IBIDI chambers) and oriented migration towards a specific gradient (transwell assays) were both reduced after chol depletion by methyl-beta-cyclodextrin (mβCD) but not after sphingolipid depletion by fumonisin B1. The effect of mβCD on myoblast migration could be related to sphingolipid opposite polarization and/or to the abundance of chol-enriched domains as both parameters were specifically reduced by mβCD treatment. To discriminate between those two possibilities, the effects of actin cytoskeleton disruption by cytochalasin D (cytoD) on myoblast migration, sphingolipid polarization and lipid domains abundance were evaluated in parallel. Compared to mβCD, this treatment induced a similar or even lower decrease of sphingolipid polarization contrasting with a stronger decrease of spontaneous migration and chol-enriched abundance, suggesting the specific implication of chol-enriched domains for myoblast spontaneous migration. To understand the mechanism behind, membrane lipid lateral diffusion and focal adhesions (FAs) abundance, size and distribution were respectively evaluated by fluorescence recovery after photobleaching (FRAP) and immunolabeling. Whereas a lipid mobility-based mechanism can be excluded, both mβCD and cytoD decreased the number of FAs in perfect correlation with the chol-enriched domain abundance and spontaneous migration. In addition, inhibition of actin polymerization abrogated the mβCD-induced impaired FAs and chol-enriched domains, supporting their interplay. This study reveals the contribution of chol-enriched domains in myoblast spontaneous migration possibly by controlling FAs.

B413/P1401

APOE Targets Astrocyte Lipid Droplets to Modulate Triglyceride Saturation and Droplet Size

I. Windham, E. D. Wallace, C. Wagner, S. Cohen; University of North Carolina-Chapel Hill, Chapel Hill, NC

APOE is the strongest genetic risk factor for late-onset Alzheimer's Disease (AD). Individuals with one or two copies of the *APOE4* variant are at an increased risk of developing AD compared to those homozygous for the *APOE3* variant. In the central nervous system, apolipoprotein E (APOE) is highly expressed in astrocytes. Astrocytes secrete APOE-containing lipoproteins, which mediate bidirectional lipid transport between astrocytes and neurons. However, the role of APOE in intracellular lipid metabolism is not understood, nor is the mechanism by which the *E4* variant predisposes individuals to AD.

Surprisingly, we discovered that a subset of APOE diverts from the secretory pathway and instead targets cytoplasmic lipid droplets (LDs) during lipogenesis. This raises the possibility that APOE plays previously unrecognized roles in cellular lipid metabolism by acting directly on LDs. Using immunogold electron microscopy and live-cell imaging, we found that APOE translocates onto LDs via membrane bridges at ER-LD contact sites. Expression of APOE truncation mutants indicates that both the N and C terminal domains of APOE play a role in LD targeting; the N-terminus is required for trafficking to LDs while the C-terminus is required for LD binding. Lipidomics studies demonstrate that *APOE* knockdown increases unsaturated triglycerides. Increased unsaturation of triglycerides stored in LDs has previously been shown to promote larger LDs. Consistent with this idea, knockdown of *APOE* causes fewer, larger LDs. Expression of a chimeric construct targeted only to LDs rescues the LD size of the knockdown, indicating that LD-localized APOE is responsible for the droplet phenotype. Like *APOE* knockdown cells, we observed that *APOE4* cells have fewer and larger LDs and more unsaturated triglyceride than APOE3-expressing or *APOE* knockdown cells. The larger LDs observed in E4 cells are more sensitive to lipid peroxidation than E3 LDs. Our data indicate that APOE can localize to cytoplasmic LDs in astrocytes,

where it promotes triglyceride saturation and modulates LD size. Loss of APOE reduces triglyceride saturation, while APOE4 is a toxic gain of function variant that causes increased triglyceride unsaturation and larger LDs. We propose that astrocytes with large, unsaturated LDs caused by *APOE4* are sensitized to lipid peroxidation or lipotoxicity, which could contribute to AD risk.

B414/P1402

Chronic Ethanol Exposure Induces an Increase in the LD Surface Enzyme HSD17B13 via a Disruption of the Ubiquitin-Proteasome Transporter Protein VCP

R. Schulze[1], S. Weller[1], S. Sen[1], C. Casey[2], P. Thomes[2], **M. McNiven**[1]; [1]Mayo Clinic, Rochester, MN, [2]University of Nebraska Medical Center, Omaha, NE.

BACKGROUND: Steatotic hepatocytes store and catabolize substantial numbers of large neutral lipid-storage organelles termed lipid droplets (LDs) upon which associate scores of enzymes and adapter proteins that regulate LD dynamics. The process by which the synthesis or utilization of these organelles is perturbed during alcohol-induced fatty liver of steatohepatitis AFLD/ASH is poorly defined. METHODS**:** Mass Spectrometry of LDs isolated from EtOH damaged rat livers, combined with immunofluorescence microscopy of cultured hepatocytes, and Western blot analysis demonstrated a substantial number of LD-associated proteins with a marked increase in ubiquitination in response to chronic EtOH exposure. From this initial finding we predict that this alteration in the LD-proteome can lead to a disruption of LD catabolism and hepatocellular steatosis. RESULTS**:** Further analysis of our Mass Spec results revealed a substantial 10- fold reduction in the Valosin Containing Protein (VCP) from the LD surface in EtOH damaged hepatocytes. VCP is a well-studied “protein segregase” that is known to remove ubiquitinated proteins from cell membranes for transport to the proteosome. Western blot analysis of LDs isolated from control or EtOH-damaged hepatocytes confirmed this loss of VCP while high resolution confocal imaging of EtOH-treated cells expressing GFP-VCP showed a dramatic 5-fold reduction in LD-association. To define how changes in VCP might lead to alteration in the ubiquitinated-LD proteome and hepatocellular steatosis we tested for changes in the LD enzyme HSD17B13, known to play an important role in hepatic steatosis. We find that HSD17B13 is a ubiquitinated protein that exhibits an increased association with LDs from EtOH-damaged cells. Importantly, disruption of VCP function by a well characterized chemical inhibitor (DBeQ), or siRNA knock down, leads to an increase in HSD17B13 association with LDs and a doubling in hepatocellular LD content. In CONCLUSION, this study demonstrates that chronic EtOH exposure disrupts the association of a known ubiquitin segregase from the LD surface leading to a marked increase in the ubiquitination of LD-surface proteins such as HSD17B13 and that this increase is concomitant with hepatocellular steatosis. These findings provide new insights into the cellular mechanisms by which EtOH exposure disrupts normal hepatic lipid catabolism.

B415/P1403

Visualizing & dissecting mechanisms driving liquid-crystalline lipid droplet (LD) biogenesis

S. Rogers[1], L. Gui[1], A. Kovalenko[1], V. Zoni[2], M. Carpentier[3], K. Ramji[3], K. Ben Mbarek[3], A. Bacle[3], P. Fuchs[3], P. Campomanes[2], E. Reetz[1], N. Ortiz Speer[1], E. Reynolds[1], A. Thiam[3], S. Vanni[2], D. Nicastro[1], **M. Henne**[1]; [1]UT Southwestern Medical Center, Dallas, TX, [2]University of Fribourg, Fribourg, SWITZERLAND, [3]Laboratoire de Physique de l'École Normale Supérieure, Paris, FRANCE.

Lipid droplets (LDs) are the primary energy storage organelles of cells, and serve as reservoirs for triglycerides (TGs) and sterol-esters (SEs). These lipids typically exist as isotropic liquids, but recent findings indicate that SEs including cholesterol-ester can transition from amorphous states into so-called smectic liquid-crystalline phases within LDs. Liquid-crystalline SE deposits are commonly observed in metabolic diseases such as atherosclerosis, obesity, NAFLD, and coronary heart disease, but the metabolic cues that trigger their formation are poorly understood. A key knowledge gap is also how changes in the phase properties of LD lipids influence the LD proteome, which ultimately gives LDs their cellular function. In this multi-disciplinary study, we combine *in situ* cryo-electron tomography, molecular dynamics simulations, cell biology, and biochemistry to reveal that TG lipolysis is a major driver in the formation of liquid-crystalline lattice LDs (LCL-LDs). Furthermore, we find that LDL-LD biogenesis selectively alters the LD proteome, with a sub-set of resident LD proteins translocating to the ER network, whereas others remain LD-associated. Mechanistically, we find that TG lipolysis alters the TG:SE ratio within LDs, promoting SE accumulation and its transition from an isotropic to liquid-crystalline phase. Molecular dynamics simulations reveal that TG depletion promotes spontaneous TG and SE de-mixing, additionally altering the lipid packing of the LD phospholipid monolayer surface. Fluorescence imaging and proteomics further reveal that liquid-crystalline phases are associated with selective remodeling of the LD proteome. A sub-set of canonical LD proteins including Erg6 re-localize to the ER network, whereas others remain LD-associated. Model Type I (ERTOLD) protein LiveDrop also redistributes from LDs to the ER, suggesting liquid-crystalline phases influence ER-LD inter-organelle transport, and may affect Type I (ERTOLD) LD protein localization. Proteomics data using isolated LDs also indicates that non-canonical proteins not normally targeted to LDs can associate with LCL-LDs, suggesting liquid-crystalline LDs may interact with non-LD protein machinery. Collectively, we propose that TG mobilization alters the TG:SE neutral lipid ratio of LDs, driving lipid demixing within the LD interior and ultimately the phase transition of LD SEs into a liquid-crystalline phase, which selectively alters the LD surface proteome.

B416/P1404

The role of lipid droplets in muscle hypertrophy

S. Hayashi, S. Yonekura; Shinshu University, Nagano, JAPAN.

Muscle hypertrophy is caused by various external factors, including exercise and the intake of some nutrients, for example vitamin D, zinc and source of protein. Recently, there has been a great deal of effort in promoting skeletal muscle hypertrophy, and *in vitro* and *in vivo* reports have shown that appropriate heat stimulation is beneficial to the hypertrophy of skeletal muscle. It was reported that C2C12 mice myoblast cultured under mild heat stimulation (39℃) show muscle hypertrophy compared to standard temperature (37℃). However, the detailed mechanisms that mild heat stimulation promotes hypertrophy are unknown. We observed heat-stimulated myoblasts form a lot of lipid droplets (LD) in inside. Therefore, we hypothesized that mild heat stimulation contributes to

hypertrophy through promoting LD formation. The aim of this study was to demonstrate whether heat-induced LD formation facilitates C2C12 skeletal muscle hypertrophy or not. Firstly, in order to track changes over time, we observed LD in muscle cell at day1, 3, 5 after differentiation using fluorescent immuno-staining. We found that LD number in heat-stimulated cell significantly increased compared to non-heat cell at day1. In addition, *Plin2*, *Plin3*, *Plin5*, which are LD membrane localized proteins, those gene expression in heat-stimulated cell also significantly increased compared to non-heat cell at 12 hours after differentiation. However, almost LD in heat-stimulated cell disappeared after day3 when myotube forms. These results suggest that LD formation at early differentiation stage is crucial event in order to promote hypertrophy by mild heat stimulation. Finally, we evaluated whether muscle hypertrophy attenuate or not by inhibiting LD formation. We confirmed that LD number under mild heat stimulation didn't increase at day1 by *Plin2* siRNA transfection, which molecule is LD membrane localized protein and essential for LD formation. Interestingly, we observed that myotube formation significantly decreases in *Plin2* siRNA group at day3 compared to non-heat cell. These results indicated that LD formation is essential for mild heat-induced muscle hypertrophy.Taken together, these findings provide evidence that mild heat stimulation contributes to hypertrophy through promoting LD formation. Currently, we investigate how mild heat stimulation promotes LD formation and how LD affects hypertrophy.

B417/P1405

Modeling Congenital Generalized Lipodystrophy in C. elegans

X. Bai[1], L. Huang[2], S. Chen[3], B. Nebenfuehr[1], B. Wysolmerski[4], J. Wu[5], S. Olson[4], A. Golden[1], C. Wang[3]; [1]National Institutes of Health, Bethesda, MD, [2]Institute of Plant and Microbial Biology, Academia Sinica, Nankang, Taipei, TAIWAN, [3]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, TAIWAN, [4]Department of Biology and Program in Molecular Biology, Pomona College, Claremont, CA, [5]Department of Clinical Laboratory Science and Medical Biotechnology, National Taiwan University, Taipei, TAIWAN.

The metabolic homeostasis of fatty acids is important for human health. The lipid droplets (LDs) serve as the major energy reservoirs to maintain fat homeostasis in nearly all cell types. Seipin, a conserved endoplasmic reticulum protein, plays pivotal roles during LDs biogenesis. Clinical studies demonstrated that SEIPIN contributes to congenital generalized lipodystrophy, which is a disorder with the excessive absence of adipose tissue and muscular dystrophy. Dysfunction of SEIPIN also affects both adipocyte development and lipid storage in non-adipose tissues. However, the mechanisms of Seipin associated diseases are poorly understood. Here, we functionally characterized *seip-1*, the sole ortholog of SEIPIN in the nematode model *Caenorhabditis elegans*, using CRISPR/Cas9 gene editing, transcriptional assay, lipidomic analyses, and other genetic tools. We observed that SEIP-1 is associated with LDs and is crucial for controlling LDs size and lipid homeostasis. The depletion of seip-1 disrupted fatty acid synthesis, and particularly reduced the ratio of polyunsaturated fatty acids (PUFAs) in the *C. elegans* embryonic fatty acid pool, suggesting an important role of SEIP-1 in maintaining fatty acid homeostasis. Homozygous *seip-1* mutants displayed penetrant embryonic lethality, which is caused by the disruption of the lipid-rich permeability barrier, the innermost layer of the *C. elegans* embryonic eggshell. Intriguingly, dietary supplementation of selected n-6 PUFAs rescued the embryonic lethality and defective permeability barrier in *seip-1* deletion mutants. Accordingly, we propose that SEIP-1 may maternally regulate LD biogenesis and lipid homeostasis to orchestrate the formation of the permeability barrier for eggshell synthesis during embryogenesis. We then edited the gene to mimic an orthologous lipodystrophy

patient variant *seip-1(A185P)* in *C. elegans*, which also resulted in embryonic lethality and could be rescued by PUFA supplementation. The expression pattern indicated that *seip-1(A185P)* may only compromise SEIP-1 protein function instead of affecting trafficking and cellular localization of SEIP-1 *in vivo*. The penetrant phenotype allowed us to perform a chemical mutagen-based suppressor screen to revert or suppress the embryonic lethality in the *seip-1(A185P)* mutant. A total of five suppressor candidate lines were isolated in a small trial screen by screening for a significant restoration of the embryonic viability. We identified a set of candidate suppressor alleles in each suppressor line from our genomic mapping and whole-genome sequencing studies. These newly identified suppressing factors may lead to the new direction of LD biology, and shed light on future therapies for lipodystrophies.

B418/P1406

New chemical genetic tools selectively illuminate choline phospholipid distribution in organelle membranes

W. Moore[1], R. J. Brea-Fernandez[2], N. K. Devaraj[1], I. Budin[1]; [1]University of California, San Diego, San Diego, CA, [2]Universidade da Coruña, Coruña, Spain

The membrane bilayer is a defining feature of cells and contains complex mixtures of individual lipid building blocks. While early models for membrane structure assumed a homogeneous distribution of lipid species within the bilayer, mounting evidence has illustrated that both lateral heterogeneity and interleaflet lipid asymmetry are key features of membrane architecture and function. However, there is still a poor understanding of how lipids are organized and transversely distributed between bilayers in eukaryotic cells. In large this has been due to a lack of non-destructive tools capable of imaging lipids in an organelle-specific manner and resolving their transbilayer orientation. Here we have developed a chemical genetic tool capable of quantitatively imaging lipid subpopulations in living cells. The method utilizes bioorthogonal click chemistry to label phospholipid headgroups with a fluorogenic dye, whose fluorescent emission in genetically controllable through the expression of a fluorogen-activating protein. As a test case we show that bioorthoganol incorporation of azido choline into cellular membranes can be used to selectively image and measure subcellular choline phospholipid (CPL) pools in live cells. Moving forward, we use this tool to investigate how lipid biosynthesis and trafficking influence CPL content and organization in specific membrane compartments.

B419/P1407

Surface Cholesterol-Enriched Domains Specifically Promote Invasion of Breast Cancer Cell Lines by Controlling Invadopodia and Extracellular Matrix Degradation

M. Maja[1], D. Mohammed[2], A. C. Dumitru[2], S. Verstraeten[3], M. Lingurski[4], M. Mingeot-Leclercq[3], D. Alsteens[2], D. Tyteca[1]; [1]de Duve Institute, UCLouvain, Brussels, BELGIUM, [2]Louvain Institute of Biomolecular Science and Technology, UCLouvain, Louvain-la-Neuve, BELGIUM, [3]Cellular and Molecular Pharmacology Unit, UCLouvain, Brussels, BELGIUM, [4]Institut de Duve, UCLouvain, Brussels, BELGIUM.

Breast cancer is the most common neoplasm and the primary cause of cancer death in women. Mortality is almost exclusively due to metastatic disease. Better molecular characterization of the primary tumor is therefore crucial for a good prediction of the clinical outcome. Although breast cancer cells present an altered cholesterol content, how this change influences plasma membrane biophysical properties and cancer cell invasion is unknown, mainly due to difficulties to investigate cholesterol both quantitatively and qualitatively and to compare isogenic cell models. In this study, we benefited from

endogenous cholesterol probes, confocal vital imaging and atomic force microscopy to investigate if and how plasma membrane cholesterol content and distribution contribute to breast cancer cell invasion. We started with the MCF10A cell line series (non-tumorigenic MCF10A, pre-malignant MCF10AT and malignant MCF10CAIa cells) as an isogenic model of breast cancer progression and showed that, compared to A and AT cells, malignant CAIa cells exhibit (i) a decreased cytocortex stiffness, (ii) an increased plasma membrane stiffness, (iii) a high cholesterol content at the dorsal cell surface and its distribution in submicrometric domains and (iv) a high invasive potential. To explore the involvement of cholesterol in cell invasion, cells were partially cholesterol-depleted with methyl-β-cyclodextrin (mβCD) and tested for Matrigel invasion assay. We found a specific and reversible inhibition of the malignant cell invasion, in perfect correlation with the residual cholesterol content. Mechanistically, dorsal cholesterol-enriched domains can be endocytosed and reach the cell ventral face where they participate in invadopodia formation and extracellular matrix (ECM) degradation, as revealed by the mβCD-induced decreased abundance and size of cortactin-positive invadopodia structures and of fluorescent gelatin degradation areas. We then extended our research to MDA-MB-231, Hs578T and BT549, 3 invasive triple-negative breast cancer cell lines, and found similar cholesterol-enriched domains and dependence of invadopodia outgrowth, ECM degradation and cell invasion to cholesterol. Our data provide new clues on the molecular mechanisms of breast cancer invasion and open the way for the development of new therapies based on specific membrane cholesterol targeting in cancer cells.

B420/P1408

Coupling of protein condensates to ordered lipid domains determines functional membrane organization

I. Levental[1], H. Wang[1], S. H. Chan[1], M. K. Rosen[2], J. Ditlev[3], K. R. Levental[1]; [1]Molecular Physiology and Biophysics, University of Virginia, Charlottesville, VA, [2]Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, TX, [3]Department of Biochemistry, University of Toronto, Toronto, ON, CANADA.

During T-cell activation, the transmembrane adaptor Linker of Activation of T-cells (LAT) forms biomolecular condensates with Grb2 and Sos1, facilitating signaling. LAT has also been associated with cholesterol-rich condensed lipid domains. However, the potential coupling between protein condensation and lipid phase separation and its role in organizing T-cell signaling were unknown. Using a combination of reconstitution and live cell imaging, we report that LAT/Grb2/Sos1 condensates reconstituted on model membranes can induce and template lipid domains, indicating strong coupling between lipid- and protein-based phase separation. Correspondingly, activation of T-cells induces protein condensates that associate with and stabilize raft-like membrane domains. Inversely, lipid domains nucleate and stabilize LAT protein condensates in both reconstituted and living systems. This coupling of lipid and protein assembly is functionally important, since uncoupling of lipid domains from cytoplasmic protein condensates abrogates T-cell activation. Thus, thermodynamic coupling between protein condensates and ordered lipid domains regulates the functional organization of living membranes.

B421/P1409

Membrane tension gradients induce membrane mechanical lysis in living bacteria

D. Gomez[1], G. Hocky[1,2], E. Rojas[1]; [1]Biology, New York University, New York, NY, [2]Chemistry, NYU, New York, NY.

Environmental fluctuations represent a continuous thread for single-celled organisms, increasing lysis probability, the phenomenon of cell membrane rupture that leads to cell death. To survive, bacteria adapt their plasma membrane mechanical properties and activate their tension-sensing mechanosensitive (MS) channels. While the mechanisms of cell survival using MS channels are well known, critical features of membrane biology linked to cell survival have been largely overlooked. Here we demonstrate that mechanical lysis in living cells is supported by tension gradients, experiencing a maximum at the heterogeneously distributed protein-lipid interface. Furthermore, changes in membrane fluidity affect the protein distribution within the membrane, which induces a more homogeneous protein distribution and higher survival probabilities. Our work highlights lipid fluidity and membrane protein's vital role in cell lysis, a potentially vulnerable feature that could be used in future antibiotic designs.

B422/P1410

Investigating the organisation of the asymmetric plasma membrane using a novel solvatochromic reporter

C. Patra[1], M. Singh[2], T. V. Zanten[1], P. P. Singh[2], R. Vishwakarma[2], S. Mayor[1]; [1]National Centre for Biological Sciences, TIFR, Bangalore, INDIA, [2]CSIR-IIIM, Jammu, INDIA.

The plasma membrane of an animal cell is a heterogenous mixture of lipids and proteins that exhibits both lateral heterogeneity and transverse asymmetry in composition with Phosphatidylserine(PS) and Phosphatidylethanolamine(PE) being predominantly in the inner leaflet and Sphingomyelin(SM) and Phosphatidylcholine(PC) in the outer leaflet[1]. The membrane is a complex mixture of lipid species with a range of headgroups and degrees of acyl-chain unsaturation, influencing the properties of the bilayer such as fluidity and ordering and consequently the function of embedded membrane proteins[2] . The outcome of particularly the leaflet composition asymmetry on membrane organisation, local physical properties and function has been explored in limited ways[3]. In this study, we have probed the presence of liquid-ordered (l_o) domains in each leaflet and their transbilayer registry that can regulate information transfer, in live cells by designing a membrane localised probe that is sensitive to lipid order. This fluorescent probe, C3L, uses the solvatochromic reporter Laurdan as a template and reports on changes in membrane lipid order of complex compositions such as Giant Plasma Membrane Vesicles(GPMVs), while exhibiting a preference for l_o-domains. C3L distributes across both leaflets in the live cell membrane, regulated by flippases and scramblases and may be selectively and robustly confined to the inner leaflet after back extraction. Using this reporter we find that depletion of SM has a more outer-leaflet restricted effect, PS depletion leads to effects on both leaflets with the inner leaflet being more affected. This suggests that PS and SM play important roles in building and maintaining domains as well as coupling them across the bilayer. The organisation of the active composite membrane is hypothesised to be driven by the activity of the juxtaposed cortical cytoskeleton along with composition[4]. Using C3L, we see preliminary evidence for this hypothesis. We find that the lipid order distribution in live cells exhibits domains of much smaller scale and broader order distribution in the membrane, whereas it is rather homogenously disordered at the same temperature in GPMVs derived from the same cells. This

provides support for active de-mixing in membranes of living cells[5]. Using this tool we intend to explore the fundamental principles of regulation of membrane properties by the membrane composition and cytoskeletal activity and its impact in tuning cellular function.
1.Verkleij, A. J. *et al. BBA - Biomembr.* **323**, 178-193 (1973). 2.Marquardt, D., Geier, B. & Pabst, G. *Membranes (Basel).* **5**, 180-196 (2015). 3.Kiessling, V., Wan, C. & Tamm, L. K. *Biochim. Biophys. Acta - Biomembr.* **1788**, 64-71 (2009). 4.Raghupathy, R. *et al. Cell* **161**, 581-594 (2015). 5.Saha, S. *et al. PNAS* **119**(2022).

B423/P1411

Sphingolipids protect ergosterol in the *Leishmania major* membrane from sterol-binding toxins

C. Haram[1], S. Moitra[1], R. Keane[1], M. Kuhlmann[2], C. Frankfater[2], F. Hsu[2], S. M. Beverley[2], K. Zhang[1], P. Keyel[1]; [1]Department of Biology, Texas Tech University, Lubbock, TX, [2]Washington University, St. Louis, MO.

Leishmaniasis causes 1.5-2 million cases and 70,000 deaths annually worldwide, and is caused by protozoan parasites in the genus *Leishmania*. One key treatment is amphotericin B. Amphotericin B suffers from drawbacks like renal toxicity and drug resistance. It targets ergosterol, which maintains *Leishmania* membrane integrity and interacts with other lipids in the membrane, including Inositol Phosphorylceramide (IPC), the major sphingolipid in *Leishmania*. Sphingolipids protect sterols from toxin accessibility in mammalian cells, but it is unclear if this is conserved in *Leishmania*. Here, we used the cholesterol-dependent cytolysins (CDCs) streptolysin O and perfringolysin O to test the hypothesis that sphingolipids restrict ergosterol plasma membrane accessibility. Using flow cytometry, we showed that CDCs bound equally well to *L. major* genetically lacking IPC synthesis enzymes compared to wild type, indicating IPC does not restrict ergosterol accessibility. While CDC binding was IPC and ceramide independent, CDC cytotoxicity was enhanced in the absence of IPC. Ceramide reduced perfringolysin O, but not streptolysin O, dependent cytotoxicity. This difference was due to membrane binding determinants in the toxin lipid binding L3 loop. We conclude that the L3 loop can sense ceramide. Ceramide was sufficient to protect *L. major* promastigotes from amphotericin B. Thus, *L. major* serves as a genetically tractable model organism for understanding toxin-membrane interactions. Furthermore, our findings suggest removing ceramide may enhance ergosterol-targeting drug efficacy.

B427/P1412

Cytoplasmic tail of the fission yeast polycystin Pkd2 regulates its clustering in the eisosomes

M. Malla, P. Chowdhury, D. Sinha, B. T. Bisesi, Q. Chen; The University of Toledo, TOLEDO, OH.

Cytoplasmic tail of the fission yeast polycystin Pkd2 regulates its clustering in the eisosomes Mamata Malla, Pritha Chowdhury, Debatrayee Sinha, Benjamin Thomas Bisesi, and Qian Chen*Department of Biological Sciences, The University of Toledo, Toledo, OH, 43606
Polycystins are a family of evolutionarily conserved ion channels. Mutations of either human polycystins, PC1 or PC2, lead to a common genetic disorder Autosomal Dominant Polycystic Kidney Disease. The unicellular model organism *Schizosacchromyces pombe* possesses a single polycystin homolog Pkd2 which localizes to the plasma membrane and regulates cytokinesis, the last step of cell division. Here, we carried out a functional analysis of Pkd2 based on its Alphafold predicted tripartite structure consisting of N-terminal lipid-binding (LBD), central transmembrane (TMD) and C-terminal cytoplasmic (CCD) domains. LBD assumes a unique immunoglobulin-fold, while TMD contains nine transmembrane

helices. Both were essential, but CCD was not. However, without CCD, Pkd2 clustered on the cell surface in eisosomes, a furrowing microdomain on the plasma membrane. Inhibiting eisosome assembly prevented the clustering, while disrupting ER-PM contacts further increased it. This eisosome-clustering of Pkd2 was further supported by the negative genetic interaction between mutants of *pkd2* and the eisosome gene *pil1*. The tripartite structure of Pkd2 is also preserved in the *S. japonicus* Pkd2 ortholog which can fully replace Pkd2. Our discovery suggests a potential mechanism of regulating polycystin function through clustering in the microdomains of the plasma membrane.

B428/P1413

Perilipin facilitates lipophagy in yeast

J. R. Monala, B. Folger, J. M. Goodman; Pharmacology, UTSW Dallas, Dallas, TX.

Lipid droplets (LDs) are ubiquitous cell organelles that are vital for energy homeostasis for which they constantly undergo anabolic and catabolic processes based on cellular metabolic need. The mechanism of LD biosynthesis is becoming understood but the molecular mechanism of LD degradation is far less clear. Since the discovery of lipophagy in liver in 2009, many studies have attempted to understand the underlying mechanism. In yeast, the predominant form of lipophagy is microlipophagy (µlipophagy), in which LDs are directly engulphed by the vacuole. We have been trying to identify LD surface proteins that mediate µlipophagy in yeast, and report here the involvement of Pln1, a protein we previously identified in yeast as a member of the perilipin family, important for LD biogenesis, morphology and stability. Perilipins have three known domains, PAT, Hydrophobic, and Repeats. We find that lipophagy, measured by fluorescence microscopy and by vacuolar release of GFP from Erg6, is delayed or blocked in the pln1 null strain under nitrogen starvation or glucose restriction. Deletions of Pln1 were generated to identify regions necessary and sufficient for lipophagy. The PAT and Hydrophobic domains were necessary for LD targeting and stability and are not easily assessed for lipophagy, while the Repeats domain could be deleted with no apparent loss of function. However, a region of ~120 residues (which we term GAP2) after Repeats lost significant lipophagy function while not interfering with LD targeting or stability. Yeast µlipophagy can be dissected into LD binding to vacuoles, engulfment, and internalization. Fluorescence microscopy suggest that Pln1 is not required for binding but is required for a subsequent step such as engulfment. Lipophagy is inhibited but not fully blocked in the absence of Ypt7 (Rab7), a known factor in vacuolar fusion events, suggesting it is important but not absolutely necessary for this lipophagy. Our studies suggest that Pln1 controls an intermediate but essential step in µlipophagy and that GAP2 is important for this function. We hope to identify the binding partner to Pln1 on the vacuolar surface.

B429/P1414

Regulation of mitochondrial derived compartments by unsaturated fatty acids

S. Wong, A. Hughes; University of Utah, Salt Lake City, UT

Unsaturated fatty acid levels in cells must be tightly regulated, as too much or too little both have adverse effects on cellular health and animal physiology. The deregulation of cellular lipids is a major cause of metabolic diseases, a hallmark of metabolic syndrome, and a risk factor for other diseases including diabetes and heart disease. However, the regulatory mechanisms that maintain unsaturated fatty acid levels are not completely understood, nor are the full extent of their impacts on the function of organelles, including mitochondria. Our lab discovered a new mitochondrial remodeling pathway that

responds to nutrient stress in yeast and mammals. In response to various metabolic stressors, cells generate mitochondrial-derived compartments (MDCs). MDCs form at ER-mitochondria contact sites and are cargo selective, only incorporating select mitochondrial outer membrane proteins. Upon formation, MDCs remove membrane proteins and lipid bilayers from mitochondria, and thus play a role in regulating both mitochondrial lipid and protein homeostasis. To date, it remains unclear as to what is the cellular function of MDCs, and whether MDCs are strictly responsive to alterations in amino acid metabolism or if other metabolic stressors, such as lipids, trigger MDC formation. Our recent studies are uncovering a role for unsaturated fatty acids and triglycerides in the MDC pathway. Overexpression or constitutive activation of *OLE1*, the yeast stearoyl-CoA desaturase, results in constitutive MDC formation, as well as elevated unsaturated fatty acids and triglycerides. Interestingly, heterozygous deletion of *OLE1* also results in constitutive MDC formation. These data suggest that MDCs may respond to an imbalance of lipids that affects metabolic pathways and/or membrane composition. Overall, these studies raise the exciting possibility that MDCs may form as a response to deregulated cellular lipids.

B430/P1415

Understanding the mechanisms of accessible plasma membrane cholesterol maintenance using a novel cholesterol biosensor

D. Koh[1], T. Naito[1], P. Rozario[1], F. Zhong[1,2], Y. Saheki[1,3]; [1]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore, [2]Skin Research Institute of Singapore (SRIS), Singapore, Singapore, [3]Department of Molecular Physiology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Cholesterol is an essential component of eukaryotic cell membranes, where it plays crucial roles in membrane integrity and cell signaling. While vesicular transport contributes to bulk transport of cholesterol in cells, this mode of transport is often slow and non-selective. Recent evidence suggests that non-vesicular lipid transport facilitated by lipid transfer proteins (LTPs) controls the transport of a biochemically defined fraction of cholesterol, termed “accessible” cholesterol, between the plasma membrane (PM) and other organelles, including the endoplasmic reticulum (ER) (where cholesterol is synthesized) to maintain cholesterol homeostasis. However, the molecular basis of accessible cholesterol transport remains poorly understood. A family of evolutionarily conserved sterol-binding LTPs known as GRAMD1s (GRAMD1a/1b/1c) plays an important role in transporting accessible cholesterol from the PM to the ER. Here, we engineered the N-terminal cholesterol sensing domain of GRAMD1b (i.e., the GRAM domain) to a highly sensitive cholesterol biosensor and successfully visualized the distribution of accessible cholesterol in several cell types in live. To elucidate the mechanisms that are responsible for transporting accessible cholesterol to the PM, we performed a mini-screen using HeLa cells and keratinocytes and identified oxysterol binding protein (OSBP) as a critical player for maintaining the levels of accessible cholesterol in the PM. OSBP is another evolutionarily conserved LTP, which transports cholesterol from the ER to the *trans*-Golgi network (TGN) against its concentration gradients in exchange for TGN-enriched phosphatidylinositol 4-phosphate (PI4P) at ER-TGN contacts. Using live cell imaging approaches, we found that drug-induced formation of aberrant enlarged vesicles induces OSBP-dependent accumulation of accessible cholesterol on these vesicles, which is accompanied by reduction of accessible PM cholesterol. Our findings provide important insights into how the cellular distribution of cholesterol is maintained by LTPs at membrane contact sites.

B431/P1416

Coupling*de novo*lipid synthesis to ER and nuclear membrane biogenesis

J. W. Carrasquillo Rodriguez[1], S. Gao[2], O. Uche[1], M. V. Airola[2], S. Bahmanyar[1]; [1]Yale University, New Haven, CT, [2]Stony Brook University, Stony Brook, NY

The endoplasmic reticulum (ER) is the largest membrane bound organelle in cells. The ER is made up of distinct structural and functional domains, which include the nuclear envelope and peripheral ER sheets and tubules. The nuclear envelope surrounds and protects the genome, while the peripheral ER is essential for protein and *de novo* lipid synthesis. The membrane architecture of the peripheral ER is generated and maintained by membrane shaping proteins and interactions with the cytoskeleton; however, it remains unclear how the *de novo* synthesis of bilayer lipids is regulated to control the organization of the ER and nuclear envelope. Here, we focus on CTDNEP1 *(Sc Nem1)* and its regulatory binding partner NEP1R1 *(Sc Spo7)* that control the activity of a key enzyme necessary for *de novo* glycerolipid synthesis, lipin 1 (*Sc Pah1*), in human cells. Regulation of lipin 1 by the CTDNEP1/NEP1R1 complex limits *de novo* glycerophospholipid synthesis throughout the cell cycle to restrict ER size and maintain NE shape. Using a combination of protein modeling, structure-function analyses, and live cell microscopy, we show that CTDNEP1 contains an amphipathic helix necessary for its targeting to ER/nuclear envelope membranes. Deletion of the amphipathic helix of CTDNEP1 reduces its interaction with NEP1R1, impairs its ability to regulate lipin 1 and decreases its protein stability. We identify a key residue in NEP1R1 essential for binding to CTDNEP1 and for regulating its protein half-life. Our data suggest a model in which the subcellular localization of CTDNEP1 controlled by its amphipathic helix and its protein stability, which depends on its interaction with NEP1R1, regulates the synthesis of bilayer lipids that generate ER/nuclear membranes. Future work will determine whether CTDNEP1's amphipathic helix senses specific types of bilayer lipids or bulk membrane properties of the ER/nuclear envelope. Together, these data suggest a model in which regulation of *de novo* lipid synthesis is coupled to the specific lipid content of ER/nuclear envelope membranes through direct lipid sensing.

Intracellular Organization by Biomolecular Condensation

B432/P1417

Metastable Phase-separated Membraneless Organelles (MLOs) of Human Myxovirus-resistance (MxA) GTPase: Rapid Reversible Osmosensing

P. B. Sehgal; Cell Biology & Anatomy, and Medicine, New York Medical College, Valhalla, NY.

Liquid-liquid phase-separation (LLPS) leads to the formation of biomolecular condensates [also called membraneless organelles (MLOs)] in the cytoplasm and nucleus of eukaryotic cells. MLOs provide a scaffold for diverse cellular functions. The replication of many viruses in mammalian cells also involves LLPS condensates [e.g. vesicular stomatitis (VSV), rabies (Negri bodies), influenza A, Ebola, measles, Epstein-Barr, and SARS-CoV-2 viruses]. Four years ago, in studies involving the interferon-α2-induced human myxovirus-resistance protein (MxA), a dynamin-family large GTPase, we made the discovery that GFP-MxA as well as IFN-induced endogenous MxA formed biomolecular condensates in the cytoplasm. As background, MxA is a major antiviral effector of IFNs-α/β against diverse RNA- and DNA-viruses. Heterogenously sized and shaped cytoplasmic MxA condensates were observed in intact human Huh7 and A549 cells, showed homotypic fusion, were membraneless by CLEM, had a gel-like consistency by FRAP, evidenced a shell and core organization by dissection using 0.03% saponin, were often tethered to

intermediate filaments, and displayed spheroid to filament metastability in response physical pressure on live cells. Functionally, cells with cytoplasmic MxA condensates showed an antiviral phenotype against VSV and SARS-CoV2-mCherry viruses. VSV nucleocapsid protein associated with MxA condensates. Previous mutational studies had shown that GTPase activity was required for this antiviral activity but not for condensate formation. Mutants that had lost oligomerization properties also lost antiviral activity. Thus, the ability to form condensates was necessary but not sufficient for antiviral activity. Unexpectedly, MxA condensates were exquisitely sensitive to hypotonic stress similar to tissue edema. Condensates of GFP-MxA or IFN-induced endogenous MxA disassembled within 1-2 min of exposing cells to hypotonic medium (in the range ¼ to ½ tonicity), but then spontaneously reassembled 10-15 min later into new structures. Antiviral activity of MxA survived hypo-osmotic stress despite a cycle of disassembly and reassembly of MxA condensates during VSV infection. The data point to (a) cell integrity and cytoplasmic "crowding" as a critical regulator of MxA condensate structure, and (b) a complex relationship between MxA condensate dynamics and antiviral activity.

B433/P1418

Constructing Synthetic Organelles for Mammalian Cellular Engineering

W. Wang, M. Garabedian, R. Welles, M. Good; Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

Intrinsic disordered proteins (IDPs) form macromolecular membraneless compartments through phase separation to regulate cellular process. Our goal is to explore the potential of synthetic organelles in modulating cellular signaling and behaviors through targeting and sequestering endogenous proteins. These organelles offer a powerful and generalizable approach to modularly control cell decision-making with broad applications for human cellular engineering. Genes encoded synthetic scaffolds were cloned into either pcDNA3.1 vector (Addgene) for transient expression or 3rd gen lentiviral vector pLJM1 (Addgene) for lentiviral transduction. The inducible versions of scaffold were generated by swapping the constitutive promoter to either Tet-on 3G promoter (Takara Bio). Transient transfections were performed through lipofection. Cell lines with CRISPR tagged dimerization motifs at C-terminus of endogenous Rac1, Erk1 and Par6. Evaluation of condensates formation, recruitment, enrichment, and client partitioning were primarily done by data acquisition from confocal microscopy. For evaluating efficiency of inducible systems, induced and uninduced cells were run through flow cytometry. Optogenetic release of client were done by 10 sec 405nm light pulse in a 40 sec acquisition cycle for consecutively 11 cycles. We observed up to 80% of scaffold proteins and up to 60% of endogenous client ended up in the synthetic organelles via cognate recruitment motifs and no recruitment with non-matching recruitment motifs, demonstrating efficient and specific client recruitment. Lentiviral generated cells showed slightly weaker scaffold and client partitioning with greater consistency compared to transient transfection. The optogenetic controlled scaffold shows more than 50% of client release within the first 10 minutes, showing robust reversibility of the system controlled by light. We observed similar scaffold and client partitioning in the Erk1 and Par6 double knock-in cell lines generated by CRSPR with indications of phenotypical changes. Our synthetic organelles have shown highly specific and efficient recruitment of endogenous clients with optogenetic controlled reversibility, reveals its potentials as a generic tool kit for cellular engineering. Furthermore, we successfully implement our platform with lentivirus, broadening its application to therapeutic development involving primary cells and stem cells. The future direction for this project will be continuing to enhance client

partitioning, evaluating and improving its safety profile as a therapeutic approach and validating its efficacy in pre-clinical model.

B434/P1419

Amphiphilic proteins coassemble into multiphasic condensates and act as biomolecular surfactants

B. Favetta[1], F. Kelley[1], R. Regy[2], J. Mittal[2], B. Schuster[1]; [1]Rutgers University, New Brunswick, New Brunswick, NJ, [2]Texas A&M University, College Station, TX.

Membraneless organelles that form due to liquid-liquid phase separation have emerged as a mechanism for cellular organization. Such organelles may have complex morphologies, including multiphasic systems and core-shell structures, that enable complex functions. For instance, Ape1 condensates are coated by a layer of Atg19 that is required for selective autophagy in yeast. Also, the nucleolus is composed of nested liquid phases of distinct compositions and functions which are required for ribosome biogenesis. Importantly, the size and structure of such condensates must be regulated for proper biological function. We leveraged a bioengineering approach to study how amphiphilic, surfactant-like proteins may contribute to the structure of membraneless organelles. We designed and examined families of amphiphilic proteins comprising one phase-separating domain and one non-phase separating domain. Proteins included the soluble structured domain glutathione S-transferase (GST) or maltose binding protein (MBP), fused to the intrinsically disordered RGG domain from P granule protein LAF-1. When one amphiphilic protein is mixed in vitro with RGG-RGG, the proteins assemble into enveloped structures, with RGG-RGG at the core and the amphiphilic protein forming the surface film layer. Interestingly, MBP-based amphiphiles are surfactants and influence droplet size, with increasing surfactant concentration resulting in smaller droplet radii. In contrast, GST-based amphiphiles at increased concentrations co-assemble with RGG-RGG into multiphasic structures. Our in vitro and computational results support a mechanism explaining these observations based on the strength of domain-domain interactions. Varying this interaction strength by modifying the environment (e.g., salt concentration, redox state) allows us to tune the behavior of the system. We speculate that surfactant proteins may be widespread in cells. Modification of the intracellular environment may be leveraged by cells to regulate how surfactant proteins and biomolecular condensates assemble, thus affecting their structure and function.

B435/P1420

Membrane surfaces regulate assembly of a ribonucleoprotein condensate

W. T. Snead, A. P. Jalihal, T. M. Gerbich, I. Seim, Z. Hu, A. S. Gladfelter; Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Biomolecular condensates compartmentalize diverse biological processes, but little is known about how cells control the spatial organization and sizes of these structures. In cells, condensates often appear as relatively small assemblies that do not grow (coarsen) into a single droplet despite their propensity to fuse, suggesting that condensate function is closely linked with size. Here we find that ribonucleoprotein condensates, composed of the Q-rich protein Whi3 and mRNA, are tethered to the endoplasmic reticulum, prompting us to examine how membrane association impacts condensate assembly and size. Recruitment of Whi3 to synthetic membrane surfaces promotes condensate formation under physiological buffer conditions and protein concentrations. However, the growth of membrane-associated assemblies rapidly arrests within minutes of formation, resulting in size distributions that

resemble native assemblies in cells. We find that condensate size can be controlled by the temporal ordering of interactions among Whi3 and mRNAs on membrane surfaces. Moreover, the slow diffusion of membrane-bound complexes limits condensate size by reducing the probability of encounter between molecular binding partners. Thus, our experiments reveal a tradeoff between locally-enhanced protein concentration at membranes, favoring condensation, and an accompanying reduction in diffusion, restricting coarsening. Given that many condensates are tethered to endomembranes, we predict that the biophysical properties of lipid bilayers are key for controlling condensate size and function in diverse biological contexts.

B436/P1421

Dr.
A. Dall'Agnese[1], J. Platt[1], M. Zheng[1], M. Friesen[1], G. Dall'Agnese[1], E. Tevonian[2], R. Jaenisch[1], L. Griffith[2], I. Cisse[2], J. Jeppesen[3], T. Lee[1], R. Young[1]; [1]Whitehead Institute, Cambridge, MA, [2]MIT, Cambridge, MA, [3]Novo Nordisk, Copenhagen, DENMARK.

Insulin receptor (IR) signaling is central to normal metabolic control and is dysregulated in metabolic diseases such as type 2 diabetes. We have found that, in insulin-sensitive hepatocytes and adipocytes, IR is incorporated into biomolecular condensates that exert liquid-like properties and that insulin stimulation further promotes IR incorporation into condensates wherein IR is functional. In insulin-resistant cells, IR condensate dynamics, insulin-induced incorporation of IR in condensates and IR activity are all attenuated, suggesting a physico-mechanical link between insulin response and the dynamic molecular behavior of IR condensates. The observed defects in IR condensate behaviors are caused, at least in part, by an increase in oxidative stress in insulin-resistant cells, which is known to interfere with normal condensate dynamics. Treatment of insulin-resistant cells with metformin, a first-line drug used to treat type 2 diabetes, can rescue accumulation and dynamic behavior of IR condensates. This rescue corresponds with metformin's effect of reducing the levels of reactive oxygen species. The observation that IR is incorporated into biomolecular condensates during the normal response to insulin stimulation, and evidence that changes in the physico-mechanical features of IR condensates contribute to insulin resistance, have implications for improved therapeutic approaches for patients.

B437/P1422

Principles Determining Coacervation Specificity of Intrinsically Disordered Domains

R. M. Welles, M. V. Garabedian, M. C. Good; Cell & Molecular Biology, University of Pennsylvania, Philadelphia, PA.

Intrinsically disordered protein (IDP) sequences lack secondary structure and often self-assemble via liquid-liquid phase separation (LLPS) into biomolecular condensates or "membraneless organelles" (MOs). There are a number of distinct MOs within cells, each with unique composition and function. Although previous studies have identified electrostatic and cation-pi interactions as key contributors to phase separation of individual IDPs, far less is known about the mechanisms that maintain condensate identity to determine which substrates are recruited and prevent promiscuous interactions. To close this gap, we identified two orthogonal non-interacting model IDPs that can be co-expressed to form distinct compartments in cells and characterized the properties that impart selectivity to their condensates. We chose the low complexity (LC) domain of FUS and the arginine and glycine rich (RGG) repeat domain of P

granule protein LAF-1. We hypothesized that amino acid sequence features and polypeptide chain-level properties of FUS LC prevent it from mixing with RGG condensates. To test these hypotheses, we characterized modified FUS LC sequences and assessed their ability to enrich as clients within RGG condensates, using budding yeast as a living test tube. We observed that the addition and segregation of charged residues significantly enhanced mixing of FUS LC with RGG. Increasing the distance, or blockiness, of opposing charges was a crucial factor in promoting this coacervation. Local clustering or declustering of these charges additionally affected the levels of enrichment, indicating the importance of both sequence composition and ordering. Interestingly, we found that the presence of tyrosine residues is necessary but not sufficient to promote mixing. Lastly, increasing the valency of FUS LC mutant sequences lowered their apparent c_{sat} and in some cases contributed to further enrichment with the RGG scaffold. Taken together, these data uncover new rules toward a predictive framework to both understand IDP partitioning into distinct organelles and engineer orthogonal microcompartments.

B438/P1423

Manipulate and study biomolecular condensate dynamics and properties in real time to understand phase separation

S. Rahmanseresht[1], T. Simpson[1], B. Groen[2]; [1]LUMICKS, Waltham, MA, [2]LUMICKS, Amsterdam, Netherlands

Membrane-less organelles and other subcellular structures result from liquid- liquid phase separation of proteins and RNA. These include stress granules, nucleoli, RNA-transport granules, and possibly heterochromatin formation. Studies have progressively contested the conventional notion that the protein machinery is homogeneously distributed in the cytoplasm - like a soup of soluble molecules and membranous organelles. Instead, they suggest a formation of structures through multivalent interactions associated with specific protein regions. Understanding the formation of these structures, their physical properties, and mechano-chemical interactions have aided our understanding of several cellular processes. On top of that, the link between droplet formation and medical conditions, such as neurodegenerative amyotrophic lateral sclerosis (ALS) and cancer, has enhanced the need for appropriate assays that can assess related properties. In these cases, proteins can aggregate to promote a successive solidification of droplets, forming gel-like or irreversible solid structures known as amyloid fibrils (plaques). While we have come a long way in understanding such processes, these droplets are extremely dynamic, dissolving and forming in response to cues that we do not yet fully understand. As a result, the proponents of liquid-liquid phase separation have been unable to capture the dynamics and properties of these droplets with the current techniques. We will introduce you to a unique approach that correlates optical tweezers and the latest imaging techniques to study phase separation properties in real time. We show you how you can use the Optical Tweezers combined with Fluorescence Microscopy (C-Trap) to investigate the assembly and disassembly of droplets and the properties that can lead to the pathological solidification of these structures. The optical tweezers enable you to trap micron-sized particles, such as protein droplets or other condensates, with a highly focused laser beam and fuse them to study their fusion dynamics with a high temporal resolution. The correlated fluorescence microscopy lets you visualize the trapped biocondensates and study their dynamics, as the droplet structure is manipulated in real time. The C-Trap is a powerful tool that complements and expands the phase separation field allowing the users to study physiologically relevant conditions in real time.

B439/P1424

Condensation of ER-Golgi interface component Trk-fused gene (TFG) into vesicle-like structures

W. R. Wegeng, S. M. Bogus, K. S. M. Noori, A. M. Ernst; University of California, San Diego, La Jolla, CA

The mechanisms that allow for specific contacts between organelles in human cells, which often coincide with a distinct membrane topology, remain largely unknown. One such contact is formed between the ER and Golgi, which together form the early secretory pathway that controls secretion of biosynthetic cargo. At this interface, both ER donor membranes as well as acceptor Golgi membranes are concave, forming a nearly 300-nm spherical interface. It is not known how this anatomy is achieved or what its purpose is. In search of candidate proteins that could structure the ER-Golgi interface, we quantified the local concentrations of multiple interface components by combining segmentation of immunolabeled HeLa cells with published proteomics data. COPII coat components achieve local concentrations in the mM range, which was rivaled only by a similarly high concentration of the protein Trk-fused gene (TFG). TFG localized between ER exit site (ERES) and cis-Golgi markers, and the number of TFG-positive foci in HeLa cells matched those observed for ERES components, suggesting that TFG serves as a cytoplasmic extension of ERES. We found that upon overexpression, TFG undergoes a liquid-liquid phase transition, forming micron-sized condensates that exhibit high protein mobility as well as hallmarks of liquidity. Surprisingly, the distribution of TFG within condensates is highly anisotropic, assuming a sponge-like appearance both in induced condensates *in vivo* and in supersaturated solutions of TFG *in vitro*. Notably, individual TFG condensates readily form under physiological conditions and at critical concentrations well below its average cellular concentration. Upon forming, individual TFG condensates assume the shape of vesicle-like hollow spheres that closely match the dimensions of the ER-Golgi interface. These condensates are permeable for dextrans that mimic the molecular weight of COPII components but are impermeable to those mimicking coatomer (COPI). We propose that a vesicle-like TFG sphere serves to connect ERES to the Golgi, thereby creating a diffusion-limited space between the two organelles. Vesicle-like TFG condensates may also aid in compartmentalizing anterograde carriers enclosed within from retrograde carriers outside the condensates, suggesting an explanation for how bidirectional membrane flux is spatially controlled at the ER-Golgi interface.

B440/P1425

Enzyme regulated biomolecular condensate structure and dynamics

S. Coupe, N. Fakhri; Massachusetts Institute of Technology, Cambridge, MA

Enzyme activity has been postulated to be central to regulating native ribonucleoprotein condensate properties within living cells. One class of enzymes believed to act as condensate regulators is the DEAD-box helicase. However, few mechanistic insights into this process exist beyond mutational studies, which show disruptions to DEAD-box helicase activity often result in disrupted host condensate localization, morphology, and dynamics. By altering RNA length, protein-RNA interactions, and RNA-RNA interactions we are able to assign two modes by which DEAD-box helicases can alter condensate dynamics: 1) ATPase-dependent RNA release and 2) ATP-dependent disruption of RNA-RNA interactions. To uncover the first mode, we alter the ability for a well-studied DEAD-box helicase to release from RNA, resulting in the effective crosslinking of RNA within the condensed phase. To study the second, we correlate RNA unwinding by the helicase to condensate dynamics and material properties. Together, these insights provide a biochemical and biophysical framework for understanding the function of DEAD-box helicases within biomolecular condensates. These results have implications for targeting condensates that contain

DEAD-box helicase for engineering and pharmaceutical purposes but also provide broader insight into the interactions that govern condensate structure and dynamics.

Peroxisomes and Chloroplasts

B441/P1426

Elucidation of Genes Regulating Peroxisome Homeostasis in Human Cells

J. T. Vu[1], K. Tavasoli[2], M. Morrissey[2], C. D. Richardson[2], B. M. Gardner[2]; [1]BMSE/MCDB, University of California, Santa Barbara, Santa Barbara, CA, [2]MCDB, University of California, Santa Barbara, Santa Barbara, CA.

The peroxisome is a conserved, membrane-bound organelle that compartmentalizes specialized metabolic reactions that enable the cell to adapt to its environment. Disruptions to peroxisome homeostasis impair development and contribute to disease in humans, yet the genetic factors that contribute to peroxisome homeostasis are understudied. To identify genes that regulate peroxisome homeostasis in human cells, we engineered a human colorectal cell line "Pex-ZeoR" to sequester the Zeocin resistance protein (ZeoR) in the peroxisome, thereby linking cell viability to peroxisomal import. We then performed a genome-wide CRISPRi screen with the goal of uncovering novel genes involved in peroxisomal biogenesis and maintenance. Our primary screen enriched both known PEX genes and new candidate genes, thereby validating our strategy and providing intriguing candidates. To further refine our results, we implemented a fluorescence microscopy secondary screen to characterize candidate genes knockdowns that influence peroxisomal morphology. We identified that CRISPRi induced suppression of the E3 ligase RNF146 dramatically reduces peroxisome import. The RNF146-mediated loss of peroxisome import depends on an increase in the poly-ADP polymerase tankyrase (TNKS), which has been previously reported to localize to the peroxisome. Intriguingly, we also found that the loss of peroxisomes alters RNF146 and TNKS activity, linking the stability of known TNKS/RNF146 substrates to peroxisome abundance. These observations not only suggest previously undescribed roles for RNF146 in peroxisomal regulation, but also implicates the peroxisome in an intricate and vital network in developmental cell biology and signal transduction.

B442/P1427

Characterization of the N-terminal domains of the Pex1/Pex6 AAA-ATPase

R. M. Judy[1], D. Castanzo[2], T. Glastetter[1], A. Martin[2], **B. M. Gardner**[1]; [1]Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA, [2]University of California, Berkeley, Berkeley, CA.

The heterohexameric AAA-ATPase Pex1/Pex6 is essential for the formation and maintenance of peroxisomes. Pex1/Pex6, similar to other AAA-ATPases, uses the energy from ATP hydrolysis to mechanically thread substrate proteins through the central pore of the motor, thereby unfolding them. In related AAA-ATPase motors, substrates and co-factors bind the motor's N-terminal domains, which positions the substrate in the central pore. Here we use structural and biochemical techniques to characterize the function of the Pex1 and Pex6 N1 domains in the budding yeast, *S. cerevisiae*. Truncation of the Pex1 N1 domain does not inhibit motor ATPase or unfoldase activity *in vitro*, but dramatically impairs peroxisome formation *in vivo*. The Pex1 N1 domain does not impair binding to the Pex1/Pex6 peroxisomal tether Pex15, suggesting that it must bind another essential substrate for

peroxisome formation, most likely the ubiquitinated PTS1 receptor Pex5. Additionally, we solved an X-ray crystal structure of the isolated Pex6 N1 domain. Integrating this structure with existing cryo-EM structures and Alphafold2 predictions, we found that the Pex6 N1 domain interacts with the D2 ATPase domain of Pex1/Pex6. This interaction may help coordinate binding of the Pex1/Pex6 substrates with Pex1/Pex6 ATPase activity.

B443/P1428

Investigating the role of peroxisomal membrane proteins and retromer proteins in peroxisomal inner membrane dynamics

A. Swearingen, Z. J. Wright, B. Bartel; BioSciences, Rice University, Houston, TX.

Peroxisomes are organelles that house metabolic reactions, including fatty acid beta oxidation and reactive oxygen species detoxification, and are essential in most multicellular eukaryotes. Despite the importance of peroxisomes for cellular health, peroxisome biogenesis and structure are incompletely understood. Indeed, instead of a simple lumen surrounded by a lipid bilayer, our lab visualized multiple inner membranes, or intralumenal vesicles (ILVs) within Arabidopsis peroxisomes. Many questions remain about the formation, protein composition, and function of peroxisomal ILVs. We are exploiting the unusually large size of Arabidopsis peroxisomes, which allows confocal imaging of peroxisome sub-structure, to characterize proteins associated with ILVs. We are investigating a subset of peroxin (PEX) proteins that are peroxisomal membrane proteins (PMPs) that function in peroxisome biogenesis and protein import. To determine whether PMPs differentially localize among peroxisomal membranes, we are imaging trifluorescent constructs with reporters for peroxisomal membranes, lumen, and individual PMPs in Arabidopsis. Using confocal microscopy, we observed a PEX3B-mTagBFP2 fusion localize to some ILVs and the outer peroxisomal membrane, suggesting that PEX3B may be involved in ILV biology. Additionally, we used CRISPR-Cas9 to generate a viable *pex3a pex3b* double mutant to determine whether ILV formation is altered when PEX3 function is reduced. To identify other proteins involved with ILV biology, we are investigating the retromer complex, which sorts and transports cargo among ILVs in the endocytic pathway. The retromer complex consists of VPS26, VPS29, and VPS35, and *vps29* and *vps35* seedlings display sucrose-dependent growth, which can reflect disruption of peroxisomal beta oxidation. We crossed *vps29*, *vps35a*, *vps35c*, and *vps35a vps35c* insertional mutants to lines expressing fluorescent reporters for peroxisomal membranes and lumen. By imaging the resulting progeny, we will determine if ILV formation or morphology is altered in retromer mutants. These experiments will reveal what proteins localize to ILVs, which may clarify ILV function(s) in peroxisome biology. (This research is supported by the NIH and the Welch Foundation.)

B444/P1429

Transport of specialized peroxisomes is not required for septal closure in filamentous fungi

L. D. Songster[1], J. R. Christensen[2], S. L. Reck-Peterson[2]; [1]Biological Sciences, University of California, San Diego, La Jolla, CA, [2]Cellular & Molecular Medicine, University of California, San Diego, La Jolla, CA.

The localization of many cellular components, such as organelles and protein complexes, must be precisely controlled. Proper distribution of components is largely achieved by motor proteins that selectively bind and transport cargos along cytoskeletal tracks. Peroxisomes are important metabolic organelles that exhibit different transport properties depending on the organism and cell type. In the filamentous fungus *Aspergillus nidulans*, peroxisomes move long distances along microtubules by

hitchhiking on motile early endosomes. Peroxisomes are the only organelle demonstrated to hitchhike on early endosomes in *A. nidulans*, but it is unclear why. Peroxisome hitchhiking requires the protein PxdA, which is conserved within the Pezizomycotina clade of filamentous fungi. This suggests that peroxisome hitchhiking may be important for a cellular process present within this fungal clade. Woronin bodies are specialized peroxisomes that are unique to the Pezizomycotina. Upon local damage to a hyphal segment, Woronin bodies "plug" septal pores to prevent widespread cytoplasmic leakage. Here, we investigated why peroxisomes hitchhike in filamentous fungi by testing if peroxisome hitchhiking is important for Woronin body motility, distribution, and function in *A. nidulans*. We found that Woronin bodies colocalized with peroxisomes during long-distance movements but moved less frequently when dissociated from peroxisomes. Loss of peroxisome motility by knocking out *pxdA* significantly affected Woronin body distribution in the cytoplasm and significantly decreased the frequency of Woronin body localization at septa. However, this altered distribution of Woronin bodies was not important for septal plugging after damage to hyphal tips. Our data suggest that Woronin bodies remain attached to peroxisomes during long-distance hitchhiking motility, then separate from peroxisomes once they reach a cortical or septal tether. Future work will address if other cellular processes require peroxisome hitchhiking.

B445/P1430

Investigating the role of peroxisomes in innate immunity during microbial infection

M. Munawar[1,2], S. Leung[1], E. Miraglia[1,2], P. Kim[1,2]; [1]The Hospital for Sick Children, Toronto, ON, Canada, [2]The University of Toronto, Toronto, ON, Canada

Background: Peroxisomes are organelles involved in the metabolism of lipids and reactive anionic species. Defects in peroxisome number and function result in a class of metabolic disorders called peroxisome biogenesis disorders (PBD). Clinical data shows that patients with PBD's have early deaths linked to infection and sepsis. In the last decade, peroxisomes were found to be signalling hubs for antiviral activity; however, their antimicrobial properties lack sufficient characterization. This project identifies that peroxisome are required for adequate antimicrobial response during murine bacterial infection. Our current work aims to elucidate the cellular processes behind peroxisomes effects on antimicrobial immunity.

Hypothesis: We suspect that peroxisomes have a role in initiating inflammatory signalling in the intestine; In peroxisome-deficient mice, lack of this initiation results in defects in inflammatory pathways that dictate immune cell recruitment and immune cell diversity. Additionally, peroxisomes or peroxisome-derived metabolites have anti-inflammatory properties, whose fluctuations result in prolonged inflammation and infection.

Methods: We generated transgenic peroxisomal knockout mice ($PEX16^{IEKO}$), which are then infected with the bacterium, *Citrobacter rodentium*, and assessed at various infection timepoints for bacterial clearance, infection severity, inflammation, and gene expression. Novel single cell technologies, such as time-of-flight cytometry (CyTOF) and single-cell RNA-sequencing, will be used to assess changes in anti-inflammatory and pro-inflammatory signalling involved with epithelial cell-immune cell communication during bacterial infection.

Results: Throughout bacterial infection, $PEX16^{IEKO}$ mice have increased pathogen load, increased colitis severity, delayed hyperplasia, and prolonged infection. Additionally, $PEX16^{IEKO}$ mice demonstrate infection mortality, which is uncharacteristic for typical *C. rodentium* infection. $PEX16^{IEKO}$ females have mortality rates of 80%, whereas males have a mortality rate of 20%, which suggests a sex-specific role of

peroxisomes during infection. Using qPCR, common inflammatory pathways displayed no difference in gene expression between control and knockout mice. The novel role of peroxisomes in bacterial infection remains to be characterized in our upcoming single-cell experiments.

B446/P1431

Loss of intestinal peroxisomes increases susceptibility to *Citrobacter rodentium* infection in mice

E. Miraglia[1,2,3], M. Munawar[1,2], S. Leung[1], R. Bandsma[3,4], P. Kim[1,2]; [1]Cell Biology, Hospital for Sick Children, Toronto, ON, CANADA, [2]Department of Biochemistry, University of Toronto, Toronto, ON, CANADA, [3]Translational Medicine, Hospital for Sick Children, Toronto, ON, CANADA, [4]Department of Nutritional Sciences, University of Toronto, Toronto, ON, CANADA.

Peroxisomes have recently been identified as a regulator of immune function, expanding the organelle's repertoire of roles in the cell beyond their classically considered metabolic processes. The role of peroxisomes as signaling platforms in antiviral immunity has been characterized, but their involvement in responding to bacterial infections is not well understood. We developed an intestine-specific peroxisome knockout mouse model to study antibacterial peroxisome function by infecting animals with the gut pathogen *Citrobacter rodentium* and characterizing their response. We found that *PEX16* knockout mice developed more severe intestinal *C. rodentium* colonization associated with a 20% mortality rate in males and 80% mortality rate in females, whereas littermates who lack the peroxisome knockout have a 100% survival rate. Histological examination of infected knockout mouse tissue showed a delay in the colonic crypt hyperplasia response as well as increased damage in the colon epithelial layer compared to controls. This correlated to an increase in the bacteria load found in other tissues such as the liver, indicating exacerbated bacterial translocation from the gut. Together, these findings reveal that peroxisomes are a necessary component of the innate response of intestinal epithelial cells to bacterial pathogens. Ongoing studies are investigating multiple aspects of epithelial cell function in knockout vs control mice using various technical approaches including Airyscan superresolution microscopy of gut sections. Our goal is to ultimately elucidate the cause of enhanced susceptibility to intestinal bacterial infections in peroxisome deficient mice.

B447/P1432

Association of single algal septin with evolutionarily related chloroplast translocons

S. Delic[1], S. Ramundo[2], M. Onishi[1]; [1]Duke University, Durham, NC, [2]Gregor Mendel Institute, Vienna, AUSTRIA.

Cell division is a fundamental process for every organism. In addition to DNA, the cell must effectively divide and distribute organelles to their daughter cells. Many organisms, including photosynthetic algae, possess single large organelles known as chloroplasts. These membrane-bound organellar compartments arose from endosymbiosis of cyanobacteria approximately 1 billion years ago. For cells with single organelles, it is imperative to coordinate both cell and plastid division. What are the cellular and molecular pathways behind this coordination? Septins are cytoskeletal GTPases that form filaments and rings at sites of membrane curvature. While initially thought to only exist in animals & fungi, our lab has identified septin sequences in a range of eukaryotes, including many algal species. *Chlamydomonas reinhardtii,* a unicellular green alga, possesses only a single septin protein (SEP1). We previously identified by coimmunoprecipitation mass spectrometry experiments that SEP1 interacts with components of the translocons at the outer chloroplast envelope (TOCs). Interestingly, septins are

evolutionarily related to TOCs and share homology among their GTPase domains, however their physical and functional interaction has never been explored. Our results suggest that the loss of septin leads to mislocalization of the plastid division protein, FtsZ2. This protein normally forms a ring with its homolog, FtsZ1, at the chloroplast division site. The loss of septin, however, impairs ring formation and leads to FtsZ2 fragments to be dispersed in the chloroplast. This data, combined with SEP1's interaction with translocons implicates its function in chloroplast translocation. Formally, this could be due to the failed translocation of FtsZ2 directly or other proteins responsible for assembling and positioning the FtsZ ring. To investigate this, we are using two orthogonal proteomic approaches. First, we are performing native coimmunoprecipitation experiments using FtsZ2 as bait in either wildtype or septin null mutants to compare the FtsZ2 interactome. Additionally, we seek to identify peptides with intact chloroplast-targeting sequences to characterize proteins that preferentially utilize SEP1-bound translocons. This project is the first functional characterization of septins in a photosynthetic organism and explores a novel interaction of septin proteins with a distantly related family of proteins.

B448/P1433

Lack of peroxisomal catalase affects heat shock response in *C. elegans*

M. Musa[1], **P. A. Dionísio**[2], R. Casqueiro[2], I. Milosevic[3], N. Raimundo[4], A. Krisko[5]; [1]Mediterranean Institute for Life Sciences, Split, Croatia, [2]MIA-Portugal, University of Coimbra, Coimbra, Portugal, [3]Wellcome Centre for Human Genetics, Nuffield Department of Medicine, NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom, [4]Penn State University College of Medicine, Pennsylvania, PA, [5]University Medical Center Göttingen, Göttingen, Germany

The exact mechanisms of heat shock-induced lifespan extension, while documented across species, are still not well understood. Here, we put forth evidence suggesting that fully functional peroxisomes, specifically peroxisomal catalase, are needed for the activation of canonical heat shock response and heat-induced hormesis in *C. elegans*. While during heat shock the HSP-70 chaperone is strongly upregulated in the wild-type (WT), as well as in the absence of peroxisomal catalase (*ctl-2(ua90)II*), the small heat shock proteins display modestly increased expression in the mutant. Nuclear foci formation of HSF-1 is reduced in the *ctl-2(ua90)II* mutant. In addition, heat-induced lifespan extension, observed in the WT, is absent in the *ctl-2(ua90)II* strain. Activation of the antioxidant response and pentose phosphate pathway are the most prominent changes observed during heat shock in the WT worm, but not in the *ctl-2(ua90)II* mutant. Preliminary work on a human cell line with silenced catalase exposed to transient heat shock did not recapitulate the blunted expression of small heat shock proteins, although different models may be needed to compare the heat shock response-dependent mechanisms between *C. elegans* and human cells. Overall, involvement of peroxisomes in the cell-wide cellular response to transient heat shock reported here gives new insight into the role of organelle communication in the organism's stress response.

B449/P1434

Study of the role of chloroplast unusual positioning 1 (CHUP1) in regulation of chloroplast and stromule movement dynamics

A. Nedo[1], K. Seward[1], N. Meier[2], Y. Liu[1], H. Liang[1], C. Kambhamettu[1], S. Dinesh-Kumar[2], J. Caplan[1]; [1]University of Deleware, Newark, DE, [2]University of California, Davis, CA

Plant innate immunity is regulated by the generation and transmission of various signals. Key examples of this are hydrogen peroxide (H2O2), a type of reactive oxygen species (ROS), and salicylic acid (SA). Chloroplasts are a major source of these signals, and it's believed that chloroplast repositioning and tubular extensions called stromules play a major role in regulating the transmission of these signals in cellular signaling. The direct mechanics behind chloroplast movement remain unclear. It is known that chloroplast movement is dependent on the actin microfilament network, however the direct causes are not known. Chloroplast Unusual Positioning 1 (CHUP1) is believed to play a major role in regulating of the blue light movement (BLM) as part of an avoidance response, however, the exact role it plays other than being required for proper BLM based repositioning in the mesophyll is not been reported in the literature. Specifically, data has been collected to show that the avoidance response as part of BLM is a behavior that is still present in mesophyll cells. Additionally, this process is still primarily regulated by phototropin 2 (PHOT2), with a complete lack of the avoidance response when PHOT2 is silenced. As such, establishing the role of CHUP1 is likely a key component for fully understanding the mechanisms behind chloroplast movement, localization and cellular signaling in plants. This work was supported by a grant from NIH-NIGMS (R01 GM097587).

B450/P1435

Giant peroxisomes: optogenetic enlargement of candidate organelle for future use as a synthetic organelle

N. Menjivar, M. Chan; San Francisco State University, San Francisco, CA

Emerging research on artificial cells and organelles shows great potential for refined production of biomolecules including pharmaceuticals. Synthetic organelles are at the forefront for deeper understanding and control of cellular reactions. In this poster, we focus on the peroxisome that has previously shown to be capable of compartmentalizing heterologous proteins, a necessary ability for usage as a synthetic organelle. Previous studies have shown that the amount of protein able to be imported into the peroxisome depends on the organelle's size, so we aim to engineer cells with larger peroxisomes to maximize capactiy. Furthermore, initial studies have shown that the size of organelle's typically scales closely with cell size. Therefore, we aim to produce larger peroxisomes in budding yeast by applying optogenetic perturbations to grow cells to artificially large sizes. Preliminary results showed that in these enlarged cells, peroxisomes do maintain a scaling relationship with cell size, and this occurs via proliferation rather than expasion in volume. Future experiments will use mutants targeting peroxisome biogenesis, fission, and fusion pathways to gain more mechanistic insight into how peroxisome size is regulated.

Signaling Scaffolds and Microdomains

B452/P1436

Hedgehog pathway modulation by glypican 3 conjugated heparan sulfate

C. Liu[1], B. Wierbowski[2], A. Salic[3]; [1]Biology, Hood College, Frederick, MD, [2]Whitehead Institute, MIT, Boston, MA, [3]Cell Biology, Harvard Medical School, Boston, MA.

Glypicans are a family of cell surface heparan sulfate proteoglycans that play critical roles in multiple cell signaling pathways. Glypicans consist of a globular core, an unstructured stalk modified with sulfated glycosaminoglycan chains, and a glycosylphosphatidylinositol anchor. Though these structural features are conserved, their individual contribution to glypican function remains obscure. Here, we investigate how glypican 3 (GPC3), which is mutated in Simpson Golabi Behmel tissue overgrowth syndrome, regulates Hedgehog signaling. We find that GPC3 is necessary for the Hedgehog response, surprisingly controlling a downstream signal transduction step. Purified GPC3 ectodomain rescues signaling when artificially recruited to the surface of GPC3-deficient cells but has dominant-negative activity when unattached. Strikingly, the purified stalk, modified with heparan sulfate but not chondroitinsulfate, is necessary and sufficient for activity. Our results demonstrate a novel function for GPC3 associated heparan sulfate and provide a framework for the functional dissection of glycosaminoglycans by in vivo biochemical complementation.

B453/P1437

Mutual Regulation between Membrane Rafts and Protein Condensates in T Cell Activation

S. Chan[1], H. Wang[1], S. Dey[1], I. Castello-Serrano[1], J. A. Ditlev[2], M. K. Rosen[3], K. R. Levental[1], I. Levental[1]; [1]University of Virginia, Charlottesville, VA, [2]University of Toronto, Toronto, ON, CANADA, [3]University of Texas Southwestern Medical Center, Dallas, TX.

T cells are central effectors of adaptive immunity, mediating critical defenses against infections and cancer. T cell activation must be tightly regulated to integrate and transduce various signals detected at the plasma membrane and elicit proper immune responses. Inappropriate T cell activation results in anergy, auto-immune disease, and inflammatory disorders. Despite decades of research, the regulatory mechanisms of normal and aberrant T cell activation are not fully understood. Recently, we reported that biomolecular condensates form at the T cell immunological synapse (IS) via liquid-liquid phase separation (LLPS) driven by multivalent macromolecular interactions. Specifically, the transmembrane protein Linker for Activation of T cells (LAT) is phosphorylated upon antigenic stimulation to multivalently interact with proteins called Grb2 (growth factor receptor-bound) and Sos1 (son of sevenless) to form condensates with fluid properties *in vitro* and in living cells. Lying near phase coexistence boundaries, membrane lipids can also undergo LLPS and de-mix to form phase-separated lateral domains, which may serve as platforms for organizing and regulating condensates. Notably, LAT is recruited to the ordered, cholesterol-rich membrane domains known as lipid rafts, and such recruitment has been implicated in T cell activation. Thus, we hypothesized that LAT may mediate interplay between protein and membrane lipid phase separation in T-cell signaling. Here, we report physical, thermodynamic and functional coupling between membrane rafts and protein condensates via LAT using both *in vitro* reconstituted membrane systems and in live cells. We have observed that LAT condensates recruit and stabilize ordered membrane domains in reconstituted membrane systems. Using Jurkat cells under activating conditions, we observe that Grb2 condensates recruit markers of raft

proteins. Stabilizing raft domains potentiates LAT condensate formation, which in turn induces T cell signaling. To study how LAT condensates and membrane rafts cooperatively regulate T-cell signaling, we perturbed membrane raft domains and observed changes in condensate density and signaling. Moreover, using fluorescent probes that are sensitive to local membrane lipid packing in living cells, we quantitatively measure changes in membrane biophysical properties induced by condensate assembly. By evaluating the effects of lipid remodeling on LAT condensate properties, and effect of condensate formation on membrane properties, we provide evidence that membrane rafts and LAT condensates mutually regulate T cell activation for optimal immune signaling.

B454/P1438

Dissecting the function of glycolytic condensates at the maternal-fetal interface

A. Skinkle, A. Gladfelter; University of North Carolina, Chapel Hill, NC.

The placenta is an organ that is indispensable in maintaining mammalian species. Aberrations of placental development have been linked to common pregnancy-related problems such as preeclampsia and miscarriage. Key to the placenta's development is formation of a large multinucleate cell, the syncytiotrophoblast (STB). The STB acts as the barrier between maternal and fetal circulation where it performs the vital task of transferring nutrients and oxygen between the mother and developing fetus. This meters-long cell is exposed to very different local nutrient microenvironments along its length. To meet its unique metabolic demands, the STB relies heavily on aerobic glycolysis. How a non-compartmentalized process such as glycolysis is differentially regulated in a continuous cytoplasm is unknown. Here, we demonstrate for the first time the organization of glycolytic enzymes into membraneless compartments termed biomolecular condensates within placental tissue. Immunostained tissue sections obtained from term placentas demonstrate glycolytic condensates are heterogeneously localized in the STB, suggesting condensates are sensitive to local environmental cues. In an STB model cell line starved of glucose, glycolytic condensates are reduced in number but grow larger. These results are consistent with studies conducted in other species where glycolytic condensates grow in size under energy stress and promote increased rates of glycolysis. We hypothesize that glycolytic condensates promote glycolysis in the STB and that their physical properties are important for their function. Future work will characterize how the physical properties of glycolytic condensates are affected by energy stress and how this relates to metabolite levels in the STB.

B455/P1439

The eisosome-associated proteins, Pil1 and tetraspan membrane proteins regulate TORC2 signaling during the stress response in budding yeast

K. Sakata[1], K. Hashii[1], K. Yoshizawa[1], Y. O. Tahara[2], N. Tanaka[1], T. Maeda[3], M. Miyata[2], M. Tabuchi[1]; [1]Faculty of Agriculture, Kagawa University, Kagawa, JAPAN, [2]Graduate School of Science, Osaka City University, Osaka, JAPAN, [3]Department of Biology, Hamamatsu University School of Medicine, Kagawa, JAPAN.

Saccharomyces cerevisiae has three distinct plasma membrane domains, MCC (Membrane Compartment occupied by Can1), MCT (Membrane Compartment occupied by TORC2), and MCP (Membrane Compartment occupied by Pma1). MCC corresponds to inner membrane furrows termed eisosome. Eisosome contains various membrane associated-proteins, transporters, and tetraspan membrane proteins, which are considered to contribute to normal eisosome structure and function. Pil1

is a core eisosome protein and is responsible for MCC-invaginated structures. In addition, six-tetraspan membrane proteins (6-Tsp) are localized in the MCCs and classified into two families, the Sur7 family, and the Nce102 family. To understand the coordinated function of these MCC proteins, single and multiple deletion mutants of Pil1 and 6-Tsp were generated. These deletion mutant cells analyzed the structure of the eisosome and assayed growth under various stress conditions. Genetic interaction analysis indicated that the Sur7 family and Nce102 function in stress tolerance and normal eisosome assembly, respectively, by cooperating with Pil1. To further understand the role of MCCs/eisosomes in stress tolerance, we screened for suppressor mutants using the SDS-sensitive phenotype of *pil1Δ 6-tspΔ* cells. The growth defect on the SDS-contained medium was suppressed by the loss of function of Tor2, which is the component of the Tor kinase complex 2 (TORC2). This data indicated that the SDS sensitivity in *pil1Δ 6-tspΔ* cells is caused by hyperactivation of TORC2-Ypk1 signaling. Interestingly, inhibition of sphingolipid metabolism, a well-known downstream pathway of TORC2-Ypk1 signaling, did not rescue the SDS-sensitivity of *pil1Δ 6-tspΔ* cells. In summary, the eisosome-located proteins, Pil1, and 6-Tsp cooperatively regulate TORC2 signaling during the stress response.

B456/P1440

ZO-2 is a scaffold for Hippo signaling that restores tight junction sealing in the steatotic liver

L. Gonzalez-Mariscal[1,2], L. González-González[3], H. Gallego-Gutiérrez[3], D. Martin-Tapia[3], J. E. Avelino-Cruz[4], C. Hernández-Guzmán[3], M. C. Gutiérrez-Ruiz[5], D. Hernández-Melchor[6], E. López-Bayghen[6]; [1]Physiology, Biophysics, and Neuroscience, Lorenza Gonzalez Mariscal y Muriel, CDMX, MEXICO, [2]Physiology, Biophysics, and Neuroscience, Cinvestav, Cdmx, MEXICO, [3]Physiology, Biophysics, and Neuroscience, Cinvestav, CDMX, MEXICO, [4]Laboratory of Molecular Cardiology, Institute of Physiology, Benemérita Universidad Autónoma de Puebla, Puebla, MEXICO, [5]Department of Health Sciences, Autonomous Metropolitan University-Iztapalapa (UAM-I), CDMX, MEXICO, [6]Department of Toxicology, Cinvestav, CDMX, MEXICO.

ZO-2 is a peripheral protein of tight junctions involved in the regulation of cell size. Previously we demonstrated the development of hypertrophy in MDCK ZO-2 KD cells and accordingly observed that in renal compensatory hypertrophy induced in rats by unilateral nephrectomy, ZO-2 is silenced. Now, we show that in another model of hypertrophy, the steatotic liver of obese Zucker (OZ) rats, ZO-2 expression is also silenced. Moreover, we demonstrate that the reduced expression of ZO-2 is accompanied by a decreased activity of the kinase LATS of the Hippo signaling route, and the nuclear concentration of YAP, the final effector of this pathway. LATS1 binds to the carboxyl segment of ZO-2 that contains the actin-binding and proline-rich regions of the protein, and the absence of ZO-2 reduces LATS activity and concentration at the cell border, thus indicating that ZO-2 acts as a platform for the Hippo signaling pathway. Silencing of ZO-2 in hypertrophic tissue is due to a reduction in ZO-2 mRNA due to a diminished expression of the Sp1 transcription factor, which is critical for *TJP2* gene expression in renal cells. Previously, we had shown that AMPK activation induces the appearance of ZO-2 at the cell borders, even in cells cultured in a low calcium condition. Now, in the steatotic liver of OZ rats, we demonstrate that the activation of AMPK with metformin augments the expression of ZO-2, activates LATS, and diminishes the nuclear concentration of YAP. This, in turn, restores claudin-1 expression and ameliorates the increased paracellular permeability of hepatocytes and the augmented content of bile acids in serum found in the steatotic liver, through a process mediated by a diminished JNK activation. These results indicate that ZO-2 silencing is a common characteristic of hypertrophic tissue, and reveal

that ZO-2 is a crucial scaffold of the Hippo pathway. This study identifies AMPK, JNK, and ZO-2 as therapeutic targets for treating pathologies with blood-bile barrier dysfunction.

B457/P1441

Tetraspanin CD82 regulates Hematopoietic Stem and Progenitor Cell Quiescence through the modulation of TGFb signaling

E. Pascetti, M. Floren, C. Saito-Reis, J. Gillette; University of New Mexico, Albuquerque, NM

The significant cellular demand of the hematopoietic system is maintained by a rare pool of tissue-specific, hematopoietic stem and progenitor cells (HSPCs) that are primarily found in a quiescent state. Upon hematopoietic stress, HSPCs undergo rapid cell cycle activation, but ultimately must return to quiescence to prevent hematopoietic exhaustion. Evidence from our laboratory demonstrates that the tetraspanin CD82 plays a critical role in the regulation of HSPC maintenance. Tetraspanins are membrane scaffold proteins with the ability to modulate signaling through the organization of membrane receptors and intracellular signaling molecules. Using a CD82 knockout (CD82KO) mouse model, we find a reduction in long term-HSPCs, resulting from increased HSPC activation and a reduction of quiescent G_0 cells, indicating a role for CD82 in the regulation of HSPC quiescence and activation. To further delineate how the CD82 scaffold modulates HSPC quiescence, we transplanted CD82 overexpressing (CD82OE) progenitor-like cells into mice. Despite the fact that CD82OE cells display similar proliferation rates to control cells in culture, once localized to the bone marrow, CD82OE cells demonstrate reduced proliferation and increased quiescence, as indicated by diminished BrdU incorporation and IVIS signal. Additional mechanistic insight is provided by analysis of cells expressing a mutant form of CD82, where three N-linked glycosylation sites are inhibited (Ngly-CD82). Ngly-CD82 cells do not exhibit the same level of quiescence as CD82OE cells when injected into NSG mice, demonstrating an important role for CD82 glycosylation in the regulation of HSPC quiescence. Mechanistically, the cytokine TGFβ plays an essential role in supporting HSPC quiescence and activation, as one of the most potent inhibitors of HSPC growth. To investigate a critical role for TGFβ signaling in the observed *in vivo* CD82-mediated quiescence phenotype, we injected a TGFβ neutralizing antibody. Interestingly, CD82OE cells are stimulated out of quiescence when TGFβ is inhibited, confirming viability of the CD82OE cells within the marrow and supporting a critical role for TGFβ signaling in CD82-mediated quiescence. Additionally, *in vitro* studies indicate that when progenitor cells or primary HSPCs with differential CD82 expression are TGFβ stimulated, cells with higher CD82 expression have increased SMAD2/3 activation when compared to lower expressing CD82 cells or Ngly-CD82 cells, but only when cells are engaged with fibronectin. Together, these data indicate that the CD82 scaffold has the capacity to modulate TGFβ signaling through niche-mediated interactions and implicate integrins in the modulation of signal transduction.

B458/P1442

Scaffold Protein RACK1 mediates plant hormone Auxin and Salt stress Cross-talk pathway

A. Webber-Deonauth[1], S. Dakshanamurthy[2], H. Ullah[1]; [1]Howard University, Washington, DC, [2]Georgetown University, Washington, DC

Auxin is a primary plant hormone directly implicated in growth, development, and differentiation of plants cells during every stage of the plant life cycle. In addition to the growth and development, auxin is reported to regulate diverse environmental stresses including osmotic stress signaling pathways.

However, the precise cellular mechanism of this auxin mediated environmental stress signaling pathways is poorly defined. Using genetic knock-out and chemical inhibitors of scaffold protein RACK1, previously we have reported that the protein regulates auxin induced lateral root development signaling. Separately, we have also reported that RACK1 protein regulates salt stress resistance signaling pathways. Here we tested our hypothesis of RACK1 mediating the cross-talk between auxin and salt stress pathways. Using transgenic *Arabidopsis* with auxin reporter construct pIAA5::GUS, we investigated the cross-talk mediating function of the RACK1 protein. While pIAA-GUS plants showed salt-induced auxin reporter gene expression, use of RACK1 small compound inhibiting RACK1 failed to induce such response. The response was found to be present in both whole seedlings and in mature leaves. To provide molecular genetic evidence for RACK1 in this pathway, construction of the double 'mutant' of pIAA5::GUS and rack1 knock-out plant is underway. Additional auxin reporter containing transgenic plants (DR5::GFP, pIAA5::Luc, and BA3::GUS) are being used to elucidate the pathway. Molecular understanding of this cross-talk between auxin and stress signaling pathway will pave a pathway to bioengineer crop plants to combat climate change induced potential environmental stresses.

B459/P1443

Receptor for Activated C Kinase 1 (RACK1) Regulates Plant Nitrogen Stress Signaling Pathways

A. K. Mascarenhas[1], S. Dakshanamurthy[2], H. Ullah[1]; [1]Howard University, Washington, DC, [2]Georgetown University, Washington, DC

Plant stress hormone Abscisic acid (ABA) regulates diverse plant responses to environmental stresses such as drought, high heat, salinity, water stress, and Nitrogen (N) availability. Nitrogen is an essential nutrient for plant growth and development such as seed germination, root branching, shoot branching, and flowering time. ABA is known to regulate nitrate uptake, metabolism and signaling pathways that result in changes of primary and lateral root architecture. Scaffold protein RACK1 is known to negatively regulates ABA signaling pathway. By using our lab developed RACK1 inhibitor compounds, the role of RACK1 in the ABA mediated nitrogen stress signaling pathway is investigated. Depending on the nitrogen availability (25% to 100%), the inhibitor compounds improved nitrogen use efficiency significantly as evident by the superior growth and development under limited nitrogen availability. Particularly, the root architecture (both primary and lateral root) and the hypocotyl length are significantly impacted by the compounds. Availability of small compounds to make crop plants utilize nitrogen efficiently can potentially lower the usage of nitrogen-based fertilizers whose wide-spread usage is known to cause myriad of negative impacts in the overall quality of the environment.

B460/P1444

The scaffold protein CNK2 drives cell migration and invasion in cancer cells

G. Serwe[1,2], D. Kachaner[2], J. Gagnon[1,2], C. Plutoni[2], D. Lajoie[2], E. Duramé[1,2], M. Sahmi[2], D. Garrido[2], M. Lefrançois[2], G. Arseneault[2], M. K. Saba-El-Leil[2], S. Meloche[3,2], G. Emery[4,2], M. Therrien[4,2]; [1]Molecular Biology Program, Faculty of Medicine, Université de Montréal, Montréal, QC, CANADA, [2]Institute for Research in Immunology and Cancer, Montréal, QC, CANADA, [3]Department of Pharmacology and Physiology, Faculty of Medicine, Université de Montréal, Montréal, QC, CANADA, [4]Department of Pathology and Cell Biology, Faculty of Medicine, Université de Montréal, Montréal, QC, CANADA.

Scaffold proteins of the Connector enhancer of KSR (CNK) family are evolutionarily conserved from invertebrates to mammals. In *Drosophila*, CNK is a positive regulator of the oncogenic Ras-MAPK

signaling pathway. In contrast, the role of its human orthologs (CNK1, CNK2, CNK3, and IPCEF1) in this pathway is unclear, and instead, they have been linked to small GTPases of the ARF and RHO subfamilies. These GTPases function in membrane trafficking and cytoskeletal remodeling and are crucial regulators of cell morphology and motility. Yet, the role of human CNKs in these processes is almost completely unknown. Interestingly, we observed that human CNKs, especially CNK2 and CNK3, are expressed in a variety of cancer cell lines of diverse tissue origin. Our objective was therefore to identify the biological functions of CNKs in cancer cells and to characterize their roles in signal transduction.
Using a proteomics approach (BioID), we identified the proximal interactomes of different CNKs in human cells to obtain a global view of their potential functions. This led us to the unexpected discovery that CNK2, which is normally expressed in neuronal tissues, is required for cell migration and invasion in multiple cancer cell lines of diverse tissue origin. Using osteosarcoma cells as a model, we found that CNK2 exerts a positive effect on motility by regulating the activity of ARF and RHO GTPases and by limiting downstream contractility and adhesion. Through immunofluorescence and biochemical assays, we then showed that CNK2 operates at the plasma membrane and forms functional interactions with several binding partners. Additionally, we found that the membrane localization of CNK2 is regulated by growth factors and membrane receptors. Finally, using a mouse xenograft model, we confirmed that the function of CNK2 in motility is conserved in vivo.
In summary, we identified the CNK2 scaffold as a novel regulator of motility in cancer cells and uncovered aspects of its mechanism of action in signal transduction. Since cell motility is a defining feature of cancer cell dissemination, the characterization of CNK2 and its associated partners could lead to the identification of novel biomarkers and therapeutic targets for metastasis.

B461/P1445

Evolution of the β-catenin destruction complex: Insights from the anthozoan cnidarian *Nematostella vectensis*

H. Sun, **A. Wikramanayake**; University of Miami, Coral Gables, FL

Axin and APC are critical scaffolding proteins in the Wnt/β-catenin (cWnt) pathway that heterodimerize and recruit several proteins including β-catenin, CK1α and GSK3β that together form the β-catenin destruction complex (DC). Work done in bilaterians has indicated that β-catenin is recruited to the DC through binding a conserved β-catenin binding site on Axin. Axin also has a GSK3β binding site and current models propose that heterodimerization of Axin and APC mediates phosphorylation of β-catenin by CK1α and GSK3β that targets β-catenin for degradation via the proteasome. Bilaterian APC also has multiple β-catenin binding domains distinct from the Axin β-catenin binding domain indicating that these motifs had separate evolutionary origins. Intriguingly, Axin proteins in all non-bilaterian taxa (Ctenophora, Porifera, Placozoa, and Cnidaria) lack the β-catenin binding domain questioning a conserved role for Axin in regulating β-catenin stability in non-bilaterians. Using loss- and gain-of-function approaches in the cnidarian *Nematostella* we show that NvAxin has a clear role in regulating cWnt. We also show that NvAxin can regulate cWnt in the bilaterian sea urchin embryo. Using a rescue assay we show that only the GSK3β binding domain of NvAxin is essential for its function in the DC in sea urchin embryos. Finally, we show that Nvβ-catenin directly interacts with NvAPC but not NvAxin. Our results indicate that NvAxin forms an essential part of the DC in *Nematostella* even though it does not directly bind to β-catenin. We propose that whereas bilaterian β-catenin directly binds to both Axin and APC, in *Nematostella,* Axin binds to GSK3β and heterodimerization of Axin and APC allows GSK3β to

phosphorylate β-catenin bound to APC targeting it for degradation. Evolution of the β-catenin binding site in bilaterian Axin may have produced a more tightly regulated DC to downregulate β-catenin. Our work is providing insight into the evolution of the β-catenin DC in metazoans.

Extracellular Vesicle Signaling

B462/P1446

Hepatocyte extracellular vesicle (EV) mRNA content is linked to EV-regulated gene expression in activated hepatic stellate cells, the principal fibrosis-producing cell in the liver

X. Li, R. Chen, S. Kemper, Z. Xu, **D. Brigstock**; Nationwide Children's Hosp, Columbus, OH.

Background: Extracellular vesicles (EVs) are membrane-limited nanoparticles that are involved in intercellular signaling via shuttling of molecular payloads between cells. In the liver, EVs produced by non-injured hepatocytes may mediate therapeutic outcomes following injury. For example, hepatocyte EVs are therapeutic in experimental hepatic fibrosis through their binding to and regulation of hepatic stellate cells (HSC), a normally quiescent cell type that becomes activated during chronic injury and produces fibrotic scar. In this study, the miRNA content of hepatocyte EVs was determined and assessed for its potential relevance to the ability of these EVs to cause the transcriptome of activated HSC (aHSC) to trend towards that of quiescent HSC (qHSC). **Methods:** EVs purified by sequential centrifugation of serum-free cultures of mouse AML-12 hepatocytes were characterized by Nanosight tracking analysis and Western blot. Small RNA or mRNA sequencing (Illumina HiSeq 4000) was performed, respectively, on RNA from AML-12 EVs or on Swiss Webster mouse Day 1 qHSC or P1 aHSC that had been treated with or without 8 μg/ml AML-12 EVs for 48 hrs. Differentially expressed genes (DEGs) were categorized by an adjusted P-value (false discovery rate (FDR)) of <0.05 and fold-change of >2. KEGG pathway enrichment, protein-protein interactions or predicted miR targets were assessed using DAVID, STRING or MiRwalk software respectively. **Results:** In comparing aHSC to qHSC, 6505 protein-coding DEGs were identified of which 3325 were upregulated (genes enriched for metabolism, oxidative phosphorylation, lysosome pathways) and 3180 were downregulated (genes enriched for complement/coagulation, cancer, Hippo pathways). EV treatment of aHSC led to 638/6505 genes being differentially regulated, of which 207 were downregulated (genes enriched for hematopoietic cell lineage, phagosome, calcium signaling) and 431 were upregulated (genes enriched for cancer, PI3K-Akt signaling pathway, rheumatoid arthritis). AML-12 EVs contained 71 miRNAs, with miR-122-5p being the most abundant (34% of total miR counts). Of the EV-regulated DEG genes in aHSC, 172/638 DEGs were identified as miR-122-5p targets, including 56/207 downregulated genes and 116/431 upregulated genes. At least 7 major protein-protein interaction clusters including ECM genes, cell adhesion, and signaling transduction were identified in the 172 targets. **Conclusion:** Addition of hepatocyte EVs to aHSC results in the altered expression of 638 genes to levels more typical of qHSC. 71 miRNAs were identified in AML-12 EVs of which miR-122-5p was the most enriched and predicted to regulate 172/638 DEGs in aHSC. Modulation of gene expression in HSC by hepatocyte EV miRs likely contributes to EV-mediated anti-fibrotic actions.

B463/P1447

Ceramide drives microglial recruitment by altering exosome cargo and secretion in retinal neurodegenerations

C. J. Germer[1], L. Tan[1], N. R. La Cunza[1], G. Rathnasamy[1], E. Iorio[2], F. Elahi[3], A. Lakkaraju[1]; [1]Ophthalmology, UNIVERSITY OF CALIFORNIA SAN FRANCISCO, San Francisco, CA, [2]Memory and Aging Center, UNIVERSITY OF CALIFORNIA SAN FRANCISCO, San Francisco, CA, [3]Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY.

Abnormal secretion of exosomes and activation of microglia have been implicated in the pathogenesis of intractable neurodegenerations such as Alzheimer's and Parkinson's diseases. Yet, the link between exosome secretion and microglial activation is not well understood. What triggers the biogenesis and secretion of pro-inflammatory exosomes from specific cell populations in disease? How is exosomal cargo modified to recruit microglia? We addressed these questions in cell-based and mouse models of macular degeneration, a complex neurodegenerative disease of the retina that causes irreversible vision loss. Using super-resolution microscopy, live-cell imaging, nanotracking analysis, and electron microscopy along with complementary molecular and cellular assays, our study identifies a novel mechanism that implicates the bioactive lipid ceramide in driving the secretion of proinflammatory exosomes from the retinal pigment epithelium (RPE), the tissue that sits beneath the photoreceptors and is the primary site of damage in macular degenerations. Excess ceramide in diseased RPE increases the biogenesis of intraluminal vesicles, induces missorting of the gap junction protein connexin43 to these ILVs, and causes expansion of Rab11-positive apical recycling endosomes. Collectively, these lead to the secretion of Cx43-containing exosomes at the apical surface of the RPE, which induce migration of microglia into the subretinal space. Small molecule therapeutics that decrease RPE ceramide prevent exosome biogenesis and secretion in mouse models of macular degeneration. By identifying how ceramide-driven exosome release leads to microglial activation, our studies establish a novel, druggable mechanism that could pave the way for exciting new therapeutics for neurodegenerations, which impact millions of people across the globe.

B464/P1448

Identification of polycystin interactors within ciliary extracellular vesicles using proximity labeling in *Caenorhabditis elegans*.

I. Nikonorova, E. desRanleau, J. Saul, J. Walsh, J. Wang, M. Barr; Rutgers University, Piscataway, NJ.

Ciliary EV shedding is evolutionarily conserved. Disrupted ciliary EV signaling is an emerging driver of the pathophysiology of many ciliopathies. The ciliary EV field lacks a basic understanding of how EVs form, what cargo is packaged in different types of EVs originating from different cell types, and how different cargoes influence the range of ciliary EV bioactivities. We use *Caenorhabditis elegans* to tackle these major challenges. In *C. elegans* evolutionarily conserved ciliary EVs are released from male-specific ciliated neurons and transferred to vulva of mating partners, suggesting a role in interorganismal communication. We recently identified the proteome of enriched ciliary EVs released by the animal into the environment. Here we present a much-improved pipeline for dissection of the proteome of individual EV subtypes using proximity-labeling approach. We engineered targeting of a proximity-labeling enzyme TurboID to the evolutionarily conserved polycystin PKD-2::GFP EVs using an anti-GFP nanobody domain. Targeted EVs were enriched using buoyant density centrifugation, followed by pulldown of biotinylated proteins. Mass spectrometry analysis revealed 20 candidate interactors of PKD-

2, as opposed to 2,888 EV cargo of the whole EV proteome. For validation, we generated CRISPR knock-in fluorescent reporters for the top 8 hits and analyzed their trafficking to ciliary PKD-2 EVs. **Results**: We discovered that PKD-2 proximity-interactors comprise two categories: (i) a genus-specific set of transmembrane adhesion proteins and (ii) an evolutionarily conserved set of soluble proteins with signaling function. All analyzed proximity-interactors colocalize with PKD-2 in cilia and ciliary EVs. The adhesion proteins are expressed and shed in ciliary EVs in non-overlapping PKD-2 ciliated sensory neurons, suggesting a role in EV targeting, whereas evolutionarily conserved soluble cargo are expressed in all PKD-2 neurons, suggesting a role in EV signaling. We are currently testing these hypotheses. **Conclusion**: Coupling density equilibration with pulldown of biotinylated proteins resulted in at least 10,000-fold specific enrichment for interactors of the targeted EV cargo with virtually no false-positive hits. This methodology may be applied systematically to many EV cargo to break the code of ciliary EV cargo sorting and combinatorial composition.

B465/P1449

The characterization and functional analysis of IRSp53-mediated secretion of the extracellular vesicles from tumor cells

H. T. Hu, T. Nishimura, S. Suetsugu; Nara Institute of Science and Technology, Ikoma, Nara, JAPAN.

The Inverse-Bin/Amphiphysin/Rvs (I-BAR) domain protein, insulin receptor substrate protein of 53kDa (IRSp53), is a membrane shaping protein that could bind to the phosphoinositide-rich membrane and generate membrane protrusions, called filopodia. Filopodia are often implicated in cancer cell metastasis, especially in cell adhesion and migration. We found that IRSp53 serves as an essential regulator for filopodia formation and positively correlated to the lifespan of head and neck carcinoma patients. The head and neck tumor cells could secrete IRSp53-containing particles, which were thought to be the vesicles, to the surrounding tissues. However, the role of these IRSp53-containing particles in the tumor has been unclear. In this study, we isolated the particles, and studied the large-EVs (l-EVs, or microvesicles) fraction to examine their physiological function on tumor growth. We found that the head and neck carcinoma cells secreted a larger amount of the l-EVs that carry integrin-α2 than the IRSp53-knockout cells. These l-EVs could promote the activation of ERK kinase and the proliferation of the recipient cells. The presence of the RGD (Arg-Gly-Asp) peptide, which binds to integrin on the l-EV, inhibited the promotion of proliferation of the recipient cells upon the l-EV treatment, suggesting that the promotion of the proliferation was dependent on the functional integrin on the l-EVs. Collectively, these results demonstrate that IRSp53 could promote the secretion of integrin-α2-containing l-EVs that trigger ERK activation and lead to tumor cell proliferation.

B466/P1450

Plasma Membrane Damage-Induced Senescent Cells Accelerate Wound Healing via Extracellular Vesicles and Soluble Molecules

H. Barbee, Y. Moriyama, K. Kono; OIST, Onna, JAPAN.

Cellular senescence is an essentially irreversible cell cycle arrest that contributes to physiological and pathological processes in vivo, including organismal aging, development, cancer progression, and wound healing. The three best-studied triggers of cellular senescence are telomere shortening, oncogene activation, and DNA damage. We previously showed that transient plasma membrane damage induces a novel subtype of senescence (PMDS) in normal human fibroblasts. However, the pathophysiological

functions of PMDS cells remain unknown. Here, we investigated the paracrine functions of PMDS cells, via extracellular vesicles (EVs) and soluble molecules, and found that PMDS cells accelerate wound healing in vitro. We first measured the number of EVs and found that PMDS cells produce more EVs than DNA damage repair-dependent senescent (DDRS) cells. Then, we conducted scratch assays with PMDS cell co-culture and PMDS conditioned media transfer and saw acceleration of wound healing in young fibroblasts, which was more significant than with DDRS cells. We next separated the EV fraction and the soluble fraction to test their ability to accelerate wound healing. We found that these two fractions were less effective on their own but synergistically contribute to wound healing. Mass spectrometry analysis of PMDS cell-derived EVs revealed that wound-healing and Ca^{2+}-signaling regulators are enriched in the EVs. Altogether, these results revealed that the PMDS cells contribute to wound healing in vitro via EVs and soluble molecules. Our findings raise the possibility that PMDS cells may accelerate tissue repair in vivo.

B467/P1451

Multivesicular Body Fusion and Exosome Secretion Links Repair of Single Cell Injuries to Tissue-Wide Health

D. C. Bittel, M. Hogarth, A. Defour, J. Jaiswal; Children's National Medical Center, Washington DC, DC

Cells of all tissues are routinely subjected to sublethal plasma membrane (PM) injury. The success and efficiency of repair of these cellular injuries influences long-term tissue health. As such, impaired or inefficient PM repair causes multiple tissue-degenerative diseases. Yet, how cellular PM injury activates tissue-wide adaptive responses remains poorly understood. Exosomes are implicated in long-range, tissue-wide intercellular signaling that regulates tissue health. While PM injury triggers vesicle fusion, MVB fusion and accompanying exosome secretion in response to PM injury - a potential link between cell repair and tissue homeostasis, has not been explored. Here we have studied the effect of PM injury on MVB fusion and exosome secretion in healthy and diseased (PM-repair deficient) muscle cells. Using live cell focal injury assays we monitored individual MVB fusion events and found that calcium stimulates robust MVB exocytosis within seconds post-injury. Moreover, MVB fusion is polarized to injury-proximal regions (5-fold higher vs. injury-distal regions). Calcium-dependent translocation of Annexin A2 on injury-proximal MVBs facilitated their fusion, and lack of Annexin A2 compromised exosome release. Using super-resolution and electron microscopy we have characterized these exosomes and found that injury-triggers a 5-fold increase in exosome release by injured cells, which was prevented by Annexin A2 deficit. In a wound closure assay exosomes secreted by injured cells, but not from uninjured cells, stimulated cell migration in a concentration-dependent manner. Using in vivo exercise-induced muscle injury, we observed that muscle fiber PM injury also triggers exosome accumulation in the injury-proximal endomysium, and causes a corresponding increase in inflammatory cell accrual. Additionally, injury-triggered MVB fusion was increased in muscle cells from PM-repair deficient Limb Girdle Muscular Dystrophy 2B (LGMD2B) patients and concomitantly, these LGMD2B mouse muscles showed greater muscle inflammation. This increased inflammation was ameliorated by the lack of Annexin A2 in the LGMD2B mouse. Thus, our results show that MVBs are previously unrecognized injury and calcium responsive secretory vesicles that undergo rapid, calcium-triggered, polarized exocytosis to secrete their exosome cargoes. This is facilitated by Annexin A2 and is enhanced in LGMD2B patient and mouse models. Reducing exosome secretion offers a target to mitigate chronic muscle inflammation and degeneration in LGMD2B and other disease where the cells show poor PM repair ability.

B468/P1452

Role of exosomal endoglin in filopodia formation and tumor cell metastasis

C. McAtee[1], D. Hoshino[2], N. Hong[3], B. Sung[1], E. Krystofiak[1], A. von Lersner[4], A. Zijlstra[5], A. Weaver[1,4]; [1]Vanderbilt University, Nashville, TN, [2]Kanagawa Cancer Center Research Institute, Yokohama, Japan, [3]Fred Hutchison Cancer Research Center, Seattle, WA, [4]Vanderbilt University Medical Center, Nashville, TN, [5]Genentech, San Francisco, CA

The objective of this study is to determine the role of extracellular vesicles and their protein cargoes on filopodia formation and metastasis in tumor cells. Exosomes are small extracellular vesicles (SEVs) that carry a variety of cargoes and have been shown to promote tumor cell motility and metastasis. Cell motility is influenced by dynamic formation and stability of filopodia: actin-rich protrusions that extend from the leading edge and perform directional sensing. Filopodia regulators such as fascin are upregulated in multiple epithelial cancers and can promote invasive phenotypes. However, how filopodia are induced and controlled by extracellular factors is poorly understood. Here, we describe a role for SEVs in regulating filopodia formation, tumor cell motility, and metastasis. We utilized B16F1 melanoma cells and HT1080 fibrosarcoma cells for fixed- and live-cell imaging to quantify filopodia numbers and dynamics in control and exosome-deplete conditions. iTRAQ proteomics was used to identify SEV protein cargoes that contribute to filopodia formation. *In vivo* experiments were performed using a chick embryo model for metastasis. Our results show that inhibition of exosome secretion in cancer cell lines, via Rab27a or Hrs knockdown, led to decreased filopodia numbers. Specificity to SEVs was demonstrated by rescue experiments in which purified SEVs but not large EVs rescued the filopodia phenotypes of exosome-inhibited cells. Live imaging of Hrs-KD cells revealed that exosome secretion regulates formation and stability of filopodia. Proteomics data and molecular validation experiments identified the TGF-beta coreceptor endoglin (Eng) as a key SEV cargo regulating filopodia formation, cancer cell motility, and metastasis. Additionally, we identified the RGD integrin binding motif present in endoglin as essential for it's effect on filopodia formation and tumor cell motility. In conclusion, our data identifies endoglin as a SEV cargo and regulator of filopodia, motility, and metastasis in tumor cells. These data are relevant to cancer as endoglin expression is altered in many cancer types. In addition, endoglin is the disease gene for hereditary hemorrhagic telangiectasia, and may influence angiogenesis. Overall, our data implicate SEV-carried endoglin as a key cargo regulating filopodia.

B469/P1453

Role of stimulus-elicited human neuronal extracellular vesicles in regulating inflammation and mitochondrial repair response

R. L. Nyunoya[1], J. G. Bauer[2], C. L. Howe[2], B. D. Clarkson[2]; [1]Soka University of America, Aliso Viejo, CA, [2]Mayo Clinic, Rochester, MN

Extracellular vesicles (EVs) are small lipid membrane particles involved in immunity. They are naturally released from cells and carry miRNA, mRNA, proteins, and cellular mitochondrial DNA. Mitochondrial components in EVs may play important roles in certain cellular contexts. For example, the packaging of mitochondrial components or mRNA may help repair dysfunctional mitochondria. Mitochondrial dysfunction increases with age and is associated with neurodegenerative diseases. This dysfunction is caused by the impairment of mitophagy, which is the degradation of mitochondria, especially damaged ones. Neuronal EVs may carry mitochondrial parts that repair dysfunctional mitochondria, acting as a therapeutic agent. To explore the therapeutic effect of human neuronal EVs, neural stem cells were

treated with a drug called CP2. This drug mildly inhibits mitochondrial complex I in human and mouse brain mitochondria. In response, mitochondrial genes are activated for mitochondrial repair. In this study, we focused on stimulus-elicited human neuronal EVs. Specifically, we investigated whether treating neurons with CP2 may cause these cells to increase their export of EVs containing mitochondrial repair parts by generating human neuronal aggregates and treating them under three conditions: DMSO (control), low dose CP2, and high dose CP2. From these cells, EVs were collected. Additionally, EVs from neurons that were electrically stimulated through a C-Pace EM machine were collected for analysis. We report changes in mitochondrial gene expression in 1) neurons treated with CP2, 2) neurons treated with EVs isolated from CP2 treated neurons, and 3) EVs isolated from CP2 treated neurons. Most notably, NDUFS2 was downregulated in high dose CP2 treated neurons after 24 hours, upregulated in EVs from high dose CP2 treated at 72 hours, and upregulated in neurons treated with 24 hr EVs. This may be due to neurons exporting EVs containing NDUFS2 mRNA. Additionally, both unstimulated and electrically stimulated EVs modulate cytokine secretion by human peripheral blood mononuclear cells and microglia in response to danger signals such as pan-bacterial or pan-viral Toll-like receptor ligands. EVs isolated at 72 hours from CP2 treated neurons may be more effective on mitochondrial repair response, and regardless of the stimulation, EVs seem to play a role in regulating inflammation.

B470/P1454

Optimization and characterization of the isolation of brain-derived exosomes from human plasma/serum: A pilot study for blood-based biomarker development for neurodegenerative diseases

J. Rack, E. Kosa, A. Agbas; Kansas City University, Kansas City, MO

Chemically modified aberrant Transactive Response DNA Binding Protein 43 (TDP-43) derivatives were found to represent a major accumulating protein in neuronal cytoplasmic inclusions and in exosomes in Frontotemporal Lobar Degeneration (FTLD), and in ALS patients. Exosomes are nano-size membranous vesicles that contain several macromolecules including aberrant pathological proteins. The size and membranous structure of exosomes allows them to pass through the blood brain barrier. These features make exosomes a potential platform in which targeted biomolecules can be analyzed. Our objective is to develop a method to isolate serum/plasma-derived brain-cell originated exosomes and their TDP-43 content as part of a surrogate biomarker development for limbic-predominant age-related TDP-43 encephalopathy (LATE). A heterogenous mixture of extracellular vesicles was obtained by running healthy human plasma through an Izon qEVoriginal size exclusion chromatography column with 70-1000nm matrix size. The exosome enriched fractions were pooled, and brain-derived exosomes were isolated by antibody cross-linked high-performance immunoprecipitation (HPIP) tips in conjunction with urea elution. For the isolation of astrocyte-derived exosomes, anti-GLAST antibody was cross-linked for the HPIP. Anti-TMEM119 and anti-MOG antibodies were cross-linked to HPIP tips to isolate microglia-derived and oligodendrocyte-derived exosomes, respectively. Western blot analysis and transmission electron microscopy (TEM) were used to confirm the presence of exosomes. Western blot results showed the presence of TSG101, GLAST, TMEM119 and TDP-43 in the HPIP eluates. TEM images confirm the presence of intact exosomes in the GLAST cross-linked HPIP eluates. These results suggest that intact astrocyte-derived exosomes and microglia-derived exosomes can be isolated from human plasma and that these exosomes contain TDP-43, a potential blood-based biomarker for neurodegenerative disease. The blood-based biomarker (a.k.a. liquid biopsy) would be a minimally invasive way to check for the progress of neurodegenerative diseases. Being able to isolate brain-derived exosomes from blood, our

prediction is that TDP-43 and pTDP-43 levels in these brain-derived exosomes will be a good analytical tool to monitor longitudinal changes of TDP-43 and its derivatives during neurodegenerative diseases.

B471/P1455

Analysis of extracellular vesicles isolated from *Sorghum bicolor* utilizing an improved isolation method.

T. Chaya[1], A. Banerjee[1], D. Adekanye[1], B. Rutter[2], J. Ross[1], G. Hu[3], J. L. Caplan[1]; [1]University of Delaware, Newark, DE, [2]Indiana University, Bloomington, NY, [3]Brookhaven National Laboratory, Upton, NY

Extracellular vesicles (EVs) are implicated in a variety of biological functions across living systems. Extensive research on EVs has been conducted in animal systems, but the field of plant EVs is relatively new. EVs isolated from plants have yielded proteins, small RNAs, and other metabolites, however the isolation methods have greatly varied across plant species. Our group has developed a method that robustly isolates EVs from the monocot *Sorghum bicolor*, an important crop for grain and energy production. This method collects the apoplastic flow-through as well as vesicles that are released during the buffer infiltration process, which are then purified via density gradient ultracentrifugation. Incorporated into this isolation process is a staining method for light microscopy that utilizes the membrane permeable dye Calcein AM Green and the membrane binding dye Potomac Gold. Correlative light and electron microscopy has shown that the staining method precisely tags isolated vesicles. Transmission electron microscopy (TEM) and cryo electron tomography (cryo-ET) and nanoparticle tracking showed a diverse population of vesicles. Proteomics yielded 437 proteins across three replicates and 404 proteins were common to all three EV isolation replicates. Gene ontology analysis shows an enrichment of proteins involved in metabolism, transmembrane transport, vesicle docking and fusion, and protein secretion.

Kinases and Phosphatases 1

B472/P1456

Regulation of the p38-MAPK Pathway by Hyperosmolarity and by WNK Kinases

Z. Liu[1,2], D. Rotin[1,2]; [1]Cell Biology, Hospital for Sick Children, Toronto, ON, CANADA, [2]Biochemistry, University of Toronto, Toronto, ON, CANADA.

p38-MAPK is a stress-response kinase activated by hyperosmolarity. Here we interrogated the pathways involved. We show that p38-MAPK signaling is activated by hyperosmotic stimulation in various solutions, cell types and colonic organoids. Hyperosmolarity sensing is detected at the level of the upstream activators of p38-MAPK: TRAF2/ASK1 (but not Rac1) and MKK3/6/4. While WNK kinases are known osmo-sensors, we found, unexpectedly, that short (2 hrs) inhibition of WNKs (with WNK463) led to elevated p38-MAPK activity under hyperosmolarity, which was mediated by WNK463-dependent stimulation of TAK1 or TRAF2/ASK1, the upstream activators of MKK3/6/4. However, this effect was temporary and was reversed by long-term (2 days) incubation with WNK463. Accordingly, 2 days (but not 2 hrs) inhibition of p38-MAPK or its upstream activators ASK1 or TAK1, or WNKs, diminished regulatory volume increase (RVI) following cell shrinkage under hyperosmolarity. Overall, our study reveals a tight connection between the p38 and the WNKs pathway and both pathways are important in regulating RVI.

B473/P1457

The DUSP Domain of the Pseudophosphate MK-STYX Interacts with G3BP1 to Decrease Stress Granules

O. Mitchell, J. Smaileys, F. Jiang, L. Zavada, S. D. Hinton; College William & Mary, Williamsburg, VA.

Mitogen-activated protein kinase phosphoserine/threonine/tyrosine-binding protein (MK-STYX) is a dual specificity (DUSP) member of the protein tyrosine phosphatase (PTP) family. In particular, MK-STYX is a pseudophosphatase, which lacks the essential amino acids histidine and cysteine in the catalytic active signature motif (HCX_5R), of the MAPK phosphatases (MKPs). We previously reported that MK-STYX interacts with G3BP1 [Ras-GAP (GTPase-activating protein) SH3 (Src homology 3) domain-binding-1], and the DUSP domain to reduce stress granules, the protective response of cells to environmental stressors. Stress granules are stalled mRNA and proteins that form cytoplasmic aggregates. They serve as temporary sites of mRNA modification, sorting, and translation of essential proteins during cellular stress. To determine how MK-STYX reduces stress granules, simplified versions of MK-STYX's two domains, CH2 (cdc 25 homology) and DUSP, were used. HEK/293 cells were co-transfected with G3BP1-GFP (stress granule nucleator) and mCherry-MK-STYX, mCherry-MK-STYX-CH2, mCherry-MK-STYX-DUSP or mCherry and analyzed with fluorescent microscopy and coimmunoprecipitation. Cells co-expressing G3BP1-GFP and mCherry (control) formed stress granules. Whereas G3BP-GFP and mCherry-MK-STYX cells showed a decrease in stress granules, which was expected. In addition, cells expressing G3BP1-GFP and mCherry -MK-STYX-DUSP showed a decrease in stress granules compared to G3BP1-GFP and mCherry or mCherry -MK-STYX-CH2. To further analyze the interaction of G3BP1 and the domains of MK-STYX, coimmunoprecipitation experiments were performed. mCherry -MK-STYX-DUSP domain and G3BP1-GFP or G3BP1 (endogenous) were coimmunoprecipitated, suggesting that it is the DSUP domain of MK-STYX that interacts with G3BP1 to prevent stress granule assembly.

B474/P1458

Hsp90 activity required for phosphorylation and nuclear accumulation of Akt1

A. Vogel, C. Corvalan, M. Doriti, O. Fida, E. Zolotareva, I. Yudushkin; University of Vienna, Vienna, AUSTRIA.

Akt localizes in the cytosol and in the nucleus in many cell types, yet whether nuclear Akt is catalytically active and how it is transported into the nucleus is not known. Previous reports suggested that Akt interacts with Hsp90. Here we propose that the nuclear accumulation of active, phosphorylated Akt is dependent on Hsp90 activity. To test this hypothesis, we compared the nuclear localization and phosphorylation of all three Akt isoforms upon inhibition of Hsp90 and upon allosteric inhibition of Akt in HeLa cells. We found that Akt2 shows higher nuclear localization in steady state conditions compared to Akt1 and Akt3. Hsp90 inhibition resulted in decreased nuclear localization of constitutively active (ca) $Akt1^{ca}$ and $Akt2^{ca}$, while the localization of the wildtype Akt1, 2 and 3 was unaffected. Hyperactive $Akt1^{ca}$ phosphorylation on T308 was decreased in a concentration dependent manner when Hsp90 was inhibited, suggesting that Akt1 in the nucleus is protected from dephosphorylation by Hsp90. Our data indicate that Akt1 but not Akt2 requires Hsp90 activity to sustain its phosphorylation and nuclear accumulation. Akt2 phosphorylation and nuclear accumulation appear to be independent of Hsp90 activity, but its nuclear localization is dependent on its conformation. Taken together these results suggest that Hsp90 can protect Akt from dephosphorylation and promote its nuclear accumulation in an isoform specific manner.

B475/P1459

Identifying the Tao kinase as an upstream regulator of the Misshapen kinase in the *Drosophila* egg chamber

J. Paras; Butler University, Indianapolis, IN.

Intercellular bridges are an essential structural feature of developing eggs and sperm in many organisms, but the pathways that regulate their formation, stability, and growth are not fully understood. The developing *Drosophila* egg chamber provides an excellent model system to study intercellular bridges. Within the egg chamber, the developing oocyte is connected to the supporting nurse cells by large intercellular bridges, or ring canals. We have previously shown that the Ste20 kinase, Misshapen (Msn), localizes to these germline ring canals and regulates their size and stability; however, it is not known how Misshapen is targeted to the ring canals or how its activity is regulated during oogenesis. Here, we test the hypothesis that the Tao kinase phosphorylates and activates Msn in the germline to regulate the size and stability of the ring canals. Our preliminary data suggest that depletion of Tao in the germline alters ring canal size and the size of the mature eggs that are produced. In addition, Western blot analysis supports a role for Tao in phosphorylating Msn. In the future, we hope to further explore a role for Tao in regulating the localization and activity of Msn in the germline.

B476/P1460

Interaction Between Pseudophosphatase MK-STYX and Vimentin

P. Singh, G. N. Wagner, L. Zavada, S. D. Hinton; Department of Biology, College of William & Mary, Williamsburg, VA.

The neuron has a unique structure required for efficient cellular communication. The specific mechanisms by which the neuron forms its unique shape still remains elusive. We previously reported that MK-STYX [mitogen-activated protein kinase (MAPK) phosphoserine/threonine/tyrosine binding-protein] induces neurite outgrowths in rat pheochromocytoma PC-12 cells and primary rat hippocampal neurons. Although MK-STYX is a catalytically inactive (pseudophosphatase) member of the MAPK phosphatase (MKP) family, it serves as a signaling molecule, which may interact with other proteins. A recent mass spectrometry interactome study revealed that MK-STYX interacts with cytoskeletal protein vimentin. Vimentin is an intermediate filament that supports growing neurites. We hypothesized that MK-STYX interacts with intermediate filament vimentin to regulate neuritogenesis. Our studies seek to validate the interaction of MK-STYX and vimentin. Here, we sought to characterize the dynamics between MK-STYX and vimentin through colocalization studies. We co-transfected HEK293 cells with MK-STYX-GFP and Vimentin-mCherry. Fluorescence microscopy showed partial colocalization of MK-STYX and vimentin. The partial colocalization was observed in the cytoplasm with a 45 percentage of cells with colocalization. Similar results were seen in cells overexpressing vimentin and the MK-STYX active mutant (MK-STYXactive) which suggests that the non-catalytic phosphatase function of MK-STYX is crucial for its function. The colocalization was observed within the cytoplasm of cells in both cases. Phosphorylated vimentin subunits are often found in the cell body of undifferentiated neurons. Thus, this cytoplasmic interaction may suggest that MK-STYX interacts with vimentin monomers to induce neuritogenesis. Taken together, these results support a previously reported protein-protein interaction, dynamics between MK-STYX and vimentin, and give new insights into the mechanisms underlying neuronal differentiation.

B477/P1461

Dsk1 and Kic1 kinases in heterochromatin dynamics

A. Sugarman[1], A. Vyas[2], Z. Ralston[2], B. Alhoch[2], P. Zhang[2], C. Ylagan[1], X. Yang[3], C. Tang[3], Z. Tang[2]; [1]Scripps College, Claremont, CA, [2]Claremont McKenna College, Claremont, CA, [3]Peking University, Beijing, CHINA.

Dsk1 is the ortholog of human serine-arginine (SR) protein-specific kinase1 (SRPK1 and Kic1/Lkh1 (LAMMER kinase homolog1) is a homolog of mammalian Clk/Sty kinases in the fission yeast, *Schizosaccharomyces pombe*. Dsk1 and Kic1 belong to the evolutionarily conserved LAMMER-related kinase family. While these kinases have been previously identified as important regulators of cellular function with roles in mitotic cycle, differentiation, and development, the molecular mechanisms through which they act remain unclear. Previous results spurred our interest in the potential involvement of Dsk1 and Kic1 in heterochromatin dynamics. Genome-scale epistasis analysis uncovered strong genetic interactions of Dsk1 with components of the DASH complex, an essential component of kinetochores. We thus tested the functional involvement of Dsk1 and Kic1 and found that Dsk1 or Kic1 kinase activity is required for complete gene silencing at centromeres, implicating their role in the dynamic state of the centromeric heterochromatin. Also, Kic1 predominantly localizes to the nucleus as 2-5 discrete dots, similar to the localization pattern of Swi6/HP1 (heterochromatin protein1), a key protein for centromeric heterochromatin and the ortholog of human HP1. We further investigated possible colocalization of Kic1 and Swi6/Hp1 at nuclear heterochromatin foci, as well as whether the Dsk1 and Kic1 kinases affect the cellular localization, modification, and overall production of Swi6/HP1 protein. Indirect immunofluorescence analysis provides evidence for the colocalization of GFP-Kic1 with Swi6 in wild-type cells, with the characteristic pattern of nuclear heterochromatic foci. Additionally, using GFP-tagged *swi6* strains, we detected some delocalization of the Swi6/HP1 signal to the cytoplasm in the *dsk1* or *kic1*-deletion strains compared with the wild-type nuclear punctate pattern. Notably, this cytoplasmic delocalization phenotype is significantly exacerbated in *Δkic1* mutants under the condition of temperature shift to 42^{o}C. Further, preliminary co-immunoprecipitation data are consistent with potential physical interactions between Kic1 and Swi6/HP1. Our results suggest that Dsk1 and Kic1 kinases may be involved in the recruitment of Swi6/HP1 to the nuclear heterochromatin foci, influencing the dynamic state of chromatin.

B478/P1462

Effects of GSK3 Isoforms on Skeletal Muscle Differentiation and Autophagy

Y. Hsu, M. Chuang, Y. Liu; Institute of Molecular Medicine, National Taiwan University, Taipei, TAIWAN.

Glycogen synthase kinase 3 (GSK3) is a ubiquitously expressed protein kinase involved in various signaling pathways, including insulin, mTOR, Wnt signaling pathways. GSK3 is comprised of two isoforms, GSK3α and GSK3β, encoded by two homologous and highly conserved genes. In the past, scientists have mostly paid attention to GSK3β, in part because of the embryonic lethality of GSK3β-knockout mice, whereas GSK3α-knockout mice were viable and fertile, leaving GSK3α neglected. Over the past few decades, increasing studies have shown that GSK3α has isoform-specific functions in distinct tissues. For instance, GSK3α was demonstrated as a critical regulator of aging in mouse cardiomyocytes potentially through autophagy regulation. In our work, we aim to investigate the role of GSK3α in skeletal muscle. We observed that the depletion of GSK3α does not affect the differentiation or insulin response in C2C12 myoblasts. On the other hand, GSK3β depletion results in less myotube

number yet with higher insulin sensitivity. On the contrary, we discover that the formation of autophagosome is promoted in GSK3α-depleted C2C12 myoblasts but restrained in cells with GSK3β depletion. In summary, our data suggest that GSK3α and GSK3β indeed have distinct functions on skeletal muscle differentiation and autophagy.

B479/P1463

A computational characterization of a novel predicted NEK10 - MAP3K1 interaction

A. Silva Dos Santos[1,2], S. Singh[1,2,3]; [1]Biology, Brooklyn College, CUNY, Brooklyn, NY, [2]The Biochemistry PhD Program, The Graduate Center of the City University of New York, New York, NY, [3]Biology, The Graduate Center of the City University of New York, New York, NY.

The NEK kinase family of proteins comprises of eleven serine/threonine kinases that participate in the disjunction of the centrosome, mitotic spindle assembly, and primary cilium formation. NEK10 is the most divergent member of the NEK family. It is unique in having a catalytic domain that is centrally positioned and flanked by two coiled-coil domains, in contrast to the N-terminal location of the catalytic domains of other NEKs. NEK10, instead has four armadillo repeats in its N-terminus. NEK10 is suggested to play a key role in carcinogenesis and has been linked to melanoma, breast cancer, and various ciliopathies. As part of our long-term goal to construct interactomes of all the NEK members, we have formerly reported data for known and predicted NEK10 interacting proteins, including novel protein-protein interactions such as HSPB1 and MAP3K1, which have not been previously reported in the literature. In this study, we focused on understanding the molecular mechanism underlying NEK10's interaction with MAP3K1 (MAPKKK1, MEKK, MEKK1) and its functional consequences. MAP3K1 is a serine/threonine kinase and ubiquitin ligase that possess a pivotal role in a network of enzymes integrating cellular receptor responses to several mitogenic and metabolic stimuli. Recent studies have shown that MAP3K1 mutations are seen in a significant number of different cancers, being most prominent in luminal breast cancer. MAP3K1 contains a protein kinase domain, PHD finger (which has a RING finger domain-like structure) that serves as an E3 ubiquitin ligase, and scaffold protein regions that mediate protein-protein interactions. Using a computational approach, we modeled and characterized the full-length NEK10 and MAP3K1 proteins. In addition, we probed the role of the NEK10 kinase domain in its interaction with MAP3K1. Our docking analysis shows that I693 of NEK10 located within its kinase domain interacts with well-known phosphosite S275 of MAP3K1. Our results, therefore, suggest a scenario in which, upon UV irradiation, NEK10 phosphorylates and activates MAP3K1, which in turn phosphorylates MAP2K1/2-ERK1/2 promoting cell survival. Overall, this study shows sheds light on NEK10's structure-function relationships, especially in the context of its interaction with MAP3K1. Furthermore, it establishes the framework for elucidating the detailed molecular mechanisms of NEK10 interactions with other proteins to further explore its potential as a therapeutic target.

B480/P1464

Probing the role of subcellular populations of Extracellular Signal-Regulated Kinase on cell fate decisions

K. Slye, C. Scott, O. Webster, J. Keyes; Pennsylvania State University Erie, Erie, PA.

Extracellular signal regulated kinase (ERK) is a MAP kinase that regulates the proliferation and differentiation of mammalian cells. Although ERK has been an area of intense research for decades, an exact understanding of how ERK is differentially regulated to perform a plethora of functions remain

elusive. Previous work from our group indicates that, in PC12 cells, ERK inhibition at the plasma membrane does not inhibit ERK activity elsewhere in the cell. Upon plasma-membrane specific ERK inhibition, we observe changes in cell morphology and EGF-induced protrusion dynamics that mimics NGF-induced protrusions. This suggests that plasma membrane pool of ERK, which has previously been overlooked, plays a critical role in cell fate decisions. Here, we report that ERK activity displays unique subcellular spatiotemporal dynamics in response to an individual signals, and we report new genetically encodable tools to probe the function of subcellular dynamics.

B481/P1465

The Target of Rapamycin TORC2 complex regulates the activity of conserved NDR kinase Orb6 to control cell morphogenesis and chronological lifespan.

D. D'Urso, L. Doyle, C. Romero, C. Chen, **F. Verde**; University of Miami, Miami, FL

Conserved NDR kinase controls cell morphogenesis from yeast to human cells and has a role in the onset of disease. In fission yeast, the NDR kinase Orb6 controls cell polarity by spatial regulation of the key morphology factor Cdc42 GTPase, by direct phosphorylation of the Cdc42 GEF Gef1. Furthermore, Orb6 kinase promotes polarized cell growth and modulates chronological cell aging, by altering the phase separation properties of RNA binding protein Sts5. We previously showed that Orb6 kinase activity responds to nutritional availability and is downregulated by nutritional stress, however the signaling mechanisms that mediate this response are still unclear. Therefore, we performed a genome-wide screen to define the functional interaction network that controls Orb6 kinase activity. We found that Orb6 kinase genetically interacts with multiple key stress-sensitive kinase pathways involved in glucose starvation, nitrogen starvation, and salt stress. In particular, we found that *orb6* mutants display genetic interactions with mutants in components of the TOR (Target of Rapamycin) complex 2 (TORC2) complex, or in components that are shared with both the TORC1 and TORC2 complexes. Consistent with these observations, we found that *orb6* mutants display strong synthetic lethality with the TORC1 inhibitor Rapamycin. However, we found that Rapamycin does not promote Cdc42 delocalization, Gef1 dephosphorylation or Sts5 aggregation, hallmarks of Orb6 kinase inhibition. Conversely, we found that exposure of fission yeast cells to Torin1, an inhibitor of both TORC1 and TORC2, leads readily to Cdc42 delocalization, Gef1 dephosphorylation and Sts5 aggregation. To further analyze the role of TOR kinases in the control of Orb6 kinase activity, we tested the effect of Rapamycin and Torin1 on the phosphorylation state of Orb6 Serine 456, a crucial site for kinase activation in AGC kinases, using custom-made phosphospecific antibodies. We found that Torin1 strongly reduces the levels of Orb6 kinase phosphorylation on Serine 456, while Rapamycin has little or no effect. Finally, consistent with TORC2 functioning in the Orb6 pathway, we found that mutants of the TORC2 complex alter the response of Orb6 substrates Sts5 and Gef1 following exposure to Torin1 or nutrient stress. In summary, our observations indicate that conserved NDR kinase Orb6 functions downstream of TORC2, highlighting a novel functional connection for TORC2 in the control of cell morphogenesis and chronological lifespan in eukaryotes.

B482/P1466

Dissecting dynamics of glycans signaling by real-time microscopy

N. Baranova, H. Vrban, C. Rademacher; University of Vienna, Vienna, AUSTRIA.

We have detailed biophysical models on how immune cells manage to find an antigenic peptide in the sea of other peptides. However, we do not have the same level of understanding on how immune cells recognise pathogen-associated glycan motifs. Why is it important to consider the dynamics of interactions when we think about the immune response to glycans? The recognition of glycan motifs by innate immune cells is mediated through a dedicated family of receptors, such as C-type lectins. The affinities of most C-type lectins to glycans are very low, and this sort of recognition is a dynamic on- and off- process. Being intrinsically transient, some of the recognition events are sufficient to activate intracellular signaling pathways. How this activation is achieved on molecular level remains largely unclear. Here we developed an assay to investigate at nm-precision recognition of pathogenic glycans by engineered immune cells. The well-controlled reconstitution on silica beads enabled us to correlate the length and density of beta-glucans with the activation of NFkB or MAPK signaling pathways. Using this assay we found that soluble small molecular weight fragments of beta-glucans are capable to activate cell signaling *via* Dectin-1 receptor without a need to form an immune synapse. Using similar reconstitution, but on a planar surface, we aim to investigate the dynamics of Dectin-1 receptor oligomerization at a single-molecule level and correlate it with the activation of cell signaling. The gained here mechanistic knowledge can instruct further development of glycomimetic drug delivery systems for controlled engineering of immune response.

B483/P1467

Characterizing the interaction between mTORC2-component SIN1 and the small GTPase Rap1

A. Orlinski[1], P. Charest[2]; [1]Chemistry and Biochemistry, University of Arizona, Tucson, AZ, [2]Molecular and Cell Biology, University of Arizona, Tucson, AZ.

The mechanistic Target of Rapamycin Complex 2 (mTORC2) is one of two evolutionarily conserved signaling complexes formed by the kinase mTOR. Whereas mTORC1 is a master regulator of cell growth, mTORC2 regulates cell survival and cell migration, as well as some aspects of metabolism. mTORC1 has been extensively studied and much is known about its biochemistry and regulation, but less is understood about how mTORC2 is regulated. Previously, we discovered that *Dictyostelium* mTORC2 is regulated by the small GTPase Rap1 through its interaction with the mTORC2 component RIP3/SIN1, an interaction that we found to be conserved with the human proteins. Here, using pull-down assays with recombinant proteins and in-cell Bioluminescence Resonance Energy Transfer (BRET) assays, we investigated the molecular determinants of the Rap1-SIN1 interaction *in vitro* and in live cells. Our results suggest that SIN1 preferentially interacts with active Rap1, and that this involves SIN1's Ras Binding Domain (RBD). Moreover, using BRET, we detected the interaction of SIN1 with both Rap1a and Rap1b isoforms in live HEK293T cells, and our analyses suggest a preference of SIN1 for the active forms of the GTPases. Ongoing work focuses on investigating the contribution of SIN1's PH domain in mediating the interaction with Rap1, and the dynamics of the interaction. mTORC2 is a key signaling hub in many pathways and has been implicated in oncogenesis. Therefore, a better knowledge of the molecular mechanisms involved in mTORC2 regulation and signaling is important to our understanding of tumorigenesis and identifying new therapeutic strategies.

B484/P1468

Design principles of Cdr2 node patterns in fission yeast cells

H. Opalko[1], S. Geng[2], A. R. Hall[2], D. Vavylonis[2], J. B. Moseley[1]; [1]Dartmouth Medical School, Lebanon, NH, [2]Physics, Lehigh University, Bethlehem, PA.

Pattern forming networks have diverse roles in cell biology from eukaryotic development to bacterial cell division. Rod-shaped fission yeast cells use pattern formation to control the localization of mitotic signaling proteins and the cytokinetic ring. During interphase, the mitotic kinase Cdr2 forms membrane-bound multiprotein complexes termed nodes, which are positioned in the cell middle due in part to the node inhibitor Pom1 enriched at cell tips. Node positioning is important for timely cell cycle progression and positioning of the cytokinetic ring. Here, we combined experimental and modeling approaches to investigate pattern formation by the Pom1-Cdr2 system. We found that Cdr2 nodes accumulate at cortical regions nearest to the nucleus, and Cdr2 undergoes nucleocytoplasmic shuttling when cortical anchoring is reduced. To explore how node patterns arise, we generated particle-based simulations based on tip inhibition, nuclear positioning, and cortical anchoring. We tested predictions from these models by investigating Pom1-Cdr2 localization patterns after perturbing each positioning mechanism, including in both anucleate and multinucleated cells. Experiments show that tip inhibition and cortical anchoring alone are sufficient for the assembly and positioning of nodes in the absence of the nucleus, but that the nucleus and Pom1 facilitate the formation of unexpected node patterns in multinucleated cells. Our results suggest that local node concentration is determined by competition between a positive (nucleus) and negative (Pom1) regulator buffered by cortical anchoring. This model has implications for spatial control of cytokinesis by nodes and for spatial patterning in other cell signaling networks.

B485/P1469

Functional Interaction between PKA and 4.1B During PDGF-signaling

P. B. Deming; University of Vermont, Burlington, VT

cAMP-dependent protein kinase A (PKA) is a serine/threonine kinase that regulates cellular processes including proliferation, survival, differentiation, and migration in response to a variety of extracellular cues. Our lab has previously identified the platelet-derived growth factor receptor (PDGFR), as a regulator of PKA activity through tyrosine phosphorylation of the catalytic subunit. However, the PKA-mediated downstream signaling events and functional impacts remain largely unknown. As tyrosine phosphorylation of proteins often generates a docking site for the recruitment of signaling proteins, we hypothesized that tyrosine phosphorylation of the PKA catalytic subunit may induce protein-protein interactions important for the cellular response to growth factors. Here we report that activation of the PDGFR in NIH 3T3 mouse fibroblasts induces a novel interaction between PKA and EPB41L3 (4.1B), a 4.1 family member known to serve an important role in cytoskeletal organization. Stable isotope labelling of amino acids in culture (SILAC) coupled with mass spectrometry identified induced binding of 4.1B to PKA in NIH3T3 cells stimulated with platelet derived growth factor (PDGF). Moreover, 4.1B was phosphorylated in PDGF-stimulated cells and this phosphorylation was blocked when cells were pretreated with the PKA inhibitor, H89. In vitro kinase assays revealed that PKA can directly phosphorylate 4.1B, and mass spectrometry identified the phosphorylation site as serine 428. Importantly, mutation of serine 428 to non-phosphorylatable alanine (S428A) ablated PDGF-induced phosphorylation of 4.1B, suggesting that this is the primary site of phosphorylation during PDGF-signaling. Taken together these results reveal a novel interaction between PKA and 4.1B during PDGF

signaling. Current studies are focused on elucidating the impact of PDGF-induced phosphorylation of 4.1B on cytoskeletal organization, cell adhesion and migration, which could shed light into the mechanism/s whereby PKA regulates these cellular processes.

B486/P1470

Research Focal Areas Associated with Understudied Kinases, GPCRs, and Ion Channels: A Portfolio Analysis

B. Hair, K. Sharma, D. Sheeley; NIH, Rockville, MD

Introduction: In 2014 the National Institutes of Health's Common Fund launched the Illuminating the Druggable Genome (IDG) program to generate information on proteins that are currently understudied within three commonly drug-targeted protein families (kinases, G protein coupled receptors (GPCRs), and ion channels). The program funded Data and Resource Generation Centers (DRGCs) to generate innovative data and tools for the understudied proteins and pilot projects to study the proteins beyond the scope of the DRGCs.
Objective: The goal of this study is to assess the major focal areas of research associated with the understudied proteins by analyzing a portfolio of grants funded by IDG.
Methods: Publications associated with the DRGCs and abstracts for the pilot project awarded grants (R03 awards) were assessed. Each publication and R03 award was assigned a research focus area. Publications and awarded grants with more than one category were double-counted. Review publications were excluded.
Results: 57 grants and 46 publications were included in the analysis. Overall, approximately 60% of publications associated with the DRGCs focused on probe discovery, assay development, or probe development. Of the publications that focused primarily on a specific disease, cancer was the most frequent research focal area, followed by neurological and sleep cycle research. When assessing research areas by the protein families individually, cancer was the most frequent focus for kinases, followed by probe discovery and resource development. For GPCRs, probe discovery and assay development were the most focused on research areas. For both kinases and GPCRs, the majority of the publications were resource focused (probe development, probe discovery, assay development, or resource development) rather than disease focused.
For the R03 awards, the research areas were primarily disease focused. About a quarter of the grants focused on cancer and neurological diseases each. Diseases or disorders of the metabolic system were the focus of 11% of the awards, followed by the lymphatic system (10%), mental health/substance use (5%), and developmental disorders (5%).
Conclusion: IDG's portfolio includes a diverse mix of resource-focused and disease-focused research, which has enabled the program to generate information of broad scientific interest.

B487/P1471

Pathogenic mutations in *TTBK2* localize at the peroxisome and modify primary cilia membrane composition

J. MUNOZ ESTRADA, S. GOETZ; Duke University, Durham, NC

Tau Tubulin Kinase 2 (TTBK2) is a kinase that localizes to the mother centriole and is required for primary cilia formation. *TTBK2* mutations cause neurodegenerative disorder Spinocerebellar ataxia 11 (SCA11), which affects the cerebellum. Our previous work showed that these pathogenic variants of *TTBK2*

dominantly interfere with primary cilium formation and Shh signaling in mice. Nevertheless, the molecular mechanisms underlying the dominant interference of mutations remain unknown. We found that the SCA11-associated mutations generate a reliable peroxisomal target sequence, and their expression in RPE cells alters the morphology of peroxisomes, reduces their numbers and alter the molecular machinery of peroxisomal division. Consistent with previous reports that peroxisomes supply ciliary membrane with cholesterol essential for Shh signaling, we find that cells expressing pathogenic *TTBK2* variants have reduced numbers of peroxisomes near the base of cilia, and defective trafficking of ciliary Smoothened. These results highlight and strengthen a novel communication between peroxisome and primary cilia and indicate that peroxisomes are necessary for maintaining ciliary membrane composition. Our findings uncover a dominant, neomorphic function of SCA11-associated mutations and shed light on the molecular mechanisms underlying SCA11.

B488/P1472

The dual roles of Pib2 in reactivating and inhibiting vacuolar TORC1 in *S. cerevisiae*

K. K. Troutman, N. V. Varlakhanova, M. G. J. Ford; University of Pittsburgh, Pittsburgh, PA

The target of rapamycin complex 1 (TORC1) is a kinase complex that is highly conserved through eukaryotes. TORC1 is essential for the control of cell growth and autophagy and dysregulation of the complex has been implicated in a variety of diseases. The TORC1 complex incorporates nutritional cues via several upstream regulators to appropriately modulate cell growth. TORC1 can be inhibited by nutrient starvation or treatment with the specific inhibitor rapamycin, which arrests cell growth and triggers an increase in autophagy. Reactivation of TORC1 can be achieved by amino acid stimulation. In *Saccharomyces cerevisiae*, this reactivation is dependent on phosphatidylinositol 3-phosphate-binding protein 2 (Pib2), which has been shown to interact with and activate TORC1 in a glutamine dependent manner. Pib2, has also been shown to have a TORC1 inhibitory function, however, the exact molecular mechanisms of Pib2 regulation remain poorly understood. Multiple sequence alignments of Pib2 from ascomycete fungi highlight several conserved regions and domains. Here we use rapamycin exposure assays and live-cell confocal imaging to functionally dissect these conserved Pib2 regions. Using these assays, we have identified regions and key residues that are essential for TORC1 reactivation and Pib2 localization. We demonstrate that the TORC1 inhibitory function of Pib2 can be pinpointed to residues in the N-terminal A and B regions, while the TORC1 activation function of Pib2 is dependent on its C-terminal helical E region, FYVE domain, and tail motif. TORC1 and Pib2 localize primarily to the vacuolar membrane as well as signaling endosomes. To determine the effects of subcellular localization of Pib2 on its ability to regulate TORC1 activity, we generated fusion proteins that target Pib2 to the vacuole or endosome. Using rapamycin exposure assays we demonstrate that vacuolar localization of Pib2 is essential for the reactivation of TORC1. We also show that while the Pib2 PI3P-binding FYVE domain is critical for vacuolar localization, it is interestingly not needed for rapamycin recovery. Here we have demonstrated that Pib2 plays a role as a dual modulator of vacuolar TORC1 activity in *S. cerevisiae* which may have implications for the regulation of TORC1 activity in higher eukaryotes.

B489/P1473

Peptide Lv promotes trafficking and membrane insertion of K_{Ca}3.1 through the PI3K-Akt signaling pathway

D. L. Pham, A. Niemi, R. Blank, J. Tham, M. L. Ko, **G. Y. P. Ko**; Veterinary Integrative Biosciences, Texas A&M University, College Station, TX.

Activation of endothelial cells (ECs) for angiogenesis requires EC membrane hyperpolarization, which is mediated by activating potassium channels. Peptide Lv is an endogenous angiogenic peptide that can elicit EC hyperpolarization by increasing the current density of intermediate-conductance calcium-dependent potassium (K_{Ca}3.1) channels. One way to enhance the current density of an ion channel is to promote its trafficking and insertion into the plasma membrane. The phosphoinositide-3-kinase (PI3K)-protein kinase B (Akt) pathway is known to mediate the trafficking and membrane insertion of various ion channels in neurons. However, it is unclear whether K_{Ca}3.1 current density increased by peptide Lv is in a similar manner in ECs. Our hypothesis was that PI3K-Akt mediates peptide Lv-elicited K_{Ca}3.1 channel trafficking and membrane insertion, leading to increased K_{Ca}3.1 current density. To test this hypothesis, we employed patch-clamp electrophysiological recordings and cell-surface biotinylation assays with cultured human umbilical vein ECs in the presence and absence of peptide Lv and pharmaceutical inhibitors of PI3K and Akt. Inhibition of PI3K or Akt diminished peptide Lv-elicited EC hyperpolarization and augmentation of K_{Ca}3.1 current density. Blocking PI3K or Akt decreased the level of plasma membrane-bound but not cytosolic K_{Ca}3.1 protein indicating that inhibition of PI3K-Akt did not affect the protein expression of K_{Ca}3.1 but dampened its trafficking and insertion to the plasma membrane. Therefore, the peptide Lv-elicited EC hyperpolarization and K_{Ca}3.1 augmentation are in part through channel trafficking and insertion mediated by PI3K-Akt signaling. These results elucidate the molecular mechanisms by which peptide Lv promotes EC-mediated angiogenesis.

B490/P1474

Inhibition of collagen production in keloid fibroblasts through GSK-3β activation

M. Carulla, A. Asker, **D. Telgenhoff**; Oakland University, Rochester, MI

Keloid scars are abnormal wound healing processes characterized by excessive collagen deposition that exceed the borders of the original wound. These scars have a higher tendency to develop in individuals with a family history of keloid scarring, are under the age of 30, or are of African or Latin American descent. Keloid scarring may inhibit movement and have significant physical and psychological effects on the individual. Inactivated glycogen synthase kinase 3-β (GSK-3β), a molecule that breaks down β-catenin which is used by the Wnt/β-catenin pathway in hypertrophic scarring, has been found to be abundant in keloid fibroblasts. The Wnt/β-catenin pathway is known to influence hypertrophic scarring, and the activation of GSK-3β has been found to inhibit other hypertrophic scarring diseases. The objective of this study was to examine the effects of an activator or inhibitor of GSK-3β on keloid cell collagen production. Human keloid fibroblasts were cultured *in vitro* and treated with sodium nitroprusside (known to activate GSK-3β), lithium chloride (an inhibitor of GSK-3β), or a vehicle control. Cultured cells were permeabilized and incubated with antibodies for collagen I or collagen III followed by incubation in fluorescent labelled secondary antibodies and imaging. In duplicate cultures proteins were isolated in buffer, quantified, separated on 8% SDS-PAGE, and assessed through Western Blot analysis for changes in collagens I and III, elastin, fibronectin, and beta-catenin (normal and phosphorylated). All experiments were repeated in triplicate. The results of this study suggest that GSK-

3β is partially inactive in keloid fibroblasts and that activation of the protein activates the Wnt signaling pathway and decreases collagen and elastin production. Fibronectin levels were found to increase with treatment, possibly due to its role in cell adhesion and contraction in wound healing. These results indicate a role for GSK-3β in the Wnt/β-catenin pathway in the formation of keloid scars, and a potential target for inhibition of keloid formation.

Cytoskeletal-Membrane Interactions

B492/P1475

The Ste-20 kinase Slik deforms membranes to regulate cytoneme biogenesis independently of its kinase activity

B. Rambaud[1], M. Joseph[2], C. De Jamblinne[1], R. Sabelli[1], E. Del Guidice[1], D. Hipfner[2], S. Carreno[1];
[1]Institute for Research in Immunology and Cancer, University of Montréal, Montréal, QC, CANADA,
[2]Institut de recherches cliniques de Montréal, University of Montréal, Montréal, QC, CANADA.

Cytonemes are specialized actin-rich filopodia in charge of long distance cell-cell communication. They have attracted much interest in the recent years, but the mechanisms regulating their biogenesis are still poorly understood. Here, we show that Slik, a drosophila Ste-20 kinase, deforms membrane to induce budding and elongation of cytonemes.
We used drosophila S2 cells, a cell line that form cytonemes in culture, to show that overexpression of Slik increases cytoneme length and number. We also found that the role of Slik on cytoneme biogenesis is independent of its kinase activity and relies on its C-terminal domain. By Correlative Light Electron Microscopy (CLEM), we also observed that Slik-Cterm induces strong membrane deformation of the cell cortex. Interestingly, Slik-Cterm contains three coiled-coil domains that are organized to form I-BAR like domain. We are currently testing the possibility that Slik-Cterm acts as a non-canonical I-BAR domain that generate negative membrane curvature.Our study opens new avenue for investigating the kinase-independent function of Slik for cytoneme biogenesis.

B493/P1476

Three-dimensionally extending filopodia of neuronal growth cones, are associated with the localization of an axon guidance receptor

M. Nozumi, M. Igarashi; Dept of Neurochem & Mol Cell Biol, Niigata University Grad Sch Medical Dent Sci, Niigata, JAPAN.

Neuronal growth cones are the highly motile structures at the tip of extending axons in the developing or regenerating neurons. The highly dynamic actin structure in the peripheral domain is crucial to the growth cone motility and its guidance. Using SIM super-resolution microscopy, we have previously revealed that local endocytosis is associated with the F-actin-bundling at the leading edge of growth cones. The F-actin-dependent endocytosis most likely contributes to the membrane retrieval, containing the lipid rafts from the nonadherent, upper surface of the growth cone [Cell Rep 2017]. Using 3D-SIM, we also found that F-actin bundles were distributed along the upper surface, and part of them were protruding, not only towards the leading edge, but also in the z-axis direction. An axon guidance receptor neuropilin-1 was transiently concentrated in this type of filopodia. The localization of neuropilin-1 was extinguished by cholesterol depletion with methyl-beta cyclodextrin. Interestingly, synaptophysin and two endocytic components, endophilin A3 and dynamin 1, also were accumulated in

such a type of filopodia. These results suggest that the formation of this type of 3D-extending filopodia effectively promotes the neuropilin-1-associated local endocytosis, and our observation indicates that the growth cone has a cooperating, sensing mechanism by actin polymerization, as well as its advancing activity.

B494/P1477

Deciphering an unconventional role fo BIN1 in filopodia-like structure formation

C. Gauthier-Rouviere[1], L. Picas[2], F. Comunale[1], C. André[2], H. Bousquet[3], F. Tsai[3], F. Rico[4], P. Maiuri[5], J. Pernier[6], S. Bodin[1], A. Nicot[7], J. Laporte[8], P. Bassereau[3], B. Goud[3], S. Miserey[3]; [1]CNRS-CRBM, Montpellier, FRANCE, [2]IRIM-CRBM, Montpellier, FRANCE, [3]Curie institute, Paris, FRANCE, [4]INSERM, Marseille, FRANCE, [5]Napoli university, Napoli, ITALY, [6]CNRS, Paris Saclay, FRANCE, [7]INSERM, Grenoble, FRANCE, [8]CNRS, Illkirch, FRANCE.

BIN1/M-Amphiphysin2 is a splice variant of Amphiphysin2 expressed in muscle that has been implicated in T-tubule biogenesis. BIN1 belongs to the BAR (Bin1-Amphiphysin-Rvs) domain family, which are known to induce membrane curvature and participate in endocytosis in certain cell types. Several BIN1 mutations are reported on centronuclear myopathies (CNMs), a heterogeneous group of inherited muscular disorders that are characterized by a compromised normal muscle function and nuclei positioning, ultimately leading to fiber atrophy and muscle weakness. The suppression of BIN1 on muscular cells has shown to impair the fusion of myoblast during muscle differentiation[1], although the exact mechanism remains elusive. Here, we report that BIN1 participates in forming filopodia-like structures at myoblasts' intercellular junctions, structures that promote adhesion and fusion of muscle cells. Furthermore, our results show that BIN1 assembles actin bundles *in vitro* and regulates plasma membrane tension. We identified ezrin as a new BIN1 partner and showed that ezrin association with $PI(4,5)P_2$-containing model membranes and the plasma membrane is favored by BIN1. Our results establish BIN1 and ezrin as central players to form long-lived filopodia-like structures, known to promote adhesion and fusion of muscle cells.

B495/P1478

Dissecting the Recruitment Kinetics of Mechanosensitive Proteins to Stress Fiber Strain Sites

S. Sala, P. W. Oakes; Loyola University Chicago, Maywood, IL

Cells respond to a variety of mechanical cues by converting them into biochemical signals, a process called mechanotransduction. This ubiquitous process underlies many fundamental functions in cell biology, including differentiation and division. The actin cytoskeleton and its associated adhesion and motor proteins play a key role in mechanotransduction. This network produces many dynamic architectures, including stress fibers (SF) and focal adhesions (FA), which enable cells to generate and transmit forces to the extracellular environment. How forces are actually sensed in the actin cytoskeleton, however, remains poorly understood. LIM domain proteins (LDPs) have emerged as cytoskeletal mechanosensors that recognize SF strain sites (SFSS) through a conserved mechanisms requiring their LIM domains. In the case of zyxin, its binding partners VASP and α-actinin are also recruited to these SFSS. These proteins are thought to stimulate SF repair through polymerization and crosslinking of actin, respectively. The role of myosin in SF strain repair, and how it contributes to the maintenance of cellular force generation during repair remains unclear. To answer these major questions, we combined laser photoablations with traction fore microscopy to locally induce SFSS and

quantify protein recruitment to these sites while simultaneously measuring cellular forces at FAs during and after SF repair. Within seconds post strain induction, we observed a massive recruitment of the LDPs testin and zyxin to SFSS which coincided with a loss of actin and myosin in the region of strain and hence a reduction in local traction forces. LDP relocation to SFSS was followed by the recruitment of the Ena/VASP family of proteins which are known to promote actin polymerization. Interestingly, only when the SFSS was fully repaired, as indicated by a complete recovery of the actin signal and concomitant loss of the LDP and Ena/VASP signals, myosin association with actin was restored, resulting in a recovery of the local traction stresses. Together, our data provide novel insights into how SF damage is detected and repaired to maintain tensional homeostasis in cells. We propose a model showing that SF damage impairs actomyosin contractility reducing local force transmission in the adjacent FAs, which is recovered by a repair mechanism mediated by LIM domain and Ena/VASP proteins.

B496/P1479

Multistability and general relations of cell motion on Fibronectin lanes

B. Amiri[1], J. Heyn[2], J. Rädler[2], **M. Falcke**[1]; [1]Max Delbruck Ct Molec Med, Berlin, GERMANY, [2]Dept. of Physics, Ludwig-Maximilians-Universität, Munich, GERMANY.

Cell motility on flat substrates exhibits coexisting steady and oscillatory morphodynamics, the biphasic adhesion-velocity relation, and the universal correlation between velocity and persistence (UCSP) as phenomena common to many cell types and observed simultaneously. Their universality and concurrency suggest a unifying mechanism to exist causing all three of them. We search for such a mechanism by investigating a large ensemble of trajectories of MDA-MB-231 cells on Fibronectin lanes. We find cells with steady or oscillatory morphodynamics and either spread or moving. We observe apparently spontaneous transitions between the dynamic regimes, spread and moving motion states and direction reversals. We formulate a biophysical theory on the basis of the force balance at the leading edge, the noisy clutch of retrograde flow and a response function of friction and membrane drag to integrin signaling. The theory reproduces the experimental results with good quantitative agreement in a large Fibronectin density range. Analysis of the experiments with the biophysical model establishes a stick-slip oscillation mechanism, explains multistability of cell states and state transitions, and shows protrusion competition to cause direction reversal events, the statistics of which explains the UCSP. The model also explains cell behavior at Fibronectin steps and the adhesion-velocity relation. We suggest a mechanism, where signaling sets the cellular parameters for a multistable dynamic regime with steady or oscillatory morphodynamics, spread or moving motion states. These dynamics are driven by F-actin polymerization and shaped by the clutch mechanism of retrograde flow friction, protrusion competition via membrane tension and drag forces.

B497/P1480

Mechano- and phospho-regulation of End4p coordinates the temporal and spatial transmission of force during clathrin-mediated endocytosis

Y. Ren[1,2], J. Yang[1], B. Fujita[1,2], Y. Zhang[1,1], J. Berro[1,2,1]; [1]Yale University, New Haven, CT, [2]Yale University, West haven, CT

During clathrin-mediated endocytosis (CME), a patch of plasma membrane is reshaped into a cargo-filled vesicle by the choreographed action of more than 50 proteins conserved from yeasts to humans. Mechanical force from the actin cytoskeleton is crucial to overcome the energy barrier to deform the

membrane during CME, especially for mammalian cells with elevated membrane tension and cells with high turgor pressure such as the fission yeast *Schizosaccharomyces pombe*. Forces are transmitted to the membrane by adaptor proteins that physically connect the membrane's lipids to actin filaments. The onset and the termination of force transmission is tightly regulated temporally during different stages of CME, and spatially over the surface of the invaginated membrane. How force transmission is regulated and coordinated at the molecular scale is unclear.
Here we show that the THATCH actin-binding domain of the endocytic adaptor protein End4p (homologous to HIP1R in mammalian cells and to Sla2p in the budding yeast) is mechanosensitive, partially unfolds under 13 pN of force, and only gains affinity for actin filaments after partial unfolding. We also identified a previously uncharacterized domain upstream of THATCH, the R_{end} domain (**R** domain of **End**4p), a five-helix bundle which is homologous of Talin's R domains, yet cannot be functionally substituted by R_3. R_{end} unfolds under 15 pN in an all-or-none manner. R_{end} promotes the localization of End4p to endocytic sites and mediates End4p protein condensation with prolonged binding to either F-actin or the membrane. Membrane anchored R_{end} dimers form condensates, which are inhibited by the phosphorylation of R_{end} at T841. By using the new coiled-coil force sensors we developed, we measured forces during CME in wild type fission yeast cells on End4p are around 19 pN before THATCH and around 15 pN before R_{end}. We propose that the unfolding of THATCH initiates force transmission through End4p, that transient condensation of R_{end} spatially coordinates the transmission of force, and that phosphorylation and mechanical unfolding of R_{end} promotes the disassembly of End4p at the end of CME.

B498/P1481

Mechanosensitive cell volume regulation via actomyosin and ion transport

Q. Ni[1], Z. Ge[1], Y. Li[2], J. Fu[1], K. Bera[1], Y. Yang[1], A. Sen[1], Y. Wang[1], K. Konstantopoulos[1], S. X. Sun[1]; [1]Johns Hopkins University, Baltimore, MD, [2]The State University of New York at Binghamton, Binghamton, NY.

The mammalian cell volume plays an important role in regulating the mechanical and physiological functions of cells. During mitosis or exposure to extracellular mechanical and physiological challenges, cells are able to dramatically change their volume within minutes. Within this timescale, cell volume change comes from cell surface water flux, which is actively regulated by cell ionic fluxes and the hydrostatic pressure difference across the cell surface. The role of ion transporters in volume regulation has been well-established. However, the role of the actomyosin cytoskeleton mediated cell mechanics is still unclear. There is likely substantial interplay between ion channels/transports and cytoskeletal activity. In this paper, we applied osmotic shocks to several cell lines and studies their sequential cell volume response. We uncover that, in addition to the ion transport dominant cell volume regulation found in HT1080 and MDA-MB-231, there exists a mechanosensitive cell volume regulation system that combines both ion transport and actomyosin activity in NIH-3T3 and MCF-10A. In the latter cases, hypotonic stress triggers a 20% delayed volume increase over the timescale of 30 minutes following the initial volume regulatory recovery. We found that the delayed volume increase is initialized by Calcium influx through stretch-activated channel Piezo1, which induces actomyosin remodeling through Calmodulin. Rather than generating forces that directly contract cells, actomyosin regulates cell volume by activating Sodium-Hydrogen exchanger 1 (NHE1) through actin binding partner ezrin. Overall, our findings reveal the central role of Calcium and actomyosin mediated mechanosensation in regulating ion transportation and cell volume.

B499/P1482

Trogocytosis in macrophages is governed by target membrane tension

C. E. Cornell, A. Chorlay, D. A. Fletcher; Bioengineering, University of California, Berkeley, Berkeley, CA.

Macrophages are immune cells responsible for detecting and eliminating pathogenic bacteria and viruses, as well as cancerous and apoptotic host cells. While macrophages will often completely engulf their target through phagocytosis, in a growing number of examples they are observed to 'nibble' fragments off their target instead. Do the physical properties of the target membrane play a role in determining whether a target is phagocytosed or 'nibbled', a process known as trogocytosis? To address this question, we developed an assay using cell-sized giant unilamellar vesicles (GUVs) with fluid membranes containing biotinylated lipids bound by anti-biotin IgG, which are recognized by macrophage Fcγ receptors and trigger the phagocytic machinery. For GUVs under high membrane tension, macrophage-like RAW 264.7 cells bind and can fully engulf them within a matter of minutes. However, when we reduce membrane tension via micropipette aspiration or osmotic shock, we find that these highly deformable GUVs are only partially engulfed, leading to the internalization of target fragments that resembles trogocytosis. High-resolution fluorescence microscopy shows that broad extension of the phagocytic cup around GUVs under high membrane tension is replaced by short phagocytic cup extensions and target membrane deformation in the case of low membrane tension GUVs. We propose a model in which mechanical feedback during extension of the phagocytic cup leads to premature constriction and scission of membrane fragments under low target membrane tension. In the case of high membrane tension, the phagocytic cup continues to grow and eventually seal around the target.

B501/P1484

Focusing the Effort: Polarized cell adhesive recognition by Junctional Adhesion Molecule C orients granule neuron migration selectivity in a mouse model for cerebellar development

L. Hallada[1], D. Solecki[2]; [1]Developmental Neurobiology, St Jude Graduate School of Biomedical Sciences, MEMPHIS, TN, [2]Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN.

The laminar organization of the cerebellum is a result of intersecting mechanisms that regulate normal neural development and suppress disease. Cerebellar granule neuron (CGNs) coordinate differentiation, orientation, and migration to exit from their germinal zone in the external granule layer (EGL) via glial-guided migration. How CGNs encode the adhesive selectivity for the Bergmann glia substrate for migration has not been answered because it requires 1) identifying surface interactions that initiates this transition and 2) developing the techniques to study these cell-cell adhesions at sufficient resolution in the developing cerebellum. Previous work from the Solecki Lab showed that the intracellular partitioning defective (PAR) protein complex is responsible for the coordination of when and where the CGNs migrate by modulating morphological asymmetry, also known as cell polarity. In this research, the polarity-dependent junctional adhesion molecule (JAM)-C enabled us to develop a modular system of experimental models to live image cell-cell adhesions, manipulate the interfaces, and parse the composition of distinct surfaces in complex environments. We used single point mutants of JAM-C to show how the *cis* and *trans* interactions of JAM-C in CGNs cue glial-guided migration. Live-imaging neurons in *ex vivo* cerebellar slice cultures showed that JAM-C specificity in *cis* influences the proportion of cells transitioning to radial migration without fully inhibiting motility. While Fluorescent Recovery after Photobleaching of JAM-C adhesions showed that *cis* interactions distinguish JAM-C adhesions from

freely diffusing membrane, both *cis* and *trans* mutants reduced JAM-C to JAM adhesive recognition. Further live imaging studies showed that this JAM recognition regulates the actin during leading process extension and stabilization. Altogether these results suggest that JAM-C interactions constrict the architecture of the leading process in migrating CGNs to govern the timing of glial-guided migration based on the probabilistic efficiency of delimiting cytoskeletal dynamics.

B502/P1485

An investigation of how the physicochemical properties of membranes impacts septin association and assembly

B. Curtis[1], C. Edelmaier[2], A. Gladfelter[3]; [1]Department of Biochemistry and Biophysics, University North Carolina-Chapel Hill, Chapel Hill, NC, [2]Department of Applied Physical Science, University North Carolina-Chapel Hill, Chapel Hill, NC, [3]Department of Biology, University North Carolina-Chapel Hill, Chapel Hill, NC.

Throughout the lifecycle of most cells, they must be able to sense, generate, and react to changes in their shape. One such shape-defining structure is the cell membrane, which can be described in terms of its curvature over a range of nanometer to micrometer length scales. While nanometer-scale curvature sensors are well-documented in processes such as in endocytosis and cell trafficking, there are few known sensors of micrometer-scale curvature. Septins are filament-forming proteins necessary for cell processes involving major changes to cell shape, such as cytokinesis and polarized growth, and are intrinsically sensitive to micrometer-scale membrane curvature. Interestingly, they possess an amphipathic helix (AH) that is necessary and sufficient for septin curvature sensitivity but is structurally similar to AH domains specific to nanometer-scale curvature. How do these small helical domains give rise to micrometer curvature sensitivity? Previous work has shown that changes to lipid composition that alter lipid packing may mimic membrane curvature, and this may be especially relevant for septins which are capable of localizing to many different membrane geometries, both in vitro and in vivo. This work focuses on revealing the impact of lipid composition on septin association and assembly by changing the physicochemical properties of the membrane that may help septins “sense” curvature, such as the identity of the head group and the degree of lipid packing. Using a combination of molecular dynamics simulations and the membrane tension dye, FLIPT-R, we have assembled lipid compositions that span from loosely packed to tightly packed. We measure septin adsorption and observe septin assembly over time on these different compositions using in vitro reconstitution assays on supported lipid bilayers to assess how modulating membrane properties may alter septin assembly independently of curvature. As lipid packing becomes tighter, septin adsorption and assembly rates decrease, signifying both membrane geometry and lipid packing may be regulating spatio-specific localization of septins.

B503/P1486

Biophysical properties governing septin assembly

E. Vogt[1], I. Seim[1], B. Woods[1], J. Liu[2], G. McLaughlin[1], K. Cannon[1], A. Gladfelter[1]; [1]University of North Carolina At Chapel Hill, Chapel Hill, NC, [2]North Carolina School of Science and Mathematics, Durham, NC.

Across the biosphere, cells utilize nanometer-scaled proteins to form micron-scaled structures. Septins are a highly-conserved class of GTP-binding proteins that dynamically assemble into higher-order structures. These higher-order structures such as rings, lattices, and gauzes provide a scaffold for signaling molecules and other cytoskeletal elements and organizing cell membranes. How the geometry

of these septin assemblies is controlled by the cell remains open for intensive study. Marrying a filament polymerization reconstitution system with cell extracts utilizing yeast genetics, we begin to investigate how regulators tune biophysical processes necessary to build higher-order septin assemblies. Filaments polymerized from extracts were paired and their flexibility could be tuned by regulators. Septin assembly is a multi-step process involving septin interactions with the membrane and between septin proteins during filament polymerization. Whereas septin adsorption onto membranes is a cooperative process, multiple aspects of filament assembly on membranes are consistent with isodesmic filament polymerization. We parameterized a physical model of septin filament assembly on membranes, laying the groundwork for future investigations into how regulators control the assembly of higher-order septin structures.

B504/P1487

Dissection of a potentially ancient septin in the emerging model black fungus *Knufia petricola*

G. E. Hamilton, A. S. Gladfelter; Biology, University of North Carolina, Chapel Hill, NC.

Septins are filament-forming, GTP-binding cytoskeletal proteins. They self-assemble into diverse higher-order structures and sense micron-scale membrane curvature, two abilities that allows septins to function in processes including cytokinesis, membrane remodeling, scaffolding of signaling pathways, and cell polarity. Though conserved across eukaryotes (excepting land plants), septins are still relatively mysterious compared to other cytoskeletal polymers such as actin and microtubules. The best-characterized septins are the mitotic septins of budding yeast, founding members of four septin "Groups" of orthologous proteins that self-assemble into orthologous, palindromic hetero-oligomers Though the number of septin genes varies between organisms, likely due to gene duplication events, phylogenetic analysis indicates that nearly all septins studied to date fall into one of those four Groups. Another septin Group, however, is missing in unicellular yeasts, but present in the genomes of specific ciliates, diatoms, chlorophyte algae, brown algae, and filamentous fungi, suggesting that this "Group 5" septin-type is likely ancestral. To our knowledge, no Group 5 septin has ever been purified, nor characterized on the biophysical level.I identified a Group 5 septin in the genome of the emerging model black fungus *Knufia petricola*. I recombinantly expressed and purified this septin, dubbed KpAspE in recognition of its Aspergillus homolog. Remarkably, *in vitro*, KpAspE alone recapitulates many of the abilities of canonical septin heteromers. Gel filtration and mass photometry indicate that KpAspE forms homopolymers in a salt-dependent manner, much like canonical septins. Similarly, in a membrane curvature-sensing assay, KpAspE appears able to both bind supported lipid bilayers and discriminate between different degrees of positive membrane curvature- just as heteropolymeric septins do . Taken together, these results suggest that KpAspE homo-oligomers may replicate some of the most vital functions of core septin hetero-oligomers: forming micron-scale higher-order structures and sensing micron-scale membrane curvature. Current and future experiments are focused on potential KpAspE interactions with septins from Groups 1-4. If physical interactions exist, I will compare the functional consequences of KpAspE incorporation into homopolymers versus heteropolymers.Because septin genes are thought to have arisen from repeated gene duplication events, septin homo-oligomers may represent an ancestral state from which now-canonical septin hetero-oligomers evolved. Thus, Group 5 septins from non-model organisms- such as KpAspE- may offer us a window into the evolutionary history of septins.

B505/P1488

Deciphering the Role of RhoA and Cell Geometry in Septin Recruitment to the Actin Cortex

S. Chandrasekar, H. Wu, J. R. Beach, P. W. Oakes; Loyola University, Chicago, IL.

The cytoskeleton provides an architectural framework which cells use to generate and respond to physical forces. Historically, actin, microtubules and intermediate filaments have been the most well studied components of the cytoskeleton. More recently, septins have been recognized as a fourth important component of the cytoskeleton. These GTP-binding proteins not only localize to a subset of actin stress fibers, but also to regions that are thought to need mechanical reinforcement (e.g., base of cilia and blebs). In many of these regions there is also a concomitant enrichment of active RhoA (e.g. the cleavage furrow). This suggests septins could be playing a role in either altering or sensing local mechanical properties of the cytoskeleton. While the preferential localization of septins to micron-scale membrane curvatures has been previously shown, the mechanism by which septins are recruited to these structures and their specific contributions to the mechanosensitive properties of the cytoskeleton are yet to be explored. Here we test whether septins are recruited in response to RhoA activation, changes in cell geometry and mechanics, or a combination of these inputs. We find that global activation of RhoA is insufficient to spur additional septin polymerization in the cytoskeleton, but that RhoA inhibition leads to relocation of septins from stress fibers to the actin cortex/membrane. Interestingly, when cells migrate through microchannels featuring constrictions, septins accumulate along the cortex at the site of constriction. This suggests that septins relocate to sites of compression along the cortex. To test this further, future experiments will utilize an optogenetic RhoA probe to test how septins respond to locally induced contraction in both adherent and non-adherent cells. Together, these experiments will help reveal the mechanism(s) that govern septin localization and function in the cytoskeleton.

B506/P1489

ERM proteins respond to extracellular matrix mechanosensing

Z. Baster[1], A. Massey[2], A. X. Cartagena-Rivera[2], C. M. Waterman[1]; [1]National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, [2]National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD

Cells sense the stiffness of their extracellular matrix (ECM) and respond by modulating their mechanical properties, behavior, or identity. Such mechanosensing behavior can be mediated by signaling pathways that alter cytoskeletal protein activity to change cell mechanics and behavior, or that effect transcriptional regulators to change gene expression. For example, when cells sense a stiff ECM, they stiffen their own surface and the transcriptional co-activator Yes-Associated Protein (YAP) translocates from the cytosol to the nucleus to affect gene expression. We sought to determine if these two responses were linked, and if so, how. Cell surface mechanics are well known to be regulated by proteins of the Ezrin, Radixin and Moesin (ERM) family, which bind to phosphatidylinositol 4,5-bisphosphate (PIP2) in the plasma membrane and to the cortical actin cytoskeleton. ERM proteins are activated by phosphorylation and recent evidence suggests that their expression can be regulated by YAP. We thus sought to explore whether ERM proteins were required for changes in cell surface mechanics in response to ECM mechanosensing, and if this was mediated by ERM phosphorylation or YAP-dependent changes in ERM expression.

To investigate this hypothesis, we plated U-2 OS cells on polyacrylamide substrates with elastic modulus of 4 kPa or 50 kPa, and on plastic or glass coated with fibronectin. We assessed ERM protein expression,

phosphorylation and distribution using molecular biology- and light microscopy-based techniques. We found that Ezrin protein abundance decreased with decreasing substrate stiffness. Interestingly, Moesin did not show this same dependence. Furthermore, with atomic force microscopy, we analyzed how these changes impacted biophysical parameters such as cell surface stiffness.
In our next step, using YAP knock-down and overexpression, and Ezrin knock-down systems, we plan to investigate the role of YAP signaling pathway in the observed changes. Moreover, we want to examine whether the lower abundance of ERM on in cells plated on soft substrates influence plasma membrane - actin cortex organization.
Our results show that the function of ERM proteins is regulated by the surrounding mechanical environment. Furthermore, the observed changes in Ezrin levels may be involved in regulation of cell surface biophysical properties in response to different matrix stiffness.

B507/P1490

Defining the structure and function of the multivalent protein network at cell-cell adherens junctions during *Drosophila* morphogenesis

A. Schmidt[1], D. Kong[2], T. Finegan[3], M. Peifer[1]; [1]University of North Carolina At Chapel Hill, Chapel Hill, NC, [2]Philipps-University Marburg, Marburg, Germany, [3]University of Rochester, Rochester, NY

Adherens junctions (AJs) mediate cell-cell adhesion, and through their linkage to the cytoskeleton, permit shape changes and rearrangements during *Drosophila* embryonic morphogenesis. AJs need to be strong yet also dynamic, allowing cell rearrangement without tissue rupture. We want to understand how proteins like the multi-domain scaffold Polychaetoid (Pyd; fly homolog of ZO-1), work together to allow AJs to react dynamically to cell shape change and force generation. We hypothesize that the junctional complex and its linkage to the cytoskeleton involves multivalent connections among multiple proteins, assembled into a structured molecular complex that mediates binding to the cytoskeleton. My goal is to explore Pyd's functional role as one player in this network. While Pyd is not absolutely essential for embryonic viability, mutants display defects in cell shape and AJ integrity during germband extension, suggesting defects in responding to elevated force on AJs. However, these occur without major tissue rupture, suggesting other proteins act in parallel. Tension-dependent enrichment of some AJ proteins is affected in *pyd* mutants. Both observations led to the hypothesis that AJs in *pyd* mutant embryos are under less tension- we confirmed this by laser cutting. We also hypothesize that AJs assemble as a structured and layered molecular complex, comparable to the structured focal adhesions connecting cells to the extracellular matrix. I used structured illumination microscopy (SIM) to explore localization of AJ proteins in high resolution. My data reveal that the core junction-associated proteins Canoe (Cno; fly homolog of Afadin) and Armadillo (Arm; fly homolog of β-Catenin) are differentially enriched in different AJ puncta along the zonula adherens. Furthermore, Pyd surrounds these core junctional proteins in a cloud-like pattern of strands and puncta. I will apply live imaging to understand if these clouds are formed by phase-separation, as ZO-1 is known to phase-separate. Strikingly, localization of Arm and Cno is not perturbed in *pyd* mutants. Together our data suggest that Pyd balances tension on AJs by acting in parallel with Cno to directly strengthen AJ-cytoskeleton connections to resist force perpendicular to the junction, and potentially by or by scaffolding and stabilizing AJ- and cytoskeleton associated proteins parallel to the junction.

B508/P1491

Planar Cell Polarity Dependent Organization of the Actin Cortex during Convergent Extension

V. Bejar-Padilla, C. Devitt, S. Weng, J. Alvarado, J. Wallingford, J. Stachowiak; University of Texas At Austin, Austin, TX.

Planar cell polarity (PCP) complexes are highly conserved macromolecular assemblies associated with the plasma membrane that confer directional polarity to cells along a specific axis. During vertebrate development, PCP coordinates proper elongation of tissues along the anteroposterior axis. It achieves this by enabling changes in cell shape and motility required for the collective cell behavior termed convergent extension. Accordingly, understanding how PCP regulates cytoskeletal mechanics is critical for understanding vertebrate tissue morphogenesis. To probe the role of PCP in regulation of the actin cytoskeleton, we disrupted PCP in the dorsal marginal zone tissue of *Xenopus laevis* embryos by knocking down expression of Vangl2 and Sept7. Using mRNA microinjection of a fluorescent actin probe in conjunction with TIRF microscopy, we visualized actin filaments in the anterior and posterior compartments of cells undergoing convergent extension. Actin filament dynamics were quantified using TSOAX, an automated image analysis program. Results showed that the persistence length of actin filaments, which reflects their bending stiffness, was enhanced in the anterior of the cell compared to the posterior. This subcellular mechanical polarization was not observed in Vangl2-KD and Sept7-KD cells, suggesting PCP is required to establish a subcellular stiffness gradient. To explore the relationship of PCP complexes and cortical actomyosin, we labeled PCP proteins using mRNA microinjection of fluorescent fusion proteins and dissociated the plasma membrane from the cell cortex using chemical vesiculation. Results showed that Vangl2 is retained in the plasma membrane following vesiculation. Interestingly, the cytoplasmic PCP protein Prickle2, which is recruited to the plasma membrane by Vangl2, does not colocalize with Vangl2 during vesiculation, suggesting that association of Vangl2 with the cell cortex is necessary for its interaction with Prickle2. These finding help clarify the poorly understood relationship between PCP and the actin cytoskeleton and highlight novel methods to probe this relationship.

Collective Chemotaxis and Directed Migration

B509/P1492

Migration of zebrafish epidermal T-cells in response to electrical signals after wounding

M. Sathe, C. K. Prinz, N. M. Belliveau, M. J. Footer, J. A. Theriot; Department of Biology, University of Washington/Howard Hughes Medical Institute, Seattle, WA.

Skin wound healing is a complex process that requires precise spatio-temporal coordination between multiple cell types (e.g. epithelial cells and leukocytes). Motivated by a desire to investigate leukocyte behaviour in a physiologically relevant environment, we developed an adult zebrafish scale explant system as a model skin tissue (‘skin-on-glass’). It retains several key features of animal skin, including the co-existence of multiple cell types (epithelial cells, mechanosensory cells, T-cells, neutrophils and macrophages) in a near-native organisation. The presence of T-cells in the ‘skin-on-glass’ explant system is particularly exciting since T-cells have not yet developed in the more easily studied embryonic and early larval stages of zebrafish development. After laser wounding, ‘skin-on-glass’ exhibits complex wound recovery features including extrusion of damaged cells from the wound and cell division in the nearby epidermis. Skin, in vivo, maintains a trans-epithelial potential (TEP) due to asymmetric ion flow.

Wounding disrupts the TEP and induces a lateral electric field (EF). EFs are such a strong migratory cue that an externally applied EF oriented in the opposite direction can even compel epithelial tissue to migrate away from an endogenous wound. In 'skin-on-glass' exposed to EFs, we observed the epithelial cells and neutrophils rapidly migrating towards the cathode (i.e. toward the wound), as expected based on previous reports. However, surprisingly, T-cells migrated towards the anode (i.e. away from the wound). Isolated primary T-cells also displayed directed migration toward the anode, indicating that this unusual directional response is a cell-intrinsic behaviour. This hypothesis is supported by preliminary data from the late larval stage, which demonstrates that T-cells in vivo migrate away from an epidermal wound, unlike neutrophils.

B510/P1493

Physical limits on galvanotaxis

A. Kim, I. Nwogbaga, **B. Camley**; Johns Hopkins University, Baltimore, MD.

Eukaryotic cells of many types can polarize and migrate in response to electric fields via "galvanotaxis"; this ability helps skin cells heal wounds. Recent experimental evidence suggests galvanotaxis occurs because membrane proteins redistribute via electrophoresis, though the sensing species has not yet been conclusively identified. We want to understand how precisely eukaryotic cells can sense the direction of an electrical field. We use a physical model to show that randomness from the finite number of sensing proteins limits the accuracy of galvanotaxis via electrophoresis. Using maximum likelihood estimation, we show how cells can best interpret this noisy signal, and how their accuracy should depend on the cell size and electric field strength. Our model can be fit well to data measuring galvanotaxis of keratocytes, neural crest cells, and granulocytes. Our results show that eukaryotic cells can likely achieve experimentally observed directionalities with either a relatively small number (around 100) of proteins that are highly asymmetric in the electric field, or a large number (around 10,000) proteins with a relatively small change in concentration across the cell (~4% change from cathode to anode). This may explain why identifying the sensor species has been difficult, as candidates need not be strongly polarized even in large electric fields. A second prediction of the model is that the accuracy of cells should only weakly depend on their size.

B511/P1494

Welcome to the Zellenstaat: learning the rules of collective cell behavior from microscopy data

J. LaChance[1], K. Suh[2], M. Heinrich[1], **D. J. Cohen**[3]; [1]Mechanical Engineering, Princeton University, Princeton, NJ, [2]Chemical and Biological Engineering, Princeton University, Princeton, NJ, [3]Princeton Bioengineering Institute; Mechanical Engineering, Princeton University, Princeton, NJ.

Groups of cells constitute communities whose complex behaviors, such as healing and development, emerge from myriad interactions amongst individual cells. Similarities between tissues and other communal structures were first recognized by Vichow (19th c.) who coined 'Zellenstaat' ('cell state'), writing that 'the body is a state and every cell a citizen.' This comparison was prescient, as data today increasingly emphasizes how complex collective cell dynamics are critical to multicellular life. Many of these behaviors are dynamic, and even a 'simple' transmitted light microscopy movie contains deep information about cellular behavior, health, and more. Extracting and interpreting collective data from large-scale timelapse movies is an exciting challenge and we have been combining tools from swarm dynamics, machine learning, and tissue engineering to explore these questions, with two examples

discussed here. At the cellular scale, we have built neural networks to convert transmitted light microscopy data into predicted fluorescence microscopy images for key biomarkers such as nuclei, cadherins, and cytoskeletal structures. Such augmented microscopy allows us to conduct massive timelapses with little-to-no phototoxicity needed to segment and track tens of thousands of cells in growing and healing epithelia. We then built 'Attention Networks' to predict for any given focal cell in a tissue which of its neighbors strongly influence how that cell will migrate. These attention heatmaps provide snapshots of the underlying rules of collective cell migration and can detect how these rules change over space and time within a tissue, across different cell types (e.g. healthy vs. metastatic), and in response to different drugs. At the tissue scale, we engineer and film the growth dynamics of arrays of large-scale epithelia as they grow and collide with each other. In addition to cellular-scale analyses, we also rely heavily on large-scale approaches from swarm theory where we use particle-image-velocimetry of transmitted light data to extract tissue flow dynamics and large-scale correlations well above the cellular scale. These methods are computationally efficient and broadly accessible, and we have coupled their outputs to biophysical continuum models to describe key collective rules governing how tissues grow, collide, and heal. Overall, our approaches are part of a growing body of tools developed by the broader collective behavior community to extract increasingly complex and, hopefully, interpretable data from collective behavior datasets

B512/P1495

Physical and post-translational regulation of vinculin controls cellular coupling during collective cell migration

T. C. Shoyer, Jr., E. M. Gates, B. D. Hoffman; Biomedical Engineering, Duke University, Durham, NC.

The coordinated movement of groups of cells, termed collective cell migration (CCM), plays an important role in morphogenesis, tissue repair, and cancer metastasis. CCM is driven by mechanical forces between cells and with the substrate, which are mediated by specialized structures known as Adherens Junctions (AJs) and Focal Adhesions (FAs), respectively. Recent work indicates that frictional forces at these interfaces are major determinants of CCM, however, the molecular mechanisms regulating friction at these structures remain poorly understood. Here, we used a combination of tension- and conformation-sensitive biosensors, site-directed mutagenesis, CCM assays, and mechanochemical models to probe the relationship between mechanosensitive protein dynamics, friction, and cellular coupling. We focused on vinculin as it can participate in mechanical linkages at both FAs and AJs, is known to stabilize these adhesions under loads, and forms a strong catch bond with actin. We found that vinculin transmitted molecular forces at both the FAs and AJs of collectively migrating MDCK cells and that vinculin's conformation and loading were regulatable thru the S1033 phosphorylation site in this context. Vinculin expression and mechanical loading controlled the speed and coordination of CCM, which were also regulatable by the S1033 site. To understand this effect, we developed mathematical models of frictional forces at the FAs and AJs based on force-sensitive binding kinetics from the literature and interpreted them in the context of existing macroscopic models of CCM. Our models demonstrated increases in frictional forces at both FAs and AJs due to vinculin mechanical loading, which was consistent with the experimentally observed effects of vinculin on CCM. This work suggests a new control paradigm for CCM, where physical and biochemical regulation of vinculin connectivity within FAs and AJs regulates frictional forces to tune the speed and coordination of multicellular migration. Broadly, this work provides a framework to bridge molecular scale dynamics to

macroscopic physical parameters, connecting biological and mechanical descriptions of CCM across scales.

B513/P1496

Cell junctional proteins are involved in straightforward directional movement and enhance cell migration in malignant gastric cancer cells

S. Kee, S. Kim, S. Park, H. Jang, Y. Park; Korea University, Seoul, KOREA, REPUBLIC OF.

Efficiency of cell migration is influenced by speed and directionality of cell movement. Here, we analyzed quantitatively cell movements by tracing individual cells in micropatterned free edge migration model using several malignant gastric cancer cells. MKN28 cells expressing high level E-cadherin with strong cell-cell adhesion showed lower migration speed than that of MKN1 cells which express low level of E-cadherin, but both cells showed similar cell migration accessed as colony expansion in our model system, suggesting that straightforward movement of MKN28 cells may be associated with efficient cell migration. Overexpression of E-cadherin in AGS (EC96) and MKN1 cells led to increase of ZO-1 expression and straightforward movement of cells, suggesting that establishment of cell junctions is important for directional cell movement. Subsequent knock-out experiments of ZO-1 reduced straightforward movement of both EC96 and MKN28 cells. Immunofluorescence analysis showed that tight junctions (TJs) of EC96 cells might play important roles in intracellular communication among boundary cells. ZO-1 is localized to the base of protruding lamellipodia and cell contact sites at the rear of cells, indicating that ZO-1 might be important for the interaction between traction and tensile forces. Overall, dynamic regulation of E-cadherin expression and localization by interaction with ZO-1 protein play a role directional movement of cells especially in a mode of collective cell migration.

B514/P1497

Optimal collective durotaxis through E-cadherin adhesions

R. Sunyer[1,2], M.-E. Pallarés[3], I. Pi-Jaumà[1], I. Corina Fortunato[3], V. Grazú[4], M. Gómez-González[3], P. Roca-Cusachs[3], J. M de la Furente[4], R. Alert[5], J. Casademunt[1], X. Trepat[3]; [1]University of Barcelona, Barcelona, Spain, [2]Institute for Bioengineering of Catalonia, Sant Just Desvern, Spain, [3]Institute for Bioengineering of Catalonia, Barcelona, Spain, [4]Instituto de Nanociencia y Materiales de Aragón, Zaragoza, Spain, [5]Max Planck Institute for the Physics of Complex Systems, Dresden, Spain

The directed migration of cellular clusters enables morphogenesis, wound healing, and collective cancer invasion. Gradients of substrate stiffness are known to direct the migration of cellular clusters in a process called collective durotaxis, but underlying mechanisms remain unclear. Durotaxis has been mainly studied when mediated by focal adhesions at the extracellular matrix (ECM) interface. However, in ECM-depleted environments cells migrate through the cell-cell adhesion protein E-cadherin. Here we show that when cell adhesion is mediated by E-Cadherin, clusters of cancer cells dewet on soft substrates and wet on stiff ones. At intermediate stiffness, clusters on uniform-stiffness substrates become maximally motile, and clusters on stiffness gradients exhibit optimal durotaxis. Durotactic velocity increases with cluster size, stiffness gradient, and actomyosin activity. We first demonstrate this new migratory mode on substrates coated with E-cadherin and then establish its generality on substrates coated with extracellular matrix. We develop a physical model of three-dimensional active wetting that explains this mode of collective durotaxis in terms of a balance between in-plane active traction and tissue contractility, and out-of-plane surface tension. Finally, we show that the distribution

of cluster displacements has a heavy tail, with infrequent but large cellular hops that contribute to durotactic migration. Our study demonstrates a new physical mechanism of collective durotaxis, through both cell-cell and cell-substrate adhesion ligands, based on the wetting properties of active droplets.

B515/P1498

Luteinizing hormone induces inward migration of granulosa cells in mouse preovulatory follicles

C. M. Owen, L. A. Jaffe; UConn Health Center, Farmington, CT

Mammalian preovulatory follicles respond to luteinizing hormone (LH) by initiating a cyclic AMP-dependent signaling cascade in the granulosa cells that surround the oocyte, resulting in ovulation. Among the complex of responses, LH is known to increase motility in isolated granulosa cells, but it is unknown whether LH causes cells to migrate within intact follicles. Here, we used mice with a hemagglutinin tag on the LH receptor (LHR) to investigate LH-induced changes in the localization of HA-LHR-expressing cells within intact follicles. Mice were injected with kisspeptin, which induces a surge-like release of LH that reaches a peak amplitude 90 minutes after injection. Ovaries were collected at 0, 2, 4, 6, 8, and 10 hours after injection, then fixed in 4% paraformaldehyde and frozen before cutting 10 µm cryosections. Immunofluorescence using an HA antibody was performed on the cryosections to observe the localization of HA-LHR-expressing cells. Before the LH surge, the LHR-expressing mural granulosa cells are localized almost entirely in the outer half of the mural granulosa region, in cells contacting the basal lamina. Within 30 minutes after the peak of the LH surge (or 2 hours after kisspeptin injection), the LHR-expressing cells begin to elongate into the inner mural granulosa layers and move farther away from the basal lamina. The proportion of LHR-expressing cell bodies in the inner half of the mural region continues to increase until ovulation. Accompanying the movement of the granulosa cells into the follicle interior, LH receptors are endocytosed from the plasma membrane into the cytoplasm, and the cells begin to detach from the basal lamina. At about 6 hours after the LH peak, the follicle undergoes a transient contraction, and at sites associated with patches of LHR-expressing cells, the basal lamina is pulled inwards. These findings suggest that the LH-induced inward migration of granulosa cells could contribute to the contraction of the follicle and breakdown of the basal lamina, which could in turn contribute to causing ovulation.

B516/P1499

COPD Alters Bronchial Epithelial Wound Closure Dynamics*In vitro*

K. T. Wilmsen, C. Swaby, E. Parajon, A. Wally, V. K. Sidhaye[1]; Johns Hopkins University, Baltimore, MD

Chronic Obstructive Pulmonary Disease (COPD), a progressive and irreversible chronic disease of the lungs, can be characterized *in vitro* through structural and functional changes in cultured bronchial epithelial cells. Patient derived COPD cells exhibit reduced barrier function, partial EMT (p-EMT), reduced ciliation and ciliary function, and the induction of collective cellular motion within the epithelial sheet. Interestingly, the collective motion of COPD cells *in vitro* occurs under normal culture conditions in intact and undisturbed epithelial cells. To better understand the functional consequences of increased collective motion in COPD we are striving to understand the cellular processes that are driving the observed motion and if these changes impact migration dependent processes like wound closure. Scratch wound closure assays of Normal and COPD patient derived cells cultured at air-liquid interface (ALI) uncovered interesting differences in the cellular dynamics of wound closure. 15-hour timelapse

imaging of wound closure showed that COPD cells exhibit increased wound closure compared to Normal cells, however, measurements of cell movement speed were unchanged between the two groups. Additionally, normal cells had an approximately 2-hour delay prior to collective migration to close the scratch, compared to COPD cells, where a slight retraction of the wound edge could be observed. Along with these findings we have observed altered localization of the focal adhesion protein vinculin coupled with increased stress fiber formation in intact COPD cells, compared to normal cells, suggesting that the collective motion observed in COPD cells may be due to cellular reprogramming that primes COPD cells for migratory processes even in the absence of a wound edge. Further research is required to understand the gene and/or protein level changes leading to the observed COPD phenotype, and to better understand if COPD cells can resolve a wound and return to their previous steady state condition.

B517/P1500

***Drosophila*NPAS4 negatively regulates JAK/STAT signaling to constraint the invasive cell population**

J. W. Wu[1], C. W. Wang[1], R. Y. Chen[1], L. Y. Hung[1], Y. C. Tasi[2], Y. T. Chan[1], Y. C. Chang[3], A. C. C. Jang[1]; [1]National Cheng Kung University, Tainan, Taiwan, [2]Tunghai University, Taichung, Taiwan, [3]National Sun Yat-sen University, Kaohsiung, Taiwan

Cell migration is a critical process for embryonic development and cancer metastasis, but the epithelial cells are selected and adapt to migratory cell fate remains elusive. To understand the underlying mechanism, we apply border cells (BCs), a small group of cells disseminating from the epithelium and migrating through germ cells in *Drosophila* oogenesis, as a model to study collective cell movement. In a forward genetic screen, we find that overexpression of *dysfusion* (*dysf*) severely impedes BC recruitment which is governed by Jak/Stat signaling. Conversely, loss of *dysf* led to extra migratory BCs. It is noteworthy that Dysf gain of function dramatically impairs the phosphorylation, transcriptional activity, and nuclear import of Stat protein. The ectopic BC phenotype caused by Jak or Upd overexpression can be suppressed by overexpression of UAS-*dysf*, indicating that *dysf* negatively regulates Jak/Stat signaling. Furthermore, Dysf protein is located at the nuclear membrane of all germline and follicle cells but specifically reduced in BCs upon migration. Moreover, when BCs reach their destination, oocyte their Dysf becomes undetectable. Further research showed that Notch is upstream of Dysf to regulate its downregulation. To elucidate how Dysf regulates Stat nuclear transport, we carried out a biochemical screen to seek proteins interacting with endogenous knock-in tagged Dysf. We found that Dysf gates Stat nuclear imports by interacting with Pendulin, a member of the Importin-alpha protein family, and that mammalian homologue of Dysfusion (Npas4) negatively regulates the Stat3 and cancer cell migration. Therefore, *dysf* may determine the size of border cell clusters by regulating Jak/Stat signaling.

Intermediate Filaments

B518/P1501

Gigaxonin is required for intermediate filament transport on microtubules

B. Renganathan, J. P. Zewe, Y. Cheng, M. Kittisopikul, P. Opal, K. M. Ridge, V. I. Gelfand; Northwestern University, Chicago, IL.

Giant axonal neuropathy, a neurodegenerative condition, causes disorganization of intermediate filaments (IFs) in multiple cell types, including neurons. Giant axonal neuropathy is caused by mutations in the *GAN* gene that encodes Gigaxonin, an E3 ligase substrate adaptor protein. Gigaxonin is required

for ubiquitination and subsequent degradation of IF proteins. Although IF disorganization is a hallmark of giant axonal neuropathy, the molecular mechanism behind IF aggregation remains unknown. We used IF proteins tagged with photoconvertible protein mEOS3.2 to study the role of Gigaxonin in the dynamics of IFs. We observed that the loss of gigaxonin halts transport of IFs along microtubules by kinesin-1. This transport inhibition was specific for IFs, as other types of kinesin-1 cargo moved normally after Gigaxonin loss. Thus, IF disorganization observed in *GAN* knock-out cells is caused by inhibition of kinesin-dependent IF transport. Interestingly, we observed a significant increase in the soluble form of vimentin with the loss of Gigaxonin protein. We hypothesize that soluble oligomers of IF protein sequester a yet unidentified adaptor that mediates IF-motor interactions, inhibiting IF transport along microtubules.

B519/P1502

Alterations to the Keratin Composition of Progenitor Keratinocytes Drive Stem Cell Exhaustion and Premature Aging

C. Redmond[1,2], R. Q. Alvarado[1], B. Albedry[1], P. A. Coulombe[1,3]; [1]Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Ann Arbor, MI, [2]Doctoral Program in Cancer Biology, University of Michigan Medical School, Ann Arbor, Ann Arbor, MI, [3]Dermatology, University of Michigan Medical School, Ann Arbor, Ann Arbor, MI.

Complex epithelia function as physical and chemical barriers in numerous organs and tissues across the body, including skin, eye (cornea), oral mucosa, and reproductive tract. Barrier function is maintained by carefully balancing the proliferation and differentiation of keratinocytes, the predominant cell type in complex epithelia, through the incompletely defined process of epithelial homeostasis. Common to all complex epithelia is the type I intermediate filament forming protein keratin 14 (K14). K14 and its type II co-assembly partner keratin 5 (K5) are highly expressed by progenitor keratinocytes, in which they provide essential mechanical integrity. Recent work in our laboratory has expanded on this mechanical function of K14 with the identification of a novel role for K14 in the regulation of epidermal homeostasis via the Hippo signaling pathway. Using the novel transgenic mouse model *Krt14*$^{C373A/C37A}$, a novel and differentiation-dependent signaling role was uncovered for a disulfide-bonded form of K14 through the cooperation of the scaffolding protein 14-3-3σ and the Hippo pathway effector YAP1. Here we build on this new finding through the characterization of *Krt14*$^{C373A/null}$ compound heterozygous mice. *Krt14*$^{C373A/null}$ mice possess 50% less K14 than WT or homozygous *Krt14*$^{C373A/C373A}$ animals, while maintaining expression of an additional progenitor type I keratin, keratin 15 (K15). *Krt14*$^{C373A/null}$ mice develop a unique aging phenotype in addition to exhibiting the intriguing molecular phenotype of *Krt14*$^{C373A/C37A}$ mice. We hypothesized that the increased contribution of K15 within the progenitor keratin network alters the sensitivity threshold for K14 disulfide-bonding in response to differentiation cues, including reactive oxygen species (ROS), which abnormally keeps progenitor keratinocytes in a pro-proliferative state. This pro-proliferative state results in both precocious development and premature exhaustion of these stem-like cells and aging of K14-expressing epithelia. Using morphological and molecular analyses, both in vivo and ex vivo, we are characterizing the aging phenotype of *Krt14*$^{C373A/null}$ mice and further defining the molecular mechanism enabling keratin intermediate filaments, ROS-catalyzed disulfide bonding, and the Hippo pathway to regulate homeostasis in complex surface epithelia.

B520/P1503

K14 and K15 Influence Airway Basal Cell Proliferative Capacity and Fate

V. Ievlev[1], T. J. Lynch[1], F. W. Kyle[2], C. B. Gries[2], A. Shah[1], A. C. Pai[2], B. A. Ahlers[3], S. Park[1], J. F. Engelhardt[1], K. R. Parekh[2]; [1]Anatomy and Cell Biology, University of Iowa, Iowa City, IA, [2]Surgery, University of Iowa, Iowa City, IA, [3]University of Iowa, Iowa City, IA.

Background: Airway basal cells (ABCs) are marked by expression of Keratin5 (K5) - an abundant cytoskeletal protein which can heterodimerize with Keratin14 (K14) or Keratin15 (K15) to make intermediate filaments. K15 is expressed in ABCs at homeostasis *in vivo,* while K14 turns on in the surface airway epithelium (SAE) during injury regeneration and in the disease setting. This altered basal cell state has been associated with a decline in stem cell function, but the causality of this trend as well as the functional differences between K14 and K15 have not been evaluated. **Methods:** In this study we used genetic knockout approaches in vitro and in vivo to study the functions of K14 and K15 in ABCs. We additionally present there a novel ex vivo ferret tracheal injury model that provides new insights into the mechanics of airway epithelial wound healing. **Results:** After evaluating the changes in basal keratin profile of cultured ABCs we conclude that it resembles that of regenerating ABCs in vivo, with enhanced K14 expression and reduced K15 expression after several passages. Knockout of K14 and K15 in primary ABCs leads to contrasting phenotypes: loss of K15 impairs colony formation efficiency, while the loss of K14 enhances it. Knockout of K14 also leads to re-activation of K15 expression in advanced-passage ABCs. We generated in vivo and in vitro knockout models of K14 and K15 and observed that loss of K14 (but not of K15) results in decreased abundance of club cells in mouse trachea and in vitro. Additionally, the nuclei of K14-KO ABCs appear enlarged and elongated during differentiation in vitro and produce fewer club cells. K14-KO cultures have reduced levels of tumor suppressor Sfn and increased oncogene p63 early in differentiation which can explain the observed phenotypes. K15-KO mice have fewer label retaining basal cells 21 days after injury potentially suggesting an importance of this keratin for stem cell maintenance. **Conclusions:** Airway repair and regeneration by basal progenitors happens in most lung and airway diseases that lead to injury to the airway epithelium, including Cystic Fibrosis. The mechanisms driving commitment of basal stem cells to regeneration are not completely understood. In this work we demonstrate that one such mechanism involves switching K15 for K14 which can affect structure, function and fate of basal progenitors. Overall, this work suggests that keratins may be actively involved in regulation of ABC behavior and fate decisions.

B521/P1504

Expression of vimentin alters cell mechanics, cell-cell adhesion, and gene expression profiles suggesting the induction of a hybrid Epithelial to Mesenchymal Transition in human mammary epithelial cells

S. Sivagurunathan[1], A. Vahabikashi[1], H. Yang[2], J. Zhang[3], K. Vazquez[4], D. Rajasundaram[5], Y. Politanska[6], H. Abdala-Valencia[6], J. Notbohm[7], M. Guo[2], S. Adam[1], R. Goldman[1]; [1]Department of Cell and Developmental Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL, [2]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, [3]Biophysics program, Department of Engineering Physics, University of Wisconsin-Madison, Madison, WI, [4]Department of Engineering Physics, Department of Mechanical Engineering, University of Wisconsin-Madison, Madison, WI, [5]Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA, [6]Department of Medicine, Feinberg School of Medicine, Northwestern University,

Chicago, IL, [7]Biophysics program, Department of Engineering Physics, Department of Mechanical Engineering, University of Wisconsin-Madison, Madison, WI.

Vimentin is a Type III intermediate filament (VIF) cytoskeletal protein that regulates the mechanical and migratory behavior of cells. Its expression is considered to be a marker for the epithelial to mesenchymal transition (EMT) that takes place in tumor metastasis. However, the molecular mechanisms regulated by the expression of vimentin in the EMT remain largely unexplored. We created MCF7 epithelial cell lines expressing vimentin from a cumate-inducible promoter to address this question. When vimentin expression was induced in these cells, extensive cytoplasmic VIF networks were assembled accompanied by changes in the organization of the endogenous keratin intermediate filament (KIF) networks and disruption of desmosomes. Significant reductions in intercellular forces by the cells expressing VIFs were measured by quantitative monolayer traction force and stress microscopy. In contrast, laser trapping micro-rheology revealed that the cytoplasm of MCF7 cells expressing VIFs was stiffer than the uninduced cells. Vimentin expression activated transcription of genes involved in pathways responsible for cell migration and locomotion. Importantly, the EMT related transcription factor *TWIST1* was upregulated only in WT vimentin expressing cells and not in cells expressing a mutant non-polymerized form of vimentin, which only formed unit length filaments (ULF). Taken together, our results suggest that vimentin expression induces a hybrid EMT correlated with the upregulation of genes involved in cell migration. **Keywords** - Vimentin, TWIST1, Desmoplakin, Hybrid/partial EMT, Intracellular mechanics, Intercellular forces, cell-cell adhesion.

B522/P1505

Using Proximity Biotinylation to Investigate the Vimentin Interactome

K. Brock[1], K. Alpha[1], E. De Jong[2], G. Brennan[1], C. Turner[1]; [1]Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY, [2]Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY.

The type III intermediate filament protein, vimentin, has been shown to be involved in a variety of cell processes including cell migration, focal adhesion dynamics, and maintenance of the mechanical integrity of a cell. Despite vimentin's role in these processes, there may potentially be novel interactors of vimentin that have yet to be identified. In an attempt to uncover more about vimentin's proximity interactome and identify possible candidates for further investigation, we utilized the BioID2 biotin ligase to label proteins in close proximity to vimentin. We developed stable lines of cells which express BioID2-tagged vimentin and confirmed that the construct localized to the vimentin filament network and was able to biotinylate proteins. We performed a pulldown of biotinylated proteins, followed by liquid chromatography - mass spectrometry to identify potential proximity interactors of vimentin. We further evaluated the potential proximity interactors identified in our mass spectrometry data using the CRAPome analysis pipeline, STRING analysis, and Gene Ontology. We found that the potential proximity interactors of vimentin were enriched for proteins that localize to exosomes, the extracellular space, focal adhesions, intracellular vesicles, and the cytoskeleton. Gene Ontology analysis of molecular function showed an enrichment for "cell adhesion molecule binding" and "cytoskeletal protein binding". This approach is a viable method for dissecting the vimentin interactome and yielded candidate proximity interactors for further investigation. Supported by NIH grant R35 GM131709 to CT. The mass spectrometry data was collected on an instrument purchased with NIH shared instrumentation grant 1S10OD023617-01A1.

Integrins and Cell-ECM Interactions

B524/P1506

Regulation of Cell Dynamics by Rapid Transport of Integrins Through the Biosynthetic Pathway

M. Lerche[1,2], M. Mathieu[1,2], L. Malerød[3,4], N. Pedersen[3,4], H. Hamidi[1,2], M. Chastney[1,2], B. Bruininks[5], S. Kaptan[5], G. Jacquemet[1,6,7], I. Vattulainen[5], P. Roca-Cusachs[8,9], A. Brech[3,4], F. Perez[10], G. Boncompain[10], S. Miserey[10], J. Ivaska[1,2,11]; [1]Turku Bioscience Centre, Turku, FINLAND, [2]University of Turku, Turku, FINLAND, [3]Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, NORWAY, [4]Institute of Clinical Medicine, University of Oslo, Oslo, NORWAY, [5]Department of Physics, University of Helsinki, Helsinki, FINLAND, [6]Åbo Akademi University, Turku, FINLAND, [7]Turku Bioimaging, University of Turku and Åbo Akademi University, Turku, FINLAND, [8]Institute for Bioengineering of Catalonia (IBEC), the Barcelona Institute of Technology (BIST),, Barcelona, SPAIN, [9]Universitat de Barcelona, Barcelona, SPAIN, [10]Institut Curie, PSL Research University, Sorbonne Université, Centre National de la Recherche Scientifique, Paris, FRANCE, [11]Foundation for the Finnish Cancer Institute, Helsinki, FINLAND.

Cells sense and respond to the extracellular matrix (ECM) milieu through integrin proteins. Integrin availability on the plasma membrane, regulated by endosomal receptor uptake and recycling, has been extensively studied and regulates cell dynamics in various normal and pathological contexts. In contrast, the role of integrin transport through the biosynthetic pathway has been considered primarily as a mechanism to replenish the receptor pool and too slow to influence cell dynamics. Here, we adopted the RUSH (Retention Using Selective Hooks) assay to synchronize integrin anterograde transport from the endoplasmic reticulum (ER), allowing spatial and temporal analysis of newly synthesized receptor traffic. We observe that the delivery of new integrins to the plasma membrane is polarized in response to specific ECM ligands, facilitates integrin recruitment specifically to the membrane-proximal tip of focal adhesions (FA) and contributes to cell protrusion and FA growth. We explain the augmented adhesion growth using a computational molecular clutch model, where increased integrin availability drives recruitment of additional integrins. Notably, a subset of newly synthesized integrins undergo rapid traffic from the ER to the cell surface to facilitate localized cell spreading, seemingly bypassing the Golgi. This unconventional secretion is dependent on cell adhesion and mediated by Golgi reassembling stacking proteins (GRASPs) association with the PDZ-binding motif in the integrin α5 cytoplasmic tail. This spatially targeted delivery of integrins through the biosynthetic pathway may propel cell dynamics by rapidly altering adhesion receptor availability, providing cells with an additional degree of plasticity to respond to their environment.

B525/P1507

Fibronectin Fibrillogenesis Is Mediated by Autocrine TGF-β1-dependent Secretion of Soluble Fibronectin

M. Sofroniou, 1st, C. Lemmon; Biomedical Engineering, Virginia Commonwealth University, Richmond, VA.

Epithelial-Mesenchymal Transition (EMT) is a cellular transdifferentiation program in which epithelial cells transform into a mesenchymal-like phenotype. Transforming growth factor - beta 1 (TGF-β1) is a potent inducer of EMT, secreted from two distinct sources: 1) Exogenous TGF-β1 originating from neighboring cells, which is upregulated during wound healing, fibrosis and cancer and 2) Autocrine TGF-

β1 which is constitutively expressed at low levels but is upregulated in response to exogenous TGF-β1. A key process of EMT is the assembly of fibronectin (FN) into insoluble fibrils, which are the primary component of the provisional extracellular matrix (ECM). While it is well characterized that exogenous TGF-β1 is required for FN fibrillogenesis, we sought to investigate whether autocrine TGF-β1 plays a role in this process. The objective of this study is to determine the effects of autocrine TGF-β1 signaling on the deposition and assembly of FN fibrils in the context of EMT. To accomplish this, the *TGFB1* gene was deleted in both benign and malignant human breast cells using CRISPR-Cas9. We compared the ability of wild-type (WT) and *TGFB1* knockout (KO) cells to secrete and assemble FN in response to exogenous TGF-β1 and explored possible mechanisms behind alterations in FN deposition. FN expression, secretion, and assembly were assayed using RT-qPCR, ELISAs and immunofluorescence (IF) imaging. EMT markers and relevant signaling pathway components were also assayed.
In both benign and malignant cells, the deletion of the *TGFB1* gene led to a decrease in both the secretion of soluble FN and the assembly of FN fibrils. Interestingly impaired FN assembly occurs at the level of gene expression benign cells, but not in malignant cells. Instead, malignant KO cells upregulate FN comparably to the WT, but still appear to have a deficiency in FN secretion. Deletion of the *TGFB1* gene led to a general downregulation of mesenchymal markers and upregulation of epithelial markers. Data also revealed that *TGFB1*-KO cells express less of the *CDC42* gene and the active form of the Cdc42 enzyme. Cdc42 activity has been previously shown to regulate the secretion and deposition of ECM components, pointing to a potential explanation for the observed deficiency in FN secretion. These results demonstrate that autocrine TGF-β1 maintains a key role in regulating both the secretion and assembly of FN.

B526/P1508

Epithelial adherens junctions regulate ECM remodeling via miRNAs

A. Daulagala[1], C. Bridges[1], J. Nair-Menon[1], D. Jiminez[1], A. Bradshaw[2], J. Jones[3], A. Kourtidis[1];
[1]Department of Regenerative Medicine and Cell Biology, Medical University South Carolina, Charleston, SC, [2]Division of Cardiology, Collage of Medicine, Medical University South Carolina, Charleston, SC, [3]Deparment of Surgery, Collage of Medicine, Medical University South Carolina, Charleston, SC.

Colon cancer is the third most common and second deadliest type of cancer. Colon cancer ischaracterized by compromised epithelial integrity and by aberrant extracellular matrix (ECM)remodeling. However, a possible connection between epithelial integrity and ECM remodelingthat contributes to the disease progression, has not been explored. The Adherens Junctions (AJs)is a cell-cell adhesion complex composed of cadherin and catenin family proteins and is essentialfor maintaining epithelial tissue integrity. We have found that the p120 catenin partner PLEKHA7is critical for epithelial integrity, and is responsible for recruiting core components of the RNAimachinery at mature apical AJs to regulate miRNA levels and activity. Loss of PLEKHA7 results indisruption of junctional localization of the RNAi machinery, miRNA dysregulation, oncogeneupregulation, and pro-tumorigenic colon cell behavior, in vitro and in vivo. Interestingly, MEP1A,MMP1, MMP9, LOX and CDX2, all of which are ECM remodeling markers, were among the topupregulated mRNAs in PLEKHA7 depleted colon epithelial Caco2 cells according to whole cell RNAsequencing results. We confirmed increased protein expression of these markers upon PLEKHA7depletion by western blot analysis. Bioinformatic analysis identified several PLEKHA7-regulatedmiRNAs as potentially targeting the above ECM-related mRNAs; among them, miR-24 and miR-30c indeed rescued the increased protein expression of aforementioned markers as confirmedby western blot results. Further, MMP1 and LOX enzyme activities as well as migration

andinvasion rates of cells increasedupon PLEKHA7 depletion and were rescued upon MMP inhibition.Moreover, preliminary examination of colon tissues from a PLEKHA7 knockout mouse modelshows increased collagen deposition which corroborates roles of MMPs and LOX in ECMremodeling upon PLEKHA7 loss. In summary, our data reveal anovel mechanism, through whichepithelial adherens junctions regulate ECM remodeling through miRNA activity, to suppress pro-tumorigenic cell behavior.

B527/P1509

Regulation of aVb6 integrin to facilitate TGFb1 activation

M. Bachmann, J. Kessler, B. Wehrle-Haller; Cell Physiology and Metabolism, Centre Medical Universitaire, Geneva, SWITZERLAND.

Integrin aVb6 activates the transforming growth factor beta 1 (TGFb1) cytokine by binding to, and mechanically pulling on, the prodomain of TGFb1 (called latency-associated peptide 1, LAP1). The signaling of TGFb1 is important for cell proliferation and differentiation, but also for protection of cancer cells from chemotherapy and for supporting invasiveness. The affinity of aVb6 integrin for LAP1 is unusually high supporting sustained, strong mechanical pull needed for activation of TGFb1. Here we asked whether this extracellular adaptation is accompanied by cytoplasmic adaptations of aVb6 integrin that allow force transmission via the actin-integrin-LAP1 axis. We developed new tools to study cell-LAP1 interaction with a variety of cell biological techniques. This allowed us to test the relevance of the intact actin cytoskeleton as well as different integrin adapters, in particular activators talin and kindlin and signaling scaffold paxillin, for LAP1 binding and TGFb1 activation. We show that dynamic cytoplasmic adapter recruitment to b6 integrin allows regulation of LAP1 binding and eventually TGFb1 activation. Given the excess amount of latent TGFb1 stored in the extracellular environment, it is crucial for cells to tightly regulate TGFb1 activation by cells. Our data highlights how regulation of aVb6 integrin activation, its stability in adhesions, and its connection to actin is involved in this important aspect of TGFb1 biology.

B528/P1510

Heterogeneous Expression of Alternatively Spliced lncRNA mediates Vascular Smooth Cell Plasticity

J. Mayner[1], E. Masutani[2], E. Demeester[3], A. Kumar[1], P. Beri[1], V. Lo Sardo[4], G. Macapugay[5], A. J. Engler[1]; [1]Bioengineering, UCSD, La Jolla, CA, [2]Bioengineering, UCSD, San Diego, CA, [3]UCSD, La Jolla, CA, [4]University of Wisconsin Madison, Madison, WI, [5]University of Wisconsin Madison, Madison, CA.

9p21 locus polymorphisms have the strongest correlation with coronary artery disease, but as a non-coding locus, disease connection is enigmatic. Vascular smooth muscle cell (VSMC) phenotype may be regulated by lncRNA ANRIL found in 9p21. We observed significant variability in induced pluripotent stem cell-derived VSMCs from patients homozygous for risk (RR) versus isogenic knockout (RR KO) or non-risk (NN) haplotypes. Sub-populations of risk haplotype cells exhibited variable morphology, proliferation, contraction, and adhesion. When sorted by adhesion using a microfluidic device, VSMCs parsed into synthetic and contractile sub-populations, i.e., weakly adherent (WA) and strongly adherent (SA), respectively. RNA-seq revealed >2000 differentially expressed genes, and of those also sorted by haplotype, >90% were co-regulated and associated with Rho GTPases, i.e., contractility. WA sub-populations expressed more of ANRIL's short isoforms, and when overexpressed in knockout cells,

ANRIL suppressed adhesion, contractility, and αSMA expression. These data are the first to suggest that variable lncRNA penetrance may drive mixed functional outcomes that confounds pathology.

B529/P1511

The *cis* association of CD47 with integrin Mac-1 regulates macrophage responses by stabilizing the extended integrin conformation

N. Podolnikova, S. Key, X. Wang, T. P. Ugarova; ASU, Tempe, AZ.

CD47 is a ubiquitously expressed cell surface integrin-associated protein. Recently, we have demonstrated that integrin Mac-1 ($\alpha_M\beta_2$, CD11b/CD18, CR3), the major adhesion receptor on the surface of myeloid cells, can be coprecipitated with CD47. However, the molecular basis for the CD47-Mac-1 interaction and its functional consequences remain unclear. Here, we demonstrated that CD47 regulates macrophage functions by directly interacting with Mac-1. In particular, adhesion of macrophages isolated from $CD47^{-/-}$ mice to fibrinogen and ICAM-1, the established physiological ligands of Mac-1, was significantly decreased compared to wild-type counterparts. Also, spreading of CD47-deficient macrophages was reduced by four- and two-fold on fibrinogen and ICAM-1, respectively. Compared to wild-type macrophages, migration of CD47-deficient macrophages was significantly decreased. The lack of CD47 on the surface of macrophages impaired their ability to fuse in the presence of IL-4. Finally, the deficiency of CD47 also reduced phagocytosis of opsonized beads, a process entirely dependent on Mac-1. The functional link between CD47 and Mac-1 was validated by co-immunoprecipitation analysis using various cultured and natural Mac-1-expressing cells. In HEK293 cells expressing individual α_M and β_2 integrin subunits, CD47 has been found to bind both subunits. Interestingly, the amount of CD47 recovered with the free β_2 subunit, which on the surface of these cells exists in the extended conformation, was higher than in complex with the whole integrin. Furthermore, activating Mac-1-expressing HEK293 cells with PMA, Mn^{2+}, and mAb MEM48 increased CD47 in complex with Mac-1, suggesting that CD47 has a greater affinity for integrin in the extended conformation. Notably, on the surface of cells lacking CD47, fewer Mac-1 molecules could convert into an extended conformation in response to Mn^{2+} treatment. The binding site in CD47 for Mac-1 was identified in its constituent IgV domain. The complementary binding sites for CD47 in Mac-1 were localized in the integrin epidermal growth factor-like domains 3 and 4 of the β_2 subunit and calf-1 and calf-2 domains of the α subunit. These results indicate that Mac-1 forms a lateral complex with CD47, which regulates essential macrophage functions by stabilizing the extended integrin conformation.

B530/P1512

Myosin 10 at the tips of filopodia-derived retraction fibers supports adhesion during mitosis when conventional focal adhesions disassemble

J. Hammer[1], A. T. Pedrosa[1], Y. Yim[1], X. Wu[1], L. Boley[1], R. Cheney[2]; [1]Cell and Developmental Biology Center, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, [2]Cell Biology, University of North Carolina Chapel Hill, Chapel Hill, NC.

Historically, adhesion during mitosis for cells grown in 2D has been attributed to retraction fibers (RFs), which are thought to arise from a combination of the cell rounding that occurs upon mitotic entry and the persistence of interphase focal adhesions (FAs). A recent study showed, however, that Talin, the main clutch component connecting actin to integrin, largely disappears from sites of adhesion in mitotic cells (Dix et al Dev Cell 2018). What then connects actin to integrin during mitosis? Here we show that

endogenously tagged Myo10, an integrin-binding MyTH4/FERM domain myosin commonly referred to as the “filopodial myosin”, localizes together with active integrin and IRM signals along the shaft and at the tips of metaphase RFs. Consistent with the results of Dix et al, and with the idea that RFs are in fact filopodia, time lapse imaging shows that Talin-rich FAs at the cell perimeter vanish upon mitotic entry while pre-existing, Myo10-positive, interphase filopodia persist, such that ~95% of them become RFs. In support of this, metaphase RFs stain for the filopodial crosslinker fascin and the filopodial tip marker VASP, and endogenous Myo10 moves out RFs at 0.7 um/s, consistent with the bundled, barbed end-out organization of actin found within filopodia. These results, together with the fact that fluorescence intensity measurements within the TIRF field as proxies for adhesion support show that Myo10 increases and Talin decreases between mitotic entry and metaphase, suggest that Myo10 at the tips of filopodia-derived RFs is replacing Talin as the main clutch component connecting actin to integrins during mitosis. Consistent with this idea, measurements of RF failure frequency and the content of active integrin within RFs indicate that adhesion is attenuated in dividing cells lacking Myo10. Moreover, these defects are rescued by WT Myo10 but not by a version that cannot bind integrin. Together, these data reveal a self-organizing property of mitotic RFs: their ability to support adhesion during mitosis is hardwired by the fact that they pre-exist as Myo10-dependent adhesive filopodia, and their barbed end-out organization licenses Myo10-dependent adhesion reinforcement during mitosis.

B531/P1513

Early phases of αV-class integrin adhesion to vitronectin under mechanical load are β-subunit-dependent

U. Sharma[1], J. Reber[2], N. Strohmeyer[1], R. Faessler[2], D. J. Mueller[1]; [1]Department of Biosystems Science and Engineering, ETH Zuerich, Basel, SWITZERLAND, [2]Department of Molecular Medicine, Max Planck Institute for Biochemistry, Martinsried, GERMANY.

αV-class integrins have been implicated in several cellular paradigms ranging from fat and bone biogenesis, angiogenesis to tumor metastasis and poor prognosis of several cancers. Given the recent efforts to develop αV-class targeting pharmacophores, delivery strategies and biomarker research, most studies do not focus on the aspect that integrins encounter varying magnitudes of extra- and intra-cellular forces during early steps of adhesion. We employ single cell force spectroscopy (SCFS) to quantify early stages (~120s) of adhesion strengthening and observe a non-monotonic behavior for αV-class integrins on vitronectin (VN) but not on fibronectin (FN), under varying mechanical load. A similar response has earlier been reported for α5β1 integrins where the catch bond behavior of α5β1 integrins is consolidated due to a crosstalk with αV-class integrins upon FN binding. Interestingly, in case of vitronectin bound αV-class integrins, we find a non-monotonic response to increasing loading rate that is β-subunit-dependent. This subunit dependence is correlated to a differential contributions from signaling molecules, like - Arp2/3 complex, Src kinase and Phosphatidylinositol 3-kinase. Our work further enquires whether the integrin heterodimers synergize or antagonize during early phases of VN binding under mechanical load and explores if this depends on their commitment to different intracellular signaling pathways.

B532/P1514

Control of Fibrotic Gene Expression in Cardiac Fibroblasts via the Rap1 GTPase

M. R. McLeod[1], J. G. Heywood[2], W. J. Richardson[2], A. D. Dubash[1]; [1]Biology, Furman University, Greenville, SC, [2]Bioengineering, Clemson University, Clemson, SC.

Fibrosis describes the excessive deposition of extracellular matrix components by cardiac myofibroblasts (CFs), a process which frequently promotes the adverse effects of pathological conditions damaging cardiac muscle, such as myocardial infarction and arrhythmogenic cardiomyopathy. Roughly 1 million Americans suffer a myocardial infarction each year, after which necrotic cardiac myocytes (CMs) are replaced by CF-derived extracellular matrix (ECM), in response to (a) biochemical signaling cues from necrotic CMs and (b) mechanical cues from the contracting myocardium and ventricle cavity pressure. Accumulation of ECM proteins (such as collagen) within the infarct scar is initially critical to prevent further tissue damage, but chronic and excessive collagen deposition is detrimental to regeneration of CMs and healing of the myocardium. While the physiological outcomes of cardiac fibrosis are well studied, the biochemical and mechano-responsive signaling mechanisms which regulate fibrotic gene expression remain poorly investigated. The GTPase Rap1 is very well-known to mediate both inside-out and outside-in signaling via attachment of integrins to ECM proteins, but less is known about its role in fibrotic gene expression. In our study, we show that expression of constitutively active (63E) Rap1A in cardiac fibroblasts results in a dramatic decrease in myofibroblast activation, measured via mRNA levels of smooth muscle alpha-actin (ACTA2). Further, Rap1A(63E) also inhibits expression of many different ECM proteins (COL1A1, COL3A1, COL4A1 and FN1), as well as other markers of fibrotic gene expression (CTGF and EGR1). In contrast, Rap1A(63E) increases the expression of matrix remodeling enzymes (such as MMP3 and MMP9) which have been shown to play a role in increasing collagen turnover, reducing ECM rigidity/stiffness and repair of damaged myocardial tissue. Moreover, we provide evidence that Rap1A(63E)-driven MMP expression is a consequence of elevated TNF-alpha/NF-kB signaling. Altogether, these data point to a significant role for Rap1A GTPase in reducing fibrotic gene expression and myofibroblast activation and suggest possible new therapeutic strategies to combat the damaging effects of cardiac fibrosis.

B533/P1515

Invasively distinct subpopulations cooperate via a laminin-332/Rac1 axis in triple-negative breast cancer

S. Yoon[1], J. K. Mouw[1], L. Chen[2], H. Wu[2], A. I. Marcus[1]; [1]Hematology and Medical Oncology, Emory University, Atlanta, GA, [2]Department of Biostatistics and Bioinformatics, Emory University, Atlanta, GA.

Intratumoral heterogeneity poses a significant hurdle for cancer treatment, yet is under-characterized in the context of tumor invasion. Cancer cells from solid tumors can invade through two predominant modes: collective invasion, whereby cancer cells invade in multi-cellular packs or streams marked by intact cell-cell junctions; and single-cell invasion, whereby cells invade independently without intercellular adhesion. We have observed that collective and single-cell invasion co-occur within the same tumor microenvironment in triple-negative breast cancer, suggesting that invasive heterogeneity supports cooperative behavior between tumor subpopulations. To test this, we used a novel, published technique developed by the lab (SaGA) to isolate pure subpopulations of 4T1 cells that collectively invade (collectives) or single cells that invade alone (singles). 3-D spheroids of SaGA-purified collectives and singles exhibited almost exclusively collective and single-cell invasion, respectively, and these

invasive phenotypes were retained over multiple passages. Integration of RNA sequencing and methylation array data obtained from RNA and DNA isolates, respectively, of collectives and singles revealed that collectives exhibit drastic overexpression and promoter hypomethylation of two laminin genes that form the laminin-332 complex, *Lama3* and *Lamc2*. Additionally, an unbiased proteomic analysis of secreted proteins also revealed an overabundance of these laminins in collectives media. We found that singles have increased expression of integrin α6 and β4, which together have been found to specifically bind to laminin-332 to activate the Rac1 GTPase. Interestingly, our RNA sequencing data revealed a binary overexpression of a Rac1 GTPase, *Prex1* in singles, suggesting that singles have enhanced Rac1 activation when compared to collectives with the potential for hyperactivation upon laminin-332 binding. Indeed, laminin-332 resulted in higher GTP-bound Rac1 in singles and subsequently increased invasion of singles in 3-D models. Additionally, laminin-332 induced cell elongation at the leading edge of singles spheroids, which was reversable by treatment with a Rac1 inhibitor. Together, our data suggests that distinct subpopulations amidst a heterogeneous tumor cooperate via laminin-332 and Rac1 to facilitate tumor invasion in metastatic triple-negative breast cancer.

B534/P1516

CRP3 ABSENCE IMPAIRS FOCAL ADHESION SIGNALING AND AORTIC SMOOTH MUSCLE CELL CONTRACTION

J. Ribeiro-Silva[1,2], M. H. Fonseca-Alaniz[2], J. E. Krieger[2], A. A. Miyakawa[2]; [1]SUNY Upstate Medical University, Syracuse, NY, [2]University of Sao Paulo (USP) Medical School, Sao Paulo, Brazil

We have previously demonstrated that Cysteine and glycine-rich protein 3 (CRP3) is a mechanosensor regulating focal adhesion kinase (FAK) activation in stretched vascular smooth muscle cells (SMCs). Here, we tested the hypothesis that CRP3 is critical for aortic SMCs mechanotransduction by modulating focal adhesion (FA) signaling. Under basal conditions, $Crp3^{-/-}$ SMCs display greater FA size, increased spreading and type I collagen secretion when compared to wild type (WT) SMCs. Upon stimulation with angiotensin II (Ang II), WT SMCs increase Y397-FAK, Y118-Paxillin and Y165-p130CAS phosphorylation, an event associated with FA growth, increased cell stiffness and collagen gel contraction. In contrast, $Crp3^{-/-}$ SMCs display neither changes in FAK-Paxillin-p130CAS phosphorylation, nor FA growth and collagen contraction, despite the greater increase in cell stiffness upon Ang II stimulation compared to WT SMCs. Analysis of MLC phosphorylation, F/G-actin ratio, cofilin phosphorylation, YAP levels and MRTF-dependent SMC gene expression showed no difference between WT and $CRP3^{-/-}$ SMCs, suggesting that CRP3 absence affects FA signaling but not the actomyosin apparatus. In agreement with this theory, PDGF-BB-dependent chemotaxis was blunted in $CRP3^{-/-}$SMCs, consistent with a deficiency in FAK signaling. FAK inhibition partially recapitulates the $CRP3^{-/-}$contraction in WT SMCs, but not cell stiffness, demonstrating that FAK signaling deficiency is responsible for the reduced contraction but not for the increased stiffness of $CRP3^{-/-}$ SMCs. CRP3 protein structure analysis evidenced the presence of two phosphorylatable tyrosines (Y57 and Y167), responsive to Ang II in cardiac cells, which may explain the dependence of CRP3 for FAK activation and contraction. In summary, we demonstrated that CRP3 is required to FAK-paxillin-p130CAS activation, a necessary and sufficient signaling for migration and contractility. In the absence of CRP3, downstream mechanosignaling is compromised, leading to deficient vascular mechanotransduction. Collectively, these evidences demonstrate that CRP3 influences FA signaling mechanisms, thus impacting SMC mechanotransduction.

B535/P1517

Integrin inactivation as a mitotic check-point

M. Lerche[1], C. M. Waterman[1], H. R. Thiam[2]; [1]NIH/NHLBI, Bethesda, MD, [2]Stanford, Stanford, CA

Focal adhesions (FAs) link the intracellular actin cytoskeleton to the extracellular matrix (ECM) via transmembrane integrin receptors and a complex network of cytoplasmic proteins. In addition to providing cells with attachment to their environment, FAs regulate cell behaviors including migration and proliferation, and are therefore assembled and disassembled in a highly regulated manner. One important process during which FAs are coordinately disassembled is cell division that occurs during M phase of the cell cycle. During mitosis, cell de-adhesion allows flat spread or columnar cells to round up, creating a radially symmetric space for mitotic spindle assembly and the equal capture and segregation of chromosomes to prevent aneuploidy. As cell division mediates growth and homeostasis of organisms, progression through each phase of the cell cycle is monitored by checkpoints that ensure completion of one phase before entry into the next. This is driven and regulated by phosphorylation of so-called checkpoint proteins by cyclin dependent kinases (CDKs) or their downstream effectors. We have found previously (Thiam et al, unpublished) that cells disassemble FAs during early prophase, and this process is dependent on phosphorylation by CDK1 in the intrinsically disordered region of the FA protein tensin 1. In addition, we found that locking integrins in their active conformation blocks FA disassembly and prevents cells from undergoing mitosis. Although high integrin activity prevents FA disassembly and mitosis, the mechanism of integrin inactivation is unknown. Here we propose that integrin inactivation, mediated by dissociation of an integrin activator or association of an activity preventer, is a novel and previously unappreciated mitotic cell cycle check-point. Our preliminary results shows that the integrin inactivators SHANK 3 and SHARPIN, undergo an electrophoretic mobility shift and an increase in expression level respectively, after exit from G2 phase of the cell cycle and entry into M phase. These results provide important initial support of the notion that integrin inactivators are regulated by mitotic kinases as cells enter mitosis, and suggests that this regulation may mediate FA disassembly, cell rounding, and progression through the cell cycle.

B536/P1518

Integrin activation and deposition of extracellular matrix are stimulated and spatially guided by adherens junctions

R. Hadjisavva[1], O. Anastasiou[1], P. Ioannou[2], M. Zheltkova[1], P. Skourides[1]; [1]University of Cyprus, Nicosia, Cyprus, [2]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Integrins and cadherins have a major role in the mechanical integrity and organization of the tissues. Adherens junctions (AJs) and focal adhesions (FAs) are intrinsically linked through the actin cytoskeleton and they mechanically interact, while the crosstalk between the two adhesion systems is crucial during embryonic development and in the adult organism. With the use of artificial cadherin glass substrates, we show that cadherin clustering can stimulate and spatially guide integrin activation, which takes place in the absence of extracellular matrix ligands. The locally generated tension at adherens junctions creates a pool of primed integrin clusters, in the extended with closed headpiece conformation, which consequently determine where FAs will be formed if ligands are present, and where ligands will be deposited when available in soluble form. Integrin activation at AJs promotes the targeting of the microtubules, which facilitate their disassembly via caveolin-based endocytosis, impacting the stability of the cadherin complex. Thus, we show that the interplay between integrins and cadherins is more

intimate than described, rooting in the capacity of integrins to become activated and stabilized by AJ-generated tension. Altogether, our data suggest a cross-regulation mechanism between cadherins and integrins.

B537/P1519

Mitotic cell responses to substrate topological cues are independent of the molecular nature of adhesion

O. Anastasiou, R. Hadjisavva, **A. Christodoulou**, P. A. Skourides; University of Cyprus, Nicosia, Cyprus

Mitotic spindle orientation is essential for a broad spectrum of processes in multicellular organisms, including cell fate decisions, tissue morphogenesis and homeostasis. Integrins and cadherins, which mediate interactions with the extracellular matrix (ECM) and between cells respectively, are implicated in the determination of spindle orientation. Here, we showed that both cadherin- and integrin-based adhesion resulted in cell divisions parallel to the attachment plane and elicited identical spindle responses to spatial adhesive cues. In addition, the use of micro patterned FN and N-cadherin Fc substrates showed that spindle orientation regulation within the plane of attachment is independent of the molecular nature of adhesion. This suggests that mechanical cues provided by adhesion topology can guide spindle orientation, independently of the molecular link or signaling from the adhesive complexes. We also demonstrated that cortical integrin activation was essential for correct spindle orientation on both cadherin and fibronectin substrates. These data suggest that spindle orientation responses to adhesion topology are primarily a result of force anisotropy on the cell cortex and show that integrins play a central role in this process that is distinct from their role in cell-ECM interactions.

B538/P1520

Contractility and adhesion to the matrix is impaired in vascular smooth muscle from Acta2 R149C mice

K. R. Ojha[1], H. Kim[2], S. Padgham[1], L. Hopkins[3], A. Chattopadhyay[4], G. Han[3], D. M. Milewicz[4], M. P. Massett[2], **A. Trache**[1]; [1]Texas A&M University Health, Bryan, TX, [2]Texas Tech University, Lubbock, TX, [3]Texas A&M University Health, College Station, TX, [4]University of Texas Health, Houston, TX.

Mutations in ACTA2, encoding smooth muscle alpha-actin, are associated with impaired vascular smooth muscle function. Vascular smooth muscle cells sense mechanical stresses from the extracellular microenvironment through cell-matrix adhesions. These adhesion structures contain clusters of integrins which connect to the actin cytoskeleton via specialized adhesion proteins. This study investigates the functional characteristics of vascular smooth muscle in aortic rings as well as isolated aortic vascular smooth muscle cells from mice presenting Acta2 R149C mutation. Functional experiments performed in aortic rings isolated from Acta2 R149C mice showed that stress relaxation was reduced at lower tensions, presumably due to reduced cytoskeletal-dependent contractility. Quantification of protein fluorescence measurements in vascular smooth muscle cells showed that smooth muscle alpha-actin mutation decreases stress fiber formation and induces a compensatory increase in smooth muscle gamma-actin. In addition, cell stiffness at the point of contact measured by atomic force microscopy showed a significant increase in Acta2 R149C cells by comparison with wild-type cells. These data suggest that upregulated smooth muscle gamma-actin may contribute to increased cell stiffness. Moreover, specific alpha5beta1 integrin recruitment at cell-matrix adhesions was reduced in Acta2 R149C cells. Taken together, the increased stiffness and reduced integrin recruitment in Acta2 R149C vascular smooth muscle cells lead to a reduced contractile phenotype.

B539/P1521

Extracellular matrix driven changes in cardiomyocyte organization and adhesion

S. Balasubramanian, A. Vallopra, J. Yang, L. Davidson, A. Kwiatkowski; University of Pittsburgh, Pittsburgh, PA

Extracellular matrix (ECM) transitions in the heart are critical for proper development, and deleterious changes in ECM composition are associated with heart disease and injury. The ECM of the embryonic and perinatal heart is dominated by fibronectin, whereas the ECM in the mature heart is primarily comprised of collagen type I. The fibronectin to collagen I ECM transition is associated with an increase in matrix stiffness and the activation of programs that coordinate cardiomyocyte maturation. However, it is not clear how the ECM regulates cardiomyocyte organization to promote heart tissue maturation and function. Integrins, adhesion proteins that bind ECM, also undergo spatiotemporal changes during heart development. Integrin-fibronectin adhesions decrease after birth and are replaced by integrin-collagen I adhesions, concurrent with junctional remodeling and cardiomyocyte maturation. Using advanced cell biology, microscopy, and bioengineering techniques, here we show that ECM composition regulates neonatal cardiomyocyte organization. On collagen I, cardiomyocytes adopt a mature, rod-shaped phenotype with myofibrils coupled to cell-cell adhesions at the longitudinal ends. In contrast, cardiomyocytes plated on fibronectin are not well organized, with basal myofibrils that largely fail to integrate at long, lateral cell-cell contacts. Our findings support a model in which the transition from fibronectin to collagen I promote junctional maturation and cardiomyocyte polarization. We speculate that cardiomyocytes recognize distinct ECM proteins to activate specific integrin-mediated signaling pathways that promote myofibril organization and cell-cell adhesion. Defining the relationship between cardiomyocytes and the surrounding ECM is critical to understanding heart biology and developing effective therapies to treat heart disease.

B540/P1522

A stiff matrix favors the mechanical cell competition that leads to extrusion of bacterially-infected epithelial cells

R. Aparicio-Yuste[1], M. Muenkel[2], A. Clark[3], M. Gomez-Benito[1], **E. Bastounis**[2]; [1]University of Zaragoza, Zaragoza, Spain, [2]University of Tübingen, Tuebingen, Germany, [3]University of Stuttgart, Stuttgart, Germany

Cell competition refers to the mechanism whereby less fit cells (“losers”) are sensed and eliminated by more fit neighboring cells (“winners”) and arises during many processes including intracellular bacterial infection. Extracellular matrix (ECM) stiffness can regulate important cellular functions, such as motility, by modulating the physical forces that cells transduce and could thus modulate the output of cellular competitions. Herein, we employ a computational model to investigate the previously overlooked role of ECM stiffness in modulating the forceful extrusion of infected “loser” cells by uninfected “winner” cells. We find that increasing ECM stiffness promotes the collective squeezing and subsequent extrusion of infected cells due to differential cell displacements and cellular force generation. Moreover, we discover that an increase in the ratio of uninfected to infected cell stiffness as well as a smaller infection focus size, independently promote squeezing of infected cells, and this phenomenon is more prominent on stiffer compared to softer matrices. Our experimental findings validate the computational predictions by demonstrating increased collective cell extrusion on stiff matrices and glass as opposed to softer matrices, which is associated with decreased bacterial spread in the basal cell monolayer *in vitro*.

Collectively, our results suggest that ECM stiffness plays a major role in modulating the competition between infected and uninfected cells, with stiffer matrices promoting this battle through differential modulation of cell mechanics between the two cell populations.

B541/P1523

The kinesin-2 Kif3AC functions as an integrin recycling motor that interacts with talin and β1 integrin

J. A. Z. Rockenbach[1], G. P. F. Nader[1,2], S. Antoku[1], G. G. Gundersen[1]; [1]Columbia University, New York, NY, [2]University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Integrins released from disassembling focal adhesions remain in an active conformation and are endocytically recycled by a Rab11-dependent process that polarizes formation of new adhesions (Nader et al., Nature Cell Biol, 2016). The molecular motor(s) responsible for recycling the active integrin are unknown. We tested whether heterodimeric kinesin-2s (Kif3AB or Kif3AC), which interact with Rab11-FIP5, contribute to the recycling of active integrins. Knockdown of kinesin-2 subunit revealed that Kif3A caused a reduction in the size of focal adhesions. We tested whether this effect was due to impaired integrin recycling using the nocodazole washout assay to synchronize focal adhesion disassembly, integrin recycling and focal adhesion reformation. Knockdown and dominant negative approaches with Kif3 tails, revealed that Kif3A and Kif3C, but not Kif3B prevented formation of new focal adhesions after adhesion disassembly. In Kif3AC disrupted cells, cell surface levels of integrins did not return after adhesion disassembly, and integrins were instead detected in the Rab11 endocytic recycling compartment where they colocalized with Kif3AC. GST-pull down from cells expressing GST-Kif3C and myc-β1 integrin showed that Kif3C interacted with β1 integrin and endogenous talin but only during endocytic recycling. Depletion of Kif3AC decreased cell migration in a wound healing assay. These results indicate that Kif3AC is a motor for recycling integrins and suggest that talin may be necessary both to keep recycling integrin in an active state and contribute to its association with Kif3AC. This work was supported by NIH grant R35 GM12858593.

Structure and Function of the Extracellular Matrix

B542/P1524

Secreted footprints organize complex cell migration patterns

E. Perez Ipina, B. A. Camley; Johns Hopkins University, Baltimore, MD.

Eukaryotic cell migration is essential to biological processes like embryonic development, immune response, wound healing, or cancer metastasis. Successful cell migration usually requires adhesion to an extracellular matrix (ECM), a network of multiple components, such as collagen and glycoproteins. Cells can also modify the matrix by depositing new matrix proteins such as fibronectin. Recent experiments with MDCK epithelial cells on 1D fibronectin micropatterned stripes observed that cells move almost persistently in regions they have previously crawled over, but barely advance into unexplored regions, resulting in oscillatory motion of increasing amplitude. These observations suggest that cells leave behind a footprint which facilitates their own or other cells' passage. Here, we explore through mathematical modeling how footprint secretion affects cell motility patterns. We simulate cell crawling on micropatterns of different geometries with a phase field model. We hypothesize that local contact with the secreted footprint activates Rac1, a polarity protein at the front of the cell, and find that this minimal assumption can recapitulate many of the experimental observations. Depending on the

footprint secretion rate and the response to the footprint, cells can display a variety of motility patterns, including self-confined, oscillatory, and persistent motion. Our model provides insight into when cells' interactions with their own footprint can lead to oscillations, and what properties of the footprint and the cell regulate this behavior.

B543/P1525

A dominant modifier scree for genetic interactor of Jagunal in Drosophila

J. Abuel, B. Riggs; Biology, San Francisco State University, San Francisco, CA.

The Endoplasmic Reticulum (ER) is a multifunctional organelle involved in protein and lipid synthesis, secretory proteins, and post-translational modification of proteins. However, the organization and inheritance of the ER during cell division is poorly understood. A recent study highlighted the highly conserved tetraspanin ER protein Jagunal (Jagn) plays a role in the organization and asymmetric partitioning of the ER in pro-neural cells during mitosis in early embryonic development of drosophila just before cell fate determination. We hypothesized that Jagn interacts with other genes that drive the generation of neural cell diversity and cell fate determinants. To identify the specific genes that interact with Jagn, we examined a collection of deficiencies covering the entire 3rd chromosome and performed a dominant modifier screen to observe changes in the compound eye phenotype. Expression of JagnRNAi construct in the Drosophila compound eye resulted in an 80% rough eye phenotype. Using these results as a baseline, we examined whether the deficiency lines on the 3rd chromosome was an enhancement or suppression of the rough eye phenotype. We have identified eight suppressors and ten enhancers of Jagn-induced rough eye phenotype. Through our screening efforts, we identified 19 gene targets based on their involvement in many predicted functions of Jagn such as organelle assembly, microtubule attachment, and organelle movements. Here, we discovered a genetic interaction with the g-secretase subunit Presenilin (Psn), a transmembrane protein involved in proteolysis and activation of Notch signaling. These findings highlight a connection between Jagn-dependent mitotic ER partitioning and the Notch signaling pathway.

B544/P1526

Eggshell membrane promotes homeostasis of skin and lung tissue associated with type III collagen and decorin expression and ameliorates pulmonary fibrosis in a bleomycin mouse model

E. Ohto-Fujita[1,2], M. Shimizu[1], A. Atomi[1], S. Horinouchi[1], N. Shibayama[1], R. Hosoda[1], H. Hiruta[1], T. Murakami[3], S. Miyazaki[3], M. Kurimoto[1], Y. Asano[4], T. Watanabe[5], Y. Hasebe[6], Y. Atomi[1]; [1]Material Health Science, Tokyo University of Agriculture and Technology, Tokyo, JAPAN, [2]Tokyo Woman's Christian University, Tokyo, JAPAN, [3]Cooperative Dep. Veterinary Medicine, Tokyo University of Agriculture and Technology, Tokyo, JAPAN, [4]Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, JAPAN, [5]Division of Applied Chemistry, Graduate School of Engineering, Tokyo University of Agriculture and Technology, Tokyo, JAPAN, [6]Almado, Inc., Tokyo, JAPAN.

The integrity of the extracellular matrix (ECM) of the dermal papilla in the skin and the interstitium in the lungs is critical for tissue homeostasis. We have previously reported that moderate amounts of eggshell membrane (ESM) provide a young and elastic ECM environment via type III collagen (COL3), decorin (DCN), and MMP2 expression by skin fibroblasts. In this study, we investigated the ESM response of lung fibroblasts and whether oral intake of ESM improves skin and lung homeostasis in mice, including the bleomycin lung fibrosis model (BLM model). The response of human lung fibroblast

cell line IMR-90 to ESM was investigated in a culture environment with solubilized ESM (SESM) surface. Changes in gene expression were investigated in mice lungs and skin after 2- and 4 weeks of oral ESM ingestion. The anti-fibrotic effect of ESM was then investigated in the BLM model and expression of COL3 in the bronchial stroma was investigated. To investigate the relationship between the induction of COL3 expression by ESM ingestion and the amelioration of pulmonary fibrosis, the activation of the mechano-transducer TAZ transcription factor, which senses extracellular matrix stiffness and regulates myofibroblast marker expression, proliferation and migration, was quantified as an indicator of nuclear migration by immunostaining of TAZ in lung fibroblasts. Lung fibroblasts cultured on ESM showed markedly higher COL3, DCN, and MMP2 levels. Oral ESM administration in mice markedly increased COL3 and DCN levels in lung tissues after 2 weeks, and COL3, DCN, and MMP2 levels in the papillary dermis after 4 weeks. To further explore the effects of ESM on the lungs, we used a mouse model of bleomycin-induced fibrosis. In these mice, ESM significantly suppressed fibrosis at 2 weeks and increased the COL3 levels in the bronchioles and DCN levels in the alveoli, which was implicated in the suppression of lung fibrosis. TGF-β induced nuclear translocation of TAZ was not increased when cultured in medium containing SESM. Oral ESM intake may prevent the age-dependent decline of the papillary dermis and pulmonary fibrosis by improving the extracellular environment in skin and lung tissues.

B545/P1527

Compartmentalization of alpha-tectorin (TECTA) through microvilli regulates the collagen attachment and the organization of the tectorial membrane

A. Niazi, J. Kim, J. Park, S. Park; Neurobiology, University of Utah, Salt Lake City, UT.

Background: The microvilli are membrane protrusions that are found on the apical surface of some epithelial cells, but their biological function is poorly understood. The tectorial membrane (TM) is an apical extracellular matrix (ECM) in the inner ear that mediates auditory transduction. The developing TM exhibits a highly organized architecture composed of parallel collagen fibrils that are attached to the tip of the microvilli of the cochlear supporting cells. The attachment of elongating collagen fibrils to the tip membrane is crucial for collagen arrangement. Previously, we showed that both surface-tethering and the release of alpha-tectorin (TECTA), a GPI-anchored collagen binding protein, play critical roles in TM morphogenesis by preventing the diffusion of secreted TM components, and mediating matrix elongation, respectively. However, the role of surface shape in the matrix organization and the mechanism by which collagens are attached to a specific membrane compartment were unknown. We identified that transmembrane serine proteases (TMPRSSs) shed TECTA from the cell surface. Noting that surface TECTA is preserved on the tip of the microvilli, we hypothesized that the microvillus structure may restrict TMPRSS sheddase activity from the distal tip, which prompts the preservation of full-length TECTA and collagen attachment sites. **Methods:** To identify the role of TECTA shedding in the matrix organization, we generated Tecta knock-in mice in which the release of TECTA is blocked. In TECTA-R2061S mice, the TMPRSS-mediated cleavage is specifically blocked. Similarly, in TECTA-ZP3 mice, a transmembrane form of TECTA was generated to block the GPI-anchor-dependent cleavage. We studied how these mutations impact the organization of the TM by immunohistochemistry and Transmission electron microscopy (TEM). **Results:** Collagen fibrils are attached to the lateral and base membrane of the microvilli in Tecta-R2061S mice. Furthermore, we observed a significant increase in the microvilli length and extracellular vesicle (EV) density in this mutant. In Tecta-ZP3 mice, the release of TECTA and EV density is dramatically reduced. Also, in Tecta-ZP3 mice, the elongated collagen fibrils

are attached to the distal tips of microvilli but are densely packed and lack crosslinking fibers.
Conclusion: Our findings indicate that the cell surface shape SCs compartmentalizes the release of the TECTA and consequently restrains the collagen attachment site and mediates the specific highly ordered organization of the TM.

B546/P1528

The Application of Fibrotic Lung Matrix Derived Hydrogels for *in-vitro* Small Molecule Intervention in Lung Fibrosis

J. Fernandez-Davila[1], D. W. Moore[1], L. R. Rodriguez[1], S. Nathan[2], G. Grant[1], J. L. Moran[3]; [1]Department of Biology, George Mason University, Fairfax, VA, [2]Advanced Lung Disease and Transplant Program, INOVA Fairfax Hospital, Fairfax, VA, [3]Department of Mechanical Engineering, George Mason University, Fairfax, VA.

Idiopathic Pulmonary Fibrosis (IPF) is a disease marked the dysregulated activation and chronic proliferation of lung fibroblasts. With only two FDA approved drugs for this devastating disease, there is an outstanding need for improved *in vitro* model systems to evaluate and identify novel therapeutics. Using decellularized lung tissue donated by patients with IPF, we present and validate a protocol for the generation of a 3D fibrotic hydrogel *in vitro* culture. The culturing of fibroblasts in fibrotic matrix closely recapitulates the cell-to-matrix signaling present in fibrosis and removes complexity of cell cultures on stiff tissue culture plastic. We use this model to explore the effects of small molecule stimulation and inhibition of profibrotic pathways.
IPF lung tissue was decellularized, digested, desalinated, and dehydrated prior to gelation at 37°C. Primary 3D fibroblast cultures were assayed for myofibroblast phenotypes after challenge of TGF-β and the antifibrotic Nintedanib (NTD). This includes quantification of myofibroblast associated gene expression, contractile capacity, and generation of ROS species. Fibroblast-immune cell cross talk is conducted via conditioned media experiments. Media from primary fibroblasts in 3D cultures is added to U937 monocyte cells which are then driven towards macrophage differentiation. Macrophage phenotypes was assayed via QPCR of macrophage polarization genes.
We observed increased rates of contractility in ECM hydrogels seeded with IPF fibroblasts (IPF-F) as compared to normals human lung fibroblasts (NHLF). This contraction was correlated to cell density and increased when stimulated with TGF-β. Expression of genes associated with myofibroblast activation was decreased in all fibroblasts when seeded in hydrogels, as compared to tissue culture plastic. However, even in 3D hydrogel cultures, IPF-F maintained their disease phenotype with increased expression of proliferation and myofibroblast activation genes as compared to NHLF. Treatment of cultures with NTD decreased expression of myofibroblast associated genes and partially mitigated TGF-β driven contraction. Differential expression of cytokines was observed in hydrogel cultures as compared to plastic, with the potential activation of a profibrotic macrophage phenotype in U937 cells treated with hydrogel culture conditioned media.
Overall, our hydrogel is a novel platform to test functional characteristics of activated fibroblasts including contractile forces, cell proliferation, expression of myofibroblast associated genes, generation of oxidative stress, and cytokine stimulation. Furthermore, the application of co-culture assays allows for the exploration of immune cell crosstalk, a key aspect to the amplification of the fibrosis.

B547/P1529

Heat-dependent regulation of collagen metabolism in mouse dermal fibroblasts

T. Nakajima, Y. Ishiuchi-Sato, T. Nedachi; Toyo University, Tokyo, JAPAN.

It has been demonstrated that heat stimulation exerts the activation of Matrix metalloproteinases (MMPs) secreted from skin cells, thereby inducing degradation of collagens in the connective tissues of the skin. On the other hands, it is still unclear detail characteristics of heat-dependent responses of epidermal and dermal cells, including signaling cascades which are activated by heat stimulation as well as the impact of heat on collagen biogenesis. In this study, we analyzed heat-dependent responses of mouse primary cultured dermal fibroblasts (MDFs), especially focused on collagen metabolisms.We cultured MDFs under the different temperature for various times and analyzed the expression of Heat shock protein 70 (HSP70) as an indicator for heat stimulation, and found HSP70 expression was clearly observed when the MDFs were cultured at 41 C for more than 3h. To set more physiological condition, MDFs were stimulated at 41 C for 3 h, then returned cells at normal condition (37 C) and keep culturing for 21 h. We next performed RNA-seq analysis to elucidate gene expression changes during this experimental schedule. The gene expression of major fibrous collagens (Collagen type I and III) was not altered by heat stimulation; however, the gene expression of MMP-2, -3, -14 and collagen fiber synthesis-related protein (BMP1, TLL1) was significantly decreased. Gene ontology analysis suggested that unfolded protein response (UPR) was apparently enhanced in response to heat stimulation. Intriguingly, although UPR activation often correlated with MMPs induction in the other cells, the present study suggested that heat stimulation on MDFs rather reduced these gene expressions.Overall, the present study suggested that the heat stimulation might delay collagen metabolism through down-regulation of BMP1, TLL1, and MMPs in MDFs, as well as exerted UPR to repair and/or remove inappropriate proteins. Further studies on heat-dependent regulation of collagen metabolism and structural changes of collagen fibers will clarify heat-dependent regulation of dermal tissues.

B548/P1530

The desmosomal cadherin Desmoglein-2 controls extracellular matrix gene expression via Src and NF-kB signaling.

J. M. Powers, M. M. Mrus, B. T. Mateo, A. D. Dubash; Biology, Furman University, Greenville, SC.

Desmosomes are protein complexes crucial for maintaining cell-cell adhesion and integrity of tissues. These complexes are made up of proteins from three families: transmembrane cadherins (Desmoglein and Desmocollin) link adjacent cells in the extracellular space, armadillo proteins (Plakophilin and Plakoglobin) stabilize the intracellular plaque, and the cytolinker Desmoplakin (DP) connects the plaque to the intermediate filament network. Desmosomal proteins have also been shown to coordinate gene expression pathways required for processes such as proliferation, differentiation and cell migration. In particular, several lines of evidence have linked desmosomal proteins to gene expression of extracellular matrix (ECM) proteins. In our study, we sought to investigate the role of desmosomal cadherins in ECM gene expression. Compared to control A431 cells, Dsg2 knockout cells (generated via CRISPR) demonstrated a dramatic increase in expression of Fibronectin (FN1) mRNA, and minor changes in expression of Collagen 1 (COL1A1) and Collagen 2 (COL2A1) mRNA. An increase in expression of Fibronectin protein levels in Dsg2-deficient cells was also observed via western blot. Increased expression of ECM genes was also observed upon siRNA-mediated knockdown of Dsg2 in both A431 cells and HaCaT keratinocytes, verifying that these changes are not clone-specific or due to off-target CRISPR

effects. An extensive analysis of signaling pathways known to be involved in regulation of ECM gene expression uncovered an important signaling role for Src kinase and the NF-kB transcription factor, as abrogation of either protein (via knockdown or pharmacological inhibition) resulted in a rescue of Dg2KO-mediated increases in FN1 gene expression. Moreover, we have documented a significant increase in localization of NF-kB to the nucleus of Dsg2-deficient cells, indicating an increase in NF-kB transcriptional activity. Taken together, our study highlights a novel role for Dsg2 in mediating ECM gene expression via Src/NF-kB signaling, adding significant insight into the mechanisms by which desmosomal cadherins control the adhesive behavior of cancer cells.

B549/P1531

Tension-suppressed degradation of collagen underlie tissue stiffness scaling with fibrillar collagen

K. Saini[1], S. Cho[1], M. Tewari[1], A. Jalil[1], M. Wang[1], M. Vashisth[1], A. Kasznel[1], K. Yamamoto[2], D. Chenoweth[1], D. Discher[1]; [1]University of Pennsylvania, Philadelphia, PA, [2]University of Liverpool, Liverpool, UNITED KINGDOM.

Extremely soft tissues such as embryonic chick hearts or adult mouse brain contain far less collagen than highly stiff adult tissues such as mouse tendons, but cell and molecular mechanisms for such homeostatic differences remain unclear. We hypothesized that cell-generated or exogenous forces combine with strain-suppressed collagen degradation to sculpt extracellular matrix (ECM) levels in tissues. For various mature tissues as well as beating chick hearts and mouse tendons, we find that label-free second harmonic generation (SHG) image intensity from collagen fibrils generally scales non-linearly with tissue stiffness, aligning well with results from cellularized gels of collagen. Chick hearts beating at ~5% strain in a spatiotemporal wave maintain collagen levels until contractile strain is suppressed by myosin-II inhibition; endogenous matrix metalloproteinases (MMPs) then degrade collagens within ~30-60 minutes. Tendons have oriented collagen fibrils but also generally exhibit heterogeneous strains in deformation, with addition of MMP or bacterial collagenase again showing degradation is suppressed at physiological strains (i.e., ~5-8%). Sequestration of collagen cleavage sites by strain is a likely mechanism because molecular permeation and mobility prove strain-independent whereas artificial collagen cross-links accelerate collagen degradation. We conclude that strain-suppressed turnover of collagen underlies tissue stiffness scaling.

B550/P1532

PAR-1 induces the TN-C expression in activated microglia in the ischemic brain

Y. Fan[1], X. Feng[1], **C. Chung**[2,1]; [1]Tianjin University, Tianjin, China, [2]Mercer University School of Medicine, Columbus, GA

Stroke is one of the most common causes for mortality and morbidity worldwide. Following acute stroke onset, biochemical and cellular changes induce further brain injuries such as neuroinflammation, cell death, and blood-brain barrier disruption. As the resident immune cells of the central nervous system, microglia rapidly respond to brain insults, including stroke and traumatic brain injury. Evidences suggest that Protease Activated Receptor-1 (PAR-1) mediates neuronal injury in cerebral ischemia. We examined if PAR-1 signaling upregulates the expression of tenascin-C (TNC) in microglia. TNC, an extracellular matrix protein, is considered to be an important inducer in promoting neuroinflammatory cascades and the resultant pathology in stroke. We found that TNC is highly expressed in cultured primary microglia treated with PAR-1 peptide, which can be effectively blocked with PAR-1 antagonist. Upregulation of

TNC exocytosis upon PAR-1 activation in microglia was also observed. To examine the expression of TNC in microglia in the ischemic brain, mice were subjected to 2 hours of transient middle cerebral artery occlusion (MCAO), followed by 12 or 24 hours of reperfusion. Consistent with results from cultured microglia, a high level of expression of TNC was observed in activated microglia in the ischemic brain. These results suggest that PAR-1 signaling plays an important role in neuronal injury in cerebral ischemia via inducing TNC expression and exocytosis in microglia.

B551/P1533

Identification of two COMP C-terminal globular domain epitopes that bind and enhance BMP/TGFbeta growth factors

J. Fraley[1], J. H. N. Yik[1], G.-y. Liu[2], D. R. Haudenschild[1]; [1]University of California, Davis Health, Sacramento, CA, [2]University of California, Davis, Davis, CA

The extracellular matrix (ECM) regulates cellular responses by binding and enhancing TGFβ-superfamily growth factors.The potency and pleiotropic effects of BMP/TGFβ growth factor activity necessitates tight regulation. The ECM plays an active role in growth factor regulation and helps fine-tune cellular responses.One such regulatory ECM component is the cartilage oligomeric matrix protein (COMP), a multi-domain pentameric glycoprotein of bone, cartilage, and most human tissues.We previously identified that the COMP C-terminal globular domain (COMP CTD) bound and enhanced the activity of bone morphogenetic protein-2 (BMP-2) and transforming growth factor beta 1 (TGFβ-1), *in vitro* and *in vivo,* however the exact growth factor binding sites on COMP remain unknown.Therefore, the objective of this study is to identify the interactions sites of the COMP CTD that bind to the TGFβ-superfamily growth factors.A synthetic peptide library of the entire 270aa COMP CTD was developed, consisting of partially overlapping 15aa peptides.Of the 28 COMP CTD peptides tested, we identified two that interfered with rhCOMP binding to immobilized BMP-2 and TGFβ-1 in competitive ELISAs.Peptide YAG corresponds to the sequence YAGFIFGYQDSSSFY, (COMP residues 606-620), and peptide CFS corresponds to the sequence CFSQENIIWANLRYR (COMP residues 726-740).The two sequences were then mapped onto the crystal structure of the COMP CTD, suggesting a potential binding pocket.Since overlapping peptides did not interfere with COMP binding to BMP-2 and TGFβ-1, the potential binding sites were further narrowed down to the inner five residues of each peptide.The inner five residues of peptides YAG and CFS were analyzed using an alanine point mutant screen in a competitive ELISA for their interference of rhCOMP binding to immobilized BMP-2.The results indicated that four residues of the YAG peptide, and one of the CFS peptides as potential sites on COMP where BMP-2 and TGFβ-1 bind.To further confirm YAG and CFS epitope sequences as binding sites on the COMP CTD, antibodies (α-YAG, α-CFS) were developed and tested in a cell-based assay for their function blocking ability. Using a BMP-2 dependent gene transcription luciferase cell line (C33A-2D2), α-YAG and α-CFS significantly blocked rhCOMP enhancement of BMP-2 in a dose dependent manner. Our data suggests that two distinct epitopes in the COMP C-terminal globular domain bind BMP-2 and TGFβ-1. Now, α-YAG and α-CFS will be further tested for their ability to block COMP enhancement of BMP-2 in other quantitative osteogenic assays. Characterization of the COMP binding sites for TGFβ-superfamily growth factors can inform the development of 2nd-generation biologics whereby BMP-2 can be combined with native ECM components to augment clinical outcomes.

B552/P1534

A transient cell population in the *Drosophila* gut synthesizes matrix components following basement membrane damage

A. Stricker, K. LaFever, K. Peebles, S. Hutson, A. Page-McCaw; Vanderbilt University, Nashville, TN

Basement membranes are the oldest, most conserved forms of extracellular matrix which serve to separate tissue layers, provide mechanical support, and direct signals to neighboring cells. Additionally, basement membranes are subject to mechanical damage and require dynamic repair mechanisms. Our work utilizes the *Drosophila* adult midgut basement membrane to probe repair dynamics. In *Drosophila*, all major basement membrane components have been conserved but with less redundancy than mammals. Our lab has developed an assay to damage the basement membrane and study the repair process by feeding the flies dextran sodium sulfate (DSS). We have found that many genes required for basement membrane repair are also required to maintain adult basement membranes over time, even without damage, suggesting that continuous synthesis of matrix components is required to maintain and repair the basement membrane. This raises the question of how these processes differ. My data suggests basement membrane damage is actively detected. Following DSS damage, a subset of cells in the *Drosophila* midgut begins transcribing the matrix components collagen IV and the collagen IV crosslinking enzyme, Peroxidasin. We have termed these cells "Matrix Makers." I performed lineage tracing to identify the cellular source and ultimate fate of the Matrix Makers. I found these cells are likely enteroblasts that die or differentiate into short-lived enterocytes once the basement membrane is repaired. In summary, I have identified a transient cell population that synthesizes long-lived matrix components required for basement membrane repair.

B553/P1535

MECHANOSENSITIVE TRPV4 channel guides maturation and organization of the bilayered mammary epithelium

T. Kärki[1], C. Guenther[2], **S. Koskimäki**[2], J. Pirhonen[2], K. Rajakylä[3], S. Tojkander[2]; [1]Aalto University, Espoo, Finland, [2]Tampere University, Tampere, Finland, [3]Tampere University of Applied Sciences, Tampere, Finland

Introduction:

Biophysical cues from the cell microenvironment are detected by mechanosensitive components at the cell surface. Such machineries convert physical information into biochemical signaling cascades within cells, subsequently leading to various cellular responses in a stimulus-dependent manner. At the surface of extracellular environment and cell cytoplasm exist several ion channel families that are activated by mechanical signals to direct intracellular events. One of such channels is formed by transient receptor potential cation channel subfamily V member, TRPV4, that is known to act as a mechanosensor in a wide variety of tissues and control ion-influx in a spatio-temporal way.

Materials and Methods:

TRPV4 depletion was conducted on MCF10A or 184A1 mammary epithelial cells (purchased from ATCC) using siRNA. Actin cytoskeleton organization was analyzed from monolayer cultures, alongside with calculation of cell-exerted tractions utilizing traction force microscopy. Maturation markers for both myoepithelial and luminal epithelial cells were screened using immunocytochemistry and western blot analysis. 3D cultures were performed with both MCF10A and 184A1 mammary epithelial cells to analyze the formation and maintenance of mammosphere structures.

Results and Discussion:
Here we report that TRPV4 is prominently expressed in the stem/progenitor cell populations of the mammary epithelium and seemingly important for the lineage-specific differentiation, consequently affecting mechanical features of the mature mammary epithelium. This was evident by the lack of several markers for mature myoepithelial and luminal epithelial cells in TRPV4-depleted cell lines. Interestingly, TRPV4 expression is controlled in a tension-dependent manner, and it also impacts differentiation process dependently on the stiffness of the microenvironment. Furthermore, such cells in a 3D compartment were disabled to maintain normal mammosphere structures and displayed abnormal lumen formation, size of the structures and disrupted cellular junctions.
Conclusion:
Mechanosensitive TRPV4 channel acts as critical player in the homeostasis of normal mammary epithelium through sensing the physical environment and guiding accordingly differentiation and structural organization of the bilayered mammary epithelium.

Autophagy

B555/P1536
WIPI2B/ATG-18 Phosphorylation Regulates Neuronal Autophagosome Biogenesis *in vivo*
H. Tsong, A. Stavoe; University of Texas Hlth Sci Ctr, Houston, TX.

Autophagy is a homeostatic mechanism that cells utilize to clear waste. Autophagy is especially important for neurons because they cannot dilute waste through cell division. However, neurons lose their ability to perform autophagy with age, and the misregulation of autophagy has been implicated in many age-related neurodegenerative diseases (NDDs). Previously, we found that we could restore the rate of autophagosome biogenesis in aged primary murine neurons through the overexpression of WIPI2B, a key autophagy component. Importantly, this rescue is contingent upon the phosphorylation state of WIPI2B. These data suggest that WIPI2B phosphorylation regulates its function in autophagosome biogenesis and that increasing dephosphorylated WIPI2B via protein regulators will upregulate neuronal autophagy. To test this hypothesis, we first verified the role of WIPI2B phosphorylation in neuronal autophagy *in vivo* in *Caenorhabditis elegans*. We generated *C. elegans* strains endogenously expressing phospho-mimetic or phospho-dead ATG-18 (the WIPI2B ortholog). We previously showed that autophagy regulates PVD axon outgrowth cell-autonomously. Using PVD axon length as a simple, visual readout of neuronal autophagy, we determined that worms with phospho-mimetic ATG-18 had longer axons, suggesting defective autophagy, while worms with phospho-dead ATG-18 had normal axon lengths, suggesting functional autophagy. Due to the importance of this phospho-site in autophagy, we used a candidate approach to identify the kinase and phosphatase that regulate ATG-18/WIPI2B phosphorylation. Overexpression of constitutively active CDK-1 in PVD yielded longer PVD axons, suggesting that CDK-1 might regulate neuronal autophagy as a kinase that regulates ATG-18 phosphorylation. Conversely, worms with mutations in a PP2A regulatory subunit SUR-6/B55 displayed longer PVD axons, similarly implicating PP2A in the regulation of neuronal autophagy. Since autophagy misregulation has been implicated in NDDs, a better understanding of how neurons utilize and regulate autophagy will uncover novel therapeutic targets for treating these NDDs.

B556/P1537

"Impact of the ER Stress Sensing Protein PERK and Autophagy On Exacerbated Strokes in Alzheimer's Patients "

H. Gudlavalleti, R. Viswanthan, B. Stoveken, H. Deborah, D. Lozano, N. Derosa, s. Weintraub, k. Bieniek, J. Lechleiter; University of Texas Health Science Center - San Antonio, San Antonio, TX.

Patients with Alzheimer's disease are at high risk of stroke and also exhibit increased stroke severity and infarct volume compared to age matched subjects. The role of autophagy in the exacerbation of strokes in these patients is poorly understood. Increased autophagy has been reported as both protective and detrimental, depending at what age stroke occurs. PKR like endoplasmic reticulum (ER) Kinase (PERK) is an early ER stress sensor that activates the unfolded protein response (UPR). Subsequent phosphorylation of eIF2a in this ER stress pathway inhibits general protein translation but can also lead to increased autophagy. PERK variants correlated with an increased risk of Alzheimer's disease exhibit decreased kinase activity and increased turnover rates. We initially investigated the underlying mechanisms of PERK turnover and its impact on autophagy. Our data indicate autophagy / lysosomal inhibition using chloroquine (CQ) treatments (50 uM) of cultured mouse embryonic fibroblasts (MEFs) significantly increased PERK levels over a 20-hour period. In contrast, treatment of cultured MEFs with the proteasomal inhibitor MG-132 (25 uM), did not appear to affect total PERK levels under our conditions. Pharmacologically inhibiting PERK phosphorylation using GSK414 and increasing cell stress with thapsigargin (1 uM) decreased autophagy markers and significantly increased PERK levels. These data suggested non-phosphorylated PERK under stressed conditions decreased PERK degradation due to decreased autophagy. A proteomics analysis of protein extracts from wildtype MEFs and PERK knockout MEFs, treated with and without chloroquine revealed both a loss (5) and gain (2) of autophagic proteins when PERK protein was knocked out. We are currently investigating the role of these PERK-dependent autophagy proteins. An initial analysis of human brain samples from patients with Alzheimer's disease and stroke infarcts showed autophagy markers were increased and PERK levels decreased compared to controls, consistent with our working model. We are also investigating the specific role of PERK-dependent autophagy on stroke severity and volume in AD mouse models. Data from Alzheimer's patients with strokes are being used to guide these experiments. Funded by NIH RF1 **AG058778.**

B557/P1538

UnVAILing the mechanisms of noncanonical autophagy signaling in STING-mediated innate immunity

T. Fischer[1], E. Bunker[1], F. Le Guerroue[1], C. Wang[1], A. Werner[2], R. Youle[1]; [1]NINDS/NIH, Bethesda, MD, [2]NIDCR/NIH, Bethesda, MD.

Autophagy machinery play a well-recognized, yet poorly understood, role in host immunity and inflammation. We have recently demonstrated that Stimulator of Interferon Genes (STING), an essential component of innate immune signaling, may confer antiviral defense through an unanticipated mechanism that is distinct from canonical autophagy. STING activation mediates V-ATPase-ATG16L1-induced LC3B (VAIL) lipidation to single membrane vesicles similarly to other noncanonical autophagy signaling in pathogen- and damage-associated responses. We now identify a novel role for ubiquitylation induced by STING in VAIL signaling and reveal key mechanistic details underlying the relationship between ubiquitin, conjugation of LC3B and mammalian ATG8s to membranes, and innate immune signaling. As ubiquitin plays an important role in both autophagy and innate immunity, our

findings position ubiquitylation as a central molecular link between these cellular processes and VAIL signaling mediated by the STING pathway.

B558/P1539

Crosstalk between Mitochondrial Quality Control and Antibacterial Pathways in *Mycobacterium tuberculosis* infected Macrophages

S. VERMA[1], S. Sarkar[2], D. Kumar[1]; [1]Cellular Immunology, ICGEB, New Delhi, INDIA, [2]Institute of Cancer and Genomic Sciences, Institute of Biomedical Research, University of Birmingham, UNITED KINGDOM.

Autophagy, the key homeostatic machinery of the cell, has emerged as a critical cellular defense mechanism against intracellular pathogens, including *Mycobacterium tuberculosis (Mtb)*. While autophagy is believed to target invading bacterial pathogens towards lysosome for degradation, virulent *Mtb* is also known to inhibit the maturation of autophagosome into auto-lysosomes. In this study, we explore the role of autophagy adaptors to understand the regulation of the homeostatic and defense arms of autophagy in the infected macrophages. We report here that autophagy adaptors in general, with an exception of NDP52, unexpectedly, had a pro-bacterial role. Depletion of autophagy adaptors, particularly p62/SQSTM1 resulted in excessive redox stress as well as lysosomal targeting of *Mtb*, which enhanced its clearance from the macrophages. We noted that in the absence of autophagy adaptor, mitophagy process gets impaired and results in the activation of alternate compensatory mitochondrial quality control measure i.e. mitochondria-derived vesicle (MDV) biogenesis. The MDVs were trafficked towards the bacterial phagosomes and enhanced the killing of *Mtb*. Thus, we disclose an unexpected crosstalk between the mitochondrial quality control pathway and the host anti-bacterial pathway, which could be further exploited for therapeutics against tuberculosis.

B559/P1540

Discovery of new *Legionella pneumophila* anti-autophagy effector proteins

K. R. Parducho, C. R. Roy; Yale University, New Haven, CT.

Legionella pneumophila is an intracellular bacterial pathogen that secretes over 300 different “effector” proteins into host cells to manipulate various host pathways. The autophagy pathway, which results in the lysosomal degradation of intracellular bacteria, is a target of effector-mediated inhibition. Although multiple effectors have been described that can interfere with autophagy, genetic studies have suggested there remain undiscovered anti-autophagy effectors. To identify these effectors, a *Saccharomyces cerevisiae* library producing individual effector proteins was screened for defects in the transport of GFP-Atg8 to the yeast vacuole, which is a process mediated by autophagy. This screen identified a *L. pneumophila* effector that prevents the starvation-dependent turnover of GFP-Atg8 and p62 in yeast and mammalian cells, respectively. By examining the localization of autophagy-related proteins, cargo and cargo-receptors, it was found that this effector interferes with an early stage of autophagy. Studies are ongoing to determine the biochemical function of this effector and the host target. Determining the function of this *L. pneumophila* effector will provide a better understanding of how bacteria evade autophagic destruction and could provide new insight into autophagy regulation.

B560/P1541

DTHIB induces autophagy in human pancreatic cancer

A. Fausey[1,2,3], A. Young[1], S. Ghai[1], **K. Su**[1,2,3]; [1]Department of Cell and Cancer Biology, College of Medicine and Life Sciences, The University of Toledo, Toledo, OH, [2]MD Program, College of Medicine and Life Sciences, The University of Toledo, Toledo, OH, [3]The Medical Student Research Program, College of Medicine and Life Sciences, The University of Toledo, Toledo, OH.

Background: KRAS mutant pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer with poor prognosis and limited treatment options. Additionally, many malignancies develop chemo-resistance, significantly complicating treatment strategies and highlighting the demand for innovative treatments. The transcription factor Heat-Shock Factor 1 (HSF1) is momentarily activated in response to a variety of cellular stressors, such as temperature fluctuations or an increase in proteotoxic stress. Proteotoxic stress is reduced within the cell by the production of different heat-shock proteins. HSF1 is continuously overexpressed in several malignancies, including KRAS-mutant PDAC, where it promotes malignancy through its transcriptional targets as well as non-transcriptionally through cytosolic protein interactions to maintain protein stability and control cellular proliferation. The intracellular process of autophagy enables the recycling and destruction of cellular organelles and components. The mammalian target of rapamycin complex 1 (mTORC1) is known to suppress autophagy, whereas HSF1 is known to control mTORC1 activity. DTHIB is an HSF1 inhibitor that directly and specifically reduces HSF1 expression. Although DTHIB has significant anticancer efficacy against prostate cancer, its contribution to the emergence of pancreatic cancer is yet unclear. Autophagy plays two distinct roles in cancer, controlling either the growth of tumors or serving as a tumor suppressor. The molecular underpinnings between HSF1 and autophagic induction in PDAC development are not yet determined. Objectives: We hypothesize that inhibiting HSF1 could lead to a autophagic-associated cell death profile in PDAC and serve as a potential therapeutic target for treating human pancreatic cancer. Methods and Results: In the human pancreatic cancer cell line MIA PaCa-2 cells, the HSF1 inhibitor DTHIB dose-dependently induced autophagy which reduced the autophagy marker P62, inhibited mTORC1 activity, and induced LC3 I/II lipidation. In gemcitabine-resistant MIA PaCa-2 cells, DTHIB also elicited autophagy responses. Overexpression of HSF1 partially inhibited DTHIB-induced autophagy. Furthermore, combination with DTHIB and chemotherapy drug gemcitabine synergistically induced MIA PaCa-2 cell death. Conclusion: DTHIB inhibits HSF1 to induce autophagy and cell death in human pancreatic cancer cells.

B561/P1542

Cell-type-specific functions for the autophagy receptor p62 in neurons and astrocytes

D. K. Sidibe, C. Miranda, M. H. Stempel, S. L. Maday; University of Pennsylvania, Philadelphia, PA.

Neurons and astrocytes have unique demands in regulating the quality and composition of their proteome. A key regulator of the proteome is autophagy, a lysosomal degradation pathway. Preliminary studies show that autophagy is regulated differently in neurons and astrocytes in paradigms of stress. However, how autophagy is regulated in each cell type to facilitate cell-type-specific functions and stress responses is largely unknown. The objective for this study is to elucidate cell-type-specific functions for the selective autophagy receptor p62 in neurons and astrocytes. P62 facilitates selective forms of autophagy by binding to ubiquitinated substrates and the autophagy marker LC3, thereby incorporating cargo into a forming autophagosome. By selecting specific cargos for degradation, p62 has multiple neuroprotective functions. P62 mitigates proteotoxic stress by targeting ubiquitinated protein

aggregates to autophagy. Additionally, p62 mitigates oxidative stress by targeting Keap1, a negative regulator of the antioxidant transcription factor NRF2, for degradation by autophagy. However, the cell-type-specific function of p62 in neurons and astrocytes is poorly understood. To examine functions of p62 in each cell type, we established a robust system to coculture neurons and astrocytes. This system recapitulates intercellular interactions found *in vivo,* and provides a manipulatable system for defining cell-type-specific p62 function with high resolution. Using our neuron-astrocyte coculture system, I found that metabolic stress increases p62-positive structures in both neurons and astrocytes, but only in neurons do these structures associate with ubiquitin and require ubiquitination for their formation. Strikingly, oxidative stress, which is known to preferentially activate astrocytes, increases p62 levels in astrocytes but not in neurons. These data support the model that p62 has cell-type-specific roles for quality control in neurons and astrocytes. Specifically, p62 may function primarily in selective autophagy in neurons, and in the antioxidant pathway in astrocytes. Intriguingly, ALS-linked mutations in p62 fall within domains that function in selective autophagy or the antioxidant response. I am testing the hypothesis that these mutations may preferentially disrupt neuronal vs. astrocytic function, thus identifying cell-type-specific contributions to ALS.

B562/P1543

Macroautophagy and Aging Synergize to Drive Dysfunctional Metabolism in the Alveolar Epithelium of Non-Resolving Lung Fibrosis

L. R. Rodriguez, J. Katzen, A. Murthy, Y. Tomer, T. Dimopoulos, S. Iyer, K. Chavez, V. Abraham, M. F. Beers; University of Pennsylvania, Philadelphia, PA.

Our understanding of the molecular changes in aging cells highlights the role of cell quality control mechanisms such as macroautophagy in age associated diseases. Using *in-vitro* models we first demonstrated that the expression of mutant surfactant protein C (*SFTPC*^{I73T}) in alveolar epithelial type II cells (AT2) yields a block in late macroautophagy further inducing a time dependent metabolic reprogramming and mitochondrial dysfunction. As proof of concept, our inducible murine model of AT2 *SFTPC*^{I73T} expression results in spontaneous non-resolving lung fibrosis, a condition typically seen in humans after the age of 55. Given these observations, we hypothesize that aging synergizes with defects in cell quality control to drive AT2 initiated fibrosis. To test this, we first induced expression of *SFTPC*^{I73T} in old 18-month-old mice and compared the fibrotic phenotype to young 3-month-old *SFTPC*^{I73T} mice followed by characterizing the observed cellular phenotype in an *in-vitro* reductionist model. The murine model is a tamoxifen responsive hypomorph with an excisable cassette in the *SFTPC* locus. A dox-inducible *in vitro SFTPC*^{I73T} expression system was generated in murine lung epithelial cells (MLE-12) and challenged with low dose bleomycin to induce senescence. Characterization of the metabolism in AT2 cells isolated from 3-month-old, 18-month-old, and in induced *Sftpc*^{I73T} MLE-12s was performed by QPCR, immunoblot, flow cytometry, YSI biochemistry, Seahorse^{TM} respirometry, high-resolution microscopy. After 4-weeks, surviving mice underwent lung physiology measurements and endpoint analysis by bronchoalveolar lavage and whole lung collection. In our aged murine model, we observed increased inflammatory cell infiltration and higher mortality versus young mice. Characterization of old *Sftpc*^{I73T} AT2 cells demonstrated that the acquired macroautophagy defects coincide with metabolic reprogramming favoring glycolysis and enhanced lactate secretion. Additionally, a decrease in mitochondrial biogenesis was associated with inhibited mitochondrial respiration. I*n-vitro* studies in the senescent *SFTPC*^{I73T} cell model supported the observed synergism of aging *in vivo* as these cells also demonstrated increased metabolic reprograming marked with increased lactate secretion, decreased

mitochondrial respiratory capacity, and decreased mitochondrial biogenesis. Collectively, these data further highlight the important role of cell quality control, mitochondrial biology, and cellular aging. Given that the AT2 cell serves as a progenitor cell in the distal lung epithelium, these data begin to elucidate an important connection between an aging lung, metabolic reprogramming, and the impaired resolution of lung injury observed in non-resolving lung fibrosis.

B563/P1544

Metabolic Regulation of Autophagy

E. Delorme-Axford[1], D. J. Klionsky[2]; [1]Biological Sciences, Oakland University, Rochester, MI, [2]Life Sciences Institute, University of Michigan, Ann Arbor, MI.

The goal of this study is to investigate how autophagy modulates cellular metabolism, and in return, how cellular metabolism affects autophagy activity. Macroautophagy/autophagy is a dynamic pathway of cellular degradation and recycling that is highly conserved from yeast to humans. Basal autophagy is low, but is upregulated during stress conditions, such as nutrient deprivation and various other forms of stress. The classical morphological feature of autophagy is the *de novo* formation of the double-membrane vesicle known as the autophagosome. In humans, perturbation of autophagy (i.e., too much or too little) can have deleterious effects on cell health and survival, contributing to disease pathogenesis. Autophagy is of key importance in the maintenance of cell health through its contribution to both metabolic and quality control mechanisms. Despite the significance and clear connections, the relationship between metabolism and autophagy is not well understood. To address the gap in our understanding of the interplay between autophagy and cellular metabolism, we utilized the yeast *Saccharomyces cerevisiae* to identify and characterize a novel metabolic regulator of autophagy through a targeted genetic screen. Using multiple assays, our studies have demonstrated that the yeast metabolic transcription factor Stb5 functions as a negative regulator of autophagy. Atg1 is a key kinase that promotes autophagy, and Stb5 protein expression decreases significantly in an Atg1-dependent manner when cells are starved for nitrogen. Loss of Stb5 results in the upregulation of select autophagy-related (*ATG*) transcripts under nutrient-replete conditions as demonstrated by real-time quantitative PCR (RT-qPCR). However, the primary effect on autophagy occurs through Stb5-mediated transcription of genes involved in the generation of cellular nicotinamide adenine dinucleotide phosphate/NADPH pools. This work provides insight into the role of Stb5 as a transcription factor that regulates both cellular metabolic responses and autophagy activity. Our findings support a model in which autophagy is stimulated when cellular levels of the metabolite NADPH decrease and may serve to provide biomolecular building blocks for cellular metabolism during nutrient-limiting conditions.

B564/P1545

SQST-1/p62 and SKN-1/Nrf2 promote Compartmentalized Cell Elimination under stress

Y. Mohamed, A. Elkhalil, P. Ghose; University of Texas at Arlington, Arlington, TX

Stress responses, such as autophagy, and developmental death programs are critical to cellular quality control. The interplay between autophagy and developmental death programs has been an intriguing question. In particular, how these processes are connected is essentially unknown in morphologically complex cells, which are characterized by distinct compartments, such as the cell body and neurites in neurons. We employ a novel in vivo system to explore this question. In this “tri-partite” killing program, Compartmentalized Cell Elimination, or CCE, three segments of the C. elegans tail-spike cell (TSC) and

the sex-specific CEM neurons die in different ways in the embryo, with the soma and process engulfed by different phagocytes. We examined CCE under heat stress conditions and found CCE takes place normally. From a forward genetic screen we found that mutants for sqst-1, which encodes the C. elegans ortholog of the mammalian SQSTM1/p62, a receptor for selective autophagy, are defective for CCE following stress. Similarly, mutants for uba-1, which encodes the C. elegans ortholog the E1 ubiquitin-ligase enzyme UBA1 that is required for SQSTM1/p62 function, show a similar defect. Interestingly, sqst-1 is not expressed in TSC, but rather in the surrounding phagocytes. Moreover, cell-specific rescue experiments suggest SQST-1 does not function cell autonomously, suggesting a role in phagocytosis. One degradation target of SQSTM1/p62 is KEAP1, a negative regulator of the stress response transcription factor Nrf2. Like sqst-1 mutants, mutants for skn-1, which encodes the C. elegans ortholog of the mammalian Nrf2, also show a similar CCE defect under stress. Both TSC and neighboring phagocytes express skn-1. Interestingly, the p62-Nrf2 axis is linked to preventative therapy for neurodegenerative disease, but a role in stress-induced phagocytosis has not been described. Together, our data suggests autophagy and cellular stress resistance mechanisms bolster a developmental death program of complex cells under stress conditions. We aim to determine whether SKN-1 and SQST-1 act in the same pathway, to determine the transcriptional target of SKN-1, and to genetically assess whether stress-induced CCE is an example of LC3-Associated Phagocytosis (LAP), an autophagy-assisted phagocytic stress response.

B565/P1546

The selective autophagy receptor p62 and the heat shock protein HSP27 facilitate lysophagy via the formation of phase-separated condensates

E. Gallagher, E. Holzbaur; University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA

Lysosomes are membrane-bound organelles that regulate cellular proteostasis. Loss of lysosomal integrity threatens cellular homeostasis and thus initiates the selective autophagy of damaged lysosomes or lysophagy. Lysophagy begins with the ubiquitination of damaged lysosomes. This ubiquitination induces the recruitment of receptors that facilitate the formation of a double-membrane autophagosome that engulfs the damaged lysosome. Proteomic studies have identified several selective autophagy receptors on the surface of damaged endolysosomes, including NDP52, TAX1BP1, and p62/SQSTM1. However, the specific contributions of each of these receptors to lysophagy remains unclear. We used a penta-KO HeLa cell line lacking five well-characterized autophagy receptors, and found that the selective autophagy receptor SQSTM1/p62 is both necessary and sufficient to promote autophagosome formation at damaged lysosomes. We find that p62 is recruited to damaged lysosomes in both HeLa cells and neurons, following lysosomal damage induced either chemically with LLOMe or by a genetically encoded lysosomal photosensitizer. We identify a specific requirement for the Phox and Bem1p (PB1) domain of p62, as loss of the PB1 domain prevents p62 recruitment and inhibits lysophagy. The PB1 domain of p62 facilitates oligomerization and the formation of liquid-like condensates that engulf damaged lysosomes. We demonstrate that the fluidity of these condensates are regulated by the small heat shock protein HSP27. HSP27 is activated by lysosomal damage, and is recruited to damaged lysosomes. Live imaging demonstrates that HSP27 recruitment coincides with autophagosome biogenesis, and that depletion of HSP27 induces p62 aggregation and inhibits lysophagy. Finally, lysosomal dysfunction is particularly injurious to neurons, as evidenced the emerging link between disruption of lysosomal health and neurodegenerative disease. Moreover, mutations in p62 have been associated with Amyotrophic Lateral Sclerosis (ALS). We find that expression of ALS-associated

mutations in p62 impair lysophagy, suggesting that deficits in this pathway may contribute to the cellular pathogenesis of neurodegeneration.

B566/P1547

Distinct Pathogenic Mutations in LRRK2 Disrupt Axonal Transport of Autophagosomes

D. Dou[1], C. Boecker[2], J. Goldsmith[1], E. L. F. Holzbaur[1]; [1]Physiology, University of Pennsylvania, Philadelphia, PA, [2]Neurology, University of Gottingen, Gottingen, GERMANY.

Dysfunctional autophagy has been repeatedly implicated in Parkinson's disease (PD) pathogenesis. Leucine-rich repeat kinase 2 (*LRRK2)* mutations are the most common genetic cause of PD and result in hyperactive phosphorylation of the Rab family of GTPases. We hypothesized that LRRK2 hyperactivity contributes to autophagic disruption by causing deficits in autophagosome transport in neuronal axons. To investigate, we live-imaged the autophagosome marker LC3B in axons of human iPSC-derived neurons with knock-in (KI) of the most common LRRK2 mutation, p.G2019S. In wild-type neurons, we observed smooth retrograde transport of autophagosomes, characteristic of physiologic axonal autophagy. In contrast, imaging p.G2019S KI neurons revealed striking increases in autophagosome pausing. Another PD-linked mutation, *LRRK2*-p.R1441H, has been reported to induce even greater magnitude of kinase hyperactivity than p.G2019S. In p.R1441H KI neurons, we observed more severe disruption of autophagosome transport, manifesting as increased pause duration and likelihood of stationary autophagosomes. Thus, magnitude of autophagosome transport deficits may scale with LRRK2 activity. In p.R1441H KI neurons, we also found that overexpression of the small GTPase Arf6, a motor protein regulator, partially rescued the deficits in axonal transport of autophagosomes. Together, our results lead us to propose a model where imbalance between Arf6 and LRRK2-phosphorylated Rabs induces aberrant recruitment of the scaffold protein JIP4 to autophagosomes. This results in an unproductive tug-of-war between molecular motors, disrupting the autophagosomal transport that is tightly linked to neuronal homeostasis. Importantly, autophagosome transport deficits were reversed by LRRK2 kinase inhibitor treatment, further reinforcing LRRK2's status as a promising therapeutic target.

B567/P1548

WIPI2 orchestrates omegasome formation at the plasma membrane by recruiting E-Syt2

D. Campisi, S. Blanchard, T. Wollert; Institut Pasteur, Paris, France

Autophagy is a cellular recycling process that degrades damaged or superfluous cytoplasmic components. The formation of autophagosomes occur at specialized domains of the endoplasmic reticulum (ER), termed omegasomes, from which cup-shaped phagophores emerge. How omegasomes are generated and how they relate to phagophores remain unknown. We found that targeting the phosphatidylinositol (3) phosphate (PI(3)P) binding protein WIPI2 to the plasma membrane (PM) induces the formation of omegasome-like membranes from which phagophores emerge. The process is independent of canonical autophagy inducers including the ULK1 kinase or PI(3)-kinase complexes, suggesting that WIPI2 is a key factor for the induction of omegasomes. The spatiotemporal uncoupling of omegasome formation and canonical autophagy allows us to identify membrane trafficking pathways that contribute lipids for the formation of omegasomes and important molecular regulators of the pathway. We combined confocal imaging and correlative light electron microscopy (CLEM) and found that overexpression of a PM-targeted WIPI2 protein induced the recruitment of extended

synaptotagmin 2 (E-Syt2). E-Syt2 recruitment at the PM formed an expanded and thin cortical ER. I will present our most recent data which suggest that E-Syt2 is a key regulator of omegasome formation.

B568/P1549

Clearance of mutant huntingtin inclusion bodies by selective autophagy

A. Folger, K. Frey, Y. Wang; Florida State University, Tallahassee, FL

Abnormal protein aggregation causes neurodegenerative diseases, such as Huntington's disease. This disease is caused by mutations that increase the number of glutamine-encoding trinucleotide repeats in the first exon of the huntingtin (Htt) gene. Mutated Htt exon 1 with polyglutamine expansion is prone to pathological aggregation and forms inclusion bodies. Normally, aggregates of misfolded proteins are cleared by the ubiquitin-proteasome system or autophagy, which alleviates their cytotoxicity. Previous work has elucidated the role of autophagy in the clearance of misfolded proteins, but autophagic clearance of inclusion bodies remains poorly characterized. Here we use mutated Htt with 103 polyglutamine (Htt103QP) as a model substrate to study the autophagic clearance of inclusion bodies in budding yeast. We found that all core autophagy proteins are required for Htt103QP inclusion body autophagy. We further found that the proteins involved in selective autophagy were required for autophagic clearance of Htt103QP inclusion bodies, indicating that this autophagy is selective. Unexpectedly, a known autophagy receptor for mutated Htt, Cue5, was dispensable for inclusion body autophagy, indicating that Cue5 likely promotes the autophagic clearance of mutant Htt aggregates but not inclusion bodies. However, three known selective autophagy receptors were shown to be essential for this inclusion body autophagy, thereby revealing a novel autophagic pathway specific for inclusion bodies. We named this type of autophagy as IBophagy.

B569/P1550

OPA-1 deficiency promotes muscle atrophy through upregulating Phago-MERCs

D. Stephens; Fisk University and Vanderbilt University, Nashville, TN

Autophagy classically operates as a physiological process to degrade cell membrane components, protein aggregates, and damaged organelle, as a mechanism for nutrient breakdown, and as a regulator of cellular architecture. Proper autophagic flux is paramount for both functional skeletal muscle, which maintains support and movement of the skeleton, and metabolism. The function of autophagy as a metabolic controller in muscle has been previously studied; nevertheless, the underlying molecular mechanisms that regulate autophagy in skeletal muscle have merely started to materialize. Notably, mitochondrial dynamics have been linked to autophagy mechanisms. For example, Fibroblasts bearing dominant-negative, mitochondrial dynamics fusion protein, Optic Atrophy Protein 1 mutations showed **increased autophagy and mitophagy in response to uncoupled oxidative phosphorylation**. Remarkably other mitochondria associated structures, such as mitochondrial endoplasmic reticulum contact sites (MERCs) can also regulate autophagy. However, it is not well understood if OPA1 regulates autophagy through mitochondrial endoplasmic reticulum contact sites (MERCs) or an independent pathway. MERCs are enriched with specific proteins and lipids that aid in specialized structural rearrangements such as the formation of autophagosomes. We hypothesized that loss of OPA1 in skeletal muscle increases Phago-MERCS and serves as the platform for autophagosome formation. To investigate the role of OPA-1 on Phago-MERCs, studies were performed in OPA-1 deficient mice, fly, and OPA-1 ablated in murine myotubes. The ablation of OPA-1 in skeletal muscle showed a decrease in soleus, gastrocnemius,

quadriceps, and tibialis anterior muscle weight. Loss of OPA-1 in gastrocnemius muscle showed an increase in the following MERC proteins that are important for Phago-MERC tethering which include: MFN-2 and PACS2. Loss of OPA-1 also increased the Phago-MERC recruiting protein, ATG5. qPCR analysis after the deletion of OPA-1 in 40-week skeletal muscle displayed an increase in MFN-2, LC3B, ATG7, and ATG5 transcripts, while p62 transcripts were shown to decrease. Primary myoblast and myotubes were generated by isolating satellite cells from OPA-1 floxed mice, differentiated, and subsequently the deletion of OPA1 by infecting the cells with adenoviral Cre recombinase. Ablation of OPA-1 in myotubes increased the following MERC proteins: MFN-2, and PACS2. TEM Analysis of OPA-1 deficient skeletal muscle and myotubes had an increase in Phago-MERCs and lysosome structures. Loss of OPA-1 Like in drosophila muscles increases Phago-MERCs. This data suggests that loss of OPA-1 increased Phago-MERCs, which may lead to decreased muscle mass, but a mechanism of action still needs further exploration.

B570/P1551

Defining the mechanisms of dysfunctional ferritinophagy in β-propeller protein-associated neurodegeneration

C. Chen[1], J. P. Chua[2,3], S. C. Stumpf[2,3]; [1]Krieger School of Arts & Sciences, Johns Hopkins University, Baltimore, MD, [2]Department of Neurology, Johns Hopkins Medicine, Baltimore, MD, [3]Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD.

Beta-propeller protein-associated neurodegeneration (BPAN) is a congenital neurodegenerative disorder and subtype of neurodegeneration with brain iron accumulation (NBIA), manifesting with childhood intellectual disability followed by progressive parkinsonism, dystonia, and dementia in adolescence or early adulthood. BPAN is X-linked and caused by mutations in *WDR45*/WIPI4, a critical effector of vesicle maturation in the highly conserved degradation pathway of autophagy. Although prior studies have found that mutations in *WDR45*/WIPI4 lead to impairments in autophagic flux and abnormal accumulation of degradative cargo, the molecular mechanisms underlying brain iron accumulation and neuron-selective degeneration in BPAN remain undefined. In the present study, we evaluate cell type-specific derangements in ferritinophagy, a selective form of autophagy that regulates intracellular iron content and turnover. To do this, we use a novel cell model of BPAN comprised of human induced pluripotent stem cells edited by CRISPR/Cas9 to knock-in a BPAN-related mutation (c.C52>T, p.Gln18X), from which we generate isogenic neurons, astrocytes, and skeletal muscle cells. In comparing mutant against wild-type cells and neurons against non-neurons, we are assessing immunocytochemical, Western, and qPCR analyses of ferritinophagy machinery: ferritin, an essential intracellular carrier of iron, and NCOA4, a selective autophagy receptor for degrading ferritin. We expect that ferritinophagy proteins fail to undergo normal autophagic degradation and are mislocalized in BPAN cells, and that these derangements are most severe in neurons. By clarifying how and to what extent ferritinophagy is dysfunctional in BPAN and on a cell type-specific basis, we will further our understanding of BPAN pathogenesis and the neurotoxic effects of dysfunctional autophagy. In so doing, our studies will provide the basis for investigating novel and neuron-specific mechanisms of rescuing dysferritinophagy and inform treatment strategies for BPAN and other neurodegenerative autophagiopathies.

B571/P1552

FKBP5, a depression-associated gene, mediates autophagy initiation

H. Park[1], S.-Y. Jung[2], J. Chang[2], S. Lee[1]; [1]Inha Universiry, Incheon, Korea, Republic of, [2]Ajou University school of medicine, Suwon, Korea, Republic of

Major depressive disorder (MDD), simply called as depression, is a common but serious psychiatric disorders caused by a combination of genetic and environmental factors. There are many antidepressants that relieve symptoms of depression, but they are ineffective for more than half of patients. Therefore, genetic factors may contribute to individual variation in antidepressant response. However, the molecular mechanisms for the pathophysiology of MDD are not yet fully understood. FK506 binding protein 51 (FKBP51, also known as FKBP5) belongs to a family of immunophilins which regulates stress response as a co-chaperone of HSP90. The scaffolding activity of FKBP5 enables it to play a modulatory role in the immune response, tumorigenesis, cell death, and stress hormone axis. Moreover, single nucleotide polymorphisms (SNPs) in FKBP5, which increase its protein levels, are significantly associated with increased recurrence of depressive episode and response to antidepressants. However, the molecular mechanism for how FKBP5 regulates depressive response remains unclear. It has been recently reported that FKBP5 not only modulates antidepressant response but also interacts with Beclin1, Akt, and PHLPP, which are critical for autophagy, an intracellular degradation system required to maintain cellular homeostasis. To investigate the relationship between FKBP5 and autophagy, we generated FKBP5 knock-out cell lines using a CRISPR/Cas9 system and analyzed them. We found that autophagy initiation step was impaired by a loss of FKBP5. The detailed mechanisms will be shown in the presentation. We hope these findings help us to understand the molecular mechanism of how autophagy is associated with depression.

B572/P1553

IGFBP-3 inhibits mTOR to promote mitochondrial hyperfusion and block mitophagy in cells under hyperosmolar stress

W. L. Stuard, I. J. Trautman, D. M. Robertson; UT Southwestern, Dallas, TX

Purpose: Mucosal epithelial surfaces protect tissues from the external environment and are subject to hyperosmolar stress in many different disease states. We have previously found that the Insulin-like growth factor binding protein-3 (IGFBP-3) protects against hyperosmolar stress-induced mitochondrial damage. The objective of this study is to determine the mechanism whereby IGFBP-3 mediates autophagy and mitophagy in mucosal epithelial cells subject to hyperosmolar stress. **Methods**: Telomerized human corneal epithelial (hTCEpi) cells, human conjunctival epithelial cells (HCjECs), and human bronchial epithelial cells (HBECs) were cultured in serum-free keratinocyte basal media (KBM) at 330 mOsm (control), 450 mOsm KBM, or 450 mOsm KBM with recombinant human (rh)IGFBP-3. Cells were treated with siRNA oligonucleotides targeting TSC1 or rapamycin to constitutively activate or inhibit mTOR. Mitochondrial bioenergetics and mitophagy were analyzed using a seahorse metabolic flux assay, TEM, western blotting, immunofluorescence, and live cell imaging. To test the effects of hyperosmolar stress on the ocular surface *in vivo*, an aqueous deficient mouse model of dry eye disease (DED) was developed by injecting botulinum toxin into the exorbital lacrimal gland. Mice were then treated topically with rhIGFBP-3 or vehicle control eye drops. **Results:** Hyperosmolar stress increased autophagic flux. This was associated with a decrease in p-mTOR. rhIGFBP-3 blocked the increase in autophagic flux at 6 and 24 hours and promoted p-mTOR. At 6 hours, rhIGBFP-3 inhibited autophagic

flux through PINK1 and BNIP3L/NIX. At 24 hours, rhIGFBP-3 selectively blocked BNIP3L/NIX. This was associated with an increase in mitochondrial fusion. There was differential expression of PINK1 and BNIP3L/NIX in the mouse corneal epithelium. Treatment with rhIGFBP-3 blocked mitophagy in our DED mouse model. **Conclusion**: IGFBP-3 blocks the hyperosmolar stress-induced increase in autophagic flux and mitophagy through the activation of mTOR. This occurs through differential regulation of PINK1 and BNIP3L/NIX mitophagy. Further studies are needed to investigate the differential expression PINK1 and BNIP3L/NIX in the corneal epithelium.

B573/P1554

Rab9 regulation of autophagic flux is mediated by changes in lysosomal positioning

E. G. Krebs[,1], C. Gomatan, A. Peddireddi, M. Ignacio, S. Shaw, A. M. Strohecker[,1]; The Ohio State University, Columbus, OH

Autophagy is a conserved cellular process that degrades damaged cytoplasmic components and supports metabolism. In the final stages of autophagy, the autophagosome fuses with the lysosome forming a hybrid structure called the autolysosome wherein contents are degraded and returned to the cytoplasm. This step requires autophagosomes and lysosomes to be in proximity. Understanding the molecular mechanisms governing lysosome trafficking in the cell will provide important insights into the regulation of autophagy. A previously executed screen demonstrated that silencing the small GTPase *Rab9* robustly increased autophagic flux, suggesting that RAB9 can negatively regulate the pathway. Similarly, silencing *RAB9* in human NSCLC cell lines via lentiviral shRNAs induces autophagic flux. To directly test the ability of RAB9 to regulate autophagy, we introduced HA tagged vector, -RAB9$^{Wild\ type}$ or its hydrolysis resistant (constitutively active) mutant -RAB9^{Q66L} into HEK293 cells and assessed autophagic flux by western blot. We found that expression of the RAB9^{Q66L} mutant is capable of impairing autophagy induced by multiple stimuli, while expression of RAB9$^{Wild\ type}$ facilitates flux. This suggests that the function of RAB9 is regulated by GTP loading status or ability to cycle. RAB9 has previously been implicated in lysosome positioning in HeLa cells. Thus, we asked whether changes in RAB9-mediated lysosome positioning were responsible for the observed autophagic outcome in our 293 and NSCLC cell lines. Lysosome positioning was profiled in *RAB9* intact or silenced A549 cells under nutrient replete or starvation conditions, using LAMP1 immunofluorescence to visualize lysosomes. We found that silencing *RAB9* in this system results in perinuclear clustering of lysosomes and that this occurred in a dose-dependent manner. We hypothesize that the increased perinuclear localization is responsible for the increased autophagic flux observed in the *RAB9* silenced cells. Ongoing work will test this hypothesis by (i) expressing the RAB9^{Q66L} or RAB9^{S21N}(dominant negative, GDP-bound) mutants in the *RAB9* silenced cells and monitoring lysosome position in the resulting cells and by (ii) identifying the effector interactions that mediate this effect. This work identifies a GTPase-dependent mechanism of regulating autophagic flux via controlling lysosome positioning within the cell.

B574/P1555

High glucose and high fat differentially regulate autophagic flux in H9C2 cardiac myoblasts

H. Chen, **Y. Hahn**, B. Silverstein, T. Kobayashi, Y. Huang, S. Kobayashi, Q. Liang; NYIT College of Osteopathic Medicine, Old Westbury, NY

Hyperglycemia and hyperlipidemia are two metabolic disorders that contribute to cardiac damage in diabetic patients. However, the mechanisms underlying cellular responses to these stressors are not

fully defined. Autophagy is a cellular protein degradation pathway that plays a key role in cellular response to various stressful conditions, including diabetes. We previously showed that hyperglycemia inhibited autophagic flux in cultured cardiomyocytes and in the hearts of type 1 diabetic mice, which was a protective response that limited hyperglycemic injury. In the present study, we compared the effects of high glucose and high fatty acid on autophagic flux in H9C2 cardiac myoblasts. H9C2 cardiomyocytes were cultured in DMEM containing either high glucose (30 mM) or high fat (25uM Palmitate-BSA) for 72 or 16 hours, respectively. A cocktail of lysosomal protease inhibitors pepstatin A (25ng/mL) and E64d (5ng/mL) were used to measure autophagic flux. For western blotting, whole-cell lysates were probed for expression of LC3-II protein, a well-established marker for autophagic vacuoles. Autophagic flux was determined by comparing the expression levels of LC3-II with and without protease inhibitors. In the immunofluorescence assays, cells were infected with an adenovirus that encodes an autophagy reporter known as tandem fluorescently tagged mRFP-GFP-LC3. Autophagic flux was determined by comparing the area occupied by mature autolysosomes (red puncta or dots) with and without the inhibitors. The western blot analysis showed that high glucose inhibited, while high fat accelerated autophagic flux, as indicated by the difference in LC3-II levels in the presence and the absence of the lysosomal inhibitors. These results were corroborated in reporter studies as shown by the area or number of puncta. In addition, 3-Methyladenine (3-MA), an inhibitor of autophagosome formation, decreased high fat-induced cell death as shown by propidium iodide staining, suggesting that high fat-induced autophagy was detrimental. Together, our study demonstrated opposite effects of high glucose and high fat on autophagy flux, highlighting the complexity of cellular responses to diabetes-like stressors in H9C2 cells. Future studies will investigate the signaling mechanisms that mediate these differential responses in cardiomyocytes and in the heart.

B575/P1556

Interplay between secretory autophagy and macroautophagy in neurons

S. D. Palumbos, J. Goldsmith, E. L. F. Holzbaur; University of Pennsylvania, Philadelphia, PA

Unlike most cell types, neurons are largely resistant to autophagy induction following starvation or stress. Instead, autophagy primarily functions as a homeostatic mechanism, constitutively degrading damaged proteins and organelles. This reduced capacity for autophagy induction could leave neurons particularly vulnerable to stress, especially considering neurons are post-mitotic, precluding them from clearing damaged proteins and organelles through cell division. Do neurons mobilize additional pathways to discard of damaged material when their capacity for autophagy is overwhelmed? Recent findings suggest that autophagy-dependent degradation and autophagy-dependent secretion can act in coordination to regulate cellular homeostasis in non-neuronal cell types. When autophagosome maturation is impeded, autophagy-dependent secretion of extracellular vesicles (EVs) can be initiated as a mechanism to unburden the degrative machinery. Whether neurons similarly extrude damaged material via autophagy dependent secretion is unclear. We propose that stressed neurons engage autophagy-dependent secretion as an alternate quality control mechanism to dispel cellular waste. This is supported by several preliminary observations. First, we observe that LRRK2 mutant neurons, which exhibit strained degradative autophagy, shunt cargo toward a secretory fate. Additionally, we observe that treating cultured neurons with Bafilomycin A1, effectively blocking lysosomal fusion, prompts the upregulation of secretion. Together, our data suggest that chronically or acutely straining macroautophagy leads neurons to upregulate secretory autophagy. These observations have important implications in neurodegenerative diseases where autophagy is strained, and the expulsion of damaged

proteins or dysfunctional organelles could heighten systemic inflammatory responses. Future directions will further describe interplay between cell-autonomous autophagy and secretory autophagy.

B576/P1557

Atlastin regulates Tor signaling and autophagy in *Drosophila* muscle through alteration of the lysosomal network

S. Srivastav, K. van der Graaf, P. Singh, A. Budi Utama, J. A. McNew, M. Stern; Rice University, Houston, TX

Aim: The hereditary spastic paraplegias (HSPs) represent a family of genetic disorders comprising at least 72 different genes with the common pathology of progressive locomotor deficits and spasticity thought to result from degeneration of upper motor neurons. *atlastin* (*atl, SPG3A*) encodes an ER fusion protein that controls ER morphology, which implicates ER morphology as a causal factor in HSP, but it is not clear how altered ER morphology might result in neurodegeneration.**Method:** Here we use *Drosophila melanogaster* to study the effects of decreased *atl* activity on properties of the larval body wall muscle. Given that one of the primary phenotypes observed in muscle lacking *atl* involves accumulation of aggregates containing poly-ubiquitin (poly-UB), we hypothesize that autophagy flux could be affected. Therefore, different approaches were used to study autophagic flux in muscle lacking *atl*.**Results:** We find that muscle *atl* loss causes accumulation of poly-UB aggregates, most bound to autophagy receptor p62/SQSTM1 (ref(2)p). Muscle *atl* loss also decreases volume and complexity of the lysosomal network as visualized with RFP linked to the lysosomal gene *spinster (spin)* and decreases the number of punctae labelled with LAMP1. To determine effects of these lysosomal deficits on progression through the autophagy pathway, we expressed *ATG8a* tagged with both *GFP* and *mCherry* in a wildtype and *atl* mutant background. We observed numerous punctae with mCherry but not GFP fluorescence in wildtype animals indicating that ATG8 was found mostly in autolysosomes. In contrast, muscles lacking *atl* exhibited a significant number of punctae marked with both mCherry and GFP. This compartment was also labelled with the late endosomal marker Rab7 but not lysosomal marker LAMP1, suggesting that these punctae were most likely amphisomes. We also found that loss of *atl* leads to inability to activate the Tor kinase, perhaps due to the altered lysosomal network.**Conclusion:** We suggest that the accumulation of amphisomes containing cargo in larvae lacking *atl* indicates an inability of autophagosomes to merge with lysosomes, causing autophagosome fusion to be diverted to endosomes. Our results provide new mechanistic insights into the role of Atl, and hence ER morphology, in maintaining cell viability and raise the possibility that certain pathologies in patients with mutations in *SPG3A* might result from altered Tor signaling.

B577/P1558

ATG2A Extracts Lipids from ER Exit Sites to Promote Autophagosome Biogenesis

D. Fuller, T. Olivas, Y. Wu, K. Melnyk, P. De Camilli, T. Melia; Yale University, New Haven, CT

Macroautophagy involves the mobilization of millions of lipids in less than ten minutes and requires the establishment of a membrane contact site between a contested donor membrane and the nascent autophagosome. Recent work demonstrated that the essential autophagy protein ATG2A is a bridge-like lipid transport protein that tethers the donor membrane to the early autophagosome to facilitate a rapid transfer of lipids. As ATG2A must interact with the donor membrane to extract lipids, we performed proximity labeling and mass spectrometry to identify proteins on this organelle. Intriguingly,

we found an abundance of ER Exit Site (ERES) proteins which have previously been implicated in autophagosome biogenesis via COPII vesicle fusion. In this body of work, we propose a new model of ERES - autophagosome interaction in which ATG2A competitively interacts with the early secretory pathway by extracting lipids from the ER exit site. We first confirmed colocalization of ATG2A and ERES proteins by live cell imaging and found by co-immunoprecipitation that the N-terminus of ATG2A interacts with ERES proteins. To perturb ATG2A recruitment to the donor membrane, we overexpressed Sar1bH79G, which distorts the ERES membrane. We found that under starvation conditions, ATG2A was occasionally recruited to tubular structures. By CLEM-FIBSEM, we found that these structures corresponded to thin, compact membranes that resembled distorted phagophores. We further found that ATG2A localized on the rim of cup-like structures and along the length of the tubular structures, suggesting a strong curvature preference. Sites of ATG2A localization also contained a high density of membrane contacts with adjacent tubular ER, which could potentially cause this phenotype. To drive autophagosome biogenesis and thereby the association between ATG2A and the donor membrane, we transiently rescued ATG2 DKO cells which contain large compartments filled with pre-autophagosome materials with ATG2A. Under these conditions, ATG2A and ERES proteins strongly colocalize at or near LC3B positive structures. These results, taken together, strongly affirm that ERES function as the donor membrane for autophagosome biogenesis.

B578/P1559

A putative disease-causing mutation in autophagy protein ATG3

E. Guinn[1], L. Wang[1], S. Yang[1,2], W. Ji[3], A. Cuentas-Condori[1,2], L. Jeffries[3], V. Knight[4], B. Asomanning[1], M. Khokha[3,5], D. Colón-Ramos[1,2], S. Lakhani[3], T. Melia[1]; [1]Department of Cell Biology, Yale University, New Haven, CT, [2]Department of Neuroscience, Yale University, New Haven, CT, [3]Department of Pediatrics, Yale University, New Haven, CT, [4]Department of Pediatric Neurology, Yale University, New Haven, CT, [5]Department of Genetics, Yale University, New Haven, CT.

Macroautophagy is a degradative cellular process that is upregulated in response to stress. Dysregulation of autophagy and associated pathways are broadly associated with several diseases including cancer, aging, and neurodegeneration. However, since this process is essential for mammalian development, diseases affecting core autophagy genes are very rare. For example, ATG3 is a core autophagy gene driving autophagosome biogenesis; there are no known disease-causing mutations within this gene. However, a patient with an undiagnosed severe neurodevelopmental disease was found to have a point mutation in a single allele of the ATG3 gene. To explore the potential disease-causing impacts of this patient mutation we tested ATG3 function in patient cells, in cell culture models, *in vitro*, and in an animal model. Fibroblasts isolated from the patient display a dysfunctional autophagy response to amino acid starvation. This mutated ATG3 cannot support the conjugation of Atg8 proteins to lipid, the formation of Atg8 puncta nor the lysosome-dependent turnover of Atg8, all suggesting that this mutant is inactive in autophagy. However, in vitro biochemistry reveals the mutant retains the ability to capture Atg8 via a thioester bond, suggesting it is only the final deposition on lipid that is inhibited. ATG3 mediates other conjugations largely independent of autophagy, including the formation of a self-conjugated ATG12-ATG3 complex and the Atg8-ylation of proteins. The patient mutation also fails support the redistribution of endosomes normally driven by ATG12-ATG3, suggesting this pathway is also inhibited. Finally, *C. elegans* with a mutation at this conserved position display significant defects in viability, development, and locomotory behavior that are consistent with autophagy gene knockouts. Thus, this appears to be the first example of an ATG3-dependent disease.

Protein Aggregation, Protein Folding and Phase Separation

B579/P1560

Linking proteostasis and the 12-hour rhythms of nuclear speckle liquid-liquid phase separation dynamics

W. Dion[1], H. Ballance[1], J. Lee[2], Y. Pan[3], S. Irfan[1], C. Edwards[1], M. Sun[1], J. Zhang[1], X. Zhang[4], S. Liu[1], B. Zhu[1]; [1]University of Pittsburgh School of Medicine, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, PA, [3]UPMC Genome Center, Pittsburgh, PA, [4]The Pennsylvania State University, State College, PA.

Organismal health requires a consistent and balanced internal environment known as homeostasis; different physiological processes maintain proper levels of biomolecules at a cellular level. Proteostasis, sustained levels of correctly folded proteins in the endoplasmic reticulum (ER), is maintained by the Unfolded Protein Response (UPR). Excessive misfolded proteins in the ER activate the three branches of the UPR, facilitating adaptive processes to restore a balanced proteome in the cell. XBP1s-dependent ultradian rhythms of ER stress and subsequent UPR-mediated stability are apparent in mammals and produce a 12-hour clock that functions independently of the 24-hour clock or the cell cycle. This 12-hour clock links the liquid-liquid phase separation (LLPS) dynamics of the nuclear speckle (NS) to the UPR through SON, the NS core protein. High SON levels create a diffuse and fluid NS, boost the expression of UPR-associated genes, and improve the cell's ability to clear aggregated proteins. In contrast, low SON levels result in a spherical and stagnant NS, blunt the expression of UPR genes, and weaken the cell's ability to clear insoluble protein aggregates. Furthermore, murine hepatic *Son* levels decrease with age, suggesting that NS LLPS dynamics may change over the course of mammalian lifespan. This link between NS LLPS dynamics and proteostasis identifies a potential target to combat diseases with dysregulated proteostasis.

B580/P1561

Investigating TDP-43 Toxicity via Human Suppressor Genes in *Saccharomyces cerevisiae*

C. Alspaugh, E. Hayden, S. Ju, S. Chen, B. Stammen, Q. Zhong; Biological Sciences, Wright State University, Fairborn, OH.

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a neurodegenerative disease that is either inherited (fALS) or occurs sporadically (sALS). A pathogenic hallmark of ALS, similar to other neurodegenerative diseases such as Parkinson's and Alzheimer's, is the mislocalization and aggregation of certain proteins. Over the years, many proteins have been implicated in the pathology of ALS, but one group of proteins has been of recent interest: RNA-binding proteins. One RNA-binding protein of interest is TDP-43, which is found to be toxic, mislocalized, and aggregated in the neurons of patients with ALS. These toxic phenotypes have been recapitulated in the well-conserved budding yeast model. To study the pathology of TDP-43 toxicity, we have conducted genetic screens to uncover human genes that are able to reduce TDP-43 toxicity in yeast. We identified fifty human genes capable of rescuing toxicity and performed growth assays, microscopy, immunoblotting, and protein-protein interaction studies to determine the mechanism of rescue. While these human genes reduced cell death in yeast, there was no alteration in TDP-43 aggregation patterns nor TDP-43 protein levels in the presence of these genes; thus our human gene suppressors must be rescuing toxicity independent of TDP-43 aggregation. By investigating the normal functions of our human suppressor genes, we can begin to

elucidate what other pathways might be at play in TDP-43 toxicity, giving a better scope of TDP-43's role in ALS pathology.

B581/P1562

Composition can buffer protein dynamics within liquid-like condensates.

S. Jelenic[1], J. Bindics[1], P. Czermak[1], B. R. Pillai[1], M. Ruer[2], C. Hoege[2], A. S. Holehouse[3], S. Saha[1]; [1]Institute of Molecular Biotechnology (IMBA), Vienna, AUSTRIA, [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, GERMANY, [3]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO.

Compartmentalization of cellular biochemistry in membrane-less condensates has emerged as a key regulatory principle of many physiological processes. Many of these condensates have a complex composition and form via liquid-liquid phase separation. Rates of biochemical reactions within these condensates depend on diffusion rate of its components. It has been unclear if condensates can deliver robust functional output despite changing conditions e.g. short-term effects of chemical modifications, intrinsic mutational changes, accumulation of high local concentration of sticky molecules.
Here, we address the mechanism that regulates diffusion rate of a scaffold protein (PGL-3) within the liquid-like P granule condensate in adult gonads of *C. elegans*. Scaffold proteins contribute to assembly of most liquid-like condensates in cells and account for a large fraction of the mass within these condensates. The protein PGL-3 is 693 residues long. We found that a folded alpha-helical domain accounting for 65% of the PGL-3 protein drives liquid-liquid phase separation *in vitro*. Using mutational analysis and biophysical perturbations, we generated PGL-3 constructs with reduced alpha-helicity that phase separate into condensates *in vitro* with significantly slower diffusion rates compared to wild-type PGL-3. In contrast, within the P granule condensate (a complex mixture of RNA and >50 proteins) *in vivo*, these PGL-3 constructs diffuse at rates similar to wild-type PGL-3. This suggests that, in liquid-like condensates, composition can buffer against large change in diffusion rates of proteins (hereafter called 'dynamics-buffering').
We investigated the molecular mechanism of dynamics-buffering using *in vitro* reconstitution. It is generally thought that chaperone proteins and/or ATP-dependent RNA helicases are essential to counteract the slow-down of diffusion rate in biomolecular condensates that might result from denaturation of components or inter-polypeptide/RNA chain entanglements. In contrast, we found that weak interactions among two or more components within P granule-like condensates can account for dynamics-buffering. As expected of a buffer, we found that diffusion rate of PGL-3 constructs within condensates remain relatively unchanged regardless of changes in intra-condensate concentration. Once the buffering capacity is exhausted i.e. beyond a threshold intra-condensate concentration, diffusion rate of PGL-3 constructs slow down at higher concentrations.
We speculate that dynamics-buffering of proteins may contribute to robust functional output of cellular liquid-like compartments.

B582/P1563

Oligodendrocyte function in health and disease is regulated by liquid condensate properties of Tubulin Polymerization Promoting Protein

S. Kemal[1], H. Richardson[1], T. McAlear[2], A. Kopach[1], J. Nowacki[1], S. Bechstedt[2], M. Fu[1]; [1]NINDS, NIH, Bethesda, MD, [2]McGill University, Montreal, QC, CANADA.

Axon myelination is carried out by oligodendrocytes, which undergo intense cytoskeletal transformation to carry out this function. We previously showed that Tubulin Polymerization Promoting Protein (TPPP) is a microtubule nucleator that localizes to Golgi outposts in oligodendrocytes. TPPP regulates microtubule polarity and branching, and *Tppp* KO mice have shorter myelin sheaths and are hypomyelinated. TPPP aggregates have been documented alongside α-synuclein inclusions in oligodendrocytes in the neurodegenerative disorder Multiple System Atrophy (MSA). There are currently no therapies for MSA, which leads to death within an average of 9 years from symptom onset. There is a critical need to understand MSA disease etiology to identify viable targets for intervention. We are now investigating the link between TPPP and MSA at the protein, cellular, and organismal level. At the protein level, we made the discovery that recombinant TPPP forms liquid condensates that are functional and robustly nucleate microtubules. TPPP shares many properties common to proteins that undergo liquid-liquid phase separation. First, TPPP contains a prominent N-terminal intrinsically disordered region. Second, TPPP droplets undergo fission and fusion. Third, recombinant TPPP droplets are dynamic in FRAP experiments. At the cellular level, GFP-TPPP expressed in cultured primary rodent oligodendrocytes also exhibit liquid condensate properties by forming droplet-like structures and exhibiting fast FRAP recovery. Surprisingly, overexpression results in aberrant aggregation along the microtubule lattice of GFP-TPPP, which does not recover by FRAP. At the organismal level, MSA patient brains contain widespread perinuclear cytoplasmic TPPP inclusions in oligodendrocytes. We identify a discrete population of TPPP inclusions that are fibril-like, far from the cell body, and colocalize with myelin markers. Contrary to prevailing hypotheses, our data suggest that aggregation initiates at distal sites where TPPP is normally localized to perform microtubule nucleation and subsequently spreads to the perinuclear cytoplasm. Also, we observed many perinuclear TPPP inclusions that lack α-synuclein, suggesting that TPPP aggregation may precede α-synuclein aggregation. Finally, to confirm causality (i.e., TPPP aggregation can cause disease), we performed stereotaxic injection of recombinant TPPP fibrils into wildtype and *Tppp* KO mouse brains. Together, our results showcase the duality of TPPP. Its normal liquid condensate properties allow it to exert precise control over oligodendrocyte microtubule architecture, thus enabling myelin sheath formation. However, these properties increase its propensity to aggregate and contribute to disease pathology.

B583/P1564

RNP condensates decrowd the cytoplasm in stress conditions

Y. Xie, D. Gresham, L. Holt; New York University, New York, NY

The intracellular environment is packed with macromolecules, and this crowded environment has profound impacts on cellular biochemistry. The cytoplasm can be further organized into condensates including ribonucleoprotein (RNP) granules, like P-bodies and stress granules. These RNP granules are crucial in both normal physiology and diseases, such as cancer and neurodegeneration, yet the precise function of these condensates remains elusive. We analyzed the motion of self-assembling fluorescent nanoparticles of 40 nm diameter (40nm-GEMs) as microrheology probes to infer macromolecular

crowding levels. Previous studies in yeast suggested that, upon acute glucose starvation, increased macromolecular crowding and reduced cytoplasmic pH lead to a protective solid-like state of the cytoplasm at the ~100 nm length-scale. Surprisingly, our probes revealed a transient reduction of macromolecular crowding at the 40 nm length-scale in response to acute environmental stresses. Using a combination of mutants, chemical perturbations, and inducible synthetic RNP granules, we found that the collapse of polysomes, and the partitioning of mRNAs into RNP-granules is required for this cytoplasmic decrowding. These findings suggest a new role of RNP granules in modulating the biophysical property of the cytoplasm.

B584/P1565

Unexpected, but conserved control of protein self-assembly by translation initiation, in eukaryotes

S. Venkatesan[1], J. J. Lange[1], B. Lerbakken[1], D. Tsuchiya[1], P. Berry[1], S. Swanson[1], Y. Hao[1], L. Florens[1], T. S. Kandola[1], M. Cook[1], B. Slaughter[1], J. Unruh[1], R. Halfmann[1,2]; [1]Stowers Institute for Medical Research, Kansas City, MO, [2]University of Kansas Medical Center, Kansas City, KS

Living proteomes are necessarily far from equilibrium. It is paradoxical, then, that reducing protein influx -- which should promote equilibration -- instead, prolongs life across numerous model systems. Protein aggregation to thermodynamically favored but kinetically limited self-assemblies like amyloids often depletes normal protein function and proteostasis, in turn. We therefore reasoned that kinetic barriers to energetic equilibration of native states to rare but probabilistic amyloid-like states decrease with protein influx.

We used Distributed Amphifluoric FRET to measure kinetic barriers by quantitatively reading out protein self-assembly at single cell resolution, over a range of the query protein's intracellular concentration in vivo. We expressed structurally diverse proteins inducible from a high-copy plasmid in living yeasts at different strengths of translation initiation controlled either globally (pharmacologically) or specifically (uORF or Kozak), while maintaining the same level of query protein expression by controlling plasmid copy number. Remarkably, all reductions in translation initiation hampered seed formation irrespective of neither the query protein structure nor the expression level.

The provision of a pre-existing seed revealed that the proteins remained fully competent for polymerization. Translation, thus, only specifically influences the kinetic barrier to aggregation, and not their ability to aggregate. Various manipulations to enhance polypeptide interaction and residence time on the polysome, such as domain positioning and ribosome collisions, downstream of the coding region of interest, also enhanced aggregation of the query proteins. Single molecule FISH revealed clusters of mRNA in the cytosol, in all experimental manipulations, potentially becoming a pre-requisite for our observations.

We also asked if a tight dodecamer translated equally from two traceable alleles, formed complexes from polypeptides of one allele in strong initiation and from both alleles in poor initiation strengths, and preliminary data agrees.

Finally, our observations are true in both yeast and mammalian cells alike, thereby underscoring the widely conserved nature of this phenomenon. Thus, translation initiation strength potentially dictates the behavior of a wide range of protein classes, across eukaryotes, in strikingly profound manner. We are now exploring both - the underlying physical mechanism, as well as the physiological implications of this phenomenon, via proteomics, mathematical modeling, computational mining of RiboSeq datasets.

B585/P1566

Dissecting the mechanics of the Hrd1-centric ERAD system

B. G. Peterson, J. Hwang, J. Russ, J. Schroeder, P. Freddolino, R. D. Baldridge; University of Michigan, Ann Arbor, MI

Endoplasmic reticulum associated degradation (ERAD) is a conserved eukaryotic system that surveils and clears misfolded proteins from the ER. In addition, ERAD regulates key enzymes in biosynthetic pathways and is exploited by a number of infectious agents. We focus on the Hrd1-centric ERAD system that degrades both luminal proteins (ERAD-L) and membrane proteins (ERAD-M) in *S. cerevisiae*. Hrd1-ERAD substrates are recognized, retrotranslocated across the ER membrane to the cytosol, ubiquitinated, extracted from the ERAD system by the Cdc48 complex, and ultimately degraded by the 26S proteasome. Despite its importance in eukaryotic physiology, the mechanics of how this system recognizes, transports, and delivers its substrates to downstream proteins remains unclear. To dissect the function of this system, we conducted total codon mutagenesis on Hrd1 coupled with a fluorescence-based assay to screen for separation of function mutants. We have identified classes of mutants that specifically fail to degrade either ERAD-L or ERAD-M substrates. Using both cell-based and reconstitution biochemical assays, we're interrogating whether the defective degradation results from errors in substrate selection, retrotranslocation, or extraction from the ERAD complex. This work has generated novel insight into how Hrd1 selects, transports, and ubiquitinates its different classes of substrate.

B586/P1567

RAGE Ligands and Protein-Degrading Systems in Animal Models of Neurodegeneration and Normal Senescence

L. A. Lysenko, N. P. Kantserova, N. L. Rendakov; Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk, Russian Federation

The accumulation of irreversibly modified (glycated, carbonylated, aggregated etc.) proteins may result from the deficiency of their clearance pathways including intracellular proteolytic degradation. In murine models of amyloid- and glutamate-induced neurodegeneration, as well as in senile brain, we detected the aggregated abnormal protein deposits, inflammatory responses, oxidative stress, dysregulation of calcium homeostasis, and elevated levels of several S100 calcium-binding proteins. Neurodegeneration and normal ageing were accompanied by a reliable change in concentration, localization, and enzymatic activities of catheptic proteases, decreased proteasome complex assemblage, hyperactivation of calpain-dependent proteolysis, and upregulation of caspase 3. Functionally, lysosomal autophagy and ubiquitin-proteasome systems control protein quality and eliminate damaged ones, and highly selective calpain and caspase systems mediate necrotic and apoptotic pathways of cell death. Senile plaque surroundings express higher levels of the receptor for advanced glycation end-products (RAGE), an immunoglobulin-like cell surface receptor. When bound diverse ligands, such as β-amyloid, advanced glycation end-products (AGEs), transthyretin, and S100 proteins, RAGE appears to initiate several signal transduction cascades resulting in oxidative stress and NF-κB activation. Advanced glycation end-products (AGEs), the proteins, lipids, and nucleic acids modified by reducing carbohydrates, could be consumed with high-temperature cooked meal (exogenous source) and generated in inflamed, ischemic, and oxidatively damaged tissues (endogenous path). We revealed negative correlation of total AGEs level with serum cholecalciferol (vitamin D3) and

endogenous antioxidants, such as GSH system. Moreover, dietary supplementation with natural antioxidants, such as dihydroquecetin, resveratrol, ascorbic acid, occurs to effectively reduce protein glycation, particularly hemoglobin glycation and even heme oxidation in model animals. So, depletion of RAGE ligand pool via dietary antioxidant supply may be considered as a path to decrease chronic inflammation and cell loss. The study was fulfilled as a part of the budgetary task FMEN-2022-0017.

Spatial Temporal Modeling & Bioinformatics

B589/P1568

The correlation between cell and nucleus size is explained by an eukaryotic cell growth model

Y. Wu[1], A. Pegoraro[2], D. Weitz[3], P. Janmey[4], S. Sun[1]; [1]Johns Hopkins University, Baltimore, MD, [2]University of Ottawa, Ottawa, ON, CANADA, [3]Harvard University, Boston, MA, [4]University of Pennsylvania, Philadelphia, PA.

In eukaryotes, the cell volume is observed to be strongly correlated with the nuclear volume. The slope of this correlation depends on the cell type, growth condition, and the physical environment of the cell. We develop a computational model of cell growth and proteome increase, incorporating the kinetics of amino acid import, protein/ribosome synthesis and degradation, and active transport of proteins between the cytoplasm and the nucleoplasm. We also include a simple model of ribosome biogenesis and assembly. Results show that the cell volume is tightly correlated with the nuclear volume, and the cytoplasm-nucleoplasm transport rates strongly influence the cell growth rate as well as the cell/nucleus volume ratio (C/N ratio). Ribosome assembly and the ratio of ribosomal proteins to mature ribosomes also influence the cell volume and the cell growth rate. We find that in order to regulate the cell growth rate and the cell/nucleus volume ratio, the cell must optimally control groups of kinetic and transport parameters together, which could explain the quantitative roles of canonical growth pathways. Finally, although not explicitly demonstrated in this work, we point out that it is possible to construct a detailed proteome distribution using our model and RNAseq data, provided that a quantitative cell division mechanism is known.

B590/P1569

Exploring mechanistic rules governing emergent hiPSC colony dynamics using iterative agent-based model development

J. S. Yu[1], .. Allen Institute for Cell Science[1], G. Johnson[1], N. Bagheri[1,2]; [1]Allen Institute for Cell Science, Seattle, WA, [2]University of Washington, Seattle, WA.

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain the robust dynamic localization of their cellular structures, and how these cells transition between states during differentiation and disease. hiPS cells grow in epithelial-like colonies where changes in individual-level cell and nuclear shape are tightly coupled to population-level colony dynamics. We interrogate the emergent dynamics of growing hiPSC colonies through the iterative development of cell colony agent-based models (ABMs)—computational modeling frameworks comprising autonomous cell agents guided by biologically-derived rules—in an effort to establish minimal sets of rules that can explain observed states we have previously quantified. We integrate an existing ABM framework (https://github.com/bagherilab/ARCADE) with a 3D Cellular Potts-based representation of both cell and nuclear shape. Initial simulations of simple models with

basic rules guiding cell growth, division, and death are parameterized and validated by comparing simulated distributions of cell and nuclear size to the hiPSC single cell image dataset (https://open.quiltdata.com/b/allencell/tree/aics/hipsc_single_cell_image_dataset). We then systematically evaluate different combinations of rule sets to identify possible explanatory mechanisms underlying observed cell shape distributions. To interrogate and predict colony dynamics with this baseline model, we initialize simulations using the segmentation of nuclei in the first frames of 3D time-lapse videos of growing colonies of mEGFP-tagged laminB1 hiPSCs from the Allen Cell Collection. Cell shapes are approximated from the nuclear shapes using a Voronoi tessellation. Spatial and temporal dynamics of the simulated cell and nuclear sizes, shapes, and positions within the colony are extracted and compared to those observed in the corresponding 3D time-lapse videos. We expect that similarities and differences between simulated and observed colony behavior will provide insight into the underlying mechanisms necessary or sufficient for the emergence of specific dynamics. Iterative model development, which tightly couples model development with experimental observations and expert feedback, provides the opportunity for hypothesis testing and hypothesis generation. The computational framework presented is modular and open-source to facilitate application to diverse systems and collaboration across disciplines.

B591/P1570

Temporal and spatial topography of proliferation in cancer

S. Kabraji[1], G. Gaglia[2], D. Rammos[2], Y. Dai[2], A. Verma[2], J. Bergholz[1], W. Wang[1], S. Wang[3], C. Mills[3], M. Chung[3], S. Coy[2], J. Lin[3], R. Jeselsohn[1], O. Metzger[1], E. P. Winer[4], D. Dillon[2], J. J. Zhao[1], P. Sorger[3], S. Santagata[2]; [1]Dana Farber Cancer Institute, Boston, MA, [2]Brigham and Women's Hospital, Boston, MA, [3]Harvard Medical School, Boston, MA, [4]Yale Cancer Center, New Haven, CT.

Cancers are complex ecosystems where intracellular and extracellular signals are integrated to modulate tumor cell proliferation. However, cell-cycle state characteristics, spatial organization, and evolution with perturbation are poorly characterized in tumor tissues. We used multiplexed tissue imaging to develop a robust classifier of proliferation, the multivariate proliferation index (MPI), and applied this to map proliferative and non-proliferative cells in 650 unique tumors across five cancer types. Next, we created a framework for studying cell cycle dynamics using images of fixed tissues, based on time inference. Finally, we used single cell RNA sequencing (scRNAseq) to probe the gene expression programs underlying cell cycle states. The MPI outperforms single markers, like Ki67, when classifying proliferative index across diverse tumor types. Markers of quiescent, stem, and dormant cancer cells identified unique populations of non-proliferating cancer cells. Proliferative and non-proliferative cancer cells are organized across microscopic and macroscopic length scales that are reshaped by therapy. Local clusters of proliferative and non-proliferative tumor cells preferentially neighbor distinct tumor-infiltrating immune cells. In high-dimensional marker space, populations of proliferative cancer cells express canonical patterns of cell cycle protein markers, a property we refer to as "cell cycle coherence". Untreated tumors exist in a continuum of coherence states, ranging from optimal coherence, akin to freely cycling cells in culture, to reduced coherence characterized by either cell cycle polarization or non-canonical marker expression. Coherence can be stereotypically altered by induction and abrogation of mitogen signaling in a HER2-driven model of breast cancer, and with neoadjuvant therapy in patients with localized breast cancer. Microscopic residual tumor cells after HER2 inhibition from patients and tumor models have similarly skewed cell cycle dynamics. scRNAseq of residual tumor cells with skewed cell cycle dynamics suggests that upregulation of the nucleolar stress response is necessary to survive

HER inhibition. In sum, the MPI robustly defines proliferating and non-proliferating cells in breast cancer tissues, with immediate implications for clinical practice and research. The coherence metrics capture the diversity of post-treatment cell cycle states directly in clinical samples from patients with breast cancer, a fundamental step in advancing precision medicine. Replacing binary metrics with multivariate traits provides a quantitative framework to study temporal processes from fixed static images and to investigate the rich spatial biology of human cancers.

B592/P1571

Community-driven FAIR data management and reproducibility for the entire image-data life cycle

J. Ryan[1], A. Rigano[2], A. Kang[3], S. C. Ogg[4], J. Lacoste[3], T. Stroh[5], C. M. Brown[6], **C. Strambio-De-Castillia**[2]; [1]Advanced BioImaging Facility - Department of Physiology, McGill University, Montreal, QC, CANADA, [2]Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, [3]MIA Cellavie, Montreal, QC, CANADA, [4]IMAGING AND CHARACTERIZATION CORE LAB, King Abdullah University of Science and Technology, Thuwal, SAUDI ARABIA, [5]Neurology & Neurosurgery, McGill University, Montreal, QC, CANADA, [6]Advanced Bioimaging Facility - Department of Physiology, McGill University, Montreal, QC, CANADA.

Significant advances in spatiotemporal resolution have led to ever-expanding microscopy datasets which, without agreed-upon community guidelines, are challenging to manage, curate, reproduce, quantitatively analyze (including AI-assisted strategies), visualize, publish, and re-use (10.1038/nrd3439-c1; 10.1038/483531a; 10.7554/eLife.67995; 10.7554/eLife.45120/).

As such, biomedical advances crucially depend on the generation of high-quality Findable, Accessible, Interoperable, and Reproducible (FAIR; 10.1038/sdata.2016.18) datasets. This, in turn, requires the seamless integration of community-specified image documentation practices within the Research Data Management (RDM) processing pipelines required to ensure the execution, tracking, and documentation of the entire life cycle of data from sample preparation to publication (i.e., data provenance).

This is important for microscopy, where data interpretation is crucially dependent on easy-to-use RDM software enabling the capture and reporting of knowledge that is collectively termed Image Metadata, and that consists of three key aspects: i) biological context (i.e., organism, growth conditions, sample-type); ii) image acquisition (i.e., microscope hardware/settings/quality-control); and iii) image processing (i.e., software, analysis steps).

To illustrate these points, this presentation will first introduce recently published 4DN-BINA-OME-QUAREP community-driven Image Metadata specifications developed in the context of international bioimaging initiatives (10.1038/s41592-021-01327-9) and how they can be applied to typical light microscopy experiments.

This will be followed by a deep dive into the importance of incorporating robust microscopy quality assessment and reporting procedures in the life cycle of light-microscopy data to ensure rigor, reproducibility, and reusability.

The discussion will identify key stages in the pathway that includes image data acquisition, management, analysis, and dissemination and provide OMERO-based concrete and practical examples of how open-source tools and protocols developed by an international consortium of community initiatives led by QUality Assessment and REProducibility in Light Microscopy (QUAREP-LiMi), are being utilized in close collaboration with Canada Bioimaging, at McGill University and UMass Medical School to capture and report the necessary quality-control metrics and metadata to support the reproducibility

and reusability of image-based datasets.
Finally, the presentation will also introduce the Micro-Meta App and MethodsJ2 software tools that allow researchers to collect detailed microscope hardware and acquisition settings metadata and generates draft methods text for publication.

B593/P1572

A Deep Learning Model to Accelerate Molecular Dynamics Simulations of Membrane Fusion for Exocytosis

H. Zhu, D. An, B. O'Shaughnessy; Columbia University, New York City, NY.

Fusion of vesicle and targetmembranes to release neurotransmitters and other contents is central toexocytosis for neurotransmission, hormone secretion and other processes. Thecore components of the cellular fusion machinery are the SNARE proteins. Fusionglycoproteins with mechanistic parallels are used by SARS-CoV-2 and otherenveloped viruses for cell entry.
The mechanisms of membranefusion are poorly understood. A major obstacle is that simulating membranes withtens of thousands of phospholipids and a multiprotein machinery on millisec timescalesis highly computationally demanding. Further, the high complexity makes itdifficult to identify patterns of molecular behavior on the fusion pathway.Previously, we developed ultra coarse-grained molecular dynamics (MD) simulationsof SNARE-driven fusion on msec timescales for the first time (Zeng et al.,2021). However, fusion required lengthy simulation, and intermediate states remainpoorly characterized.
Compared to traditionalanalytical tools, deep learning models are far better adapted to pattern recognitionin complex systems, exploited by applications from image classification tospeech recognition. Here, we developed a deep learning model to accelerate MDmembrane fusion simulations, to compute free energy landscapes and to characterizerarely visited intermediates.
We identified a novelcollective coordinate encoding the probabilistic destiny (unfused, hemifused orfused) of a molecular state on a given timescale, and we used a branchingalgorithm to generate training data. We developed and trained a deep learning basedclassifier to compute the collective variable for a given molecular state, andused the classifier to develop a bias function represented as a deep neural network.This enabled enhanced sampling, calculation of free energies and accelerated computationof fusion times. Our method is applicable to a wide range of molecular systems.
We find vesicle-planarmembrane fusion is driven by SNARE-SNARE entropic forces that press membranestogether and catalyze hemifused connections called stalks, until a stalk expandsinto a hemifusion diaphgragm whose rupture produces fusion by tension-driven formationof a pore. Consistent with measurementsat neuronal synapses, for 8-10 SNAREs mean fusion times are sub-millisecond, decreasingwith number of SNAREs due to increased entropic forces.

B594/P1573

Cellular Drift and Diffusion in *Myxococcus xanthus*

K. Gibson[1], C. Grant[1], C. Cotter[2], L. Shimkets[2], H. Schüttler[1]; [1]Physics and Astronomy, University of Georgia, Athens, GA, [2]Microbiology, University of Georgia, Athens, GA.

We apply a novel integral equation (IE) formalism for stochastic cell movements, to examine experimental microcinematography data sets of random cell trajectories, observed in *M. xanthus* populations which exhibit either collective rippling wave or collective aggregation behavior, also known

as fruiting body formation. In terms of our IE-based data analysis, these two types of cell populations are characterized by fundamental differences in their single-cell drift-diffusion dynamics. Specifically, the IE formalism allows us to extract, from the observed cell trajectory data, effective single-cell drift and diffusion coefficients, *C(rho)* and *D(rho)* respectively, including their dependence on the ambient (local) cell density, *rho*. The observed single-cell trajectories are represented in this analysis as a stochastic sequence of straight-line "runs" of random duration, *tau*, speed *v*, and bearing angle *theta*, each run being followed by a "reversal" with random turning angle, *phi*. *C(rho)* and *D(rho)* are then expressed in terms of certain averages over the random sample of observed *(tau, v, phi)*-variables. Several different types of data binning and curve fitting procedures are applied and compared in the course of this IE-based data analysis process. The diffusion coefficient, *D(rho)*, is always positive and thus always acts to disperse accumulations of cells away from higher density regions. The drift coefficient, *C(rho)*, can have either sign and hence can either assist or oppose the diffusive dispersal of cells. In the latter case, the drift tends to drive the random cell motions, on average, in the direction of higher cell density which leads to aggregation under the condition *rho|C(rho)|>D(rho)*. A crucial difference between populations observed to exhibit aggregation *vs.* populations observed to exhibit rippling wave formation is the found in their respective low-density behavior: In the aggregating populations, the drift coefficient always opposes diffusive dispersal, *i.e.*, tends to favor aggregation, at almost all cell densities. By contrast, in rippling populations, the drift coefficient favors diffusive dispersal, *i.e.*, opposes aggregation at low densities, and it favors aggregation only in some narrow intermediate density range.

B595/P1574

Biomed Resource Watch: A Knowledge Base of Performance and Issue Information for Cell Lines and Antibodies

K. Lin[1], P. L. Pascual[1], A. E. Bandrowski[1,2], C. Hsu[1], M. Pairish[2], V. Guo[1], M. E. Martone[1], J. S. Grethe[1]; [1]University of California San Diego, La Jolla, CA, [2]SciCrunch, Inc., San Diego, CA.

The NIDDK Information Network (dkNET; dknet.org) is an open community resource portal for researchers supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)[1]. dkNET has developed tools, resources, and training modules to increase the awareness of and enhance scientific rigor and reproducibility. Research Resource Identifiers (RRIDs), unique identifiers for research resources, help improve the identification of research resources in the biomedical literature[2]. Utilizing RRIDs, we developed tools such as Resource Reports to assist researchers in finding and evaluating biomedical research resources such as antibodies, organisms, plasmids, cell lines, biosamples, and software tools for use in their research. Resource Reports provide researchers aggregated resource information, alerts when resources have problems, and validation information. To support these Reports, we have developed a new service, Biomed Resource Watch (BRW, scicrunch.org/ResourceWatch), a knowledge base for aggregating and disseminating known problems and performance information about research resources. We aggregate trustworthy information from authorized sources such as Cellosaurus, which provides information from The International Cell Line Authentication Committee (ICLAC) (example: scicrunch.org/ResourceWatch/Search?q=CVCL_1906). For antibodies, the information is aggregated from sources such as the Antibody Registry, Human Protein Atlas, ENCODE, the Intestinal Stem Cell Consortium(ISCC), and YCharos (example: scicrunch.org/ResourceWatch/Search?q=AB_1078467). We also include information extracted from the literature via natural language processing [Antibody Watch, 3] (example: scicrunch.org/ResourceWatch/Search?q=AB_10013321). BRW provides researchers and curators an

easy-to-use interface to report their claims about a specific resource. As of August 2022, BRW contains the following information: Cellosaurus (14296, 15.92%), Antibody Registry (48513, 54.03%), Antibody Watch (69, 0.08%), and other databases and communities (26918, 29.98%). Researchers can check information about a resource before planning their experiments via Biomed Resource Watch knowledge base-enhanced Resource Reports. This new service aims to help improve efficiency in selecting appropriate resources, enhancing scientific rigor and reproducibility, and promoting a FAIR (Findable, Accessible, Interoperable, Reusable) ecosystem in the biomedical scientific community. References: [1] Whetzel PL et al., PLoS One. 2015; PMCID: PMC4578941. [2] Bandrowski AE et al., Neuron. 2016; PMCID: PMC5854161. [3] Hsu C-N et al., PLoS Comput Biol. 2021 May; PMCID: PMC8189493. Source of Support: NIH NIDDK Award U24DK097771.

B596/P1575

Building macromolecular assembly dynamics into cell-scale simulations

S. Guo, **M. E. Johnson**, H. Z. Liu, A. Jhaveri, M. Sang; Biophysics, Johns Hopkins University, Baltimore, MD.

Self-assembly of protein components is ubiquitous across cell biology. Ensuring that such assembly occurs at the right place and the right time is essential for cellular functions. With simulation, we can study the principles governing these processes in diverse conditions in solution and on surfaces. However, these dynamical systems are challenging to study through simulation because they are inherently spatial and structural, and within the cellular environment they are frequently coupled to active, force-responsive, and energy-consuming structures. Here we introduce a variety of new features to our NERDSS software, which builds coarse molecular structure into the reaction-diffusion framework to enable such simulations. With recent applications to dynamics in clathrin-mediated endocytosis, HIV-1 viral lattice assembly, circadian clocks and transcriptional assembly on DNA, simulations have defined mechanisms that recapitulate *in vitro* measurements while providing new quantitative predictions on *in vivo*behavior. With new tools to automate construction of models from PDB structures, we can directly test how assembly of general macromolecular assemblies depends on component stoichiometries, rates, and crucially, to localization on surfaces or DNA substrates. We have further developed python libraries to simplify analysis of our simulation output, improving visualization and comparison to experimental observables. Lastly, we demonstrate how the use of AI methods such as autodifferentiation can be used to accelerate parameter optimization of binding energies and rates.

B597/P1576

Defects Within the HIV-1 Immature Lattice Support Dynamic Remodeling and Protease Dimerization

S. Guo, M. E. Johnson; Johns Hopkins University, Baltimore, MD

For HIV virions to become infectious after budding from host cells, they must mature via the activity of the HIV-1 proteases that are embedded in the immature Gag-Pol polyprotein lattice. However, only 5% of the Gag proteins in this lattice carry the proteases, and they are not activated until forming a homo-dimer. The mechanism through which a pair of embedded proteases find one another is unknown. Here, we use reaction-diffusion simulations to assemble a spherical immature lattice with 2/3 coverage of the membrane surface, in good agreement with structures determined by cryoEM. Our simulations show that dynamics of the immature Gag lattice on the membrane is unavoidable due to the missing 1/3 of the spherical protein coat, which allows for Gag-Pol molecules carrying the proteases to detach and

reattach at new places within the lattice. These rebinding events ultimately facilitate dimerization of proteases at timescales of <1 minute. These timescales are achievable for a range of realistic binding energies and rates due to the size of the gap in the lattice and the resultant imperfect contacts along its edge. Our simulations also demonstrate that during assembly, the 5% of lattice proteins that carry proteases is too high to prevent stochastic dimerization events in solution, indicating that prior to budding, dimerization events are either 'lost' to diffusion or are actively inhibited to prevent early protease activation. We find that the speed of dimerization events is largely insensitive to diffusion, which is almost exclusively restricted to occurring efficiently along the 2D surface. Both Gag-Gag interaction stability and diffusion influence the large-scale autocorrelation times of the lattice. Both the simulated and experimentally measured correlation functions from iPALM measurements support diffusive and remodeled dynamics of the immature lattice on the membrane. These dynamics are likely essential for proper maturation, and quantifying the stability of the associated lattice is thus a key step in understanding formation of infectious viruses.

B598/P1577

Interactome models incorporating diverse data sources predict cell-line differences in protein complexes

G. R. Wilkins, Jr, R. F. Murphy, J. Lugo-Martinez; Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

The formation of protein complexes is governed by the set of expressed proteins and regulated by cellular conditions. Most prior work on measuring and predicting protein complexes has been either based on data for a specific cell type or generalized without considering cell type. We have therefore sought to model variation in complexes across cell types. We began by developing improved models for complex prediction, extending the set of proteins for which complexes can be predicted. These models started by predicting protein-protein interactions using dramatically expanded sets of features and a new technique, "Ensemble Learning using Complete Feature Subsets," that we developed to better handle missing features. For a previously described dataset derived from known complexes in the CORUM database, we achieved a predictive accuracy of 0.81 compared to 0.61 and 0.63 in previous studies. We then clustered high-confidence predicted pairs into putative complexes using graph-based techniques and observed high agreement between many of our predictions and those in the STRING database. Evaluation of our predicted complexes using F-score-based comparison metrics yielded a score of 0.528, much higher than the scores of 0.302 and 0.157 obtained in previous work. Using cell line-specific versions of these models, we ranked complexes by the difference in predicted probability of occurrence between pairs of cell lines. We found that 94% of known CORUM complexes had a probability difference of less than 0.1 (and 88% less than 0.05), which is expected since most currently identified complexes are involved in cell housekeeping and would not be expected to be different between cell lines. By contrast, we identified 201 complexes, not previously identified in CORUM, that had predicted probability differences greater than 0.1 between two of the cell lines. A number of these complexes, but not all, have been previously identified in specific circumstances. For example, one complex was identified as elevated in adolescent schizophrenic brains. Our results establish the potential impact of cell type on complex assembly and exemplify the need for capturing cellular diversity in models of the interactome.

B599/P1578

CZ CELLxGENE Discover is an online analytical platform and the largest repository of standardized single-cell data.

P. E. Garcia-Nieto, S. Chambers, H. Thomas, B. Raymor, M. Lombardo, B. Aevermann, K. Liang, M. Czerwinski, S. Badajoz, C. Megill, T. Smith, M. Dunitz, D. Hegeman, A. Mani, T. Huang, K. Katsuya, E. Bezzi, B. Martin, A. Tolopko, A. Tarashansky, N. Gloria, A. Infeld, M. Urisko, J. Cool, A. Carr; Chan Zuckerberg Initiative, Redwood City, CA.

CZ CELLxGENE Discover (cellxgene.cziscience.com) is a free-to-use online data portal hosting a growing corpus of more than 400 single-cell datasets with over 24 million unique cells from human and mouse. The portal hosts single-cell data from modalities that include gene expression, chromatin accessibility, DNA methylation, and spatial transcriptomics. All data are standardized to include raw and normalized counts, and annotated using an ontological shared vocabulary for cell and gene metadata.
Data are easily searchable and can be downloaded in multiple formats via web or by programmatic API calls. Additionally we deploy UI-based analytical tools for exploration of single datasets that do not require download. We will showcase the main tools hosted in the portal. First, the CELLxGENE explorer which displays an interactive 2-dimensional representation of cells in a dataset and allows users to color cells by gene activity or metadata (e.g. cell type, disease, technical features, etc.), subset and analyze subgroups of cells, perform differential gene expression and create scatter plots of gene expression. Second, scExpression which allows querying the expression of any gene across all human and mouse cell types available in the concatenated data from the portal.
CELLxGENE is intended for community use and contributions. By supporting multiple modalities and data generated by labs around the world, the CELLxGENE suite of tools and data aims to maximize rapid use of data. To date, we support data from over dozens of labs and consortia such as the Tabula projects, LungMap, BICCN, Allen Institute for Brain Science, KPMP and the Human Cell Atlas. New contributions are welcome, the CELLxGENE team actively supports curation of data, and we work to ensure that self-curation is easy.
We are continuously improving the usability of CELLxGENE and adding new features tailored to the needs of cell and computational biologists. Groups interested in submitting their own data can contact the CELLxGENE team at cellxgene@chanzuckerberg.com to explore whether your data would be a good fit for CELLxGENE.

B600/P1579

Structural basis for CTPS regulation

C. Guo; ShanghaiTech University, Shanghai, CHINA.

CTP synthase catalyzes the final step of de novo pyrimidine biosynthesis. As for the importance of its product CTP, CTPS was considered as a potential target for viral, protozoal, and *Mycobacterium tuberculosis* infections, as well as for cancer and immunosuppression. CTPS can accurately recognize and interact with all four basic nucleotides ATP, UTP, CTP, GTP, and is also regulated by transient internal gas tunnels, multi ligand interactions, different proteomes, different oligomers and post-translational modifications. Because of the complexity of its regulation mechanism, CTPS is considered as a classic exemplar of protein regulation. In the past decades, through X-ray crystallography and biochemical experiments, people have learned a lot about its regulation. Cryo-EM is a rapidly developing technology in recent years. Through rapid freezing technology, the state of protein in solution can be largely

retained. It is very suitable for structural analysis of dynamic and large proteins. With this technology, we obtained a series of high-resolution structures of CTPS proteins, which provided a structural basis for the study of CTPS regulation.

B601/P1580

Long term consequences of early life adversity on neural architecture: A brain-wide cell biological perspective

T. W. Uselman[1], R. E. Jacobs[2], E. L. Bearer[1,3]; [1]Pathology, University of New Mexico - Health Sciences Center, Albuquerque, NM, [2]Zilka Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA, [3]Biology and Biological Engineering, California Institute of Technology, Pasadena, CA.

Early life adversity (ELA) correlates with vulnerability to neuropsychiatric disorders later in life. That psychotropic drugs target monoamine reuptake transporters points to the noradrenergic system (NS) as one of the contributing factors. Yet how ELA influences brain biology to heighten risk and what roles NS might play remain a mystery. We hypothesize that one mechanism is defective development of NS anatomy which shifts brain network coordination. To investigate impacts of ELA we coupled longitudinal magnetic resonance imaging (MRI) with optical microscopy to map brain-wide neural activity and the NS in a preclinical model for ELA. Limited bedding, a fragmented maternal care protocol, was provided to dams with pups from postnatal day 2-9. Pups with or without ELA (n=24) were aged 10 weeks in normal housing and then subjected to longitudinal manganese-enhanced MRI, which can be paired with video recordings of movements. Images and videos were captured before, immediately and long after acute exposure to ethological threat (TMT, 2,3,5-Trimethyl-3-thiazoline). At conclusion of longitudinal imaging, mice were sacrificed, brains perfusion fixed, embedded, serially sectioned and immunohistologically stained for norepinephrine transporter (NET) or for immediate early gene cFos, to delineate NS anatomy or to confirm neural activity. MR images were aligned and normalized prior to statistical parametric mapping (SPM), segmentation, and network analysis. Recordings were analyzed with Noldus Ethovision. which revealed that ELA increased exploration at baseline. TMT provoked increased defensive-avoidance with or without ELA, attesting to its expected effect. By comparison of SPMs of cohorts with or without ELA, we found that ELA heightened baseline neural activity in ventral pallidum, and decreased in prefrontal cortex and thalamus. Network analysis found ELA decreased the coordination of neural activity between many brain regions and shifted network topology. NET-staining identified shifts in distal NS termini in the nucleus accumbens and prefrontal cortex, correlating with ELA-induced changes to neural activity. Together our data suggest that ELA reconfigures brain-wide neural activity and may do so in part through developmental defects of NS anatomy. Future work will study roles of NS activity in ELA brain-states via chemogenetic DREADD modulation of NS neuron activity. Support: RO1MH096093, Harvey Family Endowment, UNM BBHI.

B602/P1581

Mass spectrometry signal quantification using supervised deep learning

C. Gohel[1], A. Swartz[2], G. Rahnavard[1]; [1]The George Washington University, Washington, DC, [2]University of Washington, Seattle, WA

Mass spectrometry (MS) is an important and often used tool for detecting the intensity of small and large molecules (metabolites and proteins) in molecular biology experiments. Small molecules can be

further used as biomarkers for characterizing and investigating human health and disease underlying biological activities. Liquid Chromatography and Gas Chromatography processing steps are used ahead of MS analysis to separate molecules using retention time. However, molecules whose mass-to-charge ratio overlap are often ignored by detection software. One approach for the annotation of molecules with overlapped retention times is to manually assign start and end times to molecules that overlap. For this, an expert must review output curves from the mass spectrometer and locate the start and stop times of the signal of interest. To assist this, we have developed and evaluated massLens, an automated pipeline software that integrates the curve from the start to the stop time. massLens accurately identifies the quantity of the molecule that generates a given signal. Determining the quantity of each molecule in the sample is the ultimate goal of the MS analysis.

B603/P1582

Learning Representations of 3D Intracellular Structures and their Organization using Deep Generative Models

G. Pires[1], R. Vasan[1], . Allen Institute for Cell Science[1], J. Theriot[2], T. Knijnenburg[1]; [1]Allen Institute for Cell Science, Seattle, WA, [2]University of Washington and Howard Hughes Medical Institute, Seattle, WA

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. Our approach is rooted in large-scale imaging experiments followed by quantitation and modeling. To describe high-dimensional, rich 3D images using interpretable latent representations, we are investigating the use of deep generative models, namely the variational auto-encoder (VAE). The VAE is a deep learning framework composed of two networks: the encoder, which takes the input data and produces a lower-dimensional representation; and the decoder, which takes the latent representation produced by the encoder and attempts to reconstruct the original input as faithfully as possible. Our work consists of an exploration of what choices of encoder/decoder architectures, inductive biases, and data modalities best suit the modeling of different components of the cell, as well as their inter-relationships. This formulation exhibits several benefits over classical methods. Specifically, it can be extended to handle any type of input data modality; it can leverage non-linearities to expand the set of learnable representations; it can incorporate inductive biases to exploit a priori knowledge about the underlying phenomena (e.g. symmetry), or to impose certain properties in the resulting representations (e.g. disentanglement). For example: Can we exploit symmetries, like XY rotational equivariance, to disregard non-meaningful sources of variation? What are good data modalities to use for more fluid structures, like the nucleolus, or for network-like structures, like the mitochondria? Can we capture quantitative aspects of organelle interactions in our learned representations? We attempt to address (narrower versions of) these questions using our large-scale hiPSC image dataset (available for access and download at allencell.org), which comprises over 200,000 high resolution 3D images of single cells in hiPSC lines.

B604/P1583

Magnetic levitation-based viable cell enrichment minimizes isolation-induced inflammatory response signaling in mammalian immune cells

D. Wakhloo, M. Margalef, A. Kale, S. Brodie, C. Stevens, G. Facer, K. Travers, J. McKinney; LevitasBio, Menlo Park, CA

Isolation of single cells from tissue with minimal transcriptional profile changes is critical to elucidating the link between gene expression and disease. Widely used enrichment methodologies can exert undue stress on cells, altering their native transcriptomic profiles. This method-related dysregulation of genes and/or pathways often masks more biologically-relevant gene expression profiles and can be misinterpreted as a consequence of disease. Certain cell types such as brain resident microglia and the immune fraction of Dissociated Tumor Cells (DTC) are highly sensitive to stress or other stimuli, which triggers increased inflammatory responses due to cell handling. Here, we report on a novel, gentle, quick, label-free enrichment procedure that harnesses magnetic levitation to efficiently isolate a higher percentage of microglial cells from fresh rodent brains without inflicting significant cell stress. Magnetic levitation-enriched samples yield more homeostatic microglia and fewer proliferating and inflammatory microglia as compared to cells isolated by flow cytometry. Expression of genes associated with inflammatory pathways was increased (2-6 fold) in flow-processed cells compared to the magnetically-levitated cells, suggesting that flow cytometry workflows significantly alter the transcriptomic profile of these cells. Similar results were observed in the immune cell component of DTC samples, where certain inflammatory pathways were found to be upregulated when flow cytometry was used to enrich for viable cells compared to magnetic levitation. These changes in gene expression may be misinterpreted as disease-related phenotypes. In addition to significantly lowering the expression of inflammation-related genes, magnetic levitation-enriched cells had higher quality sequencing metrics after scRNAseq. Median genes per cell, fraction reads per cell, and median UMI per cell were all higher in magnetically-levitated samples. A concomitant decrease in mitochondrial content per cell was also observed. In conclusion, using two different tissue types, we unequivocally demonstrate the effectiveness of Levitation Technology to isolate and enrich cells in their native transcriptomic state, enabling researchers to study disease-related pathways and/or targets that reflect their true biology, not stress-induced activity.

Physical Approaches to Cell Biology 1

B605/P1584

Characterizing Mixing in Cytoplasmic Streaming During Motility in Amoeba Chaos

U. Diaz[1], M. Das[2], W. Marshall[1]; [1]University of California San Francisco, San Francisco, CA, [2]Rochester Institute of Technology, Rochester, NY.

The cytoplasm is a dynamic fluid where cellular building blocks and components are continuously mixing. Cytoplasmic mixing is important for transporting intracellular material ranging from nucleotides and proteins to full organelles. Although diffusion is sufficient to drive cytoplasmic mixing in relatively small cell types, large cell types rely on active transport mechanisms to facilitate cytoplasmic mixing. In large ameboid and neutrophil cell types, cytoplasmic streaming is coupled to membrane deformations formed in motility. However, it remains unclear how well material mixes within cytoplasmic flows during motility. In addition, it is also unclear whether these cytoplasmic flows are laminar or turbulent in

nature, especially considering the low Reynolds number of the cytoplasm. In relatively small volumes of viscous fluids at very low Reynolds number, such as the cytoplasm, it is hard to obtain significant mixing simply from streaming. Here we examine whether cellular activity and shape-change may facilitate chaotic mixing of the cytoplasm.
Chaos carolinensis is an excellent model system to study mixing in cytoplasmic flows during motility thanks to its large size, which makes it highly amenable to microinjection and micro-surgery. To visualize cytoplasmic flows, we microinject two fluorescent beads into the cytoplasm of each amoeba and use fluorescence time lapse microscopy to track the movement of individual beads very accurately across long periods of time (up to 12 hours). To describe mixing we use an automated pipeline to find timepoints where the two bead trajectories come very close to each other, then characterize the separation of the particle trajectories with time via power law curve fits of the inter-particle separation and estimate Lyapunov exponents. We also study mean square displacements of individual beads with time to identify regimes of diffusive and active transport. To perturb amoeba motility we modulate temperature, substrate thickness, and use perturbations against the integrity of the uropod, an actin based contractile structure at the posterior end of the cell. In our work we describe how temperature, substrate thickness, and uropod integrity influence mixing in cytoplasmic flows during active motility in the giant amoeba Chaos carolinensis.

B606/P1585

Isometric size-scaling of cell surface components in mammalian cells

T. P. Miettinen, W. Wu, S. R. Manalis; Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA.

The surface area of a cell and the components of the plasma membrane set the maximum limit to cell's ability to uptake nutrients, secrete components and deform. In animal cells, the complicated ultrastructure of the plasma membrane causes cell surface area to be high and difficult to quantify. Despite common assumptions about surface-to-volume scaling, little is known about how animal cell surface area and components scale with increasing cell size. Here, we combine single-cell measurements of cell size with surface-specific detection of plasma membrane proteome in near-spherical mammalian cells to examine the size-scaling of cell surface area and components. We find that freely proliferating cells exhibit near-isometric size-scaling of overall plasma membrane proteome that is independent of the cell cycle. This near-isometric size-scaling of plasma membrane proteome is maintained despite large size increases during polyploidization. On transcriptional level, single cells scale their plasma membrane protein transcripts proportionally to cell size. However, individual surface proteins display a range of scaling phenotypes that arise post-transcriptionally. The isometric scaling of overall plasma membrane proteome is made possible by an increasingly folded plasma membrane ultrastructure in larger cells. This isometric scaling of plasma membrane proteome may function as a design principle that reduces regulatory complexity and cell size-dependency of cell extravasation, while ensuring sufficient membrane area for nutrient uptake and cell division that is largely independent of cell size.

B607/P1586

Mesoscale molecular assembly is favored by crowded active matter in the cell

T. Shu[1], J. Alberts[2], W. Burrell[3], D. Sang[1], M. Viana[4], M. Heidenreich[5], S. Rafelski[4], E. Levy[5], G. Hocky[6], L. Holt[1]; [1]Institute for System Genetics, NYU Langone Health, New York City, NY, [2]Department of Biology, Center for Cell Dynamics, University of Washington, Seattle, WA, [3]Department of Biology, NYU, New York City, NY, [4]Allen Institute for Cell Science, Seattle, WA, [5]Department of Structural Biology, Weizmann Institute of Science, Rehovot, ISRAEL, [6]Department of Chemistry, NYU, New York City, NY.

Cells are extremely crowded at the mesoscale, with up to 40% of the cell volume taken up by macromolecules. Moreover, the intracellular environment is in a state of non-equilibrium due to energy-dependent active processes. This crowded and active cellular environment can impact both molecular diffusion and interactions, and these effects are length-scale dependent. Many biochemical processes in the cell involve assembly of hundreds of proteins, at length-scales from nanometers up to microns. We set out to investigate the impact of the active, crowded cellular environment on these mesoscale molecular assembly processes, using liquid-liquid phase separation (LLPS) as an easily observable example. To simplify this problem, we leveraged a synthetic engineered experimental model system that condenses into a mesoscale assembly from two geometrically defined nanoscale protein components in the cytoplasm, which can be expressed in both S. cerevisiae yeast and mammalian Hela cells. We then developed two independent simulation platforms to allow microscopic interpretations of our experimental results through the framework of graph theory. We discovered that macromolecular crowding increases the kon rate of biochemical bond formation, shifts the critical point for LLPS, affects the kinetics of initial condensate formation, and stabilizes the condensed state. In addition, reducing cellular activity experimentally through ATP depletion or actin network disruption inhibits the growth of mesoscale assembly while increasing effective temperature from active processes in simulations promoted the coarsening of liquid droplets. These changes in condensate dynamics were directly related to changes in mesoscale diffusion. Finally, using poly(A)-binding protein (Pab1) in yeast, a marker protein for stress granule, we found that modulating phase separation through osmotic compression promotes yeast fitness during prolonged heat stress and its effect was compromised under Pab1 mutation that impedes phase separation. Our work highlights the importance of the cellular environment for mesoscale molecular diffusion and assembly.

B608/P1587

Membrane compression from exocytosis of synaptic vesicle fusion triggers ultrafast endocytosis

J. Liu[1], H. Jing[1], S. Raychaudhuri[1], T. Ogunmowo[1], G. Kusick[1], T. Ha[1], E. Chapman[2], S. Watanabe[1]; [1]Johns Hopkins University, Baltimore, MD, [2]University of Wisconsin-Madison, Madison, WI

Compensatory endocytosis keeps the surface area of secretory cells constant following exocytosis. At chemical synapses, clathrin-independent ultrafast endocytosis maintains such homeostasis. This endocytic pathway is temporally and spatially coupled to exocytosis, initiating within 50 ms at the region immediately next to where vesicles fuse: the active zone. How synaptic vesicle exocytosis induces ultrafast endocytosis is unknown. Here, we demonstrate that actin filaments are enriched in the region surrounding active zone at mouse hippocampal synapses and that the membrane area conservation due to this actin corral is necessary for exo-endocytic coupling. Simulations suggest that flattening of fused vesicles exerts lateral membrane pressure in the plasma membrane against the actin corral, resulting in rapid formation of endocytic pits at the border between the active zone and the surrounding actin-

enriched region. Consistent with our simulations, ultrafast endocytosis does not initiate when actin organization is disrupted, either pharmacologically or by ablation of the actin-binding protein Epsin1. These data suggest that endocytosis is mechanically coupled to exocytosis at synapses.

B609/P1588

Vast heterogeneity in cytoplasmic diffusion rates revealed by nanorheology and Doppelgänger simulations

A. T. Molines[1], R. M. Garner[2], J. A. Theriot[3], F. Chang[4]; [1]Cell and Tissue Biology, University of California San Francisco, San Francisco, CA, [2]Systems Biology, Harvard Medical School, Boston, MA, [3]Department of Biology, University of Washington, Seattle, WA, [4]Cell and Tissue Biology, University of California, San Francisco, San Francisco, CA.

The cytoplasm is a complex, crowded, actively-driven environment whose biophysical characteristics modulate critical cellular processes such as cytoskeletal dynamics, phase separation, and stem-cell fate. Little is known about the variance in these cytoplasmic properties. Here, we employed particle-tracking nano-rheology on genetically encoded multimeric 40-nm nanoparticles (GEMs) to measure diffusion within the cytoplasm of the fission yeast Schizosaccharomyces pombe. We found that the apparent diffusion coefficients of individual GEM particles varied over a 400-fold range, while the average particle diffusivity for each individual cell spanned a 10-fold range. To determine the origin of this heterogeneity, we developed a Doppelgänger Simulation approach that uses stochastic simulations of GEM diffusion that replicate the experimental statistics on a particle-by-particle basis, such that each experimental track and cell had a one-to-one correspondence with their simulated counterpart. These simulations showed that the large intra- and inter-cellular variations in diffusivity could not be explained by experimental variability but could only be reproduced with stochastic models that assume an equally wide intra- and inter-cellular variation in cytoplasmic viscosity. To probe the origin of this variation, we found that the variance in GEM diffusivity was largely independent of factors such as temperature, cytoskeletal effects, cell cycle stage and spatial locations, but was magnified by hyperosmotic shocks. Taken together, our results provide a striking demonstration that the cytoplasm is not “well-mixed” but represents a highly heterogeneous environment in which subcellular components at the 40-nm size-scale experience dramatically different effective viscosities within an individual cell, as well as in different cells in the population. These findings carry significant implications for the origins and regulation of biological noise at cellular and subcellular levels.

B610/P1589

Coupling between Membrane Protein Condensates and Ordered Membrane Domains

H. Wang[1], S. Chan[1], S. Dey[1], I. Castello-Serrano[1], J. Ditlev[2], M. Rosen[3], K. Levental[1], I. Levental[1]; [1]University of Virginia, Charlottesville, VA, [2]University of Toronto, Toronto, ON, Canada, [3]University of Texas Southwestern Medical Center, Dallas, TX

Liquid-liquid phase separation of membrane proteins have been observed in many cellular machineries, including LAT/Grb2/Sos1 condensates formed at the membrane of immunological synapse and PSD95/SnyGAP/Homer3/Shank3 condensates at the membrane of neurological synapse. The presence of a membrane can influence condensate formation and conversely, condensates may influence membrane organization. Specifically, the condensate-forming protein linker for actication of T-cells (LAT) associates with membrane domains known as lipid rafts, whose formation is guided by liquid-liquid

phase behavior. Thus, while LAT has the potential to act as the biophysical and functional linker between 3D protein condensates and 2D lipid domains, such coupling not been widely studied. Here we provide evidence that LAT condensates can induce and stabilize lipid domains, and in turn that raft domains can nucleate and stabilize LAT condensates. First, reconstituted LAT condensates formed exclusively in Lo domains on phase-separated supported membrane bilayers, and their location and morphology were dictated by condensates. Protein condensates could induce phase separation in biomimetic giant unilamellar vesicles (GUV), with liquid ordered (Lo) domains being recruited to condensates. Inversely, phase separated membranes concentrate LAT molecules in Lo regions, facilitating condensate formation. Moreover, we found that protein condensation could change their raft affinity: when monomeric LAT was included in both ordered and disordered domains, LAT condensates were still exclusively observed in ordered domains. Finally, these mechanisms were confirmed in living T-cells, where activation induces protein condensates that associate with and stabilize raft-like membrane domains. This coupling of lipid and protein assembly was essential T-cell activation. Altogether, we conclude that LAT protein condensates are thermodynamically coupled to ordered membrane domains to regulate the organization of T-cell membranes that facilitates immune cell signal transduction.

B611/P1590

Post-injury hydraulic fracturing drives fissure formation in the zebrafish basal epidermal cell layer

M. Sathe[1], A. S. Kennard[2], E. C. Labuz[1], C. K. Prinz[1], J. A. Theriot[1]; [1]Biology, University of Washington/Howard Hughes Medical Institute, Seattle, WA, [2]Biology, University of Massachusetts, Amherst, MA.

The skin epithelium acts as the barrier between an organism's internal and external environments. In zebrafish and other freshwater organisms, this barrier function requires withstanding a large osmotic pressure differential. Here we show that, following acute injury, the larval zebrafish epidermis undergoes a dramatic fissuring process that resembles hydraulic fracturing, driven by the influx of external fluid. The fissuring starts in the basal epidermal layer nearest to the wound, and then propagates at a constant rate through the tissue spanning over one hundred micrometers. Interestingly, the basal cells of the skin still remain connected via thin tethers after fissuring while the outermost superficial epidermal layer remains intact. Fissuring is completely inhibited when larvae are wounded in an isotonic external media, suggesting that osmotic pressure gradients drive fissure. Additionally, fissuring partially depends on myosin II activity, as its inhibition reduces fissure propagation away from the wound. During and after fissuring, the basal layer forms large macropinosomes (with cross-sectional areas ranging from 1-10 μm^2), presumably to clear the excess fluid. We conclude that excess external fluid entry through the wound and subsequent closure of the wound through actomyosin purse string contraction in the superficial cell layer causes fluid pressure buildup in the extracellular space of the zebrafish epidermis. This excess fluid pressure causes tissue to fissure, and eventually the fluid is cleared through macropinocytosis.

B612/P1591

In vivo*nanorheology reveals a novel role for the outer nuclear membrane KASH protein ANC-1 in regulating cytoplasmic macromolecular crowding in*Caenorhabditis elegans

G. Luxton[1], H. Hao[1], S. Kalra[1], X. Ding[1], E. F. Gregory[1], L. J. Holt[2], D. A. Starr[1]; [1]Molecular and Cellular Biology, University of California, Davis, Davis, CA, [2]Institute for Systems Genetics, New York University School of Medicine, New York, NY.

Macromolecular crowding considerably influences biochemical reaction rates and the physical properties of intracellular environments. Recent advances have made progress on how crowding is modulated in individual cultured cells by regulating ribosome concentrations. However, the mechanisms that regulate molecular crowding throughout development in multicellular *in vivo* contexts are largely unexplored. We used genetically encoded multimeric nanoparticles (GEMs) to study the physical properties of the cell interior within developing tissues of the multicellular organism, *C. elegans*. GEMs are homomultimeric scaffolds fused to a green fluorescent protein that self-assemble into bright, stable particles 40 nm in diameter. To use GEMs to perform single-particle tracking nanorheology experiments *in vivo*, we engineered *C. elegans* strains stably expressing 40 nm GEMs under the control of tissue-specific promoters in the hypodermis, intestine, and neurons. GEMs exhibited tissue-specific effective diffusion coefficients. We observed more immobile GEMs in the hypodermis and intestine than we observed in neurons or previously reported in individual yeast, or mammalian tissue culture cells. Previous nanorheology studies performed in cultured mammalian cells identified the conserved nuclear envelope-spanning linker of nucleoskeleton and cytoskeleton (LINC) complex as a key determinant of cellular mechanical stiffness. We asked if LINC complex components were required to regulate macromolecular crowding and cellular mechanics in *C. elegans*. The effective GEM diffusion coefficients were considerably increased in *C. elegans* intestinal or hypodermal cells lacking the KASH protein ANC-1. In contrast, no significant effect on GEM mobility was observed in strains null for the SUN protein UNC-84, or harboring muscular dystrophy-associated mutations in the nuclear lamin protein, LMN-1. Furthermore, we demonstrate that ANC-1 is required for the homogeneous cytoplasmic distribution of ribosomes *in vivo*. Collectively, these results establish a LINC complex-independent role for ANC-1 in controlling the mesoscale biophysical properties of the cytoplasm *in vivo* across several different tissues.

B613/P1592

The uniformity and stability of cellular mass density in mammalian cell culture

X. Liu, S. Oh, M. W. Kirschner; Harvard Medical School, Boston, MA.

Cell dry mass is the sum of the biosynthesis and degradation activities that act on a time scale of hours, whereas cell volume can be instantaneously altered by external osmotic stress in minutes. How the two are coupled is a fundamental question in cell size control. It has been proposed that cell volume is proportional to cell dry mass during growth and proliferation, resulting in a constant cellular mass density defined as cell dry mass dividing by cell volume. However, the goodness of the proportionality and its stability against perturbations has never been characterized. Here, using Normalized stimulated Raman Imaging (NoRI), we systematically investigated the uniformity of mass density in three cultured mammalian cell lines and their response to pharmaceutical and physiological perturbations. We found a remarkably narrow mass density distribution within and among cell lines, independent of the cell cycle. Furthermore, we found that cellular mass density can be modulated directly by extracellular osmolytes or membrane tension but is highly resistant to the inhibition of protein synthesis or degradation,

suggesting the existence of feedback controls to maintain mass density homeostasis. These findings shed light on the understanding of how and why mass density changes during the transition of cell physiological states and pave the road to discovering molecular mechanisms.

B614/P1593

How Cells Program Mineral Shape in Sea Cucumbers - Part 1

P. Vyas, C. Brannon, L. Formery, C. Lowe, M. Prakash; Bioengineering, Stanford University, Stanford, CA.

Biomineralization is ubiquitous in both unicellular and multicellular living systems and has remained elusive for several centuries due to limitations in the simultaneous understanding of both physical and biomolecular processes. Specifically, in holothurians, biomineralization occurs in the form of discrete ~100 um length scale structures called ossicles that diversify in shapes not only across holothurian species but even within an individual animal. To understand the fundamental processes enabling cellular cooperation at multi-cell length scales during the growth of ossicles, we directly observe them through live and fixed microscopy techniques. We establish that these structures grow from individual crystalline seeds inside a multi-cell syncytial complex with the biomineralized phase completely covered with a membrane-coated cytoplasmic sheath. We demonstrate that the initial seed transforms into a fully formed holed structure through 4 key steps - instability in the seed, tip extension, tip splitting, and merging of two tips. Through large-scale statistics on microCT data, we probe the robustness of the processes described. Throughout the growth, we demonstrate that cytoplasmic and membranous activity restricted to the surface of the biomineralized phase rather than motility of participating cell bodies regulate the material transport and directional growth of the structure. By observing distinct developmental niches within the animal we demonstrate differential symmetry breaking and seed cell-cluster dispersion as the structure grows, which acts as an additional layer of control over the fundamental growth processes. The system thus serves as a unique playground merging non-equilibrium solidification growth in chemical soups and classical branching morphogenesis in living systems.

B615/P1594

The role of hair pigmentation in thermoregulation and heat adsorption

G. A. Jimenez-Jordan[1], J. Roldan-Kalil[2], J. Alves[1], M. Inyushin[1]; [1]University of Central Del Caribe-School of Medicine, Bayamon, PR, [2]University of Puerto Rico Medical Sciences Campus, San Juan, PR

Studies show that men find women with blonde hair more attractive on average and women find men with dark hair more attractive on average[1]. This shows the social importance of hair color in human social interactions. It has also been historically key for camouflage and protection against predators in early human development[2]. However, hair phenotype has another important function, which is its role in protection from light and thermoregulation. This function has not been studied in detail in comparison to the previous ones discussed and it may be the most important one in terms of human physiology. The difference of temperature adsorption between different types of pigmentation of hair holds a lot of answers to many questions when it comes to the reasons why humans form distinct patterns of hair phenotypes. Melanin is the main pigment in hair, it has a propensity to bind metals and it is present in specialized organelles called melanosomes[3]. Using Transmission Electronic Microscopy (TEM), melanin with adsorbed metal ions is visible as dark spots that are quantifiable for the three types of hair pigmentation (blonde, brown, black) that are being studied. Low energy blue (441 nm) and green (532 nm) lasers were used to study the temperature change between irradiated and the unirradiated

hair shaft in samples of different hair types. It was determined that different hair pigmentations have significant differences in melanin granule quantities and in the changes in heat that occur when lasers are shone at the strands. It was determined that black hair has the most amount of melanin granules, followed by brown hair, and lastly blonde hair. Blue light adsorption also follows the same trend, with black hair absorbing the most, followed by brown hair and lastly blonde hair, while green light equally adsorbed by brown and black hair. Conclusion and discussion: These results show the reason why different hair pigmentations are common in different parts of the world. They provide a quantifiable explanation of why most people in sun-deprived areas, such as Scandinavia, have blonde hair. On the other hand, these results are consistent with the opposite effect, why most people located close to the Equator have dark, black hair. This study demonstrates the role of hair pigmentation in human physiology and evolution utilizing TEM microscopy and Infrared Thermal microscopy.
Acknowledgements: Supported by Universidad Central Del Caribe (UCC) and the office of the Associate Dean for Research and Graduate Studies, The Alliance-NIMHD-NIH, Expanding Undergraduate Students Education, Opportunities and Options in Clinical and Translational Research Supported by the US Department of Education: Title V Grant Award #P031S160068 and MAC-FRED Program 2018.

B616/P1595

Computational Extraction of Cytoplasm Oscillations in Slime Mold *Physarum polycephalum*

P. Chudinov; UCSF, San Francisco, CA

Physarum polycephalum is a single cellular slime mold that is famously capable of solving complex optimization problems. The mechanisms of decision-making in the slime mold have been actively studied in the past 2 decades, but we still do not completely understand of exact decision-making process of the cell. Our initial data suggested that cytoplasmic oscillations are involved in Physarum's change of movement direction behavior, yet verification with fluorescence microscopy poses a challenge due to the phototoxicity. We propose a novel imaging method to extract cell parameters such as weight centroid, cytoplasm oscillation frequency and local plasmodium thickness estimate which provide an insight into fluid mechanics of the cytoplasm. The approach consists of bright field microscopy with even amount of light across the cell body (to avoid phototoxicity) followed by a computational pipeline - segmentation and pixel intensity analysis. Using this method we can see changes in cytoplasm oscillation patterns at decision-making points.

B617/P1596

Physical principles behind strong sensitivity of temperature receptors

R. Chou[1,2], Q.-X. Jiang[3,4]; [1]Case Western Reserve University, Cleveland, OH, [2]University of Buffalo, Buffalo, NY, [3]University At Buffalo, SUNY, Buffalo, NY, [4]University of Florida, Gainesville, FL

Temperature is a ubiquitous environmental variable for all cellular processes. Living organisms have evolved specific mechanisms for temperature sensing in order to seek optimal inhabiting temperatures and avoid danger from noxious heat or frigid cold. One of the mechanisms in mammals involves a group of ion channels, thermal transient receptor potential (thermoTRP) channels, which act as sensors of ambient temperatures in peripheral sensory neurons. Functions of these channels necessitate exquisite temperature sensitivity, but the underlying mechanisms remain elusive. Thermodynamics implies that thermoTRP opening by temperature requires energetic changes in the regime of hundreds of kcal/mol. Such a change far exceeds what most enzymatic reactions can provide, which usually is in the range of 5-

30 kcal/mol. Thus, one central question on temperature receptors has been on how they attain the large energetics needed for their physiological functions. In this work we introduce differential scanning calorimetry (DSC) to directly measure energetic changes accompanying temperature-induced conformational transitions in a group of prototype heat-sensitive receptors. The DSC scans unravel that thermoTRPs undergo thermal transitions at an energy scale that is significantly larger than what was inferred from functional measurements, and reaches the regime of (partial) protein unfolding. Thermodynamic analysis reveals that the thermoTRPs bear intrinsic thermal instability and that their heat-induced opening represents an early event of protein unfolding. Our findings suggest that coupling functional transitions to (partial) protein denaturation may represent a general physical principle for living cells to achieve exceedingly high temperature sensitivity.

B618/P1597

Hydrodynamic forces during microtubule aster movement

T. Mitchison, L. Bai; Harvard Medical School, Boston, MA

The cytoplasm of animal cells is a complex material that can be though of as a gel made of entangled cytoskeleton and endomembrane networks percolated by a sol made of water, small molecules and macromolecules that span a large range of sizes. If these components move collectively, continuum approximations such bulk viscoelasticity may suffice to describe the mechanics. If the sol moves relative to the gel, counterintuitive phenomena may occur whose description requires more complex physical models. We previously used a poroelastic formalism to account for slow equilibration of hydrostatic pressure gradients across tissue culture cells, driven by actomyosin or osmotic forces, and proposed an effective pore size of approximately 30nm for the network component of cytoplasm (PMID: 17921219, PMID: 19690051). Our current focus is on centrosome positioning during early cleavage divisions in frog eggs. After mitosis, microtubule asters move apart in response to pulling forces from dynein and actomyosin. Current models typically assume that asters are rigid objects moving through a static, viscous liquid. Our observations in frog egg extract, which is undiluted, living cytoplasm, showed that all the cytoplasmic networks inside asters move collectively. When asters moved rapidly over dynein attached to a glass surface, they advected a small molecule probe, and presumably also water. Whether advection of water also occurs at physiological rates of aster movement in eggs is unknown, but it seems likely given the large size of asters and small effective pore size of cytoplasm. These observations suggest that a realistic model of aster movement must take hydrodynamic forces into account. Preliminary results from microneedle perturbation of egg extract indicate that F-actin is the predominant elastic element, even inside asters where microtubules instruct spatial organization. We propose that F-actin networks, percolated by large macromolecule complexes such as ribosomes and glycogen, determine the effective pore size of animal cell cytoplasm. This pore size is small enough to advect water during changes in cell shape or internal organization, which must be considered in physical models.

B619/P1598

Tracking Chromatin Across Temporal Scales Shows Variation in Dynamics in Response to DNA Damage

F. Iqbal, J. Liu; IUPUI, Indianapolis, IN

The movement of chromatin in human cells is highly complicated. Such movement is dependent on the molecular size of the chromatin domain and time lag of Single Particle Tracking (SPT) measurements. It

is unclear why such behavior exists. Most theoretical models which are mainly based on the polymer model fail to explain this phenomenon. We have proposed a single molecule imaging strategy that can track the dynamics of single nucleosomes in the human cell. By varying the temporal scale of imaging, our observations show that two types of motion are present in chromatin dynamics: a fast local fluctuation of the nucleosome, and a slow chromatin chain movement. A theoretical model that was developed from ChromoShake validated this conclusion. We also investigated the response to DNA damage from the nucleosomes by varying temporal scales. It was noted that bleomycin (BLM) induces the accelerated motion of nucleosomes at short time scale (interval ~ 30 ms), while the motion of nucleosomes at long time scale (interval ~seconds) is reduced. This disparity discloses that the DNA damage accelerates the local chromatin motion, while slows down the chromatin chain movement.

B620/P1599

Metal ion doping of melanin compounds enhances xray absorption

S. A. Baranowitz[1], L. Malone[2], M. F. Kiani[3]; [1]Epitek, Marlton, NJ, [2]Drexel University, Philadelphia, PA, [3]Temple University, Philadelphia, PA

Melanin is a biopigment widespread in animals and humans. It has been shown to absorb ultraviolet light, x-rays, and gamma rays. Lead shields are the most common protection against x-rays. Since lead is extremely toxic, non-toxic alternatives would be desirable to protect against radiological damage. The objective of this study is to compare the x-ray absorption of metal ion doped melanin compounds to lead controls. The melanin was extracted from a natural source, cuttlefish ink. Three metal ion doped melanin compounds were tested: bismuth - synthetic melanin (disk A), bismuth - natural melanin (disk B) and silver - synthetic melanin (disk C). Each compound's absorption was tested and compared to lead samples of the same size. Both the bismuth - synthetic melanin and the bismuth- natural melanin had absorption not substantially different than the lead controls.

B621/P1600

Force-induced site-specific enzymatic cleavage of DNA probes reveals serial mechanical engagement boosts T cell activation

J. Rogers, R. Ma, Y. Hu, K. Salaita; Emory University, Atlanta, GA.

T cells use their T cell receptors (TCRs) to identify antigens presented on the surface of virtually all cell types in structures called peptide-major histocompatibility complexes (pMHCs). The interaction between a TCR and pMHC is critical for the identification and eradication of virally infected or cancerous cells. A mystery in the field concerns how the weak affinity between the pMHC and TCR can be highly sensitive and discriminatory. To explain how TCR-pMHC interactions initiate potent signaling, models of TCR triggering have been introduced, which include the serial engagement and the mechanosensory models. Mechanical forces and the number of engagements between the TCR and pMHC are factors that have each individually been linked to TCR triggering; however, the extent of their contributions toward T cell activation in tandem remains poorly understood. Herein, we introduce **f**orce-ind**u**ced **s**ite-specific **e**nzymatic cleavage of DNA (FUSE DNA) probes, which allows the tension *duration* along the TCR-pMHC bond to be controlled experimentally. With FUSE DNA, the tension duration can be tuned by varying the concentration of a restriction endonuclease, which is responsible for selectively cleaving mechanically sampled DNA probes. This new capability provides a method to study how molecular mechanisms, such as TCR tension and serial engagement, contribute to TCR triggering. First, we show

this assay can visualize and disrupt mechanical interactions greater than 7.1 pN between OT-1 TCRs and anti-CD3ε. The average lifetime of the FUSE DNA probe (τ_p) was determined to be 1.6 min at 1000 U/mL of restriction endonuclease *AseI* and can be increased by decreasing the nuclease concentration. We define this lifetime as the average tension duration experienced by a TCR. T cells challenged with probes presenting ovalbumin (OVA) pMHC demonstrated dampened expression of pYZAP70 in the presence of the *AseI* endonuclease, thus demonstrating the perturbation of TCR mechanotransduction. This activation differential was exacerbated at lower antigen densities, wherein the largest decline in activation was observed with single molecule densities of pMHC. Finally, T cells experience a greater level of activation as the average tension duration increases, which leads to the conclusion that TCRs must serially, mechanically engage with pMHCs to boost subsequent activation. These results also demonstrate the potential for FUSE DNA to investigate the role of force duration in more general mechanosensitive pathways, such as Notch signaling and integrin-ligand interactions.

Tissue Development and Morphogenesis 1

B623/P1601

Evaluating airway ALI model fabrication methods and comparing differentiation potential of primary and hTERT-immortalized epithelial cells.

K. Tyo, F. Tian; ATCC, Gaithersburg, MD.

Human respiratory research encompasses a variety of fields including drug development, disease modeling, and toxicology testing. Despite the availability of traditional in vitro airway models, there is a persistent concern with their lack of physiological relevance to the human lung. Within the past decade, several advanced in vitro airway models have been constructed, which promises to provide more relevant applications in human respiratory research. However, the numerous variables associated in the generation of these advanced models can cause incomplete or inconsistent differentiation, resulting in research delays or cost overruns. In these studies, we showcase an optimal method of fabricating airway models consisting of human bronchial tracheal epithelial cells (HBECs) grown in collagen-coated 24-well plate inserts and cultured under air-liquid interface for 5 weeks. Model generation using different lots of primary HBECs as well as hTERT-immortalized HBECs were compared. In addition, various commercial media designed to promote epithelial differentiation were evaluated. Next, primary HBECs from ATCC and other commercial companies were evaluated and compared on epithelial differentiation and model morphology using optimized processes validated during the first phase of the study. All airway models were evaluated via weekly microscopy and transepithelial/transendothelial electrical resistivity measurements. Additionally, H&E and alcian blue imaging and MUC5AC and α-tubulin immunohistochemical analysis from histological samples of mature airway models were generated. These studies elucidate techniques and procedures to reliably generate 3D airway models with consistent full epithelial differentiation across replicates using both ATCC and other commercial primary HBECs.

B624/P1602

RNase L Acts as a Regeneration Repressor

N. Islam[1], **C. S. Kirby**[1], E. Wier[2], A. Li[2], M. P. Alphonse[2], E. Sweren[2], G. Wang[2], D. Kim[3], H. Liu[2], S. Reddy[4], L. Miller[2], J. Yu[5], W. Huang[6], J. W. Jones[5], S. Lee[1], S. Kim[1], M. Kane[5], R. H. Silverman[7], L. A. Garza[2]; [1]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, [2]Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, MD, [3]Department of Biochemical Engineering, Dongseo University, Busan, KOREA, REPUBLIC OF, [4]Department of Plastic Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, [5]Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD, [6]School of Biomedical Sciences, University of Queensland, St. Lucia, AUSTRALIA, [7]Department of Cancer Biology, Cleveland Clinic, Cleveland, OH.

Mammalian tissue injury response is usually characterized by fibrosis and scarring rather than functional regeneration. This limited regenerative capacity in mammals could reflect a loss of pro-regeneration programs or active suppression by genes functioning akin to tumor suppressors. To uncover programs governing regeneration in mammals, we performed comprehensive transcriptome screening in human subjects after laser rejuvenation treatment and cross-referenced these transcripts to those found in mice with enhanced Wound Induced Hair Neogenesis (WIHN), a rare example of mammalian organogenesis. We find the anti-viral endoribonuclease RNase L to be a powerful suppressor of regeneration. Rnasel-/- mice exhibit a remarkable regenerative capacity with elevated WIHN ($n=10$, $p<0.0001$) through enhanced IL-36α ($n=3$, $p<0.01$). Consistent with the known role of RNase L to stimulate caspase-1, we find that pharmacologic inhibition of caspases promotes regeneration ($n=3$ versus 4, $p<0.001$) in a novel IL-36-dependent manner ($n=4$, n.s.). This occurs by a potential negative feedback loop, where Rnase L activated Caspase-1 inhibits the pro-regenerative dsRNA-TLR3 pathway through the cleavage of TRIF. Additionally, these responses are not limited to skin but extend to other organs, such as the colon ($n=4$, $p<0.05$), suggesting that suppression of regeneration is a fundamental characteristic of epithelial wound healing. Taken together, this work suggests that RNase L functions as a regeneration repressor gene in a functional tradeoff that prioritizes host antiviral abilities and as a potential target to enhance healing in multiple epithelial organs, perhaps even during viral infection.

B625/P1603

An Unbiased Characterization of Epithelial Monolayer Development in Culture

N. Dawney[1], C. Cammarota[2], Q. Jia[1], A. Shipley[1], J. Glichowski[2], T. Finegan[3], D. Bergstralh[1,2]; [1]Department of Biology, University of Rochester, Rochester, NY, [2]Department of Physics and Astronomy, University of Rochester, Rochester, NY, [3]Department of Physics and BioInspired Institute, Syracuse University, Syracuse, NY.

Epithelial tissues are the most common tissue type, and the function of these tissues is frequently linked to a monolayered cell architecture. Our work aims to address the question of how epithelial monolayers form, and in particular how they develop organization with respect to the apical-basal axis (meaning tissue depth). We have developed a computational tool, *A*utomated *L*ayer *An*alysis (ALAn), that characterizes epithelial architecture in this axis. We find that that MDCK and MCF-7 cell lines, which can develop polarized monolayers in culture, form four distinct architectures with different structural characteristics and material properties. Three of these architectures (termed Immature, Intermediate and Mature) are organized, whereas the fourth (termed Disorganized) is a category of exclusion. We find

that epithelial architecture in the apical-basal axis is governed by multiple factors, including but not limited to cell density. Interestingly, we find cells are capable of reorganization in the apical-basal axis to form monolayers from cell aggregates. This behavior is governed by substrate availability; upon seeding, cells quickly land and spread to cover the substrate, preventing new cells forming substrate connections. Our results show that monolayers grown in culture can vary substantially, and highlight the importance of taking all three dimensions into account when analyzing experimental results.

B626/P1604

Whole-embryo analysis of local cell arrangement links differential cadherin expression to specific tissue architecture

M. Brambach[1,2], D. Gilmour[1]; [1]Department of Molecular Life Sciences, University of Zurich, Zurich, SWITZERLAND, [2]Life Science Zurich Graduate School, Zurich, SWITZERLAND.

Tissue architecture, the three-dimensional arrangement of cells, is a key feature of tissues that enables their functions. While it has been extensively studied how tissues are specified molecularly and consequently how morphogenic processes are induced, it is still largely unclear how cells generate their local arrangement. Many modern omics approaches require that samples are dissociated, and thus dispose of this tissue-architectural information, with the result that general mechanisms that link the organisation of cells in tissues to patterned gene expression remain elusive.
Here we use a quantitative morphometric approach to investigate how cells generate and maintain specific tissue architectures on a whole-organism scale. We use *in toto* light sheet microscopy of zebrafish embryos and nuclear segmentation to characterise individual cells based on the organisation of their tissue neighbourhood. This uncovers that the complexity of tissue architecture can be reduced to two broad classes: regular, low-density organisation and a more irregular, high-density configuration. To understand how these different types of architecture emerge, we define the expression patterns of the most abundantly expressed cadherin family members and classify them based on the organisation of their constituent cells. This reveals a striking correspondence to tissue architecture, with cadherins of the 'Cdh1 class' correlating with a regular, low-density organisation whereas cadherins of the 'Cdh2 class' correlate with irregular, high-density tissues. Time course analysis demonstrates that these two modes of organisation exist throughout early development and that downregulation of Cdh2 coincides with cells transitioning between architectural modes. Targeted perturbations of cadherin expression support a model where differential cadherin expression underlies the architectural diversity of tissues.

B627/P1605

The LINC complex controls global breast acini architecture through connections to the actin cytoskeleton

M. Rayer[1], K. Mui[1], A. Nickerson[2], T. P. Lele[3], G. G. Gundersen[1]; [1]cell and pathology, Columbia University, New York, NY, [2]Division of Genes & Development, Department of Pediatrics,, University of Massachusetts Medical School, Worcester, MA, [3]Department of Biomedical Engineering, Department of Chemical Engineering, Department of Translationa, Texas A & M, College Station, TX.

Lumen formation of a glandular epithelium is essential for normal breast function, and its absence is a hallmark of cancer. Recently, dominant negative approaches revealed that the linker of nucleoskeleton and cytoskeleton (LINC) complex is required for proper formation and maintenance of acinar lumens in breast epithelia grown in 3D culture (Zhang Q et al., *Curr Biol* 2019). The LINC complex is downregulated

in breast tumors (Matsumoto A et al., *Cancer Medicine,* 2015), and we confirmed that this was an acute response to transformation using the inducible Src-ER MCF-10A cell system. To test whether downregulation of the LINC complex affects normal breast acinar architecture in 3D culture. We knocked down specific LINC complex components. This revealed that SUN1, SUN2 and nesprin-2, but not nesprin-1 were required for proper formation of a lumen in breast epithelial acini. In nesprin-2 depleted cells, re-expression of the actin binding domain of nesprin-2G, but not its microtubule motor binding domain, rescued normal acini formation. The formins FHOD1 and FHOD3 interact with nesprin-2 in fibroblasts and muscle cells to enhance actin coupling to the LINC complex (Kutscheidt, S et al, *Nature Cell Biol* 2014; Antoku S et al, *Dev Cell* 2019). We found that knockdown of either FHOD 1 or FHOD3 also prevented normal acini formation. By confocal microscopy of fixed or living breast acini, we identified a small zone of F-actin accumulation in close proximity to the nucleus on the basal side of the epithelium. We have generated a nesprin-2 degron to test whether nesprin-2 is also required to maintain normal epithelial architecture. Our results show that specific LINC complex components mediate the connection of nuclei to actin filaments in breast epithelial and that disrupting this connection results in abnormal acinar architecture, a hallmark of cancer.

B628/P1606

Pulsed Apical Constriction during Embryogenesis Is Driven by Self-Assembled and Intrinsically Disordered Actomyosin Networks

H. Zhu, T. Zhu, B. O'Shaughnessy; Columbia University, New York City, NY.

During embryogenesis, cells exert active forces to sculpt tissues into complex shapes that are precursors of organs and other specialized structures. Apical constriction is a conserved cell deformation mode that contracts and folds tissues. In many organisms, apical constriction is driven by contractile pulses generated by apical actomyosin cellular networks (Martin and Goldstein, 2014). These actomyosin networks have radial polarity: formins and actin filament barbed ends localize to the peripheral cell-cell junctions of the apical surface, while actin filaments point inwards with their pointed ends and myosin II localizing to the medial apical zone (Mason et al., 2013).

The mechanism of network formation and pulsed contraction is unclear. In Drosophila, medial-apical accumulation of upstream regulators such as RhoA determine the radial polarity, and RhoA may be the pacemaker for the pulsed network contractions (Mason et al., 2016). However, other studies suggest mechanical feedback from myosin to upstream signaling molecules drives radial polarity and pulsing (Munjal et al., 2015).

Here we built a detailed, molecularly explicit simulation of apical actomyosin networks in Drosophila embryos, incorporating polymerizing actin filaments, myosin II clusters, formins and actin crosslinkers, with amounts constrained by experiment. In simulations, myosin clusters and actin filaments spontaneously self-assembled into a disordered network with radial geometry. Myosin bundled actin filaments, and myosin density was greatest in the medial apical region, consistent with the observed myosin coalescence during apical constriction (Martin et al., 2009).

Interestingly, simulations exhibited spontaneous stochastic pulsing, with cyclic accumulation of actin filaments and myosin in the medial apical zone, reminiscent of cyclic bridging instabilities in fission yeast cytokinetic actomyosin rings with deficient actin severing activity (Cheffings et al., 2019). However, in simulations incorporating a RhoA pacemaker that regulated myosin on- and off-kinetics, pulsed contractions were far stronger.

Our results suggest actomyosin networks driving apical constriction use mechanical forces to self-

organize, that their intrinsic disorder leads to spontaneous stochastic pulsing, and an upstream pacemaker may render the pulsing dynamics far stronger and more coherent.

B629/P1607

Actomyosin Pulsing Protects Embryo Tissue Folding from Unavoidable Fluctuations due to Inherent Stochasticity in Cellular Systems

H. Zhu, B. O'Shaughnessy; Columbia University, New York City, NY.

A feature common to many morphogenetic processes across diverse organisms is pulsatile time-dependence of actomyosin contractile structures and contraction response. During embryogenesis myosin patterning with pulsatile time-dependence generates contractile stresses that reshape tissue into the precursors of organs and other structures, but the function of pulsing is not understood. *Drosophila* ventral furrow formation is a classic example, when tissue contraction and folding are driven by coordinated apical constriction of ventral cells, driven by apical actomyosin networks with pulsatile time-dependence (Martin et al., 2009). Without myosin pulsing, tissue contraction is inhomogeneous, with failure of tissue folding and furrowing, as observed in embryos with C-GAP depletion (Mason et al., 2016) or in myosin phosphomimetic mutants (Vasquez et al., 2014). Why furrowing requires pulsing is unexplained.

Here we used biophysical modeling and analysis of extensive experimental data to show pulsing protects tissue folding from unavoidable stochastic fluctuations in cellular myosin. Such variations are inherent to cells and multicellular organisms, and originate in stochasticity of upstream regulating networks and other processes. During Drosophila ventral furrow formation, myosin levels have large fluctuations from cell to cell (Heer et al., 2017). We show that the multicell contraction profile across tissue is highly sensitive to these cell-scale myosin fluctuations about the envelope, and when frozen in time they disrupt ventral furrowing in Drosophila, as seen experimentally (Mason et al., 2016; Vasquez et al., 2014). However, with pulsatile time-dependence the fluctuations are effectively time-averaged and dramatically weakened by a low-pass filter mechanism, and furrowing is rescued as observed in wild type embryos (Martin et al., 2009). We also explain why pulsing fails to rescue furrowing in Twist-depleted embryos (Xie and Martin, 2015): since a myosin envelope fails to ramp up, fluctuations are unopposed by tissue-scale envelope curvature that contracts cells and folds tissue. In conclusion, myosin pulsing is a low pass filter mechanism protecting tissue folding from unavoidable fluctuations in myosin due to the inherent stochasticity of upstream regulatory networks and other processes.

B630/P1608

Exploring the dynamic mechanical interactions of extruding cells and their neighbors: Do apoptotic cells leave? or are they pushed?

S. Anjum[1], Y. Atieh[2], G. T. Eisenhoffer[2], L. A. Davidson[1]; [1]Bioengineering, University of Pittsburgh, Pittsburgh, PA, [2]Genetics, University of Texas MD Anderson Cancer Center, Houston, TX.

Epithelial tissue homeostasis is necessary to maintain barrier function and to prevent the onset and progression of cancer. Damaged or dying cells are removed from epithelial tissues by the process of extrusion. The epithelium of larval zebrafish enables experimentally controlled cell death that allows apoptotic cell extrusion to be visualized *in vivo* and in real time. In these experiments, cells exhibit stochastic area oscillations before being removed from the tissue. Extrusion is thought to be driven by both cell-autonomous and non-autonomous processes that are difficult to test by traditional

experimental techniques alone. Furthermore, cell and tissue-scale mechanics are frequently assumed to be coupled, but these interactions are difficult to test *in vivo*. To address these issues, we develop and present a cell-based model that can capture the dynamics of oscillations prior to cell extrusion using amplitude, duration, and areas of contractile activity measured from *in vivo* live imaging. Starting with mechanically interacting cell centroids, we trigger extrusion of a single cell by reducing its interactions with neighbors such that the area change and ultimate extrusion of the triggered cell match rates of cell extrusion observed in living epithelia. To capture the dynamics associated with extrusion, we move centroids over time and tessellate them. To represent changes in cell area found experimentally, we develop a hybrid weighted Voronoi tessellation method capable of encoding the magnitude of cell-cell mechanical interactions into cell areas. Time-sequences of simulated epithelial tissues are then segmented to identify cell boundaries from which we analyze local and global shape changes. Now we can vary biophysical programs of both extruding and neighbor cells such as frequency and amplitude of area oscillations, as well as tissue-wide viscosity and confinement. To query autonomy, we can keep the properties of either an extruding cell, or its local neighborhood fixed over time. Our results demonstrate that cell autonomous properties dominate rates of cell area loss even as surrounding cells oscillate in area. This model illustrates how kinetic programs of cell oscillation and extrusion can be mechanically coupled to tissue-level stability and highlights the power of combining modeling and experimentation to characterize cellular and tissue mechanics.

B631/P1609

Revealing the cellular mechanisms of tracheal-esophageal separation through live imaging

R. Yan, C. Tabin; Department of Genetics, Harvard Medical School, Boston, MA.

The respiratory system of humans and other amniotes derives from the foregut during early embryonic development. Initially emerging as a pair of buds attached to the ventral side of the foregut tube, the respiratory system separates from the digestive tract through the septation of the trachea from the esophagus in concert with the elongation of the bronchi. In humans, defects in foregut patterning can disrupt this process, leading to congenital malformations impacting 1 in 3,000 newborns. Contrasting the current knowledge about the genetic components of tracheal-esophageal separation (TES), its cellular mechanisms, including what determines the narrowing of the epithelium and how the tracheal-esophageal junction moves anteriorly over time, remain largely elusive. To solve these questions, we developed explant culture systems for embryonic chick and mouse foreguts to recapitulate TES ex vivo, which allowed us to observe the cellular dynamics at unprecedented spatiotemporal resolution as well as to perturb the cellular pathways with pharmacological treatments. We identified actomyosin contraction as an essential component of TES, driving the bilateral indentation of the epithelial tube by the surrounding mesenchyme. As the opposite epithelial layers join, the junctional cells lose their apicobasal polarities and undergo apoptosis, further facilitating the mesenchymal invasion to split the foregut tube into the esophagus and trachea. Pharmacological inhibition of actomyosin contractility or apoptosis precludes TES and thus the formation of the trachea in explants. Our results reveal the essential cellular processes for TES, whose dysregulation potentially causes tracheal-esophageal malformations in patients. Combining ex vivo culture and quantitative live imaging, we are further investigating the physicochemical underpinnings of the cellular dynamics during normal and pathophysiological TES.

B632/P1610

Decoding morphogenic instruction by Notch receptors

M. White, K. Jacobs, T. Singh, **M. L. Kutys**; Cell and Tissue Biology, University of California, San Francisco, San Francisco, CA.

The coordination of genetic programs and the physical organization of cells sculpt developing tissues, drive regeneration and, when dysregulated, facilitate disease. Exchange of chemical and mechanical information at cell-cell interfaces necessarily coordinates these processes, though molecular details remain unclear. The ubiquitously important Notch pathway mediates cell-to-cell communication in a variety of tissues across developmental stages. Interestingly, recent work from our group and others have contributed to an emerging model where Notch receptor activation has the capacity to regulate both cell mechanics and transcriptional changes. Here, by employing an organotypic tissue-engineered model of human mammary ducts in combination with genetic approaches to specifically isolate the cortical and transcriptional arms of Notch signaling, we demonstrate that deletion of *NOTCH1* results in the morphogenic dysregulation of duct tissue architecture that is not observed with loss of Notch1 transcriptional activity. Live cell imaging reveals that loss of Notch1 cortical signaling leads to defects in epithelial polarization, adherens junctions and actin organization, and proliferation, which ultimately impairs tissue architecture through lumen filling and duct occlusion. Mechanistically, we identify that as epithelia reach confluence and initiate apical-basal polarization, Notch1 localizes to lateral membrane contacts via previously unappreciated protein-protein interactions. Lateral contact localization and complex assembly is coincident with proteolytic activation of Notch1, which occurs without any downstream transcriptional change, yet is necessary for epithelial cell-cell adhesion, polarity maintenance, and growth arrest. Further, we demonstrate the increase in cell proliferation and putative impaired contact inhibition in Notch1 knockout cells are driven by the aberrant internalization and signaling of EGFR. Together, these results establish new roles and mechanisms by which cortical Notch1 signaling influences the homeostatic maintenance of epithelial tissues.

B633/P1611

Elucidating the role of copper and ATP7A in skeletal myogenesis

T. J. Whitlow, 1st Gen, Y. Zhang, N. J. Ferguson, A. M. Perez, H. Patel, J. Link, E. Larson, A. T. Mezzell, K. E. Vest; Molecular Genetics, Biochemistry, and Microbiology, UNIVERSITY OF CINCINATTI MED. SCHOOL, Cincinnati, OH.

Copper (Cu) is an important trace nutrient that functions as an enzyme cofactor and signaling molecule. Cu is also toxic, so intracellular Cu must be regulated by transporter and chaperone proteins. The trans-Golgi Cu transporter ATP7A provides Cu to secreted cuproenzymes and exports Cu when levels are high. Thus, ATP7A is a major determiner of intracellular Cu levels. Though the basic mechanisms of Cu handling are known, the tissue specific functions are less understood. Skeletal muscle accounts for approximately 23% of total systemic Cu and is required for movement, breathing, and swallowing. Skeletal muscle is a post-mitotic tissue comprised of multi-nucleated myofibers that can be regenerated by an associated pool of stem cells. Few studies have focused on ATP7A in muscle stem cells and muscle regeneration. Our previous work showed that total Cu, ATP7A protein, and *Atp7a* mRNA stability increase during muscle cell differentiation (myogenesis). Thus, we hypothesized that ATP7A is important for myogenesis. Proliferating C2C12 cells, an immortalized muscle cell line, and primary myoblasts can be induced to form multinucleated myotubes in a process that resembles in vivo myogenesis. We found

that ATP7A deficiency impairs myotube formation in both, suggesting that it is a critical component of myogenesis. ATP7A loads copper onto secreted cuproenzymes and also pumps copper directly out of the cell as the primary copper detoxification pathway. Using BCS-mediated Cu chelation, we determined that the differentiation defect from ATP7A loss was not due to copper overload. This led us to hypothesize that the primary role of ATP7A is to provide copper to secreted enzymes. We determined that co-differentiating ATP7A knockdown cells with wild type cells in trans-wells rescues myotube formation. We then sought to determine which secreted cuproenzyme is necessary for myogenesis. Previous literature suggests that the amine oxidase lysyl oxidase (LOX) is required for myogenesis. We found that knocking down ATP7A reduced LOX activity, and that addition of recombinant LOX rescues differentiation in ATP7A knockdown cells. These results confirm that ATP7A is important for providing Cu to secreted factors that are required for differentiation thus adding to our knowledge of tissue specific Cu requirements.

B634/P1612

Stage specific copper prioritization during muscle cell differentiation

A. M. Perez, Y. Zhang, T. J. Whitlow, K. E. Vest; Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, Cincinnati, OH.

Copper (Cu) is an essential trace nutrient that acts as a cofactor in enzymes required for fundamental processes like energy production. Cu can be toxic and therefore must be tightly regulated. The primary importer of Cu into the cell is CTR1. In the cytoplasm, Cu can be bound by intracellular Cu proteins, bound by small molecule metabolites in labile Cu pools, exported via ATP7A, or imported into mitochondria. Mitochondrial Cu is important for oxidative phosphorylation (OXPHOS) for both energy generation and metabolite synthesis. Cu enters the mitochondrial matrix via the transport protein SLC25A3 before transport to the IMS. Impairment of any of these pathways can lead to systemic or cellular Cu deficiency or overload and cause diseases with tissue specific defects, which are poorly understood. Skeletal muscle is a regenerative tissue that contains ~23% of total Cu, which is largely attributed to the function of Cu in OXPHOS. However, the function of Cu in muscle regeneration is poorly understood. Skeletal muscle is made up of bundles of multinucleated myofibers. Residing along the myofibers are satellite cells (SC), adult stem cells that drive muscle regeneration. Skeletal muscle regeneration, known as myogenesis, requires activation, proliferation, and differentiation of SC. This process requires coordinated activity of intracellular and extracellular signaling pathways. Our published and unpublished data suggest that Cu is required for muscle cell differentiation and that ATP7A-mediated Cu export to secreted enzymes is required at the intermediate stages of myogenesis. However, the importance of mitochondrial Cud during myogenesis is not known. We hypothesized that mitochondrial Cu needed in mature skeletal muscle rather than for myogenesis. Using siRNAs, we knocked down *Slc25a3* in C2C12 myoblasts and found that myotube formation is not affected. This result suggests that although we know that mitochondrial Cu is required for OXPHOS, it is not required for differentiation of C2C12 myoblasts. Our future studies will focus on understanding when mitochondrial Cu is loaded during myogenesis and how it is delivered from CTR1 to mitochondria.

B635/P1613

Evaluating neuroprotective effects of sulforaphane in a VPA-induced autism model

R. Bessetti[1], V. Koonce[1], A. Papariello[1], C. Camacho[1], K. McCoy[2], K. Litwa[1]; [1]East Carolina University/Anatomy and Cell Biology, Greenville, NC, [2]Florida Atlantic University, Boca Raton, FL.

In the last decade, U.S. Autism Spectrum Disorder (ASD) diagnoses have increased. Pregnant women are commonly exposed to environmental factors that can increase the likelihood of offspring developing an ASD. A potential avenue for reducing the adverse effects of environmental contaminants is to enhance the body's own detoxification and antioxidant pathways, ultimately reducing cellular oxidative stress. NF-E2-related factor 2 (Nrf2) is a transcription factor thatpromotes the expression of cytoprotective and antioxidant genes. The phytochemical, sulforaphane (SFN), potently activates the Nrf2 pathway by decreasing Nrf2 proteasomal degradation mediated by Keap1 (Kelch-like ECH-associated protein 1). Our investigation aims to test the hypothesis that SFN will protect developing neural circuits from the detrimental effects of environmental contaminants. We generated a human fetal brain model of chemically-induced autism by exposing human cortical spheroids (HCSs) to the anti-epileptic drug valproic acid (VPA). Fetal exposure to the VPA has been linked to an increased risk of developing an ASD in humans and autism-like behavior in rodents. At a molecular level, VPA functions as an inhibitor of histone deacetylases and is known to increase oxidative stress. In HCSs, either VPA or SFN alone impair synapse formation. Whereas in combination, they restore synapse formation. Nrf2 nuclear translocation is increase by both VPA and SFN. We are also using CRISPR interference to establish whether sulforaphane's effects are mediated via the Nrf2 pathway. We have successfully used this technology to decrease Nrf2 expression in HCSs. Based on these findings, we are currently testing the hypothesis that synapse formation requires a delicate balance between oxidative stress and antioxidant buffering. Our data supports that sulforaphane can restore synapse formation and oxidative homeostasis in the presence of environmental stressors.

B636/P1614

Organ size and shape control by potassium-channel mediated bioelectricity in zebrafish

G. Zhang; Comparative Pathobilogy, Purdue University, West Lafayette, IN.

Organ size determination and growth regulation are critical in animal embryogenesis and larva growth. Vertebrate limbs and fins have been served as a model for studying this fundamental question. The zebrafish fin development has been extensively studied. However, the signals that determine fin size and shapes remain largely unexplored. We identified two long-fin zebrafish mutants of inwardly rectifying potassium channel (*kir*) genes. These mutants show allometric growth during the larva stage and significant longer fins in adults in a genetically dominant manner. We mapped a viral DNA insertion into the noncoding region of the two *kir* genes, and this insertion led to a transient and ectopic expression in somite, from which the fin ray progenitors originate. Moreover, we showed that ectopic expression of additional *kir* genes in the somite of transgenic zebrafish phenocopied the long-fin phenotype in a gene dosage-sensitive manner. Furthermore, this long-finned phenotype was only able to be reversed by a CRISPR-induced *kir* gene loss-of-function mutation in an allelic-specific manner. Taken together, our results reveal that potassium-channel mediated bioelectricity is an emerging vital regulator for fin size determination in zebrafish.

B637/P1615

Multiscale mechanical reinforcement for the propagation of a morphogenetic process

S. Weng, R. J. Huebner, C. C. Devitt, B. Nyaoga, J. R. Alvarado, J. B. Wallingford; University of Texas At Austin, Austin, TX.

Force propagation integrates cell intercalation and tissue-scale convergent extension

Convergent extension (CE) is an evolutionary conserved morphogenetic process by which repeated polarized cell intercalations drive convergence in one axis and elongation of the tissue in an orthogonal direction. We recently showed that the poorly characterized catenin Arvcf is largely dispensable for cell intercalation but is absolutely required for mechanically effective CE. Here, we explore the mechanisms by which Arvcf links cellular behaviors and tissue-scale shape change, using new methods for image-based mechanical assessment at different scales. We found that Arvcf-dependent converging forces were required to fully resolve individual cell intercalation events and that local converging forces can propagate in the direction of tissue extension. These data suggest a multiscale mechanical feedforward loop underpinning the propagation of CE across the tissue. By contrast, in the absence of Arvcf, converging forces were reduced and cells took on aberrant packing configurations that effectively blocked force propagation. Together, these data are significant for providing new biomechanical and cell biological insights into a fundamental morphogenetic process that is implicated in human neural tube defects and skeletal dysplasia.

B638/P1616

The spindle orienting machinery requires activation, not just localization

T. M. Finegan, K. E. Neville, D. T. Bergstralh; Department of Biology, University of Rochester, Rochester, NY.

The orientation of the mitotic spindle at metaphase determines the placement of the daughter cells. Spindle orientation in animals typically relies on an evolutionarily conserved biological machine comprised of at least four proteins - called Partner of Inscuteable (Pins), Gαi, Mushroom body defective (Mud), and Dynein in flies - that exert a pulling force on astral microtubules and reels the spindle into alignment. The canonical model for spindle orientation holds that the direction of pulling is determined by asymmetric placement of this machinery at the cell cortex. In most cell types, this placement is thought to be mediated by Pins, and a substantial body of literature is therefore devoted to identifying polarized cues that govern localized cortical enrichment of Pins. In *Drosophila* neuroblasts, for example, this cue is thought to be Inscuteable, which helps recruit Pins to the apical cell surface. In this study we revisit the canonical model. We find that spindle orientation in the follicular epithelium requires not only Pins localization but also activation, which relies on direct interaction between Pins and the multifunctional protein Discs large. This mechanism is distinct from the one mediated by Inscuteable, which we find also has an activating step. Together our results show that the canonical model is incomplete. Local enrichment of Pins is not sufficient to determine spindle orientation; an activation step is also necessary.

B639/P1617

The tricellular junction protein tricellulin is involved in multiciliated cell epithelial insertion during Xenopus skin development

C. Savvidou, K. Miti, N. Christodoulou, P. A. Skourides; UNIVERSITY OF CYPRUS, Nicosia, Cyprus

Epithelial cell insertion is a crucial process necessary for early embryo development and tissue homeostasis. To accomplish this, cells become polarized, move apically anchoring at the epithelial tight junctions, and subsequently remodel these junctions generating an apical surface. Multiciliated cell (MCC) intercalation into the epidermis during *Xenopus laevis* embryogenesis can be used as a model system to understand the cellular and molecular mechanisms orchestrating cell insertion into an epithelium. Previous studies have revealed that MCCs anchor preferentially at the contacts of three epithelial cells, a structure known as tricellular junction (TCj). Lipolysis-stimulated lipoprotein receptor (LSR) and tricellulin (Marveld2) are the two main components of vertebrate tricellular junctions. LSR was recently shown to be necessary for MCCs insertion into the skin epithelium. However, the role of tricellulin during MCC intercalation has not been addressed. Here our aim is to elucidate the role of tricellulin in MCC radial intercalation. Our data show that tricellulin has a crucial role during MCCs epithelial insertion. Specifically, we show that the majority of MCCs expressing dominant negative constructs of tricellulin fail to emerge at the surface of the epithelium. The few cells which do emerge display severe ciliogenesis defects. Analysis of the apical actomyosin network in MCCs expressing the tricellulin mutant constructs suggests that the cell intercalation and ciliogenesis defects stem from defective apical actin organization. Overall, our findings reveal a novel role of tricellulin in MCCs radial intercalation which can serve as the basis for future studies aiming to examine the role of tricellular junction proteins in the insertion of cells into an epithelium in development and disease.

B640/P1618

PSPC1 deficiency retards angiogenesis by downregulating VEGFR2 and Dll4/Notch1 signaling

C. Y. Chang, S. S. Lee, Y. S. Jou; Institute of Biomedical Sciences, Academia Sinica, Taiwan, Taipei, Taiwan

Paraspeckle component 1 (PSPC1) has been known to be the contextual determinant of pro-metastatic switches of TGFβ and subcellular translocations of oncogenic proteins in tumor progression. PSPC1 was expressed during early developmental stage in multiple tissues. However, the biological functions of PSPC1 during development remain largely unknown. Here, we generated *Pspc1* knockout (*Pspc1* KO) mice and discovered the impacts of PSPC1 in development. *Pspc1* KO mice showed dilated hearts with smaller size, decreased reticulocyte number, reduced height of intestinal villus, abnormal cerebellar foliation, and increased lipid drop in adipocyte tissue, and about 75% *Pspc1* KO mice died in early postnatal stage. Among multiple *Pspc1* tissue-specific knockout mice, endothelial-specific *Pspc1* knockout (*Pspc1* Tie2-cKO) mice had higher postnatal death rate and resembled the smaller heart size phenotype of *Pspc1* KO mice. In *Pspc1* Tie2-cKO heart, retarded coronary vasculature was detected. In retinal angiogenesis assay, the vessel progression was significantly retarded with diminished endothelial cell proliferation and filopodia formation in *Pspc1* Tie2-cKO mice. Moreover, knockdown of *Pspc1* in mouse endothelial MS1 cells significantly abolished angiogenic sprouting in 3D fibrin gel bead assay. By transcriptome analysis, knockdown of *Pspc1* significantly downregulated Dll4 and VEGFR2 mRNA levels, and also the Dll4/Notch1 and receptor tyrosine kinase signaling pathways. By Western blots assay, knockdown of *Pspc1* largely reduced the VEGFR2 phosphorylation and Notch1 activation respectively stimulated by VEGF treatment and increasing cell density. Interestingly, *Pspc1* knockdown induces the

tip cell gene expressing signature in MS1 cells. Together, our results indicated that expression of *Pspc1* is required for angiogenic sprouting, especially in tip cell induction, vasculature development and heart morphogenesis of mouse.

B641/P1619

Using multiphoton fluorescence lifetime imaging to visualize NADH/FAD endogenous autofluorescence in *Drosophila* early embryogenesis

M. España, A. C. Paré, K. Quinn; University of Arkansas, Fayetteville, AR

Tissue remodeling occurs in the context of both normal development and wound healing. By studying tissue remodeling events across a range of organisms--notably convergent extension (CE) during head-to-tail axis elongation--researchers have identified conserved contractile and adhesive proteins that alter cell morphology. In addition, the processes that drive CE share striking similarities at the molecular level with epithelial wound healing, and many view epithelial regeneration as a reactivation of normal developmental mechanisms. Notably, wound healing is also dependent on mitochondrial dynamics (i.e., fusion and fission) and the production of reactive oxygen species. It is therefore compelling to think that changes in cellular metabolism or mitochondrial signaling may be necessary to drive the dynamic cellular processes of CE, although this has not been demonstrated *in vivo*. One of the premier models for studying CE is the *Drosophila* embryo, because 1) cell movements are easy to visualize, 2) CE occurs rapidly over the course of 30 minutes, and 3) tissue remodeling occurs in the absence of cell division (which complicates analysis in other systems). To address whether there are changes in cellular metabolism over the course of CE in living embryos, we are using non-destructive multiphoton microscopy and fluorescence lifetime imaging (FLIM) to visualize the endogenous autofluorescence of mitochondrial cofactors before, during, and after CE. We are also using fractal image analysis to probe mitochondrial network architecture to ask whether mitochondrial dynamics are correlated with CE. These experiments will reveal the role of cellular bioenergetics during tissue remodeling in the context of normal development, and may contribute to our understanding of the cellular basis of wound healing and CE-based birth defects, such as spina bifida.

B642/P1620

Visualization of cytoskeletal proteins reveals the architecture of the cortical actomyosin network driving apical constriction in C. elegans

P. Zhang, **T. N. Medwig-Kinney**, B. Goldstein; University of North Carolina At Chapel Hill, Chapel Hill, NC

Apical constriction results in cell shape changes that drive key morphogenetic events during development, including Drosophila ventral furrow formation, C. elegans gastrulation, and vertebrate neural tube formation. Apical constriction is primarily driven by contraction of apical actomyosin networks. It has been reported that the actomyosin networks of the epithelial cells within the ventral furrow of Drosophila exhibit a radial sarcomere-like pattern, where nonmuscle myosin II, myosin-activating kinase, and pointed end-capping proteins all exhibit enrichment in the center of the apical domain. To determine if this is a broadly conserved actin architecture during apical constriction, we examined actomyosin dynamics during C. elegans gastrulation, in which two endodermal precursor cells internalize from the surface of the embryo. We observed enrichment of nonmuscle myosin (NMY-2) and a myosin regulatory light-chain kinase (MRCK-1) in the apical cortex, as expected. However, we did not observe enrichment of NMY-2 or MRCK-1 in the center of the apical domain; instead, both proteins

exhibited punctate localization throughout the apical cortex. To visualize the polarity of actin filaments, we used CRISPR to generate endogenously tagged alleles of several capping proteins and focused on those that were readily visible in the endoderm precursors. CAP-1, a barbed-end capping protein, was enriched at cell-cell contacts, consistent with what was observed in Drosophila. However, UNC-94, a pointed-end capping protein, was not enriched in the center of the apical cortex and instead showed a more diffuse pattern throughout the apical cortex. Our observations suggest that C. elegans endoderm precursors employ a mixed-polarity actin network with myosin and a myosin activator distributed throughout this network, in contrast to the polarized actomyosin network reported in Drosophila epithelial cells. Taken together, these results suggest that diverse strategies of actomyosin organization are used in animal cells to accomplish apical constriction.

B643/P1621

Investigating horizontal cell ultra-structure through 3-D electron microscopy in the pure-rodskate retina

Y. Yusuf, I. Anastassov; San Francisco State University, San Francisco, CA

The overall goal of this project is to determine what molecular and structural adaptations have allowed the pure-rod skate (L. erinacea) retina to achieve functionality over a full range of brightness conditions. The visual systems of most vertebrate species have two different kinds ofphotoreceptors: rods and cones. Vertebrates can distinguish color and bright light by combining and comparing signals from cones sensitive to different wavelengths of light. Rods are sensitive to low light levels and drive vision in dim light.

L. erinacea only possesses rods, which can function across large ranges of light intensity. We aim to understand how the skate retina achieves functional duality with a monotypic photoreceptor population. Specifically, we aim to describe how the diversity of horizontal cells (i.e., a downstream partner of rods and cones) and their structural characteristics contribute to the ability of the skate retina to transmit visual information across vastly different levels of illumination. We analyze differences between internal and external horizontal cells (IHC and EHC, respectively) by using serial section EM data to obtain 3D representations of cell structure and cell connectivity.

SB-3DEM was performed on skate retina; the dataset analyzed here was from adult animals with width=57.344µm, height=315.392µm, and depth=34.79µm. Voxel size was 7x7x70nm.

Preliminary 3-D reconstructions of an IHC show a length of ~55.41µm, while reconstructions of an EHC show a length of 54.14µm. Volume and surface for both cells appear to be significantly different from each other, with IHCs having a larger surface area (~1755µm 2) and a smallervolume (~2173 µm 3), and EHCs having a smaller surface area (~1566µm 2) and larger volume (~3963 µm 3). Additional reconstructions of connections of each cell type to rods is ongoing, but preliminary data suggests EHCs contact significantly more rods than IHCs.

Large differences in structural characteristics between IHCs and EHCs suggest a possible division along functional lines. Given that in mixed rod-cone retinas, horizontal cells contact rods or cones very selectively, we hypothesize that IHCs and EHCs also contact rods selectively. However, we propose that this selectivity is based on the numbers and functional range of the contacted rods within the dendritic field of each type of horizontal cell.

B644/P1622

Investigating cell type specific modes of Wnt dispersal *in vivo*

M. Favichia[1], B. Goldstein[2], A. M. Pani[1]; [1]University of Virginia, Charlottesville, VA, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC

Wnts are secreted signaling proteins with essential roles in development, homeostasis, and disease. How Wnt signaling is regulated in space and time is a key question in developmental and cell biology. Wnt ligands can move between cells to activate signaling across a range of distances, and in different contexts Wnts can utilize distinct mechanisms to disperse between cells. However, the cell-biological bases for Wnt dispersal in living animals remain unknown in many cases, and the extent to which dispersal mechanisms vary by cell type, organism, and/or ligand remain uncertain. We are using *C. elegans* as a tractable system for *in vivo* cell biology that is amenable to live imaging and rapid genome engineering. *In vivo* imaging of multiple endogenously tagged Wnts and architectures of Wnt-expressing cells revealed cell-type specific modes of Wnt transport during larval development and a potentially novel mechanism for long-range Wnt signaling. Live imaging of two Wnt homologs, CWN-1 and EGL-20, revealed two scenarios where we hypothesize that neurons, or neuron-like cells, utilize different transport processes to deliver Wnt proteins over long distances to non-neuronal cells. We observed that CWN-1 is highly expressed in Canal Associated Neuron (CAN) cells during larval development, and endogenously tagged CWN-1 localizes to mobile punctae within the bipolar, axon-like CAN projections. Time-lapse imaging revealed anterograde transport for CWN-1 punctae in CAN cells at speeds consistent with kinesin motor proteins. However, CWN-1 appears to spread between cells by extracellular diffusion during embryonic development, which provides an example of cell-type specific Wnt dispersal mechanisms. To test the extent to which Wnt transport in neurons may generally resemble CWN-1 tranport in CAN, we examined Wnt/EGL-20 transport in *egl-20*-expressing neurons with projections to the ventral nerve cord. We observed that endogenous EGL-20 accumulates on cells adjacent to axons of *egl-20*-expressing neurons, and EGL-20 distribution in neighboring cells tracks outgrowth of these axons during development. However, we did not observe clear EGL-20 punctae in axons, which suggests the mode of transport differs from CWN-1 transport in CAN. To test whether differences in transport depend on cell type vs. ligand, we expressed EGL-20::mNG in CANs using a short fragment of the *cwn-1* promoter. Transgenically expressed EGL-20 localized to punctae in CAN cell extensions similar to endogenous CWN-1, which indicates that transport modes for these proteins in their endogenous contexts likely depends on cell type rather than properties of the different ligands. We are currently investigating how CWN-1 is trafficked and secreted from CAN and the functions for Wnt signaling from these cells.

B645/P1623

Urotensin-II-related peptides, Urp1 and Urp2, control zebrafish spine morphology

E. E. Bearce; University of Oregon, Eugene, OR

The spine provides structure and support to the body, yet how it develops its characteristic morphology as the organism grows is little understood. This is underscored by the commonality of conditions in which the spine curves abnormally such as scoliosis, kyphosis and lordosis. Understanding the origin of such spinal curves has been challenging in part due to the lack of appropriate animal models. Recently, zebrafish have emerged as promising tools with which to understand the origin of spinal curves. Using zebrafish, we demonstrate that the Urotensin II-related peptides (URPs), Urp1 and Urp2, are essential

for maintaining spine morphology. Urp1 and Urp2 are 10-amino acid cyclic peptides expressed by neurons lining the central canal of the spinal cord. Upon combined genetic loss of Urp1 and Urp2, adolescent-onset planar curves manifested in the caudal region of the spine, akin to a lordosis-like condition. Highly similar curves were caused by mutation of Uts2r3, an URP receptor. Quantitative comparisons revealed that Urotensin-associated curves were distinct from other zebrafish spinal curve mutants that more closely reflected idiopathic scoliosis or kyphosis. Last, we found that the Reissner fiber, a proteinaceous thread that sits in the central canal and has been implicated in the control of spine morphology, breaks down prior to curve formation in an idiopathic scoliosis model but was unperturbed by loss of Uts2r3. This suggests a Reissner fiber-independent mechanism of curvature in Urotensin-deficient mutants. Overall, our results show that Urp1 and Urp2 control zebrafish spine morphology and establish new animal models of lordosis-like curves.

B646/P1624

The role of Pericentrin in Kupffer's vesicle (KV) formation

Y. WU[1], K. Rasmussen[1], L.-E. Jao[2], H. Hehnly[1]; [1]Syracuse University, Syracuse, NY, [2]University of California Davis School of Medicine, Sacramento, CA

Human diseases such as dwarfism, mental disorders, and ciliopathies are linked to loss-of-function mutations in the centrosome gene, Pericentrin. Pericentrin is a largely conserved centrosome-localized coiled-coil protein that serves as a multifunctional scaffold for protein anchoring (Delaval and Doxsey, 2009). Pericentrin has been linked to regulating cilia formation, spindle assembly and positioning, centrosome positioning, and tissue morphogenesis. Correct spindle positioning, followed by strategic placement of the cytokinetic bridge within the confines of a developing tissue, can ultimately determine where daughter cells are positioned. A report from our lab identified that cell division, specifically the final stage of cell division, cytokinetic bridge placement and cleavage, could potentially be employed for lumen formation *in vivo* (Rathbun et al., 2020). In our current work, we again took advantage of Danio rerio's (zebrafish) left-right organizer, the Kupffer's vesicle (KV), to test the role of Pericentrin in directing KV cell patterning events. To do this, we compared wild-type ($pcnt^{+/+}$) to $pcnt^{-/-}$ as well as $pcnt^{+/-}$ embryos. KV development requires a population of migratory-like progenitor cells (15-20 cells) to potentially proliferate, then self-assemble into a rosette-like structure. This structure then transitions into a cyst of ciliated cells with a fluid-filled lumen located at the base of the notochord (approximately 100-200 cells in total). Our studies identified that $pcnt^{-/-}$ as well as $pcnt^{+/-}$ embryos have significantly fewer cilia and a decreased lumen area compared to the control animals ($pcnt^{+/+}$). To determine if this was due to defects in mitotic progression, we first needed to better characterize the mitotic and post-mitotic population of KV cells under control conditions. Interestingly, we not only identified a significant mitotic (phospho-Histone3B$^+$) and post-mitotic (BrdU$^+$) population of KV cells, but also that both populations were enriched towards the notochord. This finding suggests that mitotic activity is spatially confined during KV development. A significant decrease in KV cells number was found in $pcnt^{+/-}$ embryos compared to control. This finding suggests that the loss of Pericentrin results in KV cell proliferation defects. Our future directions are to carefully characterize $pcnt^{+/-}$ and $pcnt^{-/-}$ KV cell mitotic behavior and organization during KV development, and further characterize downstream developmental defects that may occur (e.g., congenital heart defects, laterality defects, and overall size differences).

Embryogenesis

B647/P1625

Optogenetic rescue of a patterning mutant

H. E. Johnson[1], N. J. V. Djabrayan[2], S. Y. Shvartsman[3], J. E. Toettcher[4]; [1]Biological Sciences, The University of Hong Kong, Hong Kong, HONG KONG, [2]Visiting Assistant Professor, Clark University, Boston, MA, [3]Chemical and Biological Engineering, Princeton University, Princeton, NJ, [4]Molecular Biology, Princeton University, Princeton, NJ.

Animal embryos are partitioned into spatial domains by successive patterns of intracellular signaling and gene expression. Yet in most cases it is unknown which pattern features are required to support normal development. In one such example early in *Drosophila* development, receptor tyrosine kinase (RTK) activity leads to an evolving gradient of Erk activity, patterns gene expression, and the specification of tissues / morphogenic movements. The ideal experiment to determine the required features of signaling would be to abolish this signaling to create a “blank canvas” and “paint” arbitrary signaling patterns back onto it. To accomplish this, we developed a light-inducible system activate Erk in early Drosophila embryo and expressed it in a mutant background devoid of a forementioned RTK signaling. In this background, we systematically test the requirements of early embryo Erk signaling for cell fates, gene expression, and the formation of a viable embryo. Surprisingly, we find that the embryo is quite robust to spatial perturbations in the signal, as simple all-or-none light inputs were able to completely rescue normal embryogenesis, generating viable larvae and fertile adults from an otherwise-lethal genetic mutant. Systematically varying illumination parameters further revealed that at least three distinct developmental programs are triggered at different signaling thresholds and that the morphogenetic movements of gastrulation are robust to a 3-fold variation in the posterior pattern width. These results have opened the door to studies using these techniques to reveal the role of more complex signaling dynamics during development. Through live imaging of signaling biosensors, we have begun to map intricate signaling dynamics during development. We are now extending these optogenetic techniques to these complex developmental events and signaling dynamics to understand their function.

B648/P1626

How conserved signaling pathways build the unique tardigrade body

K. L. Heikes[1], B. Goldstein[2]; [1]Genetics and Molecular Biology, UNC Chapel Hill, Chapel Hill, NC, [2]Biology, UNC Chapel Hill, Chapel Hill, NC.

Embryonic development depends on precise communication between cells to establish patterns, such as germ layers and tissue morphology. Most knowledge of developmental patterning mechanisms stems from studies using only a small number of model systems. Emerging models provide means to explore how the same signaling pathways are used to construct organisms of diverse shapes. The tardigrade *Hypsibius exemplaris* is one such system. Although tools to study *H. exemplaris* are limited, methods do exist for fluorescence in situ hybridization (FISH). We studied the Bone Morphogenetic Protein (BMP) and Fibroblast Growth Factor (FGF) pathways at 24 hours post laying, using FISH to reveal expression patterns of *H. exemplaris* homologs to build a picture of where these pathways function. We found that the BMP receptors thickveins (*tkv*) and punt (*put*) are ubiquitously expressed, which suggests that most cells are receptive to BMP signaling. The BMP ligand decapentaplegic (*dpp*) is enriched in a set of cells laterally along the left and right sides of the embryo in the outermost (presumptive ectoderm) layer of

cells, and the BMP antagonist short gastrulation (*sog*) is enriched in a similar pattern. We examined *dpp* and *sog* in combination using double FISH and found that the *dpp* pattern is more dorsal than the *sog* pattern. Applying knowledge of BMP signaling from other systems, we hypothesized that BMP signaling functions laterally in *H. exemplaris* to either establish ectodermal fate, pattern the cuticle, pattern the limbs, or a combination of these roles. We also examined expression of the FGF ligand (*fgf8*) and the most highly expressed FGF receptor (*fgfr1*). We found that *fgf8* is expressed in the outermost layer of cells in a striped pattern, likely overlapping with the posterior end of each segment boundary, and *fgfr1* is expressed in a more internal layer of cells, overlapping with the regions where the presumptive endomesodermal pouches form within each segment. We hypothesized that FGF signaling specifies the fate of the cells in these pouches. We will address these hypotheses using chemical inhibitors of BMP and FGF signaling to see how disruption of these pathways impacts development. This work collectively sheds light on how the unique tardigrade body develops and expands our knowledge of the ways conserved signaling pathways are used to build diverse organisms.

B649/P1627

ATF6 responses to ER stress under high temperature during early porcine embryonic development

M. SUN, X. Cui; Chungbuk National University, Cheongju, KOREA, REPUBLIC OF.

Activating transcription factor 6 (ATF6), one of three sensor proteins in the endoplasmic reticulum (ER), is an important regulator in the ER stress-induced apoptosis pathway. ATF6 resides in the ER and upon activation is translocated to the Golgi apparatus, where it is cleaved by site-1-protease (S1P) to generate an amino-terminal cytoplasmic fragment. Although recent studies have made some progress in elucidating the regulatory mechanisms of ATF6, the function of ATF6 during early porcine embryonic development under high temperature remains unclear. In this study, zygotes were divided into 3 groups: control, high temperature (HT) group, and HT+ATF6 knockdown group. The results showed that ATF6 is expressed at all stages during embryonic development, with ATF6 clustered around the nucleus at 2- and 4-cell stages and localized in the nucleus at BL stage. Meanwhile, the HT decreased blastocyst rate, and increased levels of ATF6 protein expression and lead to a nuclear localization of ATF6 upon induction of 4-cell stage. Next, the expression level of ATF6 was knocked down in embryos by microinjection of ATF6 dsRNA, and the results showed that the reduction of ER marker Calnexin expression, Ca^{2+} release and abnormal ER distribution caused by HT can be alleviated by reducing ATF6 expression. In addition, ATF6 knockdown also reduced the level of embryonic apoptosis mediated by elevated AIFM2 expression in heat-exposed embryos. In conclusion, the results suggest that ATF6 is an important mediator of HT-induced ER stress as well as apoptosis during early porcine embryonic development. Key words: ATF6; embryo; high temperature; ER stress; apoptosis

B650/P1628

Effect of YBX1 on Early Embryonic Development in Pigs

W. Jiang, X. Cui; Chungbuk National University, Cheongju, KOREA, REPUBLIC OF.

Y-box binding proteins, including YBX1, are a group of proteins found in bacteria, plants, and animals. YBX1 is a member of the family of DNA- and RNA-binding proteins, that plays vital roles in multiple aspects, including RNA stabilization, translational repression, and transcriptional regulation, but its roles remain less known during pre-implantation development. In this study, to investigate the function of YBX1 and its mechanism of action in porcine embryo development, YBX1 was knocked down by

microinjecting double-stranded RNA of YBX1 at the 1-cell stage. YBX1 was located in the cytoplasm during embryo development. The mRNA level of *YBX1* was increased from 4-cell stage to blastocyst but it was significantly decreased in YBX1 knockdown embryos compared with the control. Moreover, the percentage of blastocysts was decreased in following YBX1 knockdown compared with the control. Defecting YBX1 expression decreased the level of m^6A, METTL3, and YTHDF2 at 4-cells and blastocyst. Additionally, YBX1 depletion reduced histone modification (H3K9ac and H3K4me2) at 4-cells. YBX1 knockdown increased the unfolded protein response and endoplasmic reticulum stress. In conclusion, YBX1 is essential for early embryo development by regulating mRNA stability and translation.

B651/P1629

GRP78 is Required for the Stabilization of MC4R in Porcine Embryos

G. Heo, X. S. Cui; ChungBuk National University, Seowon-gu, Cheongju-si, Chungcheongbuk-do, KOREA, REPUBLIC OF.

GRP78(Glucose-Regulated Protein 78) is a protein known to be overexpressed in the context of glucose deficiency. Currently, many studies have been conducted on the increase in expression of GRP78 due to Endoplasmic Reticulum (ER) stress. However, there are no studies examining the role of GRP78 in germ cells, particularly porcine embryos. Therefore, the purpose of this study was to investigate the role of GRP78 in porcine embryonic development. HA15, an inhibitor of GRP78, was added to the culture medium and embryos were cultured for 7 days. Inhibition of GRP78 reduced the activity of MC4R and subsequently decreased the activity of Gαs, a type of G protein. As evidence, it was confirmed that the amount of cAMP was decreased in porcine blastocyst in which GRP78 was inhibited. Also, CDK1, CDK2, p-ERK 1/2, and APC/C are proteins related to cell division, and their expression was reduced in the HA15-added group compared to the control. As expected, the expression of p-DRP1 was increased in GRP78-inhibited blastocyst. PGC1α and SIRT1, which induce mitochondrial biogenesis, were decreased in porcine embryos lacking GRP78. Furthermore, inhibition of GRP78 led to mitochondrial dysfunction including decreasing ATP content, mitochondrial membrane potential, and active mitochondria. In conclusion, GRP78 is essential for the development of porcine embryos due to GRP78 regulates the G protein-coupled receptor pathway and cell proliferation by contributing to the internalization and stabilization of MC4R and it also helps to maintain the function of mitochondria.

B652/P1630

Effect of Zscan4 on porcine embryonic development

X. Li, X. Cui; Chungbuk National University, Cheongju, KOREA, REPUBLIC OF.

Zinc finger and SCAN domain containing 4 (Zscan4) is a DNA-binding protein which involved in the regulation of telomere elongation and genomic stability in mouse embryos and embryonic stem cells. However, the effect of Zsacn4 on telomere length in porcine preimplantation embryos remains less known. In this study, to investigate the function and underlying mechanism of Zscan4 in porcine embryo development, Zscan4 was knocked down by microinjection of dsRNA and Dnmt1 was inhibited by the inhibitor at the 1-cell stage. Zscan4 was highly expressed at the 4-cell and 5-8-cell stage of porcine embryos. The percentage of 5-8 cell stage embryos and blastocyst were decreased in Zscan4 knockdown group compared to control. Notably, depleting Zscan4 induced the protein expression level of Dnmt1 as well as DNA methylation (5mC) at the 4-cell stage. Meanwhile, the expression levels of Tet2 and H3K9me3 were decreased following Zscan4 knockdown. The q-PCR results showed that Zscan4

knockdown led to decrease of *Trf1* mRNA expression level and the shorten telomere length compared to control. In addition, the Hinokitiol, a kind of Dnmt1 inhibitor, was used to reduce DNA hypermethylation in Zscan4 knockdown embryos. Dnmt1 inhibition rescued the blastocyst defects caused by Zscan4 knockdown. In conclusion, Zscan4 is essential for maintain of telomere length and genomic stability by regulating Dnmt1 expression during early porcine embryonic development.

B653/P1631

Alpha-lipoic acid Attenuates Heat stress-induced Oxidative Stress in Porcine Embryos

S. Lee, X. Cui; Chungbuk National University, Cheongju, KOREA, REPUBLIC OF.

Heat stress (HS) is one of the long-standing barriers that animals face in the living environment and has deleterious effects on reproductive performance and may ultimately lead to infertility. Alpha-lipoic acid (ALA) is a strong antioxidant synthesized by plants and animals, known as a cofactor for mitochondrial bioenergetic enzymes. Previous studies have shown that ALA has a protective effect on oxidative stress and endoplasmic reticulum stress caused by heat damage in mammalian reproductive system. However, the mechanism of ALA in HS-induced early porcine embryonic development has not been investigated. In this study, parthenogenetically activated porcine zygotes were divided into 3 groups: control, high temperature (HT) group (42℃ for 11 h, treated from 24 h to 35 h after activation), and ALA+HT group (with 10 μmol/L ALA). The results showed that HT treatment significantly reduced the rate of blastocyst compared to the control. Addition of ALA partially recovered the rate of blastocyst and improved the quality of blastocyst, which indicating large in diameter of blastocyst, more total cell numbers, and lower number of TUNEL positive cells in blastocysts were detected by ALA treatment compared to the HT group. Moreover, supplementation of ALA significantly reduced reactive oxygen species level and higher glutathione level, suggesting ALA reduced oxidative stress induced by HS. This study showed that the mRNA expression of heat shock protein 70 (*HSP70*), heat shock protein 40 (*HSP 40*), and the protein level of heat shock factor 1 (HSF1) were increased in ALA+HT group compared to HT group. Collectively, this study revealed that supplementation of ALA ameliorates the HS-induced oxidative stress by increasing the expression of heat shock response genes, which eventually improve the quality of HS-induced porcine embryo.

Keyword: Temperature, Alpha-lipoic acid, Oxidative stress, Heat shock protein

B654/P1632

Nascent Transcriptome Reveals Orchestration of Zygotic Genome Activation in Early Embryogenesis

H. Chen[1], M. C. Good[1,2]; [1]Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [2]Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA.

Early embryo development requires maternal-to-zygotic transition, during which transcriptionally silent nuclei begin widespread gene expression during zygotic genome activation (ZGA). ZGA is vital for early cell fating and germ layer specification, and ZGA timing is regulated by multiple mechanisms. However, controversies remain about whether these mechanisms are interrelated and vary among species and whether the timing of germ layer-specific gene activation is temporally ordered. In some embryonic models, widespread ZGA onset is spatiotemporally graded, yet it is unclear whether the transcriptome follows this pattern. A major challenge in addressing these questions is to accurately measure the timing of each gene activation. Here, we metabolically label and identify the nascent transcriptome using 5-

ethynyl uridine (EU) in *Xenopus* blastula embryos. We find that EU-RNA-seq outperforms total RNA-seq in detecting the ZGA transcriptome, which is dominated by transcription from maternal-zygotic genes, enabling improved ZGA timing determination. We uncover discrete spatiotemporal patterns for individual gene activation, a majority following a spatial pattern of ZGA that is correlated with a cell-size gradient. We further reveal that transcription necessitates a period of developmental progression and that ZGA can be precociously induced by cycloheximide, potentially through elongation of interphase. Finally, most ectodermal genes are activated earlier than endodermal genes, suggesting a temporal orchestration of germ layer-specific genes, potentially linked to the spatially graded pattern of ZGA. Together, our study provides fundamental new insights into the composition and dynamics of the ZGA transcriptome, mechanisms regulating ZGA timing, and its role in the onset of early cell fating.

B655/P1633

A Natural Cell-size Gradient Dictates Patterning of Genome Activation and Vertebrate Early Development

W. Qian[1,2], H. Chen[1], M. C. Good[1,2,3]; [1]Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [2]Cell and Molecular Biology Graduate Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [3]Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA.

Early embryogenesis features cell division without growth. After rapid reduction in cell volume, the cell size or the DNA-to-cytoplasm ratio plays a pivotal role in thresholding the onset of zygotic genome activation (ZGA) in multiple species. However, the functional importance of the pattern of cell size in early development is largely unknown. To address this question, we use the early *Xenopus* embryo because it contains a natural gradient of cell size along the animal-vegetal (AV) axis, varying over 1000-fold in volumes between the smallest cells at the animal pole and the largest cells at the vegetal pole. This variation displays a cell-size-dependent spatial pattern of ZGA onset, which initiates transcription first at the animal pole and delays in the vegetal pole. Here we leverage a temperature-gradient controller to alter the cell-size gradient in the early *Xenopus* embryo by changing cell division rate along the AV axis. Interestingly, we discover that ZGA onset inversely correlates with the cell size regardless of the location of cells in space, corroborating a critical role of a cell-size threshold in regulating ZGA. We further characterize the phenotypes of the embryos with an inverted cell-size gradient along the AV axis, depending on the extent of inversion. Strikingly, extremely inverted embryos lyse before the gastrula stage, potentially linked to an incongruous ZGA pattern caused by the inversion of the cell-size gradient. Moreover, targeted transcriptional inhibition at the animal pole, where ZGA firstly occurs in a normal embryo, phenocopies the embryonic lysis, suggesting a critical role of a natural cell-size gradient in early development depending on the proper spatiotemporal order of zygotic transcription. Finally, moderately inverted embryos show defects in eyes and heads at the tadpole stage, which are ectodermal tissues derived from the animal pole. Together, our study reveals the considerable significance of cell-size gradient in coordinating genome activation in space and shaping early embryo development.

B656/P1634

Arvcf catenin links subcellular actomyosin dynamics to tissue scale force production during vertebrate axis extension.

R. J. Huebner, S. Weng, C. Lee, S. Sarıkaya, O. Papoulas, R. M. Cox, E. M. Marcotte, J. B. Wallingford; The University of Texas at Austin, Austin, TX.

Anterior-posterior (head-tail) axis extension is an early and essential step in animal development and this process requires the coordinated movement of proteins, cells, and whole tissues. Thus, a challenge to understanding axis extension is connecting events that occur across these various length scales. Here we describe how a poorly characterized adhesion effector, Arvcf catenin, controls head-to-tail axis extension during gastrulation in *Xenopus laevis*. We find that Arvcf is required for extension of the intact embryo but surprisingly is not required for extension of isolated tissues. Next, we determined that the organism scale phenotype results from a modest defect in tissue scale force production that was only observable when the tissue experienced external resistance (e.g., surrounding tissues in the animal). Finally, we link the tissue scale force defect to a dampening of the pulsatile recruitment of cell adhesion and cytoskeletal proteins to membranes. These results not only provide a comprehensive understanding of ARVCF function during axis extension but also provide insight into how a cellular scale defect in adhesion results in an organism scale failure in development.

B657/P1635

Filopodia-mediated morphogenic cytokinesis underlies *C. elegans* embryonic handedness

Y. Xiong, K. Sugioka; University of British Columbia, Vancouver, BC, Canada

Chirality is one of the fundamental molecular and cellular properties that controls animal morphogenesis. In organisms with bilateral symmetries, molecular and cellular chirality specify the larger scale organismal handedness, wherein organ's relative positioning and shape exhibit left-right asymmetry. In *Caenorhabditis elegans* and pond snails, body handedness is first specified during early embryonic cell division in a CYK-1/Formin-dependent manner (Middelkoop *et al.*, PNAS, 2021; Davison *et al.*, Curr. Biol., 2016; Kuroda *et al.*, Sci. Rep., 2016). However, the mechanism underlying cell division-dependent chiral morphogenesis remains elusive. Here, we found that the contractile ring tilting and filopodia-dependent ring-cell surface coupling underly chiral morphogenesis in *C. elegans*. *C. elegans* organismal handedness is specified during cytokinesis of ABa and ABp blastomeres at the 4-cell stage. Division axes of ABa and ABp are orthogonal to the anterior-posterior axis until early anaphase, but they tilt due to the clockwise rotation during cytokinesis. To identify the intrinsic and extrinsic cues that regulate chiral morphogenesis, we performed the blastomere isolation assay to eliminate and reconstitute cell-cell interactions. We found that adhesion between ABa and ABp is sufficient for chiral morphogenesis and that the attachment of adhesive polystyrene bead to either ABa or ABp induced the clockwise cell rotation. Consistently, the contractile rings of ABa and ABp in intact embryos undergo clockwise rotation relative to the ABa-ABp boundary, resulting in non-parallel alignment of the two rings. Furthermore, we found the Formin-dependent formation of interlocking filopodia at the ABa-ABp boundary. Interestingly, filopodia extending from ABp is pulled by the ABa contractile ring and vice versa. Because of the non-parallel ring alignment, ABa and ABp contractile rings pull ABp and ABa cell surfaces toward the anterior right and posterior left, respectively, leading to chiral blastomere arrangement. Our results reveal the novel contractile ring-dependent morphogenesis mechanism critical for shaping the embryonic body plan.

B658/P1636

Role of mechanical forces during morphogenesis of the left-right organizer

E. M. Retzlaff[1], P. C. Sanematsu[2,3], J. D. Amack[1], M. L. Manning[2]; [1]Cell and Developmental Biology, SUNY Upstate Medical University Library - Syracuse, NY, Syracuse, NY, [2]Department of Physics, Syracuse University, Syracuse, NY, [3]BioInspired Syracuse: Institute of Material and Living Systems, Syracuse, NY.

In vertebrates, several internal organs develop asymmetries along the left-right (LR) body axis. Defects in LR asymmetry result in a broad spectrum of clinical complications. A transient structure referred to as the 'left-right organizer' (LRO) directs LR patterning of vertebrate embryos. During morphogenesis of the zebrafish embryo's LRO, which is called Kupffer's vesicle, LRO cells bearing motile cilia undergo regional shape changes along the anterior-posterior (AP) axis. Anterior cells take on columnar shapes that allow tight packing, whereas posterior cells become cuboidal-like and more widely spaced apart. These shape changes create an AP asymmetric concentration of beating cilia that is critical for establishing LR asymmetric fluid flows and patterning cues. However, the mechanisms that control LRO cell shape changes are not understood. We are using a combination of quantitative live imaging and 3-dimensional (3D) computational modeling to test the hypothesis that coordinated biophysical forces provide a mechanism that regulates LRO cell shape changes. During early somite stages when the embryonic AP axis extends, we find that the LRO moves faster towards the posterior of the embryo than surrounding cells, possibly being 'pushed' by convergence and extension of cells in the rod-like notochord. 3D modeling simulations indicate mechanical drag forces generated as the LRO moves through the surrounding tissue can drive LRO cell shape changes. To experimentally test the impact of these forces on LRO cell shapes *in vivo*, we use light-activated small molecules to alter actomyosin contractility in selected cells surrounding the LRO. This optical approach allows spatial and temporal of control of mechanical properties of tissues involved in generating 'pushing' and drag forces that can be used to systematically define the role of extrinsic mechanical forces on LRO morphogenesis and function. This work is supported by NIH grant R01HD099031.

B659/P1637

Mouse nodal immotile cilia sense bending direction for left-right determination: Mechanical regulation in initiation of symmetry breaking

T. A. Katoh[1], T. Omori[2], K. Mizuno[3], T. Itabashi[4], A. H. Iwane[4], T. Ishikawa[2], Y. Okada[5,6], T. Nishizaka[7], H. Hamada[1]; [1]RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, JAPAN, [2]Grad. Sch. Eng., Tohoku Univ., Sendai, JAPAN, [3]Fac. Med. Sci., Univ. of Fukui, Fukui, JAPAN, [4]RIKEN Center for Biosystems Dynamics Research (BDR), Hiroshima, JAPAN, [5]RIKEN Center for Biosystems Dynamics Research (BDR), Osaka, JAPAN, [6]Grad. Sch. Med., Grad. Sch. Sci., UBI, WPI-IRCN, The Univ. of Tokyo, Tokyo, JAPAN, [7]Fac. Sci., Gakushuin Univ., Tokyo, JAPAN.

How is the left-right (L-R) asymmetry of a mammalian body determined? Cilia at the ventral node of mouse early embryos play an essential role in symmetry breaking. While motile cilia located at the central region of the node generate the leftward nodal flow, immotile cilia located at the periphery of the node sense flow-dependent signals, and only L-side ones are activated. The cation channel Pkd2 localized at immotile cilia is required for sensing the flow and triggers *Dand5* mRNA decay, however, it has long remained elusive how cilia sense the flow and why only the L-side ones are activated. **Asymmetric passive deformation along the dorsoventral axis in response to the flow:** How immotile

cilia behave in response to the nodal flow *in vivo*: High-speed imaging revealed low-frequency motion is important for symmetry breaking. Thus, the steady-state deformation of cilia was examined under controlling nodal flow by UV laser. The shape of the same cilium in the presence or absence of the flow were observed by 3-D high-resolution microscopy: the L-side cilia showed ventral bending (5.0±9.2°; *n*=21), whereas the R-side ones showed dorsal bending (4.2±7.4°; *n*=18). **Mechanical stimuli to immotile cilium trigger *Dand5* mRNA degradation and Ca^{2+} transient:** We next tested whether immotile cilia of *iv/iv* embryos (which lack nodal flow) respond to mechanical force with the use of optical tweezers. Significant degradation of *Dand5* mRNA was induced at 2h after the mechanical stimulation (53±21%; *n*=28), while it was not detected with *Pkd2*$^{-/-}$ embryo (110±14%; *n*=7). The cytoplasmic Ca^{2+} frequency is increased significantly from 0.83±0.71 to 1.39±1.65 spikes/min (*n*=42) in response to mechanical stimuli. **Immotile cilia sense bending direction in a manner dependent on polarized localization of Pkd2:** We found that the localization of the Pkd2 channel, a candidate of mechanosensitive channel, was significantly biased toward the dorsal side (0.54±0.12; *n*=50), which could explain how cilia sense the direction. In the presence of the nodal flow, the ventral bending of the L-side cilium imposes a strain of 0.014±0.013 (*n*=8) to the dorsal side of them, which is sufficient to activate dorsally localized Pkd2 and trigger the Ca^{2+} response. In contrast, strain at the R-side cilium is 0.000±0.001 (*n*=8), would not support a response. Indeed, the induction of Ca^{2+} transients was observed preferentially by mechanical stimuli directed toward the ventral side. These observations explain the differential response of immotile cilia to the directional flow [1]. **Reference:** [1] Katoh, Takanobu A., et al. "Immotile cilia of the mouse node sense a fluid flow-induced mechanical force for left-right symmetry breaking." *bioRxiv* (2022).

B660/P1638

Identifying the role of SOX9 in neural crest EMT

H. R. Menendez, 1st; Anatomy, Physiology, and Cell Biology, University of California, Davis, Davis, CA.

Neural crest cells (NCC) are a population of stem-like, transient, multipotent cells in developing vertebrate embryos. These cells begin as tightly adherent epithelial cells and then undergo an epithelial to mesenchymal transition (EMT) where they invade distant tissues and differentiate. NCCs become more than 30 different tissue and cell types in adults including craniofacial bone and cartilage and the peripheral and enteric nervous systems. Sox9 is a definitive NC marker that plays a role in NCC development and EMT; however, the functional mechanism by which SOX9 regulates NC EMT and migration remains unknown. Our work aims to define the level of functional conservation between SOX9 and other NC gene regulatory network (GRN) markers across species. We will additionally identify how perturbations of SOX9 alter the expression of other NC GRN markersand the formation of craniofacial structures. Using Gallus gallus and Coturnix japonica, my work uses SOX9 perturbations prior to NC EMT to identify the necessity and sufficiency of SOX9 to drive NC behaviors and embryonic craniofacial development. I hypothesize that Sox9 expression is activated by PAX7 and SOX10 transcription factors and that SOX9 drives NC migration by regulating the expression of genes coding for cadherin proteins involved in EMT. Using a combination of gain and loss of function experiments followed by immunohistochemistry and histological analysis, my findings show that a reduction of SOX9 stunts NC migration distance, but SOX9 alone is not sufficient to drive differences in migration. Overall, my project will illuminate the role that SOX9 plays in NC cell development, migration, and craniofacial morphogenesis.

B661/P1639

The role of signaling sensitivity in congenital heart defects and heterotaxy

J. H. Kong[1], C. B. Young[2], G. Pusapati[3], F. H. Espinoza[3], C. B. Patel[3], J. F. Bazan[4], T. M. Gunn[5], C. W. Lo[6], R. Rohatgi[1]; [1]Biochemistry and Stanford Cardiovascular Institute, Stanford University, Stanford, CA, [2]Princeton University, Princeton, NJ, [3]Stanford University, Stanford, CA, [4]ħ Bioconsulting llc, Stillwater, MN, [5]McLaughlin Research Institute, Great Falls, MT, [6]University of Pittsburgh, Pittsburgh, PA.

Hedgehog signaling is essential for the development of many tissues and organs. Hedgehog ligands are morphogens and thus direct cell fate decisions in a manner dependent on signaling strength. Precise regulation of this signaling strength is critical for embryonic development, as even modest disruptions in the signaling amplitude can result in severe birth defects. Previous studies have shown that signaling strength is influenced by both the morphogen concentration and the duration of morphogen exposure. However, an equally important layer of regulation remains unrecognized and understudied -- How does a target cell regulate its sensitivity to a morphogen? The central focus of our research examines the mechanisms that modulate a cell's sensitivity to extracellular morphogens. Initially, genome-wide CRISPR screens were used to identify a novel protein complex (the MMM complex) that suppresses sensitivity to Hedgehog ligands. The MMM complex consists of three proteins: the tetraspan MOSMO, single-pass transmembrane protein MEGF8, and E3 ligase MGRN1. We discovered that the MMM complex is a membrane-tethered ubiquitination complex that promotes the degradation of the Hedgehog pathway transducer Smoothened. Remarkably, developing embryos are exquisitely sensitive to mutations in the MMM complex and the gradual loss of functional MMM alleles corresponds with a gradual increase in the prevalence of left-right patterning defects and severe heart malformations. Overall, our work has identified a novel cellular pathway that regulates morphogen signaling sensitivity and provides insight into the mechanisms and complex genetics that underlie defects in left-right patterning and heart development.

B662/P1640

An epigenetic factor *KANSL1* is involved in neural progenitor proliferation in the adult mouse brain

B. Jeon[1], T. Ford[2]; [1]Kent State University, Kent, OH, [2]Department of Biological Sciences, Kent State University, Kent, OH.

KAT8 regulatory NSL complex subunit 1 (KANSL1) is a component of a chromatin remodeling complex and plays a role in histone modifications such as methylation and acetylation. While KANSL1 dysfunction is associated with neurodevelopmental conditions, its neurobiological function in the brain remains unclear. We found a significant decrease in neural progenitor proliferation in the hippocampal dentate gyrus of *Kansl1* heterozygous knockout mice compared to wild type controls. In addition to the impairment in the hippocampus, progenitor proliferation in the subventricular zone appeared to be disrupted in *Kansl1* knockout mice as the olfactory bulb size was markedly reduced compared to the control bulb size. The number of dividing progenitors identified by BrdU tracing or phospho-histone H3 immunostaining was reduced in the mutant mice. We also examined neurodevelopmental behaviors in *Kansl1* knockout mice and found learning and memory impairments. The Morris water maze test revealed that *Kansl1* mutant mice spent more time in locating the hidden platform over the learning period than control mice. The probe trial at the end of the learning process showed defective memory maintenance or retrieval in the mutant mice. Our results demonstrate a critical role of KANSL1 in brain

cell proliferation and shed light on the potential link between neurodevelopmental pathology and abnormal neurogenesis mediated by chromatin remodeler dysfunction.

B663/P1641

Connexin Function in Zebrafish Lens Development

T. Liang, S. Y. Choi; Biomedical Sciences, Chonnam National University Medical School, Hwasun 58128, KOREA, REPUBLIC OF.

A functional visual system is essential for species such as humans and zebrafish, in which vision is the primary sensory pathway for seeking food. Zebrafish is frequently used in ophthalmology research because of its amenability to genetic manipulation and easy assessment of visual function. There are two types of cells in zebrafish lens: lens epithelial cells (LECs) and lens fiber cells (LFCs). LECs form a monolayer covering the spherical LFCs, and an organelles-free zone begins in the center when the lens becomes a functional optical element in the visual pathway. Gap junctions are intercellular channels that mediate the transfer of small molecules between adjacent cells, and Connexins are vertebrate-specific gap junction proteins. Thirty-nine zebrafish *connexin* genes have been reported to date. We have screened 30 zebrafish *connexins* for lens-specific expression and found that *cx23, cx44.1, cx48.5,* and *cx50.5* were expressed in developing zebrafish lens. To explore roles for these Connexins in the development of zebrafish lens, we downregulated expression of these genes using CRISPR/Cas9 technology.Whole mount in situ hybridization (WISH) using *cx23, cx44.1, cx48.5,* and *cx50.5* riboprobes showed that all four *connexins* were expressed in primary LFCs and LECs at one day post-fertilization (dpf). *cx23, cx44.1,* and *cx48.5* were expressed at both LFCs and LECs at 2-5 dpf, whereas *cx50.5* was expressed only in LFCs at 2-5 dpf. In addition, the *cx44.1* and *cx48.5* crispants showed reduced lens size than control at 1-5 dpf, whereas *cx23* and *cx50.5* did not. Generation of mutant zebrafish lacking these genes, either alone or in combination, is ongoing. In conclusion, *cx44.1* and *cx48.5* may play a vital role in the development of the zebrafish lens.

B664/P1642

Dysregulated trophoblast invasion and differentiation in Preeclampsia results from a reactive oxygen species-mediated endoplasmic reticulum stress pathway

I. Mukherjee[1,2], S. Singh[1], J. B. Sharma[3], S. Biswas[4], R. Dhar[1], S. Karmakar[1]; [1]Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, INDIA, [2]Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, INDIA, [3]Department of Obstetrics & Gynecology, All India Institute of Medical Sciences, New Delhi, INDIA, [4]Amity Institute of Molecular Medicine & Stem Cell Research, Amity University, Noida, Uttar Pradesh, INDIA.

Pre-eclampsia (PE) is a leading cause of maternal and fetal mortality and morbidity. This high-risk condition needs close monitoring, possibly an early delivery if beyond 34 weeks as per ACOG guidelines. PE is a pregnancy-related disorder having trophoblast-mediated poor vascular modification with inadequate perfusion resulting in insufficient uterine blood flow, which in turn induces hypoxia leading to placental ischemic-reperfusion injury. This results in reactive oxygen species (ROS) mediated oxidative stress (OS) and tissue injury. PE is often associated with placental pathology defects possibly resulting from ROS-driven aberrant differentiation-invasion program, details of which are largely unknown. Therefore, we aimed to delineate the molecular pathogenesis of ROS on trophoblast and its effect on placenta function. Post-delivery placenta with paired blood samples was collected from 25 clinically

established PE patients and 20 healthy subjects for making organoids. To recapitulate the ROS-mediated effect, trophoblast-derived cell line HTR-8/SV neo and BeWo were treated with a sub-lethal dose of H_2O_2 *in vitro*. Cell viability was determined, and the impact of ROS was confirmed by estimating lipid peroxidation. Elaborate transcriptomic and GSEA analyses were performed to identify the differentially expressed genes (DEGs) between exposed and control groups. Electron microscopy (EM) based ultrastructural changes in placental tissues were studied. Results showed OS significantly affected the differentiation and syncytialization in trophoblasts, both of which are crucial placental functions. ROS also severely enhanced the invasiveness and motility of these cells as evident from the Boyden chamber assay. TEM showed ROS-driven signatures of intracellular injury as swollen endoplasmic reticulum (ER), cell membrane damage, reduction of glycogen granules, and mitochondrial disintegration. Interestingly, these were also found abundantly in PE organoids, thereby validating our ex-vivo PE model. ROS also induced the classical unfolded protein response (UPR) in trophoblasts through the engagement of the BiP-XBP1s axis which was in accordance with the endoplasmic reticulum (ER) stress response reported earlier. Our findings elucidated the molecular pathogenesis in PE subjected to OS that has profoundly influenced trophoblast differentiation and invasion. We are currently validating these in our organoids to explain the effect of ROS on transcription and to modulate the function of uterine immune cells, thereby affecting the cytokine milieu at the feto-placental unit. Our study will be helpful for mechanistic details and future intervention for the basic scientists and clinicians for effective disease management.

B665/P1643

Role of CHD7 in radiation-induced fetal malformations

A. Noda, K. Muramoto, S. Mishima; Radiation Effects Research Foundation, Hiroshima City, Japan

In the process of studying the biological effects of unrepairable DSBs caused by radiation exposure, we found ATM-dependent phosphorylation of the transcription factor CHD7. CHD7 is a transcription factor in early development, governing the morphogenesis of the heart and cranial sensory organs such as the eyes, mouth, nose and brain from neural crest cells. CHD7 gene haploinsufficiency induces fetal malformations and is known in humans as the causative gene of CHARGE Syndrome. Homozygous mutation causes embryonic death. CHD7 is strongly expressed during morphogenesis stage. We found that CHD7 was phosphorylated by radiation exposure, released from the native promoter binding, and relocated to DSBs in mouse embryos. Therefore, it was found that CHD7 has dual role; transcription factor for morphogenesis and DSB repair foci factor for stress response. Based on these results, we propose that a morphogenesis coupled DSB repair mechanism exists in early development of higher animals. For radiation-induced malformation, a possible mechanism is that a large amount of CHD7 protein is devoted to DSB repair during high-dose radiation exposure, resulting in incompleteness of morphogenesis.

B667/P1644

Optogenetic manipulation of endogenous Rho signaling in the *Drosophila melanogaster* germband

A. D. Countryman[1], M. S. Puri[1], C. A. Doherty[2], S. Y. Shvartsman[2], K. E. Kasza[1]; [1]Columbia University, New York, NY, [2]Princeton University, Princeton, NJ

Despite significant insight into the physical mechanisms of animal development, the precise reconstitution of morphogenetic processes for science and engineering applications remains a challenge. Optogenetic manipulation of Rho signaling provides a promising avenue to achieve spatial,

temporal, and quantitative control over actomyosin-generated contractile forces and tissue folding processes in epithelia. Here, we use CRISPR/Cas9 gene editing in *Drosophila melanogaster* to generate a novel optogenetic tool via the endogenous tagging of RhoGEF2 with components of the iLID/SspB optogenetic system. This endogenous tagging approach is designed to produce flies that have normal levels and patterns of RhoGEF2 protein. Spatially localized blue light activation of the tool in the embryonic ectoderm recruits RhoGEF2 to the cell membrane and induces an invagination in the targeted area. Local activation of the tool in the converging and extending germband is sufficient to drive invagination in the intercalating tissue, demonstrating the ability to override the existing morphogenetic program and providing the opportunity to explore how out-of-plane deformations in a tissue interact with in-plane remodeling processes. In addition, we show that quantitative control of spatial light patterns influences the tissue invagination process, providing the opportunity to dissect how gradients in contractility affect tissue invagination. Notably, the observed tissue invaginations are accompanied by multiple distinct modes of cell shape changes, suggesting a spectrum of tissue mechanical responses that are capable of producing invaginations. Taken together, our findings lay a foundation for the use of calibrated optogenetic perturbations of contractility to reconstitute morphogenetic events and precisely fold tissues into a range of desired shapes and structures.

B668/P1645

Molecular analysis of Toll receptor function during the polarization of contractile and adhesive proteins during *Drosophila* embryogenesis

C. A. Kuebler, A. Paré; University of Arkansas, Fayetteville, AR

Elongation of the head-to-tail axis is a conserved process during animal development, and it occurs through remodeling of epithelial sheets via cell intercalation. The process of cell intercalation is driven by the polarization of contractile proteins (e.g., myosin) and adhesive proteins (e.g., Par-3) within the plane of the epithelium. An outstanding question is how planar polarity is established consistently across large groups of cells. In *Drosophila*, three Toll receptors--*Toll-2, Toll-6*, and *Toll-8*--are expressed in a complex pattern of vertical stripes, and planar polarity in triple mutants lacking these three receptors is severely disrupted. However, it is unclear how these three receptors function together to generate uniform polarity across the tissue. Preliminary data suggest that these three receptors can function independently, but it is still not known whether they play distinct roles in specific cells. All Toll family receptors (including vertebrate Toll-like receptors) contain extracellular leucine-rich repeats and a highly conserved intracellular TIR (Toll/Interleukin-1) domain. In addition, *Drosophila* Toll-2, Toll-6, and Toll-8 contain extended C-terminal regions--the functions of which are not well understood. To consistently quantify which receptor regions are required for polarity, we have created a genetic system in which endogenous planar polarity is absent and can be induced in response to an ectopic horizontal stripe of transgene expression. Currently we are performing structure-function analyses of the Toll-2 receptor using this system, and we are characterizing the ability of specific Toll-2 protein regions to induce planar polarity. Narrowing down the protein regions required for proper cell intercalation will help reveal the downstream interactions involved in tissue remodeling during *Drosophila* embryogenesis.

B669/P1646

Secretome of differentiating trophoblast stem cells and urine proteomics identifies functional candidates for pregnancy detection in ruminants

C. R. Detrick, P. Koganti, K. E. Gingrich, K. M. Schalich, V. V. Pillai, B. T. Nguyen, V. Selvaraj; Cornell University, Ithaca, NY

Trophectoderm development is the first lineage-separation during early embryonic differentiation with a sole purpose of setting up the placental interface in mammals. Unlike most species, epitheliochorial attachments in ruminants are restricted to regions of uterine caruncles necessitating an extended 3-week period of embryo elongation prior to implantation. Therefore, early embryo-maternal communication and maternal recognition of pregnancy relies on embryo-secreted humoral factors released by trophoblast stem cells (TSCs). In this study we sought to profile unique factors secreted by the bovine preimplantation TSCs and in early stages of differentiation for a systems biology investigation of fetal-driven humoral communication using TSCs and differentiated trophoblasts transcriptomics data. Concurrently, we investigated the urine proteome of pregnant cows to identify, clone and develop trophoblast-secreted candidate biomarkers for pregnancy detection in ruminants. This was performed using tandem predictions using SignalP and TargetP for the classical secretory pathway and SecretomeP for the non-classical secretory pathway, followed by eliminating transmembrane domain containing proteins using Phobius. We identified 600 and 617 transcripts destined to be secreted in TSCs and differentiated trophoblasts respectively. Shotgun proteomics of urine samples from pregnant cows identified 2134 unique proteins, of which 671 proteins were identified as secreted. Analysis of overlap between the secretome of cells and urine resulted in 180 candidates. Unique to the trophoblast/placenta, pregnancy-associated glycoproteins (PAGs) were identified in both urine (PAG1) and in the transcriptome (PAG2, PAG8, PAG11 and PAG12). Levels of PAG2, PAG8 and PAG12 decreased with TSC differentiation, and PAG11 appeared only after differentiation. Although their functions remain unknown, the high abundance of PAG1 and PAG2 agree with the temporal expression patterns from early pregnancy to parturition in cows. In preparation to study the physiological effects of PAG1 and PAG2, we cloned and expressed these genes in HEK293T cells for purification and functional assessment in uterine and ovarian signaling. In summary, transcriptomics and proteomics analysis identify PAG1 and PAG2 as secreted biomarkers for functional analysis and testing in bovine reproductive function.

Host Anti-viral Responses

B671/P1647

SARS-CoV-2 Spike Protein Induces Cell-Cell Fusion of Endothelial Cells

K. V. Tieu, D. E. Conway; Ohio State University, Columbus, OH.

SARS-CoV-2 Spike protein mediated cell fusion plays an important role in host cell infection. Cell-cell fusion is seen in several human viruses such as herpes simplex virus, human immunodeficiency virus, hantavirus, and in the previous severe acute respiratory coronavirus (SARS). The SARS and SARS-CoV-2 Spike protein are known to cause cell-cell fusion in epithelial cells, and is characteristic to infect epithelial cells. In addition, recent studies have shown SARS-CoV-2 can also infect endothelial cells, but how this affects endothelial cells, including syncytia formation is not well understood the replication process in endothelial cells is not fully understood. Thus, we sought to examine the effects of Spike protein expression in if cell-cell fusion also occurs in endothelial cells, including whether or not this

would result in syncytia formation. In this study, human umbilical vein endothelial cells (HUVEC) were transfected with SARS-CoV-2 D614G variant adenovirus. We observed that in HUVEC, Spike protein expression results in cell-cell fusion in HUVEC, including the formation of larger syncytia with >6 nuclei, as well as gaps forming between cells that suggest a compromised barrier. Syncytium size and frequently is similar to what we and others have observed in epithelial cells. Interestingly we observed that cells treated with arterial shear stress (12 dynes/cm^2) do not undergo syncytia formation when Spike protein is expressed, whereas cells exposed to lower levels of shear stress (characteristic of smaller blood vessels such as capillaries) still were able to form syncytia. We also observed gaps between cells near syncytia, suggesting that the formation of syncytia compromises the vascular endothelial barrier. These observations support the hypothesis that COVID-19 infection may affect the endothelium, with the possibility that endothelial cell-cell fusion may be an important factor in vascular dysfunction in severe COVID-19 infection.

B672/P1648

Assessing SARS-CoV2 Neutralizing Antibody Activities in Sera with a Cell-Based ELISA

B. Saepoo[1], J. Hines[2], Z. Assar[3], J. Bickel[4], P. Good[5], **W. Ho**[5]; [1]Assay Service, Cayman Chemical, Ann Arbor, MI, [2]Protein Core, Cayman Chemical, Ann Arbor, MI, [3]Structural Biology Service, Cayman Chemical, Ann Arbor, MI, [4]Antibody R&D, Cayman Chemical, Ann Arbor, MI, [5]Cell & Molecular Biology, Cayman Chemical, Ann Arbor, MI.

Neutralizing antibodies produced after COVID-19 vaccination or infection interfere with the binding of SARS-CoV2 to the angiotensin-converting enzyme 2 (ACE2) on the cell surface. A cell-based ELISA has been developed using reverse transfection to produce a more native platform to monitor these protein-protein interactions. The receptor binding domain of the spike protein (S1-RBD) with a rabbit Fc-tag was used as a tracer for measuring its binding to the ACE2 receptor on the cell which can be detected with a HRP-conjugated anti-rabbit Fc antibody in a colorimetric read-out. By using a fixed concentration of S1-RBD at submaximal signal level, inhibitors for S1-RBD binding on ACE2 can be identified in the cell-based ELISA. The assay was first validated using a soluble fragment of ACE2 (sACE2) and human recombinant neutralizing antibodies as inhibitors. After pre-incubation with S1-RBD and then applied to the cells, these proteins displayed dose-dependent suppressions of S1-RBD binding up to 100% effectiveness. The assay protocol was further simplified to increase throughput by pre-mixing the HRP-conjugated anti-rabbit Fc antibody, the Fc-tagged S1-RBD, and test sample before adding to the cell. Including the 1 hour incubation on cell, plate washing, and incubation with HRP substrate, the whole assay can be completed within 2 hours. Using this assay protocol, sera collected from volunteers before and after vaccination were examined in parallel with a positive control neutralizing antibody. All volunteers received the vaccines from Pfizer or Moderna exhibited very strong neutralizing antibody activities within 2-3 weeks after the first injection. A lower but still significant inhibition of S1-RBD binding was also observed in the sample from a patient with COVID-19 diagnosed within a few months. To examine if these sera with neutralizing antibodies can inhibit the binding SARS-CoV2 variants, S1-RBD proteins containing N501Y (B.1.117) and K417N/E484K/N501Y (B.1.351) mutations were examined with these samples in the cell-based ELISA. The neutralizing antibodies were found to produce weaker inhibition in the binding of N501Y S1-RBD, and even more so with the triple mutant, than the original S1-RBD to the ACE2. This progressive reduction in the effectiveness of the neutralizing antibody binding among variants were confirmed in SPR studies.

B673/P1649

TFEB and TFE3 contribute to the cellular response againts ß-coronavirus

P. Contreras, P. Tapia, E. Jeong, R. Puertollano; National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.

IntroductionThe transcription factors EB and E3 (TFEB and TFE3) are master regulators of lysosomal biogenesis and autophagy. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the ß-coronaviruses family. Recently it was described that ß-coronaviruses use lysosomal trafficking for egress the cell. The aim of this work was to analyze if ß-coronavirus infection promotes TFEB/3 activation and to evaluate their impact on the cellular response to viral infection. **Methods**We used mouse hepatitis virus (MHV) as a model of ß-coronavirus. We infected Hela-mCC1a cells that stably overexpress the receptor for MHV virus and evaluated TFEB/TFE3 nuclear translocation by immunofluorescence. In addition, we used siRNAs to deplete TFEB/TFE3 and evaluated changes in gene expression upon viral infection by RNA-seq and q-PCR, and performed a Chip-seq analysis to assess TFE3 promoter occupancy in MHV-infected cells. We also evaluated the contribution of TFEB/3 to immune response and cellular death pathways induced by MHV infection using antibody arrays for human cytokines, western blots against PARP1 and Caspase-3, and flow cytometry measuring Annexin V and 7-ADD. Finally, we evaluated viral infectivity in control and TFEB/TFE3-depleted cells using crystal violet experiments. **Results**MHV infection caused a robust and persistent TFEB/TFE3 nuclear translocation. TFEB activation occurred in the absence of mTORC1 inactivation but required calcineurin. At later infection times, MHV induced TFEB and TFE3 degradation that was mediated by the E3 ubiquitin ligase subunit DCAF7. TFEB and TFE3 regulated expression of several immune genes under MHV infection. Concordantly, TFEB/TFE3 depletion significantly reduced the upregulation of immune and inflammatory genes induced by viral infection. Furthermore, TFEB/TFE3 depletion prevented MHV-induced cellular death and decreased MHV infectivity. **Discussion**Our data strongly suggest that MHV infection induces TFEB and TFE3 activation, which greatly contributes to the anti-viral inflammatory and immune host response, but may also result in increased cellular death. The degradation of TFEB/TFE3 induced by MHV virus seems to be part of a viral strategy to maintain a restricted immune response and favor cellular survival for its own benefit.

B674/P1650

CP47, an Autophagy Inhibitor, Reduces the Replication of Feline Coronavirus *in vitro*

Y. Park[1], R. Park[2], Y. Park[1], S. Lee[1], J. So[1], C. Kim[1], J. Park[1]; [1]Yonsei University, Wonju, KOREA, REPUBLIC OF, [2]Yongin University, Yongin, KOREA, REPUBLIC OF.

Feline coronavirus(FCoV) is a common viral pathogen of domestic and wild cats. FCoV includes two subtypes, feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV). FIPV is known to cause fatal systemic diseases and considered as the virulent mutant form of FECV. However, the effective treatment is not available and effective treatments are required. Recent studies showed that autophagy is required for the efficient replication of coronavirus, and AMPK plays an important role in autophagy regulation. Here, we used Feline enteric coronavirus(FECV) to measure the feline coronavirus replication and reported treatment of CP47, an autophagy inhibitor, reduced the replication of FECV. Quantitative RT-PCR analysis and titration assay demonstrated that the viral titer of FECV was reduced by treatment of CP47. In addition, CP47 treatment decreased the cytotoxicity of FECV infection in a dose

dependent manner. These results suggest that CP47 is potentially useful to treat feline coronavirus disease.

B675/P1651

EGCG, Green Tea Polyphenol, Treatment Reduces Human Coronavirus Replication *in vitro* and *in an Animal Model*

Y. Park[1], R. Park[2], Y. Park[1], S. Lee[1], J. So[1], C. Kim[1], J. Park[1]; [1]Division of Biological Science and Technology, Yonsei University, Wonju si, KOREA, REPUBLIC OF, [2]Department of Life Science, Yongin University, Yongin si, KOREA, REPUBLIC OF.

<META NAME="author" CONTENT="박예인">**EGCG, Green Tea Polyphenol, Treatment Reduces Human Coronavirus Replication in vitro and in an Animal Model.**

Yea-In Park[1], Rackhyun Park[2], Yeonjeong Park[1], Si-Yun Lee[1], Jaeyeon So[1], Chansoo Kim[1] and Junsoo Park[1*]

[1]Division of Biological Science and Technology, Yonsei University, Wonju, Republic of Korea [2]Department of Life Science, Yongin University, Yongin, Republic of Korea

COVID-19 pandemic has resulted in huge number of deaths from 2020 to 2022. Although a vaccine for SARS-CoV-2 is now available, herbal medicine can be very useful to treat and protect COVID-19. Many studies showed that natural compounds have therapeutic effect to treat COVID-19. Recently, We showed that EGCG treatment inhibits SARS-CoV-2 3CL-protease. However, the effect of EGCG on coronavirus replication remains unclear. Herein, we examined the inhibitory effect of EGCG on coronavirus replication. Human beta corona virus (HCoV-OC43) and human alpha corona virus (HCoV-229E) were used to examine the effect of EGCG on human coronavirus replication in vitro. EGCG treatment reduced the level of human coronavirus RNA level in infected cell media. EGCG treatment also decreased HCoV-OC43 induced cytotoxicity. Scanning electron microscopy analysis results showed that EGCG treatment reduced coronavirus protein and virion particle production. In addition, we verified the effect of EGCG on coronavirus replication using an animal model. Quantitative RT-PCR analysis showed that EGCG-fed mice exhibited reduced levels of coronavirus RNA in mouse lungs. These results demonstrate that EGCG, green tea polyphenol, treatment inhibits human coronavirus replication effectively in vitro and in vivo.

B676/P1652

A Rare Case of Kaposi Sarcoma in an Immunocompetent Heterosexual Hispanic Male - A Potential Budding Clinical Subtype

A. Fijany[1], M. Chalman[1], K. Givechian[2]; [1]TCU School of Medicine, Fort Worth, TX, [2]Yale School of Medicine, New Haven, CT.

Purpose: Kaposi sarcoma (KS) is a malignancy associated with endothelial spindle cell proliferation. This vascular neoplasm is due to an infection by human herpesvirus 8 (HHV-8), which is also known as Kaposi sarcoma-associated herpesvirus (KSHV). At the cellular level, KS is composed of spindle cells derived from vascular endothelial cells along with a variable cocktail of inflammatory cells. Historically, four subtypes of KS have been described based on the clinical circumstances by which they develop: classic (Mediterranean descent), endemic (African origin), iatrogenic (immunosuppressive therapy-associated), and AIDS-associated. A fifth recently described variant includes men who have sex with men (MSM) without HIV infection. We present an unusual case of classic KS in an immunocompetent, heterosexual

Hispanic male lacking any of the risk factors found in the literature. Design: A literature review on PubMed was performed on May 12th, 2022, which searched for articles that included the terms "Kaposi sarcoma" AND "immunocompetent" or "HIV-negative" or "HIV negative". The literature was then surveyed for other relevant cases of Kaposi sarcoma in individuals lacking immunodeficiency to identify associated risk factors and confirm the paucity of this patient's KS subtype.
Findings: Here, we present the case of a middle-aged, heterosexual Hispanic male with a single violaceous, non-blanching patch on the plantar surface of his foot. The patient is immunocompetent, HIV-negative, and with no other identifiable risk factors of HHV-8 exposure or (re)activation. He has no significant medical history, steroid use, IV drug use, or prior blood transfusion. Biopsy was performed and histopathology findings were consistent with classic Kaposi Sarcoma. The patient was worked up and found to be negative for any potential immunodeficiency. The lesion's full-thickness excision was successful, and the patient has had no recurrence to date. Given this patient's lack of risk factors, clinical suspicion of KS prior to biopsy was extremely low. Few similar cases have been described; however, a potential unrecognized Hispanic KS variant has been proposed in recent literature. Here we overview the viral cell entry mechanism and propose a genetic susceptibility in this subgroup.
Summary: Hispanic males with biopsy-proven Kaposi sarcoma may represent an infrequently recognized subtype of KS. As such, clinicians should have a lower threshold when considering biopsy of suspicious violaceous lesions, even in the absence of other KS risk factors. The utilization of appropriate biopsies and histological analyses may lead to Kaposi sarcoma diagnoses in patients who do not fall into the currently accepted four clinical subtypes.

B677/P1653

Generation of a Cas9-Expressing Macrophage Line to Facilitate Ebolavirus Research

T. McGee[1], J. A. Aguilar Briseño[2], J. Elliff[3], W. Maury[2]; [1]Hampden-Sydney College, Hampden Sydney, VA, [2]Department of Microbiology and Immunology, University of Iowa, Iowa City, IA, [3]University of Iowa, Iowa City, IA.

Ebolavirus (EBOV) is a highly virulent negative-stranded RNA virus endemic to Western and Central Africa, with mortality rates ranging between forty and ninety percent. There are limited effective antiviral treatments, and the efficacy of the FDA-approved vaccine using a recombinant Vesicular Stomatitis Virus expressing the EBOV glycoprotein protein has recently been called into question by evidence of breakthrough infections in the Democratic Republic of Congo among vaccinated individuals. Macrophages are important cell targets during early EBOV infections. Among the many contributions of macrophages to EBOV pathology are their capacity to serve as vehicles for viral dissemination and to produce pro-inflammatory mediators. However, mature macrophages cannot be easily genetically manipulated to characterize host genes responsible for these activities. Our previous research has identified Interferon gamma (IFN-γ) as highly protective against EBOV infection in macrophages but mechanistic means by which this protection is mediated remains unclear. To study antiviral host responses mediating the effects of IFN-γ, we identified a murine Kupffer cell line that recapitulates most characteristics of primary mature macrophages. The *Streptococcus pyogenes* Cas9 gene governed by an EF-1a promoter was introduced into murine Kupffer cells in preparation for Cas9/guide RNA knockout of genes to improve the mechanistic understanding of EBOV infection and the protection IFN-γ can provide. The lentivirus transfection and blasticidin selection protocol were successfully optimized to generate and subsequently select transduced cell populations. Bioinformatics tools were also employed to identify guide RNA sequences that are being used to edit genes relevant to IFN-γ protection during

EBOV infection of macrophage populations. Western blotting was used to confirm Cas9 Expression. We successfully developed this important cellular reagent for future studies to elucidate host genes in macrophages that control EBOV infection.

B678/P1654

Characterizing the infection dynamics of a novel nematode virus variant

S. Khan[1], C. Fujii[2], L. van Sluijs[3], D. Wang[4], J. Sowa[1]; [1]West Chester University of Pennsylvania, West Chester, PA, [2]Washington University in St. Louis, St. Loius, MO, [3]WAGENINGEN, Wageningen University, Wageningen, NETHERLANDS, [4]Washington University in St. Louis, St. Louis, MO.

The model organism *Caenorhabditis elegans* is a nematode that is commonly used to study the interactions between pathogens and host innate immunity. One branch of the *C. elegans* innate immune system is the Intracellular Pathogen Response (IPR). The IPR is a transcriptional response that is activated in response to intracellular pathogens, such as viruses, entering the cells. To date, there is only one virus that is known to naturally infect *C. elegans*, the Orsay virus. In an effort to identify novel viruses, wild nematode samples were collected from the Netherlands and screened for infections that could be transmitted to *C. elegans.* In this process, a putative virus (V2) was discovered. RNA sequencing revealed that V2 is likely a new variant of Orsay. In order to compare the infection dynamics of V2 to standard Orsay, a 72-hour infection time course was run by infecting N2 *C. elegans* with V2 and Orsay in parallel. The viral transcript and IPR activation levels from each time point were quantified using RNA extraction, cDNA synthesis, and qPCR. Future studies will investigate the tissue tropism of both viruses using FISH. Further studying these two viruses could lead to a better understanding of the genetic factors that influence infection dynamics.

B679/P1655

The effect of Orsay virus protein expression on *C. elegans* Intracellular pathogen response

N. Martinez, B. Chen, E. Cooper, J. N. Sowa; Biology, West Chester University of Pennsylvania, West Chester, PA.

C. elegans are a popular genetic model organism used by many fields to investigate fundamental questions of cell biology. *C. elegans* innate immune signaling shares many similarities with that of humans, making them a useful model system to study how viruses interact with innate immunity. There is one known virus capable of naturally infecting *C. elegans*, the Orsay virus, a positive sense ssRNA virus that infects the *C. elegans* intestine. Using a transcriptional reporter strain, we found that the *C. elegans* innate immune response is suppressed by Orsay virus infection. This is particularly interesting in light of the fact that the Orsay genome only encodes four proteins: capsid, delta, capsid-delta fusion, and RNA-dependent RNA polymerase. In order to find out which of these proteins mediates the suppression of *C. elegans* immunity, we created transgenic *C. elegans* strains expressing each of the four Orsay proteins individually. Our next steps are to test each of these single Orsay protein transgenic strains to see which (if any) show suppression of *C. elegans* innate immunity. If we are able to identify which one of the proteins is responsible for the suppression of *C. elegans* immunity, then future studies will focus on determining the mechanism of action of the immunosuppression. This could provide a new system for studies of the evolution and mechanisms of viral suppression of host immunity.

B680/P1656

Antiviral function and viral antagonism of the rapidly evolving dynein activating adapter NINL

D. Stevens[1], C. Beierschmitt[2], S. Mahesula[1,3], M. Corley[2], J. Salogiannis[4,1], B. Tsu[2], B. Cao[2], A. Ryan[2], S. Reck-Peterson[5,3,6], M. Daugherty[2]; [1]Department of Cellular and Molecular Medicine, University of California San Diego, San Diego, CA, [2]Department of Molecular Biology, University of California San Diego, San Diego, CA, [3]Howard Hughes Medical Institute, Chevy Chase, MD, [4]Department of Molecular Physiology and Biophysics, University of Vermont, Burlington, VT, [5]University of California San Diego, San Diego, CA, [6]Department of Cell and Developmental Biology, University of California San Diego, San Diego, CA.

Viruses interact with the intracellular transport machinery to promote viral replication. Such host-virus interactions can drive host gene adaptation, leaving signatures of pathogen-driven evolution in host genomes. Here we leverage these genetic signatures to identify the dynein activating adaptor, nineinlike (NINL), as a critical component in the antiviral innate immune response and as a target of viral antagonism. Unique among genes encoding components of active dynein complexes, NINL has evolved under recurrent positive (diversifying) selection, particularly in its carboxy-terminal cargo binding region. Consistent with a role for NINL in host immunity, we demonstrate that NINL knockout cells exhibit an impaired response to interferon, resulting in increased permissiveness to viral replication. Moreover, we show that proteases encoded by diverse picornaviruses and coronaviruses cleave and disrupt NINL function in a host- and virus-specific manner. Our work reveals the importance of NINL in the antiviral response and the utility of using signatures of host-virus conflicts to uncover new components of antiviral immunity and targets of viral antagonism.

B681/P1657

Discovery of new viral pathogens of *C. elegans* from wild nematode isolates

I. Cannon[1], S. Lancaster[1], N. Diarra[1], L. van Sluijs[2], J. N. Sowa[1]; [1]West Chester University, West Chester, PA, [2]Wageningen University, Wageningen, Netherlands

Caenorhabditis elegans are a popular model system for studying host-pathogen interactions because their short life cycles, optically transparent bodies, and ease of genetic screening make them powerful tools for discovery. To date, there are only three known viruses that naturally infect *Caenorhabditis* nematodes and of those, only the Orsay virus infects *C. elegans*. Through collaboration with colleagues in the Netherlands, wild nematode communities were collected from various sampling sites. We then tested these wild nematodes for the presence of intracellular infections that could be transmitted to *C. elegans* using a co-culture method. In this method, wild nematodes are co-cultured together with transgenic *C. elegans* engineered to express fluorescent reporters when infection-induced genes are activated. To prioritize potential viral infections specifically, co-cultures that showed reporter activation were homogenized and filtered through 0.22µm filters to size-exclude non-viral pathogens. These filtrates were then tested for their ability to transmit the infection. For infections that did transmit via the 0.22µm filtrates, samples of infected worms were taken and stained using Fluorescence in Situ Hybridization (FISH) probes for each of the known *Caenorhabditis* viruses. Through this process, at least one new viral isolate, has been identified: V2, which appears to be a novel variant of Orsay. The discovery of this and other novel virus variants will provide scientists with new research opportunities to explore mechanisms of host immunity and pathogen virulence.

B682/P1658

CoV2PIM: Establishment of a Virus-Host and Virus-Virus Interaction Map of SARS-CoV-2

P. Tafelmeyer[1], F. Moisant[2], V. Collura[2], R. Benarous[2], K. Wijesiriwardana[3], P. Diamantis[4], L. Lane[3], J.-C. Rain[2]; [1]HYBRIGENICS Corp., Boston, MA, [2]HYBRIGENICS Services, Evry, France, [3]Faculty of Medicine, University of Geneva and SIB-Swiss Institute of Bioinformatics, Geneva, Switzerland, [4]current address : Hengrui Europe Biosciences AG, Zuerich, Switzerland

In the fight against the SARS-CoV-2 pandemic, huge progress has been made by the scientific and industrial community to bring to the market efficient vaccines and antiviral drugs. However, many details about the viral biology and the interaction of the virus with the human host remain unknown. The identification of protein interactions between the virus and the host is often crucial to understand the mechanisms of viral replication and transmission. Moreover, knowledge of the molecular details of these protein interactions can lead to the discovery of new therapeutic targets. We have therefore used our high-throughput platform that allows exhaustive and reproducible yeast two-hybrid (Y2H) screening to establish a protein interaction map (PIM) between 29 proteins or protein fragments of SARS-CoV-2 and proteins from a human lung cells library. Our PIM connects 780 CDS by 979 protein interactions, and also determines the sites of interactions. The validation of a subset of these interactions is in progress. The fundamental knowledge provided by a large-scale PIM aims to better understand SARS-CoV-2. Moreover, the convergence between virus and host cell-control makes these efforts useful for the understanding of infections with other (Corona)viruses. A detailed comprehension of the molecular levels of the host-virus interactions will help to pave the way for the identification of new targets of therapeutic interest and will contribute to better control similar events that will likely occur in the future.

B683/P1659

Identification of critical residues for palmitoylation of human cytomegalovirus tegument protein pp28 and their functions during viral infection

J. B. Lee, J. Y. Seo; Severance Biomedical Science Institute, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, KOREA, REPUBLIC OF.

Human cytomegalovirus (HCMV) tegument protein pp28 is crucial for the final envelopment during viral maturation. This protein contains covalently bound fatty acids including myristic acid, which affects the activity and subcellular targeting of proteins. The pp28 protein localizes to the viral assembly compartment (AC) late in infection and its localization depends on myristoylation. Defect in this modification of pp28 leads to failure in infectious progeny virus production. Meanwhile, plamitoylation of pp28 remains poorly understood. Here, we showed that pp28 is palmitoylated at cysteine residues (aa 6, 10, and 11). Inhibition of its palmitoylation by site-directed mutagenesis or palmitoylation inhibitor 2-bromopalmitate treatment disrupted the subcellular localization of pp28 and also decreased the stability of the protein *via* a proteasome-dependent pathway in transiently transfected cells. When the three cysteine residues were substituted individually or in combination with alanine residues, the level of palmitoylation was significantly reduced. The recombinant HCMVs producing pp28 mutants in which two or three cysteine residues are substituted with alanine residues, exhibited delayed viral growth kinetics and a decrease of infectious virus secretion. However, these recombinant HCMVs did not exhibit defects in cell entry. In addition, the number of intracellular viral particles produced from cells infected with the recombinant HCMVs was similar to that from wild type HCMV. Collectively, our

data indicate that palmitoylation at the N-terminal cysteine residues of pp28 plays an essential role in its subcellular localization and the release process of infectious virions during HCMV infection.

Protists and Parasites

B684/P1660

Investigating the molecular basis of learning by a single cell

D. Rajan, W. Marshall; University of California, San Francisco, San Francisco, CA.

Learning is usually associated with a complex nervous system, but there is increasing evidence that life at all levels, down to single cells, can display intelligent behaviors such as decision-making and learning. In both natural and artificial systems, learning is the adaptive updating of system parameters based on new information, and intelligence is a measure of the computational prowess that facilitates learning. *Stentor coeruleus* is a unicellular pond-dwelling organism that exhibits habituation, a form of learning in which a behavioral response decreases following a repeated stimulus. *Stentor* contract in response to mechanical stimulation, an apparent escape response from aquatic predators. However, repeated low-force perturbations induce habituation, demonstrated by a progressive reduction in contraction probability. Habituated *Stentor* still contract after receiving high-force mechanical stimulation or photic stimulation. These observations, which align with Thompson and Spencer's classic criteria for habituation, strongly suggest that the original contractile response decrement is due to learning rather than fatigue or ATP depletion. As a free-living cell, *Stentor* can be studied without much interference from surrounding cells as would be the case in a multicellular tissue. Several additional features make *Stentor* a tractable system for studying learning: its large size (1 mm), quantifiable habituation response, ease of injection and micromanipulation, a fully sequenced genome, and the availability of RNA interference (RNAi) tools. But how can a cell learn without a brain or nervous system?
Here, we explore potential molecular mechanisms of *Stentor* learning. We find that protein phosphorylation likely plays a role in habituation at short timescales (under 60 minutes). We also further characterize *Stentor* learning in response to different forces and frequencies of mechanical stimuli. Intriguingly, high frequency stimulation significantly increases the speed of habituation. Furthermore, *Stentor* can habituate to high-force stimuli after longer timescales of mechanical training (overnight), calling into question the decades-old idea that high-force stimuli preclude habituation in *Stentor*. By understanding habituation at the level of a single cell, we are beginning to uncover learning paradigms that are independent of complex circuitry.

B685/P1661

Cell cycle genes and oral regeneration in *Stentor coeruleus*

C. Yan, P. Sood, A. Lin, R. McGillivary, W. Marshall; University of California, San Francisco, San Francisco, CA.

Regeneration and wound healing are hallmarks of life and are fundamental for tissues and cells to regain their integrity and functionality after injury from external perturbations. While regeneration has been extensively studied at the level of tissues and organs, much less is known about how cells themselves are able to regenerate. The giant single cell ciliate *Stentor coeruleus* is a classical model system that has unique advantages for studying wound regeneration on a single cell level, including large size and effective wound healing. When the cell's oral apparatus is removed, the cell can regenerate a new oral

apparatus in a series of defined, visible morphological steps. This regeneration paradigm provides an opportunity to study regeneration of complex structures and how this process is initiated. It is known that different geometric perturbations of the cortex can trigger regeneration, but the molecular mechanisms underlying this process are unknown. A transcriptional analysis of regeneration and proteomics produced candidate genes which are differentially expressed during regeneration. One approach for identifying signals that trigger regeneration is to determine which genes are expressed early in the pathway, then move upstream to identify signals required to turn these genes on. Among early-expressed genes, the transcription factor E2F was identified as a potential regulator of oral regeneration based on the expression pattern of its predicted target genes. The Rb-E2F-DP1 module plays important roles in regulating cell cycle dependent processes in many organisms. Phosphorylation of Rb by CDK4 releases binding from E2F, allowing for transcription of E2F target genes. RNAi-mediated knockdown experiments show that E2F is involved in the completion of regeneration, specifically the formation of the oral primordium, the first step of oral regeneration. Similarly, the addition of a CDK4 inhibitor, Palbociclib, produces the same phenotype. It is possible that cell cycle machinery might help regulate the timing of regeneration, as the morphological steps of forming a new oral apparatus appear identical to morphological changes that occur during cell division. This highlights a question in the biology of regeneration of whether regeneration is a distinct process, or does it reflect a re-activation of development?

B686/P1662

The role of a robust cytoskeleton in wound resistance and healing in the ciliate *Stentor coeruleus*

A. V. Nadkarni[1,2], K. S. Zhang[1], W. F. Marshall[2], S. K. Y. Tang[1]; [1]Stanford University, Stanford, CA, [2]University of California San Francisco, San Francisco, CA

Wound healing is a fundamental property of living systems. It is especially important for free-living, single-celled organisms such as the freshwater ciliate, *Stentor coeruleus*. Wounds must be sealed promptly to prevent both the leakage of cytoplasm, and the influx of undesirable molecules. Concurrently, these cells must possess properties that allow them to resist wounding in the first place. *S. coeruleus* has a robust capacity to heal from large mechanical wounds within a single cell. It can seal bisection wounds within 100 -1000s and can regenerate a new cell from a fragment $1/27^{th}$ its original size. We hypothesize that the cytoskeleton of *Stentor coeruleus* plays an important role not only in wound healing but also in resisting wounds. However, since the molecular players and arrangement of the *Stentor* cytoskeleton is not fully elucidated, the mechanism by which the cytoskeleton influences wound healing in these cells remains an open question. By using microfluidic methods of cell wounding and cytoskeletal perturbation (using RNAi and small molecule inhibitors), we find that disruption of the microtubule cytoskeleton makes cells more susceptible to shear-induced wounds. Once wounded, disruption of cytoskeletal elements, such as microtubules and septin, alters the cell's wound response and the healing rate. Thus far, we have not observed actomyosin purse-string contraction in *S. coeruleus* wound healing. The significance of our work lies in the elucidation of the role of the cytoskeleton and the identification of novel wound resistance and healing biology in the non-model system *Stentor* as well as its general relevance for the study of free-living, giant cells. Our work also has potential to inform the engineering of a new function—wound resilience—in synthetic cell systems.

B687/P1663

Actin and Myosin contribute to cell wall integrity in the pennate diatom Phaeodactylum tricornutum

P. Avasthi, C. D. MacQuarrie, A. Radkov; Arcadia Science, Berkeley, CA.

Diatoms are well known for their unique and intricate shapes. The pennate diatom *Phaeodactylum tricornutum* exists in 3 main morphotypes: fusiform, triradiate, and ovular. Recent work has uncovered that the actin cytoskeleton localizes proximal to the cell wall in this species. Additionally, transcriptomic studies revealed altered expression of actin-binding proteins between cells of different morphotypes, suggesting the actin cytoskeleton could be contributing to cell morphology. Here, *Phaeodactylum tricornutum* cells were treated with a panel of cytoskeletal inhibitors to determine what role, if any, actin plays in diatom shape determination. Surprisingly, many of these treatments resulted in the formation of protoplast-like, naked round cells. The myosin II inhibitor (-)-blebbistatin and the Arp2/3 complex inhibitor CK-666 had the most drastic effect. Because *Phaeodactylum tricornutum* express 10 different myosin proteins, it was unclear which protein (-)-blebbitation was targeting. Predicted structures of the *Phaeodactylum* myosins were compared to the solved structure of (-)-blebbistatin bound *Dictyostellium* myosin II to determine that *Pt*MyoE, which is upregulated in fusiform and triradiate cells, is likely to be targeted and inhibited by (-)-blebbistatin. To determine if these round cells were protoplasts, we treated cells with the cell wall degrading enzyme Alcalase and observed similar round cells leaving their cell wall shell, forming a protoplast. Because disruption of actin regulators and motors results in a phenotype similar to direct cell wall degradation, we speculate that the actin cytoskeleton contributes to cell wall integrity through cell wall anchoring, polarized secretion of cell wall components, transcriptional regulation of cell wall biosynthetic enzymes, or through other uncharacterized mechanisms.

B688/P1664

Lipid organization protects GPI-anchored proteins from membrane perturbing agents in *Leishmania major* promastigotes

R. M. Keane, C. Haram, S. Moitra, E. Breslav, K. Zhang, P. Keyel; Biology, Texas Tech University, Lubbock, TX.

Leishmaniasis is the fourth-largest neglected tropical disease and is caused by the kinetoplast *Leishmania major*. The *L. major* plasma membrane is a key therapeutic target. It contains key virulence factors like lipophosphoglycan (LPG) and the GPI-anchored metalloproteinase gp63, yet the accessibility of membrane components to toxic extracellular substances is poorly understood. Prior work suggests that membrane components are modified relative to their mammalian counterparts to reduce toxin access. The GPI-anchor in *L. major*, gp63, has reportedly bound to the beta pore-forming toxin (PFT) aerolysin when expressed in mammalian cells, but not in *L. major*, due to differences in GPI anchor structure. However, our recent observations conclude that PFTs depend on the absence of sphingolipids for cytotoxicity, raising the possibility of other membrane components sheltering the GPI-anchor in *L. major*. We hypothesize that LPG prevents cytotoxicity by sheltering gp63 from aerolysin. We genetically removed or altered membrane components, including sphingolipids, sterols, gp63, or LPG, to test their susceptibility to aerolysin. Using flow cytometry, we found that trypsin-activated aerolysin and pro-aerolysin bound and killed wild type *L. major*. We tested the impact of sphingolipids in protecting *L. major* from aerolysin and found no change in cytotoxicity. Disruption of sterol synthesis had mixed

results on cytotoxicity. Deletion of sterol methyltransferase was less susceptible to aerolysin than deletion of c14-demethylase. We next examined aerolysin's ability to bind GPI-anchored proteins, including gp63, on *L. major*. Knockout of gp63 or LPG did not block binding of fluorescently-labeled pro-aerolysin, indicating aerolysin can bind to multiple determinants in *L. major*. Aerolysin killed LPG-/- *L. major* 75-fold more effectively than wild type, suggesting LPG protects *L. major* from aerolysin. While trypsin-activated aerolysin killed gp63-/- *L. major*, pro-aerolysin did not. This indicates that gp63 can cleave pro-aerolysin into aerolysin. We conclude that *L. major* uses LPG and ergosterol to reduce clustering of GPI-anchored proteins following aerolysin binding to prevent cytotoxicity. Thus, targeting virulence factors in combination with sterols may enhance the susceptibility of *L. major* promastigotes to therapeutic drugs.

B689/P1665

The Characterization of Two Novel Calcium Signaling Proteins in Toxoplasma gondii

A. Delapenha[1], E. A. Sizemore[2], S. Agrawal[1]; [1]Biological Sciences, University of Mary Washington, Fredericksburg, VA, [2]Biological Sciences, University of Mary Washington, King George, VA.

Toxoplasma gondii is an opportunistic apicomplexan parasite infecting humans and livestock. Infection in immunocompromised individuals can cause neurological damage and infection during pregnancy can lead to fetal death. These parasites engage in a complex life cycle, involving repeated invasion of host cell and egress from the host cell. Calcium signaling is an important regulatory mechanism for many essential processes in the parasite including gliding motility (actomyosin-dependent mode of motion), invasion, and egress. Our work focused on two previously uncharacterized calcium regulator proteins. The proteins of interest were modified using the CRISPR-Cas 9 system to express the Auxin Degron system downstream of each gene. Our work demonstrates the localization of these proteins and the role of these proteins on parasite growth and fitness.

B690/P1666

Energetics dissection of the ultrafast microsporidian polar tube invasion machinery

R. Chang[1], A. Davydov[2], P. Jaroenlak[2], B. Budaitis[2], D. Ekiert[2], G. Bhabha[2], M. Prakash[1]; [1]Department of Bioengineering, Stanford University, Stanford, CA, [2]Department of Cell Biology, New York University School of Medicine, New York, NY.

Microsporidia is a group of eukaryotic obligate intracellular parasites which causes huge economic burden worldwide. Their infection process is characterized by the rapid ejection of polar tube (a 100-nm wide thin cylinder shooting at a speed of 300 um/sec, creating a shear rate of 3000 1/sec) and passage of sporoplasm and entire nucleus through a narrow long tube (~60 um long, 100nm wide) into the host cell. Considering the large resistance in extremely thin tube and the low Reynolds number nature of the process, it is not known how exactly can microsporidia achieve this ultrafast shooting event without a functional mitochondria. In this study, we propose a theoretical framework accounting for all topological possibilities and systematically dissect them for their energy requirement. These geometries are classified and compared against each other for required energy, power, and pressure requirement. We compared the prediction from our theoretical framework to polar tube ejection experiments in varying viscosity and high-resolution serial block-face scanning electron microscopy (SBF-SEM) data. Our study provided the first comprehensive biophysical analysis of the energetics and dissipation of the polar tube ejection process in microsporidia and can point out new directions for future experiments.

B691/P1667

Water is not sufficient to reanimate Colpoda steinii after desiccation and solutions to quantitate complex lifestyles of protists

G. Garcia, III, P. Avasthi; Arcadia Science, Berkeley, CA.

Global climate change and increasing human population strain fresh water resources. Many protists, including the ciliate Colpoda steinii, live in ephemeral bodies of water and can survive for extended periods without water. Active Colpoda swim in dew that forms on the surface of plants. As the dew evaporates, Colpoda encyst and become dormant to resist desiccation. Colpoda harbor evolutionary innovations that shield the organism from desiccation and other harsh environments.Three questions guide our research to uncover these protective innovations. What is the composition of the resting cyst wall and how is it built? Which stimuli reanimate cells from the resting cyst? What is the evolutionary history of the resistance of cysts to desiccation? To begin to answer these questions we first needed to determine culturing conditions and how to reproducibly induce resting encystment and reanimation. We find that rehydration in water is not sufficient for reanimation. Instead, we find that reanimation occurs upon exposure to bacterized hay medium. After reanimation, Colpoda steinii divides in reproductive cysts which are distinct from resting cysts. The complex life cycle of Colpoda steinii - with a motile state and at least two static states- poses a challenge to microscopy-based experiments. Cells move out of, or encyst out of, the field of view. To solve these challenges, we developed microchambers to visualize behavior over long time scales while maintaining cells within a field of view. Currently, we are sequencing the genome and transcriptomes of Colpoda steinii to aid in molecular analysis of the resting cyst and desiccation resistance. We are also developing assays to phenotype Colpoda steinii across its life cycle in the context of drug screening or other experimental perturbation to uncover mechanisms of encystment and reanimation. Evolution has solved the problem of living with limited access to fresh water, and understanding Colpoda encystment offers strategies for management of freshwater resources in the context of global climate change.

B692/P1668

Molecular characterisation of two species of Lecithocladium (Hemiurid trematode) from two marine fish species in India.

R. Singh, A. Saxena; zoology, University of Lucknow, Lucknow, INDIA.

Genomic sequencing and molecular data on PCR-based methods are new sources to explore the identification and discrimination among helminth parasites and are helpful in resolving taxonomic and hierarchical issues. The rDNA is particularly useful for molecular studies because it contains regions with varying rates of evolution, from highly conserved (18S, 5.8S, and 28S) to highly variable. The aim of the study was to evaluate the genetic relationship between two species of Lecithocladium, from two marine fish hosts, based on 18s nuclear DNA. Genomic DNA was extracted from individual worms using Qiagen'sDNeasy Tissue Kit (Qiagen Hilden, Germany), as specified by the manufacturer. DNA concentration and purity were determined spectrophotometrically by measuring their absorbance at 260 and 280 nm. PCR amplification, the 18S region was amplified with the universal primer (Forward): 5'AACCTGGTTGATCCTGCCAGT -3' and (Reverse): 5'TGATCCTTCTG CAGGTTCACCTA -3'. Sequences of both species of Lecithocladiumhave been submitted to NCBI. (GenBank accession numbers; OM838287,ON855066). Sequences are subjected to Mfold for the reconstruction of secondary RNA

structure which brings our knowledge one step ahead. Secondary RNA structures are based on minimum free energy. The minimum negative free energy (G) of each RNA secondary structure has been measured in Kcal/mol. The minimum negative free energy (dG) ofLecithocladium sp. 1 and Lecithocladium sp. 2 are -348.06, and-293.84 respectively. The evolutionary trend of Indian representatives of the genusLecithocladium parasitizing on the Intestine of two marine fish was inferred for the first time, based on molecular data set of the 18S rDNA region using MEGA 6. The amplicon size of the sequences of both species of Lecithocladium were long 1064 & 1214 bp and G+C content was 47% and 48% respectively. Conclusion of the study: The present molecular investigation revealed only 63% similarity between both species of Lecithocladiumshowing they are clearly distinct species differing at variable nucleotide positions. The phylogenetic tree shows that both Indian species are clearly distinct and formed a sister clade with L. excisum from the UK. These species are also different in terms of morphological aspects such as the shape and size of the body, the ratio of oral sucker, and the position of the genital pore. Both the Indian species also have different fish hosts, locality as well as sites of infection. These differences may be due to variant nucleotide bases, consequences in the lineage of mutations, especially base substitutions and additions of nucleotide basepairs.

B693/P1669

Composition, Biogenesis, and Sensory Architecture of the *Podophrya collini* Feeding Tentacle: a Novel Microtubule-based Organelle with Ultrafast Prey Recognition

Z. Xu, S. Coyle; University of Wisconsin-Madison, Madison, WI.

Microtubules are conserved cytoskeletal filaments that assemble into higher-order biochemical machines to perform diverse cellular functions, from motile cilia to the mitotic spindle. However, our understanding of how these microtubule-based machines are built and function is largely derived from metazoan systems, which represent only a small fraction of the Eukaryotic design space. Here we investigate the composition, biogenesis, and sensory architecture of a microtubule-based feeding tentacle from *Podophrya collini*, a single-celled predatory ciliate. While juvenile *P. collini* are highly ciliated, mature cells replace their cilia with dozens of feeding tentacles that extend hundreds of microns out from their body into the environment. The tips of these tentacles react within milliseconds of contact with specific prey, triggering membrane fusion and transport of prey cytoplasm down the tentacle into the cell body. To understand the biogenesis of these structures, we developed a high-throughput tentacle severing and regeneration assay that enabled us to track the *de novo* assembly of these microtubule structures over time and the transcriptional programs supporting their construction. We found that tentacles first assemble in the cytoplasm before being delivered to the cell periphery and identified time-specific transcriptional patterns that correlate with these stages of biogenesis. Differential gene expression analysis revealed that many cilia-associated and cilia-specific proteins are upregulated during tentacle assembly, and immunostaining confirmed a novel distribution for many of these factors in the tentacle structure. This suggests that tentacles may be assembled in part through the alternative use of canonical ciliary proteins. To investigate the tentacle sensory activity, we sought a soluble protein factor that could trigger a feeding reaction in the absence of prey. We identified that lectins could potently induce *P. collini* tentacle contraction, grabbing behavior, and vesicle discharge. Fluorescent lectins strongly stained the tentacle tips, suggesting these effects are mediated by direct interaction with molecules involved in prey recognition. These lectins provide a tool for studying the quantitative behavior of the feeding reaction and can also serve as baits to identify receptors responsible for *P. collini* tentacle ultrafast prey recognition. Through understanding the novel

microtubule-based structure of the suctorian *P. collini* tentacles, our results provide new insights into the design principles of microtubule-based architectures and molecular machines.

B694/P1670

Three-dimensional reconstructions of the microsporidian parasite niche in infected mammalian cells

C. Lam, N. Antao, C. Petzold, J. Sall, F.-X. Liang, G. Bhabha, D. Ekiert[1]; New York University School of Medicine, New York, NY

Microsporidia are single-celled, obligate intracellular parasites closely related to fungi, which can infect a wide range of animals and can cause fatal illnesses in immunocompromised patients. *Encephalitozoon intestinalis* is a human-infecting species of microsporidia that initiates infection in the small intestine, where it develops and replicates in parasitophorous vacuoles (PV). As obligate intracellular parasites, *E. intestinalis* have evolved a highly reduced genome, about 2.3 megabase pairs, and are dependent on host metabolites for successful development and replication. *E. intestinalis* lack many key metabolic pathways and eukaryotic organelles, including a functional mitochondria that can perform oxidative phosphorylation. There is limited research that shows how this pathogen exploits their intracellular niche in order to survive and propagate. To understand the physical basis for how the parasite occupies and manipulates the host cell, we used serial-block face scanning electron microscopy (SBF-SEM) to generate a three-dimensional reconstruction of the intracellular niche for *E. intestinalis* in infected host cells. This technique allows us to examine the interactions between the parasite and host cell organelles at nanometer resolution. The three-dimensional reconstructions give us insights into how *E. intestinalis* interacts with host organelles, including the host cell mitochondrial network. Coupled with optical microscopy, our data show that microsporidia impact mitochondrial fusion and fission dynamics. An analysis of the aspect ratio of mitochondria from our SBF-SEM data show that while uninfected control cells have elongated, interconnected mitochondrial networks, infected cells contain a high number of small, disconnected, and spherical mitochondria, providing higher resolution insights into the increased fission phenotype observed in optical microscopy experiments. Our SBF-SEM data shows findings on how microsporidia manipulate and remodel mitochondria and other host cell organelles.

B695/P1671

A novel Peroxin is involved in the maintenance of mitochondrial morphology

E. Knight, A. Gianos, L. Crowe, M. Morris; Clemson University, Clemson, SC

Kinetoplastid parasites have peroxisome like organelles called glycosomes that are unique in that they harbor many glycolytic enzymes that are cytosolic in other eukaryotes. Proteins known as peroxins (Pexs) coordinate peroxisome (and glycosome) biogenesis. Homologs for only 50% of the peroxins characterized in eukaryotes have been identified in kinetoplastids. We are interested in identifying and characterizing additional Pexs. We queried the trypanosome genome for open reading frames that contain peroxin functional domains with the reasoning that functional domains would be more conserved than overall protein sequences. We found a single ORF (Tb927.9.11350) containing a putative Pex19 binding domain (P19BD; SNKLEIWEDLKIISFTR). Pex19 is a soluble chaperone that delivers peroxisome membrane proteins, many of which are Pexs, to the peroxisome via interactions with the glycosome membrane protein Pex3. Silencing Tb927.9.11350 in the model kinetoplastid, *Trypanosoma brucei*, resulted in slower growth rates. We were surprised that Tb927.9.11350-deficient *T. brucei* had swollen mitochondria while gross glycosome morphology (size, number, and shape) was unchanged. We

named the protein encoded by Tb927.9.11350, PIMM (Peroxin Involved in Mitochondrial Morphology). Immunofluorescence assays with epitope-tagged PIMM are inconclusive. We are working to define the mechanism by which PIMM influences mitochondrial morphology. PIMM-deficient cells have increased reactive-oxygen species and are more susceptible to apoptotic stimuli. We are currently working on measuring the extent to which mitochondrial metabolism (as opposed to mitochondrial structure) is altered. Understanding the function of this protein may shed light on novel ways in which mitochondria and these specialized peroxisomes communicate. While mitochondria-peroxisome communication in other eukaryotes is an active field of study, little is known about how these organelles interact in Kinetoplastid parasites.

B696/P1672

Targeting *Plasmodium* protein synthesis—the relationship between translation inhibitor efficacy and reversibility during *P. berghei* liver stage schizogony.

J. L. McLellan, K. K. Hanson; University of Texas At San Antonio, San Antonio, TX

Protein synthesis is a critical cellular process that is regulated in response to changing external stimuli and causally linked to cell growth and proliferation. Given the essentiality of protein synthesis throughout the complex lifecycle of *Plasmodium* parasites, the causative agents of malaria, parasite-specific translation inhibitors are emerging amongst the leading candidates for next generation antimalarials— capable of targeting both the asymptomatic liver stage and the disease-causing blood stage of infection. The liver stage is the obligate first step in mammalian infection and amplifies the parasite population, with each invading sporozoite generating thousands of progeny that initiate infection of the erythrocytes. Though the reversible effects of novel antimalarial translation inhibitors, including an elongation factor 2 (eEF2) inhibitor and several tRNA synthetase inhibitors, have been characterized in blood stage parasite populations, we know little about the effects of acute translation inhibition during the much more rapid growth happening during liver stage schizogony, and whether or not these effects are reversible. We hypothesized that a compound's translation inhibition efficacy, regardless of potency or mechanism of action, would determine the extent of liver stage growth and downstream developmental success after compound removal. To investigate, we developed a single cell bioimaging assay to quantify translation in hepatic schizonts of the model parasite, *P. berghei*, as well as the HepG2 hepatoma host cells. We selected a panel of reversible translation inhibitors, each having a distinct mechanism of action, with both *Plasmodium*-specific and pan-eukaryotic inhibitors included. After determining the translation inhibition EC_{50} for each compound, infected cell monolayers were treated for 4 hours at 5x and 10x EC_{50} concentrations early in the rapid schizogonous growth phase. Even at equivalent effective concentrations, we detected differences in the in efficacy of translation inhibition between the compounds. Unexpectedly, compound efficacy did not appear to determine either the speed or extent of translation recovery after thorough compound washout, or subsequent parasite growth and differentiation, as quantified by parasite size, expression of late liver stage maturation markers, hepatic merozoite formation, or release of merozoite-filled merosomes. Individual parasites from most treatments were capable of completing liver stage development, but with altered kinetics and widely variable numbers. Treatment with the least efficacious translation inhibitor resulted in only partial translation recovery, which did not appear sufficient for most parasites to resume growth, let alone complete differentiation.

B697/P1673

Toroidal cellular topology enables vertical migration of a non-motile plankton

A. Larson, R. Chajwa, H. Li, M. Prakash; Stanford University, Stanford, CA

The mass of our planet, and its daily rotation on its axis, set universal conditions that shape every cell in every organism on this planet. The impact of earth's motion on evolution and behavior is generally appreciated in the context of molecular clocks and the ubiquity of circadian processes. However, the influence of earths gravitational pull on cellular anatomy is lesser realized. It is understood that the size and shape of individual cells are defined by their environment. However, this is a dynamic process, and its understanding requires quantifying cellular behavior within a cell's native environment. Single-celled eukaryotic plankton, with a vast diversity of cellular geometry, evolved to navigate the gravitational and hydrodynamic pressures of a vertically stratified ocean and provide a unique platform for ecophysiological studies - including cellular levitation. Here we present how cellular topology can enable sinking and rising dynamics of seemingly non-motile cells in the open ocean. Since the downward gravitational pull has been a constant throughout the origin of life on our planet, the density of cellular components presented a fundamental limit on the growth of a cell living a planktonic lifestyle, allowing gravity to directly shape evolution. Several successful adaptations have enabled cells to survive under the gravitational pull; including environmental sensing, osmoregulation, and size control- mechanisms ubiquitous in higher life. How these systems determined optimal parameters however, is unknown. For Pyrocystis integrating these adaptations is crucial. By measuring the rising and sinking dynamics of the non-motile dinoflagellate Pyrocystis noctiluca throughout its life cycle with a vertical tracking microscope, we uncover a unique role of a toroidal cellular topology in regulating buoyancy via a rapid volume increase. Combining field and lab measurements with system modeling, we present a general framework for how non-motile planktonic cells escape the gravitational sedimentation trap, and how precise regulation of cell size becomes crucial to survival. We believe our model is broadly applicable to a large class of organisms. As daily planktonic migrations constituting the `biological pump' fix from 40-60% of earth's carbon emissions, understanding of this process and our anthropogenic impact will only become more important.

Adipocytes, Metabolism, and Metabolic Disorders

B703/P1674

Development of standard in vitro models of human white and brown adipose tissue.

C. Cero[1], W. Shu[2], A. Reese[3], A. R. Zhu[1], J. M. Katz[1], A. E. Pierce[1], D. Douglas[2], F. Tian[2], J. Jacobs[3], A. M. Cypess[1]; [1]NIH/NIDDK, Bethesda, MD, [2]ATCC, Biological Content, Gaithersburg, MD, [3]ATCC, Sequencing and Bioinformatics Center, Gaithersburg, MD.

The presence of bona fide thermogenic brown adipose tissue (BAT) in adult humans makes it an attractive therapeutic organ to treat metabolic diseases. However, limited access to patient-derived BAT or validated brown adipocytes (BA) cell lines hampers research of human BAT physiology. To overcome these limitations, we have established immortalized, clonal, brown (hTERT-hBA) and white pre-adipocytes (hTERT-hWA) cell lines derived from the peri-renal and subcutaneous abdominal regions, respectively, from a single donor. Differentiated BA have a higher gene (RNA-sequencing and RT-PCR) and protein (western blotting and immunohistochemistry) expression of BA-specific, thermogenic (*UCP1*, *PPARGC1A*) and mitochondrial markers (*TFAM*, *MT-CO2*). In contrast, differentiated WA express

established white adipocyte markers, including *HOXC8* and *TCF21*. Consistent with the molecular data, compared to WA, the BA have a higher metabolic rate as measured by cellular respiration. Since glucose and fatty acids are used to fuel UCP-mediated thermogenesis in brown adipocytes, we demonstrated that BA displayed higher glucose oxidation, glycolysis, and endogenous fatty acid oxidation compared to WA. Lipolysis measured by phosphorylation of hormone sensitive lipase and glycerol release was similar in differentiated WA and BA. Despite similar lipolysis, the released fatty acids produced an immediate increase in maximal respiration in BA compared to WA, indicating a higher respiratory capacity that is consistent with greater expression of transmembrane ion channels. This respiration was repressed by GDP, an inhibitor of UCP1, suggesting that the higher metabolic rate in BA is UCP1-mediated. RNA-seq transcriptomic studies revealed the β3-adrenregic receptor as clustering closest to UCP1, supporting a functional connection between the two. In summary, we have generated a well-characterized pair of WA and BA that are functional and scalable and can be used as standard models to study human adipocyte biology and physiology.

B704/P1675

Ameliorating effects of processed Curcumae longae rhizome on high-fat diet induced obese mouse model

T. Gil[1], H. Kim[1], H. Kim[1], H. An[2], Y. Cha[3]; [1]Department of Pharmacology, Sang Ji University, Wonju-si, KOREA, REPUBLIC OF, [2]Department of Oriental Pharmaceutical Science, Kyung Hee university, Seoul, KOREA, REPUBLIC OF, [3]Department of Rehabilitation Medicine of Korean Medicine and Neuropsychiatry, Sang Ji University, Wonju-si, KOREA, REPUBLIC OF.

Continuing obesity pandemic leads to constant request for preventative and therapeutic agents against the manifestation or its accompanied diseases. As a popular herbal medicine, the rhizomes of *Curcuma longa* L. (PCLR) has been used for various therapeutic applications in inflammatory disorders, wound healing, or kidney injury. This rhizome is known for promoting weight loss and reducing the possibility of obesity-related diseases. Based on the property, PCLR enhancing drug delivery was evaluated its effect on high-fat diet induced obese mouse model. C57BL/6 mice were fed 45% high-fat diet for 11 weeks. To compare the effects of processed rhizome and non-processed one, we administrated them orally and used orlistat as a positive control. At the end of the experiment, the weight of total body and different adipose tissues weight were evaluated showing slightly decreased body weight. Subcutaneous and epididymal white adipose tissues are significantly enlarged in high-fat diet group and reduced in PCLR group. Interscapular brown adipose tissues showed similar tendency of size reduction in PCLR treated group. Not only the differences of the naked eyes, the histological images of adipose tissues were different using hematoxylin and eosin staining. Bigger adipocytes were presented in the high-fat diet group and the adipocytes were dwindled in the PCLR treated ones. At the same time, the adipogenesis markers were detected in subcutaneous white adipose tissue as protein expression using western blotting assay. PCLR treatment showed decreased protein expressions on peroxisome proliferator-activated receptor γ, CCAAT/enhancer-binding protein α, and sterol regulatory element-binding protein 1 which are comparable differences with high-fat diet induced obesity group. These results suggested that PCLR could be a potential therapeutic agent for obesity.

▣ This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government Ministry of Science and ICT (MSIT) (Grant No. NRF-2021R1A2C3011862).

B705/P1676

A Study on the Anti-Obesity Effects of Lythri Herba Water Extracts *In Vivo and In Vitro*

Y. Park[1], H. Kim[1], Y. Cha[2], H. An[3]; [1]Pharmacology, Sangji University, Wonju, KOREA, REPUBLIC OF, [2]Rehabilitative Medicine of Korean Medicine and Neuropsychiatry, Sangji University, Wonju, KOREA, REPUBLIC OF, [3]Oriental Pharmaceutical Science, Kyung Hee University, Seoul, KOREA, REPUBLIC OF.

Although several studies have been reported that Lythri herba has anti-inflammatory, anti-oxidative, anti-nociceptive, anti-microbial, and haemostatic activity, there are no evidences on the effects of Lythri herba in obesity. Therefore, in this study, we aimed to investigate the anti-obesity effects of Lythri herba on high-fat diet (HFD)-induced obese mice and differentiated 3T3-L1 cells. Male C57BL/6 mice were fed either a regular chow diet or 45% HFD. The HFD-fed mice were administered with distilled water, orlistat (20 mg/kg), garcinia cambogia extracts (375 mg/kg), or Lythri herba (50, 100 or 200 mg/kg) for 8 weeks. The body weight and weight gain were significantly decreased in the 100 or 200 mg/kg Lythri herba-administered mice; however, there were no differences in food intake among HFD-fed groups. Lythri herba administration significantly reduced the concentration of serum leptin in HFD-fed obese mice. In addition, Lythri herba administration showed decrease of the mass and adipocyte size in epididymal white adipose tissue of HFD-fed obese mice. Furthermore, Lythri herba treatment markedly inhibited lipid droplets in the differentiated 3T3-L1 adipocytes. Our results demonstrate that Lythri herba could be used as a potential natural agent to treat obesity.

▣ This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ016132012022)" Rural Development Administration, Republic of Korea.

B706/P1677

The Regulatory Role of MAO-B Pathway in Obesity-associated Diseases

Y. SUNG[1], J. Kim[1], E. Kim[1], M. Lee[1], D. Yoon[1], H. Min[1], Y. Hwang[1], E. Park[2], S. Kim[2], E. Chang[1]; [1]University of Ulsan College of Medicine, Seoul, KOREA, REPUBLIC OF, [2]NeuroBiogen., Co. LTD, Seoul, KOREA, REPUBLIC OF.

Monoamine Oxidase-B (MAO-B) is a regulator of neurotransmitter which MAO-B inhibitors are known as treatment in various neurological disorders such as depression, Alzheimer's disease, and Parkinson's disease. Recent studies found that MAO-B inhibitors had correlation with metabolic syndromes, but little is yet known about the underlying therapeutic mechanism in obesity. Here we discovered the effect of newly developed reversible MAO-B inhibitor, KDS2010, on obesity *in vivo* and *in vitro*. We corroborated the effect on weight loss by oral administration(20mg/kg) of KDS2010 in diet induced obesity (DIO) mouse, while there was no significant difference in food intake between DW and KDS2010 treatment. Accordingly, we analyzed mouse activity by comprehensive lab animal monitoring system (CLAMS) whether KDS2010 supervises mouse mobility. However, mobility rate of KDS2010 treatment group was comparable with that of DW treatment group. Adipogenic differentiation of adipose tissue derived stem cell (ASC) from epididymal white adipose tissue cultured with KDS2010 showed augmented expression of brown fat related genes which is known to increase energy expenditure. These findings provide evidence for the possible role of MAO-B pathway in ameliorating obesity-associated diseases.

B707/P1678

Local fat content determines global and local stiffness in fatty livers

D. Li[1,2], P. A. Janmey[2,3,4,5], R. G. Wells[1,2,3]; [1]Division of Gastroenterology and Hepatology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, [2]NSF Science and Technology Center for Engineering MechanoBiology, University of Pennsylvania, Philadelphia, PA, [3]Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, [4]Institute for Medicine and Engineering, Philadelphia, PA, [5]Department of Physiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA.

Nonalcoholic fatty liver disease (NAFLD) is the most common form of liver disease globally, and refers to a spectrum of pathology characterized by steatosis and other changes to the liver not associated with heavy alcohol use. The contribution of mechanical changes resulting from fat accumulation to NAFLD progression is unclear and the effects of steatosis on the mechanical properties of the liver has yet to be defined. Specifically, although histology indicates a preferential accumulation of fat near the central veins of the liver, it is unknown whether this fat accumulation is associated with local variations in stiffness. Indeed, the study of any effects of tissue microarchitecture and histological features on local tissue stiffness is hampered by the inability to correlate visualized microarchitecture with measured mechanical properties in sub-millimeter regions of tissue. We studied the livers of ob/ob and wild-type littermate mice using parallel plate rheometry as well as a sub-millimeter scale microindentation technique to define the stiffness of steatotic livers at both the whole organ and meso-scale. The microindentation technique was adapted to enable determination of local fat content and identification of other local features in the indented region. We found that steatotic livers and steatotic regions of fatty livers were softer than non-steatotic livers/regions. Higher lipid content in a region significantly correlated with decreased stiffness. The finding that steatosis is associated with both global and local liver softening has significant implications for understanding the progression of early stage NAFLD, given that stiffening is a mechanical stimulus for the development of fibrosis. The ability to correlate submillimeter-scale stiffness measurements with histological features can also be used to study other pathological states, such as steatohepatitis and fibrosis, and to investigate development and disease in other tissues and organ systems.

B708/P1679

Multiomics Analysis for Characterization of the Contribution of High Fructose Corn Syrup to Non-Alcoholic Fatty Liver Disease in Obesity

G. Papadopoulos[1], A. Legaki[1], P. Vorkas[2], G. Stamatakis[3], I. Moustakas[1], H. Gika[4], M. Samiotaki[3], **A. Chatzigeorgiou**[1]; [1]Medical School, Department of Physiology, National and Kapodistrian University of Athens, Athens, GREECE, [2]Institute of Applied Biosciences, Centre for Research and Technology, Thessaloniki, GREECE, [3]Institute for Bio-innovation, Biomedical Sciences Research Center "Alexander Fleming", Athens, GREECE, [4]Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki, GREECE.

Objective: High Fructose Corn Syrup (HFCS), a sweetener rich in glucose and fructose, is nowadays widely used in beverages and processed foods; its consumption has been correlated to the emergence and progression of Non-Alcoholic Fatty Liver Disease (NAFLD). Nevertheless, the exact molecular mechanisms by which HFCS impacts hepatic metabolism remain scarce, especially in the context of obesity. In contrast, the majority of current studies focus either on the detrimental role of fructose in

hepatic steatosis or compare separately the additive impact of fructose versus glucose in high fat diet-induced NAFLD. Additionally, studies investigating the role of fructose in NAFLD utilize molecular fructose, rather than HFCS, thus not simulating properly the role of HFCS on human NAFLD. By engaging combined omics approaches, herein we aimed to characterize the role of HFCS in obesity-associated NAFLD and reveal molecular processes, which mediate the exaggeration of steatosis under these conditions. Methods: C57BL/6 mice were fed a normal-fat-diet (ND), a high-fat-diet (HFD) or a HFD supplemented with HFCS (HFD-HFCS) and upon examination of their metabolic and NAFLD phenotype, proteomic and lipidomic analyses were conducted to identify HFCS-related molecular alterations of the hepatic metabolic landscape. These results were further evaluated with enzymatic assays, which focused on specific mitochondrial functions. Results: HFD and HFD-HFCS mice displayed comparable obesity; nevertheless HFD-HFCS mice showed aggravation of hepatic steatosis, while their hepatic proteome was characterized by an upregulation of the enzymes implicated in de novo lipogenesis (DNL). Besides, palmitic and stearic acid-containing diglycerides were significantly increased in the HFD-HFCS hepatic lipidome, as compared to the HFD group. Integrated analysis of the omics datasets suggested that TCA cycle overactivation is likely contributing towards the intensification of steatosis during HFD-HFCS-induced NAFLD. Conclusion: Overall, our results imply that HFCS may significantly contribute to steatosis aggravation during obesity-related NAFLD, with its fructose-rich properties being the main suspect.

B709/P1680

A Rapid NASH Model and its Application in Candidate Drug Screening for Metabolic Syndrome and Hepatic Steatosis

C. Ju[1], Z. Chen[1], A. Zong[1], J. Zhao[1], X. Gao[1], **Z. Li**[2], M. Gao[3], X. Zhao[3]; [1]GemPharmatech Co. Ltd., Nanjing, CHINA, [2]GemPharmatech, Wilmington, DE, [3]GemPharmatech, Nanjing, CHINA.

<META NAME="author" CONTENT="陈中">

Non-alcoholic steatohepatitis (NASH) is the more aggressive form of non-alcoholic fatty liver disease (NAFLD) that may progress to cirrhosis and hepatocellular carcinoma (HCC), and so far, there is no FDA approved therapies for NASH. For therapeutic development, animal models of NAFLD/NASH are crucial for preclinical evaluation of efficacy and safety. Given the rapidly growing recognition that NAFLD/NASH is the hepatic manifestation of metabolic syndrome, scientists are paying a lot more attention to the metabolic characteristics of NASH animal models they choose to work with. Among most classical NASH animal models, the long duration of diet treatment or the lack of metabolic phenotypes limits their application in candidate drug screening. To fill the gap, GemPharmatech developed a rapid NASH model based on Alms1 mutant mice (B6-Alms1-del mice). B6-Alms1-del mice develop a plethora of early onset metabolic phenotypes on chow diet, such as obesity, hyperglycemia, hyperlipidemia and fatty liver. To accelerate the NASH progression, B6-Alms1-del mice were fed with western diet (high fat, high fructose and high cholesterol), which exacerbated obesity, hypercholesterolemia and liver injury within 6 weeks. Furthermore, major clinical features of NASH patients were observed in western diet fed B6-Alms1-del mice, including hepatic steatosis, inflammation, ballooning, mild fibrosis as well as significantly increased NAFLD activity score. To verify the applicability of this Alms1 NASH model in preclinical research, two promising preclinical drugs MGL-3196 and Semaglutide were used to treat Alms1 NASH mice. Our results indicate that both drugs lead to benefit on NASH, including body weight control, improvement in hyperlipidemia, liver injury, hepatic steatosis and fibrosis. In conclusion, we have

established a rapid mouse model for NASH. This model exhibits clinically relevant metabolic phenotypes and responds to candidate drugs that have recently showed promising phase II clinical results. This rapid NASH model provides a much needed preclinical capability to assess the efficacy of novel NASH therapeutics in a timely manner.

B710/P1681

Axon guidance molecule Slit3 is essential for cold-induced remodeling of BAT neurovasculature and thermogenesis

T. Duarte Afonso Serdan[1], H. Cervantes[1], F. Mousovich Neto[1], Y.-H. Tseng[2], **F. Shamsi**[1]; [1]New York University, New York, NY, [2]Joslin Diabetes Center, Harvard Medical School, Boston, MA

Brown adipocytes are embedded in a dense network of blood vessels and sympathetic nerves that supports their thermogenic activity. Cold acclimation enhances brown adipose tissue (BAT) thermogenesis through the coordinated induction of brown adipogenesis, angiogenesis, and sympathetic innervation. Increasing the density of blood vessels and sympathetic innervation is essential for enhanced thermogenesis in cold. However, how these distinct processes are spatiotemporally coordinated remains unclear. We have recently used single-cell RNA-sequencing (scRNA-seq) to build a network of ligand-receptor interactions involved in the cold-induced remodeling of BAT. We identified Slit guidance ligand 3 (Slit3) as a new adipokine that mediates the crosstalk among adipocyte progenitors, endothelial cells, and sympathetic nerves. Slit3 is a member of the Slit family of secreted axon guidance molecules. Using a combination of in vivo gain and loss of function studies, we showed that Slit3 is essential for BAT thermogenesis. Mice lacking Slit3 exhibited severe cold intolerance and decreased energy expenditure concomitant with abnormal BAT morphology and reduced expression of thermogenetic and mitochondrial genes. Conversely, the overexpression of Slit3 enhanced BAT thermogenesis. Analysis of BAT vasculature using imaging and molecular analyses revealed that loss of Slit3 expression significantly impaired cold-induced angiogenesis in BAT, indicated by the lower density of vasculature and reduced expression of endothelial-specific transcripts. Additionally, BAT-specific loss of Slit3 led to a drastic reduction in the density of Tyrosine Hydroxylase (TH) expressing sympathetic neurites in BAT. Our mechanistic studies identified the key regulatory events that control Slit3 signaling in vascular endothelial cells and sympathetic nerves. Collectively, these findings established the essential role of Slit3 signaling in the regulation of BAT angiogenesis, sympathetic innervation, and thermogenic function and introduced Slit3 as a new niche factor that promotes adipose tissue health. This work introduced a potential node of intervention for improving systemic metabolisms by stimulating the healthy expansion of thermogenic fat.

B711/P1682

Gonadotropin insensitivity mediates obesity-associated hypotestosteronism in mouse Leydig cells

C. Chuang, M. Hsu, C. Chiu; National Taiwan University - Taipei, Taipei city, TAIWAN.

Obesity is a public health issue in the current society and threatens the humanbody in multiple ways. One of obesity-linked reproductive disorders ishypotestosteronism which shows the lower testosterone levels in the male patient.Since how the obesity causes hypotestosteronism is still unclear, this study aims toidentify the factors that result in steroidogenic impairment in both mouse model andcultured cell study. To test the gonadotropin sensitivity in the obese mice, the controldiet (CD) or high fat diet (HFD)-fed C57BL/6 mice were injected with humanchorionic gonadotropin (hCG) to stimulate

testosterone production before sampling.The results shown that the plasma testosterone level in the HDF mice wassignificantly lower than CD group after hCG stimulation. In the cell experiments, tomimic the high level of free fatty acid in the blood in the HFD-fed mice, we treatedthe mouse Leydig cell line MA-10 with high concentration BSA-conjugated fattyacids. The results shown that the steroidogenic ability was significantly decreased inthe fatty acid-treated cells. We further examined the activity of steroidogenic enzymesby supplying the cells with different steroid intermediates. The progesteroneproduction was significantly lower in the fatty acid-tread cells when the cells given22R-OHC but not pregnenolone, which suggests that CYP11A1 was impaired underthe fatty acid-overloading condition. Moreover, both mRNA and protein levels ofCYP11A1 is significantly decreased after the fatty acid treatment. To summarize,obesity-induced hypotestosteronism may be caused by the gonadotropin insensitivityin the Leydig cells. The excessive level of free fatty acids plays an important role inimpairing steroidogenic enzyme expression; however, the transcriptional regulationinfluenced by fatty acid overloading needs more studies to elucidate.

B712/P1683

Optimizing Immunostaining for Adipose Tissue in Adult Drosophila Melanogaster

R. K. Ott, A. R. Armstrong; University of South Carolina, Columbia, SC

Obesity prevalence and increased risk for several comorbidities, such as diabetes mellitus, coronary artery disease, and several cancers, highlight the importance of multisystem-based research aimed at addressing complex health problems. The Armstrong laboratory uses Drosophila melanogaster, commonly known as the fruit fly, to investigate how adipose tissue communicates dietary input to peripheral organs, such as the ovary. While it is clear that adipocytes in adult Drosophila females use nutrient-sensing pathways like mTOR to dynamically influence the ovarian germline stem cell lineage, we know relatively little about how nutrition and signaling pathway activity impact the adipose tissue. One crucial step toward filling this knowledge gap is establishing a protocol to robustly and reproducibly assess changes in the adipose tissue at the cellular level. By modifying various steps of routine whole mount immunofluorescence labeling, we report a contemporary process for obtaining optimal cellular imaging of adult Drosophila adipose tissue, or the fat body. Specifically, I find that carrying out fixation, washes, and antibody incubations on splayed and pinned abdominal carcasses enhances endogenous fluorescence reporters and immunoreactivity. We are currently altering additional steps to optimize the immunostaining protocol - fixative type (e.g. paraformaldehyde versus glutaraldehyde) and concentration, primary antibody concentration, and detergent type (Triton X-100 versus Tween 20) and concentration. This optimized whole mount immunofluorescence protocol will be employed in future studies investigating the dietary response of Drosophila adipose tissue.

Defining Therapeutic Targets and New Therapeutics- For Neurological Diseases

B713/P1684

Discovery of a novel dual-action small molecule that improves multiple Alzheimer's disease pathologies

H. Jin[1], M. Park[2], K. Park[2], B. Choi[1], H. Yoon[2], H. Jung[2], J. Bae[2]; [1]College of Veterinary Medicine, Kyungpook Natl Unive, Daegu, KOREA, REPUBLIC OF, [2]Department of Physiology, School of Medicine, Kyungpook Natl Unive, Daegu, KOREA, REPUBLIC OF.

Alzheimer's disease (AD) is characterized by complex, multifactorial neuropathology, suggesting that small molecules targeting multiple neuropathological factors are likely required to successfully impact clinical progression. Acid sphingomyelinase (ASM) activation has been recognized as an important contributor to these neuropathological features in AD, leading to the concept of using ASM inhibitors for the treatment of this disorder. Here we report the identification of KARI 201, a direct ASM inhibitor evaluated for AD treatment. KARI 201 exhibits highly selective inhibition effects on ASM, with excellent pharmacokinetic properties, especially with regard to brain distribution. Unexpectedly, we found another role of KARI 201 as a ghrelin receptor agonist, which also has therapeutic potential for AD treatment. This dual role of KARI 201 in neurons efficiently rescued neuropathological features in AD mice, including amyloid beta deposition, autophagy dysfunction, neuroinflammation, synaptic loss, and decreased hippocampal neurogenesis and synaptic plasticity, leading to an improvement in memory function. Our data highlight the possibility of potential clinical application of KARI 201 as an innovative and multifaceted drug for AD treatment

B714/P1685

Generation of CRISPR- and AID-based reagents to assess the neuroprotective effects of HUM-2 and SPAS-1 loss in *C. elegans*

J. Feduccia, **F. Akoachere**, E. S. Luth PhD; Simmons University, Boston, MA.

Parkinson's disease (PD) is associated with excessive alpha-Synuclein (αS) buildup, which induces the death of dopaminergic neurons. There's no cure for PD, and identification of new therapeutic targets would be impactful. Preliminary data suggests that pharmacologic inhibition of cytoskeleton-associated proteins myosin-V and spastin in cell culture reduced αS aggregations and improved neuroblastoma cell growth. Additional preliminary evidence indicates that genetic disruption of protein homologs in *C. elegans*, HUM-2 and SPAS-1, promotes dopaminergic neuron survival in a model with αS-induced neuronal loss. However, lifelong knockout of myosin-V and spastin in humans is not a viable therapeutic strategy. To assess whether myosin-V/HUM-2 and spastin/SPAS-1 are truly viable therapeutic targets, we must determine whether reduction of protein activity in adults is sufficient to confer neuroprotection. To test this cleanly, we designed a strategy that uses the auxin-inducible degron (AID) system that allows for spatial and temporal control of protein degradation in the presence of auxin and the enzyme TIR1. We generated a set of CRISPR- and AID-based reagents that will allow us to insert an AID tag to endogenous HUM-2 and SPAS-1 proteins, and disrupt their expression in adult *C. elegans* neurons. This includes guide RNA to direct the CRISPR-Cas9 machinery to the *hum-2* or *spas-1* locus and specific donor repair DNA to be inserted into the cut site. To generate single guide RNAs, we used templateless PCR to combine the DNA for locus-specific crRNA with a universal tracrRNA DNA sequence, followed by in vitro transcription. To create linear DNA repair templates, we performed PCR using

primers with homology surrounding the HUM-2 or SPAS-1 translational start sites to amplify, from a plasmid, DNA containing an AID sequence and fluorescent tag. We then used magnetic bead purification to remove excess primer. Overall, we have generated and purified the reagents necessary to use the AID system and combined our products with purified Cas9 protein for injection into worms that express TIR1. Upon confirmation of successful CRISPR editing, we will introduce auxin to adult worms to assess the degree of neuroprotection at this late stage. This work will support the evaluation of myosin-V and spastin as potential drug targets to treat aSyn-induced dopaminergic neuron death in PD.

B715/P1686

Eclipse, an automated CRISPR platform for the large scale generation of cell models for the iPSC Neurodegenerative Initiative (iNDI)

P. Deng, A. King, M. Morell, A. Chair, D. Sailor, A. G. Sommer, K. Holden; Synthego, Redwood City, CA.

The National Institutes of Health led iPSC Neurodegenerative Disease Initiative (iNDI) is the largest iPSC genome engineering project attempted with the goal of generating a widely available and standardized set of diseased cell models for over 100 single nucleotide variants (SNV) mutations associated with Alzheimer's disease and related dementias (ADRD) in isogenic IPSC lines. The standardization of cell models is of vital importance for the generation of reproducible and actionable data in therapeutic development. As part of a multi-institution collaboration, Synthego was selected for the generation of 25 SNVs in the candidate KOLF2.1 iPSC line. Toward these goals, we describe the use of our automated, high throughput CRISPR editing platform, ECLIPSE, for the rapid generation of knock-in iPSC models of ADRD. We leveraged our state-of-the-art knock-in methods and automated pipelines for the design, experimental optimization, and clonal isolation of 23 of the candidate target mutations in iPSCs. For each SNV target, at least 3 clonal homozygous and 6 clonal heterozygous mutation lines were generated for a total of 264 clonal cell lines over a 6-month period. The utilization of automated systems such as our ECLIPSE platform are critical catalysts for the rapid development of relevant cell models in large scale disease initiatives such as iNDI.

B716/P1687

Vitamin B5 rescues TANGO2 deficiency-associated defects in flies and human cells

M. Sacher[1], P. Asadi[1], M. Milev[1], D. Saint-Dic[1], C. Gamberi[2]; [1]Concordia University, Montreal, QC, CANADA, [2]Coastal Carolina University, Conway, SC.

Mutations in Transport and Golgi Organization 2 (TANGO2) are associated with intellectual deficit, neurodevelopmental delay and crisis-induced phenotypes including rhabdomyolysis and cardiac arrhythmias, the latter of which are potentially lethal. These phenotypes contribute to the morbidity and mortality rate in affected individuals and preventing a metabolic crisis would reduce the mortality rate. The function of TANGO2 remains unknown but is suspected to be involved in some aspect of lipid metabolism. Here, we employ insect and human cells to examine the effects of vitamin B5, a Coenzyme A (CoA) precursor, on alleviating the cellular and organismal defects associated with TANGO2 deficiency. We demonstrate that this vitamin specifically positively affects multiple behavioural aspects of TANGO2 deficiency in *Drosophila melanogaster* and rescues membrane trafficking defects in human cells. We also observed a rescue of some of the fly defects by vitamin B3, though not to the same extent or level as vitamin B5. Our data suggest that a B complex supplement containing at least vitamin B5 may have therapeutic effects in individuals with TANGO2-associated disease.

B717/P1688

Identification of β-III-spectrin actin binding modulators for treatment of spinocerebellar ataxia

P. Guhathakurta[1], S. A. Denha[2], A. R. Keller[2], A. L. Carter[1], A. E. Atang[2], R. T. Rebbeck[1], B. Svensson[1], D. D. Thomas[1], T. S. Hays[1], **A. W. Avery**[2]; [1]University of Minnesota, Minneapolis, MN, [2]Oakland University, Rochester, MI.

β-III-spectrin is a key cytoskeletal protein that localizes to the soma and dendrites of cerebellar Purkinje cells, and is required for dendritic arborization and signaling. Mutations in the *SPTBN2* gene encoding β-III-spectrin cause the neurodegenerative disorder spinocerebellar ataxia type 5 (SCA5). Currently, there is not cure or therapy for SCA5. Numerous SCA5 mutations localize to the β-III-spectrin N-terminal actin-binding domain (ABD). We previously showed that an ABD-localized L253P mutation causes a 1000-fold increase in actin-binding affinity. Here we report the molecular characterization of ten additional ABD-localized mutations, and our progress towards developing a SCA5 therapeutic. Significantly, we found that increased actin binding is a shared molecular consequence of all tested SCA5 mutations. Further we developed a novel fluorescence-based high throughput screening platform that uses purified L253P β-III-spectrin ABD protein and F-actin. To validate the assay, we screened a 3,000-compound library of FDA approved drugs. Significantly, numerous compounds were identified that decreased FRET between fluorescently labeled L253P ABD and actin. The activity of multiple Hit compounds was confirmed in orthologous co-sedimentation actin-binding assays. The activity of a subset of compounds results from compound-induced aggregation of the mutant L253P ABD, but not wild-type ABD. Our results suggest that a small molecule that reduces actin binding of mutant β-III-spectrin may be broadly effective as a SCA5 therapeutic. Future medicinal chemistry to optimize our current Hit compounds may lead to a SCA5-specific therapeutic.

B718/P1689

Evaluation of Blood-Based, Extracellular Vesicles as Biomarkers for Aging-Related TDP-43 pathology

C. N. Winston-Gray[1], S. Sukreet[1], V. M. Lee[2], D. M. Wilcock[3], P. T. Nelson[3], R. A. Rissman[1,4]; [1]Neurosciences, UC San Diego, LA Jolla, CA, [2]University of Pennsylvania, Philadelphia, PA, [3]University of Kentucky, Lexington, KY, [4]VA San Diego Healthcare System, La Jolla, CA.

ABSTRACT

INTRODUCTION: Limbic predominant age related TDP-43 encephalopathy neuropathological change (LATE-NC) is a recently characterized brain disease that mimics Alzheimer's disease (AD) clinically. To date, LATE-NC is difficult to diagnose antemortem using clinical information or biomarkers. Recent studies suggest concentrations of extracellular vesicle (EVs) protein cargo derived from neuronal and glial cells may serve as useful diagnostic biomarkers for AD and other neurodegenerative diseases. **METHODS**: TDP-43 was evaluated in neuronal (NDEVs), astrocyte (ADEVs), and microglial derived extracellular vesicles (MDEVs). EV preparations were isolated from the plasma of research subjects with autopsy-confirmed diagnoses, including many with LATE (n = 22). Quantified TDP-43 concentrations were compared to cohort that included healthy controls, mild cognitively impairment (MCI), and AD dementia with diagnoses other than LATE-NC (n = 42). **RESULTS**: TDP-43 was significantly elevated in plasma ADEVs derived from autopsy confirmed LATE-NC subjects, with or without comorbid AD pathology. Measurable levels of TDP-43 were also detected in EV depleted plasma; however, TDP-43 levels were not significantly different between persons with and without eventual autopsy confirmed

LATE-NC. No correlation was observed between EV TDP-43 levels with cognition-based variables, sex, and APOE carrier status. **DISCUSSION**: Blood-based EVs, specifically measuring TDP-43 accumulation in ADEVs, may serve as a potential diagnostic tool to rapidly identify subjects who are currently living with LATE-NC.

B719/P1690

Genipin rescues sensory neuron defects in the peripheral nervous system

K. Saito-Diaz[1], P. Dietrich[2], A. Patel[1], C. G. Wzientek[1], A. R. Prudden[1], G. Boons[1], S. Chen[3], L. Studer[4], I. Dragatsis[2], N. Zeltner[1]; [1]University of Georgia, Athens, GA, [2]The University of Tennessee, Memphis, TN, [3]Weill Cornell Medical College, New York, NY, [4]Sloan Kettering Institute, New York, NY.

The peripheral nervous system (PNS) allows us to sense temperature, pain, regulate blood pressure, detect the position of our body, and is essential for proper body function. Diseases of the PNS are inflicted by various causes, including injury, infection, toxins, and mutations. Currently, there are no treatments for peripheral neuropathies. Familial Dysautonomia (FD) is an extremely devastating, genetically defined childhood disorder that specifically affects the PNS, and serves as a model disorder to study PNS dysfunction. FD currently has no available cure. It is caused by a homozygous point mutation in the *ELP1* gene leading to developmental and degenerative defects in the sensory and autonomic lineages, which are associated with loss of pain perception, heart-rate instability, and dysautonomic crisis, a severe dysregulation of the autonomic nervous system. Current FD treatments are only supportive and do not address the disease mechanism. We previously employed human pluripotent stem cells to show that peripheral sensory neurons (SNs) are not generated efficiently and degenerate over time in FD. Here, we conducted a chemical screen to identify compounds able to rescue this SN differentiation inefficiency. We discovered genipin, a compound that is prescribed in the form of a Traditional Chinese Medicine for neurodegenerative disorders. We describe genipin's neurogenic and neuroprotective properties. Genipin restores neural crest and SN development in FD, both in the hPSC model as well as in the FD mouse model. Importantly, genipin was able to cross the placenta, exert its restorative action and showed no toxicity to mother or pups. It further protects against neurodegeneration in FD, an important finding since prevention of degeneration is a treatment that could be offered to FD patients and possibly other patients suffering from PNS neurodegenerative disorders. We found that genipin's mode of action is exerted via crosslinking of the extracellular matrix. Finally, we show genipin's potential to enhance PNS regeneration in an *in vitro* axotomy model. Our results suggest genipin as a promising drug candidate for treatment of FD neurodegeneration. They further suggest that genipin may be further developed to enhance regeneration of PNS neurons.

B720/P1691

CRISPR/Cas9 mediated generation and characterization of a zebrafish epilepsy model with mutant *slc25a22a*

S. H. Lee[1], M. K. Kim[2], S. Y. Choi[1]; [1]Biomedical Sciences, Chonnam National University Medical School, Hwasun 58128, KOREA, REPUBLIC OF, [2]Neurology, Chonnam National University Medical School, Gwangju 501759, KOREA, REPUBLIC OF.

Epilepsy is a group of neurological disorders characterized by abnormal neuronal activity in the central nervous system (CNS). Approximately 3% of the population is affected by epilepsy in the world. The etiology of epilepsy remains elusive, yet epilepsy has been reported to have a strong genetic

component. Nevertheless, genetic abnormalities that may cause epilepsy remains mostly unknown. To identify epilepsy-related genes, we analyzed genome sequences of 400 Korean epilepsy patients by Whole Exome Sequencing (WES) and identified 171 candidate genes linked to epilepsy. Among them, the role of 17 candidate genes in epilepsy remains elusive. Whole-mount in situ hybridization revealed that all the 17 candidate genes are expressed in the zebrafish brain. Among them, only the *slc25a22a* morphants displayed epileptic-like hyperexcitability phenotype. Slc25a22a, a mitochondrial glutamate carrier 1 (GC1), was localized to the zebrafish forebrain's mitochondria. Further, to explore a role for *slc25a22a* in epilepsy, *slc25a22a* zebrafish mutant was generated using the CRISPR/Cas9 system. The *slc25a22a*$^{-/-}$ embryos recapitulated the morphant phenotype and showed hyperactive and convulsive behavior. In addition, the GABAergic/glutamatergic balance and excitatory/inhibitory post-synaptic balance were disrupted in *slc25a22a*$^{-/-}$. Interestingly, the *slc25a22a*$^{-/-}$embryos showed excess lipid accumulation similar to the human *SLC25A22* mutant epilepsy patients. Further, we intend to perform electrophysiological recordings and real-time neuronal activity to uncover the precise molecular mechanisms leading to the onset of epilepsy in *slc25a22a*$^{-/-}$. Taken together, our *slc25a22a*$^{-/-}$ line can be used for extensive epilepsy-related studies and to identify novel small molecules for therapeutic intervention in epilepsy.

B721/P1692

ARID1B plays a role in the development of social attachment behavior after early life adversity in mice

T. L. Ford, B. Jeon, W.-Y. Kim; Kent State University, Kent, OH

Adverse living environments can disrupt maternal behavior, leading to early life stress for infants. However, the molecular and cellular mechanism underlying adversity-induced behavioral changes in infants are unclear. AT-Rich Interactive Domain 1B (ARID1B) is a chromatin modifying protein and is involved in early cell development. Although ARID1B haploinsufficiency is known to cause autism spectrum disorder, nothing is known about whether ARID1B haploinsufficiency contributes to behavioral alteration after early life adversity. Using Arid1b heterozygous mice as a model of ARID1B haploinsufficiency, we created an early scarcity-adverse (S-A) environment for pups for a few days by reducing wood chip bedding and nesting materials by 80% in the cage. Then we assessed social bonding behavior of the pups by a battery of behavioral tests including the rodent strange situation paradigm (rSSP). Results showed that the wild type pups that underwent the S-A condition displayed decreased social attachment to their mother compared to wild type non-S-A pups. Importantly, the Arid1b haploinsufficient S-A pups showed a moderate increase in attachment behavior compared to the non-S-A Arid1b group. We further examined adolescent mouse behavior after weaning. The open field and three-chamber tests revealed a decrease in social exploration and an increase in anxiety-like behavior in the wild type S-A group compared to non-S-A wild type mice. However, both Arid1b haploinsufficient non-S-A and S-A groups showed decreased anxiety, which was not reflective of adult Arid1b mutant behavior, suggesting a potential phenotypic change throughout development. Together, our data reveals an impact of early adverse environment on pup social bonding and further suggests ARID1B as a mechanistic factor for the development of early social behavior.

B722/P1693

Inhibition of Notch signaling in endothelial cells preserves cognitive function in a model of familial Alzheimer's disease

S. R. Villa-Niemczyk, L. A. Naiche, J. I. Arowolo, J. K. Kitajewski; University of Illinois At Chicago, Chicago, IL

Alzheimer's disease (AD) afflicts over 6 million people in the United States and is expected to grow to 13 million by 2050. The development of effective therapeutics is hampered not only by an incomplete understanding of AD pathogenesis, but also the challenges of identifying disease-modifying therapies that can penetrate the blood-brain barrier (BBB). Increasing evidence implicates cerebrovascular dysfunction in the pathogenesis of AD. Brain endothelial cells act simultaneously as a barrier and signal transducer between the brain and peripheral environment and contribute to the regulation of neurogenesis and neuroinflammation via expression of angiocrine signaling factors. Notch proteins play critical roles in vascular development and function. Dysregulation of brain endothelial Notch (EC-Notch) signaling leads to dysregulation of neurogenesis and BBB integrity, and AD-associated alterations in γ-secretase activity have been shown to affect Notch signaling. Here, we investigate changes in EC-Notch signaling during normal aging and AD and test the effect of EC-Notch inhibition on AD onset and progression. We found that EC-Notch signaling in the brain changes with age, is disrupted in AD, and inhibition of EC-Notch signaling is protective against AD-induced cognitive impairment. Although Notch is a well-described regulator of angiogenesis, we did not find overt changes in vascular morphology or density in response to Notch inhibition, suggesting that preservation of cognitive function may result from altered angiocrine signaling in the brain. These data suggest that precision targeting of EC-Notch could be therapeutically beneficial without requiring BBB penetration.

B723/P1694

Dopamine-beta-hydroxylase Short Interfering Nucleic Acid Overcomes the Short-term Efficacy of Timolol and Bimatoprost in Noradrenergic Overactivated Glaucomatous Eye

C. Carneiro[1], L. Carvalho[2], M. Moutinho[2], S. Bessa[1], P. Kennedy[1], P. Correia-de-Sá[2,3], **P. Soares-da-Silva**[4,5,6]; [1]Phyzat Biopharmaceuticals, PORTO, PORTUGAL, [2]Laboratório de Farmacologia e Neurobiologia, Instituto de Ciências Biomédicas Abel Salazar - Universidade do Porto (ICBAS-UP), PORTO, PORTUGAL, [3]MedInUP – Center for Drug Discovery and Innovative Medicines, University of Porto, Porto, PORTUGAL, [4]MedInUP – Center for Drug Discovery and Innovative Medicines, University of Porto, PORTO, PORTUGAL, [5]University Porto / Faculty Medicine, Porto, PORTUGAL, [6]Phyzat Biopharmaceuticals, Porto, PORTUGAL.

We report a novel anti-glaucoma short interference Nucleic Acid Therapy (GLAUsiNAT) targeting dopamine-β-hydroxylase (DBH) to specifically and selectively attenuate sympathetic noradrenergic overactivity in the glaucomatous eye. Human neuroblastoma SK-N-SH cells were transfected with 0.1-10 nM of anti-DBH double-stranded nucleic acid (dsNA) complexed with a commercial liposomal transfection agent. DBH gene and protein expression were analysed by RT-qPCR and Western blot, respectively. DBH activity was quantified as the conversion of dopamine to noradrenaline by HPLC-EC. The effects of anti-DBH dsNA, timolol and bimatoprost eyedrops were evaluated in a rat model of intraocular hypertension induced by topical application of tyramine 0.1% (all experiments involving animals were approved by DGAV and the ICBAS-UP Animal Ethics Committee; decision 316/2019/ORBEA). The intraocular pressure (IOP) was measured hourly for 6 hours in rats using a

rebound tonometer (TonoLab). Human and rat dsNA sequences were selected from human (NM_000787.4) and rat (NM_013158.3) DNA target sequences. In SK-N-SH cells, human dsNA sequences decreased DBH mRNA expression (IC_{50}=1.3 nM) causing an 80% knockdown of DBH protein expression, associated with significant reductions (49.1±4.1%) in DBH activity and noradrenaline content. Tyramine eyedrops raised the IOP by 40-30% above baseline. Increases in IOP were prevented by timolol eyedrops (0.5%, 30 min before) and by reserpine IP (1 mg/kg, 24h before). Eyedrops containing the anti-DBH rat dsNA or the vehicle (PBS) were applied twice (12 h apart) in the day before the first tyramine test to right and left eyes. The topical application of anti-DBH rat dsNA (0.004-4.0µg/10µL) prevented tyramine-induced IOP increases in a dose-dependent and sustained manner up to 7 days. This contrasts with the limited efficacy of timolol (0.5%) and bimatoprost (0.1%) commercial eyedrops, which drastically declined 24h after topical application using the same experimental procedure. Data show that anti-DBH GLAUsiNAT produces a long-lasting decrease in DBH expression and activity and restrained noradrenaline availability, which allow it to overcome the short-term efficacy of timolol and bimatoprost in the noradrenergic overactivated glaucomatous eye. *Work partially supported by SIRNAGLAU project (#39743), co-funded by NORTE2020, PT2020 and the European Union.*

B724/P1695

Exploring the Effect of Aurora Kinase Inhibitors on Growth Suppression in a Yeast ALS Model

G. Cruz[1], M. Kozlov[1], R. Segal[1], S. Cobos[1,2], M. P. Torrente[1,2]; [1]CUNY Brooklyn College, Brooklyn, NY, [2]CUNY Graduate Center, New York City, NY.

Amyotrophic Lateral Sclerosis (ALS), or Lou Gehrig's Disease, is a fatal neurodegenerative disease characterized by the degeneration of motor neurons. Neuronal death results in muscle atrophy, loss of motor control in the limbs, and paralysis. Currently, ALS has no cure and FDA-approved treatments fail to control symptoms.

ALS is associated with various genes, most notably chromosome 9 open reading frame 72, or *C9orf72*. Hexanucleotide repeat expansions in the *C9orf72* gene are the most common cause of ALS. These hexanucleotide expansions result in dipeptide repeat proteins, which aggregate into inclusions in the motor neurons of ALS patients.

Exploiting a yeast ALS model overexpressing the dipeptide repeat protein PR_{50}, comprising 50 repeats of the dipeptide Proline-Arginine under a galactose-inducible promoter, we have previously discovered that overexpression of PR50 is associated with an increase in the phosphorylation level of Histone H3 on Serine 10 (H3S10ph). Furthermore, we previously found that knockdown of Ipl1, the yeast homolog of Aurora B Kinase responsible for H3S10ph in yeast, leads to a decrease in the levels of H3S10ph and growth rescue in yeast overexpressing PR_{50}.

Histone modifications are highly accessible targets for pharmaceutical intervention. Therefore, with the aim of preventing H3S10 phosphorylation increases and ameliorating PR_{50} toxicity without the need for genetic manipulation, we investigated the effects of chemical inhibition of Aurora Kinase in cell survival in the context of PR_{50} overexpression. We performed serial dilution growth assays on PR_{50} yeast in the presence of two Aurora B Kinase inhibitor drugs, Jadomycin B or Barasertib, at varying concentrations. However, we found that there was minimal rescue from growth suppression. Moving forward, we will test out other drugs that may be more specific for Ipl1 inhibition and able to penetrate yeast. We will also carry out virtual screening to uncover more potent inhibitors of Ipl1/Aurora B kinase. After successful inhibition of Ipl1 and growth rescue of PR_{50} yeast, we will establish the significance of epigenetic drugs in the treatment of neurodegenerative disease.

Monday, December 5, 2022

Scholarship of Diversity

B1/P1696

Increasing Diversity and Re-engaging Undergraduates in Course-Based Research Experiences (CURE) in a COVID-19 World

L. Schramm; Department of Biological Sciences, St. John's University, Queens, NY.

Undergraduate research is a high-impact learning practice. COVID-19 has severely debilitated participation in undergraduate research for many undergraduate students. Further, higher education experienced a significant change in the full-time faculty available to mentor undergraduate independent research. This project aims to combine the technical skills of undergraduate students and faculty to re-engage students in research projects in various STEM disciplines based on student interests and faculty expertise while increasing diversity and inclusion. Using publicly available datasets and an open-source web-based scientific analysis platform, we created an online independent research experience for undergraduates tailored to students' interests. For example, we had students interested in the opioid crisis in the United States, pesticides in fish, and the biological effects of xenoestrogens. All students, with one faculty member, completed a mini-research project with data to present at a research conference. The methodology has the advantage of scaling up to a larger venue such as a major elective for credit and provides real-time assessment data. Of greater significance, our method provides a means to discuss diversity and inclusion in the public datasets (old versus newer datasets) while creating a research space conducive to best diversity and inclusion practices in the student researchers participating in our customizable research experience.

B2/P1697

A transatlantic perspective on LGBTQ+ retention in STEM

C. Chinowsky[1], S. Coffield[2], K. Clark[3], A. Dye[4], J. O'Connor[5], R. Prawer[6]; [1]Salk Institute for Biological Studies, La Jolla, CA, [2]University of California Irvine, Irvine, CA, [3]Washington University at St Louis, St Louis, MO, [4]North Carolina State University, Raleigh, NC, [5]California Council on Science and Technology, Sacramento, CA, [6]United Kingdom Science and Innovation Network, Boston, MA.

Increasing LGBTQ+ retention is vital to improving the diversity of perspectives and experiences necessary to drive high-quality scientific innovation. The issue of LGBTQ+ attrition has become more apparent in recent years, with some studies estimating that LGBTQ+ people across STEM professions are 17-21% less represented, drop STEM majors at high rates, and face clear limitations and harassment. However, the issue has received very little attention, resources, and policy interventions, partly due to a lack of widespread demographic data on sexual orientation and gender identities. Our group of early-career scientists in the US partnered with the UK Science and Innovation Network via the National Science Policy Network's (NSPN) Science Diplomacy Exchange and Learning (SciDEAL) program. We took a multifaceted approach to understanding and addressing the data on LGBTQ+ attrition. This included a comprehensive literature review, interviews, and roundtables with stakeholders across universities, governments, and non-profit organizations from the US and UK. We present a set of policy recommendations on how to improve data collection, combat toxic professional environments, and

support LGBTQ+ scientists in various settings. This synthesis will help provide clear actions universities, funding agencies, and professional societies can take to better understand and address LGBTQ+ attrition.

B3/P1698

Reverse Mentoring in STEM: Changing Perspectives to Foster Dialogue

Y. R. Seger, **F. Banda**; Federation of American Societies for Experimental Biology (FASEB), Rockville, MD.

By pairing a senior-level mentee with a junior-level mentor, reverse mentoring provides organizations with key insights into the personal and professional needs and preferences of staff members from a variety of backgrounds and experiences. Although successfully utilized by the corporate world for over a decade to help employees gain appreciation of colleagues' lived experiences, reverse mentoring has not been adopted in academic research settings. Given the well-documented barriers to participation and attrition of individuals from historically excluded groups from the professoriate, we established a reverse mentoring program for scientists and staff of scientific societies to assess the ability of this strategy to foster a greater understanding and appreciation of differences between individuals at different career stages and lived experiences. Our presentation will summarize key findings from our pilot cohort of six reverse mentoring pairs, including applicant statistics, reverse mentoring curriculum, and preliminary participant feedback, demonstrating the utility of reverse mentoring in dismantling traditional hierarchies in academic research environments.

B4/P1699

Growing the Pipeline of Men of Color in Medicine

M. C. Pickron-Davis, Administra, J. Gerhart, Researcher, M. Martin, Student; Office of Diversity and Community Relations, Philadelphia College of Osteopathic Medicine, Philadelphia, PA.

Healthcare professions suffer from an imbalance in the number of culturally and ethnically diverse providers and the populations they serve. This poster presentation illustrates our commitment to mitigating the dearth of underrepresented men in medicine. Currently, African Americans make up 13% of the U.S. population, but only 4% of U.S. doctors and less than 7% of U.S. medical students. (Of active U.S. doctors in 2013, 48.9% were white, 11.7% were Asian, 4.4% were Hispanic or Latinx, and 0.4% were American Indian or Alaska Native.) (HBR, August 10, 2018). In 1986, 57% of black medical school graduates were men — but by 2015 that number had declined to just 35%, even as the total number of black graduates in all fields had increased. Although Latinos are the fastest growing ethnic group in the nation, the rate of Latino doctors as compared to the population has steadily declined in recent years. In 1980, there were 135 Latino physicians for every 100,000 Latinos, but by 2010 the number had dropped to 105 for every 100,000, a 22% decline. (nbcnews, February 11, 2015). Since minority health professionals are more likely than Whites to practice in minority and medically underserved areas, a more diverse health workforce could help to improve access to healthcare. (KFF, October 2008).
In summer 2019, the Office of Diversity and Community Relations and Division of Research and Science at the Philadelphia College of Osteopathic Medicine (PCOM) partnered with Cabrini University, an emerging Hispanic-Serving Institution, to pilot the inaugural African American and Hispanic Male Undergraduate Research initiative. Studies show that African American and Hispanic males are under-represented in science, technology, engineering and mathematics (STEM) educational programs and careers. Existing research has suggested that a lack of access to advanced science courses and

professional environments could be one reason for the disparity. Addressing this crises and challenge requires intentionality, innovation, resources, and partnerships. Our program aims to offer opportunities for African American and Hispanic male undergraduate science majors at Cabrini University to engage in research at PCOM and to expose them to the intricacies of working in a professional laboratory. Since launching this initiative, we have paired six Cabrini interns with PCOM faculty mentors. One of our graduates is currently employed as a Lab Assistant at PCOM and another is pursuing a graduate degree in Pharmaceutical Chemistry. The other interns are completing their senior year in Biology at Cabrini University and plan to pursue careers in medicine. We will share lessons learned about the power of partnerships in leading this social change work.

B5/P1700

Pathways to academic success of underrepresented student groups- access to undergraduate research

P. Callahan, J. Sanjeevi, B. Porter, **J. Fernandes**; Miami University, Oxford, OH.

A National Science Foundation award made possible through the Undergraduate Research and Mentoring [URM] program, was used to mentor approximately 50 students in the biological sciences, with a goal of improving retention outcomes of underrepresented student groups. The award lasted from 2008-2014, and involved four departments, Botany, Chemistry and Biochemistry, Microbiology and Zoology. Students were accepted into the program having successfully completed their first year in the major, and were provided with scholarships for up to 3 years of their college education. The research mentoring program involved enrichment such as monthly meetings, one-on-one advising, peer mentoring, outreach activities and a number of skills based workshops such as literature searches, grant writing and opportunities for on-campus as well as conference presentations. 67% of the cohort graduated in 4 years, which was a significantly higher [4 year] degree completion rates and final GPA when compared with matched controls. Ethnic under-representation was a risk factor for degree completion, only when additional factors were present, such as pell eligibility, high financial need and first generation to college. Qualitative data obtained from student interviews revealed that the cohort experience and access to mentors and professional development, raised their awareness of research based career opportunities, and that the enrichment activities offered by the program prepared them to be confident about careers after college. Based on the success of this program, a first year research experience program has been developed to provide early access to research experiences, and to raise awareness about research opportunities across disciplines. The undergraduate research office has collaborated with institutional programs aimed at retention to provide pathways for engagement in research and scholarly activities.

B6/P1701

BioImaging North America: supporting bioimaging scientists through professional development and educational opportunities.

N. Bialy[1], V. L. Orr[1], K. W. Eliceiri[2], C. M. Brown[3]; [1]BioImaging North America, Morgridge Institute for Research, Madison, WI, [2]Departments of Biomedical Engineering and Medical Physics, University of Wisconsin at Madison, Madison, WI, [3]Department of Physiology and Advanced BioImaging Facility, McGill University, Montreal, QC, CANADA.

BioImaging North America (BINA) is a network of optical imaging scientists across Canada, Mexico and the United States, dedicated to supporting and strengthening light microscopy-based bioimaging, while

partnering and contributing to global bioimaging efforts. BINA is a non-profit association founded in 2018, with active, volunteer-based working groups focusing on communications, corporate partnership, diversity, equity and inclusion, image informatics, quality control and data management, and training and education (https://www.bioimagingnorthamerica.org/). BINA has supported the professional development of more than 70 imaging scientists in the past 18 months, 47% of whom self-identified as belonging to an underrepresented group. Imaging scientists that were trained came from the USA (75%), Canada (20%) and Mexico (4%). The list of supported 2022 training courses and those which BINA members can apply for support to attend in 2023 are found at https://tinyurl.com/BINA-PD. BINA membership is required to receive financial support or participate in any of BINA's programs, but membership is free - https://www.bioimagingnorthamerica.org/join/. Members receive a monthly newsletter where we highlight opportunities that are available. The BINA Training and Education working group hosts a monthly virtual Exchange of Experience meeting (EoE, https://tinyurl.com/BINA-Virtual-Group-EoE) where imaging scientists share their experience and expertise. In-person EoEs will kick off in late 2022, so imaging scientists interested in learning about facility management, a new instrument or technique or image analytics can visit the facility of a BINA member to acquire the new skills/knowledge. BINA has also coordinated an international effort to develop a community-driven, global database for training and education materials in light-microscopy, that can be searched easily to retrieve pertinent resources through a user-friendly web interface. MicroscopyDB launched in June of 2022 and researchers and microscopy organizations interested in sharing their resources or displaying content from the database on their organization's website should visit https://microscopydb.io/ to learn more and get involved.
This project has been made possible in part by a grant from the Chan Zuckerberg Initiative DAF, an advised fund of Silicon Valley Community Foundation.

B7/P1702

An analysis of Course-based Undergraduate Research Experience (CURE) efficacy at community colleges

M. J. Wolyniak[1], J. Zhang[2], S. DeChenne-Peters[3], D. A. Hecht[4], M. L. Kuhn[5], C. Koletar[6], N. Galport[6], R. Eddy[6], J. J. Provost[7], J. K. Bell[7], J. E. Bell[7]; [1]Hampden-Sydney College, Hampden-Sydney, VA, [2]University of Nebraska, Lincoln, NE, [3]Georgia Southern University, Savannah, GA, [4]Southwestern College, Chula Vista, CA, [5]San Francisco State University, San Francisco, CA, [6]Cobblestone Applied Research and Evaluation, Inc., La Verne, CA, [7]University of San Diego, San Diego, CA.

Course-based Undergraduate Research Experiences (CUREs) have emerged as a well-supported high-impact practice for improving both retention of students in science, technology, engineering, and mathematics (STEM) disciplines and overall achievement of students in STEM coursework. While considerable scholarship has been performed on the overall efficacy of CUREs as a teaching approach, comparatively little work has been done to uncover the more nuanced data on how CUREs serve students at two-year institutions, especially students in minoritized groups (MGs). In this study, we leveraged the Malate Dehydrogenase CURE Community, a CURE network focused on bringing research experiences in protein biochemistry to undergraduates, to collect data on student achievement at two-year institutions. Using the Test of Scientific Literacy Skills (TOSLS), a validated assessment instrument, we considered how the CURE experience effected the overall scientific literacy of students enrolled in the CURE at two-year institutions when compared to students enrolled in control classes with traditional labs. Our data revealed that the CURE approach to instruction was especially effective with respect to

MG student scientific literacy relative to control students. Importantly, we noted that two-year college students exposed to a CURE experience were 24% more likely to be retained in the STEM disciplines relative to their peers. Taken together, the data suggest that CUREs may present an effective means for the providing of equitable opportunity for scientific literacy and retention for students at two-year colleges. The outcomes of this research will hopefully enable these institutions to provide biochemistry-focused high-impact educational experiences for their undergraduates tailored to enable them to join a well-equipped next generation of life scientists.

B8/P1703

Linking biological and sociological reflections to cultivate STEM research experiences.

M. Juarez; DNA Learning Center, Cold Spring Harbor Lab, Brooklyn, NY.

DNA Learning Center NYC offers a series of 9 week-longsummer camps for students entering 6th grade thru 12thgrade. The goal of these summer camps is to promote Biology laboratoryexperiences for the New York City public school community of over 1,000,000students. The recent demographic data for the region reports that 90% of thestudent population are science PEERs (persons excluded because of theirethnicity or race). The impact of intentional efforts to increase retention forDNALC NYC camp participants can provide a case study on effective strategies tofoster early development of a diverse community of future STEM scholars. Theprevious assessments of summer camp participants focused on a pre- andpost-test of students' basic knowledge about grade-level matched Biologyconcepts. As an extension of the evaluation on learning gains, the goal of thecurrent study is to determine how the summer camp students rank their attitudesand awareness with careers in Biology and STEM. Through a collaboration withCUNY City Tech and College Now, two high school summer research internsinitiated a research project to explore survey methodology to determinestudents' perspectives on their summer camp experiences. The summer internsobserved four different weeks of summer camps. Concurrent with learning thebiological concepts and lab techniques, the summer interns conducted aliterature survey to identify questions that may determine summer campstudents' attitudes toward STEM. Preliminary data was collected from 68 summercamp respondents using an online Google form. Overall, the summer camp studentsreported high interest in Biology but rated lower confidence in pursuing a STEMcareers. Complementing this initial study with the perspectives of the summerresearch interns, as both summer camp participants and survey designers,provided unique insights into the data analysis. Comparing the survey data ofcamp participants and the weekly reflections written by the summer internshighlighted what specific activities can develop student self-efficacy andconfidence with a potential career in STEM. The future goal of this project isto adapt our teaching methods to better support a broader range of studentbackgrounds and focus on instructional activities that promote scienceidentity.

B9/P1704

A model for diversity, equity and inclusion workshops in STEM: Designed for science culture, by scientists

L. Dahlberg[1], R. Barber[1], B. Coleman[2], K. Christiani[1], R. Kodner[1]; [1]Western Washington University, Bellingham, WA, [2]Clark University, Worcester, MA

Increasing the diversity of Science, Technology, Engineering, and Math (STEM) fields has become a priority at many colleges and universities. However, efforts to build inclusive institutions can be

hampered by a lack of understanding of how current structures reinforce inequity. We describe a series of workshops designed “by scientists, for scientists” to discuss race, racism, and inclusion in scientific cultures and communities. We outline the four-part workshop curriculum and activities, which were presented as interconnected workshops to STEM faculty and staff at a predominantly white, mid-sized regional state university. We used quantitative and qualitative methods to assess the impact of the workshops on participants. Surveys of participants prior to and following workshops showed that participants increased their knowledge and understanding of topics that were the explicit focus on the workshop, for example: intersectionality, microaggressions, and bias. A qualitative evaluation to assess focus group interviews of participants in the workshops defined five themes, which point to the complex layering of scientific “objectivity” with an increasing emotional and social awareness in the mostly white participants. While participants were open to learning more about racism and how science culture intersects with systemic racism, many also remained largely unaware of their own conflicting views and tendencies to center whiteness in their narratives. These findings are reflected in the scientific community, at large, and are part of the deepening conversation on access, diversity, equity, and inclusion in STEM cultures and communities.

B10/P1705

Leveraging, Enhancing and Developing Biology (LED-BIO) Scientific Societies Shedding Light on Persistent Cultural Challenges: A Research Coordination Network

V. A. Segarra; Goucher College, Baltimore, MD

Each field in the sciences has historically been represented by its own scientific society, bringing together individual researchers for regular meetings where they can network, share discoveries, and collaborate. While it is rare for multiple distinct scientific societies to interact, they often perform similar functions, such as promoting the professional development of their members and promoting diversity among the next generation of researchers in their respective fields. We have established a collaborative network of experts to identify evidence-based inclusion strategies to accomplish the following: (1) collect consistent demographic data of society memberships, (2) better integrate scientists in transitional career stages into scientific society activities, and (3) diversify the ranks of scientific society leaders. By fulfilling these goals, this project aims to overcome persistent challenges that frequently undermine diversity efforts within independent communities of scientists and to broadly share this information for the benefit of all scientific communities. This project is an extension of the work of the Alliance to Catalyze Change for STEM Success (ACCESS), and its member societies in the life sciences (the American Society for Biochemistry and Molecular Biology, the American Society for Cell Biology, the American Society for Pharmaceutical and Experimental Therapeutics, the Endocrine Society, and the Biophysical Society), joined by the Quality Education for Minorities Network, the Marine Biological Laboratories in Woods Hole, and the NSF INCLUDES Aspire Alliance. We share the progress made during the first year of the three-year grant.

B11/P1706

ASCB and ACCESS+: A cohort-based program for STEM professional societies to advance diversity, equity, and inclusion

V. Segarra; Goucher College, Baltimore, MD

The Amplifying the Alliance to Catalyze Change for STEM Success (ACCESS+) is an ADVANCE-funded initiative working with STEM professional societies to create intersectional gender equity throughout their organizations. ASCB is part of the first cohort that participated in this program, joining a Community of Practice of societies and evaluating its diversity and equity efforts using the a comprehensive assessment of different society functions. In this poster we share ASCB's progress through the ACCESS+ program.

B12/P1707

The gene editing research lab — a classroom-based research experience at UC Merced

G. Ligunas, S. Materna, **S. Woo**; University of of California, Merced, Merced, CA

The University of California Merced is a designated Hispanic- and Minority-Serving Institution (HSI and MSI, respectively). Our undergraduate student population is majority nonwhite (54.3% Hispanic, 20.6% Asian/Pacific Islander, 9.5% White, 4.2% African American/Black, <1% Native American, and 3.3% Multiracial), and over 70% of our students are the first in their family to attend college. Especially for underrepresented minority (URM) and first-generation students, authentic research experiences are an effective way to improve retention and learning outcomes in the sciences. We have developed a Course-based Undergraduate Research Experience (CURE) that introduces students to discovery-based research through hands-on experience with CRISPR/Cas-based gene editing techniques, which have quickly become standard in biology and biomedical research. Our first pilot cohort consisted of 6 students who all identified as URM and/or first-generation. Each student was assigned one gene and designed and executed a CRISPR-based knock-in strategy for each gene. Course activities were a combination of lectures, structured training demonstrations, and self-paced lab work. Throughout the semester, students were surveyed on their perceptions and attitudes towards scientific research. In terms of scientific outcomes, we are currently in the process of identifying germline transmission for 4 of the initially targeted 6 genes. In terms of educational outcomes, all students have presented their work at departmental, campus-wide, and national conferences. Two students have since matriculated into Master's degree biology programs and another two students are currently working as research lab technicians. We are currently running a second cohort of an additional 6 students.

B13/P1708

Diversifying academia: Understanding and implementing equitable and inclusive hiring practices through faculty learning communities

E. Arevalo[1], E. Fuentes-Lopez[1], M. Wilton[2], **S. M. Lo**[1]; [1]University of California San Diego, La Jolla, CA, [2]University of California Santa Barbara, Santa Barbara, CA

Academia has historically been a space where majoritized individuals have been represented. More recently, student demographics are becoming more diverse; however, faculty demographics have not followed suit at the same rate. This presents a critical issue as the lack of representation within faculty limits not only the success of students from diverse communities but also the diversity of ideas in

academia. To address this issue, we utilize the context of search committees to examine how equitable and inclusive hiring practices are understood and implemented by faculty in science, technology, engineering, and mathematics (STEM) disciplines at four research-intensive institutions. Using an iterative design-based research approach, a total of about 10 faculty on search committees from the four campuses each year engage in faculty learning communities, peer-led structured groups that support collaborative discussion and reflections. Specifically, for the first two years of the project, we examined research literature on how to write the job advertisement and discussed the potential barriers and support in different departmental contexts for implementation. We identified four dimensions relating to equity and inclusion in the advertisement: use of language to describe the ideal candidate; description and placement of the institutional commitment to diversity, equity, and inclusion (DEI); biases and assumptions built into the evaluation criteria; and promotion and dissemination of the advertisement. In terms of barriers and support for implementation, we found that the job advertisement can be constrained by institutional structures on what is allowed to be included. However, the values and visions of a department are symbols that can be reflected in the language choice, placement and description of a DEI statement, and the inclusivity of how requirements of the position are evaluated. Individuals on a search committee are people with goals, agency, needs, and identities that may be embedded into the advertisement, and they also have strategies for distributing the advertisement that are reflective of their professional networks. We also found that the writing of the advertisement is influenced by power, i.e. the status, positioning, and political coalitions of members of the committee, department, and institution. While structures can be represented in the form of policies and symbols can guide how the structures are spoken about, power ultimately determines how specific implementations are enforced. Through this work, we are able to make key recommendations for all aspects of the hiring process that could support the diversification, inclusion, and belonging of minority faculty from minioritized communities across STEM disciplines at different institutions.

B14/P1709

Fostering An Appreciation for Research: The Meharry-Stanford Program Initiative As A Strategy for Eliminating Barriers To Career Transitions

T. Clemons; Meharry Medical College, Nashville, TN

Introduction/Statement of Problem/Background/Question: The Meharry-Stanford Program Initiative began in 2017 in an effort to partner Meharry students with scientists at Stanford who would mentor the students through a hypothesis-driven project. Since its inception in 2017, the program has evolved into a stronger program and the question we will continue to ask is how can we make the initiative even stronger than the year prior in order to help eliminate barriers to career transitions. **Goals/Objectives of the Project or Intervention**: The objective of the initiative is to allow medical students to engage in research at Stanford in an effort to foster long-lasting mentor-mentee relationships and to provide students with an opportunity to strengthen their application for their future endeavors. **Methods/Description of Project/Intervention**: The initiative has been followed since 2017 with certain critical questions being asked of the Meharry students after they have completed the program. Based on the student's responses, the goal is to improve the program each year. **Findings to Date/Evaluation to Date**: The findings to date will be shared on the poster and include valuable responses, such as do students think that their research experience at Stanford contributed to them submitting a successful application to any specific programs or positions. The data suggests that

students have found conducting research at Stanford to be a worthwhile endeavor that has aided them in their future success. **Questions still unanswered**: Although there are several questions that need further exploring, one major question that has yet to be captured in the responses is do students believe that their time at Stanford contributes to their critical thinking abilities in their medical school pre-clerkship and clerkship work.

B15/P1710

Use of community listening sessions to disseminate research findings to past participants and communities

J. Erves, E. Stewart, J. Duke, L. Alexander, **J. Davis**, D. Wilus, K. Wythe-Etheridge, S. Miller-Hughes; Meharry Medical College, Nashville, TN

The purpose of this sequential, explanatory mixed methods study is to determine changes in attitudes towards research, trust in medical researchers and the process, and willingness to participate in research among African Americans immediately after receiving past study findings in a community listening session (CLS). We developed and implemented four CLSs with a total of 57 African Americans who were either past research participants or members of the community-at-large. In the quantitative (dominant) phase, 32 participants completed pre-post surveys and 10 of those participants completed the follow-up semi-structured interviews. Paired samples t-tests and McNemar's test determined bivariate differences between pre- and post-surveys. Thematic analyses determined emerging themes to further understand these differences. There was a significant increase in: (1) perceived advantages of clinical trials pretest ($M = 26.63$, $SD = 5.43$) and post-test ($M = 28.53$, $SD = 4.24$, $p < .01$); and (2) in trust in medical researchers from pre to post ($M = 36.16$, $SD = 10.40$ vs. $M = 27.53$, $SD = 9.37$, $p < 0.001$). There was no significant difference in pre- and post-tests as it relates to perceived disadvantages of clinical trials and willingness to participate. Qualitative analysis yielded the following themes: (1) sharing research results and the impact on attitudes towards research; (2) community listening sessions: a trust building strategy; and (3) satisfaction with the community listening session. Community listening sessions hold promise as a method that researchers can use to simultaneously disseminate research findings and positively impact research perceptions and potentially participation among racial and ethnic minorities.

B16/P1711

Striving for inclusive excellence through critical reflection of teaching practices

L. R. Vega, C. J. Hegartner, B. Schoffstall, P. V. Banaji, S. M. Bingham, H. Del Rio, R. Jimenez, M. Painter, M. P. Robinson, Z. Zajickova; Barry University, Miami Shores, FL

Barry University is a mission-driven institution founded by Adrian Dominican Sisters in 1945 that now operates as a private non-profit institution with a Catholic tradition for social justice. It is recognized as a Hispanic-Serving Institution and most of its undergraduate student body originates from cultural, ethnic, or racially underrepresented minorities that are often at socioeconomic disadvantage. In 2020-2021, over 70% of our undergraduates were eligible for means-tested financial tuition assistance (IPEDs). Inclusivity, collaborative service, and social justice are key components of Barry University's mission that are infused into courses and co-curricular activities. Moreover, community engagement and collaboration are also important components of the continuing contract and promotion process for faculty. The College of Arts & Sciences at Barry University is home to our undergraduate STEM programs

(Biology, Computer Sciences, Chemistry, Physics, Psychology, and Mathematics) with full-time faculty teaching the majority of lectures and labs. STEM faculty hold advanced degrees from research-intensive intuitions and records of scholarship, but these do not necessarily guarantee that they will be effective educators for our undergraduate body. Like research, effective teaching requires experimentation and continual reflection for growth. Striving for excellence in teaching and inclusive practices is vital for institutions who focus on the undergraduate education of a diverse student body. Here, we describe how a group of faculty volunteers across STEM disciplines and beyond utilized an iterative process of study/learning, practice, and reflection to address this goal. Our fundamental premise was that broadening participation and retention within the sciences should start with careful examination of how our educational practices might unintentionally include or exclude Barry STEM students. To support STEM student persistence and success, and to improve the pedagogical practices to support student learning and inclusivity, the Barry Faculty Discussion Group was formed in 2019. We describe how our group of diverse and committed faculty, mostly in STEM, examined, discussed, and explored inclusive excellence through the framework of Paolo Freire's critical pedagogy. Faculty whose identities aligned or did not align with traditional Persons Excluded because of Ethnicity or Race (PEER) both realized how they fell back on a “banking model” of instruction because of learned practices and ease. In this poster, we also describe some critical reflections on our own teaching philosophies and practices and discuss our attempts to support and empower our students as co-investigators in their professional development in our classrooms.

B17/P1712

Cultivating Ensembles: Inclusive Interdisciplinary Community at the Intersection of Science, Theater and the Humanities.

S. Borinskaya[1], A. K. Lancaster[2], C. Sealfon[3], N. Gross[4], R. M. Holmes[5], B. J. Natalizio[6], J. E. Martinez[7]; [1]Saint Elizabeth University, Morristown, NJ, [2]Ronin Institute, Boston, MA, [3]University of Toronto & Ronin Institute, Toronto, ON, Canada, [4]Boston University, Boston, MA, [5]improvscience, Cotati, CA, [6]University of Massachusetts Chan Medical School, Worcester, MA, [7]New York Institute of Technology, New York, NY

What CE is about -> Cultivating Ensembles (CE) is an interdisciplinary and inclusive community where we co-create playful, interactive, collaborative spaces for practitioners and scholars to explore, demonstrate, and share how they build and advance science and research. Each bi-annual CE conference is a unique professional development opportunity, produced by a combination of previous organizers, recent attendees and new associates. The format is highly collaborative and participatory with a variety of different components, such as interactive, hands-on sessions; intensive workshops; lightning talks; and share fair presentations. We are educators, researchers, performers, artists, students, activists, advocates and entrepreneurs from diverse areas - academia, the arts, community organizations, business, industry, and nonprofits. We are bringing people together (ensembles) to transform the human/cultural experience of professional and social identities in STEMM (science, technology, engineering, mathematics, medicine).

History -> Historically, the CE conference is a 2 to 3-day experience of sharing research, education, and science communication practices in the sciences, arts, and humanities. We’ve hosted four conferences with more than 200 attendees from 2012 to 2019 and are currently planning the CE 2022 conference. These gatherings inspired new work and collaborations within and beyond the life sciences that have led to publications, conference presentations, and performance based workshops that inform our cultures

and advance collaboration skills. Past conferences also offered us opportunities to grow as a community of practice that cultivate ensembles across disciplines, cultures and identities.
Recent Events (Coffee Chats, CE 2022) **->** In response to the pandemic, we pivoted to a new interactive performatory form - Coffee Chat Series, a free and informal bi-monthly gathering where the Cultivating Ensembles community meets online to hear from innovative practitioners at the intersection of science and the arts and discuss topics that elevate inclusive and collaborative STEMM cultures. In our poster we will share highlights from our Coffee Chat Series, as well as the upcoming CE 2022 Conference (Nov 10-11, 2022; hosted in collaboration with the Lloyd International Honors College at the University of North Carolina-Greensboro). We will discuss how these events are part of creating inclusive and equitable ensembles where connection, play, and building relationships redefine and enrich established STEMM environments.

B18/P1713
BlackMatters: Anti-black racism in science, a film project
K. Maar[1], V. King[1], B. Yadegari[2], R. Heald[1]; [1]University of California, Berkeley, Berkeley, CA, [2]Science Communication Labs, Berkeley, CA

Black hands built the buildings of many of the most prestigious academic institutions in USA. Despite a great interest in science, Black students leave STEM fields at high rates and only a small percentage receive PhDs compared to other groups. Black people were experimented on, mutilated, and used without their consent to produce some of the most important research of our time. Black people still experience extreme racism and prejudice in the very institutions that pride themselves on being forward thinking and having inclusive cultures; they are pushed out of the science academy under the pressure of white supremacy. In this film, two Black Women graduate students, Valeria King and Khansaa Maar, provide a mirror through which science in USA has an opportunity to observe itself. Having faced racial aggressions that made them feel unwelcome, it is time to look at these BlackMatters under the microscope of social justice and expose the inequities that permeate our scientific research culture. Through interviews with Black scientists and trainees in academia and industry, we explore the effects of anti-Black racism in science in the USA. Our film production has started with the first trailer in post-production, and we are now reaching out to the larger community of Black scientists and allies for support. This is a two-year commitment with UC Berkeley's MCB program and the help of group of talented, strong Black scientists as advisory board to address all aspects of anti-Black racism in science. We need your help spreading the word and providing support for our documentary film project that directly tackles this important issue, which continues to hinder scientific progress and the well-being of our Black colleagues.

B19/P1714
Using the largest diverse human dataset to increase underrepresented biomedical researchers in research careers
D. Murray, S. Ferbach, L. Rosales, B. Lee; Baylor College of Medicine, Houston, TX

Introduction: According to the U.S. Department of Education, in Fall 2018, full-time faculty in degree-granting postsecondary institutions were 3% each Black males, Black females, Hispanic males, and Hispanic females.[1] Under-representation is one of the barriers to success for African American and Hispanic faculty. Barriers to women's career advancement from underrepresented groups in academic

medicine, science, and engineering and obtaining external research funding support for African American faculty do not occur in a vacuum and co-exist with barriers to research productivity overall.[2] This data establishes the necessity of increasing representation, creating more accessible research opportunities, building networks, mentoring relationships in STEM-related fields, obtaining a diverse scientific workforce, and ensuring the success of all underrepresented biomedical researcher (UBR) trainees and early career faculty. The *All of Us Evenings with Genetics* Research Program developed the *All of Us* UBR Faculty Summit to train early career faculty and senior postdoctoral trainees on the *All of Us* data platform. The AoUEwG Research Program aims to increase research productivity among UBR early career faculty and support their promotion to the next career level. **Results**: The *All of Us Evenings with Genetics* (AoUEwG) Research Program recruited *All of Us* UBR Scholars to the *All of Us* UBR Faculty Summit. There were 34 participants with the following demographics: 26 female, 7 male, 1 gender non-conforming, and 14 African American, 2 Asian, 13 Hispanic, 9 White, and 9 Other. The Scholars represented a variety of Carnegie Classified Institutions ranging from Doctoral/Very High (8) to Special Focus Research Institution (6), to Master's/Larger Programs (2). Six multidisciplinary research teams were formed, and these teams will develop research projects using the *All of Us* data. **Conclusion**: This researcher engagement program provides research opportunities available through the *All of Us* research hub. The research teams form a community that works toward a common goal that fosters collaboration beyond the summit's focus. Several Scholars were interested in incorporating the *All of Us* researcher workbench into their teaching curriculum. This experience will be translated to their students through didactic courses where this platform is introduced and in the Scholar's research labs.

Science Education: Primarily Undergraduate 1

B21/P1715

Analyzing student performance and problem-solving barriers in open-ended genetic problems

M. Ellefson; Molecular and Cellular Biology, University of California, Davis, Davis, CA.

The need to build problem solving skills in STEM undergraduates has been widely reported. This study seeks to better understand undergraduates' STEM problem solving performance to develop a model that will guide instructional support of students' problem-solving skill development. Specifically, the project is investigating the role of student knowledge structure, knowledge retention, and barriers in solving open-ended genetic problems. Study participants were enrolled in an upper-division genetics course that covers transmission, molecular, and applied genetics. This four-unit course consists of two 110 minute lectures and an optional 50 minute discussion session each week of a 10-week quarter. 95% of the 293 enrolled students were majoring in STEM and required to take the course. To gather a baseline of students' genetics knowledge, a Genetics Diagnostic Test (GDT) was administered to students in the first and last week of class. Students answered open ended genetic problems on an in-class summative assessment, from which ten high performing and ten low performing students were identified and invited to participate in one hour think aloud interviews (TAInt). The TAInt were conducted during weeks 7 and 8 of the quarter and entailed participants speaking through their thought processes while solving several open ended genetic linkage problems. To assess levels of knowledge retention, participants completed a follow up TAInt 8-9 weeks after the end of course during which they solved the same open-ended problems. Several developed instruments were used to analyze participant responses on the open-ended genetic problems from the in-class assessment and transcribed TAInt. Specifically, the COSINE (Coding System for Investigating Sub-problems and the Network) method is an

in-depth analysis of the difficulties students have during the problem-solving process. To apply the COSINE method, sub-problems that correlate with specific steps of genetic linkage problems are assigned a code based on student performance on a particular task. Quantitative analysis of the results uncovered that low-performing students not only spend less time on higher-level cognitive tasks but also attempt less tasks, indicating the lack of awareness of steps in the problem-solving process. Together, these results provide specific knowledge to inform important areas to model and develop improved problem-solving skills across STEM classrooms.

B22/P1716

Project CUREOS: Characterizing Undergraduate Research Experiences and Outcomes in STEM

A. L. Goodman[1], A. F. Somoza-Norton[2], D. G. Bachrach[3]; [1]California Polytechnic State University, San Luis Obispo, CA, [2]School of Education, California Polytechnic State University, San Luis Obispo, CA, [3]The Culverhouse College of Business, The University of Alabama, Tuscaloosa, AL.

Undergraduate research experiences (UREs) are among the most impactful practices promoting student persistence, self-efficacy, and science identity in science, technology, engineering, and mathematics (STEM) careers. Social factors, like mentoring, can drive positive student outcomes from UREs. But, the role played by the structure of the work, or the way that students' activities are designed, in undergraduate research has not been characterized. We use Job Characteristics Theory (JCT) as a theoretical framework to characterize how the work design of UREs generates positive student outcomes in STEM fields. Based on JCT, core job characteristics (e.g., skill variety, task identity, task significance, autonomy, and feedback) affect work-related outcomes, including motivation, satisfaction and performance (Hackman, J. R. & Oldham, G. R., 1975). In this early stage of the project, we are adapting existing survey items from the literature used to measure job characteristics to the undergraduate research context. We are using cognitive interviews to validate adapted survey items and to identify related outcomes of students' research experiences. For survey item validation (or revision), we use coding provided by multiple raters using three criteria, which Karabenick et al. (2007) defined as item interpretation congruency, coherent elaboration congruency, and answer choice congruency. Transcripts are also analyzed for emerging themes related to outcomes and benefits, as well as challenges of undergraduate research in different work contexts (e.g, fieldwork, lab work, computational work). We focus on characterizing the work design and implementation of UREs during the academic year at undergraduate-focused institutions. UREs during the academic year pose unique challenges for students, including balancing the demands of research with coursework, employment, student clubs, sports, and family obligations. URE work design structure intentionally addressing these challenges has the potential to improve student outcomes. Based on the first round of interviews, we present emerging models for different work structures in the UREs. In the future, we will explore the relationships between structural facets of URE design and key student outcomes. One of our goals is to provide recommendations for improving the design of UREs, including course-based undergraduate research experiences, ultimately improving the experiences of students in pursuit of STEM degrees, for all students, and increasing the diversity of the STEM workforce. This work was supported by NSF IUSE #2142404.

B23/P1717

Harvard Systems & Quantitative Biology REU: Fostering Student Engagement and Learning in Virtual and In-person Formats

A. Jambhekar[1], B. N. Queenan[2]; [1]Systems Biology, Harvard Medical School, Boston, MA, [2]Quantitative & Synthetic Biology, Harvard University, Cambridge, MA.

To remain competitive in science and technology, the US must recruit and retain an intellectually diverse scientific workforce. Research experiences for undergraduates (REUs) are a critical component of these recruitment and retention efforts, increasing the odds of earning a Bachelor's degree in a STEM field and acceptance to graduate school.
The Harvard Systems & Quantitative Biology REU has been running as a cross-campus program between Harvard College (Cambridge, MA) and Harvard Medical School (Boston, MA) for the past three summers, the first two virtually and the last in-person. Measures were developed and implemented during the virtual phase to promote student engagement, facilitate interactions within the REU cohort, and reduce structural barriers to participation; these have been successfully transitioned to the in-person program. The REU includes research and didactic components. The research activities foster skills in hypothesis-generation, creation of a research plan, rigorous data analysis, and understanding fundamental concepts in the research field. Interns are selected based on their motivation for research and coursework in math and biology, with the goal of providing interns unique opportunities to participate in cutting-edge systems biology research. Every intern is assigned to a laboratory and works with a dedicated mentor on a research project, with increasing autonomy and project ownership as the summer progresses. Interns develop technical skills in coding and/ or wet-lab approaches. The goals of the didactic component are to foster rigorous scientific thinking and communication skills and to build a network of peers and senior researchers. Didactics include highly-structured cohort activities such as writing workshops and journal clubs, which feature individualized feedback from peers and faculty. The training is enhanced by guest talks, departmental seminars, and social hours. To reduce barriers to participation, students receive funds and necessary computer supplies in time to arrange for travel, living expenses, and conducting program activities. Interns find the REU a transformative experience, citing the exposure to diverse research topics, structured didactics, and networking opportunities as unique elements. Alumni have gone on to receive competitive science fellowships and attend graduate programs in science and medicine.

B24/P1718

Effective multimodal science communication in a cancer biology seminar course

K. Johnson; UNH Manchester, Manchester, NH.

Science literacy and effective communication skills are remarkably important. Yet, it has been aptly stated that the language, structure, and conventions of most scientific publications might almost have been created to obscure information, using words that the general public will have never heard or used. Undergraduate students are often trained in scientific writing skills, communicating their research through a variety of scientific papers in traditional text formats. Yet, there exists a gap in scientific training with exposure to a variety of communication styles that enables both accessibility and inclusivity. Thus, it is imperative that we facilitate opportunities for students to practice making science more accessible. This is directly addressed in an advanced cancer biology seminar course in which students are given assignments that require them to step back from published scientific work and create

a variety of accessible visualizations, including graphical abstracts and video abstracts. Positive outcomes indicate students can process complex scientific concepts and translate them into simple graphical outputs accessible to a wider audience.

B25/P1719

Developing a multi-institutional, cross-disciplinary parasitology CURE (Course Undergraduate Research Experience

S. Agrawal; University of Mary Washington, Fredericksburg, VA.

Course based undergraduate (CUREs) courses provide an opportunity to develop essential research skills for undergraduates especially in primarily undergraduate institutions. Over the last two years, University of Mary Washington, Georgia State University and Albright University students and faculty have collaborated in a Molecular parasitology CURE. This collaboration across three institutions with different expertise, resources and student populations is unique among other multi-institutional CUREs in that students, not just PIs - from all three institutions interact to collaboratively work on original research problems involving the Kinetoplastid parasite *Crithidia fasciculata*. Highlights of the courses included diverse speakers on molecular parasitology, direct interaction of PI's with students at other institutions, shared project reports and joint conference presentations. Our model for collaborative CURE research can be helpful to other faculty and provide ideas to productively overcome challenges of asynchronous engagement, accelerate research process, and engage a larger number of undergraduates in the research community.

B26/P1720

Lessons Learned for Post-COVID Application from a Pandemic-resilient, Computer-based, Nationwide Genomics CURE

D. Lopatto[1], **C. S. Silver Key**[2], M. Van Stry[3], J. Siders[4], A. L. Goodman[5], W. Leung[6], M. S. Santisteban[7], K. M. Sandlin[8], C. P. Rele[9], L. K. Reed[9]; [1]Grinnell College, Grinnell, IA, [2]North Carolina Central University, Durham, NC, [3]Lane College, Jackson, TN, [4]Ohio Northern University, Ada, OH, [5]California Polytechnic State U, San Luis Obispo, CA, [6]Washington University in St. Louis, St. Louis, MO, [7]University of North Carolina at Pembroke, Pembroke, NC, [8]The University of Alabama, Tuscaloosa, AL, [9]University of Alabama, Tuscaloosa, AL.

The objective of this study is to identify the lessons learned from implementing a nationwide Course-based Undergraduate Research Experience (CURE) during the COVID-19 pandemic that can be applied post-pandemic. Established in 2006, the Genomics Education Partnership (GEP; https://thegep.org/) is a computer-based CURE in which students experience "hands-on" how to use web-based genome browsers, bioinformatics tools, and databases to investigate eukaryotic genes and genomes. The student CURE research project involves analyzing multiple sources of evidence (e.g., sequence similarity, computational gene predictions, RNA-Seq data) to construct a probable gene model in one or more Drosophila species. The efficacy of the program was assessed using student pre-/post-surveys and quizzes. In Spring 2020, the COVID-19 pandemic forced many courses to change their modality from in-person to virtual, and the courses in subsequent semesters were usually delivered in a hybrid format. During this period, GEP membership grew from pre-pandemic levels of approximately 100 faculty members to more than 260. Despite the shift from in-person to fully-virtual or hybrid delivery, the hands-on GEP student research experience persisted. Additionally, the number of annotation projects

submitted by GEP students has remained high. Furthermore, when comparing pre-COVID assessment results and more recent results, we find similar learning and attitudinal gains. We believe that the following lessons contributed to the robustness of the GEP CURE: 1) synchronous live sessions with the use of screen-sharing enhanced communication with students on genome annotation; 2) pre-recorded classes and help sessions allow more instructional time than the traditional "one-and-done" approach; 3) an increase in "flipped classroom" teaching enabled students to engage in problem-solving during the synchronous sessions; and 4) hybrid delivery of help sessions featuring both in-person and virtual teaching assistants facilitated student success. Continued application of these lessons to computer-based CUREs may provide increased access to science research for underrepresented minorities and non-traditional students as called for by Vision and Change — leading to the democratization of access to STEM careers. Supported by NSF IUSE-1915544 and NIH IPERT-R25GM130517.

B27/P1721

No Major, No Problem: Developing a Life Sciences Curriculum from Scratch at a Small Liberal Arts College

S. Walsh; Soka University of America, Aliso Viejo, CA.

Most 4-year colleges have a system of majors that includes both foundational and advanced courses where enrollment is restricted by multiple prerequisites. However, our small liberal arts college offers concentrations where students graduate with a minimum of 5 courses in an area to complement their liberal arts degree. Together with my colleagues, we developed a streamlined Life Sciences concentration that enables students to meet the prerequisites for health professions and graduate school or to explore a broad variety of topics within Life Sciences. Because of this flexibility, our curricular emphasis shifted from content mastery to authentic research experiences, experimental design, and scientific and science communication. For example, we designed an introductory, co-taught course to integrate both biology and chemistry concepts using the hallmarks of cancer as a theme, and we require at least 1 semester-long, 3-credit laboratory course-based undergraduate research experience (CURE) where students explore a faculty member's research. Rather than an independent weekly laboratory period, content-based courses have embedded laboratories where active learning is sometimes exchanged for related hands-on experience. In contrast to a traditional physics laboratory, our physics courses teach Python to investigate biological problems through coding. As part of general education (GE), students are also required to take 2 science courses so students interested in maximizing their upper level science courses can immediately engage in GE courses designed to fulfill prerequisites for upper level Life Sciences courses or instead explore other topics, such as Astronomy or Nutrition. The GE program requires all students to take cross-concentration courses so some upper level courses, such as Evolution, minimize prerequisites, allowing interested students focused on other disciplines to learn a specific scientific topic in-depth. All students across the college complete a capstone thesis, and Life Sciences thesis projects range from wet or dry laboratory projects with Soka faculty or external mentors to social sciences surveys interrogating health professionals to literature-based analysis of a specific scientific problem. The Life Sciences concentration officially launched in fall 2020, and we continue to revise the curriculum to both meet student demand and align with Vision and Change.

B28/P1722

The CRISPR IN THE CLASSROOM NETWORK: A SUPPORT SYSTEM FOR INSTRUCTORS TO BRING GENE EDITING TECHNOLOGY TO THE UNDERGRADUATE CLASSROOM

M. J. Wolyniak[1], D. L. Pattison[2], T. Hoage[3], S. K. Olson[4], J. N. Pieczynski[5], M. S. Santisteban[6], L. Hammonds-Odie[7], D. Carter[8], N. J. Ruppell[9], A. K. Challa[10]; [1]Hampden-Sydney College, Hampden-Sydney, VA, [2]University of Houston, Houston, TX, [3]University of Wisconsin-Stout, Menomonie, WI, [4]Pomona College, Claremont, CA, [5]Rollins College, Winter Park, FL, [6]University of North Carolina at Pembroke, Pembroke, NC, [7]Georgia Gwinnett College, Lawrenceville, GA, [8]Rochester Institute of Technology, Rochester, NY, [9]Randolph-Macon College, Ashland, VA, [10]University of Alabama at Birmingham, Birmingham, AL.

CRISPR-Cas9 technology represents a once-in-a-generation advance that allows precise gene editing and has become a mainstream technique in research. However, most undergraduate instructors do not have the training or support to integrate CRISPR-Cas9 into their courses. To remedy this, we formed the "CRISPR in the Classroom" Network and are facilitating workshops and mentoring activities designed to provide instructors with the skills, support, and confidence needed to introduce and implement CRISPR-Cas9 technology in undergraduate classrooms (NSF RCN-UBE #2120417). Our summer workshops provide participants with a flexible, easily-adapted curriculum and start-up kits to overcome the hurdles associated with implementing a new technology. Assessment data from previous workshops show that over 80% of attendees indicated that the design and organization of the workshop facilitated their understanding of CRISPR and that two-thirds of attendees had implementation plans for CRISPR-based activities for their courses within one year of the workshop. The CRISPR in the Classroom Network represents a dynamic community of practice for integrating CRISPR-Cas9 into courses and across model systems.

B29/P1723

Implementation and evaluation of a CURE in which students use CRISPR in budding yeast cells

K. B. Shannon; Biological Sciences, Missouri S&T, Rolla, MO.

Course-based Undergraduate Research Experiences (CUREs) are designed so that students in a class can research a question or problem for which the outcome is unknown. The opportunity for students to conduct original research is important for developing their critical thinking skills and identity as scientists. In the redesign of a Molecular Genetics lab, I used the Yeast ORFan project as a jumping off point for students to choose a gene of interest. Students then design an experiment to modify their chosen gene using CRISPR and perform an in vitro assay before attempting to modify yeast cells. Students make predictions and design assays to test their hypotheses. Student course evaluations and the Measure of College Student Persistence in the Sciences (PITS) survey were used to evaluate the effectiveness of this lab experience on student outcomes. Preliminary results show that students thought incorporating CRISPR made the lab more interesting and relevant and was useful to prepare them for careers at the bench. Students expressed feelings of ownership of the project and enjoyed working towards a self-determined goal.

B30/P1724

Harnessing MARC professional development concepts to close the gap in STEMM graduate program preparedness between Underrepresented non-MARC and MARC students.

E. Spencer, A. Hinton, Z. Vue, T. Barongan, K. Neikirk, S. Murray, H. Beasley, A. Marshall, E. Garza-Lopez; Vanderbilt University, Nashville, TN.

Providing persons excluded because of their ethnicity or race (PEERs) with professional development opportunities to help them smoothly transition into science, technology, engineering, and mathematics (STEM) fields can be incredibly powerful for institutions to implement. Programs such as Maximizing Access to Research Careers (MARC) aid in creating pipelines and preparing undergraduate PEERs to obtain foundational skills in research. It also aids PEERs to navigate the challenges of applying to graduate school and cultivate networking and mentoring relationships in STEM. To improve the graduate school preparedness of PEERs, we provided non-MARC students (i.e., non-MARC-affiliated students with no background in the subjects that MARC programs educate students on) with workshops based on professional development provided to MARC students at Winston-Salem State University. These workshops included topics on graduate school and professional programs, the application process, and the tools for graduate school success. We assessed the efficacy of these workshops by pre- and post- surveys of both non-MARC and MARC cohorts. Upon completion of these workshops, the non-MARC cohort increased their understanding of professional programs, the application process, scientific tools, and soft skills needed to thrive in STEM. Furthermore, the workshops significantly closed the gap in understanding of the same subjects between the non-MARC and MARC students. These results suggest that, given the high financial needs to implement the MARC program, institutions can consider using these workshops to meet student graduate preparedness goals more economically.

B31/P1725

Improving students' understanding of the scientific process through a complimentary multi-approach molecular genetics lab

R. L. Adams; Biology, Belmont University, NASHVILLE, TN.

Undergraduate labs serve many purposes for students: to improve their laboratory skills, to expose them to experimental techniques, to improve their scientific communication, and ideally, to help them gain an understanding of the scientific process. However, many traditional undergraduate labs often fall shy of achieving all of these goals. For example, when all students in a classroom perform the same experiment to test a hypothesis, they can struggle to understand the distinction between a scientific hypothesis from an experimental prediction. Additionally, scientific communication is challenging when all students perform the same experiment as they struggle to be motivated to explain their approach clearly. Instead, it is often the instructor who first explains the method and theory of an experimental approach for the entire class. To provide a more realistic scientific experience that would better achieve all goals, I developed a multi-week experiment for a sophomore-level Genetics course. Students initially perform a bacterial conjugation experiment to develop a hypothesis regarding antibiotic resistance genes on a plasmid. Following this, students then choose one of three different molecular approaches (PCR, restriction enzyme digest, or bacterial transformation) to test their hypothesis, forming a prediction of experimental outcome based on their own research of the approach. After the lab work, the students develop a group oral presentation that focuses on the theory of the approach to explain anticipated and actual outcomes in regard to their hypothesis. Because students do their own research

to explore the technique and they are tasked with teaching the approach to the class (i.e. each group presents a different approach they took), students are motivated to develop strong, explanatory presentations, which enhances their ownership and understanding of their lab work. Qualitative assessment of a piloted lab has indicated that having various experimental options available improves student understanding of the scientific process and communication skills. A pre- and post-survey will be conducted on an expanded group this fall to quantitatively assess the outcomes of this lab. Overall, this lab provides an inexpensive method to both improve understanding of the scientific process and allow students to compare several complimentary experimental approaches.

B32/P1726

Computer-based "virtual" labs and attainment of learning objectives and attitudes in introductory cell biology at a large, public university

L. D. Hutson, S. Kapoor; University At Buffalo, Buffalo, NY

Over the course of several years prior to the pandemic we overhauled our introductory cell biology lab program, which serves approximately 1100 Biological Sciences and related majors per year, doubling the number of labs from six to twelve and increasing the biology content of the labs. As these increases did not come with added staff, space, or equipment, we contracted with an educational software company to develop computer-based simulations of all of the labs. The computer-based labs were designed to serve students in majors that do not require much in the way of hands-on lab experiences, while the parallel traditional lab program served students in majors that are more likely to lead to research or technical careers. Because there have been limited, if any, well-controlled studies comparing the effectiveness of an entirely computer-based lab program as compared to an entirely hands-on lab program, we assessed the learning outcomes of students enrolled in each type of program over the course of two consecutive years. We found that there were some predictable and some unexpected differences between groups in both scientific and technical learning objectives of the labs. These differences, however, were relatively small, and neither group consistently attained learning objectives at a higher rate than the other. On the other hand, there were considerable differences in attitudes toward the labs between the two groups, even with respect to the activities that were identical between the two groups. The differences in attitudes that we observed have implications for all students, but it may impact minoritized groups and first-generation college students disproportionately. We have since leveraged our limited resources to minimize the reliance on computer-based labs for all students.

B33/P1727

Learning biological physics via modeling and simulation: A course for undergraduates

P. Nelson; University of Pennsylvania, philadelphia, PA

Undergraduate physical-science curricula are heavily mathematicized, despite research showing that learning should begin with qualitative phenomena of broad current interest. Meanwhile, undergraduate life-science curricula are often rooted in descriptive approaches, despite the fact that much current research involves quantitative modeling. I'll describe an alternative, an intermediate-level course now embodied in the 2022 edition of the textbook Physical Models of Living Systems. The course is the foundation of Penn's biophysics undergraduate major, as well as a response to rapidly growing interest among students in a broad range of science and engineering majors. Students acquire several research skills that are often not addressed in traditional undergraduate courses. The combination of

experimental data, modeling, and physical reasoning used in this course represents a new mode of "how to learn" for many of our students. These basic skills are presented in the context of case studies from cell biology, including: •Virus dynamics in single patients, and in populations; •Bacterial genetics and evolution of drug resistance; •Statistical inference, with applications to superresolution microscopy and cryo-EM; •Mechanobiology, for example catch bonding in immune cell receptors; •Cellular control circuits. Outcomes include student reports of improved ability to gain research positions as undergraduates, and greater effectiveness in such positions, as well as students enrolling in more challenging later courses than they would otherwise have chosen. [NSF EF-0928048; CMMI-1548571; PHY-1607611.]

B34/P1728

A CURE for the Teaching Blues: A Collectivist Framework to Move a Research Project from Conception to Manuscript in a Single Semester of an Undergraduate Laboratory Course

S. K. Olson; Molecular Biology, Pomona College, Claremont, CA.

Investigative course-based undergraduate research experiences (CUREs) are known to promote the engagement and retention of students in STEM disciplines. They are also more exciting for instructors to teach than traditional cookbook labs that do not expose students to the authentic nature of science. In the Fall semester of 2021, we challenged students in my Advanced Cell Biology class to engage collectively with the full cycle of scientific inquiry in a single semester - to start with a new project idea, generate preliminary data, propose a mechanistic model, refine the model through iterative rounds of experimentation, and draft a full-length manuscript. We chose the project based on an exciting observation made by a previous Advanced Cell lab group - that a subset of proteins required for *C. elegans* eggshell formation appears to be secreted *independently* of the conventional COPII-dependent ER-to-Golgi trafficking pathway. This year's students began by repeating the original experiments, which reinforced the importance of reproducibility in science, and allowed them to learn the standard cell biological techniques typically taught in the course. The students then shared their preliminary data and collectively developed a model to explain their findings, outlining a potential storyline for a manuscript. Next, they designed experiments to test possible explanations for the unexpected findings and to fill any gaps in knowledge that would allow them to complete the storyline, and spent the remainder of the semester conducting those experiments. At the end of the semester, students shared their findings in formal oral presentations and posters, and convened to discuss how to put the manuscript together and divide the work equitably among the lab groups. While we originally sought to complete a full manuscript in a single semester, we fell a bit shy of this ambitious goal. However, the paper is ~2/3 complete, and one student from the course joined our lab to complete the remaining experiments over the summer. Exit surveys showed the students were highly engaged with the lab, felt ownership over their project, became more confident in their ability to carry out scientific research, and came to value the collaborative and collective nature of research. This pedagogical experiment shows that significant progress toward a full-length publication can be made by undergraduates in a single semester. This model could help instructors at primarily undergraduate institutions (PUIs) improve research productivity during the busy academic year, and increase publication rates and student authorships.

Proteomics and Genomic Methods

B36/P1729

Perturb-seq analysis reveals key mediators of TNFα-induced transcriptional response

D. Tedesco[1], T. Liu[2], D. Hu[2], N. Isachenko[2], D. Deng[2], **P. Diehl**[2], A. Chenchik[2]; [1]Cellecta, Inc., Mountain View, CA, [2]Cellecta, Inc., MOUNTAIN VIEW, CA.

To identify genes involved in TNFα induced transcriptional response, a 10X single-cell Perturb-Seq screen was run on HEK293-Cas9 cells with a 88-sgRNA pooled library. As a benchmark, a parallel screen was run with cells transduced in arrayed format with a subset of the sgRNAs of the pooled library. The 10X Perturb-Seq screen was carried out in triplicate with increasing amounts of cells/10X reaction, in order to determine: a. the minimum number of cells/sgRNA required to cover the full complexity of the pooled library, and b. the maximum number of cells that can be loaded in the 10X reaction without losing single-cell resolution due to encapsulation of cell doublets. Transcriptional profiling identified TNFRSF1A, IKBKG, RELA, and CHUK as key mediators of TNFα transcriptional response in both the arrayed and pooled library screens. Increasing the number of cells per 10X reaction had a positive effect on the sgRNA library coverage, without adversely affecting the magnitude of the observed effect of TNFRSF1A, IKBKG, RELA, and CHUK knockout (KO) on TNFα mediated transcriptional response. Perturb-seq is a promising technology for the efficient and scalable interrogation of many individual genes in one single experiment, enabling the identification of transcriptional profiles linked to specific gene knockouts.

B37/P1730

Total-sync ultra-content microscopic opto-biotinylation enables high-sensitivity hypothesis-free in situ subcellular protein discovery

C. Chang[1], Y. Chen[1], C. H. Y. Cheung[1], C. Chung[1], H. Chang[1], Y. Sie[1], Y. Liu[1], Y. Lin[1], H. Kai[1], W. Chong[1], **J. Liao**[1], H. Wu[2]; [1]Syncell Inc, Taipei, TAIWAN, [2]National Taiwan University, Taipei, TAIWAN.

Localizing proteins at specific subcellular regions without using target-specific antibodies or fluorescent proteins is challenging. Laser capture microdissection is able to achieve hypothesis-free in-situ protein discovery, but the resolution cannot reach a subcellular level limited by the laser beam size, and the sensitivity is limited by the sensitivity of mass spectrometry, where only high copy-number proteins at a specific dissected region can be identified. Mass spec imaging with localized ionization can achieve a subcellular resolution, but the sensitivity is also highly limited. A major obstacle of in situ proteomic studies versus in situ transcriptomic studies is that proteins lack a PCR-equivalent amplification method to boost sensitivity. Here, in order to achieve hypothesis-free in situ subcellular protein discovery with high sensitivity and high specificity, we integrated methods of microscopy, artificial intelligence, photochemistry, and automation control to perform microscopy-guided targeted opto-biotinylation. Total-sync ultra-content biotinylation of proteins in thousands of fields of view (FOV) with similar morphological features was achieved by FPGA-based mechatronics. Convolutional neural networks-based deep learning is applied to the image to determine the regions of interest (ROIs) in real time. Multifunctional molecules that contain a photochemical warhead (such as $Ru(bpy)_3^{2+}$ or benzophenone) and a tagging molecule (such as biotin) were used for protein labeling. The three-step process of imaging-image processing-opto-biotinylation was repeated for thousands of FOVs automatically to enrich biotinylated proteins in similar ROIs enough for avidin bead purification and successive mass

spectrometry analysis, effectively beating the limit of protein amplification. With this platform, we were able to validate the technology by showing a >90% specificity and a sensitivity of >1000 species for nuclear proteins. We were also able to identify novel protein players for specific biological problems, including EIF3CL, DDX17, PPIA, RPSA, and RPLP0 for stress granules positively validated by antibody staining. Together, our total-sync ultra-content microscopic opto-biotinylation method can be applicable to widely diverse cell biology problems to identify novel protein players in the ROIs, enabling hypothesis-free subcellular protein discovery with high sensitivity and specificity.

B38/P1731

Directed evolution of new enzymes for proximity labeling in living cells

S. Lee, J. Cheah, H. Roh, A. Ting; Stanford University, Stanford, CA.

Proximity labeling (PL) catalyzed by genetically-encoded promiscuous enzymes has enabled high-resolution proteomic mapping of organelles and interactomes in living cells. The two major classes of PL enzymes are peroxidases (such as APEX2) and biotin ligases (such as BioID and TurboID). Each class has advantages but also notable limitations: peroxidases require H_2O_2 which is toxic to living organisms, while biotin ligases exhibit background leak. Both enzyme classes have spatial specificity limited by existing genetic targeting schemes. To address these limitations, we used structure guided protein engineering and directed evolution to generate two new PL enzymes: Laccase and LOV-Turbo. Laccase generates biotin-phenoxyl radicals, like APEX2, but uses non-toxic O_2 rather than H_2O_2, making it far more compatible with living organisms. LOV-Turbo is a single-chain variant of TurboID that can be reversibly turned on and off with visible light. We demonstrate multiple applications for each enzyme: PL in various organelles and cell types, pulse-chase labeling to map proteome trafficking, and electron microscopy. These new PL enzymes expand the toolkit of PL methods and enable novel applications in cell and organismal biology. <!--EndFragment-->

B39/P1732

Mapping proteome trafficking in living cells via tandem proximity labeling

J. Cheah, W. Qin, S. Lee, A. Ting; Stanford University, Stanford, CA.

Proximity labeling has been a cornerstone technique for mapping organelle proteomes and interactomes at single timepoints in living cells. However, proteins are highly dynamic, and their locations within a cell will vary through their lifetimes. To capture such changes, we developed a tandem proximity labeling platform to track protein movements through multiplexing the orthogonal promiscuous enzymes TurboID and APEX2. We apply our technique to map the trafficking proteome between membraneless organelles, stress granules and nucleolus, during stress induction and recovery. We discovered that during cellular stress, a subpopulation of the transcription factor JUN relocalizes to stress granules, and that this helps to protect JUN from aggregation and degradation to promote rapid recovery from stress. Our tandem proximity labeling platform is widely applicable to studies of proteome trafficking in intracellular and intercellular signaling.

B40/P1733

An Optically Activated Proximity Labeling Method for Spatially and Temporally Resolved Proteomics

C. M. O. Davis, O. Shafraz, S. Sivasankar; Biomedical Engineering, University of California, Davis, Davis, CA.

Membrane protein interactions are essential for cell proliferation, differentiation, and survival as well as for maintaining tissue homeostasis. However, due to the lack of appropriate technologies, the interactomes of membrane proteins, especially at distinct spatial locations within the cell and at different stages of the cell cycle, are poorly understood. Here, we have developed a light-activated proximity labeling method for high precision mapping of membrane protein interactions with high spatial and temporal resolution. Our method combines a split version of the proximity biotinylation enzyme TurboID with the optically activated protein dimerization pair CRY2 and CIB1. As a proof of principle, we engineered cells expressing CIB1 and an inactive TurboID fragment on the cell membrane along with the complementary TurboID fused to CRY2 in the cytoplasm. Using immunofluorescence imaging and mass spectrometry, we demonstrate that the split-TurboID fragments assemble, become functional, and biotinylate proteins on the cell surface only upon exposure to light. Next, we tagged E-cadherin, an essential cell-cell adhesion protein, with our optically activated proximity labeling system in order to validate our tool by comparing the measured interactome upon light exposure with known E-cadherin binding partners. Our results demonstrate that light-triggered proximity labeling can map protein interactomes with high precision. We anticipate that our optically activated technique will serve as a revolutionary tool to map junctional protein interactions within cells with high fidelity, in a previously unprecedented spatially and temporally resolved fashion.

B41/P1734

Development of LipoID: an orthogonal proximity labeling enzyme with substrate promiscuity

P. E. Cavanagh[1], W. Qin[2], A. Y. Ting[3]; [1]Stanford University, Stanford, CA, [2]Genetics, Stanford University, Stanford, CA, [3]Biology, Genetics, and by courtesy, Chemistry, Stanford University, Stanford, CA.

Enzyme-catalyzed proximity labeling (PL) has enabled the characterization of unpurifiable organelles, identification of transient protein-protein interactions, and profiling of protein translocation *in vivo*. However, all *in vivo*-compatible PL enzymes use biotin as a chemical handle, limiting the range of applications and allowing background from endogenously biotinylated proteins. Development of PL enzymes utilizing orthogonal substrates could enable proteome tagging with other functional groups such as degrons, multiplexing for studies of proteome trafficking, and background reduction. To meet this need, we are developing LipoID, an engineered promiscuous variant of *E. coli* Lipoate-protein ligase A (LplA). LipoID is engineered via a combination of structure-guided mutagenesis, machine learning, and directed evolution. We have shown that first-generation LipoID has activity with a range of substrates, including a clickable azide handle. We are characterizing LipoID for spatially specific proteomic profiling in living cells across a range of organelles.

B42/P1735

Cyto R1 Platform Enriches Samples' Viability for Single Cell Sequencing and Downstream Assays

D. Thomass, K. E. Degen, R. Barua, D. Keck, A. R. Hyler; CytoRecovery, Inc., Blacksburg, VA.

Maintaining stable sample heterogeneity with sample co-cultures during dead cell removal and sample enrichment assays is an important process for DNA and RNA sequencing, protein analyses, and phenotypic studies. These single-cell level studies require an input sample with a high rate of viability. If too many dead cells are present in the input, degraded proteins and ambient DNA or RNA can increase background noise that may lead to missing identification of crucial targets. Since the cost for single-cell sequencing experiments remains high, it is critical to ensure the input sample is optimized for high viability to ensure cost-effective and reproducible data. Current bead assays or other sample viability enrichment techniques used in preparation for single-cell analyses typically result in a significant sample loss and bias of sample heterogeneity. These beads or kits can be biased and change the cell population, subject cells to unwanted stresses, and diminish sample integrity with the time needed for preparations. Here, we investigate sample cell viability enrichment on the Cyto R1 Platform, a label-free, cell enrichment, sorting, and recovery platform. At the core, the Cyto R1 uses Cyto Chips, microfluidic technologies utilizing contactless dielectrophoresis (cDEP), to phenotypically enrich and sort various cells based upon unique physical structures and subcellular features. Thus, the Cyto R1 ensures native cell recovery without any unwanted cell tagging to maximize sample integrity. Initial experiments characterized the change in concentration for several co-cultures undergoing batch processing at a voltage of 600 Vpp and frequency of 300 kHz on the Cyto R1 Platform. Co-cultures included combinations of EL4, stem cell population, slow-developing ovarian cancer, fibroblasts, THP-1 monocytes, highly aggressive ovarian cancer, and K562 cells. For all cell types listed the change in concentration of a single species in the co-culture was less than 5%. Notably the change in concentration was less than 2% for THP-1 cells, FFLs, and K562 natural killer cells in their respective co-culture. In parallel studies comparing the EL4 cells batched on the Cyto R1 platform to the current industry standard, magnetic bead assays, the magnetic bead assays altered the heterogeneity of the sample by an average of 20% more when compared to the Cyto R1 Platform. Advancements are being made to characterize the change in concentration of heterogenous cultures for different batch voltages and frequencies to ensure that the sample heterogeneity and viability are maintained.

B43/P1736

Long-read sequencing methods for studying centromere organization, evolution, and function

N. Altemose; Molecular & Cell Biology, UC Berkeley, Berkeley, CA.

Reference genome assemblies have historically excluded repetitive satellite DNA sequences found within and near centromeres, limiting the ability to study these regions using modern genomic and epigenomic tools. Recently, long-read sequencing and assembly methods have enabled reconstruction of complete centromeric and pericentromeric sequences, providing an unprecedented opportunity to study their organization, evolution, and function. In order to fully leverage these complete assemblies, we have created and applied new sequence analysis tools to reveal the organization and evolutionary relationships of human satellite DNA sequences. We also developed DiMeLo-seq, a long-read, single-molecule method for mapping protein-DNA interactions, and we applied it to measure the density of CENP-A containing nucleosomes across human centromeres. These efforts revealed strong associations between low CpG methylation, high CENP-A density, and the very recent expansion of underlying

satellite repeats, raising important questions about the molecular and evolutionary mechanisms responsible for these associations.

B44/P1737

A Rapid and Noninvasive Method That Extracts Polymerase Chain Reaction-Ready Genomic DNA from Adult Zebrafish

J. Y. Jang[1], T. Liang[1], M. K. Kim[2], K. W. Kang[2], B. Lee[3], S. Y. Choi[1]; [1]Biomedical Sciences, Chonnam National University Medical School, Hwasun 58128, KOREA, REPUBLIC OF, [2]Neurology, Chonnam National University Medical School, Gwangju 61469, KOREA, REPUBLIC OF, [3]Biochemistry, Chonnam National University Medical School, Hwasun 58128, KOREA, REPUBLIC OF.

Genotyping usually entails analysis of the products of polymerase chain reaction (PCR) carried out with genomic DNA (gDNA) as template, and is employed for validation of mutant or transgenic organisms. For genotyping of adult zebrafish, gDNA is often extracted from clipped caudal fin or skin mucus through either alkaline lysis using NaOH or proteinase K (PK) treatment. Further purification of the gDNA using ethanol precipitation was optional. To develop a rapid and noninvasive method that extracts PCRready gDNA from adult zebrafish, we combined skin swabbing with PK treatment and demonstrated its efficiency. This method could be applied to a wide range of fish.

B45/P1738

Evaluating RNA Isolation Methods for Spheroids and Organoids

J. Bhandari, A. Gupta, E. Quandt, L. Chapman; ThermoFisher Scientific, Austin, TX.

Three-dimensional (3D) cultures such as spheroids and organoids have recently gained the attention of the scientific community because they have been found to more closely model *in vivo* natural systems. However, due to their inherent 3D structure, these culture types present unique challenges to existing nucleic acid extraction methodologies that were primarily developed for 2D cultures. In this study, we evaluated the efficacy of several commonly used extraction methods such as phenol/chloroform-based extraction, solid-phase-based extraction using spin columns or magnetic beads, as well as a direct-to-PCR lysate-based method. Extraction performance was evaluated by performing RT-qPCR with the isolated RNA to measure and compare gene expression levels in spheroids and organoids. Our results showed efficient RNA recovery from spheroids and organoids could be achieved with all four methods. Hence, the selection of an appropriate extraction method depends on end-user requirements. For instance, direct cell lysate would be ideal for high-throughput users who need to process individual spheroid and organoid samples for large-scale gene expression studies. The spin column-based extraction may be most suitable for users who process <24 samples at once. The magnetic bead-based method is desirable for processing a large number of samples with minimal hands-on time. Organic solvent-based extraction may be suitable for processing a pool of spheroids and organoids.

B46/P1739

A CNN-Based Computer Vision approach for CRISPR Genomic Edit Detection

T. Patterson, H. Farris, K. Rosfjord, E. Mace, P. Hinson; MITRE, McLean, VA.

Clustered regularly interspaced short palindromic repeat (CRISPR) technology provides an inexpensive method for making genomic modifications within organisms. The field is rapidly expanding, creating

tools with increased efficiency and precision in performing these genomic edits. Editing the genome with CRISPR introduces regions of abnormally increased methylation around the edit location. These variances in normal methylation patterns can be leveraged to identify edited locations within a genome. In this project, provide a computer vision-based method of identifying CRISPR edits in mouse genomes, through visual representations of methylation variance data between edited and unedited mice. We describe an image-generation algorithm to create usable images for training a convolutional neural network (CNN), using bisulfite whole genome-sequenced mouse epigenomes. Our method implements a Convolutional Neural Network model that was trained on generated images of epigenome methylation calls of CRISPR-edited and unedited mice, to identify methylation patterns in the epigenome that are indicative of CRISPR edit locations. The application of CRISPR technology has potential repercussions, such as altering gene regulation through methylation changes, that are passed to offspring. The faster and unsupervised method for identifying CRISPR edits presented in this paper provides a path towards the rapid detection of CRISPR edits and their effects on biological processes. This is necessary to mitigate unintended consequences of CRISPR in the epigenome that may persist over generations.

B47/P1740

High-throughput genetics and essential gene discovery in the human fungal pathogen*Cryptococcus neoformans*

R. Billmyre, C. Craig, M. Eickbush, S. Zanders; Stowers Institute for Medical Research, Kansas City, MO.

Advances in sequencing technologies have revealed dramatic variation in gene content and sequence between different species and even between different isolates within individual species. However, our ability to understand the functional consequences of these changes has lagged behind significantly. The *Cryptococcus* genus is an ideal model fungal system to explore these questions. There are at least seven species within this genus amenable to lab experimentation that diverged around the same time as humans and birds. In addition, *Cryptococcus* species are pathogens of humans, causing nearly 200,000 deaths annually. We have developed a high-throughput quantitative genetics approach in the human fungal pathogen *Cryptococcus neoformans* by using massively parallel insertional mutagenesis coupled with targeted sequencing and machine learning to determine the set of genes important for growth under a given condition. Using this method, we have identified the set of essential genes in *C. neoformans*. This set includes dozens of genes that are nonessential in other fungi but required in *C. neoformans* and vice versa. These differentially essential genes include genes important for establishing cell polarity and nuclear organization in other fungi, but which are unstudied and essential in *Cryptococcus*. In addition, we have used this same approach to map genome-wide resistance to existing antifungal agents. Taken together, this work will produce a global understanding of drug resistance and a map of essential genome function to guide future drug development in *C. neoformans*. In addition, this project will establish an experimental tool and framework that can be used to analyze other non-model fungal pathogens and help bridge the gap between sequence and function.

B48/P1741

Single-cell transcriptomic network combined with imaging unveils the heterogeneity of the clonal macrophage cells during phagocytosis

S. Janewanthanakul[1], H. Ikeda[2], K. Kurimoto[2], T. Nishimura[1], K. Yakura[1], F. Garcia[3], P. Sri-iesaranusorn[3], K. Ikeda[3], S. Suetsugu[1]; [1]Molecular Medicine and Cell Biology laboratory, School of Biological Science, Nara Institute of Science and Technology, Nara, JAPAN, [2]Department of Embryology, Nara Medical University, Nara, JAPAN, [3]Mathematical Informatics laboratory, School of Information Science, Nara Institute of Science and Technology, Nara, JAPAN.

High content imaging and single-cell sequencing are the two most high throughput technology for studying gene functions related to cellular morphology and behavior. Investigating how single-cell transcriptomic profiles affect cell behavior has been a great challenge in cell biology to understand cellular heterogeneity in behaviors. Phagocytosis is a process of membrane deformation and cytoskeleton rearrangement for engulfing a foreign particle. Even by using the clonal cell lines, each macrophage cell does not undergo phagocytosis. Single-cell macrophage behaviors were recorded under a confocal microscope while adding zymosan, and then each single cell transcriptome was analyzed after recording. Out of ~10,000 genes, several hundred genes were identified to be significantly differentially expressed depending on the ability of the phagocytosis. The interactome data among these genes showed the genes that are well connected to each other. The weight gene co-express network analysis (WGCNA) analysis showed the 4 modules of correlated genes (correlation > 0.55) to phagocytosis, which are the genes encoding the toll-like receptor signaling proteins, pattern recognition receptor, SH3-domain-binding proteins, and PDZ-domain-binding proteins. These genes in each module that were retrieved from the transcriptomic profile will be further identified and studied for the regulation of phagocytosis. The finding of this study provides new insight into complex regulations in the phagocytosis process while suggesting candidate genes that can be involved in phagocytosis mechanism.

B49/P1742

Unfolding the whole transcriptome of the American Type Culture Collection Cell Line Land for its application as a molecular reference standard in the next-generation biomedicine research

A. P. Singh, A. L. Reese, C. Tabron, J. Duncan, R. Marlow, J. Kirkland, S. King, A. Fernandes, J. Bagnoli, B. Benton, J. L. Jacobs; ATCC, Manassas, VA

In the cell biology research space, the use of unauthenticated biological reference materials, irregularities in crucial related metadata and frequent gaps in data provenance often raise questions as to the credibility and reproducibility of the experimental data. To address this problem, we have begun efforts to characterize whole transcriptomes of all cell lines and primary cells held within ATCC's (American Type Culture Collection) cell biology collection to develop an authenticated application data resource to be used as a molecular reference standard. Here we present our initial analysis of authenticated whole transcriptome (RNAseq) datasets produced from 62 human kidney cell lines including primary cells found in ATCC's biorepository. These diverse datasets, represented by the ATCC Cell Line Land, include the first comprehensive collection of reference transcriptomes for use by kidney biology researchers and is available through QIAGEN's OmicSoft platform. Multiple biological replicates for each cell line are included to help establish a baseline for a wide range of cell lines under typical cell culture conditions. The ATCC Cell Line Land includes transcript and gene expression count data, RNAseq variant calls, raw sequencing data, and detailed metadata on laboratory methods employed, such as the

total cell counts, RNA extraction methods, RIN scores, number of replicates, tissue/cell-type information, disease association, passage number, catalog number, lot number, growth media, culture conditions, and cryopreservation conditions. Furthermore, all biological replicate data included in this resource is produced using a common, standardized RNA extraction, library prep, sequencing, and bioinformatics workflow - thereby enabling comparative transcriptomics of these data at scale. The ATCC Cell Line Land is a first-of-its-kind joint venture between ATCC and QIAGEN Digital Insights aimed at providing reference-grade whole transcriptome data that is authenticated, standardized and traceable to physical source materials available in ATCC's biorepository.

Regulation of Actin Dynamics 1

B51/P1743

Quantifying cellular and cytosolic levels of actin binding proteins in *S. cerevisiae*

S. Gonzalez Rodriguez, A. C. E. Wirshing, B. L. Goode; Brandeis University, Waltham, MA

Animal and fungal cells express a conserved set of 15-20 actin binding proteins (ABPs), which together orchestrate the assembly, disassembly, and reconfiguration of the actin cytoskeleton. Understanding how these proteins work in concert in living systems requires quantitative information about their cellular abundance, physical interactions with actin and each other, and specific effects on actin. We are addressing this problem in the budding yeast *S. cerevisiae*, where the activities and interactions among these ABPs are now well established, but their cellular levels have remained undefined. To close this gap, we measured both the total cellular levels and cytosolic (freely available) concentrations of 15 conserved ABPs. To measure cellular levels, we used quantitative western blotting with antibodies specific to each ABP. To measure levels in the cytosol versus bound to F-actin structures, we performed two-color live imaging on strains co-expressing GFP-tagged ABPs and RFP-tagged cortical actin patches (Arc15) or linear actin cables (Lifeact). Total cellular concentrations of ABPs ranged from 0.4-11 µM, and the percentage of each that was in the cytosol ranged from 60-90%. Remarkably, these levels remained relatively constant throughout the cell cycle, despite dramatic changes in F-actin reorganization and cell size. Together, these results show that yeast maintain high concentrations of free ABPs in their cytosol, which may be important for keeping them in a 'primed state,' responsive to internal and external signals and able to rapidly reorganize their F-actin structures. Further, they provide the necessary information required to build more accurate and complete quantitative models of how ABPs control cellular actin organization and dynamics.

B52/P1744

The Molecular Basis for Cell Migration in 3D

N. R. Martin[1], M.-H. Tang[2], W.-J. Rappel[2], O. D. Weiner[1]; [1]University of California, San Francisco, San Francisco, CA, [2]University of California, San Diego, La Jolla, CA

Immune function relies on cells that crawl through our tissues to neutralize threats. However, the overaccumulation of immune cells at damaged tissues and tumors can drive inflammation and cancer progression. Therefore, understanding the fundamental process of cell migration will provide us with strategies to bolster immune function, and to reduce damage from excessive immune cell migration. The canonical model for cell migration centers on a force-generating molecular clutch that depends on surface adhesion, but immune cells do not require adhesion to migrate *in vivo*. Thus, our current

understanding of how immune cells reach their target is incomplete. One reason may be that most studies of migration are done on 2D surfaces that fail to capture the complex 3D geometry of tissues. The Wiskott-Aldrich syndrome protein (WASP) is dispensable for migration on 2D surfaces but WASP mutations cause severe migration defects *in vivo* suggesting that WASP plays a critical role in 3D migration. Recently, we discovered that the actin nucleator WASP is curvature sensitive and integrates topographical information into the polarity program that steers cell migration. I will leverage this insight to uncover how cells engage with surface geometry to generate forces and steer their migration. To understand how substrate geometry influences migratory force generation, we are using model 3D substrates to measure WASP-dependent traction forces. We find that WASP localization and actin nucleating activity are correlated with force generation supporting the hypothesis that WASP plays a role in migratory force generation. To dissect the role of WASP in orienting migratory forces we will use optogenetics to spatially control WASP activity and measure the effects on cell polarity signals. Together, this work will uncover how and expand the model of cell migration to include geometry-guided migration in 3D.

B53/P1745

Length regulation of stereocilia by the formin inhibitor SMIFH2

C.-y. Tung, X. Liao, B. Perrin; Indiana University Purdue University Indianapolis, Indianapolis, IN

Stereocilia are actin-based protrusions at the apical surface of auditory sensory hair cells. Several stereocilia of precisely regulated length and width form a bundle with a staircase-like structure that is required for detecting sound. Stereocilia are formed around a core of parallel, unbranched actin filaments. We speculated that formin proteins may contribute to elongating these filaments. To test this idea, we studied the effect of the formin inhibitor SMIFH2 on stereocilia length. Cochlear explants from mice at postnatal day 4 were cultured with SMIFH2. If formins elongate the F-actin in the core, then inhibition would result in shorter stereocilia. Instead, we observed a rapid and dose-dependent increase in the length of stereocilia in the shorter rows of the bundle. Actin incorporated at stereocilia tips, suggesting that F-actin in the stereocilia core elongated from their barbed ends. FMN1 and DAAM1 are the most highly expressed members of the formin family in auditory hair cells according to publicly available RNAseq data. Immunostaining revealed that FMN1 and DAAM1 localize just above the base of stereocilia and that each is mislocalized with SMIFH2 treatment. We also observed that actin binding proteins that normally localize to stereocilia tips were reduced as formins were inhibited. Together, these data suggest that formins can decrease actin polymerization and indirectly regulate the protein composition of stereocilia tips.

B54/P1746

Activity and mechanism of the pan-formin inhibitor SMIFH2

C. Vizcarra; Barnard College, New York, NY

The small-molecule drug SMIFH2 was discovered in a biochemical screen for inhibitors of formin-stimulated actin polymerization (Rizvi, et al. 2009). While the *in vitro* screen was carried out against the mouse formin mDia1, SMIFH2 was also found to be a cell permeable inhibitor that targeted both yeast and mammalian formins. Since the original report, at least 340 published papers have reported experiments in which SMIFH2 was used to investigate formin function. Our research group is interested in the molecular basis of formin inhibition, specifically the potential for isoform-specific inhibition and

the effect of SMIFH2 on the myriad biochemical functions that formins carry out. In this study, we found that SMIFH2 inhibits actin assembly stimulated by formins from six of the seven families of mammalian formins. To explore the relationship between chemical structure and biological activity, we synthesized and assayed 17 analogs of SMIFH2 against a panel of five human formins (DIAPH1, DIAPH2, FMNL3, FMN2, and INF2). While some modifications improved inhibitory potency, none imparted specificity, indicating that the alkylidene thiobarbiturate core of SMIFH2 may not be an ideal platform for distinguishing among highly conserved FH2 domains. Computational studies point to reactivity instead of molecular geometry as a determinant of inhibitory potency. We are currently exploring the effect of SMIFH2 on the FH2 domain's structure and interactions with microtubules.
Rizvi, S. A., Neidt, E.M., Cui, J., Feiger, Z., Skau, C.T., Gardel, M.L. Kozmin, S.A., Kovar D.R., Chemistry & Biology 2009, 16: 1158-1168.

B55/P1747

Universal Length Fluctuations of Actin Fibers

A. Rosario, S. G. McInally, B. L. Goode, J. Kondev; Brandeis University, Waltham, MA

Actin is one of the most important and abundant cytoskeletal proteins in most eukaryotic cells. The central property of actin is its ability to polymerize into helical filaments and bundling into higher order structures *in vivo* to form parallel actin bundles which is influential in various cellular functions such as motility, signaling, and cell division. Cells tightly control the sizes of these bundles as their size is closely associated with their function even though individual filaments within these bundles are dynamic. Balance point model where two competing rates, the length dependent assembly and/or disassembly can match to produce a single, stable fixed point has been proposed as possible mechanism of length control in the context of both actin and microtubule filaments. Using a master equation approach, we find that length fluctuations, small deviations in length produced by stochastic addition and removal of monomers/fragments during filament assembly and disassembly, are Gaussian independent of the nature of feedback. The variance of this distribution is given by the product of the steady state filament length and the size of the fragments added or removed. Comparing our calculations to experimentally measured length perturbations of stereocilia and microvilli, we find that the variance scales with the square of the mean length. We propose an alternate model of length control for parallel actin bundles where individual filaments in a bundle are polymerized and can be removed at specific rates and the length of the bundle is set by the longest filament in the bundle. This leads to a peaked distribution of bundle length even though individual filaments are exponentially distributed. The variance of bundles length is proportional to the square of the steady state length consistent with experimental data.

B56/P1748

Zyxin localization and dynamics in neurons

N. Sele[1], A. B. Varona Ortiz[1], B. R. Schuldt[1,2], S. A. Kim[1]; [1]Amherst College, Amherst, MA, [2]Icahn School of Medicine at Mount Sinai, New York City, NY

Cytoskeletal reorganization occurs in response to environmental stimuli and leads to structural changes in synapses. Actin-regulatory proteins transduce signals received at the postsynaptic density to the cytoskeleton. In some cell types such as fibroblasts and cancer cells, zyxin has been well characterized as a promoter of actin polymerization but has not been described in the central nervous system. Here, we establish zyxin as a neuronal protein and describe its recruitment to dendritic spines in hippocampal

neurons. Zyxin was found localized in the soma and dendrites throughout neurodevelopment with expression in the growth cone and dendritic spine. Zyxin displayed robust recruitment to dendritic spines under various conditions. Mechanical force applied by shear stress triggered mobilization of zyxin to spines that was myosin IIB-dependent. Extracellular signals, such as depolarization and elevated zinc, also induced zinc recruitment to spines. These results suggest that zyxin may regulate F-actin reorganization in dendritic spines through its recruitment and may coordinate activity-dependent changes in postsynaptic structure.

B57/P1749

Probing molecular interaction interface between neuronal drebrin and mDia2 formin

S. Srapyan, E. Grintsevich; California State University, Long Beach, Long Beach, CA

Actin cytoskeleton is critical for neuronal shape and function. Neuron-specific drebrin A and ubiquitous diaphanous formin-2 (mDia2), are key regulators of the actin cytoskeleton. Drebrin A is highly enriched in postsynaptic terminals of mature neurons (dendritic spines) and mDia2 formin is upregulated during dendritic spine initiation. Decreased levels of drebrin in dendritic spines is a hallmark of Alzheimer's disease, epilepsy, and other complex disorders. Drebrin A was previously identified as an interacting partner and inhibitor of mDia2 formin. Characterizing the molecular binding interface between formin and drebrin is necessary to better understand functional consequences of this interaction and its biological relevance. Prior work suggested that the N-terminal sequence of drebrin (Drb1-300) contains a weak interacting site of its multi-pronged interface with mDia2. The model of the complex predicted that Drb1-300 interacts with C-terminal tails of mDia2 which is involved in formin's autoinhibition and microtubule binding. Employing biochemical assays, we show that drebrin binding cannot relieve autoinhibition of mDia2 and it does not preclude formin binding to microtubules. The model of the drebrin-mDia2 complex also predicted a high affinity mDia2 binding site in C-terminal sequence of drebrin (301-706). Guided by mass spectrometry and in silico analysis we generated an array of deletion mutants in neuronal drebrin A to identify this binding site. We employed these mutants in pull-down assays with GST-tagged mDia2-FH2 domain. Consistent with the proposed model, our results identified a highly conserved sequence in the C-terminal region of drebrin as the principal site of mDia2 binding. This work contributes towards a molecular level understanding of the formin-drebrin interface.

B58/P1750

Mechanical activation of Dia1 protects the cytoskeleton from damage, enabling cell fate commitment

F. R. Valencia[1], E. Sandoval[2], J. Du[1], E. Iu[1], J. Liu[2], S. V. Plotnikov[1]; [1]University of Toronto, Toronto, ON, Canada, [2]Johns Hopkins University, Baltimore, MD

Plasticity of cell mechanics underlies a wide range of cell and tissue behaviors allowing cells to migrate, to resist shear forces, and to upregulate contractility during wound healing. Such plasticity depends on the regulation of the actin cytoskeleton, but mechanisms of adaptive change in cell mechanics remain elusive. By combining live-cell imaging and genetic and pharmacological manipulation, we assessed how suppression of major actin elongation factors affect the dynamics of actin polymerization at focal adhesions. We found that suppression of actin elongation factor mDia1 leads to a 70% decrease in actin polymerization. Moreover, we showed that suppression of myosin contractility results in a dose-dependent decrease in actin polymerization, demonstrating mDia1's propensity to be regulated by mechanical force. By using a super-localization microscopy, we showed that actin polymerization at

focal adhesions exhibits pulsatile dynamics where the spikes of Dia1 activity are triggered by mechanical force. By combining mathematical modeling and laser nano-surgery, we showed that suppression of force-dependent actin polymerization at focal adhesions results in two-fold increase in mechanical tension on the stress fibers. Such elevated tension increases the frequency of spontaneous stress fiber damage and decreases the efficiency of stress fiber repair. Moreover, the excessive stress fiber damage in Dia1 depleted cells abrogates the differentiation of myofibroblast, highly contractile cells essential for wound healing, by suppressing nuclear localization of the SMAD family transcription factors. Remarkably, suppression of stress fiber damage in Dia1 depleted cells through downregulation of myosin contractility rescued myofibroblasts differentiation. These results demonstrate the key role of mDia1 in fine-tuning actin organization to prevent cytoskeleton damage, safeguard cells against abrupt mechanical damage and for successful differentiation of myofibroblasts.

B59/P1751

Yeast coronin synergizes with GMF to prune actin filament branches

N. Koundinya, R. Aguilar, B. L. Goode; Brandeis University, Waltham, MA

The rapid assembly and turnover of branched F-actin networks drives a number of cellular processes, including cell motility, endocytosis, and phagocytosis. While we have gained a strong mechanistic understanding of how F-actin branches are assembled in cells by the Arp2/3 complex and its nucleation promoting factors (NPFs), we know comparatively little about how branch junctions are subsequently destabilized to promote rapid network turnover. The F-actin branches formed by Arp2/3 complex are inherently stabile structures, persisting for 10-30 minutes in vitro before spontaneously debranching, yet in vivo they can turnover in 2-20 seconds. This suggests that additional cellular factors are required to drive debranching. Previously, we identified glia maturation factor (GMF) as a conserved and high affinity ligand of the Arp2/3 complex that catalyzes F-actin debranching (Gandhi et al., 2010; Ydenberg et al., 2013). Here , we show that another binding partner of Arp2/3 complex, yeast Coronin (Crn1), works ini conjunction with GMF to promote rapid debranching. Specifically, using TIRF microscopy we show that Crn1 alone has no debranching activity; however, Crn1 enhances the effects of GMF by almost 10-fold. This synergy is mediated by the disordered ‘unique domain’ of Crn1, which varies in length and sequence across different species. The unique domain alone is both required and sufficient to synergize with GMF, and these activities depend on its Arp2/3-binding ‘CA’ like motif, related to ‘VCA’ regions of Arp2/3 NPFs. Together, our results define a new role for Crn1 in driving actin network turnover through ‘collaborative debranching’. Further, they contribute to the emerging view that both the assembly and destabilization of F-actin branches in vivo are controlled by the coordinated effects of multiple Arp2/3 ligands acting in concert.

B60/P1752

CAP1 modulates activity of Src and changes cytoskeleton dynamics.

H.-D. Jang, D.-S. Shin, E. Choi, M. Fukai, G. No, H.-S. Kim; Seoul Natl University Hosp, Seoul, Korea, Republic of

Cyclase-associated protein 1 (CAP1) has a role in inflammation as a functional receptor of human Resistin, and is implicated in the invasiveness of human cancers as an actin-regulating protein. In this study, we identified that CAP1 is a novel interacting partner of Src and regulates its membrane localization and activation. CAP1-/- MDA-MB231 cells were defective both in cell growth and migration

even treated with recombinant human epidermal growth factor (rhEGF). In vivo, the tumor growth measured by volume in NOD/SCID mice was significantly smaller with engrafting CAP1-/- MDA-MB231 cells than with CAP1+/+ MDA-MB231 cells ($p<0.0001$). CAP1-/- MDA-MB231 cells were arrested at G1 phase with induction of p53, p21, and p27 expression, and showed low phosphorylation of Src and high phosphorylation of LIMK and cofilin, leading to significantly shorter phalloidin and less lamellipodia than CAP1+/+ MDA-MB231 cells. CAP1 plays a key role for cancer cell growth and migration by binding to Src leading to membrane localization and phosphorylation of Src.

B61/P1753

A central role for cofilin in maintaining cortical actin network integrity through filament turnover

R. S. Kadzik, D. R. Kovar[,1], E. M. Munro[,1]; University of Chicago, Chicago, IL

During polarization and cell division in the early C. *elegans* embryo, the actin cortex must undergo large scale deformations while maintaining structural integrity. Rapid turnover of cortical actin filaments is thought to play a key role in facilitating large-scale cortex deformation and flow by allowing local relaxation of network stress while preventing catastrophic tearing. ADF/Cofilin is a central agent for actin filament disassembly in eukaryotic cells. However, while much is known about cofilin function *in vitro*, it remains a major challenge to understand how cofilin controls actin filament disassembly in vivo, and how its activities are tuned for physiological performance by spatiotemporal patterning, network architecture, and contractile forces. To bridge this gap in our understanding, we are combining *in vivo* single molecule imaging of actin dynamics in the early *C. elegans* embryo with reconstitution of actin network disassembly *in vitro*. We found that increased stabilization of actin filaments, via the small molecule inhibitor Jasplakinolide, has a dramatic effect on the cortical actin network, causing catastrophic tearing of the actin cortex, and ultimately resulting in delayed or failed cytokinesis. Furthermore, strong cofilin knockdown causes similar cortical network instabilities, identifying cofilin as a central regulator of filament disassembly and turnover in the early embryo. By combining TIRF microscopy with single molecule imaging and particle tracking analysis of transgenically expressed Actin::GFP, we measured rapid turnover of cortical actin filaments, revealing a dominant filament lifetime of ~8 sec. We found that the average filament lifetime is increased at least two-fold in conditions where actin filament stability is increased, indicating that filament disassembly is inhibited. To understand how inhibition of filament disassembly induces the observed cortical tearing, we focused on how new filament assembly is affected by reduced filament turnover. Using TIRF-M of single molecule dynamics of formin (CYK-1) at the cortex, we found that formin-mediated actin filament assembly rates are significantly reduced with inhibition of filament turnover by cofilin RNAi, suggesting that a tight coordination between filament disassembly and assembly is required to maintain the structural integrity of the actin cortex. *In vitro* TIRF-M reconstitution assays with purified UNC-60A revealed that UNC-60A promotes both severing and depolymerization from both the barbed and pointed ends of actin filaments. We are currently expanding these assays to determine the mechanism for cofilin/UNC-60A mediated turnover in the early embryo on single and bundled actin filaments as well as its effect on actin monomer recycling and new filament assembly.

B62/P1754

Synthetic control of dynamic pattern formation at the cell cortex

L. A. Sepaniac, A. Michaud, W. M. Bement; Center for Quantitative Cell Biology, University of Wisconsin-Madison, Madison, WI.

The cell cortex, including the plasma membrane, underlying cytoskeleton, and various cytoskeletal regulators, undergoes dramatic remodeling to drive cell shape changes necessary for cell division, polarization, motility, and morphogenesis. Cortical dynamics are often regulated by Rho family GTPases such as Rho, Rac, and Cdc42. Rho family proteins cycle between an active, GTP-bound state, and an inactive, GDP-bound state. This cycling is primarily regulated through Guanine nucleotide exchange factors (GEFs) which promote activation of Rho via nucleotide exchange, and GTPase activating proteins (GAPs) which promote Rho inactivation via GTP hydrolysis. Rho family GTPases are often deployed in generating discrete patterns at the cortex such as patches, rings or stripes. One type of dynamic pattern that may arise is propagating, complementary waves of Rho GTPase activity and F-actin disassembly - referred to as cortical excitability. Cortical excitability is subject to both internal and external regulation, including internal signaling which prompts cell cycle progression and developmental processes. Importantly, the cytoskeletal remodeling needed for essential cellular functions such as cell migration, mitosis and cytokinesis is supported by cortical excitability. We have previously shown that cortical excitability can be experimentally induced in *Xenopus* oocytes (which are not normally excitable) via co-expression two Rho regulators essential for cytokinesis: Ect2 (a GEF) and RGA-3/4 (a GAP). To further our understanding of this excitable circuit and cortical excitability in general, I have used a synthetic biology approach. Specifically, I employed a synthetic GEF construct designed to provide Rho-based positive feedback, including the Rho GEF domain of LARG fused with an rGBD, a domain that binds specifically to active Rho. When co-expressed with RGA-3/4, a natural participant in Rho-based negative feedback, the synthetic positive feedback construct, *Rho Positive Feedback 1 (RhoP1)*, produces semi-synthetic waves of Rho activity and F-actin. The waves appear elongated and propagate slowly, with a relative amplitude of 1.18 and period of 436 sec, compared with non-synthetic waves produced via Ect2 and RGA-3/4 (relative amplitude: 0.82, period: 152 sec). Similarly, a synthetic inhibitor, dubbed "*Rho Negative Feedback 1 (RhoN1),*" containing a Rho GAP domain and tropomyosin (to bind F-actin), supports semi-synthetic, pulsatile waves when co-expressed with the natural cytokinetic GEF, Ect2. These waves show a relative amplitude of 0.17 and period of 320 sec. Further study of this circuit system and development of additional synthetic constructs will provide added information about the regulation and propagation of excitable dynamics of this circuit.

B63/P1755

SUP-13/ARRD-15 Controls the Transition of AIP1 Isoforms in *C. elegans* Body Wall Muscle

M. Lewis[1], K. Ono[1], Z. Qin[2], R. C. Johnsen[2], D. L. Baillie[2], S. Ono[1]; [1]Emory University, Atlanta, GA, [2]Simon Fraser University, Burnaby, BC, Canada

Actin-interacting protein 1 (AIP1) (also known as WDR1) is a conserved WD repeat protein that regulates cytoskeletal remodelling by promoting actin filament severing in the presence of actin-depolymerizing factor (ADF)/cofilin. In *Caenorhabditis elegans*, the *unc-78* gene encodes AIP1 that is essential for sarcomeric actin assembly in striated muscle. Previously, Francis & Waterston isolated *sup-13* as an extragenic suppressor of *unc-78* (Waterston, 1988). The *sup-13* mutation suppresses motility defects and sarcomere disorganization of the *unc-78* mutant, such that the *unc-78;sup-13*double mutant

exhibits nearly normal muscle contractility and sarcomere assembly. We identified that *sup-13* has a nonsense mutation in *arrd-15* encoding an α-arrestin. α-arrestins are typically involved in protein trafficking, with some serving as adaptors to recruit ubiquitin ligases in regulating the degradation of specific protein targets. However, α-arrestins have not been associated with sarcomere regulation previously, and the function of ARRD-15 remains to be characterized. ARRD-15 that was endogenously tagged with green fluorescent protein (GFP) was expressed in body wall muscle and localized diffusely in the cytoplasm. Transgenic expression of GFP-ARRD-15 in the *unc-78;sup-13* mutant enhanced sarcomere disorganization, as observed in the *unc-78* mutant. *C. elegans* has a second AIP1 isoform, AIPL-1, which is partially redundant with UNC-78 and normally downregulated in mature muscle. However, when AIPL-1 was depleted by RNA interference (RNAi), the *sup-13*/*arrd-15* mutation no longer suppressed actin disorganization of the *unc-78* mutant, indicating AIPL-1 is required for the phenotypic suppression. We found AIPL-1 protein level was higher in adult body wall muscle of the *sup-13/arrd-15* mutant, suggesting the normal function of SUP-13/ARRD-15 is to downregulate AIPL-1, and the high level of AIPL-1 may be compensating for the function of UNC-78. Depletion of several components of the ubiquitin-proteasome system by RNAi partially suppressed sarcomere disorganization in the *unc-78* mutant. In addition, ubiquitinated AIPL-1 was increased in the *sup-13/arrd-15* mutant. Taken together, these data suggest the function of SUP-13/ARRD-15 is to control the transition of AIP1 isoforms in *C. elegans* body wall muscle cells by specifically targeting AIPL-1 for ubiquitin-dependent degradation.

B64/P1756

Regulation and function of nuclear actin filament dynamics

S. Ulferts[1], R. Grosse[1,2]; [1]University of Freiburg, Freiburg, Germany, [2]Centre for Integrative Biological Signalling Studies - CIBSS, Freiburg, Germany

Although recent years allowed for the first visualizations of dynamic nuclear actin filaments in mammalian cells, their regulation, roles and functions remain poorly understood. We have previously described mainly two independent processes that involve tightly regulated and transient nuclear actin assembly: (i) as a response to ligand-induced calcium elevations, and (ii) at the exit from mitosis during daughter cell nuclear expansion. Despite the fact that both processes differ in structural appearance, temporal dynamics as well as in the participating actin-regulating factors, they converge on the level of chromatin dynamics, such as variations in the degree of chromatin compaction and mobility. The first mentioned process involves rapid GPCR-mediated elevations of cellular calcium levels propagating through the nucleus, thereby stimulating actin polymerization from the inner nuclear membrane. We identified the formin inverted formin 2 (INF2) as the downstream nucleator initiating actin polymerization. Using a nuclear-targeted actin probe in order to visualize dynamic nuclear F-actin assembly, we performed a siRNA screen for inner nuclear membrane (INM) proteins to identify a potential receptor involved in the signal transduction from GPCRs to intranuclear actin polymerization. Results showed that knockdown of SAD1/UNC84 domain protein-2 (SUN2), member of the SUN domain protein family and key component of the linker of nucleoskeleton and cytoskeleton (LINC) complex, led to a significant reduction in the number of cells susceptible to calcium mediated nuclear actin assembly. Using high resolution imaging techniques and Proximity Ligation Assay (PLA) technology, we found that INF2 indeed co-localizes with SUN2 at the nuclear envelope possibly allowing for transient protein protein interactions and signal transduction towards the nuclear compartment. Ongoing work focuses on identifying transcriptional targets controlled by SUN2-INF2 mediated nuclear actin polymerization.

B65/P1757

The GxcM-Fbp17/RacC-WASP signaling cascade regulates polarized cortex assembly in migrating cells

D. Li; Institute of Biophysics, Chinese Academy of Sciences (CAS), Beijing, China

The actin-rich cortex plays a fundamental role in many cellular processes. Its architecture and molecular composition vary across cell types and physiological states. The full complement of actin assembly factors driving cortex formation and how their activities are spatiotemporally regulated remain to be fully elucidated. Using *Dictyostelium* as a model for polarized and rapidly migrating cells, we show that GxcM, a RhoGEF localized specifically in the rear of migrating cells, functions together with F-BAR protein Fbp17, a small GTPase RacC, and the actin nucleation-promoting factor WASP to coordinately promote Arp2/3 complex-mediated cortical actin assembly. Over-activation of this signaling cascade leads to excessive actin polymerization in the rear cortex, whereas its disruption causes defects in cortical integrity and function. Therefore, different from its well-defined role in the formation of the front protrusions, the Arp2/3 complex-based actin carries out a previously unappreciated function in building the rear cortical subcompartment in rapidly migrating cells.

B66/P1758

Determining the Role of RhoG in Macropinocytosis

B. Kasu; South Dakota State University, Brookings, SD

Macropinocytosis is non-specific bulk-fluid uptake of fluids and solutes. CRISPR-cas9 whole genome screens identified RhoG, a small g-protein, as a negative regulator of macropinocytosis. Previous studies showed RhoG silencing reduces Rac1 activation in the context of cell motility. Rac1 has also been previously established as a key regulator of macropinocytosis. Therefore, we hypothesize that RhoG regulates macropinocytosis via Rac1. Experiments with targeted knockouts are ongoing to validate macropinocytosis efficiency and test the hypothesis.

B67/P1759

Human cyclase-associated protein (CAP1) regulates barbed end actin dynamics using its WH2 domain

N. Alimov, B. L. Goode; Brandeis University, Waltham, MA

Regulation of actin turnover is critical for the formation and reshaping of filamentous actin networks with a wide range of in vivo functions. Recently, cyclase-associated protein (CAP) has emerged as a central player in the regulation of F-actin turnover through interactions of its N-terminal helical folded domain (HFD) with the pointed ends of filaments (Shekhar et al., 2019; Kotila et al., 2019). Here, using microfluidics-assisted TIRF microscopy, we show that human CAP1 also regulates dynamics at the opposite (barbed) ends of actin filaments. In the absence of actin monomers, CAP1 increased the rate of brabed end depolymerization by approximately 4-fold compared to control reactions, and in the presence of actin monomers CAP1 slowed barbed end growth. These effects were mediated by the C-terminal half of CAP (C-CAP), whereas the N-terminal half of CAP (N-CAP) mediates the previously-described pointed end effects. Thus, different regions of CAP interact with the two ends of the filament to control F-actin dynamics. A further dissection of C-CAP revealed that its actin-binding WH2 domain was necessary but not sufficient for the barbed end effects, whereas actin-binding by the CARP domain did not appear to be involved. Our current efforts are aimed at understanding whether these activities depend on the specific sequence of the WH2 domain in CAP, or instead the specific molecular

architecture of C-CAP. Further, we are exploring how the regulatory effects of CAP1 at the barbed end influence other barbed end regulators such as capping protein and formins. Together, this work identifies a new function for CAP1 in F-actin turnover, mediated by its WH2 domain interacting with the barbed ends of actin filaments.

B68/P1760

More complex decisions: IQGAP1, formin, and capping protein regulate actin filament ends

M. L. Pimm, M. Alcaide Eligio, B. Haarer, A. G. Marcin, J. L. Henty-Ridilla; SUNY Upstate Medical University, Syracuse, NY

Actin filaments are intricately regulated at their barbed ends by specific proteins and complexes that promote or inhibit filament growth through dynamic competitive interactions. Using purified proteins and TIRF microscopy we examine the mechanism underlying IQGAP1's capping activity and how IQGAP1 and the formin, mDia1, regulate actin assembly. IQGAP1 significantly slows actin filament elongation through 20-25 s transient capping events regardless of the concentration of IQGAP used. Using truncation analysis, we determined a minimal ~300 aa construct of IQGAP able to cap filaments and further identified two amino acids responsible for this activity. Next, using fluorescently labeled IQGAP or capping-deficient mutant proteins, we learned the oligomeric state. Using step photobleaching analysis and functionally tagged IQGAP proteins we determined that both IQGAP and the capping deficient mutant were functional dimers. The mutant had less association with filament ends and no change in side-binding or bundling activities. In three-color TIRF microscopy experiments, we examined single molecules of IQGAP1 and constructs of mDia1 directly interacting. In the presence of actin filaments IQGAP-mDia1 complexes were observed on filament ends and ultimately slowed actin assembly to similar levels as reactions lacking formin. Two-color TIRF assays containing actin filament seeds and unlabeled proteins allowed us to investigate the open question of whether IQGAP1 could influence formin-capping protein interactions at the barbed end. Reactions containing capping protein, IQGAP1, and formin resulted in fewer blocked ends and net growth compared to reactions containing only capping protein and mDia1. Thus, IQGAP1-mDia1 interactions may protect barbed ends from capping protein. We investigated the impacts of IQGAP1-mediated filament capping on cell morphology, actin filament structure, and cell migration. Introducing IQGAP1 on a plasmid fully rescues CRISPR knockout 3T3 cell morphology and actin filament structures similar to cells expressing endogenous IQGAP1. However, a plasmid harboring the capping deficient IQGAP did not. Cells expressing only mutant IQGAP1 also displayed significantly slower wound closure. Combined this suggests that IQGAP1's capping functions are important for regulating cell morphology, actin filament structure, and migratory behaviors.

B69/P1761

Identification of Cdc42-ITSN modulators as novel partial agonists

S. Malasala, T. Penwell, C. Boykin, Y.-H. Chen, S. Nik Akhtar, Q. Lu; East Carolina Univ/Brody Sch Med, Greenville, NC

Small GTPases of Rho family control actin cytoskeleton organization. They also play a crucial role in the regulation of cellular functions such as cell morphology, migration, endocytosis and cell cycle progression involved in a wide variety of human diseases. Their activation and inactivation are controlled by various modulators like guanine nucleotide exchange factors (GEFs). We have developed

small molecule modulators (SMMs) that target Cdc42 interaction with its selective GEF intersetcin (ITSN). Although previous studies identified their inhibitory functions, further analyses revealed that some of the SMMs display partial agonistic features. N-MAR-GTP fluorophore-based Cdc42-GEF assays demonstrated that ZCL278 and ZCL279 promoted GTP loading in the absence of Cdc42-GEF *in vitro*. However, in the presence of GEF, they reduced GTP loading. By using Swiss 3T3 cell cultures, the Cdc42 G-LISA® assay revealed time- and concentration-dependent SMMs regulation of Cdc42-GTP interactions with immobilized Cdc42 binding protein. In these cells, bradykinin activated Cdc42 and induced the characteristic actin-based microspikes (filopodia). Interestingly, both ZCL278 and ZCL279 mimicked the activating effects of bradykinin. However, they showed inhibitory effects when the cells were concurrently activated by bradykinin. Therefore, these SMMs are unique Cdc42-ITSN protein-protein interaction (PPI) modulators that are partial agonists and may have important applications in investigating small GTPase-mediated cell signaling. This study is in part supported by NIH NIGMS/OD GM146257.

B70/P1762

The P38 kinase family gene, PMK-1, has a role in *C. elegans* Embryonic Development.

A. Wallace, S. Parkinson; Fairleigh Dickinson University, Teaneck, NJ

In order for organisms to correctly develop, it is critical for them to undergo proper cell migration. The process of cell migration is meticulously regulated but all the details on how this happens have not been fully described. Abnormalities in cell migration have been linked to numerous disorders. For example, individual and collective cell migration mechanisms are altered in cancer cells. Defective cell migration has also been linked to improper neuronal development contributing to the occurrence of numerous neurodegenerative diseases. In Caenorhabditis elegans, the WAVE/SCAR pathway has been shown to regulate epidermal cell migration during embryonic morphogenesis. WAVE receives signals from three axonal guidance receptors (SAX-3, UNC-40 and VAB-1) to nucleate branched actin and initiate cell movement. We performed an RNAi screen to identify genes that function in the WAVE pathway during embryonic morphogenesis and found PMK-1 as a potential WAVE pathway regulator. PMK-1 is a member of the P38 MAP kinase family and is an ortholog of human mitogen-activated protein kinases 11 and 14 (MAPK11 and MAPK14). PMK-1 has been suggested to play a regulatory role in cell development and differentiation. We found that loss of pmk-1 in C. elegans causes embryonic lethality, and a portion of these embryos die due to a failure in morphogenesis. The ventral cells of the epidermis fail to properly migrate and seal in the internal organs leading to dead embryos. This phenotype resembles what is observed when known WAVE pathway genes are mutated. Loss of PMK-1 in vab-1 and sax-3 mutant backgrounds showed enhanced embryonic lethality while no change was observed in unc-40 mutants. The enhanced lethality results could mean that PMK-1 is functioning in a pathway parallel to SAX-3 and VAB-1 while the unchanged lethality in an unc-40 background could mean that they are functioning together in the same pathway. We will further investigate to verify that PMK-1 is functioning in the WAVE pathway and determine where in the WAVE pathway PMK-1 functions. We will also perform experiments to find out how PMK-1 affects branched actin nucleation.

Actin and Actin Associated Proteins 2

B71/P1763

What makes an actin an actin?

P. Avasthi, **B. M. Bigge**; Arcadia Science, Berkeley, CA

Actin mediates a very long list of cellular processes that are required for life. Because of the essentiality of actin in the cell, it is widely conserved, existing in organisms from humans to plants to protists and beyond. However, most of what we know about actin comes from a fairly small selection of cells, mostly Opisthokonts with actins that are highly conserved and some others, leaving a gap in our understanding of the actin cytoskeleton, how it's regulated, how it functions, and how it evolved. To truly understand actin biology, we should embrace the diversity of actin across organisms. This could allow us to gather more information about interactions between actin and its binding partners, to discover novel actin-based functions, and to uncover insights into the evolution and emergence of the actin cytoskeleton. Before we can do that, we must take a step back to define the clear and testable properties that make an actin a bona fide actin as opposed to an actin related protein or actin like protein. Here, we investigate properties including sequence conservation, structural conservation, polymerizability, ATPase function, post translational modifications, cellular functions, predicted binding partners, and phylogeny. While some of these criteria are clear and useful for defining an actin, others are more complicated and even questionable. There might be others still that we have not yet considered. Thus, this is an ongoing process and an open discussion, and we want to hear from you: what properties do you think make an actin an actin?

B72/P1764

Mechanisms of crawling motility in chytrid fungi

S. M. Brody; University of Massachusetts Amherst, Amherst, MA

Crawling motility is critical to many aspects of animal biology, including embryonic development, immune system function and tumor metastasis. Cell motility typically is dependent on the dynamic restructuring and turnover of the actin cytoskeleton to create protrusive and contractile forces necessary to movement. For example, fibroblasts and epithelial cells move by extending their lamellipodia, a thin sheet-like protrusion, and forming tight focal adhesions with their environment. Many amoeboid cells crawl with broad actin-filled pseudopods that form weaker, non-specific interactions with their environment. Alternatively, cells can crawl utilizing thin, linear "filopodia" filled with bundled actin or plasma membrane "blebs" devoid of F-actin. Beyond animals many species of fungi and algae use crawling to navigate their environments; however, it is poorly understood how these mechanisms have evolved and diverged across phyla. By studying chytrids, early-diverging fungi that retain characteristics of animal cells lost is other fungi, we aim to better understand the evolution and conservation of mechanisms of cell motility. To this end, I am adapting assays to observe chytrid crawling under a variety of conditions, allowing me to observe the formation and dynamics of cellular structures involved in crawling and determine which types of motility chytrid species employ. Using adhesion and 2-dimensional cell-confinement assays I am also exploring how the physical environment alters cell crawling as well as characterizing the molecular and protein components necessary to crawling in each species. By elucidating the structures and components chytrid species use to crawl and

understanding their relationship to well-studied organisms of animals and fungi, we aim to develop a better understanding of the origin and evolution of crawling.

B73/P1765

The actin networks of chytrid fungi reveal evolutionary loss of cytoskeletal complexity in the fungal kingdom

S. M. Prostak, K. A. Robinson, L. K. Fritz-Laylin; University of Massachusetts Amherst, Amherst, MA.

Across Eukarya, cells use actin polymer networks for a variety of conserved functions including endocytosis, cytokinesis, and cell migration. Despite this functional conservation, the actin cytoskeleton has undergone significant diversification, including within the opisthokont lineage that encompasses animals and fungi. The actin cytoskeleton of animal cells is complex, with distinct structures including cell cortex, lamellipodia, filopodia, stress fibers, and cytokinetic rings, all regulated by a subset of over 30 proteins. In contrast, budding yeast have about half the actin regulatory proteins and fewer distinct structures—cables, patches, cytokinetic rings, and fusion foci. The many differences between the actin networks of two sister lineages raises questions about the complexity of the ancestral actin network. Chytrid fungi diverged before the emergence of the Dikarya (multicellular fungi and yeast), providing a unique opportunity to study actin cytoskeletal evolution. Chytrids have two life stages: flagellated, motile zoospore cells, and sessile sporangial cells encased in a chitinous cell wall- like the Dikarya. We discovered that zoospores of the amphibian-killing chytrid *Batrachochytrium dendrobatidis* (*Bd*) build dynamic actin structures resembling those of animal cells (actin cortex, pseudopods, and filopodia-like spikes). In contrast, *Bd* sporangia assemble perinuclear actin shells and actin patches similar to those of yeast. Treatment with small molecule inhibitors reveals that nearly all of *Bd*'s actin structures are dynamic and some require the same nucleator as their animal or yeast counterparts. Pseudopods, actin patches, and actin spikes require at least Arp2/3 complex function, while the actin cortex forms independently from Arp2/3 complex function. Our analysis of multiple chytrid genomes reveals the presence of actin regulators and myosin motors genes typical of animals, as well as those specific to fungi. The presence of animal- and yeast-like actin cytoskeletal components in the genome combined with the intermediate actin phenotypes in *Bd* suggests that the simplicity of the yeast cytoskeleton may be due to evolutionary loss. Using early branching fungi, like chytrids, has revealed a previously underappreciated evolutionary pattern, further solidifying chytrids as powerful model organisms for studying actin cytoskeletal evolution.

B74/P1766

Differential N-terminal processing of beta and gamma actin

L. Chen[1], P. Vedula[1], H. Tang[2], D. Dong[1], A. Kashina[1]; [1]University of Pennsylvania, PHILADELPHIA, PA, [2]The Wistar Institute, PHILADELPHIA, PA

Cytoplasmic beta- and gamma-actin are ubiquitously expressed in every eukaryotic cell. They are encoded by different genes, but their amino acid sequences differ only by four conservative substitutions at the N-termini, making it difficult to dissect their individual regulation. Here, we analyzed actin from cultured cells and tissues by mass spectrometry and found that beta, unlike gamma actin, undergoes sequential removal of N-terminal Asp residues, leading to truncated actin species found in both F- and G-actin preparations. This processing affects up to ~3% of beta-actin in different cell types. We used CRISPR/Cas-9 in cultured cells to delete two candidate enzymes capable of mediating this type

of processing. This deletion abolishes most of the beta-actin N-terminal processing and results in changes in F-actin levels, cell spreading, filopodia formation, and cell migration. Our results demonstrate previously unknown isoform-specific actin regulation that can potentially affect actin functions in cells

B75/P1767

The role of evolutionary amino acid conservation in cytoplasmic actin function

P. Vedula[1], M. E. Fina[2], B. A. Bell[3], S. S. Nikonov[2], D. W. Dong[1], C. M. Schroeder[4], A. S. Kashina[1]; [1]Biomedical Sciences, University of Pennsylvania, Philadelphia, PA, [2]Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [3]F.M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [4]Pharmacology, University of Texas Southwestern, Dallas, TX.

Cytoplasmic actins are ubiquitously expressed and are essential components of the cytoskeleton. All vertebrates express two cytoplasmic actins, β- and γ-actin, which are highly similar to each other with only several amino acid changes within their N-terminus and the C-terminal region. In anamniotes, an evolutionarily more ancient class of organisms including fish and amphibia, the cytoplasmic actins show divergence in several residues in the N- and C-terminus. However, in amniotes, which include birds, reptiles, and mammals, β- and γ-actin are nearly identical to each other, except for 4 conservative substitutions in the N-termini. These differences have been completely conserved over 320 million years of evolution. The underlying reason for this conservation, and the exact role of the 4 amino acid changes in cytoplasmic actins' function, are unknown. Here, we addressed the role of these N-terminal substitutions using a gene-edited mouse model expressing γ-actin both natively and from the nearly intact β-actin gene. These mice completely lack β-actin protein but retain the nucleotide-level elements of both cytoplasmic actin genes. Thus, any phenotypes in these mice are caused by the replacement of β-actin-specific N-terminal amino acids with those of γ-actin, making this model uniquely suited to address the biological role of these amino acid differences *in vivo*. Strikingly, while these mice undergo normal embryogenesis, the absence of β-actin protein leads to reduced long-term survival, indicating an evolutionary cost due to the loss of a highly conserved cytoplasmic actin. One of the consistent defects in these mice includes disorganization of the microvilli, actin-rich cytoplasmic protrusions present in many tissues throughout the body, prominently including the retina and the small intestine. Our results demonstrate crucial functions for the evolutionarily conserved amino acid differences between cytoplasmic actins in amniotes and indicate potentially distinct selective pressures on the evolutionarily older cytoplasmic actin orthologs in anamniotes.

B76/P1768

Evolutionary tuning of capping protein-formin competition allows simultaneous construction of two distinct actin networks

A. C. E. Wirshing, S. Gonzalez Rodriguez, B. L. Goode; Biology, Brandeis University, Waltham, MA.

Vertebrate capping protein (CapZ) and formins join each other at actin filament barbed ends in vitro and catalyze each other's displacement through associative competition. However, the physiological consequences of this mechanism and whether it is conserved in other organisms have remained unclear. To address these questions, we studied the relationship between *S. cerevisiae* capping protein (Cap1/2) and formins in vitro and in vivo. Using microfluidics-TIRF microscopy we show that yeast Cap1/2 only weakly displaces formins from barbed ends compared to CapZ. In vivo, formin-dependent actin cable

assembly was strongly attenuated by overexpression of CapZ but not Cap1/2. Further, loss of Cap1/2 resulted in Arp2/3 complex-nucleated actin patches over time taking on cable-like features, including recruitment of formins and tropomyosin. These results suggest that the properties of *S. cerevisiae* Cap1/2 have been tuned across evolution to allow robust cable assembly by formins in the presence of high cytosolic levels of Cap1/2 used to limit patch growth and shield patches from formins.

B77/P1769

An optimized F-actin probe reveals dynamic organelle-associated F-actin networks in budding yeast

A. C. E. Wirshing, B. L. Goode; Brandeis University, Wlatham, MA

Live-cell actin probes can be biased in the actin networks they label and fail to accurately detect all actin networks in a cell. Here, we show that the actin binding protein Abp140, which has been used in the budding yeast field for 20 years to visualize formin-generated F-actin cables (bundles), decorates cables in the mother cell but is largely excluded from cables in the daughter cell (bud). On the other hand, cables in both compartments are clearly visualized by phalloidin in fixed cells and by labeled tropomyosins (Tpm1 and Tpm2) in live cells. Thus, there is a population of actin cables assembled by the formin Bni1 in the bud that is poorly decorated by Abp140. Further, by expressing integrated formin chimeras (Bni1-Bnr1 and Bnr1-Bni1), we find that the lack of Abp140 on cables in the bud compartment is not dependent on a specific formin building cables in the bud. Surprisingly, cables in both compartments are clearly detected by a 17 residue fragment of Abp140 ('LifeAct') fused to a 3x-mNeonGreen tag, and the signal is much brighter than Abp140 with the same tag. We show that our optimized LifeAct probe does not alter cellular growth or actin network dynamics. Finally, using this new probe coupled with super-resolution live-cell imaging, we detect dynamic actin cables associated with organelles, which were poorly detected using Abp140. This work describes an optimized actin probe for budding yeast and opens new avenues for investigating actin-organelle interactions in this system.

B78/P1770

Direct observation of the interaction of cyclase-associated protein with actin or cofilactin filaments by high-speed atomic force microscopy

P. D. N. Nguyen[1], **S. Ono**[2], H. Abe[3], N. Kodera[1]; [1]Kanazawa University, Kanazawa, JAPAN, [2]Emory University, Atlanta, GA, [3]Chiba University, Chiba, JAPAN.

Cyclase-associated protein (CAP) is present widely among eukaryotes and regulates multiple key aspects of actin dynamics. Mutations in CAP often cause severe defects in cytoskeletal dynamics in many eukaryotes. Mutations in human CAP2 cause myopathies in cardiac and skeletal muscles. CAP binds to actin monomers and promotes exchange of actin-bound nucleotides. CAP also binds to cofilin-bound actin (cofilactin) filaments and enhances severing or depolymerization from the pointed ends. However, whether CAP binds to actin filaments at the side or only at the pointed ends is still under debate. Here, we characterized the interaction of *Xenopus* cyclase-associated protein 1 (XCAP1) with actin or cofilactin filaments directly by using high-speed atomic force microscopy (AFM). We found that XCAP1 bound to the side of bare actin filaments with no preference to the filament ends. The binding was transient with an average dwell time of ~0.7 s. The presence or absence of ATP did not affect this interaction. We often found that XCAP1 bundles actin filaments most likely by binding sides of multiple filaments as an XCAP1 tetramer. When XCAP1 was added to cofilactin filaments, dissociation of cofilin from actin filaments was induced. This resulted in enhanced filament severing by producing patches of actin and cofilactin

clusters within the filaments. Under these conditions, XCAP1 predominantly induced severing of cofilactin filaments and only rarely promoted depolymerization from the ends at much slower rates than previously published studies using microfluidics-assisted total internal reflection fluorescence microscopy. Genetic studies in the nematode *Caenorhabditis elegans* were consistent with the *in vitro* activities of CAP to promote cofilactin disassembly. Previously, we reported that mutation in *cas-1* encoding a muscle-specific CAP caused formation of excessive cofilactin aggregates (Nomura et al., 2012). In addition, we found that mutation in *cas-2* encoding a non-muscle CAP caused formation of large cofilactin rods in the syncytial gonad. These results suggest that CAP is essential for disassembly of cofilactin filaments either by depolymerization from ends or severing.

B79/P1771

Molecular control of actin cortex architecture during cell division

N. Vadnjal[1,2], S. Nourreddine[3,4], G. Lavoie[3], M. Serres[1,5], R. Peters[2], A. Jégou[6], G. Romet-Lemmone[6], F. Parmeggiani[7], P. Roux[3], E. Paluch[2]; [1]University College London, London, United Kingdom, [2]University of Cambridge, Cambridge, United Kingdom, [3]University of Montreal, Montreal, QC, Canada, [4]University of California San Diego, San Diego, CA, [5]Institut Pasteur, Paris, France, [6]Institut Jacques Monod, Paris, France, [7]University of Bristol, Bristol, United Kingdom

Animal cell shape is controlled by the actin cortex, a thin actomyosin network supporting the plasma membrane. By pulling on actin filaments, myosin motors generate contractile tension in the cortex and gradients in this tension lead to cell shape changes, such as those driving cell division. Alongside myosin, actin network connectivity, which is controlled by crosslinker levels and actin filament length, has recently been shown to be key to cortical tension regulation. Yet, how cortical network organisation is controlled at the molecular level, and how this contributes to tension regulation, remains poorly understood. To identify potential regulators of cortex tension, we firstly compared proteic composition of interphase and mitotic cortices. To isolate cortical fractions, we developed a protocol to isolate cortex-enriched blebs from synchronised cells. We detected over 922 proteins in interphase and mitotic blebs of which 238 were actin-related. Among actin-related proteins, our analysis highlighted a role for septins in the regulation of the mitotic cell shape. Interestingly, our mass spectrometry analysis indicates that cortical levels of crosslinkers, key determinants of actin network connectivity, do not display strong changes between interphase and mitosis. We thus hypothesised that other properties of actin crosslinkers might be important for the regulation of cortical actin organisation and mechanics. We focused on investigating the role of crosslinker size. Indeed, actin crosslinkers display a large variety of sizes, and protein size could affect localisation within the dense cortical actin network. To test this, we created “artificial crosslinkers” for which size can be modulated independently of other features. Artificial crosslinkers localise to the actin cortex during cell division and affect cortical mechanics, cell shape, and division dynamics. We are currently investigating how crosslinker size affects cortical localisation during cortex tension gradient formation in cytokinesis. Finally, we are testing how artificial crosslinkers modulate actin network architecture *in vitro*. Together, this work unveils new levels of regulation of cortical organisation and tension.

B80/P1772

Particle-Based Model of Mechanosensory Contractility Kit Assembly

A. I. Plaza-Rodriguez[1], L. Nguyen[2], D. N. Robinson[2], P. A. Iglesias[3]; [1]Biophysics, Johns Hopkins University, Baltimore, MD, [2]Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, [3]Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD.

Cell shape-change processes, such as proliferation, polarization, migration, and cancer metastasis, rely on a dynamic network of macromolecules. The proper function of this network enables mechanosensation, the ability of cells to sense and respond to mechanical cues. Myosin II and cortexillin I, critical elements of the cellular mechanosensory machinery, preassemble in the cytoplasm of Dictyostelium cells into complexes that we have termed contractility kits (CKs). Two IQGAP proteins then differentially regulate the mechanoresponsiveness of the cortexillin I-myosin II elements within CKs. To investigate the mechanism of CK self-assembly and gain insight into possible molecular means for IQGAP regulation, we developed a coarse-grained excluded-volume molecular model in which all protein polymers are represented by nm-sized spheres connected by spring-like links. The model is parameterized using experimentally measured parameters acquired through Fluorescence Cross-Correlation Spectroscopy (FCCS) and Fluorescence Correlation Spectroscopy (FCS), which describe the interaction affinities and diffusion coefficients for individual molecular components. Interaction affinities were verified via computational emulation of FCCS and fractional saturation curves obtained from simulations. Diffusion coefficients were corroborated via computational emulation of FCS and Mean Squared Deviation (MSD) analysis of particles in simulation. Simulations of wild-type and null-mutant conditions implied that the temporal order of assembly of these kits is dominated by myosin II dimer formation and that IQGAP proteins mediate cluster growth. Additionally, our simulations predicted the existence of “ambiguous” CKs that incorporate both classes of IQGAPs, and we confirmed this experimentally using FCCS. The model serves to describe the formation of the CKs and how their assembly enables and regulates mechanosensation at the molecular level.

B81/P1773

Engineering Platforms for Visualizing Mechanical Regulation of the Actin Cytoskeleton

J. Levin, A. Pan, G. M. Alushin; The Rockefeller University, New York, NY

Myosin generated forces have recently been shown to modulate interactions between individual actin filaments (F-actin) and actin binding proteins (ABPs). *In vivo*, F-actin and myosins are incorporated into higher-order multi-filament networks, such as the cortex and contractile stress fibers, whose network geometry could also impact F-actin-ABP interactions. To date, it has been difficult to disentangle the contributions of force and multi-filament avidity to mechanosensitive ABP localization in cells. To address this problem, we have developed an *in vitro* system for producing varied actin filament network geometries in the presence of active myosin forces while monitoring ABP engagement with TIRF microscopy. In our modified gliding filament assay, we arrange filaments in a head-to-head configuration using two covalently conjugated capping protein heterodimers. We combine this with protein micropatterning to produce dozens of individual networks in different arrangements in parallel on a coverglass. We simultaneously observe network dynamics in the presence of myosin force generation while monitoring soluble ABP binding. We apply this method to the mechanosensor α-catenin, which exists in two different states in cells: as member of the heterotrimeric cadherin-catenin complex (a core component of adherens junctions), and as a cytoplasmic homodimer which suppresses ARP2/3 branch

formation. We observe that a monomeric α-catenin construct preferentially binds filaments under tension as previously reported. However, in a homodimeric construct, this force-activated binding competes with an avidity-based preference for binding to bundles of filaments experiencing force in parallel, where the load per filament is reduced. This could mediate distinct actin network binding preferences for α-catenin's different forms in cells, highlighting how ABPs integrate biochemical and biomechanical signals including filament tension, filament bundling, and their own oligomerization status, to tune their behavior. Our experimental platform should widely enable dissecting such multimodal activities of ABPs.

B82/P1774

Engineering Orthogonal Actin-binding Protein Switches

U. M. Effiong, H. Khairandish, B. Belardi; University of Texas At Austin, Austin, TX.

Actin is a prominent feature of eukaryotic cells and plays critical roles in numerous subcellular processes such as endocytosis, cell motility and division. Actin's engagement with subcellular structures involves actin-binding proteins (ABP) that interface the filamentous form of actin (F-actin) with specific molecular complexes and regulate F-actin's organization and mechanics. While ABPs have been studied in detail in cells, these experiments often rely on the use of small-molecule F-actin disrupters or protein depletion. Unfortunately, neither of these tools is ideal for monitoring how ABPs-of-interest contribute to individual cellular processes as they either affect F-actin or the protein-of-interest globally. Thus, a means of directly manipulating actin binding with temporal control is a pressing need. We propose using protein switch domains that toggle between an "off" and an "on" state to accomplish this task. Here, we describe the design and engineering of three F-actin binding switch domains triggered by different stimuli to achieve switching activity over a broad range of timescales. Each design relies on installing an intramolecular association to drive the "off" state of actin-binding sequences (ABS). After the introduction of either a small molecule, a peptide, or incident light stimulus, the switch transitions to an "on" state, leading to F-actin binding. For peptide and small molecule stimuli, we successfully engineered switches that conformationally constrain two ABSs in an inactive state and that can be activated in the presence of their respective stimulus. For light-based stimulation, we engineered steric inhibition of ABSs by incorporating a light-responsive protein domain into the switch, allowing for blue light-mediated activation. We quantified i) the "off" state of the switches as the fold inhibition compared to wildtype activity and ii) the extent of activation after stimuli introduction. Our switches achieved 50-95% fold inhibition and binding activations up to 85% upon the addition of stimuli in cells. These switch designs were then utilized in cell junction and filament cross-linking studies. Actin-binding switches offer a new method for controlling actin binding in a direct, specific, and temporal manner, which can be applied to experiments seeking to probe and control the influence of actin binding on a subcellular process.

B83/P1775

The regulatory mechanisms of dynamin-actin bundling activity

C. Wang, M. Chuang, Y. Liu; Institute of Molecular Medicine, National Taiwan University, Taipei, TAIWAN.

Dynamin is best-known as a membrane fission mechanochemical GTPase essential for endocytosis in cells. In addition to plasma membrane, dynamin is also recruited to a unique actin structure, podosome,

where it interacts with actin polymerization factors or directly bundles actin filaments. We found that the actin bundling ability of dynamin-2 (Dyn2), ubiquitously expressed dynamin isoform, could only be observed in buffer with low ionic strength *in vitro*. This result indicates that the actin bundling ability of Dyn2 needs to be activated in cells under physiological, higher ionic strength condition. Previously, a podosome scaffold protein, Tks5, has been found to bind directly to Dyn2, whereas the Src kinase-mediated phosphorylation of Dyn2 at Y597 regulates its podosome-targeting in cells. Nonetheless, how Tks5 interaction and Dyn2^{Y597} phosphorylation regulates the actin bundling activity of Dyn2 remains unclear. In this study, we found Tks5 binds directly to Dyn2 with two SH3 domains. Interestingly, the "mini-Tks5", two SH3 domains that directly bind to Dyn2, promoted the actin bundling activity of Dyn2 in buffer with high ionic strength. Moreover, mini-Tks5 prevented Dyn2 disassembly induced by GTP addition and even increased the GTP-hydrolysis activity of Dyn2. Mini-Tks5 was also observed to increase the diameter of Dyn2-actin bundles under transmission electron microscopy. Additionally, we discovered that Dyn2^{Y597} phosphorylation and mini-Tks5 synergistically prevented GTP-induced Dyn2 disassembly and resulted in Dyn2-actin bundles with larger diameter in buffer with high ionic strength. Together, we discover that Tks5 interaction and Src kinase-mediated Dyn2^{Y597} phosphorylation collaboratively enhance the activities of Dyn2 on bundling actin filaments thus promote podosome maturation in cells.

B84/P1776

Tropomyosin and engagement of the integrin LFA-1 cooperate to promote actomyosin-dependent force generation at the T cell immune synapse

D. C. Schrock[1], M. Heydecker[1], R. Weigert[1], E. Hardeman[2], P. Gunning[2], J. A. Hammer[1]; [1]NIH, Bethesda, MD, [2]UNSW, Sydney, Australia

Efficient target cell killing by CD8+ T cells requires integrin-dependent adhesion to, and the exertion of force on, the target cell (Basu et al Cell, 2016). T cell: target cell interaction is mediated by a highly-organized interfacial structure called the immunological synapse (IS) that is composed of three concentric zones termed the distal, peripheral, and central supramolecular activation clusters (dSMAC, pSMAC, and cSMAC). This organization is established and maintained by an Arp2/3 complex-generated branched actin network in the dSMAC and a formin-generated linear actin network in the pSMAC. Bipolar filaments of myosin 2A (M2A) organize the linear pSMAC actin network into concentric, contractile actin arcs, and this structure co-localizes with the ring of LFA-1 that drives T cell: target cell adhesion by binding to ICAM-1 on the target cell surface. Inhibition of either formin (mDia1) or M2A impairs the movement of T cell receptor (TCR) microclusters toward the cSMAC, dampens TCR signaling, and impairs T cell: APC adhesion. Tropomyosins are actin-binding proteins that associate with linear, formin-generated filaments, where they serve to promote actomyosin force generation by recruiting and activating type 2 myosins. Here we show that mouse CD8+ T cells express tropomyosins 3.1 and 3.2 (Tpm3.1/3.2) and that Tpm3.1/3.2 colocalize with the pSMAC actomyosin arcs. Importantly, abrogating Tpm3.1/3.2 function using either a small molecule inhibitor (ATM3507) or CRISPR-mediated gene deletion attenuates the recruitment of M2A to the IS and disrupts the concentric organization of the pSMAC actin arcs. Conversely, ligating LFA-1 with ICAM-1 dramatically increases the amount of M2A recruited to the synapse and the concentric organization of the pSMAC actin arcs. Together, these results argue that Tpm3.1/3.2 and LFA-1 ligation cooperate to promote actomyosin-dependent force generation at the T cell IS. These results also add to the growing appreciation for the role that integrin ligation plays in promoting the function of both T cells and B cells. Finally, we hypothesize that the

pSMAC actomyosin arcs are responsible for the exertion of force on the target cell that promotes target cell lysis. Current efforts are directed, therefore, at evaluating the efficiency of target cell killing by CD8+ T cells isolated from mice lacking Tpm3.1/3.2.

B85/P1777

Effects of tropomodulin 2 on dendritic spine reorganization

B. Kuruba[1], N. Starks[1], G. Wayman[1], M. Mikhaylova[2,3], **A. Kostyukova**[1,3]; [1]Washington State University, Pullman, WA, [2]Humboldt Universität zu Berlin, Berlin, Germany, [3]University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Neurons communicate through a structure between an axon and a dendrite termed a synapse. The ability of the brain to adapt to environmental or activity-dependent changes is regarded as synaptic plasticity. Dendritic spine morphology is tightly connected to the synaptic plasticity and the strength of the input. Actin remodeling is required for the formation, maturation and reorganization of spines. Actin filaments are highly dynamic structures with a slow growing (pointed) and fast growing (barbed) ends. Very few studies have been conducted on the role of pointed-end binding proteins in regulation of dendritic spine morphology. In this study, we focus on highlighting the role played by tropomodulin 2 (Tmod2), a brain-specific isoform, on the dendritic spine post-development reorganization. Tmod2 regulates actin polymerization by binding to the pointed end via several distinct actin and tropomyosin (Tpm) binding sites. Tmod2 can also nucleate actin polymerization. We studied the effects of Tmod2 overexpression in primary hippocampal neurons on number, morphology and mobility of spines and number of other actin-based structures, e.g., filopodia and spinules. We showed that Tmod2 overexpression led to the decrease in number of thin, mushroom, and stubby spines but did not affect number of branched spines. The overexpression resulted in the increase in lengths of thin and mushroom spines. While there was no effect on number of filopodia, number of spinules increased. Eliminating the actin nucleation ability of Tmod2 by mutating one of the two actin-binding sites resulted in the increase in the number of mushroom spines. Destroying Tpm-binding ability of Tmod2 by mutating both Tpm-binding sites increased the mobility of thin spines/filopodia drastically. We assume that the ability of Tmod2 to bind at the pointed ends that depends on the presence of both Tpm-binding sites affects positively spine maturation.

B86/P1778

Delving into Tropomyosin-Actin Species Specificity and Physical Consequences of Novel Heterodimerization

C. Nie[1], R. D. Mullins[1], N. A. Petek-Seoane[1], J. Rodriguez[2]; [1]Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA, [2]University of California San Francisco, San Francisco, CA.

The actin cytoskeleton mediates a wide breadth of functions in the cell such as adhesion, cell motility, and tension sensing. Non-muscle tropomyosins are dimeric coiled-coil proteins that are critical regulators of nearly all actin cytoskeleton dynamics. In humans, four tropomyosin genes code for upwards of 40 tropomyosin isoforms through differential splicing. Fascinatingly, these isoforms are non-redundant in their regulatory roles within the cell, but the structure-function relationship of these non-muscle isoforms is not well understood. Recently, our lab has described three isoforms expressed by Drosophila Schneider 2 (S2) cells, named 1A, 1J, and 2A. In vitro experiments revealed to us that isoform

1A can bind actin as a homodimer, typical of tropomyosins. However, 1J and 2A were both unable to bind actin until they were made to heterodimerize (one coil of each). Tropomyosin heterodimerization was only reported before in the context of cardiac smooth muscle, but in the smooth muscle it merely improves binding and is not a prerequisite for binding outright as it is for our isoforms. We also observed a large species-specific affinity discrepancy for actin in these tropomyosins. This is novel because the high sequence identity of eukaryotic actins typically allow for most actin-binding protein (ABP) studies to utilize easy-to-purify heterologous systems (actin and ABP from different species). Our goal in this work is to investigate the rationale behind heterodimerization and the species-specificity of our S2 tropomyosins. To achieve this we will use a combination of in vitro biochemistry techniques and cryo-electron microscopy (cryo-EM) to investigate how the structure and interactions of novel tropomyosins allow them to bind actin. Our cryo-EM work has so far demonstrated that tropomyosin isoform 1A interacts with actin in the exact fashion as muscle tropomyosins, binding along a positive groove of actin. But we are experiencing difficulty in reconstructing the heterodimer, possibly due to low affinity in our heterologous system. Concluding from our current results and looking forward, we aim to purify S2 actin and perform homologous studies wherein the actin and tropomyosin are sourced from the same species. This will likely yield more biologically relevant results and improve our cryo-EM data collections.

B87/P1779

Characterization of a novel *Caenorhabditis elegans* tropomyosin isoform with poor actin affinity

K. Morisaki[1], E. Watabe[2], K. Ono[1], H. Kuroyanagi[2], S. Ono[1]; [1]Departments of Pathology and Cell Biology, Emory University, Atlanta, GA, [2]Laboratory of Gene Expression, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, JAPAN.

Tropomyosin is an actin-binding protein found in fungi and metazoans, which regulate actin filament stability and actomyosin contraction. In metazoans, multiple tropomyosin isoforms are expressed, and many of them are involved in diverse actin-dependent processes in an isoform-specific manner. In mammals, more than 40 tropomyosin isoforms are produced, and point mutations in several isoforms cause cardiovascular and skeletal muscle diseases. However, many tropomyosin isoforms remain poorly characterized. To investigate the biological significance of tropomyosin isoforms, we use the nematode *Caenorhabditis elegans* as a model organism. In *C. elegans*, the *lev-11* gene is the sole tropomyosin gene that is essential for embryonic development, reproduction, and regulation of muscle contractility. The *lev-11* gene is controlled by two separate promoters and extensive alternative splicing, and previous studies have demonstrated production of six tropomyosin isoforms with two alternative seventh exons (E7a and E7b). Here, we report identification of a novel seventh exon, E7c, of the *lev-11* gene. We cloned a full-length cDNA encoding a novel low-molecular-weight tropomyosin isoform, LEV-11U, containing the E7c sequence. Interestingly, LEV-11U poorly bound to actin filaments *in vitro*, whereas other isoforms strongly bound to actin filaments. Tropomyosin is a coiled-coil dimer, and several charged residues have been identified as actin-binding sites in mammalian isoforms. Sequence alignment showed that several actin-binding residues were not conserved in the E7c-encoded sequence. Tropomyosin also contains conserved non-canonical residues at hydrophobic core, such as alanine clusters, which are reported to be important for its flexible coiled-coil structure. Molecular dynamics simulations showed that the radius of an unstable core of E7c was statistically larger than that of E7b, indicating that the E7c-encoded region poorly formed stable coiled-coil structure. These results indicate that alternative splicing contributes to production of a biochemically distinct tropomyosin isoform.

B88/P1780

The binding and elongation properties of tropomyosin Tm1A to actin filaments are highly context dependent

N. A. Petek-Seoane, J. Rodriguez, D. Mullins; University of California, San Francisco, san francisco, CA.

Tropomyosins are coiled-coil proteins that copolymerize along actin filaments and therefore regulate the accessibility of actin filaments to actin-binding proteins. This gatekeeping property of nonmuscle tropomyosin effectively regulates the morphology and dynamics of actin networks in cells. Despite the abundance of nonmuscle tropomyosins, little is known about the binding properties or elongation dynamics of tropomyosin along actin filaments. Recently a number of groups, including our own, have begun to study the biophysical properties of tropomyosin assembly on actin filaments using both single filament TIRF and microfluidic assays. We find that unlike any other known actin-binding protein, tropomyosin shows a strong preference for the origin (species) of the actin. We therefore created a system derived entirely from *Drosophila melanogaster* to study the binding of *Drosophila* tropomyosin Tm1A. We show that Tm1A binding to *Drosophila* actin filaments is dependent on the experimental set up. In a microfluidic assay, we use a variety of tethers (mDia, capping protein, or phalloidin-stabilized seeds) and show that Tm1A preferentially binds to the pointed end of filaments when actin is tethered by mDia, however both the capping protein and seed tethered actin filaments do not show any preferential region for initial tropomyosin binding. We show that tropomyosin binds more rapidly to filaments tethered by the pointed end of the actin filament, and slowest to capping protein tethered filaments. Additionally, elongation of Tm1A on capping protein tethered actin filaments is considerably slower than either seed or mDia tethered actin filaments. Interestingly, when we look at untethered actin by TIRF we see that capped filaments are also slower to bind and elongate tropomyosin filaments suggesting that this is not an artifact of the microfluidic assay. Together these data suggest that tropomyosin binding and elongation to actin filaments cannot be widely compared and contrasted in the literature, due to the large amount of variability in experimental outcome when only a few, seemingly minor, assay components are altered.

Actin Dynamics in Disease

B89/P1781

SEPT9 isoform-specific interactomics in colorectal cancer

K. Makhmudova, G. Hu, E. Jeffery, S. Bandyopadhyay, G. Sheynkman, S. Ebrahim; University of Virginia, Charlottesville, VA

Background: *SEPT9* codes for a GTP-binding protein septin 9 (SEPT9) that is involved in cytoskeletal organization and membrane dynamics. *SEPT9* is associated with many human cancers, and acts as a tumor suppressor gene in colorectal cancer (CRC). There are multiple alternatively spliced mRNA variants of *SEPT9*, but hypermethylation of the promoter region of only one isoform, *SEPT9_i2*, abrogates this tumor suppressor function, ultimately resulting in malignant tumor formation. However, the underlying mechanism by which SEPT9_i2 contributes to CRC progression remains unknown. Objective: We seek to identify binding partners unique to SEPT9_i2 that will provide new insights into the mechanisms that underlie its strong association with intestinal pathology. Methods: Different SEPT9 isoforms have distinct molecular interaction domains at their N-termini. An N-terminal extension (NTE) is present in the longer SEPT9 isoforms, including Sept9_i2, but not the shorter isoforms such as

Sept9_i5. The NTE encodes binding domains that mediate direct interactions with actin, microtubules, myosin and membranes. SEPT9_i2 and SEPT9_i5 show distinct subcellular localization. We dissected the isoform-specific interactomes of SEPT9_i2 and SEPT9_i5 in DLD-1 colon adenocarcinoma cells. Briefly, cells were transfected with GFP-tagged SEPT9_i2 and _i5-cDNA as well as a GFP-only control. The successful expression of the tagged SEPT9 constructs was verified by Western blotting and immunofluorescence. Affinity-based co-immunoprecipitation was performed for each isoform-specific and control cell lysates, followed by mass spectrometry analysis. Results: Major hits unique to SEPT9_i2 included F-actin-capping protein subunit alpha-2 (CAPZA2) - a stabilizing protein of growing actin filaments, and multivesicular body subunit 12 (MVB12A) protein. Given that MVB12A is potentially involved in down-regulation of oncogenic epithelial growth factor receptor (EGFR), this is a promising clue on tumor suppression mechanism by SEPT9_i2. Our future plans include validation of direct interaction using reciprocal Co-IP. Conclusion: Our preliminary data indicate distinctive isoform-specific binding patterns that correlate with the hypothesis of antitumorigenic potential of SEPT9_i2. To our knowledge, this is the first attempt to profile isoform-specific interactome of SEPT9 in the context of CRC.

B90/P1782

Potential biophysical role of actin-binding protein TRIOBP in metastasis of human pancreatic cancer

M. J. McCormick, A. E. Massey, R. Olszewski, T. B. Friedman, A. X. Cartagena-Rivera; National Institutes of Health, Bethesda, MD

TRIOBP (TRIO and F-actin-Binding Protein) encodes three protein isoform classes (TRIOBP-1, TRIOBP-4 and TRIOBP-5). Variants of this gene have been implicated in deafness, schizophrenia and cancer. TRIOBP binds to and stabilizes actin filaments, thus influencing cytoskeletal structure. The actin cytoskeleton plays a crucial role in epithelial-mesenchymal transition and cancer progression, notably through adhesion, motility, contractility, and invasion. TRIOBP is often studied in neuroblastoma, but little has been explored about its activity in other cancers. The purpose of this study is to investigate a possible role of TRIOBP in pancreatic cancer metastasis. We selected a panel of human pancreatic adenocarcinoma (PDAC) cell lines, including primary tumor cells (BxPC-3 and PANC-1) and metastatic cells derived from patient ascites (HPAF-II and AsPC-1). These were cultured alongside the normal pancreatic ductal epithelial cell line, HPDE. We characterized TRIOBP expression with immunofluorescence (IF), western blot (WB), droplet digital PCR (ddPCR) and atomic force microscopy (AFM). ddPCR results revealed that TRIOBP-1 mRNA levels, when compared to normal cells, were significantly higher for metastatic cells and lower for primary tumor cells. These expression patterns were consistent with WB results, which show a significant increase in TRIOBP protein of metastatic cells when compared to primary tumor cells. ddPCR also revealed a remarkably higher amount of TRIOBP-1 expression compared to TRIOBP-4 and TRIOBP-5. Therefore, observed measurements in WB and IF using the TRIOBP antibody are thought to represent changes mostly in TRIOBP-1. Cells were IF-stained to visualize TRIOBP protein and F-actin. Confocal imaging showed that *in vitro* monolayers had TRIOBP mainly located in the perijunctional actin cytoskeleton. In isolated and polarized migratory cells, TRIOBP seemed to be mainly localized to retracting F-actin bundles at the trailing edge of the cell. Therefore, TRIOBP may preferentially localize to contractile F-actin structures such as these. AFM measurements of monolayers revealed that stiffness of the primary tumor cell perijunctional actin was the same as that of normal cells or lower, just as WB results showed that these cells had similar-to-less TRIOBP protein.

However, these regions were softer for metastatic cells. This analysis of PDAC cell lines shows a potential role of TRIOBP in cancer migration and invasion, possibly involving F-actin architectures.

B91/P1783

Disruption of MRTF-SRF interaction impairs membrane dynamics and motility of breast cancer cells

V. Yu, I. Eder, D. Gau, D. Boone, P. Roy; University of Pittsburgh, Pittsburgh, PA

Mortality associated with breast cancer (BC) is due to the metastatic spread of tumor cells. Dysregulated actin cytoskeleton is a feature of tumor cells that leads to aberrant cell motility and cancer metastasis. Myocardin-related transcription factor (MRTF) belongs to a family of actin-regulated transcriptional co-factors of serum-response factor (SRF) that control gene expression in both SRF-dependent and -independent manners. In the present study, we sought to investigate the role of SRF-dependent function of MRTF in motility of breast cancer cells. Using sub-lines of MDA-MB-231 breast cancer cells that are genetically engineered to express either wild-type or functionally defective mutants of MRTF, we herein demonstrate that disruption of MRTF-SRF interaction leads to a prominent inhibition of random as well as chemotactic cell migration. Kymography analyses of membrane dynamics showed that blocking MRTF-SRF interaction dramatically suppresses the dynamic behavior of the leading edge of migrating cells. In order to gain insight into potential molecular mechanisms of MRTF's regulation of cell migration, we next performed RNA sequencing of various MRTF-engineered sublines of MDA-MB-231 cells followed by Ingenuity Pathway Analysis (IPA) which revealed "Actin Cytoskeleton Signaling" pathway and the "Actin Nucleation" pathways to be significantly perturbed by MRTF expression. We further identified transcriptional repression of the formin family of actin nucleating and elongating proteins in breast cancer cells when MRTF-SRF interaction is abrogated, suggesting that formin-directed actin assembly may be one of the potential mechanisms of how MRTF-SRF interaction promotes cell motility.

B92/P1784

Vesicle-associated actin assembly by formins promotes TGF-beta- induced ANGPTL4 trafficking and secretion

D. Frank[1], C. Moussie[2], L. Lorenzen[1], C. Schwan[1], R. Grosse[1,3]; [1]Unisversity of Freiburg, Freiburg, Germany, [2]University of Marburg, Marburg, Germany, [3]Centre for Integrative Biological Signalling Studies - CIBSS, Freiburg, Germany

Vesicle trafficking has emerged as an important process driving tumor progression through various mechanisms, such as cargo endocytosis, recycling and secretion into the tumor microenvironment. TGFβ-mediated secretion of Angiopoietin-like 4 (ANGPTL4) is important for cancer development. Here we identify the formin Formin-like 2 (FMNL2) to be necessary for ANGPTL4 trafficking and secretion in response to TGFβ signaling. We show that protein kinase C-dependent phosphorylation of FMNL2 downstream of TGFβ stimulation is required for cancer cell invasion as well as ANGPTL4 vesicle trafficking and secretion. Moreover, using live-cell super-resolution microscopy, we could observe that ANGPTL4 trafficking is actin-dependent with FMNL2 polymerizing actin directly at ANGPTL4-containing vesicles. These secretory vesicles are associated with Rab8a and myosin Vb. This work uncovers a formin-controlled process that transiently polymerizes actin directly at intracellular vesicles to facilitate their mobility. This mechanism may be important for the regulation of cancer cell metastasis and tumor progression.

B93/P1785

L-plastin Knockdown reduces the invasion but increases the migration of PC3 prostate cancer cells

S. Majumdar, H. Aljohani, L. T. Senbanjo, **M. A. Chellaiah**; University of Maryland SOD, Baltimore, MD.

L-plastin Knockdown reduces the invasion but increases the migration of PC3 prostate cancer cells

Chellaiah MA, Majumdar S, Aljohani H, Senbanjo LT Department of Oncology and Diagnostic Sciences, School of Dentistry, University of Maryland, Baltimore Metastatic cancer cells use the actin-bundling process to constantly remodel the actin cytoskeleton for adhesion, migration, and invasion. L-plastin is an actin-bundling protein that belongs to the plastin family. L-plastin has been identified in several malignant colon, prostate, and breast tumors and contributes to cancer cell invasion in a phosphorylation-dependent manner. Our initial characterization of the PC3 prostate cancer (PCa) cell line derived from bone metastases demonstrated L-plastin expression in PCa cells but not in other PCa cell lines tested. Hence, this study aimed to identify L-plastin's role in the migration and invasion of PC3 cells. Immunostaining analysis demonstrated a punctate distribution of L-plastin and patchy actin staining in PC3 cells with a minimal colocalization between L-plastin and actin at the invadopodia. However, overexpression in PC3 cells increased L-plastin's colocalization and actin at the invadopodia and during the invasion. In a wound-healing assay, these cells displayed a significant reduction in migration. L-plastin and invadopodia connections were confirmed using the L-plastin knockdown strategy in PC3 cells (PC3/Si). PC3/Si cells demonstrated an increased migration, corresponding to punctate podosome-like structures. However, a decrease in the number of invadopodia contributed to a significant reduction in the invasion. Moreover, tumorsphere formation was significantly reduced in PC3/Si cells than in PC3 cells. In conclusion, our observations suggest that L-plastin regulates the formation of invadopodia required for prostate cancer invasion. Furthermore, our results highlight that it could be a novel therapeutic target for androgen-independent metastatic prostate cancer.

B94/P1786

TRIM9 modulates cytoskeletal dynamics, membrane remodeling, and motility in melanoma

K. Lukasik, S. Gupton; UNC Chapel Hill, Chapel Hill, NC.

Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Arial",sans-serif;} Cell shape change and motility are essential in development, immune responses, wound healing, and metastasis. Cell shape change involves remodeling of both the actin cytoskeleton and the plasma membrane in response to extracellular cues, like netrin. How these cellular systems are regulated and coordinated is unknown. Here we are investigating *how the brain-enriched E3 ubiquitin ligase TRIM9 regulates actin and plasma membrane dynamics in response to the morphogen netrin in melanoma.* We previously identified TRIM9 as a potent regulator of netrin-dependent actin dynamics and exocytosis in developing neurons. We found that TRIM9 negatively impacts the actin polymerase VASP by non-degradative ubiquitination. Deletion of murine *Trim9* impairs neuronal migration, axon turning, axonal and dendritic branching, VASP ubiquitination, and VASP dynamics at filopodia tips, and increased exocytosis and filopodial stability. TRIM9 is expressed in other motile cells, but the non-neuronal role of

TRIM9 remains unknown. In addition, TRIM9 has been identified a possible prognostic biomarker in melanoma. Here we demonstrate that many human melanoma lines express TRIM9, supporting published work from other groups. Using Total Internal Reflection Florescence microscopy (TIRF) and confocal microscopy, we show that TRIM9 and VASP colocalize at lamellipodia, filopodia tips, and focal adhesions. TRIM9 also localizes to lysosomes in human 1205Lu melanoma cells. In addition, 1205Lu cells lacking TRIM9 exhibit an increased velocity but reduced directional persistence. Current studies are investigating how loss of TRIM9 alters parameters of lamellipodia, filopodia, focal adhesions, and exocytosis to define the role of TRIM9 in melanoma motility.

B95/P1787

The Role of the mDia Formins in Breast Cancer Invasion

A. Ramirez; Albert Einstein College of Medicine, Bronx, NY

The actin cytoskeleton is regulated by the p21 Rho-family of small GTPases through downstream effectors including the mammalian diaphanous-related formins 1 and 2 (mDia1, mDia2). The mDia formins modulate the actin cytoskeleton by nucleation and assembling linear actin filaments, but they also recruit proteins containing SH3 domains such as Src-family of non-receptor tyrosine kinases, important in driving cell motility, including cancer invasion. To successfully invade and metastasize, breast cancer cells form actin-based, invadopodia protrusions that are capable of degrading the extracellular matrix. There is limited understanding in the control of mDia formins by upstream RhoGTPase isoforms during cancer cell invasion that contribute to breast cancer metastasis. Here, we determined the upstream RhoGTPase paralogs regulating mDia1 and mDia2 at invadopodia and their subsequent downstream functions during breast cancer cell invasion. We show that mDia1 and mDia2 are localized in discrete and differential compartments at invadopodia and impact invadopodia formation and function. We show specific upstream paralog GTPases regulate invadopodia through targeting mDia1 versus 2 within these compartments, by controlling cell adhesion to the substratum and by modulating Src-kinase mediated function. Our approach to studying this mechanism utilized a novel, direct multiplex Förster Resonance Energy Transfer (FRET) biosensor approach, wherein, a pair of spectrally orthogonal FRET biosensors was visualized simultaneously in single living cells. We developed a set of FRET biosensors capable of reporting the activity status of mDia1 and mDia2, and have spectrally shifted these biosensors into near infra-red (NIR) so that they may be used simultaneously with our cyan-yellow FRET biosensors for the upstream GTPases. We report for the first time, specific, localized activities of mDia1 and 2 and their upstream regulator RhoGTPase activities in single breast cancer cells at invadopodia. We delineate the functions of these molecular signaling nodes using our newly developed multiplex biosensor approach and report differential roles of mDia1 versus mDia2 at breast cancer invadopodia, that control cell-substrate adhesion and matrix degradation, respectively, mediated by specific upstream RhoGTPases, RhoA, RhoC and Rac3. In summary, our multiplex FRET biosensor technology for the mDia formins makes it possible to directly visualize the activity of RhoGTPases together with mDia1 or mDia2 at invadopodia. Our results point to regulation of differential invadopodia functions by mDia1 versus mDia2, signaled by different upstream RhoGTPase paralogs that control integrin-substrate adhesion and matrix degradation, which ultimately impact breast cancer cell invasion.

B96/P1788

Characterization of the transfer of mitochondria via different types of tunneling nanotubes (TNTs) and its role in the progression of Glioblastoma

I. Sáenz-de-Santa-María[1], J. Y. Tinevez[2], A. Pepe[1], C. Zurzolo[1]; [1]Cell Biology and Infection, Institut Pasteur, Paris, FRANCE, [2]Head of Image Analysis Hub, Institut Pasteur, Paris, FRANCE.

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults and lacks effective therapy. Tunnelling nanotubes (TNTs) that connect cancer cells point to a new mechanism of molecular interconnection of tumors. TNTs are membranous bridges that directly connect the cytoplasm of distant cells and exchange molecules such as mitochondria. It has been shown the diversity of TNT morphology and composition between and within cell types. Here, we investigated what roles the compositional elements of the TNTs have in the transfer of mitochondria and address the relation between the formation, the structure, and the function of TNTs in GBM. We show, for the first time the presence of different types of TNTs in GBM cell line vs GBM stem-like cells (GSLCs) in classical 2D-culture and in 3D-tumor organoids. We analyzed the ultrastructure organization of TNTs using correlative fluorescence cryo-electron microscopy (cryo-EM). Furthermore, we characterized the dynamics of the transfer of mitochondria through thick and thin TNT in GB/GS-cells by fluorescence live-cell imaging using cells transduced with different plasmids for visualizing the microtubules, actin filaments and mitochondria in different colors. Here we provide a detailed analysis of this phenomenon using software TrackMate-Kymograph extension to perform 2D automated mitochondria tracking inside the TNT and provide fully quantitative readouts, such as, average and maximal velocity, and directionality of the transfer of mitochondria, and other parameters related to the TNT: e.g., thickness and length over time. Our data in U251 GBM cell line indicate the presence of two different types of TNTs, thin actin-based TNTs and thick TNTs formed by both microtubules and actin, in contrary to the observed in GSLCs, formed only by actin TNTs. The transfer of mitochondria occurs principally via microtubules positive TNTs with an average of transfer 5.14 nm/s and a max velocity of 17.5 nm/s. However, the transfer of mitochondria via thin TNTs is faster reaching a max velocity 74.5 nm/s. The ultrastructure of the TNT via cryo-EM indicates that thin TNTs are composed of a bundle of open-ended individual tunneling. These results provide new evidence about the different composition and dynamics of the transfer of mitochondria via these structures in GBM and could be facilitate the development of effective therapy.

B97/P1789

Actin-Binding Protein Profilin-1 is an Important Regulator of Vascular-Immune Cell Crosstalk

A. E. A. Gondringer, A. Larregina, P. Roy; University of Pittsburgh, Pittsburgh, PA

Dysregulated expression of actin-binding protein profilin-1 (Pfn1) is associated with a number of vascular pathologies. The objective of the present study was to examine the immunological consequence of endothelial loss of Pfn1 function *in vivo*. For these studies, we utilized a novel mouse model engineered for tamoxifen-inducible endothelial cell (EC) specific deletion of the *Pfn1* gene ($Pfn1^{-/-(EC)}$), and performed serum-analyses of metabolic function and circulating cytokines/chemokines, immune cell composition analyses in whole blood as well as in spleen by fluorescence activated cell sorting (FACS), and immunohistochemistry-based assessment of organ pathology. Our studies showed that widespread deletion of endothelial Pfn1 leads to severe health complications as evidenced by labored breathing, lethargy, and abnormal peritoneal fluid accumulation within 3 weeks of gene ablation ultimately compromising survival. $Pfn1^{-/-(EC)}$ mice exhibited reduced liver and kidney function,

findings further supported by evidence for structural alterations, vascular collapse, EC death and prominent immune cell infiltration in the liver of these animals. Loss of endothelial Pfn1 resulted in elevated circulating levels of pro-inflammatory cytokines and chemokines (VEGF, IL1β, IL6, G-CSF, CCL2, CCL5) with concomitant reduction of anti-inflammatory cytokines and chemokines (IL-1α, IL5, CXCL5). Consistent with these findings, $Pfn1^{-/-(EC)}$ mice exhibited a dramatic increase (~10x) of circulating leukocytes alongside a significant increase in splenic myeloid cells as well as circulating macrophages. In vitro co-culture studies further support a role for endothelial Pfn1 in negatively regulating macrophage-like differentiation of monocytes through a paracrine action, with additional preliminary evidence pointing to a role of cell-secreted extracellular Pfn1 itself as a suppressor of monocyte-to-macrophage differentiation. Collectively, these findings highlight a previously unrecognized function of Pfn1 as an important mediator of vascular-immune cell crosstalk.

B98/P1790

Flotillin-1/-2 localizes at enteropathogenic *Escherichia coli* pedestals

P. Mcleod, J. Guttman; Simon Fraser University, Burnaby, BC, CANADA.

Flotillin-1/-2 localizes at enteropathogenic *Escherichia coli* pedestals

Petra McLeod and Julian A. Guttman
Department of Biological Sciences, Centre for Cell Biology, Development, and Disease (C2D2), Simon Fraser University, Burnaby, BC, Canada

Enteropathogenic *Escherichia coli* (EPEC) are extracellular pathogens that manipulate the actin cytoskeleton of epithelial cells to generate actin-rich pedestal-like structures at sites of bacterial/host cell contact. Key to pedestal formation is the bacterial effector protein Tir, and more specifically Y474 in Tir, as bacteria mutated at that site are defective for pedestal formation. Although pedestals protrude from the natural surface of the epithelial cells, they require the endocytic protein clathrin and an assortment of clathrin-associated protein for their formation. Flotillin-1/-2 is a clathrin-independent endocytic protein found within lipid rafts of the plasma membrane. Here we examined transfected GFP-tagged Flotillin-1/-2 during EPEC infections and found this protein localized along the sides of the pedestal stalk. EPEC mutants that cannot form pedestals did not recruit flotillin even when bacteria were in contact with the epithelial cell surface. Our results indicate that Flotillin-1/-2 are additional endocytic proteins hijacked by EPEC for non-endocytic functions.

B99/P1791

A genetic complementation approach examining antimicrobial sensitivity of fungal actin orthologs

M. A. Fagbolade, D. M. Gordon; Biological Sciences, Mississippi State University, Mississippi State, MS.

Actin is a highly conserved cytoskeletal protein present in all eukaryotic cells. In the yeast *Saccharomyces cerevisiae*, *ACT1* is an essential gene as it codes for the sole form of actin with roles in endocytosis, nuclear segregation, and polarized cell growth. Recent studies centered around the antifungal compound, occidiofungin, have indicated that the biological target of this natural product is actin. Whereas this compound demonstrates fungicidal activity against many yeast and filamentous fungi, occidiofungin has low efficacy against *Fusarium oxysporium* and *Penicillium digitatum* fungal species exhibiting a >10-fold resistance profile relative to *S. cerevisiae*. At the amino acid level, *F. oxysporium* and *P. digitatum* actin share 92% and 91% identity with *S. cerevisiae* actin, respectively. To determine if these amino acid differences, contribute to the altered susceptibility measured for

occidiofungin, a haploid *ACT1* shuffle strain was generated for testing the functionality of *ACT1* gene products from *C. glabrata*, *C. albicans*, *F. oxysporium*, and *P. digitatum*. Functional complementation of the *ACT1* gene products was found for cells expressing *C. glabrata* and *C. albicans* actin as determined by measuring growth kinetics, actin protein expression levels, and cell morphology and nuclear positioning analysis. Data for susceptibility testing to occidiofungin by minimum inhibitory concentration also indicate a similar sensitivity profile as *S. cerevisiae*. Expression of *ACT1* from *F. oxysporium* and *P. digitatum* resulted in a poor growth profile with the accumulation of abnormal sized cells. Analysis identified codon usage differences as a potential contributing factor, and studies using codon optimized *ACT1* genes are ongoing. This scholarship was funded by the American Society for Cell Biology through the International Federation for Cell Biology.

B100/P1792

Cofilin-actin Rod Dynamics and Efficacy of CXCR4 and CCR5 Receptor Antagonists in Prevention and Reversal of Rod Pathology in Cultured Rodent and Human Neurons

L. H. Tahtamouni[1], T. B. Kuhn[2], S. A. Alderfer[3], L. S. Minamide[2], K. P. Walsh[4], A. E. Shaw[2], O. Yanouri[5], M. Ruff[6], **J. R. Bamburg**[2]; [1]Biology and Biotechnology, The Hashemite University, Zarqa, JORDAN, [2]Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO, [3]Chemical and Biological Engineering and School of Biomedical Engineering, Colorado State University, Fort Collins, CO, [4]Biochemistrya and Molecular Biology, Colorado State University, Fort Collins, CO, [5]Molecular, Cellular and Integrative Neuroscience, Colorado State University, Fort Collins, CO, [6]Creative BioPeptides, Inc., Potomac, MD.

Cofilin-saturated F-actin (cofilactin) bundles (rods) are a pathological feature in brains of individuals who suffered from Alzheimer's and other neurodegenerative diseases and disorders. Rapid rod formation (minutes) is triggered in >80% of cultured rodent hippocampal neurons by excitotoxic glutamate or ATP depletion by a rapid pathway implicating mitochondria. In contrast, slower (hours) rod induction is triggered in ~20% of hippocampal neurons by disease-specific factors (e.g. soluble oligomers of Amyloid-β (Aβ), proinflammatory cytokines, and others), which require cellular prion protein (PrP^C) and active NADPH oxidase (NOX). Using live-cell imaging with fluorescently tagged rod components, we measured an extrapolated time to 50% recovery from photobleaching for core cofilin and actin at ~1 and ~4 hr, respectively. Concentrations for 50% inhibition of rod formation (EC_{50}) by FDA-approved CXCR4 and CCR5 antagonists (30 -100 nM) are over 3000-fold higher than those of all-D-amino acid penta/octa peptides (RAPs) targeting these receptors. RAPs reverse preformed rods with a time to 50% reduction of ~45 min. For more clinically relevant applications, we developed a human neuronal culture system for rod analysis. Neurons derived from H1 human ES cells lack NOX expression and do not form rods when treated with PrP^C-dependent rod inducers. However, a human iPSC line, WTC-11, in which neuronal differentiation by Ngn2 is readily inducible with doxycycline, express NOX, but during their first several weeks of culture, have insufficient PrP^C for rod induction. PrP^C-insufficiency was overcome by its adenoviral-mediated expression, which by itself induces rods but the response is enhanced by Aβ treatment. Long-term cultures are maintained in glial-conditioned medium. By culture day 35, axonal and dendritic markers show process distinction with spines and synapses beginning to form. By day 55, the WTC-11 rod response to Aβ is not significantly different with or without expression of exogenous PrP^C. RAPs inhibit and reverse Aβ-induced rods in human neurons with the same efficacy (sub-pM EC_{50}) as in rodent neurons, suggesting these orally administered, inexpensive small peptides that can cross the blood-brain barrier would be highly effective in reducing brain rod pathology in human disease.

B101/P1793

Determining the effect of a patient mutation in moesin on protein phosphorylation and T cell migration

A. Albis, O. Stebbins, L. Avery; Biology, Saint Michael's College, Colchester, VT.

X-linked moesin associated immunodeficiency (X-MAID) is a rare immunodeficiency characterized by lymphopenia and recurrent opportunistic infections. X-MAID is caused by a single point mutation in the protein moesin (moesin^{R171W}). Moesin is an actin-binding protein that connects the cell membrane to the cortical actin network. When phosphorylated and in its open conformation, moesin is actively linking the cytoskeleton to the plasma membrane. When dephosphorylated and in its closed conformation, the protein is autoinhibited, no longer providing linker activity. Changing conformation regulates cell shape, rigidity, and thus migratory capabilities. The ability to migrate is a critical role of T cells in the body, as they must move through tissues to reach sites of inflammation and infection. T cells expressing moesin^{R171W} exhibit an abnormally round and rigid structure when stimulated with chemokine, predicted to inhibit T cell transmigration. Therefore, the aim of this study is to investigate the effect of the X-MAID mutation on protein phosphorylation status and T cell transmigration, a critical gap in knowledge. To address this gap, we use a Jurkat T cell line that expresses moesin^{WT} and moesin^{R171W} under an inducible promoter. When treated with chemokines and analyzed by western blot, we revealed differences in phosphorylation between the mutant and wild type T cells. Furthermore, we performed transwell assays to assess the migratory capabilities of mutant and wildtype T cells in response to chemokines. Understanding of the molecular and cellular phenotypes of X-MAID T cells is essential for better understanding cytoskeletal regulation during migration and developing potential therapeutics.

B102/P1794

cd13 controls myeloid cell- cell fusion

M. Ghosh, F. Mcgurk, S. Nair, K. Morais, L. Shapiro; Center for Vascular Biology, UCONN Health, Farmington, CT.

While over a million total joint arthroplasties take place per year to treat end-stage arthritis, 40-70% of these will eventually undergo painful aseptic revision surgery due to implant degeneration. Despite advances in biomaterials, implants ultimately corrode to produce particles that initiate a foreign body response characterized by macrophage fusion into destructive, pro-inflammatory, multinucleated giant cells (MGCs) that directly define the pathology of osteolytic loosening of prosthetic implants. MGC formation initially requires cytokine-induced differentiation of precursors into macrophages, leading to membrane fusion to form very large, active MGCs with multiple nuclei sharing a common cytoplasm. While some fusion-regulatory proteins are known, mechanistic understanding of the underlying molecular events is largely theoretical and somewhat controversial, emphasizing the need for further study. Our previous observations have clearly shown that the myeloid cell transmembrane protein, CD13 is a remarkably multifunctional molecule that assembles the molecular machinery enabling diverse cellular processes such as cell-cell adhesion and the endocytosis and recycling of cell surface proteins. In the current study, we show that *in vitro* induction of MGC formation from isolated CD13^{KO} progenitors generated considerably larger MGCs with many more nuclei and increased cytokine secretion than those generated from controls. We confirm these studies in an *in vivo* implant model, demonstrating that a lack of CD13 exacerbates MGC formation and inflammatory cytokine levels both at the implant site and

systemically. Mechanistically, we identified that the post-translational degradation of the critical fusion proteins dynamin, Dendritic Cell-Specific Transmembrane Protein (DC-STAMP) and tetraspanins, CD9 and CD81 was dysregulated in $CD13^{KO}$ MGC, leading to sustained expression levels that promote macrophage fusion. This agrees with our published studies implicating CD13 in osteoclast fusion during bone resorption, suggesting CD13 acts as a component of a common fusion machinery. Therefore, we have identified CD13 as a negative regulator of cell-cell fusion in both osteoclast and MGC formation, and thus is a novel target for therapeutic intervention in pathological conditions mediated by destructive cell-cell fusion such as osteoporosis and medical implant failure.

B103/P1795

A Human Disease-Causing Mutation Results in Loss of Leiomodin 2 Protein Through Nonsense-mediated mRNA Decay.

C. T. Pappas, G. P. Farman, T. M. Larrinaga, R. M. Mayfield, C. C. Gregorio; University of Arizona, Tucson, AZ.

Leiomodin 2 (Lmod2) is an actin-binding protein involved in the assembly of actin filaments in muscle. Lmod2 plays a critical role in muscle function as loss of Lmod2 in murine models results in dilated cardiomyopathy (DCM) and rapid death. We previously described the first known human disease-causing mutation in LMOD2 (pW398*), which led to severe neonatal DCM requiring heart transplantation (Ahrens-Nicklas, Pappas *et al*., 2019). Analysis of the explanted heart revealed extremely short thin filaments, and cardiomyocytes with greatly reduced maximal calcium-activated force output. The mutation is predicted to result in expression of a truncated protein, however, we did not detect LMOD2 protein in the patient's explanted heart. We further discovered that nonsense-mediated mRNA decay underlies the loss of mutant LMOD2 protein. We also found that introduction of GFP-Lmod2 into *Lmod2* constitutive KO mice just after birth prevents onset of DCM, with the mice living to adulthood, rather than dying at ~ day 21. A surprisingly small amount of Lmod2 (<20% of endogenous levels) is required to maintain *in vivo* cardiac function. Additionally, introduction of GFP-Lmod2 with a mutation (p.W405*) homologous to that found in the patient (generated from cDNA so it is not susceptible to degradation due to *nonsense-mediated mRNA decay*) partially restores cardiac morphology and function in *Lmod2* KO mice. Therefore, recovery of even a small amount of wild type, or mutant, LMOD2 could have therapeutic potential for patients with *LMOD2* nonsense mutations.

B104/P1796

A model for endolysosome genesis, trafficking, and phagosome maturation in mammalian photoreceptors

S. Ramachandra Rao[1,2], J. Slone[1], J. Vu[1], T. Huang[1], S. J. Fliesler[1,2]; [1]State University of New York, University At Buffalo, Buffalo, NY, [2]VA Western New York Healthcare, Buffalo, NY

Purpose: Retinal photoreceptors (PRs) are polarized, light-sensing neurons that depend on the endolysosomal system to maintain homeostasis, and vision. The unique arrangement of PRs in the retina allows rigorous investigation of neuronal organelle trafficking. Using an autophagy reporter mouse line (CAG-RFP-EGFP-LC3B), we previously demonstrated rapid PR phagosome-lysosome fusion. Mature PR phagolysosomes are distributed along the cell body, and in the F-actin-rich PR inner segment (IS)-myoid region. Here, we studied actin and microtubule-dependent mechanisms of endolysosome genesis/trafficking and phagosome maturation in mammalian PRs. **Methods:** C57BL/6J, C57BL/6NTac,

and CAG-RFP-EGFP-LC3B mice were treated with vinblastine sulphate (a microtubule-depolymerizing agent) for 6 h. Eyes from control and vinblastine-treated mice were harvested, fixed, and retinal wholemounts were prepared. Wholemounts were treated with anti-LAMP2 (a lysosome marker) and a fluor-tagged secondary IgG, and phalloidin-AF568 (to detect F-actin). We developed a high-throughput confocal image acquisition/analysis workflow to assess phagosome and autolysosome distribution in the PR IS-myoid region. **Results:** Control C57BL/6J retinas exhibited the typical distribution of lysosomes, limited to the PR IS-myoid and cell body. Vinblastine treatment inhibited microtubule-dependent lysosome trafficking in those retinas, and caused lysosome accumulation exclusively in the F-actin-rich IS-myoid region. Retinas of C57BL/6N mice (a model of *Crb1*-associated retinopathy) are characterized by loss of PR tight junctions and exhibit disrupted F-actin (lesions) in the IS-myoid region. Those retinas exhibited normal lysosome distribution in lesion-free regions, while lesioned areas showed significantly lower endolysosome content. Vinblastine treatment of C57BL/6N mice confirmed loss of PR endocytic capacity in the IS-myoid region of lesion areas, indicated by complete loss of endolysosome accumulation. Surprisingly, vinblastine treatment of CAG-RFP-EGFP-LC3B mice caused accumulation of RFP/LAMP2-positive mature phagolysosomes in the IS-myoid, without causing phagosome maturation defects. **Conclusions:** We propose a model wherein F-actin-dependent endocytosis occurs specifically in the PR IS-myoid, followed by microtubule-dependent trafficking throughout the cell body. Phagosome-lysosome fusion occurs rapidly in the IS-myoid region. Our data implicate lysosomal pathway defects in *CRB1*-associated retinopathy.

B105/P1797

Localised actin dynamics at the ciliary tip regulate the pace of photoreceptor disc formation

R. Megaw[1], A. Moye[2], Z. Zhang[3], F. Newton[1], F. McPhie[1], L. McKie[1], F. He[4], V. Arshavsky[5], L. Machesky[6], T. Wensel[4], P. Mill[1]; [1]MRC Human Genetics Unit, University of Edinburgh, Edinburgh, UNITED KINGDOM, [2]Baylor College of Medicine, houston, TX, [3]Vera and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Edinburgh, TX, [4]Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Baylor College of Medicine, Houston, TX, [5]Duke University School of Medicine, Edinburgh, NC, [6]The Beatson Institute for Cancer Research, University of Glasgow, Glasgow, UNITED KINGDOM.

As signalling organelles, primary cilia regulate G protein-coupled receptor (GPCR) concentration on their membrane by ectocytosis, a process which requires highly-localised actin dynamics at their tip to alter membrane shape. Mammalian photoreceptor outer segments are an expanse of folded membrane at the tip of a highly-specialised connecting cilium, in which photosensitive GPCRs like rhodopsin are concentrated. In an extraordinary feat of biology, outer segments are shed and remade every week. Failure to do so, due to genetic mutations causes retinitis pigmentosa (RP), an untreatable, blinding disease. The mechanism by which photoreceptor cilia generate outer segments is therefore fundamental for vision yet poorly understood. Here, we show the membrane deformation required for outer segment disc genesis is driven by dynamic changes in the actin cytoskeleton in a process akin to ectocytosis. Further, we show *RPGR*, a leading causal gene of RP, regulates activity of actin binding proteins crucial to this process. Humanized disease-causing mutations in *Rpgr* mouse models compromise this, disrupting the actin dynamics required for disc formation, leading to photoreceptor death and visual loss. Manipulation of actin dynamics partially rescues the phenotype, suggesting this pathway can be targeted therapeutically. These findings help define how actin-mediated dynamics control outer segment turnover.

B106/P1798

Salmonella invasion protein A (SipA) binds F-actin with high affinity, potently inhibits depolymerization, and increases thermal stability

L. A. Runyan[1], E. Niedzialkowska[2], E. Egelman[2], D. S. Kudryashov[1]; [1]The Ohio State University, Columbus, OH, [2]University of Virginia, Charlottesville, VA

Salmonella enterica subspecies *enterica* causes over 100 million human infections annually, with cases ranging from food poisoning to typhoid fever. An essential part of *Salmonella's* pathogenicity is its ability to invade and proliferate within non-phagocytotic host intestinal cells, which is accomplished by hijacking the host cell's cytoskeleton. Host cell invasion by *Salmonella* is endowed by its pathogenic effector proteins, many of which are delivered directly to the host cell's cytoplasm via the type III secretion system. Among these effector proteins is *Salmonella* invasion protein A (SipA), which contributes to the formation of lamellipodia-like ruffles in the host cell, enveloping the bacteria and leading to its engulfment and entry into the cell. SipA's C-terminal part (425 - 684) has been identified as the actin-binding region. It has been demonstrated that SipA decreases critical concentration, induces polymerization, and inhibits depolymerization when applied in roughly equimolar concentrations to actin. However, during infection, bacteria are unlikely to deliver a sufficient amount of toxins to reach equimolar ratios with actin, even locally. Using a combination of cryo-EM reconstruction and biochemical approaches, we found that SipA binds F-actin with nearest neighbor exclusion at a 1:2 SipA:Actin stoichiometry with a K_d in the nanomolar range. The C-terminal tail of SipA is inserted in between the two actin strands, accounting for its ability to potently displace phalloidin and jasplakinolide. SipA increased the thermal stability of F-actin and notably inhibited the depolymerization of pyrene actin by ADF in a cooperative manner when SipA concentration was ~100 times lower than F-actin, likely due to combined inhibition of severing and depolymerization. These data indicate that SipA cooperatively stabilizes the actin filament structure and, while being present in low doses, may affect F-actin interactions with host partners and pathogenic effector proteins necessary for the invasion and proliferation of *S. enterica*.

B107/P1799

Spinocerebellar ataxia mutations localizing to the spectrin-repeat domains of β-III-spectrin disrupt actin binding and dendritic arborization

S. A. Denha, N. R. DeLaet, A. W. Abukamil, A. E. Atang, A. W. Avery; Oakland University, Rochester, MI

Mutations in the *SPTBN2* gene encoding the cytoskeletal protein β-III-spectrin cause the neurodegenerative disease spinocerebellar ataxia type 5 (SCA5). SCA5 mutations localize to the β-III-spectrin actin-binding domain (ABD) and the neighboring spectrin-repeat domains (SRDs). Recently, we found that increased actin-binding affinity is a shared molecular consequence of ABD-localized mutations. Here, we report the molecular consequences of SCA5 mutations in SRDs 2 and 3, and study the functional impact of SRD mutations in neurons using *Drosophila*. Biochemically, we show that a 13 amino acid deletion in SRD 3, termed the American mutation (AM), increases actin-binding affinity. A five amino acid deletion in SRD 3, termed the French mutation (FM), also increases actin-binding affinity, but additionally results in a significant decrease in B_{max}. In contrast, R480W missense mutation in SRD 2, has little or no impact on actin binding. In *Drosophila* genetic rescue assays, pan-neuronal expression of fly β-spectrin transgenes containing human SRD 2 and 3 mutations rescue lethality caused by a *βspec*

loss-of-function allele. Interestingly, this indicates that the SRD mutants retain sufficient functionality in neurons to support animal viability. In contrast, conditional expression of SRD 2 and 3 β-spectrin mutants in class IV dendritic arborization sensory neurons dominantly induce arborization defects, suggesting a cellular mechanism of pathology. Altogether, our current analyses of SRD 2 and 3 mutations indicate that these mutations differentially impact actin binding and dendritic arborization. Moreover, our data suggest the interesting possibility that SRD 3 may play an important role in directly mediating or indirectly regulating β-III-spectrin actin binding. We are currently exploring the molecular mechanism of how SRDs influence actin binding. Additionally, we are testing if the SRD 2 and 3 mutations impact other properties of β-III-spectrin, such as heterodimerization with α-II-spectrin.

Motors: Myosins in Cellular Protrusions

B109/P1800

Impact of MYO3A Motor Properties on Actin Protrusion Length and Dynamics

J. A. Cirilo, Jr.[1], X. Liao[2], B. J. Perrin[2], C. M. Yengo[1]; [1]Department of Cellular and Molecular Physiology, Penn State Univ/Milton S Hershey Medical Ctr, Hershey, PA, [2]Department of Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN.

Vertebrate class III myosins exist in two isoforms, MYO3A and MYO3B, and localize to the tips of stereocilia in the inner ear. Mutations within the motor domain of MYO3A that alter its intrinsic motor properties have been associated with non-syndromic hearing loss, suggesting that the motor properties of MYO3A are critical for its function within stereocilia. Indeed, class III myosin transgenic knockout mouse models display dysregulated stereocilia length and morphology. In this study, we aimed to understand how the motor properties of MYO3A are fine-tuned for stereocilia elongation and length regulation. To address this question, we generated chimeric MYO3A constructs that lack the kinase domain (ΔK) and replace the MYO3A motor and first two IQ motifs with the motor and first two IQ motifs of other myosins (5A, 10, 15A, 1A, NM2A, and 7A). The resulting chimeras contained an N-terminal GFP, followed by the new motor and 2IQ of the other myosins and the MYO3A tail. Transfection into COS7 cells revealed duty ratio as a critical motor property that dictates the ability to tip localize within filopodia, while in vitro actin gliding velocities correlated with filopodial extension velocities. Filopodia length was unaltered, suggesting membrane tension becomes a dominant opposing force at longer lengths. Furthermore, we utilized similar constructs for two MYO3A hearing loss mutants, H442N and L697W. Interestingly, we found that H442N demonstrated an increase in tip localization within filopodia and an increase in filopodia extension velocity, while maintaining similar filopodial lengths. However, L697W resulted in similar tip localization, slower extension velocity, and shorter filopodia. Taken together, our data suggests a model in which tip-localized myosin motors exert force that slides the membrane tip-ward, which, along with actin polymerization forces, combat membrane tension to lead to protrusion elongation. We propose that both “gain of function” and “loss of function” mutations in MYO3A can impair stereocilia length regulation, which is crucial for stereocilia formation during development and normal hearing.

B110/P1801

Mechanistic Insights into the Regulation of Human Myosin-7a

A. Hollo[1,2], N. Billington[1,3], Y. Takagi[1], A. Kengyel[2], J. R. Sellers[1], R. Liu[1,3]; [1]NIH, Bethesda, MD, [2]University of Pecs, Pecs, HUNGARY, [3]Department of Biochemistry and Molecular Medicine, Morgantown, WV.

Human myosin-7a (Myo7a) is an actin-based motor protein essential for vision and hearing. It plays critical roles in the development and functions of actin rich protrusions such as filopodia, microvilli, and stereocilia. Previous studies using *Drosophila* homolog showed that myosin-7a is a monomeric, high duty ratio motor and can move processively upon dimerization. However, characterization of full-length mammalian myosin-7a has been limited by the difficulty of expressing and purifying stable, intact protein. Here, we report the production of a complete human myosin-7a holoenzyme in insect cells and study its regulation by intra- and intermolecular mechanisms. Distinct from *Drosophila* myosin-7a which primarily associates with calmodulin, we found that human myosin-7a utilizes regulatory light chain (RLC), calmodulin and calmodulin like protein 4 (CALML4) as the light chain subunits. CALML4 is recently discovered highly enriched in stereocilia and identified as a deafness candidate gene. We show that CALML4 does not bind to Ca^{2+} but plays a key role in regulating the dynamic binding of calmodulin to myosin-7a in response to Ca^{2+} signaling. The cochlea expresses two myosin-7a splicing isoforms differed by a short N-terminal extension. Using combined optical trapping, biochemistry, and *in vitro* motility assays, we show that the N-terminal extension greatly influences the enzymatic and mechanical behaviors of mammalian myosin-7a. We propose that the hair cell regulates its mechanosensitivity by adjusting the expression levels of the two myosin-7a isoforms. Finally, using single molecule motility assays, we show that purified full-length myosin-7a alone does not move processively on actin *in vitro*, but in the presence of MyRIP, a known myosin-7a binding protein in neuroretina, it exhibits processive movements. The motor-adaptor complex moves slowly along actin filaments (~ 4 nm/s) with prolonged actin attachment time (~ 150 s). Together, our results provide new molecular insight into how myosin-7a functions in hair cell stereocilia and neuroretina. The production of intact human myosin-7a protein enables future studies to understand the molecular details of human vision and hearing loss caused by myosin-7a defects.

B111/P1802

Select autosomal dominant DFNA11 deafness mutations activate Myosin 7a

P. Acharya, G. Thapa, T. Nguyen, S. W. Crawley; Biological Sciences, University of Toledo, Toledo, OH.

Myosin-7a (Myo7a) is a motor protein that plays a critical role in the organization and function of stereocilia, specialized actin-based microvilli found on the apical surface of sensory epithelial hair cells of the inner ear that mediate hearing. Mutations in Myo7a underlie the pathology of various forms of hearing loss including autosomal recessive USH1B and DFNB2 and autosomal dominant DFNA11. Despite its importance, the cellular conditions that promote human Myo7a activity are not well understood. We used cultured kidney epithelial cells (LLC-PK1-CL4) as a model to investigate the mechanisms governing Myo7a activation, since they build prominent apical microvilli that act as surrogates to the native stereocilia found on inner ear hair cells. We observed that under basal conditions, Myo7a exhibited poor targeting to the apical microvilli of LLC-PK1-CL4 cells. In contrast, the addition of a calcium ionophore to the cells promoted Myo7a trafficking to the tips of microvilli, suggesting that this myosin may be regulated by calcium through the calcium-sensitive light chains associated with its lever arm. To explore this, we screened a directed site saturation library of lever arm mutants of Myo7a and identified specific

point mutations within the terminal fifth IQ motif (IQ5) that potently activated Myo7a targeting. Within IQ5, mutation of positions G849, I851, A852, and R853 had the most profound effect on promoting activity. This led us to test and discover that the autosomal dominant DFNA11 mutation R853C activates Myo7a, redefining the molecular etiology of this form of genetic deafness. Finally, we have identified that other autosomal dominant DFNA11 mutations found directly in the motor domain of Myo7a can also potently activate this myosin, giving us a possible structural roadmap to understand how the lever arm could inhibit motor activity. Together, these studies will shed light on the molecular basis of Myo7a-based sensory mutations, which may spur new clinical strategies for patient treatment.

B112/P1803

Myosin-7a mesa trail mutations activate the motor to cause autosomal dominant deafness

G. Thapa, P. Acharya, T. Nguyen, S. W. Crawley; Biological Sciences, University of Toledo, Toledo, OH.

Myosins are a large family of actin-based motor proteins that are involved in a diverse array of physiological functions, including muscle contraction, hearing, and vision just to name a few. As a result of this, mutations in this family can result in a variety of diseases depending on which myosin is impacted. Myosins are defined as a family by a conserved N-terminal motor domain that hydrolyzes ATP, converting chemical energy into mechanical work. Following the motor domain, myosins have a neck region comprised of a variable number of “IQ” motifs that associate with light chains. Upon ATP hydrolysis, the light chain-stabilized neck acts as a rigid lever arm that amplifies the small conformational changes that occur in the motor domain into a large displacement of the C-terminal tail. Importantly, the lever arm can also act as a point of regulation of certain myosins, through either phosphorylation or calcium binding to the light chains. Our lab has been studying Myosin-7a (Myo7a), a motor protein that plays critical role in the organization and function of the actin-based stereocilia of the inner ear that mediate hearing. We have discovered that the motor activity of Myo7a is potently regulated by its lever arm in a calcium-dependent manner, and that there are mutations that cause autosomal dominant deafness within the lever arm that interfere with this regulation. These mutations leave the myosin “constitutively on” even under low calcium conditions. We further discovered that there are autosomal dominant deafness mutations directly within the core motor domain of Myo7a that have the same effect of activating the motor, suggesting that the lever arm may fold back and directly interact with the motor to control its activity. Interestingly, these Myo7a motor domain mutations map to identical structural regions that lead to hypertrophic cardiomyopathy (HCM) in β-cardiac myosin. One of these regions in β-cardiac myosin forms a structural face known as the “mesa trail” that is involved in holding the myosin in an inactive state. Specifically, the mesa trail of β-cardiac myosin is proposed to interact with the lever arm-proximal S2 region of the myosin, locking it in an off state. Accumulating data suggests that HCM mutations in the β-cardiac myosin mesa trail are pathogenic because they prevent this interaction, resulting in a myosin that is constitutively available for muscle contraction. Given the similarities we see between Myo7a and β-cardiac myosin, we are currently testing the hypothesis that the lever arm of Myo7a folds back and interacts with a similar mesa trail found in Myo7a. These studies are expected to show that two physiologically divergent myosins, Myo7a and β-cardiac myosin, use a quasi-conserved structural basis to control their motor output.

B113/P1804

Protrusion growth driven by myosin-generated force

G. N. Fitz, M. L. Weck, C. Bodnya, O. L. Perkins, M. J. Tyska; Vanderbilt University, NASHVILLE, TN

Actin-based protrusions are found on the surface of all eukaryotic cells, where they support diverse biological activities essential for life. Models of protrusion growth hypothesize that actin filament assembly provides the mechanical force for bending the plasma membrane outward. However, membrane-associated myosin motors are also abundant in protrusions, though their potential for contributing growth-promoting force remains unexplored. Using a novel inducible system that docks myosin motor domains to membrane binding modules with temporal control, we found that the application of myosin-generated force to the plasma membrane is sufficient for driving robust elongation of protrusions. Protrusion growth scaled with motor accumulation, required active, barbed end-directed force, and was independent of cargo delivery or the recruitment of canonical barbed end elongation factors. Application of growth-promoting force was also supported by structurally distinct myosin motor domains and membrane binding modules. We conclude that myosin-generated force can drive protrusion growth and this mechanism is likely active in diverse biological contexts.

B114/P1805

Ectopically expressed, "stiff-necked" MYO10 is capable of carrying out some of the functions of wild-type MYO10

E. L. Mecchi, D. F. Carpio, A. N. Snyder, M. Kim, O. A. Quintero-Carmona; Department of Biology, University of Richmond, Richmond, VA.

Myosin X (MYO10) is an unconventional myosin important in the formation and maintenance of actin-based protrusions known as filopodia. Like other myosins, it is hypothesized that the coordination of the release of ATP hydrolysis products, actin-binding, and a conformational change moves the lever-arm element relative to the motor domain leading to force-generation and motility. The lever arm consists of two distinct regions. The first region at the N-terminus is an alpha-helical region containing three IQ motifs and is capable of binding accessory proteins known as light chains, such as calmodulin and calmodulin-like proteins. It is hypothesized that the occupancy of the IQ motifs may be regulated by cytoplasmic calcium concentration, and that light-chain binding stiffens the lever arm allowing for more productive force-generation. The second region of the lever arm consists of a an additional extended stable single alpha-helix, hypothesized to extend the length of the lever arm, providing MYO10 with the appropriate geometry for efficient movement along the fascin-bundled actin found in filopodia. To begin to explore the role of the light chains, IQ motifs, and calcium in the regulation of MYO10 function, we generated a chimeric, GFP-tagged *Bos taurus* MYO10 molecule where the IQ-motif containing region of the lever arm was swapped for an equivalent length of the *Mus musculus* stable single alpha-helix (GFP-MYO10^{SN}). We hypothesized that if stability/stiffness of the IQ-domain region of the lever arm is important to MYO10 function, then swapping out the IQ domains for a similar length of stable alpha-helix would generate a protein with similar functional properties to what has been reported for wildtype MYO10 (GFP-MYO10^{WT}). When expressed in multiple cell types, GFP-MYO10^{SN} labeled the tips of filopodia in a manner similar to GFP-MYO10^{WT}. Filopodial extension velocities were similar between GFP-MYO10^{SN} and GFP-MYO10^{WT}. Expression of GFP-MYO10^{SN} in COS7 cells led to an increase in the number of filopodia at the periphery of the cell, as had been observed with GFP-MYO10^{WT} expression, but to a lesser extent than GFP-MYO10^{WT}. This is potentially due to some impairment in function, as the

filopodial initiation rate for GFP-MYO10^{SN} was less than what was observed for GFP-MYO10^{WT}. We hypothesize that this version of MYO10 may be less sensitive to changes in intracellular calcium concentration and/or light chain function, allowing us to make use of it to investigate the role(s) of the IQ motifs and the light chains in regulation of MYO10 functions as they relate to filopodia formation and regulation in cultured cells.

B115/P1806

Investigations into the calcium and light-chain regulation of MYO10 function in filopodial formation

A. N. Snyder, K. E. Reda, D. F. Carpio, E. L. Mecchi, M. Kim, O. A. Quintero-Carmona; Department of Biology, University of Richmond, Richmond, VA.

Myosin functions are regulated via light chains proteins, which interact with the IQ-motif containing regions of the lever arm. MYO10 is an unconventional myosin thought to play a functional role in the establishment of actin-rich protrusions known as filopodia. The force-transducing lever arm domain of MYO10 contains 3 IQ motifs capable of binding light chains such as calmodulin and calmodulin-like protein, & we chose to investigate whether alterations in intracellular calcium concentration or calmodulin function resulted in changes in MYO10 activity or filopodial stability. To test this hypothesis, we treated cells with ionomycin (a calcium ionophore) or W7 (a calmodulin antagonist) & assayed MYO10 and filopodial properties. Time-lapse imaging of a fluorescent calcium sensor (G-GECO1.2) verified that the treatment of cells with ionomycin increased intracellular calcium concentrations, while W7 treatment did not. Using scanning electron microscopy, we observed that filopodia on the dorsal surface of HeLa cells retract in response to ionomycin or W7. In both instances the effect can be partially reversed by washing out the drug. MYO10 localizes to the tips of filopodial protrusions, & quantitative immunofluorescence microscopy revealed that endogenous MYO10 localization to filopodial tips is sensitive to ionomycin or W7 treatment. Similar to the SEM results, washout of either drug resulted in an increase in MYO10 localization to filopodial tips. To investigate the potential role of the IQ motifs and light chains in regulating MYO10 localization, we used quantitative fluorescence microscopy to examine the localization of ectopically expressed MYO10 after ionomycin and W7 treatment using two different GFP-MYO10 constructs. The first construct was wildtype GFP-*Bos taurus* MYO10. The second construct was a chimeric GFP-*Bos taurus* MYO10 where the IQ motif containing region of the lever arm was replaced with an equivalent length of the stable single alpha-helix from the *Mus musculus* MYO10 lever arm. We predicted that swapping the IQ motif region with a stable alpha helix would make the chimeric MYO10 resistant to perturbations caused by changes in calcium concentration or light chain function. Tip localization of wild type GFP-MYO10 was sensitive to either ionomycin or W7 treatment, and could be recovered with washout, similar to what was observed for dorsal filopodia and endogenous MYO10 tip localization. “Stiff-necked,” chimeric GFP-MYO10 did not behave like wild type GFP-MYO10 with respect to tip localization, and was not completely resistant to ionomycin or W7 treatment. Taken together, these results indicate that more investigation is required to fully understand the role of calcium and light-chain function in the regulation of MYO10 activity in filopodial formation.

Motors: Myosins in Intracellular Transport

B116/P1807

Regulation of myosin V association and dissociation with an organelle-specific adaptor complex

H. Hahn[1], N. I. Pashkova[2], M. Cianfrocco[1], L. Weisman[1]; [1]University of Michigan, Ann Arbor, MI, [2]Department of Biochemistry, University of Iowa, Iowa City, IA.

Motor proteins contribute to the regulated distribution of intracellular cargoes. Here we focus on Myo2, an essential myosin V in the yeast *S. cerevisiae*. Myo2 moves multiple cargoes in yeast, which are carried to distinct locations at specific times. Myo2 achieves these itineraries via cargo-specific adaptor complexes. Myo2 is essential for the inheritance of the yeast vacuole. Early in the cell-cycle, Myo2 attaches to a portion of the vacuole via the Vac17 adaptor protein to bring it to the bud. However, little is known about how the assembly is regulated at the molecular level. To determine how Myo2 binds to Vac17, we focused on a region of Vac17 that is important for interaction with Myo2. We previously identified eight surface residues on Myo2 that bind specifically to Vac17. To gain insight into how this region interacts with Vac17, we used AlphaFold Multimer to predict the interface of this interaction. Notably, the model identified the eight known residues on Myo2 and predicted an additional five new sites in this region. This large region may contribute to the stabilization of the complex. The model also predicted that Vac17 directly binds to Myo2 in an extended state with twelve residues directly contacting Myo2. This region includes Vac17-I140 which was previously identified in a genetic screen designed to identify residues on Vac17 that directly contact Myo2. We also tested two additional residues on Vac17. Mutation of one, Vac17-R135E, resulted in a 35% defect in vacuole inheritance and when combined with an adjacent predicted contact site, Vac17-R135E, K138E resulted in 100% inheritance defect. Together these findings provide additional support for the AlphaFold model of the contact interface. Notably, the stretch of residues within the predicted Vac17 contact site include two serines, and similarly the predicted binding site on Myo2 includes a threonine. We are generating phospho-defective and phosphomimetic mutations of these residues to test whether phosphorylation of residues within the predicted contact contribute to the regulation of Myo2 association with Vac17. Thus, tests of the AlphaFold model suggest that aspects of the model are accurate and likely to reveal amino acids that contribute to regulation of Myo2 association and dissociation with the yeast vacuole.

B117/P1808

Cingulin and Paracingulin Recruit Nonmuscle Myosins II to junctionsto Mechanoregulate the Apical membrane of Epithelial Cells

F. Rouaud[1], W. Huang[2], A. Flinois[1], K. Jain[1], E. Vasileva[1], T. DiMattia[1], M. Mauperin[1], D. A. D. Parry[3], V. Dugina[4], C. Chaponnier[1], I. Mean[1], S. Montessuit[1], A. Mutero-Maeda[1], J. Yan[1], **S. Citi**[1]; [1]University of Geneva, Geneva, SWITZERLAND, [2]University of Singapore, Singapore, SINGAPORE, [3]Massey University, Palmerston North, NEW ZEALAND, [4]Moscow State University, Moscow, RUSSIAN FEDERATION.

The apical junctional complex of epithelial cells consists of tight and adherens junctions and is associated with a highly organized actomyosin cytoskeleton, which instructs the shape and mechanical properties of the apicolateral plasma membrane. Nonmuscle myosin-2 (NM2) isoforms show distinct localizations at apical junctions, but the mechanisms underlying the spatial sorting of NM2s are unknown. We show that cingulin and paracingulin, cytoplasmic protein components of apical junctions, interact with nonmuscle myosins-2 through their C-terminal coiled-coil rod sequences. Cingulin interacts with NM2B

in an antiparallel arrangement to inhibit the assembly of the NM2B rod. In cultured epithelial cells cingulin recruits NM2B to tight junctions and binding of cingulin to NM2B is required for the junctional accumulation of ZO-1 and phalloidin-labelled actin filaments. Overexpression of either paracingulin or cingulin promotes the junctional accumulation of either NM2A and NM2B, or NM2B alone, respectively, whereas neither KO or overexpression of cingulin and paracingulin affect NM2C. Binding of cingulin, and to a lesser degree, of paracingulin to NM2s is required for the maintenance of TJ membrane tortuosity and apical membrane stiffness in MDCK cells. Finally, analysis of paracingulin-KO Eph4 cells shows that interaction of paracingulin with NM2s prevents tension-dependent fragmentation of AJ complexes into distinct junctional puncta. These results provide a molecular mechanism for the localization of NM2A and NM2B at junctions and indicate that cingulin and paracingulin transmit force from the actomyosin cytoskeleton to the apical junctional complex to fine-tune junctional architecture and the mechanics of the apical and junctional plasma membranes.

B118/P1809

Automated serial section electron microscopy and 3D reconstruction reveals role for Nonmuscle Myosin II (NM2) proteins in regulation of organelle structure and cargo transport within the thick ascending limb of the mouse kidney

B. Busselman[1], K. Otterpohl[1], J. Zimmerman[1], M. Mukherjee[1], M. Terasaki[2], I. Chandrasekar[1]; [1]Sanford Research, Sioux Falls, SD, [2]University of Connecticut Health Center, Farmington, CT

Mutations in *MYH9*, the gene that encodes the molecular motor nonmuscle myosin IIA protein (NM2A), result in kidney disease in one-third of human patients. Based on our previous work that demonstrated a novel role for NM2 motors in mammalian endocytosis, we hypothesized that NM2 isoforms might play a critical role in renal epithelial transport. To test our hypothesis, we generated an inducible pan renal tubular *Myh9&10* conditional knockout (cKO) mouse model. Loss of *Myh9&10* in adult mice resulted in progressive kidney disease of tubular origin. We determined that accumulation of the urinary glycoprotein uromodulin within expanded ER tubules and loss of ion cotransporter NKCC2 in epithelial cells of the thick ascending limb (TAL) of Henle's loop were the main cellular mechanisms that led to upregulation of ER stress/UPR pathways and kidney disease. We attempted to understand the molecular mechanisms involved in organelle transport *in situ* using mouse kidney sections. We employed immunostaining, light microscopy, and standard transmission electron microscopy, as well as an innovative automated serial section scanning electron microscopy method. TEM confirms ER tubule expansion as well as the unique nature of the organelle structure within the thick ascending limb epithelia in control kidneys. Additionally, RNA scope of kidney sections reveals UMOD mRNA throughout the TAL epithelia in control kidneys, while *Myh9&10* cKO kidneys with excessive UMOD protein accumulation in expanded ER tubules exhibit downregulated UMOD mRNA expression. To explore ER structure in an unbiased manner, we performed three-dimensional reconstructions of serial section SEM images and confirm that in control mouse kidneys, TAL epithelial cell mitochondria are partially encapsulated within basolateral plasma membrane infoldings that are positive for Na+ K+ ATPase by immunostaining. ER tubules are regularly present in the narrow space between mitochondria and membrane infoldings and are continuous with the expansive ER network present throughout the cell. In control TAL cells, we also observe widely distributed tubular vesicles in close contact with ER tubules. In *Myh9&10* cKO kidneys, spherical vesicular bodies with similar electron density are seen in close contact with morphologically abnormal ER, which appears to contain a larger number of sheets than control cells. While the plasma membrane appears intact in *Myh9&10* cKO TAL cells, the organization of

mitochondria within membrane infoldings is perturbed and ER-mitochondria-PM contact appears reduced. Overall, our findings indicate a distinct and complex ER-Mitochondria-PM structure interaction within the TAL epithelia *in situ* which requires the molecular motor nonmuscle myosin II for its organization and function.

B119/P1810

A type I myosin critical for clathrin-mediated endocytosis is a force-generating motor

R. T. A. Pedersen[1,2], A. Snoberger[3], S. Pyrpassopoulos[3], D. Safer[3], D. G. Drubin[1], E. M. Ostap[3]; [1]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, [2]Department of Embryology, Carnegie Institution of Washington, Baltimore, MD, [3]Pennsylvania Muscle Institute and Department of Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

Actin filaments generate force through assembly, which produces pushing force, and through associated myosin motors, which produce pulling forces or tension. These modes of force generation work together in a wide variety of cellular processes, but how they are coordinated in these processes is an important, unanswered question. Clathrin-mediated endocytosis depends on both actin assembly and type I myosin activity to bend the plasma membrane into a pit that is subsequently released as a cytoplasmic vesicle, but the role of myosin motor activity in this process is unknown. Some myosin-1 motors generate forces of biologically relevant magnitude. If the myosin-1 motors involved in clathrin-mediated endocytosis possess this property, they might actively assist actin assembly in invaginating the plasma membrane. Other myosin-1 motors behave as tension-sensitive anchors. If endocytic myosins have this property, they are more likely to participate in endocytosis through organizing or anchoring actin filaments.
To distinguish between these possibilities, we studied the force-dependent kinetics of the ATPase cycle for the *Saccharomyces cerevisiae* endocytic myosin-1 Myo5, a motor whose role in endocytosis has been meticulously studied *in vivo*. Transient kinetic experiments conducted on Myo5 in solution revealed that Myo5 is a low-duty-ratio motor that is activated about 10-fold by heavy chain phosphorylation. Myo5 has a rapid working stroke compared to related myosins: its ADP release rate, which is rate limiting for detachment, is 74-100 per second, around 20 times faster than the related motors Myo1b and Myo1c. Single molecule analysis of Myo5 using an optical trap revealed that the Myo5 step size is 3.4 nm. The kinetics of the working stroke agree well with the bulk kinetic measurements. By applying resistance to the Myo5 working stroke using an isometric force clamp, we found that Myo5 stepping kinetics are relatively force insensitive. Quantitatively, the force sensitive kinetics of Myo5 are more comparable to muscle myosin than to related type I myosins. We propose that Myo5 acts as a force producer to augment actin assembly forces rather than as a tension-sensitive anchor during clathrin-mediated endocytosis.

B120/P1811

Conserved ankyrin repeat proteins promote myosin-1 localization and function in fission and budding yeast

A. H. Willet, J. Chen, L. Ren, K. L. Gould; Cell and Developmental Biology, Vanderbilt University, Nashville, TN.

Endocytosis is an essential cellular process where extracellular contents such as nutrients are taken up by the cell. To do so in yeast, the cell forms pits in the plasma membrane with force generated by a branched actin network nucleated by the Arp2/3 complex. One component of endocytic patches is the

myosin-1 family of motors. These are monomeric membrane tethering proteins with a N-terminal motor domain and C-terminal TH1, TH2, SH3 and in yeast a CA domain that activates Arp2/3. To better understand the interactions and function of fission yeast Myo1, we performed a large-scale purification coupled with mass spectrometry analysis and identified an uncharacterized non-essential protein containing ankyrin repeats that we named Mai1 (myosin-1 ankyrin repeat interactor). Coimmunoprecipitation of a series of Myo1 C-terminal truncations showed that the Myo1 motor domain alone is sufficient for Mai1 interaction. Further, whereas Myo1-mNG normally localizes to endocytic actin patches, a dramatic relocalization of Myo1 to coat the entire plasma membrane occurred in cells lacking *mai1*. Additionally, in *mai1Δ* cells, the Myo1 light chains, Cam1 and Cam2, also redistributed along the plasma membrane, but other endocytic proteins (Fim1, Wsp1 and F-actin) did not. Preliminary in vitro experiments confirmed previous work showing that immunopurified Myo1 bundles F-actin in vitro. However, Myo1 immunopurified from *mai1Δ* cells does not although it still binds F-actin, via a second actin binding motif in the C-terminus of Myo1, as found in other myosin-Is. Myo1 membrane binding is thought to be mediated by the TH1 domain. Thus, we hypothesize that Mai1 promotes the interaction of the Myo1 motor domain with F-actin and in the absence of F-actin binding, Myo1 localization defaults to the membrane in a TH1 domain-dependent manner. We found that a Mai1 ortholog exists in *S. cerevisiae* and it regulates the localization of both *S. cerevisiae* myosin 1s. Thus, Mai1 represents a novel, conserved myosin-I regulator.

B121/P1812

In Vitro Characterization of Myosin 5 on Biomimetic Actin Networks Using Micropatterning

D. Han[1,2], P. Bleicher[1], Y. Takagi[1], J. Sellers[1]; [1]National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, [2]Brown University, Providence, RI.

Myosin 5 is a processive motor protein involved in cellular transport processes along actin filaments. In order to study myosin 5's biophysical characteristics in networks of actin similar to those found within cells, we used micropatterning techniques to grow organized actin networks on a glass coverslip. GFP-labeled myosin 5 was added to the assay after full polymerization of the actin. Its activity was recorded using TIRF microscopy, and ImageJ's TrackMate was used to quantitatively characterize its movement. We compared myosin 5's velocity, run length, and dwell time in the micropatterned assay to a single filament assay under the same conditions. While there were not significant differences in the measurements between the assays, the velocities and run lengths were found to be consistently higher in the micropatterned assay. The paths that were generated by TrackMate also showed jagged trajectories in the micropatterned assay which were absent in the single filament assay. By capturing the movement of the actin filaments in tandem with myosin 5's movement under greatly reduced ATP concentrations, we showed that the thermal fluctuations of the actin network had an additive effect to the apparent velocity and run length in the micropatterning assay. Additionally, these thermal fluctuations are the most likely reason for the jagged paths taken by myosin 5. Unlike myosin 2, myosin 5 did not show any ability to alter the structure of the actin network while interacting with it. In conclusion, we found that micropatterned assays are an effective system to study actin-myosin interactions. Myosin 5's velocity, run length, and dwell time in the micropatterned assay was comparable to single filament assay studies, and differences in myosin 5's movement could be attributed to thermal fluctuations of the actin network.

Motors: Kinesins in Organelle Dynamics

B122/P1813

Optogenetic motor inhibitors reveal the contributions of kinesins-1, -2, -3, and dynein to endosome and lysosome transport

S. Nagpal, S. Wang, K. Swaminathan, A. Hendricks; Department of Bioengineering, McGill University, Montreal, QC, CANADA.

Multiple kinesins and dynein are often simultaneously associated with intracellular cargoes. To dissect the roles of these motors, we developed optogenetic tools to temporally control the activity of kinesin-1 (KIF5), -2 (KIF3A/B), -3 (KIF1), and dynein. Inhibitory peptides were cloned onto the LOVTRAP optogenetic system that is comprised of two components, the mitochondria-bound Z-dark and the photo-responsive LOV2 which unbinds from Z-dark when illuminated with blue light. The inhibitory peptides for kinesin motors are based on their native autoinhibitory domains. For dynein inhibition, we used the CC1 domain from p150glued that prevents the formation of an active dynein-dynactin complex. In agreement with previous studies, early endosomes are most sensitive to inhibition of kinesin-1, kinesin-3, and dynein, while late endosome and lysosome motility is altered by inhibition of kinesins -1, -2, -3, and dynein. However, while sustained inhibition of either kinesins or dynein results in reduced motility in both directions, transport is enhanced on both early and late endosomes when kinesin-1 or dynein is transiently inhibited. These results indicate that on vesicles transported by many motors, motility can be directed by modulating the activity of a single type of motor on the cargo.

B123/P1814

The sets of kinesins and dyneins transporting endocytic cargoes governs their response to tau

A. G. Hendricks[1], D. Beaudet[1], L. Balabanian[1], C. L. Berger[2]; [1]McGill University, Montreal, QC, CANADA, [2]University of Vermont, Burlington, VT.

As endosomal cargoes progress through the degradative pathway, the types and numbers of kinesins and dyneins driving their transport changes. In turn, these sets of motors dictate how cargoes move and respond to microtubule-associated proteins. We isolated early (30 minutes) and late (90 minutes) phagosomes and counted the motors bound to them using immunofluorescence and stepwise photobleaching. While kinesin-1 (KIF5B), kinesin-2 (KIF3A/B), kinesin-3 (KIF16B), and dynein associate with both early and late phagosomes, early phagosomes have fewer kinesin-1, kinesin-2, and dynein bound. The sets of motors on early phagosomes are heterogenous and there are fewer motors bound to each phagosome compared to late phagosomes. Early phagosomes exhibit long, directed runs towards the microtubule minus end *in vitro*, in contrast to late phagosomes which switch directions frequently. On tau-decorated microtubules, the long, dynein-driven runs of early phagosomes and are strongly inhibited. While on late phagosomes, tau preferentially inhibits kinesin forces to bias their bidirectional motility towards the microtubule minus end. In agreement, high-resolution tracking of organelles in cells expressing tau show that early endosomes are inhibited more strongly by tau than lysosomes. Further, a phosphomimetic (Y18E) mutation to tau partially relieves inhibition of lysosomes, while early endosomes are strongly inhibited by both unphosphorylated and phosphomimetic tau. Through counting the motors bound to cargoes, reconstituting their motility *in vitro*, and tracking their motility in living cells, we show that sets of motors associated with endocytic cargoes changes throughout maturation, altering the effect of tau on their motility.

B124/P1815

Role of microtubule motor adaptor proteins in controlling mitochondrial inheritance and quality

M. J. Gillen[1,2], R. Cox[1]; [1]Biochemistry and Molecular Biology, USUHS, Bethesda, MD, [2]Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD.

Conflict of Interest The authors have no conflicts of interest

Mitochondria are an essential cell component that are exclusively maternally inherited. Mitochondria have their own genomes, mitochondrial (mt) DNA and accumulating mtDNA mutations is a significant cause of mitochondrial disease. However, studies have shown mitochondria with deleterious mtDNA are typically not passed on to progeny at a rate expected if mitochondrial inheritance was random, suggesting there is a purifying mechanism from one generation to the next. Drosophila and mouse studies have shown this potential selection occurs early in development and at least partly happens during oogenesis. Germline cyst formation is conserved from humans to Drosophila. Since mitochondria are moved via motor proteins along microtubules, we are interested in whether mitochondrial movement during follicle development in Drosophila potentially contributes to a quality control mechanism to filter damaged organelles deposited into the oocyte. To do this, we are studying the Milton/Miro cargo adaptor complex that links mitochondria to the motor protein kinesin and dynein. Miro is an atypical GTPase with a transmembrane domain that inserts in the mitochondrial outer membrane. Milton is an adaptor protein that binds to Miro. Our previous work showed that Class II *milton* alleles cause uncontrolled mitochondrial movement into the oocyte starting with region 3 of the germarium and continuing until approximately mid-oogenesis. This phenotype is phenocopied by germ cells lacking Kinesin heavy chain. Studies of the vertebrate Milton homolog, TRAK1 and 2, have shown TRAK1 binds Kinesin and Dynein/Dynactin to control mitochondrial movement, however, this has not yet been established for Drosophila Milton. We will present our data examining all the Milton isoforms and how altering isoform levels controls mitochondrial movement during oogenesis. One goal of this study is to understand the basic mechanism of how Milton and Miro control mitochondrial movement along microtubules. In addition, this study could help identify part of the mitochondrial quality control system governing mitochondrial inheritance. This could have implications for maternally inherited forms of mitochondrial disease.

B125/P1816

Endoplasmic reticulum and peroxisomes hitchhike on motile endosomes.

S. Abeling, **A. Morrissey**, B. Driscoll, E. Wagner, J. Salogiannis; Molecular Physiology and Biophysics, Larner College of Medicine At the University of Vermont, Burlington, VT.

Cargos (mRNA, organelles, and vesicles) are driven long distances on microtubules via molecular motors. The canonical view of microtubule-based transport is that adaptor proteins are required to recruit these cargos to the motors. Our group along with others discovered an alternative mode called membrane

hitchhiking: some organelles achieve motility by tethering to or 'hitchhiking' on moving endosomes. The mechanisms of hitchhiking are not well understood, but likely require tethering between membranes at contact sites. Hitchhiking represents a paradigm shift in how we think about cargo motility. Here, we present molecular and genetic data to determine how it is achieved. In the filamentous fungus Aspergillus nidulans, peroxisomes cotransport on Rab5-positive early endosomes. To accomplish this, peroxisomes utilize novel endosomal binding proteins, PxdA and DipA. We performed a mutagenesis screen and identified additional factors involved in peroxisome hitchhiking. In mammalian cells, we find that coordinated movement of peripheral ER tubules rely on attachment to Rab1- and Rab6-positive endosomes. Using a rapalog-inducible system, stopping the movement of specific endosomal populations concomitantly reduces the movement of ER tubules. Taken together, our data suggests that membrane hitchhiking occurs in multiple organisms and represents a novel mechanism for how cargos move along microtubules.

B126/P1817

Co-operation of KIF1C and dynein in bidirectional cargo transport

A. J. Zwetsloot[1], C. Stone[2], C. Lau[2], F. Abid Ali[2], A. Carter[2], **A. Straube**[1]; [1]Centre for Mechanochemical Cell Biology, University of Warwick, United Kingdom, [2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

The kinesin-3 KIF1C and cytoplasmic dynein are fast long-distance transporters with opposite polarity. Here we show that both motors can bind simultaneously to cargo adapters hook3, BICD2 and BICDR1 to form co-motile complexes, with quaternary complexes of dynein, dynactin, hook3 and KIF1C (DDHK) forming most efficiently. Using a rapamycin-induced cargo transport assay in cells, we found hook3 driven intracellular transport is rapid, bidirectional, and sensitive to the concentration of active KIF1C and dynein. DDHK complexes show bidirectional motility in single molecule microscopy assays, and provide evidence for co-dependence of opposite polarity motors as KIF1C increases both the initiation and the distance of minus end-directed runs, both by acting as a processivity tether and because KIF1C facilitates the recruitment of a second dynein to dynactin and hook3. Based on intensity measurements of motile complexes we propose a stoichiometry of two dynein dimers, two KIF1C dimers, two hook3 dimers and one dynactin in DDHK complexes. Directional switching of DDHK complexes was relatively rare, suggesting that the tight coupling of the motors primarily supports processive unidirectional transport and prevents a tug-of-war.

B127/P1818

Regulation of kinesin-2 motors in the sensory cilia of *C. elegans*

W. Mul, A. Mitra, E. J. G. Peterman; Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Most eukaryotic cells contain a primary cilium, which acts as an antenna to sense the environment and is involved in a wide range of signalling pathway essential for development. Cilia consist of a microtubule-based axoneme that acts as a track for bidirectional intraflagellar transport (IFT) of ciliary components, necessary for correct functioning of the cilia. In *C. elegans*, movement of anterograde IFT trains from ciliary base to tip is driven by the concerted action of two kinesin-2 motor proteins, kinesin-II and OSM-3. The slower kinesin-II navigates the IFT trains through the densely packed transition zone and proximal segment, handing over the IFT trains to the faster OSM-3 motors for transport further along the cilium. The regulatory mechanism that controls the cooperation between kinesin-II and OSM-3

remains elusive, although previous studies have suggested that the kinases DYF-5 and DYF-18 (RCK and CCRK orthologs) play a crucial role. Here, we perform quantitative fluorescence microscopy in mutant *C. elegans* strains that lack DYF-5, in order to understand how this kinase affects kinesin-2 motor cooperation. We find that the cilia of these dyf-5 knockout (KO) animals are longer and vary markedly in length. In addition, kinesin-II is distributed differently along the cilia of these animals: throughout the cilium in the mutant, in contrast to the beginning of the proximal segment in wild-type. Moreover, we find that kinesin-II often accumulates at the tip of the cilium in the mutant. Next, we looked into the single-molecule dynamics of kinesin-II in WT and dyf-5 KO animals. We observed that the velocity of the individual kinesin-II motors appears to be reduced in the absence of DYF-5 and that motors pause at the accumulation sites. These results provide mechanistic understanding in the role of kinases in kinesin-2 motor regulation.

B128/P1819

KIF3B promotes a perpendicular PI3K signaling gradient that regulates the Shh protein gradient and suppresses polydactyly

S. Wang, **Y. Tanaka**, Y. Xu, S. Takeda, N. Hirokawa; University of Tokyo Grad Sch Med, Tokyo, Japan

Digit determination in limb buds is driven by a posteriorizing Sonic hedgehog (Shh) protein gradient; however, the formation mechanism is largely unknown. Here, we propose a diffusion-and-trapping hypothesis for Shh nanoparticle gradient formation by analyzing the preaxial polydactyly phenotype of KIF3B motor hypomorphic mice. In the limb buds, a distal-to-proximal gradient of fibroblast growth factor (FGF)-phosphatidylinositol 3-kinase (PI3K) signaling and a posterior-to-anterior gradient of Shh nanoparticles were disorganized. This phenotype was reproduced by transplanting FGF8b-soaked beads. At the subcellular level, KIF3B transported the phosphatase and tensin homolog (PTEN)-like phosphatase Talpid3 to terminate PI3K signaling. High and low PI3K signaling strengths differentially sort endocytosed Shh nanoparticles toward unconventional exosomes and cytonemal punctata, respectively. These results indicate that the distal and proximal layers constitute diffusion-and-trapping kinetics of Shh nanoparticles, which sustainably form a horizontal gradient along the edge of developing limb buds. (W.S. and Y.T.: Equal contribution)

B129/P1820

Kinesin-1 autoinhibition facilitates the initiation of dynein cargo transport

R. Qiu, J. Zhang, X. Xiang; USUHS/HJF, Bethesda, MD

Kinesin-1 is normally autoinhibited, but the functional significance of kinesin-1 autoinhibition would need to be further dissected. In filamentous fungi and neurons, kinesin-1 transports multiple cargoes including cytoplasmic dynein to the microtubule plus ends. In filamentous fungi, dynein motors accumulated at the microtubule plus ends interact with early endosomes transported there by kinesin-3 rather than by kinesin-1. Dynein then drives the movement of early endosomes away from the microtubule plus ends. From a genetic screen for *Aspergillus* mutants defective in dynein-mediated early endosome transport, we identified a kinesin-1 mutation *kinA*^{K895*} that affects the C-terminal IAK motif well-known to be involved in kinesin-1 autoinhibition. To verify that dynein-mediated early endosome transport is negatively affected by the loss of IAK-mediated autoinhibition rather than the loss of other C-terminal residues, we made the *kinA*$^{\Delta IAK}$ and the *kinA*^{K895E} mutants. These mutants exhibited a defect in dynein-mediated early endosome transport similar to that of the *kinA*^{K895*} mutant. All the mutant

proteins accumulate abnormally near the microtubule plus ends, indicating a loss of kinesin-1 autoinhibition. Besides dynein, RabE-labeled secretory vesicles are also cargoes of kinesin-1 in *A. nidulans*. We show that kinesin-1 autoinhibition does not play a critical role in transporting secretory vesicles to support hyphal growth. Dynein also accumulates normally at the microtubule plus ends in the *kinA*^{K895*} mutant. However, the frequency but not the speed of dynein-mediated early endosome transport is significantly decreased, indicating that kinesin-1 autoinhibition facilitates dynein to initiate its cargo transport. Furthermore, kinesin-1 autoinhibition promotes dynein cargo initiation in a way mechanistically distinct from LIS1-promoted dynein switching from its autoinhibited form. Thus, while dynein activation involves dynactin, cargo adapter and LIS1, this study adds kinesin-1 autoinhibition as a new regulatory factor in vivo.

B130/P1821

Rapid accumulation of RhoGEF at the cleavage furrow is driven by centralspindlin-mediated transport and retention at plus-end tips

B. Warecki, **L. Tao**; University of Hawaii at Hilo, Hilo, HI

Cytokinesis, the physical division of one cell into two, requires accurate positioning of the cleavage furrow between segregating chromosomes. Failure to properly position the cleavage furrow can result in aneuploidy and is a hallmark of cancer cells. However, key aspects of the molecular mechanisms that govern where the cleavage furrow forms remain poorly understood. Furrow formation is specified by localization of the RhoA-activator RhoGEF, which leads to local membrane ingression. RhoGEF binds to the conserved motor protein complex centralspindlin. This has led to multiple models for how centralspindlin activity positions RhoGEF at the cleavage furrow: 1) centralspindlin sequestration of RhoGEF at microtubule plus-end tips, or 2) direct centralspindlin-mediated RhoGEF transport along microtubules. Here, using a combination of in vivo and in vitro experiments, we show that centralspindlin utilizes both mechanisms to position RhoGEF. We find that purified full-length centralspindlin directly transports its native cargo RhoGEF along single microtubules. Furthermore, we find that RhoGEF binding to centralspindlin may stimulate centralspindlin motor activity. Following transport, centralspindlin retains RhoGEF at the plus-end tips for an extended time. Additionally, we find that this retention on microtubules is an inherent behavior of centralspindlin. Thus, centralspindlin can stockpile at microtubule plus-ends to bind diffuse RhoGEF. Collectively, these results demonstrate that centralspindlin's motor activity and strong association with microtubules rapidly generate a high concentration of RhoGEF at areas where plus-end tips are abundant, such as at overlapping aster microtubules at the cell equator, to specify the position of cleavage furrow formation.

B131/P1822

Armadillo repeat-containing kinesin ARK represents the versatile plus-end-directed transporter in plants

M. Yoshida[1], M. Hakozaki[1], G. Goshima[2]; [1]Nagoya University, Nagoya, Japan, [2]Nagoya University, Toba, Japan

Kinesin-1 (also known as conventional kinesin) and cytoplasmic dynein are widely utilised for microtubule-based transport in animal cells. Intriguingly, plants do not possess kinesin-1, despite that bidirectional transport along microtubules is still observed in plant cells. A plant motor required for plus-end-directed ("anterograde") transport of various cargos, similar to kinesin-1 in animals, has not been

identified. In this study, we investigated the function of plant-specific armadillo repeat-containing kinesin (ARK) in the moss *Physcomitrium patens* and provided evidence that ARK is the long sought-after versatile anterograde transporter of plants. Firstly, ARK was shown as a processive motor *in vivo* and *in vitro*. Next, in *ARK* mutants, we found that microtubule plus-end-directed motility of nuclei, chloroplasts, mitochondria, and secretory vesicles is drastically suppressed. Ectopic expression of the rigor mutant or tail-deleted ARK in the mutant indicated that organelle distribution requires ARK's motility and the tail. Furthermore, the suppression of cell tip growth, the most prominent macroscopic phenotype of the *ARK* mutants, was attributed to the mislocalisation of actin regulators, including GEFs of Rho-type GTPase. Expression and forced apical transport of RopGEF3 suppressed the growth phenotype of the *ARK* mutant. The result indicates that the anterograde motility of ARK supports the polarised cell growth through localising actin regulatory molecules to the growth site. This scheme is similar to what has been revealed in other cell types, including fission yeast, filamentous fungus, and epithelial cells. Thus, long-distance transport of polarisation factors along microtubules may be a general scheme for polarised growth in a wide range of eukaryotic cells.

B132/P1823

A DNA tensiometer technique for studying detachment kinetics of microtubule motors involved in bidirectional transport.

C. R. Noell, R. Jiang, A. Gicking, W. O. Hancock; Pennsylvania State University, State College, PA

Bidirectional transport is essential for cargo trafficking in cells and is required for proper growth and cell division. Kinesin and dynein are microtubule motors responsible for bidirectional cargo transport in cells. Defects in microtubule motor-based transport are linked to many neurodegenerative diseases including Alzheimer's, Parkinson's, spinal muscular atrophy, amyotrophic lateral sclerosis, and Huntington's disease; thus, understanding the mechanisms underlying bidirectional transport is crucial to understanding transport deficiencies in disease states and developing potential treatments. Despite important advances in understanding the mechanochemical properties of individual motors, many questions remain regarding how motors work as teams, and how kinesins and dyneins coordinate with one another. The 'tug-of-war' model is a widely supported model for bidirectional transport; however, this model cannot account for the motor coordination and other regulatory factors involved. Previous modeling work identified the load-dependent detachment rate as the key parameter that determines whether kinesin or dynein wins in a motor tug-of-war. Recent experimental and theoretical work showed that vertical forces inherent to the geometry of the widely-used single-bead optical tweezers significantly accelerated motor detachment rates. Consistent with this, when kinesin and dynein were connected through DNA linkages such that forces are only parallel to the microtubule, these two-motor complexes remained attached for much longer times than seen in optical tweezer experiments. In this work we propose a novel technique that uses ssDNA as a pN-scale spring, to accurately determine motor stepping characteristics and detachment kinetics in the absence of vertical forces, mimicking physiological conditions. Here we show preliminary data of our DNA tensiometer work in the study of bidirectional transport.

B133/P1824

The kinesin KIF16B regulates MT1-MMP fast recycling inmacrophages

S. Hey, C. Wiesner, **S. Linder**; University Clinic Eppendorf, Hamburg, Germany

Membrane-Type 1 Matrix Metalloproteinase (MT1-MMP) serves critical functions during many physiological processes (e.g. vascular development, immune cell migration), through cleaving of components of the extracellular matrix (ECM) and also of cell surface-associated proteins. It is thus crucial for the capability of macrophages to proteolytically invade into different tissues. However, matrix proteolysis allows also cancerous cells to spread faster during metastasis, most likely by using the microchannels created by spreading macrophages. These tumor associated macrophages (TAMs) are linked with poor prognosis for cancer patients. Limiting the capability of TAMs to degrade the ECM thereby could serve as a potential therapy to limit cancer invasion. By using primary human macrophages, we aim to unravel the processes that regulate MT1-MMP surface exposure in more detail. One major regulatory step in this regulation is the recycling of endocytosed MT1-MMP back to the cell surface. Our work focuses on identifying the protein network that regulates the fast recycling processes of MT1-MMP. Previously, we could show that exocytosis of MT1-MMP is controlled by Rab8a and driven by kinesin-1 and -2. We could also show that the fast recycling by Rab14 positive vesicles is crucial for regulating the surface associated pool of MT1-MMP. However, the respective motor protein driving these vesicles remained unidentified. We now identify KIF16B as the major kinesin regulating MT1-MMP fast recycling processes in primary human macrophages. Using live cell imaging, siRNA mediated depletion, expression of mutant proteins and also of rescue constructs, as well as FACS measurements and surface biotinylation assays, we demonstrate that KIF16B-mediated recycling of MT1-MMP is a critical mechanism for regulation of MT1-MMP surface levels and thus also of the ECM degradation ability of macrophages. Furthermore, we show that a KIF16B depletion in macrophages can also reduce the growth of tumor spheroids *in vitro*, highlighting the close relation between immune cells and cancer cell invasion.

Microtubules in Development

B135/P1825

Microtubule organization regulation by SCAPER homolog in *C. elegans* DA9 neuron

Y. Xie, S. Yogev; Yale University, New Haven, CT.

Neurons rely on the highly dynamic microtubule (MT) cytoskeleton to execute essential processes such as neuronal development, axon guidance, synapse formation, intracellular trafficking, and signal transduction. Neurodegenerative diseases like Alzheimer's disease, and neuronal injuries are often associated with gradual loss of dendritic and axonal microtubule mass. Extensive studies have shown how neuronal MT are regulated, however, how MT organized, specifically how MT numbers are precisely regulated for specific functions in individual neurons is not well understood. To study how MT mass is regulated, we performed a forward genetic screen for disorganized microtubule networks and found a mutant in the *C. elegans* homolog S Phase Cyclin A-associated protein in the ER (SCAPER), displayed microtubules disorganization. Here, we use genetic and superresolution microscopies to illustrate the essential functions of SCAPER on microtubule organization regulation. We find that endogenous and overexpression of SCAPER show punctate structures overlapping with MT minus-end marker Patronin (PTRN-1) in cell body and neuron projections. The loss-of-function allele of *scaper* we

generated by CRISPR causes MT number decrease in *C. elegans* DA9 neuron. Specifically, *scaper* loss-of-function causes enlarged spacing of the MT minus-ends labeled by PTRN-1. In the distal axon, discontinuance of MT has been observed. We propose that the sparse MT minus-end and distal MT gaps are due to lack of MT mass. Meanwhile, overexpression of SCAPER induces microtubule number increase. Moreover, we also determine that SCAPER N-terminal domain is required for MT organization function, and there are uncharacterized domains essential for SCAPER specific localization in C-terminus. Therefore, we hypothesize that SCAPER functions for MT nucleation and regulates MT organization.

B136/P1826

Doublecortin contributes to neuronal migration through suppression of neurite branching and modification of the tubulin code

M. Sébastien, E. Prowse, H. Mary, A. Hendricks, G. J. Brouhard; McGill University, Montréal, QC, CANADA.

Doublecortin is a neuronal microtubule-associated protein (MAP) that binds directly to microtubules via two Doublecortin (DC) domains. The DC domains are very sensitive to the nucleotide state, longitudinal curvature, and protofilament number of the microtubule lattice, indicating a role in the regulation of microtubule structure in neurons. Mutations in Doublecortin cause lissencephaly and subcortical band heterotopia (also known as double-cortex syndrome), which are diseases caused by impaired neuronal migration. To better understand the role of DCX in neuronal migration, we developed a model system based on induced pluripotent stem cells (iPSCs). We knocked out the *Dcx* gene in a male iPSC line using CRISPR/Cas9 gene editing and differentiated the cells into cortical neurons. Compared to control neurons, the DCX-KO neurons showed reduced velocities of nuclear displacements, consistent with a neuronal migration phenotype. The reduced velocities correlated with an increase in the number of branches early in the neuronal development process, a result that is consistent with data from a DCX-KO mouse model. Neurite branching is regulated by a host of MAPs and other protein factors, as well as by microtubule dynamics itself. Microtubule dynamics were unchanged in DCX-KO neurons, however, with similar growth rates, lifetimes, and numbers, as measured by live-cell imaging of EB3-mCherry electroporation. Rather, we observed changes in microtubule post-translational modifications, also known as the tubulin code, namely a significant reduction in polyglutamylation. Polyglutamylation is usually abundant in neurons and regulates, for example, microtubule severing enzymes and intracellular trafficking by molecular motors. Consistently, we observe that lysosomes in DCX-KO neurons show an increase in anterograde processive motility. We propose that the reduction of polyglutamylation leads to increased neurite branching and thus reduced neuronal migration. Our results indicate an unexpected role for DCX in the homeostasis of the tubulin code.

B137/P1827

The Role of Kinesin-1 Mediated Microtubule Sliding in Regulation of Insulin Secretion

K. M. Bracey, M. Fye, G. Gu, I. Kaverina; Cell and Developmental Biology, Vanderbilt University, Nashville, TN.

Pancreatic beta cells have to secrete only a sub-population of available insulin granules to reduce blood sugar to normal levels but yet avoid hypoglycemia. This requires tight coordination between intracellular insulin storage and secretion. We have previously shown that cytoskeletal polymers microtubules (MTs) critically govern this coordination.Molecular motors use MT tracks to transport and park insulin granules

at specific cellular locations. In beta cells, MT network is complex, with interlocked, non-radial network in the cell interior, where secretory insulin granules are trapped by looped transport, and the sub-membrane MT bundle, which promotes insulin granule withdrawal from the secretion sites. Due to these features, MT network limits insulin secretion in healthy beta cells. Given a spike in glucose concentration the microtubule network is remodeled to allow for robust secretion. Here, we report that motor-dependent repositioning of existing MTs is an essential feature of glucose-triggered MT remodeling.Using real-time imaging of microtubules labeled with single-molecule fiducial marks, we demonstrate that high levels of glucose rapidly induce MT movements. Kinesin-1, a molecular motor that is highly expressed in beta cells and activated downstream of glucose signaling, is capable of transporting MTs along MT tracks, a process known as MT sliding. We found that glucose-triggered MT movements are abolished by inactivation of kinesin-1 or introducing mutations preventing attachment of MTs as a cargo. We show that microtubule sliding is important for cellular distribution of MT minus ends and is influencing the overall MT directionality and architectural features. This means that kinesin-1 likely has a dual role in insulin secretion regulation: directly, via insulin granule transport, and indirectly, via remodeling MT network.Ongoing work will utilize kinesin-1 mutant incapable of microtubule sliding but capable of insulin granule transport, combined with computational simulations, to reveal the specific contributions of MT sliding into regulation of insulin granule positioning and secretion.Overall, here we show a novel mechanism whereby glucose stimulation induces MT network remodeling, and, subsequently, MT-dependent control of insulin granule transport for secretion.

B138/P1828

ER network stability promotes organized microtubule disassembly during Compartmentalized Cell Elimination

K. Juanez, M. P. Jones, P. Ghose; University of Texas At Arlington, Arlington, TX

Programmed cell death is vital for development and homeostasis. Morphologically complex cells are characterized by elaborate processes, such as axons and dendrites in neurons. While complex cells are common, their programmed as well as pathological or injury induced elimination is poorly understood. Microtubule (MT) disassembly is linked to region-specific neurite pruning, but the exact nature of this relationship is unknown. We discovered a ‘tripartite’ killing program with stereotyped cellular dynamics that eliminates the morphologically complex tail-spike cell (TSC) and the sex-specific CEM neurons in the *C. elegans* embryo. This program, called Compartmentalized Cell Elimination (CCE), is characterized by three cell regions dying in three disparate ways. Notably, the single process/dendrite of these cells displays two very different elimination morphologies in its two segments. The proximal segment fragments in a manner strikingly reminiscent of developmental pruning or injury-induced Wallerian degeneration of axons; whereas the distal segment retracts, much like nutrient-deprived axons. Here we report that MTs have stereotyped dynamics throughout the development and death of the TSC. Through forward genetic screens, we found that genes promoting endoplasmic reticulum (ER) network stability, *atnl-1*/Atlastin and *lnp-1/*Lunapark, which encode the homologs of human Atlastin GTPase and Lunapark, promote process dismantling during CCE. We find that *atnl-1*/Atlastin and *lnp-1*/Lunapark promote the function of the conserved MT-severing ATPase SPAS-1/Spastin in facilitating CCE. Human Atlastin, Lunapark and Spastin are all associated with neurodegenerative conditions. Fluorescent reporters for the ER, SPAS-1/Spastin and MTs dynamically change in distribution as the TSC develops and dies and MTs show abnormally gross enrichment in the TSC process in ER network stability and SPAS-1/Spastin mutants, suggesting hyperstability of MTs. We propose that the stable ER network and

ER network stability proteins anchor SPAS-1/Spastin to allow for precisely targeted and organized MT disassembly, culminating in the highly defined dynamic demise of the TSC process during CCE. Our findings shed new light on the localized elimination of complex cells and illuminate the link between MTs, pruning and neurodegeneration through an unexpected connection with the ER.

B139/P1829

A distinct gamma-tubulin complex is required for spermiogenesis in Drosophila

E. Wight[1], J. V. Chen[1], C. Zheng[1], G. Callaini[2], T. L. Megraw[1]; [1]Florida State University, Tallahassee, FL, [2]University of Siena, Siena, ITALY.

We investigated the functions of variant γ-tubulin complex proteins that are expressed in the Drosophila testis, and their potential to form unique complexes that function distinctly from conventional γ-tubulin complexes. The γ-Tubulin Ring complex (γ-TuRC) is the primary microtubule nucleator at centrosomes, the main microtubule organizing center (MTOC) in dividing cells. The molecular structure of the conserved γ-TuRC was recently solved at high resolution. The γ-TuRC is comprised of a ring of γ-tubulin and Gamma-tubulin Ring Interacting Proteins, (GRIPs, aka GCPs). While γ-TuRCs are highly conserved throughout eukaryotes and associate with a variety of proteins that bind/anchor them at MTOCs, heterogeneity in the γ-TuRC core has been relatively unexplored. Three unique GRIP proteins that contains conserved GCP interacting domains are expressed in testis, collectively called t-GRIPs. In the testes, t-GRIPs form a distinct complex whose subunits favor interactions with each other over their conventional counterparts, and t-GRIPs have a distinct function in the maturation of post-meiotic spermatids into motile sperm. The 'conventional' GRIP proteins are also expressed in testis, and mutations in those genes disrupt cell division. In contrast, mutations in *t-GRIPs* do not impact cell division but impair post-division spermiogenesis and male fertility. Moreover, ectopically expressed t-GRIPs are absent from spermatocyte centrosomes, indicating that they do not associate with conventional GRIPs *in vivo*. However, when over-expressed in early embryos, we detect weak interactions between GRIPs and t-GRIPs, resulting in centrosome recruitment and disruption of division cycles. In post-meiotic spermatids, a splice variant of centrosomin, CnnT, recruits γ-TuRCs to the surface of the mitochondria converting them to a MTOC. However, CnnT recruits conventional GRIPs but not t-GRIPs to spermatid mitochondria. The t-GRIPs localize to an MTOC called the centriolar adjunct and are required to recruit γ-tubulin there. In *t-grip* mutants, no overt perturbations of the flagellar axoneme account for the lack of spermatid motility; rather, the maturation and integrity of the major mitochondrial derivative is impaired. Overall, this work indicates that variant γ-TuRCs may serve functional roles in diverse MTOCs to assist the specialized needs of different cell types.

B140/P1830

Cep215/Cdk5rap2 is essential for morphological differentiation of astrocytes

D. Kang[1], W. Shin[1], G. Kim[2], Y. Sung[2], K. Rhee[1]; [1]Biological Sciences, Seoul Natl University, Seoul, KOREA, REPUBLIC OF, [2]Medical Science, University of Ulsan College of Medicine, Seoul, KOREA, REPUBLIC OF.

Cep215/Cdk5rap2 is a centrosome protein which is involved in microtubule organization. Cep215 is also placed at specific subcellular locations and organizes microtubules outside the centrosome. Here, we report that Cep215 is involved in morphological differentiation of astrocytes. Cep215 was specifically localized at the glial processes as well as centrosomes in developing astrocytes. Morphological differentiation of astrocytes was suppressed in the *Cep215*-deleted P19 cells and in the Cep215-

depleted embryonic hippocampal culture. We confirm that the microtubule organizing function of Cep215 is critical for the glial process formation. However, Cep215 is not involved in the regulation of cell proliferation nor cell specification. Based on the results, we propose that Cep215 organizes microtubules for glial process formation during astrocyte differentiation. We also generated the knockout mice in which the *Cep215* gene was totally removed. The *Cep215* KO mice showed microcephaly as previously reported in *Cep215*$^{an/an}$ mutant mice.

B141/P1831

Drug-binding sites on tubulin as potential targets for novel anti-parasitic drugs

R. F. Luduena[1], J. A. Tuszynski[2], J. Zattoni[3]; [1]Biochemistry and Structural Biology, University of Texas Hlth San Antonio, San Antonio, TX, [2]Physics, University of Alberta, Edmonton, AB, CANADA, [3]Ingegneria Meccanica e Aerospaziale, Politecnico di Torino, Turin, ITALY.

The amino acid sequences of α-and β-tubulin are highly conserved in evolution, perhaps due to the complex conformational properties of tubulin that could mediate the dynamic properties that play a major role in microtubule function; also, tubulin has to interact with adjacent tubulin molecules. The colchicine binding site (CBS), largely located on β, is highly conserved. Nevertheless, there has been some divergence in the sequence of the CBS. There are differences in the CBS sequence in the human β isotypes and we have previously made colchicine analogues that can distinguish among them. It is noteworthy that the *Colchicum* plant, which makes colchicine, and the bees that pollinate it, have evolved alterations in the CBS, presumably to make these tubulins more resistant to colchicine. We propose here that organisms with unique sequences in the CBS could be vulnerable to colchicine analogues that might interact poorly with mammalian tubulin. One such is *Naegleria*, the "brain-eating ameba", which has two β isotypes, one flagellar and one mitotic, whose CBS sequences differ from each other and from those of other eukaryotes. There may be other pathogenic eukaryotes whose CBS could be examined. It is possible that appropriate modeling and designing of drugs to bind to the CBS of the tubulins from these organisms might result in drugs relatively non-toxic to humans and other mammals but quite toxic to the target organisms. At the same time, the complex functional requirements of the tubulin molecule may make it difficult for the target organism to evolve resistance to these drugs and still retain viability. The CBS is not the only promising target. We have used the amino acid sequences of the two α and two β tubulin isotypes of *Naegleria* to compare and contrast with the corresponding sequences for the human isotypes of tubulin whose binding sites for several ligands are well-characterized. We have identified the following compounds as providing potential scaffolds for derivatization: chalcone, epothilone, cevipabulin, dictyostatin, peloruside and dinitroaniline. With a homology model currently under development, candidate drug entities with specific and selective mode of action against *Naegleria* are being designed.

B142/P1832

Understanding how cancer cells hijack TUBB3 as a mechanism of chemoresistance

L. Wood, J. Moore; University of Colorado, Aurora, CO.

The microtubule network is integral to several hallmarks of a cancer cell such as migration, invasion, and uncontrolled proliferation. These cellular activities rely on remodeling of the microtubule network to perform specific functions and because of this reliance one of the most successful and potent chemotherapeutic classes is microtubule targeting agents. Tubulin isotypes are α and β proteins,

encoded by different genes, that vary in amino acid sequence and cell-type specific expression patterns. Ectopic expression of the β-tubulin-III (*TUBB3*) isotype accounts for 5-10% of the total tubulin pool in some cancers and correlates with resistance to taxane-based chemotherapies. Despite the correlation between *TUBB3* mRNA levels and taxane resistance, two major questions remain: 1) do isotype mRNA levels accurately reflect isotype protein levels in cancer cells? And 2) how might increased TUBB3 protein levels promote resistance to paclitaxel? To address the first question, we created GFP fusions to human β-tubulin isotypes and used these to validate a panel of isotype specific antibodies through immunoblotting. These antibodies allowed us to determine relative levels of TUBB3 and other β-tubulin isotypes in cancer cell lines known to have different levels of *TUBB3* mRNA . To address the second question, we used our panel of cell lines to test whether the level of TUBB3 protein determines response to physiological doses of paclitaxel in proliferation assays and the formation of the mitotic spindle. Our results show that concentrations of paclitaxel that inhibit proliferation also induce defects in mitotic spindle formation, with the magnitude of these defects being inversely proportional to the level of TUBB3 expression. We hypothesize that paclitaxel resistance is related to a cell's basal microtubule dynamics, which in turn are determined by the blend of β-tubulin isotypes. We are testing this hypothesis by measuring microtubule dynamics in cells lines with different isotype blends and in cells where we shift the isotype blend through knockout or ectopic expression of TUBB3, or alternative isotypes. These studies will define the impact of TUBB3 in cancer cells and give new insights into how changes in isotype expression translates to polymer composition and overall activity of the microtubule network.

B143/P1833

Diversification of alpha-tubulin for germline roles with functional consequences in cancers

R. Chen[1], L. M. Rice[2], C. M. Schroeder[1]; [1]Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX, [2]Department of Biophysics and Biochemistry, UT Southwestern Medical Center, Dallas, TX.

Microtubules are polymers required for fundamental cell processes in eukaryotes, including DNA segregation, cell motility, and cargo transport. The polymers are composed of αβ-tubulin dimers, and the ability of microtubules to dynamically grow and shrink depends on proper lateral and longitudinal contacts between individual dimers. The requirement for tubulin to preserve these polymerization interfaces and maintain dynamics imposes a strong selective constraint on tubulin structure. Most organisms express multiple paralogs of α-tubulin and β-tubulin, which consistently have very little variation in amino acid sequence. However, there is unexpected, recurrent evolution of α-tubulin paralogs that are highly divergent in amino acid sequence and specifically expressed in the germline. Although their function is unknown, the misexpression of these divergent α-tubulins in cancer correlates with decreased probability of survival. **We hypothesize that divergent α-tubulins tune microtubule dynamics for germline-specific processes and may endow cancerous cells with increased proliferative and migratory activity.** To test if sequence divergence in α-tubulin has led to distinct microtubule dynamics, we focused our studies on divergent α-tubulins found in humans: TUBA3E and TUBA3D. We identified their syntenic loci among many species and determined that TUBA3E and TUBA3D originated at least 160 million years ago and were largely retained across evolutionary time, implying a well-conserved function. We next exogenously expressed TUBA3D and TUBA3E in a cancer cell line to test if they have functionally diverged from canonical tubulin in a somatic context. Surprisingly, we discovered that TUBA3D readily incorporates into microtubules whereas TUBA3E does not. Furthermore, we found that both TUBA3E and TUBA3D alter microtubule growth speeds in distinct ways, suggesting that

sequence divergence has resulted in functional diversification from canonical α-tubulin. Interestingly, TUBA3E and TUBA3D differ by only six residues. To determine the molecular basis of TUBA3E and TUBA3D's distinct effects, we individually mutated TUBA3E residues to their TUBA3D counterparts and discovered that a single point mutation accounts for differences in TUBA3E localization when compared to TUBA3D. **Given both TUBA3D and TUBA3E'S functional diversification from canonical tubulin, we hypothesize divergent α-tubulins evolved specialized cytoskeletal dynamics for germline-specific processes and we are currently uncovering how their adaptation impacts cancer cell biology.**

B144/P1834

Analysis of factors required for *Centrocortin* mRNA localization to centrosomes in *Drosophila* embryos

C. Husbands, H. Zein-Sabatto, D. A. Lerit; Department of Cell Biology, Emory University, Atlanta, GA.

Centrosomes are highly organized and dynamic organelles and are the primary microtubule organizing center in animal cells that play a key role in ensuring proper cell division. Within centrosomes, a compartmentalized matrix of protein and RNA, known as the pericentriolar material (PCM), surrounds the centrioles. Centrosomes function in a multitude of roles throughout the cell cycle, including intracellular trafficking, spindle assembly, and polarization. Recent work from our group implicates mRNA localizes to the PCM and contributes to centrosome function, raising the possibility of post-transcriptional links between centrosome dysfunction and disease. One of these centrosomal localizing mRNAs within syncytial *Drosophila* embryos, *Centrocortin* (*Cen*) mRNA, is significantly enriched at the centrosome, where it is organized into micron-scale ribonucleoprotein complexes (RNPs). We previously showed *Cen* mRNA mislocalization disrupts nuclear division due to disorganization of microtubules and increased nuclear fallout that resulted in embryonic lethality. We are investigating the contribution of the minus-end directed dynein motor complex and associated regulatory cofactors to *Cen* mRNA localization to centrosomes. It is also possible that one or more specific sequences within the *Cen* gene are responsible for localization. Prior work, for example, indicates Cen translation is required for *Cen* mRNA localization to centrosomes, and *Cen* mRNA and protein colocalize within *Cen* RNPs. We are investigating this model and identifying cis-sequences within the *Cen* coding region important for *Cen* mRNA localization to centrosomes. This study aims to identify possible interacting sequences imperative for *Cen* centrosomal localization, aspects of which may be impaired in centrosomal disease and dysfunction.

B145/P1835

miR-1regulates mitotic spindles to mediate mitosis in early development

K. Hammond[1], M. Testa[1], N. Sampilo[2], J. Song[1]; [1]University of Delaware, Newark, DE, [2]Invitae, Iselin, NJ.

Title: miR-1regulates mitotic spindles to mediate mitosis in early development

Kayla Hammond[1], Nina Faye Sampilo[2], Michael Testa[1], and Jia L. Song[11]Department of Biological Sciences, University of Delaware, Newark, DE 19716[2] Invitae - Metropark (formerly Genosity), Iselin, NJ

AbstractMicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by binding to the 3'UTR and silencing translation of their target genes. miR-1 is a highly conserved miRNA that is known to regulate cardiac development, skeletal muscle proliferation and differentiation, blood vessel formation. However, not much is known about miR-1's role in early development. In the sea urchin embryo, miR-1 overexpression (OE) significantly delayed development, leading to a high percentage of embryos arrested at the cleavage stage, as well as embryonic lethality. Preliminary results show that

miR-1 OE results in misaligned mitotic spindles and defective cytokinesis, potentially leading to embryonic arrest. Using a candidate approach, we bioinformatically identified *Cdc42* and the *NuMA-LGN-Gαi* complex as potential miR-1 targets. Cdc42 has been known to play a part in the binding of microtubules to the kinetochores of chromosomes, as well as to play a role in actin polymerization, which is crucial for proper cytokinesis. The NuMA-LGN-Gαi complex is involved in anchoring astral microtubules to the cell cortex. Both miR-1 and its potential target transcripts have been shown to localize in close spatial proximity within the dividing cell. Cdc42 and LGN localize to the mitotic spindles, while NuMA and Gαi localize to the Microtubule Organizing Center (MTOC). miR-1 has been shown to localize to the mitotic spindle and cell cortex. We hypothesize that miR-1 regulates mitosis in part by its suppression of its targets *Cdc42* and the *NuMA-LGN-Gαi* complex. Using dual luciferase assay and site-directed mutagenesis, we demonstrated that miR-1 directly suppresses *Cdc42*. This work provides a deeper understanding of post-transcriptional regulation of an evolutionarily conserved miRNA and its role in mitosis.

B146/P1836

Centriole asymmetry break and inactivation in skeletal muscle cell

M. R. Pimentel, M. Faria, M. Bettencourt-Dias; Instituto Gulbenkian De Ciencia, Oeiras, PORTUGAL.

Skeletal muscle cells are the largest in our body, composing up to 40% of its mass. These postmitotic cells nucleate microtubules mainly from the Golgi and Nuclear Envelope, but what happens to their centrosome remains unknown. We followed centrioles during skeletal muscle differentiation and observed that Plk1 is promptly lost, prior to PCM decrease and eventual centriole disappearance. Interestingly, we noticed a break in mother-daughter centriole asymmetry with several of the respective components lost or relocalized. Depletion of proteins that were relocalized partially rescues centriole loss in differentiated cells. Our data suggest that centrosomes might be actively shut down during muscle differentiation.

B147/P1837

Muscle mechanisms that regulate postsynaptic development at the *Drosophila* neuromuscular junction: microtubules and BMP signaling

V. E. von Saucken[1,2], N. J. Torres-Santiago[2], S. E. Windner[2], M. K. Baylies[2]; [1]Tri-Institutional MD-PhD Program, Weill Medical College/Cornell University, New York, NY, [2]Developmental Biology Program, Sloan-Kettering Institute for Cancer Research, New York, NY.

Muscle-neuron communication at the neuromuscular junction (NMJ) has largely focused on the motor neuron's presynaptic machinery. However, the factors regulating specialized postsynaptic structures in the muscle are unclear and critical to understanding synaptic development and function. The postsynapse at the NMJ consists of membrane infoldings called the subsynaptic reticulum (SSR) in invertebrates. A potential regulator of SSR size is microtubule (MT)-based trafficking, as studies reveal that disrupting MT-mediated transport in the neuron or muscle effectively blocks NMJ growth during development. In addition, BMP (bone morphogenetic protein) pathway activation and signaling via downstream effector Mad in the motor neuron is known to regulate NMJ size and maintenance. In the muscle, *Mad* knockdown leads to reduced NMJ size; however, the mechanisms of how BMP signaling shapes the postsynapse is incomplete. Here we investigate the roles of the muscle's intracellular trafficking machinery and BMP receptor signaling in regulating postsynaptic development using the

simple and highly accessible *Drosophila* larval NMJ. In the multi-nucleated larval muscle, each nucleus serves as a MT organizing center, resulting in nuclear MT asters/arrays. We studied perinuclear MT density and orientation in established mutants with disrupted axon guidance ($DLar^{C12/2127}$), which have high variability in NMJ size and position. We show that NMJ proximal nuclei (<15 µm to the NMJ) are smaller and surrounded by fewer MTs than more distal nuclei (15-50 µm). However, the proportion of MTs oriented toward the NMJ is increased in NMJ proximal nuclei. MT projection to the NMJ strongly correlates with NMJ size, indicating that this pattern depends on NMJ signaling. To explore postsynaptic BMP signaling, we used muscle-specific *Mad* knockdown via RNAi. We find that, in addition to the change in SSR size, *Mad* knockdown alters the size of NMJ proximal nuclei. Our initial findings suggest preferential allocation of the muscle's MT network to the postsynaptic region and a critical role for the BMP pathway in shaping the postsynaptic environment. This ongoing work will provide important insights to the regulation of postsynaptic growth and maintenance and its dysfunction with disease and aging.

B148/P1838

Microtubule polymerase XMAP215/Mini spindles and cytoplasmic dynein are required for the oocyte determination in Drosophila

W. Lu, M. Lakonishok, V. I. Gelfand; Cell and Developmental Biology, Northwestern University Feinberg Sch Med, Chicago, IL.

In many species, only one oocyte is specified among a group of interconnected germline sister cells. In *Drosophila melanogaster*, 16-cell interconnected cells form a germline cyst, where one cell becomes the oocyte, while the rest become nurse cells that provide the oocyte with mRNAs, proteins, and organelles through intercellular cytoplasmic bridges via microtubule-based transport. In this study, we find that a microtubule polymerase Mini spindles (Msps), the *Drosophila* homolog of XMAP215, is essential for defining and maintaining of the oocyte fate determination. Knockdown of *msps* blocks the oocyte growth and causes gradual loss of oocyte determinants. *msps* knockdown abolishes microtubule polymerization in the oocyte and results in the absence of microtubules growing from the oocyte to the nurse cells. We demonstrated that cytoplasmic dynein, the main microtubule minus-end directed motor, is required for *msps* mRNA transport to the oocyte, and thus accumulation of translated Msps protein in the oocyte. The dynein-dependent concentration of Msps causes more microtubule plus-ends to grow from the oocyte to nurse cells, further enhancing dynein-dependent nurse cell-to-oocyte transport. Thus, the dynein-Msps duo creates a positive feedback loop that transforms a slight stochastic difference in microtubule polarity among sister cells into a clear oocyte fate determination.

B149/P1839

Self-organized intracellular twisters in *Drosophila* oocytes

R. Farhadifar[1], S. Dutta[2], G. Kabackaoglu[1], R. Blackwell[1], W. Lu[3], V. Gelfand[3], S. Shvartsman[1], M. Shelley[1]; [1]Center for Computational Biology, Flatiron Institute, New York, NY, [2]Lewis-Sigler Institute, Princeton University, Princeton, NJ, [3]Department of Cell and Developmental Biology, Northwestern University, Chicago, IL.

Cytoplasmic streaming is essential for transporting and mixing nutrients, proteins, and organelles within large plant and animal cells. The large ~300um *Drosophila* oocyte has recently gained attention for experimental and theoretical studies of this phenomenon. We present a quantitative study of streaming

in *Drosophila* oocytes that combines PIV of 3D time-lapse movies, with biophysical modeling and simulation. We observe a diverse family of 3D vortical flows across different oocytes, which differ in position and orientation, and which last tens of minutes. We show that a model of cytoskeletal activity at the periphery, organized by its interaction with interior fluid, explains the observed streaming structures. The emerging picture sheds light on a class of intracellular flows in large cells and highlights the wealth of questions at the interface of geometry, active matter, and basic biology.

B150/P1840

Tau and MAP6 Organizes Individual Microtubules into Stable and Labile Domains

K. Kirimtay, W. Huang, D. V. Wang, P. W. Baas; Drexel University, Philadelphia, PA

Previous studies on cultured juvenile rodent neurons documented that each microtubule in the axon consists of two distinct domains. The domain toward the plus end of the microtubule is dynamic and labile, while the domain toward the minus end of the microtubule is more stable. These two domains differ in their content of post-translationally modified tubulin subunits as well as their interactions with myriad proteins such as molecular motors and microtubule-severing enzymes. Curiously tau, long believed to be a critical stabilizer of axonal microtubules, was found to be enriched on the labile domain of the microtubule and crucial for enabling that domain to become long without being stabilized. Our goals in the present study were two-fold, first to investigate whether the same is true of tau in adult axons (which is more relevant to tau's prominent role in neurodegenerative diseases) and secondly to better understand mechanistically how tau keeps a substantial portion of the axonal mass from being stabilized. For the former, we introduced contemporary antisense oligonucleotides into the adult mouse brain, documented the diminution of tau over several days, and studied the effects on microtubule levels and stability. Even with the different tau isoforms in adult neurons compared to juvenile (3R and 4R in adult, but only 3R in juvenile), the results are consistent with labile domains of axonal microtubules becoming shorter and less dynamic when tau levels were lowered. Next, we ectopically co-expressed in fibroblasts fluorescently tagged tau and MAP6, a protein enriched on the stable domains of axonal microtubules and found these two proteins to segregate into domains on microtubules, with tau-rich domains being relatively labile and MAP6-rich domains being relatively stable. This was also consistent with the photobleaching experiments in which MAP6 bound to microtubules stably but binding and unbinding of tau was highly dynamic. This was true regardless of the which isoforms of tau or MAP6 were expressed. On this basis, we propose that in both juvenile and adult neurons, tau's principal role in regulating microtubule stability in the axon is to cooperatively concentrate on dynamic regions of microtubules and keep them dynamic by outcompeting genuine stabilizers such as MAP6.

B151/P1841

Cytoplasmic microtubule stability does not influence ciliary assembly

L. L. Dougherty, P. Avasthi; Dartmouth College, Hanover, NH

Cilia are dynamic antenna-like structures protruding from the plasma membrane that provide an important signaling and sometimes motile component to cells. Both the structure and assembly of cilia are heavily microtubule-based. Upon exiting the cell cycle, the microtubule superstructure of the cilium will assemble and disassemble upon cell cycle re-entry. Since proteins needed for cilium structure and function need to be synthesized in the cell body and trafficked to the cilium, microtubule-based trafficking mechanisms within the cell body is of interest. When there is an acute need for cilium

assembly, cytoplasmic microtubules are known to depolymerize. It has been speculated that this depolymerization occurred for the purpose of freeing tubulin dimers for availability to assemble into ciliary structures. Here we have found that cytoplasmic microtubule disassembly is not required for ciliary regrowth following ciliary excision due to the inability to affect cilium growth when depolymerization is inhibited. Using mechanical and chemical perturbations, we find that cytoplasmic microtubule organization is independent of ciliary assembly.

B152/P1842

Microtubule glutamylation is dispensable for centriole stability in *C. elegans*

N. Peel, B. Shah, J. Lee, D. Chawla; THE COLLEGE OF NEW JERSEY, Ewing, NJ

Glutamylation, the covalent attachment of glutamic acid to tubulin in the polymerized microtubule, is enriched on long-lived microtubules. It is thought to contribute to centriole stability, cilia motility and axon function. Glutamylation of the microtubules is catalyzed by a family of tubulin tyrosine ligase like (TTLL) enzymes. Comprehensive in vivo analyses of the function of tubulin glutamylation have proved challenging because of the existence of a large family of TTLL enzymes in many species. To investigate the function of tubulin glutamylation we have generated a *ttll-4(tm3310)*; *ttll-11(tm4059); ttll-15(tm3871) ttll-5(tm3360) ttll-9(tm3889)* 'quint' mutant that lacks all five *C. elegans* glutamylating TTLL enzymes. The objective of our study is to use this quint mutant to determine the essential functions of tubulin glutamylation. We find that the quint mutant shows normal embryonic viability and brood size. The localization of centriole markers ZYG-1, SPD-2 and SAS-4 is normal, moreover the centrosome organizes a spindle that is competent for cell division. Our data therefore suggest that, contrary to expectations, in *C. elegans* microtubule glutamylation is not essential for centriole stability. Interestingly we have found, that our quint mutant shows enhanced colchicine and cold-sensitivity, suggesting that microtubule stability in inclement conditions is altered by the loss of glutamylation. We are currently undertaking a detailed analysis of microtubule dynamics to determine the underlying cause of altered microtubule properties in the quint mutant.

B153/P1843

Specific domains of Ninein and its associations with partners Dynein and Ensconsin are necessary for Ninein localization and microtubule organization in the *Drosophila* larval fat body

M. M. L. Tillery[1], Y. Zheng[2], T. L. Megraw[1]; [1]Florida State University, Tallahassee, FL, [2]Xiamen University, Fujian, China

The rich diversity of non-centrosomal MTOCs (ncMTOCs) has begun to be appreciated in a range of cell types across a variety of organisms. The composition, function, and subcellular localization of ncMTOCs is unique to meet the specific needs of the cells in which they are located. Our lab recently reported a novel perinuclear ncMTOC in *Drosophila* larval fat body cells that organizes circumferential and radial microtubules (MTs) and is required for maintaining nuclear centricity and coordinating endosomal trafficking. MT assembly in fat body cells requires the parallel activities of the MT minus-end stabilizer Patronin/CAMSAP and the MT anchor Ninein (Nin).

Nin functions at both centrosomal and non-centrosomal MTOCs. In the fat body, Nin localizes to the nuclear surface mostly independent of MTs while a fraction localizes intranuclearly. Neither of the *Drosophila* Nesprins Msp300 or klarsicht are required for Nin localization. In other systems, Nin interacts with the dynein complex and Ensconsin/MAP7 (Ens), so we explored their relationship with Nin in the

fat body. We found that loss of core dynein subunits, but not the inhibition of dynactin, causes Nin to localize inside the nucleus of fat body cells. Domain mapping revealed this localization relies on the presence of a central domain of Nin (aa 454-570). Interestingly, this region is also responsible for localizing Nin intranuclearly in a wild-type dynein background. Nin physically interacts with Ens via a region in the C-terminus of Nin (aa 550-777). In the fat body, overexpression of Nin forms ectopic ncMTOCs that recruit Ens. When Nin constructs containing amino acids 550-777 are overexpressed with Ens, significantly more robust ncMTOCs are formed adjacent to the nucleus that affect nuclear integrity, resulting in the apparent leakage of chromosomes into the cytoplasm. Our preliminary genetic and cell biological data suggests that Nin requires both dynein and Ens for its role at the fat body ncMTOC.

B154/P1844

Investigating the role of tubulin-tyrosine ligase (TTL) in DREADD-mediated enhancement of sensory axon growth

A. Islam, V. J. Tom; Drexel University, Philadelphia, PA

Injury to the mature nervous system often results in permanent functional deficits. One factor limiting recovery is that neurons lose the intrinsic ability to regenerate their axons after development. Our lab showed that activating adult dorsal root ganglia (DRG) neurons using chemogenetics, i.e., designer receptors exclusively activated by designer drugs (DREADDs), in vivo, increases the ability to regenerate axons into the spinal cord after a dorsal root crush injury. This was associated with more axons turning off white matter and into grey matter and other neurons, potential synaptic partners. This increased growth and turning may be attributed to microtubules, components of the cytoskeleton important for development, shape and motility, and organelle transport. Microtubules have two distinct domains, either stable or labile, depending on the type of post-translational modifications. While mature neurons contain a high concentration of stable (acetylated) microtubules in their axons, increasing stable microtubules may lead to forced polymerization of tubulin, causing abnormal axon growth. Meanwhile, labile (tyrosinated) microtubules are much more dynamic and is necessary for normal growth-cone motility and axon extension. Our findings showed that when neurons are activated via DREADDs, there is an increase in labile, tyrosinated microtubules that mediates the improved axon outgrowth from activated neurons. This increase in tyrosination after neuronal activation may be caused by tubulin-tyrosine ligase (TTL), a protein which tyrosinates tubulin. We hypothesize that TTL is involved in improved axon outgrowth upon neuron activation. We are investigating how TTL expression is affected by DREADD-mediated neuronal activation and its role in enhanced axon regeneration. Elucidating the mechanisms involved in axon regeneration after neuron activation will identify potential molecular targets to increase the regenerative capability of adult neurons after injury.

B155/P1845

Characterization of a novel microtubule-binding protein CLIPR76 and its role in cardiomyocyte development

A. T. Bryant, J. S. V. McCourt, G. Ugrinova, C. Whalen, H. V. Goodson; University of Notre Dame, Notre Dame, IN

Characterization of the Novel Microtubule-Binding Protein CLIPR76 and Its Role in Cardiomyocyte Development

Microtubules (MTs) are critical filaments in the eukaryotic cytoskeleton and contribute to cellular

functions such as cellular migration, intracellular trafficking, and key processes in the cell cycle, including chromosomal segregation and cytokinesis. These functions are achieved through the coordination of MT-based motor proteins, such as dynein and kinesin, and non-motor proteins, such as cytoplasmic linker proteins (CLIPs) and their relatives (CLIPRs). We have identified a new CLIPR protein called CLIPR76 that is encoded by the *CLIP4* gene and spans 166 kb with the longest open reading frame encoding a 76 kDa protein. Bioinformatics analysis of the NCBI Reference Sequence Database suggests that there are at least three isoforms of CLIPR76 in humans, though which isoforms exist as protein is unclear. Overexpression of isoform-specific CLIPR76 constructs in the mouse fibroblast cell line NIH3T3 indicate that isoforms 1 and 3 localize to MTs whereas isoform 4 localizes to the endoplasmic reticulum (ER). Preliminary RT-qPCR data using a primer common to all isoforms of CLIPR76 shows that CLIPR76 mRNA is most highly expressed in striated muscle, such as cardiac and skeletal muscle, in murine tissues. Furthermore, CLIPR76 protein is highly upregulated when murine C2C12 myoblast cells are induced to differentiate into skeletal muscle. In order to further elucidate the role of CLIPR76 in striated muscle development, we have shifted our model system from murine C2C12 cells to human induced-pluripotent stem cells (iPSCs) differentiated into beating cardiomyocytes (a cardiac version of striated muscle). We are conducting immunofluorescence experiments on differentiating cardiomyocytes using our CLIPR76 antibody and markers for various organelles, such as ER, and cytoskeletal filaments, such as actin and modified MTs, to assess CLIPR76 localization. CRISPR knockout of *CLIP4* is currently underway in the lab to assess the effects of depleting CLIPR76 in developing cardiomyocytes. Additionally, we are utilizing qualitative real-time PCR to determine the relative levels of CLIPR76 mRNA at various developmental timepoints with primers specific to each isoform. In parallel, we are planning experiments to identify peptides specific to each isoform via immunoprecipitation with our CLIPR76 antibody followed by mass spectrometry analysis. Microtubules are a key element in the cardiomyocyte cytoskeleton where they function as scaffolds for myofilaments and aid in the positioning and organization of organelles; our work with CLIPR76, a novel MT-binding protein that also binds membranes (ER), seeks to further elucidate the role of CLIPR76 in cardiac muscle development.

B156/P1846

Stem cell pluripotency - a microtubule cytoskeleton regulated state?

J. Greaney[1], A. Hawdon[1], Y. Li[1], A. Aberkane[1], G. Stathatos[2], R. Parton[3], **J. Zenker**[1]; [1]Monash University, Clayton, Australia, [2]University of Melbourne, Melbourne, Australia, [3]University of Queensland, Brisbane, Australia

How equipotent cells develop into complex tissues containing many diverse cell types is one of the most fundamental as well as most important question in biology. The organisation of a cell's interior, the cytoskeleton and organelles, is pivotal for every cell's functionality. However, unlike most differentiated cells, our knowledge about the contribution of the sub-cellular architecture to pluripotency remains scarce.Using cutting-edge live imaging, we discovered polarised non-centrosomal microtubules as central player for the pluripotent state of the *in vivo* early mammalian embryo and human induced pluripotent stem cells (hiPSCs). Contrary to the textbook view that microtubules radiate symmetrically from the centrosome to the periphery of a cell, the microtubules of the preimplantation embryo grow from the so called "interphase bridges" asymmetrically from one side of the cell to the other. These non-centrosomal microtubules, anchored and nucleated by the non-centrosomal microtubule minus end marker calmodulin-regulated spectrin-associated protein 3 (CAMSAP3), initiate an asymmetry of organelles and molecules, including RNAs, as cells become more specialised *in vivo* and *in vitro*. We

further establish that the CAMSAP3-dependent microtubules rearrange upon differention in a germ layer-specific manner. Dissecting how intrinsic cellular regulation contributes to pluripotency might lead to a revolutionary era of regenerative and reproductive medicine.

Microtubule Dynamics: Post-translational Modifications

B157/P1847

Differential modifications of the C-terminal tails of α-tubulin isotypes and their importance for kinesin based microtubule transport *in vivo*

M. Bao, R. Dörig, D. Beuchle, P. M. Vazquez-Pianzola, B. Suter; University of Bern, Institute of Cell Biolody, Bern, SWITZERLAND.

Microtubules (MTs) are built from α-/β-tubulin dimers and used as tracks by kinesin and dynein motors to transport a variety of cargos, such as mRNAs, proteins, and organelles, within the cell. Tubulins are subjected to several post-translational modifications (PTMs). Glutamylation is one of them, and it is responsible for adding one or more glutamic acid residues as branched peptide chains to the C-terminal tails of both α- and β-tubulin. However, very little is known about the specific modifications found on the different tubulin isoforms *in vivo* and the role of these PTMs in cargo transport along MTs *in vivo*. In this study, we found that in *Drosophila*, glutamylation of the α-tubulin isoforms occurs specifically on the C-terminal ends of TBA1 and TBA3 in the ovaries. In contrast, the ovarian isoform TBA4 is not glutamylated. The C-terminal ends of TBA1 and TBA3 are glutamylated at several glutamyl side chains in various combinations. *Drosophila TTLL5* is required for the mono- and poly-glutamylation of ovarian TBA1 and 3. Furthermore, glutamylation of the α-tubulin is essential for the efficient localization of Staufen/*osk* mRNA and to give directionality to the fast ooplasmic streaming, two processes known to depend on kinesin-mediated processes during oogenesis. In the nervous system, the kinesin-dependent neuronal transport of mitochondria also depends on *TTLL5*. Additionally, α-tubulin glutamylation affects the pausing of the transport of individual mitochondria in the axons. Our results demonstrate the *in vivo* role of *TTLL5* in differential glutamylation of α-tubulin isoforms and point to the *in vivo* importance of α-tubulin glutamylation for kinesin-dependent processes.

B158/P1848

Genetic manipulation of α-tubulin detyrosination/tyrosination reveals a direct role in the regulation of chromosome congression and segregation fidelity during human cell division

H. Girão[1,2], H. Maiato[1,2,3]; [1]Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal, Porto, PORTUGAL, [2]Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal, Porto, PORTUGAL, [3]Cell Division Group, Experimental Biology Unit, Department of Biomedicine, Faculdade de Medicina, Universidade do Porto, Porto, Portugal, Porto, PORTUGAL.

Tubulin undergoes several enzymatic posttranslational modifications (PTMs) that generate chemical landmarks that affect microtubule behavior and protein interactions. One key PTM consists on the removal of the C-terminal tyrosine residue of α-tubulin on microtubules by tubulin carboxypeptidases. Free detyrosinated tubulin released from microtubules is then re-tyrosinated by tubulin tyrosine ligase (TTL). Several studies relying on TTL overexpression or unspecific α-tubulin carboxypeptidase inhibitors have implicated the α-tubulin detyrosination/tyrosination cycle in important cellular processes, such as mitosis, neuronal function, and muscle contraction. However, indirect side effects associated with these

approaches cannot be excluded. Recently, the identification of tubulin detyrosinating enzymes Vasohibin 1 and 2 (VASH1/2) opened the way for the genetic manipulation of α-tubulin detyrosination. To determine how the detyrosination/tyrosination of α-tubulin impacts mitosis, we challenged human U2OS cells to grow in extreme tubulin detyrosination or tyrosination conditions. This was achieved by ectopic overexpression of GFP-tagged detyrosinated or tyrosinated α-tubulin in cells knockout (KO) for TTL or VASH1/2, respectively. Super-resolution CH-STED microscopy confirmed that TTL KO cells overexpressing detyrosinated α-tubulin showed high levels of this PTM on mitotic spindle microtubules, especially k-Fibers and astral microtubules. In contrast, VASH1/2 KO cells overexpressing tyrosinated α-tubulin showed extremely low detyrosinated α-tubulin in all mitotic spindle microtubule subpopulations. Live-cell imaging revealed that mitotic cells growing under extremely high detyrosinated α-tubulin conditions have enlarged mitotic spindles and show a dramatic increase in chromosome segregation errors. On the other hand, cells growing under extremely low α-tubulin detyrosination showed penetrant chromosome congression defects, including the formation of chronically misaligned chromosomes at the poles. These findings, provide direct genetic evidence for a role of the α-tubulin detyrosination/tyrosination cycle in the regulation of mitotic spindle length, error correction and chromosome congression during human cell division.

B159/P1849

Microtubule stability reinforces stochastic detyrosination to define functional microtubule subsets

Q. Tang[1], C. Bond[1], S. Sensale[2], A. Qiao[2], A. Arab[1], S. Hugelier[1], G. Arya[2], M. Lakadamyali[1]; [1]Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, [2]Mechanical Engineering and Materials Science, Duke University, Durham, NC.

Microtubules in cells are segregated into functionally diverse subpopulations carrying distinct post-translational modifications (PTMs). Akin to histone code, the tubulin code regulates myriad microtubule functions ranging from intracellular transport to chromosome segregation. However, how individual PTMs only occur on subsets of microtubules to contribute to microtubule specialization is not well understood. In particular, microtubule detyrosination, which is the removal of the C-terminal tyrosine on α-tubulin subunits, marks the stable population of microtubules and modifies how microtubules interact with other microtubule-associated proteins to regulate a wide range of cellular processes. Previously, we found that only a small subpopulation of microtubules are highly enriched with the detyrosination mark (~30%) and that detyrosination spans most of the length of a microtubule, often adjacent to a completely tyrosinated microtubule. How a cytosolic detyrosinase, Vasohibin (VASH), builds up and maintains only a small subpopulation of highly detyrosinated microtubules is unclear. Here, using quantitative super-resolution microscopy, we visualized nascent microtubule detyrosination events in cells consisting of 1-3 detyrosinated α-tubulin subunits after Nocodazole washout. Microtubule detyrosination accumulates slowly and in a disperse pattern across the microtubule length. By visualizing single molecules of VASH in live cells, we found that VASH engages with microtubules stochastically on a short time scale with no apparent cooperativity, indicating limited removal of tyrosine per interaction, consistent with the super-resolution results. Combining these quantitative imaging results with simulations incorporating parameters from our experiments, we propose a stochastic model for cells to establish a subset of detyrosinated microtubules via a detyrosination-stabilization feedback mechanism.

B160/P1850

A novel probe illuminates the chemical logic of microtubule detyrosination

Y. Yue, T. Hotta, K. J. Verhey, R. Ohi; Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI.

Detyrosination is a post-translational modification (PTM) of α-tubulin that is critical for cell function in many physiological contexts. The carboxypeptidases responsible for this modification, VASH/SVBP and MATCAP, have been identified recently. How these enzymes select specific microtubules for detyrosination remains elusive. To study this, we generated an Alexa488-labeled fragment antigen-binding (Fab) protein that specifically recognizes detyrosinated microtubules (ΔY-Fab^{488}). We validate the ΔY-Fab^{488} as a probe by showing that taxol treatment, which is known to increase detyrosination, results in the efficient decoration of microtubules by the ΔY-Fab^{488} probe in living cells and rapid decoration of taxol-stabilized microtubules upon incubation with VASH1/SVBP in *in vitro* assays. Thus, the ΔY-Fab^{488} probe allows us to visualize the process of microtubule detyrosination in living cells and *in vitro* with high spatial and temporal resolution. We then turned to single-molecule imaging assays to examine the mechanism of detyrosination by VASH1/SVBP. We show that VASH1/SVBP undergoes frequent short interactions with taxol-stabilized microtubules regardless of their detyrosination state. These interactions result in rapid detyrosination of the entire microtubule lattice. In contrast, VASH1/SVBP activity is sensitive to the nucleotide state of the microtubules as VASH1/SVBP rapidly detyrosinates GMPCPP-stabilized but not GDP-tyrosinated microtubules. Collectively, our work suggests the underlying mechanism by which VASH1/SVBP detyrosinates microtubules and also provides a novel tool to monitor microtubule PTMs in living cells and *in vitro*.

B161/P1851

Physical properties of the cytoplasm tune tubulin post-translational modifications

Y. Shen, K. M. Ori-McKenney; Department of Molecular and Cellular Biology, University of California, Davis, University of California, Davis, CA.

Microtubules tune the physical properties of the cytoplasm; however, it is poorly understood how microtubules could be reshaped by the complex physical environment present in a living cell, which is often crowded, heterogenous and fluctuating. A recent study has demonstrated that the rates of both microtubule polymerization and depolymerization scale linearly and inversely with cytoplasmic crowdedness. Here, we ask how tubulin post-translational modifications (PTMs) and microtubule associated proteins (MAPs) respond to changes in the cytoplasm by applying osmotic challenges to three types of epithelial cells and evaluating the interplay between tubulin PTMs and MAPs and their effects on cargo transport. By using single-particle tracking of genetically encoded multimeric nanoparticles (GEMs), we characterize the spatial heterogeneity of the crowdedness of the cytoplasm and monitor these changes upon osmotic challenges. We find that the α-tubulin C-terminal tail modification, detyrosination, and the α-tubulin luminal modification, acetylation, which both mark stabilized microtubules, respond differently to an osmotic challenge. Increasing cytoplasmic crowdedness gradually increases the acetylation level, whereas decreasing the cytoplasmic crowdedness causes the opposite. However, a sharp increase of detyrosination is only observed upon decreasing crowdedness, whereas α-tubulin tyrosination is insensitive to the osmotic challenge. Interestingly, these changes of PTMs are correlated with changes in the association of MAP7, a major MAP in epithelial cells that is important for kinesin-1 driven transport. MAP7 and acetylated microtubules largely colocalize and

enrich at perinuclear region where the local crowdedness is higher than that of the cell periphery. Upon decreasing cytoplasmic crowdedness, MAP7 drastically disassociates from microtubules. Over-expression of MAP7 does not affect tyrosination, but causes an increase of acetylation and lessens the effects of osmotic challenge to acetylation levels. Taken together, our findings suggest that the physical cue from the cytoplasm may signal deeper to the microtubules than we previously appreciated, which may help explain the spatial heterogeneity of tubulin PTMs and MAPs and their interplay in cells under various physiological and pathological conditions.

B162/P1852

Polyglutamylation of microtubules drives motor axon remodeling

A. Gavoci[1], M. Wang[1], N. Marahori[1], C. Janke[2], M. Magiera[3], T. Misgeld[1], **M. S. Brill**[1]; [1]Technical Universtiy Munich, institute of Neuronal Cell Biology, Munich, GERMANY, [2]Institut Curie, PSL Research University, CNRS UMR3348, F-91401 Orsay, France; Université Paris-Saclay, CNRS UMR3348, F-91401 Orsay, France, Orsay, FRANCE, [3]Institut Curie, PSL Research University, CNRS UMR3348, F-91401 Orsay, France; Université Paris-Saclay, CNRS UMR3348, Orsay, FRANCE.

Motor axons undergo activity driven synapse elimination of terminal branches. Loss of microtubules in early phases of axon competition is a critical factor, which is in part mediated by the microtubule severing enzyme spastin recruited to polyglutamylated microtubules. The underlying regulatory mechanisms for these branch-specific cytoskeletal re-arrangements are unknown. Consistent with an instructive role of polyglutamylation, motor neurons ablating the deglutamylases CCP1&6 accelerated axon dismantling, while deleting the chain elongating glutamylase TTLL1, delayed axonal remodeling. Further measurements of polyglutamylation, microtubule mass and dynamics corroborate the predicted branch-specific regulation of microtubule stability, hinting at a rheostatic regulation of spastin-mediated severing and hence axonal remodeling. Surprisingly, deleting TTLL7, which 'seeds' the first glutamate residue, had no effect on polyglutamylation, suggesting several layers of regulation that are ingrained in parallel or consecutive steps of editing the 'tubulin code'. Neurotransmission coordinates glutamylases and deglutamylases function, as blockade of synaptic activity to mimic a dismantling branch, modulated polyglutamylation and reduced tubulin mass. Hence, a specific posttranslational modification of tubulin, polyglutamylation, acts as an instructive signal for spastin-mediated severing, which in turn paces developmental axon pruning. The 'tubulin code' could thus control specific morphogenetic events during nervous system development and could similarly determine axon stability in central neurons and during disease.

B163/P1853

Tubulin arginylation regulates microtubule dynamics via MAP1S

B. MacTaggart, A. Kashina; University of Pennsylvania, Philadelphia, PA.

Arginylation is the post-translational addition of arginine to a protein by arginyl transfer enzyme 1 (ATE1). Previous studies have found that ATE1 targets multiple cytoskeletal proteins, and *Ate1* deletion causes cytoskeletal defects, including reduced cell motility and adhesion. Some of these defects have been linked to actin arginylation, but the role of other arginylated cytoskeletal proteins has not yet been studied. Here, we characterize tubulin arginylation and its role in the microtubule cytoskeleton. Mass spectrometry of Taxol-purified cellular microtubules identified arginylation of α- (E77 of *TUBA1B*) and β-tubulin (D74 of *TUBB*), which is absent in *Ate1*$^{-/-}$ cells. While *Ate1* deletion did not visibly affect the level

or distribution of tubulin, *Ate1*$^{-/-}$ cells showed a reduction in EB1 comet velocity compared to wildtype, indicating reduced microtubule growth. Moreover, when treated with nocodazole, *Ate1*$^{-/-}$ cells showed an increased fraction of depolymerization-resistant microtubules compared to wildtype, indicating increased microtubule stability. These phenotypes were rescued by exogenous ATE1, but not by exogenous arginylated β-actin, supporting a specific role for tubulin arginylation. Comparison of the microtubule associated protein fractions from wildtype and *Ate1*$^{-/-}$ cells showed that *Ate1* knockout results in an increased amount of MAP1S associated with microtubules. Knocking down *Map1s* in *Ate1*$^{-/-}$ cells was able to rescue microtubule growth rate and stability to wildtype levels. These effects are consistent with the previously demonstrated roles of MAP1S in limiting microtubule growth rate and promoting microtubule stability. Furthermore, overexpression of non-arginylatable α-tubulin (E77A) recapitulated the microtubule defects observed in *Ate1*$^{-/-}$ cells, namely decreased microtubule growth rate, increased microtubule stability, and an increased fraction of MAP1S associated with the polymerized tubulin fraction. Together, these results demonstrate a new type of tubulin regulation by post-translational arginylation, which regulates microtubule growth rate and stability through the microtubule associated protein, MAP1S.

B164/P1854

Acetylation regulates multiple functions of the TOG protein Stu2, an XMAP215/CKAP5 homologue.

M. A. Greenlee, B. Witt, J. Sabo, S. C. Morris, R. K. Miller; Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK.

Stu2 is the yeast member of the XMAP215/Dis1/CKAP5/chTOG family of microtubule associated proteins. These proteins have multiple functions in controlling microtubules, including microtubule polymerization, microtubule depolymerization, linking chromosomes to the kinetochore, and assembly of γ-TuSCs at the SPB. Whereas phosphorylation has been shown to be critical for Stu2 localization at the kinetochore, other regulatory mechanisms that control Stu2 function are still poorly understood. Here, we report that acetylation is a novel form of Stu2 regulation. Using western blotting and mass spectrometry, we identified three acetylated lysine residues at K252, K469, and K870, located in three distinct domains of Stu2, in TOG1, in TOG2, and in the MAP interacting domains, respectively. Homology alignments showed that lysines 469 and 870 are highly conserved across evolution, from fungi, plants, and vertebrates to humans. To study functional significance, we generated a series of K to Q substitutions in Stu2 that act as acetyl-mimetics, nullifying lysine's positive charge. We also made a similar series of K to R substitution that act as acetyl-blocking mutations. Several combinations of double and triple mutations were also constructed. In extracts prepared from logarithmically growing cultures, these mutants were abundant at levels equivalent to WT Stu2. These acetyl-mimetic and acetyl-blocking mutations did not impact the essential function of Stu2. However, mutational analyses revealed distinct functions associated with the three different sites. Some of these mutations lead to a decrease in chromosome stability. Other mutant combinations lead to increased resistance to the microtubule depolymerization drug, benomyl. Based on published crystallography coordinates from other labs, we developed an *in silico* model that predicted altered interactions between Stu2p mutants and γ-tubulin. In agreement with this model, western blotting showed that several acetylation-mimetic mutants displayed increased interactions with γ-tubulin. Our analysis of the mutants suggests a hierarchy of intragenetic epistatic regulation for Stu2 acetylation at the SPB and kinetochore. Together, these data suggest that acetylation is a previously unappreciated mechanism that governs multiple Stu2 functions, including chromosome stability and interactions at the SPB. Funding by NIH-R15GM119117-01.

B165/P1855

alpha-tubulin H11' is a key site for the intrinsic and extrinsic regulation of microtubule dynamics

K. J. Hoff, J. K. Moore; University of Colorado, Aurora, CO.

Microtubule dynamics in cells are regulated both by extrinsic factors, such as microtubule-associated proteins (MAPs), as well as intrinsic mechanisms from the tubulin heterodimer itself. Free tubulin heterodimer exists in a curved conformation that straightens as it is assembles into the microtubule lattice. This transition between conformational states represents a potentially powerful point of regulation; however, much remains unknown about how this transition might be controlled by different regions of the heterodimer and interactions with MAPs. We previously identified the helix 11' (H11') region of alpha-tubulin as a strong candidate for regulating this transition. Mutations in H11' cause human brain malformations and alter tubulin's intrinsic polymerization activity and interactions with XMAP215/Stu2. In this study, we use an evolutionary approach to further our understanding of the relationship between H11', regulatory MAPs, and tubulin conformation. By aligning alpha-tubulin sequences from over 500 species, we find that H11' is highly conserved, except notably in the mitotic tubulins expressed in *Naegleria fowleri* and *Naegleria gruberi*. These divergent tubulins have unique properties, suggesting that the variants found in H11' may contribute to these distinctions. We hypothesize that H11' plays a key role in the regulation of tubulin conformation, and that the divergent *Naegleria* H11' disrupts normal conformation changes, resulting in a straightened heterodimer. To measure the impact of these H11' variants on tubulin function, and ultimately decipher the regulatory role of H11', we modeled the *Naegleria* H11' by introducing mimetic mutations at the corresponding residues in *Saccharomyces cerevisiae*. Our results reveal that these variants impact cellular fitness, create hyper-stable microtubules, and disrupt normal microtubule regulation, similarly to what we expect from a straightened heterodimer. This work highlights the role of alpha-tubulin H11' in intrinsic heterodimer conformation dynamics, as well as how it impacts conformation-sensing extrinsic MAPs. Both intrinsic and extrinsic factors play crucial roles in microtubule dynamics regulation throughout development and disease, and this work emphasizes how these mechanisms are not separate control points, but rater are intricately linked.

Assembly and Disassembly of Cilia/Flagella

B169/P1856

Testing the ion-current model for *Chlamydomonas* flagellar length sensing

H. Ishikawa[1], J. Moore[2], D. Diener[3], M. Delling[1], W. Marshall[1]; [1]University of California, San Francisco, San Francisco, CA, [2]Yale University, New Heaven, CT, [3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, GERMANY.

Eukaryotic cilia and flagella are microtubule-based organelles whose relatively simple shape makes them ideal for investigating the mechanisms of fundamental size regulation. Most of the flagellar materials are transported from cell body via an active transport process called intraflagellar transport (IFT) because no protein synthesis occurs in the flagellum. The rate of IFT injection has been shown to negatively correlate with flagellar length. However, it remains unknown how the cell measures the length of its flagella and controls IFT injection. Here we experimentally tested one theoretical model of flagellar length-sensing: the ion-current model. This model posits that there is a uniform distribution of

Ca^{2+} channels along the flagellum and that the Ca^{2+} current from the flagellum into the cell body increases linearly with flagellar length. In this model, the cell uses the Ca^{2+} current to negatively regulate IFT injection. To test this model, we measured and manipulated the levels of Ca^{2+} inside of *Chlamydomonas* flagella and quantified IFT injection. Although level of Ca^{2+} inside of flagella was weakly correlated with the length of flagella, we found that IFT injection was reduced in calcium-deficient flagella, rather than increased as a model predicted, and that variation in IFT injection was uncorrelated with the occurrence of flagellar Ca^{2+} spikes. Thus, Ca^{2+} does not work as a negative regulator of IFT injection, hence it cannot form the basis of a stable length control system.

B170/P1857

IFT-A structure reveals carriages for membrane protein transport into cilia

S. J. Hesketh, A. G. Mukhopadhyay, D. Nakamura, K. Toropova, A. J. Roberts; Department of Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck College, London, UNITED KINGDOM.

Intraflagellar transport (IFT) trains are molecular machines that traffic proteins between cilia and the cell body. With a molecular weight over 80 MDa, each IFT train is a dynamic polymer of two large complexes (IFT-A and IFT-B) and motor proteins - posing a formidable challenge to mechanistic understanding. Here, we reconstituted the human IFT-A complex and overcame its conformational flexibility to obtain its structure using cryo-EM. Combined with AlphaFold analysis and genome-edited mammalian cell data, our results illuminate how IFT-A polymerizes; interacts with IFT-B; and uses an array of β-propeller and TPR domains to create “carriages” of the IFT train that engage TULP adaptor proteins. These data establish a structural link between IFT-A’s different functions; provide a blueprint for the IFT-A train; and shed light on how IFT evolved from a proto-coatamer ancestor.

B171/P1858

Role of Sfi1 proteins in *Stentor coeruleus* membranellar band regeneration

C. Yan, P. Sood, R. McGillivary, A. Lin, W. Marshall; University of California, San Francisco, San Francisco, CA.

In response to injury and external perturbations, it is vital for individual cells to be able to heal and re-build complex structures. The giant ciliate *Stentor coeruleus* is a classical model system for studying regeneration, patterning and morphogenesis at a single cell level. At the anterior of the cell is the membranellar band (MB), which consists of organized clusters of cilia. When induced to shed its oral apparatus with an osmotic shock, the cell will generate a new oral structure in a series of defined morphological steps. This raises the question of what molecular signals are involved in building complex cellular structures and re-establishing normal patterning of the cell. A previous transcriptional study and proteomic analysis of *Stentor coeruleus* revealed several Sfi1 proteins involved in MB regeneration. While Sfi1 proteins had not previously been identified in Stentor, Sfi1 in other ciliate systems contain centrin-binding repeat motifs and are implicated in basal body orientation, stability and Ca2+ induced contraction. RNAi knockdown of Sfi1 shows that Sfi1 is involved in the completion of regeneration, with earlier expressed Sfi1 proteins being more critical than those expressed later. Immunofluorescence shows Sfi1 localizes as lateral fibers in between the cortical rows throughout the cell, as well as the cortical rows in the posterior of the cell. Similarly, centrin localizes as a mesh-like network in the anterior of the cell, and only in the cortical rows in the posterior of the cell. Sfi1 and centrin also localize to the developing oral primordium as well as the completed oral apparatus. Interestingly, in

regenerating RNAi knockdown Sfi1 cells, centrin does not appear to localize to the oral primordium. In non-regenerating Sfi1 knockdown cells, the networks of centrin are less dense and more localized to the cortical rows. This indicates that Sfi1 regulates the localization of centrin in static cells, as well as cells during regeneration, and these interactions may play a role in building and organizing the oral primordium. These concepts will shed light on sub-cellular regeneration paradigms in other systems and will give rise to insights to how individual cells are able to recover from external injury.

B172/P1859

High Temporal Resolution Mapping of the Phosphorylation Footsteps of Ciliogenesis

R. E. Turn[1], M. Aziz-Zanjani[1], T. Kanie[2], J. Demeter[1], P. K. Jackson[1]; [1]Stanford University, Stanford, CA, [2]University of Oklahoma Health Sciences Center, Oklahoma City, OK.

The primary cilium serves as a pivotal signaling hub to drive essential life functions, ranging from development, metabolism, neural transmission, vision, olfaction, and more. This small, specialized organelle is highly organized, and failure to coordinate the generation of a functional cilium leads to severe pathologies, ranging from developmental disorders, diabetes, blindness, and even cancer. To date, though, the cilium is largely understudied in part because of its size (~3µm long, ~250nm diameter) and limited tools to study it. By deciphering the stages of ciliogenesis, we will lay the groundwork for much needed insight into the mechanisms that are disrupted in the context of disease. The field has made great strides in defining key steps driving ciliogenesis. Yet, little is known concerning the dynamics of these early events, much less the post-translational modifications (notably phosphorylation events) that drive many of these protein-protein interactions and their function. This is in large part due to 1) the failure to induce synchronized ciliogenesis in cell culture, and 2) under-developed approaches for performing high-sensitivity phosphoproteomics. To address this gap, our lab has monitored ciliogenesis after releasing RPE cells from mitosis, therein systematically synchronizing ciliogenesis in a single, ~2-hr long wave. Key signaling steps are monitored by detailed immunofluorescence microscopy and by data dependent-parallel accumulation-serial fragmentation DDA-PASEF mass spectrometry. So far, we have captured critical signaling events, including the final stages of abscission, the recruitment of early factors to prime ciliary vesicle recruitment and basal body docking, and axonemogenesis. We have identified ~30,000 phosphopeptides and have correlated kinase activities to specific linked substrates to critical stages of ciliogenesis. We further validated these candidates in ciliogenesis-synchronized RPE cells via immunofluorescence in combination with pharmacological and genetic approaches. For instance, we discovered that autophosphorylation of critical ciliogenesis kinase TTBK2 is pivotal for removing CP110 and licensing early ciliogenesis. Together, our work will lay the foundation for future studies studying the temporal and spatial mechanisms driving ciliary assembly.

B173/P1860

Towards a unifying mechanism for trafficking of proteins to the primary cilium membrane.

V. Palicharla[1], S. Hwang[1], K. Liem[2], S. Mukhopadhyay[1]; [1]Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, [2]Pediatrics, Yale University School of Medicine, New Haven, CT.

The membrane of the primary cilium has a distinct protein and lipid composition, despite being contiguous with the plasma membrane. To understand the role of cilium-generated signaling in homeostasis, it is necessary to decipher the mechanisms that drive such unique compartmentalization. The tubby family protein, TULP3, transports integral membrane proteins into cilia through binding with

the intraflagellar transport complex-A (IFT-A) and phosphoinositides. These transmembrane cargoes have short motifs that are necessary and sufficient for TULP3-mediated trafficking. However, mechanisms of ciliary compartmentalization of non-integral, membrane-associated proteins are poorly understood. In addition, the mechanism by which such diverse cargos are trafficked to cilia by TULP3 is not well understood. ARL13B is a palmitoylated atypical GTPase, that is highly enriched in cilia. We recently reported that cilia in kidney tubular epithelia deleted for *Tulp3* lacked ARL13B preceding renal cystogenesis. Here, using *Tulp3* knockout mouse embryonic fibroblasts and cell lines, we show that TULP3 is required for ciliary transport of ARL13B. By promoting ARL13B trafficking, TULP3 also determines ciliary enrichment of ARL13B-dependent lipidated cargos, including farnesylated INPP5E, and myristoylated NPHP3 and CYS1. Trafficking of transmembrane cargoes, such as G-protein coupled receptors, by TULP3 requires both IFT-A and phosphoinositide binding. In contrast, we found that lipidated protein trafficking to cilia required TULP3 binding to IFT-A but not to phosphoinositides, reflective of increased affinity of interactions between TULP3 and ARL13B, compared to transmembrane cargoes. Furthermore, we identified that an N-terminal amphipathic helix in ARL13B preceding the GTPase domain and including the palmitoylation site mediated direct ARL13B binding to TULP3. This helix was required for ciliary trafficking of ARL13B irrespective of palmitoylation and RVxP sorting motifs previously implicated in ciliary trafficking. We also identified critical residues in the β-barrel of TULP3's tubby domain that mediated direct ARL13B binding and promoted ciliary trafficking of both lipidated and transmembrane cargoes. Some of these residues are mutated in human subjects with renal and liver cystogenesis and have been implicated in causing renal cystogenesis in mouse models. These findings indicate an expanded role of a shared tubby domain surface in TULP3 that captures short sequences of diverse cargoes to directly mediate their transport into cilia in concert with IFT-A. Targeting the TULP3-cargo interactions could provide therapeutics in ciliary trafficking-regulated diseases such as polycystic kidney disease and obesity.

B174/P1861

Molecular and anatomical transformations in cilia and centrosomes underlie ciliary deconstruction during cerebellar granule cell differentiation

C. Ott[1], S. Constable[2], A. Lemire[1], A. Lim[3], J. Lippincott-Schwartz[1], S. Mukhopadhyay[4]; [1]Janelia Research Campus, Ashburn, VA, [2]Adelaide Centre for Epigenetics, Adelaide, AUSTRALIA, [3]Wellesley College, Wellesley, MA, [4]UT Southwestern Medical Center, Dallas, TX.

Granule cells (GCs) in the cerebellum are the most abundant neurons and abnormal GC proliferation causes sonic hedgehog (Shh)-subtype medulloblastoma. GC progenitors divide in response to cilia transduced Shh signals prior to differentiating and migrating. Reduced ciliation in post-mitotic GCs has been described, however how cilia change as GCs mature is not well understood. The migration-induced separation of GCs during neurogenesis enabled us to explore the mechanism of cilia deconstruction using parallel analysis of ultrastructure, transcript levels and protein localization in the developing cerebellar tissue. Analysis of ciliary structure in a large-scale 3D electron microscopy volume revealed that cilia in GC progenitors formed through an intracellular biogenesis pathway during which many were concealed from external signaling. As GCs differentiated, most cilia remained internal and gradually reduced in length and frequency. Intermediate structures suggested active recovery of the cilia-related membranes as well as internal components. To identify molecular candidates contributing to the observed anatomical changes, we queried single cell transcriptomic data from developing cerebella for expression changes in centrosome and cilia related genes. Several genes important for cell cycle

dependent cilia resorption, including Aurka, were diminished in differentiating and mature cells, suggesting that alternative pathways likely regulated ciliary deconstruction during neurogenesis. We investigated gene expression changes and found that maturing GC progenitors had reductions in genes coding for pericentriolar material, centriolar satellite components, and intraflagellar transport proteins. We then used immunofluorescence imaging and found that changes in protein levels of these components accompanied decreased transcription. Immunostaining revealed that Shh signaling competent cilia-centrosomal complexes persisted in proliferative GC nests prior to tumorigenesis. These results indicated that ciliogenesis initiates early in differentiation and then cilia were deconstructed as a programmed part of neurogenesis using a pathway that includes diminution of proteins required for cilia maintenance. The regulated abatement of cilia mediated signaling is likely important for GC maturation and is dysregulated during medulloblastoma pathogenesis.

B175/P1862

Three-dimensional ultrastructural analysis reveals stepwise asymmetric initiation of ciliogenesis

Q. Lu, H. Zhao, Z. Khan, I. Saha, K. Narayan, C. Westlake; NCI/NIH, Frederick, MD.

Ciliogenesis requires a highly complex yet poorly understood sequence of membrane and cytoskeletal assembly processes. In cells that build the cilium partially in the cytoplasm, the earliest steps of ciliogenesis involve the docking of pre-ciliary membrane vesicles (PCV) to distal appendages on the mother centriole. PCVs are fused to form a larger ciliary vesicle (CV) that covers the distal end of the mother centriole. The CV subsequently seeds the ciliary membrane, that surrounds the axoneme, and the ciliary pocket membrane, which connects the cilium to the plasma membrane. The formation of the CV is associated with the removal of CP110/CEP97 (MC cap) from the distal end of the MC which is essential for axoneme elongation and transition zone (TZ) formation. How the CV and TZ formation is coordinated at the early stage of ciliogenesis is not clear. We investigated these ciliogenesis events using super-resolution live-cell imaging and volume-based electron microscopy. The 3-D ultrastructural analysis reveals that the mother centriole is surrounded by a large number of vesicles before CV formation. Distribution correlation analysis suggests that these vesicles are associated with the adjacent Golgi apparatus. Surprisingly, we found that vesicles docked on the distal appendages of the mother centriole asymmetrically fused to form a novel “C”-shaped vesicle. The “C”-shaped vesicle then fuses its ends into a toroidal ciliary vesicle before the CV is formed. Next, we correlative TZ formation to vesicle fusion and found that TZ proteins are recruited to the “C” and toroidal vesicles, which would create a link between the early vesicles and the microtubule doublets of the basal body. Remarkably, we show the removal of the MC cap correlates with this asymmetric ciliary membrane assembly process. Moreover, we find that membrane shaping and fusion directed by EHD1 functions in CP110 removal from the. MC. Together our work provides new insight into how the ciliary membrane assembles and directs ciliogenesis events on the centriole.

B176/P1863

Cell-specific α-tubulin TBA-6 and pan-ciliary IFT cargo RAB-28 generate a non-canonical transition zone

J. Akella[1], M. S. Silva[2], K. C. Q. Nguyen[3], D. H. Hall[3], M. M. Barr[1]; [1]Rutgers University, Piscataway, NJ, [2]Human Technopole, Milan, ITALY, [3]Albert Einstein College of Medicine, Bronx, NY.

Identifying mechanisms of ciliary specialization is crucial to understanding tissue-specific phenotypes of ciliopathic mutations. In *C. elegans* inner labial type 2 (IL2) neurons, the ciliary transition zone (TZ)

reorganizes from a canonical 9 microtubule (MT) arrangement to a non-canonical arrangement with 7 or fewer MTs. Here, we examined the mechanism and potential function of IL2 TZ reorganization. IL2 TZ reorganization involves the termination and disassembly of ciliary MTs and requires cell-specific α-tubulin TBA-6. Pan-ciliary IFT cargo and EV regulator RAB-28 controls IL2 TZ reorganization after axoneme assembly. IL2 TZ reorganization is important to properly localize the cell-specific motor KLP-6 but is not required for ciliary EV shedding and release. Our results identify a novel role for tubulin isotypes in generating TZ diversity. We also extend previous findings identifying a relationship between TZ and IFT. Our results highlight differences in the requirements for EV shedding and release from ciliated cells and suggest that cell-specific effects of ciliary gene mutations on EVs could contribute to differences in phenotypic severity in ciliopathies. Our simple *C. elegans* model allows us to uncover the molecular mechanisms that govern cilia specialization and plasticity. This developmental plasticity suggests that some ciliary defects (ciliopathies) may be corrected at later times, and that this represents a point of therapeutic intervention.

B177/P1864

Transcriptional regulation of flagella-specific genes during flagellar assembly in Chlamydomonas

K. Perlaza, **W. Marshall**; University of California, San Francisco, San Francisco, CA.

Length regulation of cilia and flagella represents a tractable model for investigating the general question of organelle size control. In Chlamydomonas, flagellar length results from a balance of assembly and disassembly, leading to the question of how these processes are coordinated. Most studies of flagellar assembly and length regulation have focused on the transport of proteins to the flagellar tip, but it is known that transcription and new protein synthesis are required for flagella to grow to full length. Most genes encoding flagellar proteins are upregulated during flagellar assembly, but the mechanism of this regulation is not known. Using quantitative measurements of mRNA levels by qPCR and Nanostring detection, we find that flagellar gene upregulation is partially triggered by the loss of flagella, but that full expression requires the active regrowth of flagella. We propose that flagellar genes are regulated by a repressor molecular that is sequestered into the growing flagellum, such that when flagella grow rapidly, repressor is depleted from the cell body, leading to gene induction. Because induction of flagellar genes should stimulate further flagellar growth, this scheme represents a positive feedback loop with the potential for instability. However, computational analysis indicates that in fact the system is stable and robust.

B178/P1865

Assembly of FAP93 at the proximal end of *Chlamydomonas reinhardtii* cilia depends on total ciliary length

S. Gordon[1], J. Hwang[2], H. Yanagisawa[3], E. L. Hunter[2,4], L. A. Fox[2], A. R. Jimenez[1], R. E. Goodwin[1], C. D. E. Stuart[1], K. C. Davis[5], R. Bower[6], M. E. Porter[6], S. K. Dutcher[4], W. S. Sale[2], L. M. Alford[1]; [1]Oglethorpe University, Atlanta, GA, [2]Emory University, Atlanta, GA, [3]University of Tokyo, Tokyo, JAPAN, [4]Washington University in St. Louis, St. Louis, MO, [5]Clayton State University, Morrow, GA, [6]University of Minnesota, Minneapolis, MN.

Cilia are highly-conserved, threadlike organelles required for cell motility and sensation, found on the surfaces of nearly all human cells. Defects in ciliary structures can lead to a wide array of ciliary related diseases, termed ciliopathies. Cilia are composed of over 700 proteins that are highly organized into

complex structures, but not symmetrically (Dutcher, 2019). FAP93 is a novel ciliary protein located at the proximal end of the cilium, where the bend for movement is initiated. To learn more about the function of FAP93, we are investigating its localization via immunofluorescence microscopy. FAP93 assembles at the proximal 1.10 ± 0.1µm of wild-type cilia, but is significantly shorter in the short flagella mutants *shf1* (0.69 ± 0.2µm, $p < 0.01$) and *shf2* (0.72 ± 0.2µm, $p < 0.01$), where the total ciliary length is nearly half that of wild-type. Additionally, because FAP93 is a proximally located ciliary protein and the *fap93* mutant phenotype is not overt, it is possible that its function is redundant or associated with other proximally located ciliary proteins, such as ODA10 and VFL3 (Dean & Mitchell, 2013 & Ochi et al., 2020). To assess whether there is a relationship between FAP93 and these proximal proteins, we localized FAP93 via immunofluorescence microscopy in *oda10* and *vfl3* mutants. The prediction is that FAP93 will be mis-localized, reduced, or absent if the defective protein is required for FAP93 assembly at the proximal end of the axoneme. FAP93 localization does not appear to be affected in *vfl3* mutants, but does appear to be reduced in *oda10*. To further investigate FAP93 in *oda10*, axonemes will be isolated from wild-type and *oda10* mutant cell types for FAP93 quantification via immunoblot. These results indicate the length of FAP93 assembly correlates with ciliary length, demonstrating ciliary length-dependent assembly of FAP93. Moreover, FAP93 may interact with ODA10 and play a role in proximal axoneme assembly.

B179/P1866

Axonemal dynein light intermediate polypeptide 1 (DNALI1) plays a role in MEIG1/PACRG Manchette association and sperm differentiation in mammalian spermatogenesis

Y. Yap[1], W. Li[1], Q. Huang[2], D. Zhang[3], L. Mladenovic-Lucas[1], J. Granneman[1], D. Williams[4], R. Hess[5], A. Toure[6], Z. Zhang[1]; [1]Wayne State University, Detroit, MI, [2]Wuhan University of Science and Technology, Wuhan, CHINA, [3]College of William and Mary, Williamsburg, VA, [4]University of North Carolina, Chapel Hill, NC, [5]University of Illinois, Urbana, IL, [6]Université Grenoble Alpes, Grenoble, FRANCE.

Axonemal dynein light intermediate polypeptide 1 (DNALI1) was originally cloned from *Chlamydomonas reinhardtii* in an effort to find motor proteins essential for flagellar motility. We discovered that DNALI1 is a binding partner of parkin co-regulated gene 1 (PACRG), which forms a complex with meiosis expressed gene 1 (MEIG1) in the manchette, a transient and unique structure only present in elongating spermatids and required for normal male germ cell differentiation. DNALI1 recruits the PACRG protein in transfected CHO cells, and also stabilizes PACRG in bacteria and transfected mammalian cells. The untagged DNALI1 could also be co-purified with His-tagged PACRG when the two proteins were co-expressed in bacteria. Immunofluorescence staining on isolated male germ cells revealed that DNALI1 was present in the manchette of elongating spermatids, and colocalized with PACRG in this structure. In *Pacrg* mutant mice, localization of DNALI1 in the manchette was not changed, suggesting that DNALI1 and PACRG form a complex in the manchette, with DNALI1 being an upstream molecule. Mice deficiency in DNALI1 specifically in male germ cells showed dramatically reduced sperm numbers, immotile sperm and were infertile. In addition, majority of the sperm exhibited abnormal morphology including misshapen heads, bent tails and enlarged midpiece, discontinuous accessory structure, and loss of sperm individualization, emphasizing the importance of DNALI1 in sperm development. Examination of testis histology revealed impaired spermiogenesis in the conditional *Dnali1* knockout mice. Electron microscopy revealed disrupted ultrastructure in sperm of the *Dnali1* mutant mice. Testicular levels of MEIG1, PACRG and SPAG16L proteins were not changed in the *Dnali1* mutant mice. However, MEIG1 and SPAG16L were no longer present in the manchette in the absence of DNALI1. These findings

demonstrate that DNALI1 is involved in the connection of the MEIG1/PACRG complex to the manchette microtubules for sperm flagella formation. Given that *Dnali1* mutant mice showed impaired sperm individualization that was not observed in the MEIG1 nor PACRG-deficient mice, DNALI1 might fulfill multiple roles in sperm cell differentiation and function.

B180/P1867

Galectin-8 induces primary cilia loss by calcium influx and Aurka/HDAC6 activation impacting on leptin signaling in POMC hypothalamic cell lines.

C. Herrera-Cid[1], B. Kerr[1], A. Soza[1,2,3], A. González[1,2,3]; [1]Centro de Biología Celular y Biomedicina (CEBICEM), Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, CHILE, [2]Centro de Envejecimiento y Regeneración (CARE), P. Universidad Católica de Chile, Santiago, CHILE, [3]Fundación Ciencia y Vida, Santiago, CHILE.

Introduction. Primary cilium is a cell surface antenna-like organelle where several transduction systems are located and is lost when cells enter the cell cycle and proliferate, mostly involving activation of aurora kinase A (Aurka)/HDAC6 and Ca^{2+} signaling, among other regulators. However, whether and how the primary cilium structure is regulated during physiological processes beyond cell proliferation remains little known. POMC neurons control satiety and energy balance in the hypothalamus, triggering anorexigenic signals in response to leptin, which activates the Leptin Receptor B (LepR-B) and downstream STAT3 present in the primary cilium. The loss of cilium in these neurons leads to leptin resistance, metabolic syndrome and obesity in mice models. Galectin-8 (Gal-8) is a carbohydrate binding protein that interacts with beta-galactosides present in cell surface glycoproteins, through which it can modulate a variety of cellular processes. In the brain, Gal-8 has neuroprotective properties and its expression regions include thalamus, hypothalamus and choroid plexus. Proinflammatory conditions similar to those described in obesity, diabetes and metabolic syndrome can upregulate Gal-8 expression. Here we study the role of Gal-8 on primary cilia and leptin signaling in POMC neurons. **Material and Methods.** POMC cell line Clu-177 treated with human recombinant Gal-8 was analyzed by confocal immunofluorescence for acetylated α-tubulin for cilium, FURA-2 signals for intracellular calcium changes and immunoblot for phosphorylated HDAC6 and pSTAT3. **Results.** Independently of cell proliferation, Gal-8 treatment decreased the ciliated cells by 20% and reduced cilia length by 25%, diminished leptin signaling associated with increased HDAC6 activation and calcium influx, while Aurka inhibition and extracellular calcium chelation prevented cilia loss. Cilium resorption seemed to be the main mechanism of deciliation induced by Gal-8, as shown by live-cell imaging and conditioned media analysis. **Discussion.** Gal-8 has the potential to regulate the primary cilum impacting on leptin receptor signaling, which in the brain may contribute to energy balance modulation and its alterations.

Acknowledgements. FONDECYT#1221796 and ANID Basal Projects: Centro Ciencia & Vida FB210008 and Center for Aging and Regeneration (CARE-UC) ACE210009. ANID Becas Doctorado Nacional 21211974. USS VRID_INTER22/18

B181/P1868

A Novel Cby1-like/ciBAR1 Complex Mediates Precise Positioning of the Annulus, a Septin-based Fibrous Ring, Along the Flagellum in Sperm Tail Segmentation

M. Hoque[1], J. Chen[1], Y. Fuchs[2], F.-Q. Li[1], K.-i. Takemaru[1]; [1]Stony Brook University, Stony Brook, NY, [2]Technion – Israel Institute of Technology, Haifa, Israel

The sperm flagellum is a specialized motile cilium divided into three regions: midpiece (MP), principal piece (PP), and end piece. The annulus, a septin-based ring structure, is positioned at the MP/PP boundary, where it acts as a structural support and a diffusion barrier, analogous to the transition zone of the cilium. Failure to form an annulus leads to male fertility defects. However, its components and assembly and migration mechanisms are poorly understood. The annulus is initially formed at the flagellar base and physically migrates distally to the MP/PP junction as spermatids mature. We previously reported that Chibby 1 (Cby1) localizes to the base of cilia and interacts with the membrane-binding ciBAR1 and 2 (Cby1-interacting BAR domain-containing 1 and 2; formerly known as FAM92A and B) to facilitate ciliogenesis. Cby1-like (Cby1L) is the closest family member to Cby1, but Cby1L's biological function remains unknown. We found that Cby1L is abundantly and exclusively expressed in mammalian testes, suggesting its important role in spermatogenesis. In differentiating spermatids, Cby1L localizes to the annulus at the base of the flagellum and then co-migrates to the MP/PP junction. To investigate the role of Cby1L in male germ cell differentiation, we generated a $Cby1L^{-/-}$ mouse model and found that $Cby1L^{-/-}$ males are completely infertile with markedly reduced sperm counts and kinked tails, strikingly similar to sperm from mice lacking septins. Interestingly, the annulus is present in $Cby1L^{-/-}$ sperm but fails to stop at the MP/PP junction and progresses into the PP. $Cby1L^{-/-}$ sperm also show defective membrane diffusion barrier. In addition, TEM analyses revealed that, although the annulus is firmly attached to flagellar membranes in WT spermatids, $Cby1L^{-/-}$ spermatids exhibit signs of impaired annulus-membrane attachment. Furthermore, we found that, like Cby1, Cby1L binds to ciBAR1, and ciBAR1 colocalizes with Cby1L at the annulus. Consistent with these findings, $ciBAR1^{-/-}$ male mice show fertility defects with kinked sperm morphology and annulus displacement into the PP. Moreover, the annulus localization of Cby1L and ciBAR1 is mutually dependent. Collectively, our findings suggest a working model in which a membrane-binding Cby1L/ciBAR1 complex localizes to the annulus to ensure stable annulus-membrane attachment and precisely position the annulus at the MP/PP junction, thereby contributing to proper sperm tail segmentation.

B182/P1869

Mechanisms of centrosome positioning and cilia formation during Left-Right Organizer development

F. Ononiwu, N. Krishnan, C. Taveras, J. Manikas, E. Ingram, N. Hall, J. Freshour, H. Hehnly; Syracuse University, Syracuse, NY.

An essential process for cilia formation during epithelialization is for the centrosome to move and dock with the cell's forming apical membrane. Our study examined centrosome positioning using *Danio rerio's* left-right organizer (Kupffer's Vesicle, KV) as a model. We found that when KV mesenchymal-like cells transition into epithelial cells that are organizing into a rosette-like structure, KV cells move their centrosomes from random intracellular positions to the forming apical membrane. During this process, these cells' centrosomes were constructing cilia intracellularly and were surrounded by Rab11-associated membranes containing MyoVa while the centrosome was repositioning towards the rosette center. Once the centrosome with associated cilia reaches the rosette center, the intracellular cilia

recruits Arl13b and waits to extend into the forming lumen until the lumen reaches an area of approximately 300 μm^2. Using optogenetic strategies we identified that the small GTPase, Rab11 and its associated membranes, regulates not only cilia formation, but centrosome movement towards the forming apical membrane, whereas Rab8 was primarily involved in cilia elongation once cilia extends into the KV lumen. These studies present both conserved and unique roles for Rab11 and Rab8 function in cilia formation during KV lumenogenesis.

B183/P1870

Vertebrate model to study cilia assembly and repair

V. G. Rao[1], V. Subramanianbalachandar[1], M. M. Magaj[2], S. Redemann[3], S. Kulkarni[4]; [1]Department of Cell Biology, University of Virginia, Charlottesville, VA, [2]Ctr for Membrane & Cell Phys, Dept of Molecular Phys & Biological Physics, Dept of Cell Biology, University of Virginia, Charlottesville, VA, [3]Center for Membrane & Cell Physiology, Department of Molecular Physiology and Biological Physics Dep, University of Virginia, Charlottesville, VA, [4]Department of Cell biology, Department of Biology, University of Virginia, Charlottesville, VA.

Motile cilia beating is critical for the mucociliary barrier to function as the first line of defense in the lungs. Due to exposure to several factors (such as acid reflux, infection, and inflammation), these cilia can undergo damage, that can be repaired by regenerating cilia. Though cilia regeneration mechanisms are well studied in unicellular models such as *Chlamydomonas* and *Tetrahymena*, however our understanding of cilia regeneration remains poor in vertebrate models. We use *Xenopus tropicalis* embryos as a model to study motile cilia regeneration of the mucociliary epithelium. We induce deciliation by calcium shock and show that the motile cilia completely regenerate within 6 hours. Using live imaging we demonstrate that multiciliated cells (MCCs) grow their cilia rather than undergo stem cell-based renewal. To understand if cilia regeneration depends on the preexisting pool of unassembled ciliary proteins or requires translational/transcriptional upregulation, we exposed deciliated MCCs to cycloheximide to block new protein synthesis. Our results showed that the precursor pool of ciliary proteins can drive the initial regeneration of all cilia, however, in the absence of new protein synthesis, MCCs prioritize fewer but longer cilia. MCCs do that by accumulating IFT proteins only at the few basal bodies to build longer cilia. We further studied the site of deciliation and observed that the deciliation does not affect the apical actin lattice and the basal body number, organization, or polarity in the MCCs. Unlike *Chlamydomonas*, we observe that the transition zone (TZ) is lost along with cilia during deciliation. We studied the transition zone assembly during cilia regeneration using electron tomography and by observing the dynamics of known TZ protein B9D1 during regeneration. While all ciliogenesis models predict that TZ is assembled first followed by before the assembly of axoneme assembly, we observed that these cilia begin to regenerate in the absence of TZ. Moreover, we show that B9D1 is not a part of the ciliary precursor pool in the cytoplasm during the initial stages of regeneration. The current work opens possibilities to pose new questions such as what the spatial and temporal sequence of transition zone assembly during cilia regeneration is. In conclusion, our work provides novel insights into cilia repair mechanisms in MCCs using a vertebrate model organism.

B184/P1871

The WAVE complex drives the morphogenesis of the photoreceptor outer segment cilium

W. J. Spencer[1], N. F. Schneider[2], N. P. Skiba[2], V. Y. Arshavsky[2]; [1]Upstate Medical University, Syracuse, NY, [2]Duke University, Durham, NC

The photoreceptor outer segment is a modified cilium filled with hundreds of flattened "disc" membranes responsible for efficient light capture. To maintain photoreceptor health and functionality, outer segments are continuously renewed through the addition of new discs at their base. This process is driven by branched actin polymerization nucleated by the Arp2/3 complex. To induce actin polymerization, Arp2/3 requires a nucleation promoting factor. Here, we show that the nucleation promoting factor driving disc morphogenesis is the pentameric WAVE complex and identify all protein subunits of this complex. We further demonstrate that the knockout of one of them, WASF3, abolishes actin polymerization at the site of disc morphogenesis leading to formation of disorganized membrane lamellae emanating from the photoreceptor cilium instead of an outer segment. These data establish that, despite the intrinsic ability of photoreceptor ciliary membranes to form lamellar structures, WAVE-dependent actin polymerization is essential for organizing these membranes into a proper outer segment.

B185/P1872

Uncovering principles of rod photoreceptor outer segment size control by genetically increasing growth

A. Jensen, L. Gilliam, J. Willoughby; University of Massachusetts, Amherst, MA

The sensory compartment of rod photoreceptors, the rod outer segment (ROS), is a highly modified primary cilium where photons of light are captured by the Rhodopsin protein and transduced into changes in membrane potential that alter synaptic neurotransmitter release. The ROS has the typical ciliary microtubular axoneme but additionally holds hundreds of densely packed, stacked, discrete intramembranous discs containing the phototransduction machinery (1300-1500 discs in a human ROS, Krstic, 1997). Further, compared to a typical mammalian primary nonmotile cilium, the ROS length is about 10 times longer, volume about 250-300 times greater, and membrane area nearly 1000 times greater (Marshall et al., 1979; Praetorius and Spring, 2005). Another major difference between photoreceptor outer segments and primary cilia is that the ROS is continuously regenerated or renewed through the combined processes of proximal growth and distal shedding (Young, 1967; Young and Droz, 1968; Young and Bok, 1969; Young, 1971; LaVail, 1973). It is postulated that photoreceptors renew their outer segments because the very narrow connecting cilium cannot accommodate the retrieval of worn outer segment disc membrane and associated proteins for disposal and recycling in the inner segment compartment. Since its discovery, progress in understanding the renewal process and principles of ROS size control has been slow. To accelerate progress, we developed multiple genetic tools in zebrafish that allow us to temporally manipulate transgene expression specifically in rods and also quantitatively measure both ROS growth and shedding rates (Willoughby and Jensen, 2012). To better understand how mature homeostatic ROS length is controlled, we used the TetOn rod system we created (Campbell et al., 2012) to overexpress Rheb, an effector of the central cellular mTOR growth pathway (review, Laplante and Sabatini, 2012). We find that following 14 days of doxycycline (DOX), Rheb-overexpressing ROS grow faster by about 26% (Wt growth = 11.11um; Rheb growth = 14.03um), while the ROS shedding rates are similar. We examined the ROS ciliary axoneme, the microtubule-based structure that extends

about 63% of the length of the ROS. The axoneme is a good candidate to control ROS homeostatic length by serving to deliver material/molecules to support ROS structure, function and integrity. We measured axoneme length in Wt and Rheb-overexpressing ROS and find no significant difference (Wt, 12.64um sem 0.86; Rheb, 13.67um sem 0.79). These results suggest that ROS homeostatic length is not controlled by the number of discs, distance from the inner segment or axoneme length.

B186/P1873

JNK is a novel ciliogenesis regulator controlling actin driven processes

M. Chatzifrangkeskou, P. Skourides; University of Cyprus, Nicosia, Cyprus

c-Jun N-terminal kinases (JNKs) are a family of protein kinases that play a central role instress signaling pathways and are involved in the development of a number of diseases, such as diabetes, neurodegeneration and liver disease. They have been implicated in various cellular processes including gene expression, neuronal plasticity, regeneration, cell death, and regulation of cellular senescence. We show that JNK is closely associated with the basal bodies of both primary and motile cilia. Using super-resolution imaging, we demonstrate that JNK is localized at the transition zone and is specifically associated with the transition fibers. We further show a critical role of JNK in the development and function of motile multiciliated cells (MCCs) in Xenopus laevis. Loss of JNK prevents the association of IFT52 with the basal bodies and leads to deregulation of the actin cytoskeleton. This in turn inhibits basal body migration and docking leading to severe ciliogenesis defects. JNK inhibition in mature MCCs leads to the disorganization of the apical and subapical actin networks and elicits defects in basal body spacing and cilia driven flow generation. In summary, our work uncovers a novel kinase dependent role of JNK in ciliogenesis and ciliary function.

B187/P1874

EHD1 controls centriolar satellite delivery of HERC2 to the mother centriole to promote CP110 ubiquitination and degradation

S. Xie, N. Naslavsky, S. Caplan; University of Nebraska Medical Ctr, Omaha, NE

Primary cilia are sensory organelles that coordinate diverse signaling pathways, controlling development and homeostasis. Progression beyond the early steps of ciliogenesis requires removal of a distal end protein, CP110, from the mother centriole, a process mediated by Eps15 Homology Domain protein 1 (EHD1). However, it remains unclear how CP110 is removed from the mother centriole. In this study, we show that EHD1 regulates CP110 ubiquitination during ciliogenesis, and identify two E3 ubiquitin ligases, HECT domain and RCC1-like domain 2 (HERC2) and mindbomb homolog 1 (MIB1), that interact with and ubiquitinate CP110. We determined that HERC2 is required for ciliogenesis and localizes to centriolar satellites, which are peripheral aggregates of centriolar proteins known to regulate ciliogenesis. We reveal a role for EHD1 in the transport of centriolar satellites and HERC2 to the mother centriole during ciliogenesis. Taken together, our work showcases a mechanism whereby EHD1 controls centriolar satellite movement to the mother centriole, thus delivering the E3 ubiquitin ligase HERC2 to promote CP110 ubiquitination and degradation.

B188/P1875

Understanding IFT train location in ciliated eukaryotic cells by structural studies

A. Marteil[1], T. Blisnick[2], A. Araujo Alves[2], P. Bastin*[2], A. Mallet*[1]; [1]Ultrastructural BioImaging Core Facility (UBI), C2RT, Institut Pasteur, Paris, FRANCE, [2]Trypanosome Cell Biology Unit, University of Paris, INSERM U1201, Institut Pasteur, Paris, FRANCE.

Cilia are cytoplasmic extensions found in almost all eukaryotic cell types. Defects in these structures can cause diseases termed ciliopathy, whose etiology is little understood. Cilia are constructed by the action of IntraFlagellar Transport (IFT) trains of protein complexes which allow the delivery of structural proteins thanks to kinesin and dynein molecular motors. Here, we are using protists of the Kinetoplastid order as model organisms to study IFT. They possess a flagellum composed of a canonical axoneme constituted of 9 doublet microtubules and a central pair, and a paraflagellar rod (PFR), a lattice-like structure attached to the axoneme via doublets 4 to 7. In *Trypanosoma brucei,* IFT trains are always localized next to doublets 3-4 and 7-8 of the axoneme, whereas they are found on at least 7 doublets in *Chlamydomonas,* an organism that lacks PFR. What could explain the restricted location of IFT trains in trypanosomes ? Is it related to the presence of the PFR that occupies a large volume in the flagellum and could generate intraflagellar congestion, hence restricting the location of IFTs to doublets 3-4 and 7-8. To address this question, IFT train location will be studied in presence and absence of a PFR thanks to different kinetoplastid models. First, we will produce a *T. brucei* strain genetically modified by RNAi to artificially delete the PFR and then observe if there is a repositioning of IFT trains. Second, we will investigate species which either lack the PFR such as *Strigomonas oncopelti* or where the PFR is extremely reduced in length and thickness such as *Angomonas deanei*. Control species will be *T. brucei* wild-type and *Crithidia fasciculata* that possess a fully developed PFR. Data will be obtained by the use of 2D light and electron microscopy techniques which will allow cell observation at different levels. The first results obtained with Scanning Electron Microscopy have shown the global morphology and size of each species, as well as the cytoskeleton and the PFR after membrane stripping. Results obtained by Transmission Electron Microscopy have confirmed the ultrastructural organization of the intraflagellar components : PFR, IFT trains and axoneme. Moreover, first fluorescence light microscopy results have shown different protein locations between each species and confirm the presence and the absence of the PFR based on detection (or not) of signal with the primary antibody L13D6 that recognizes two major PFR proteins.Other techniques can be used to complete data, like 3D electron microscopy (FIB-SEM) to visualize the volume and the spatial organization of IFT trains.

Kinetochore Assembly and Functions

B190/P1876

Investigating the role of dynein in eviction of distinct kinetochore populations of spindle assembly checkpoint proteins

A. H. Ide[1], L. Allan[2], A. Saurin[2], S. M. Markus[1], **J. G. DeLuca**[1]; [1]Biochemistry, Colorado State University, Fort Collins, CO, [2]Division of Cellular Medicine, School of Medicine, University of Dundee, Dundee, UNITED KINGDOM.

Kinetochore-microtubule attachment status is monitored by the spindle assembly checkpoint, a molecular surveillance system that generates a “wait anaphase” signal at unattached kinetochores. In metazoa, the minus end-directed motor protein cytoplasmic dynein-1 localizes to kinetochores at high

levels in early mitosis, and to progressively lower levels as mitosis proceeds. Upon stable microtubule attachment, kinetochore localized dynein has been proposed to transport checkpoint proteins away from the kinetochore and toward the spindle poles using its microtubule-dependent motor activity, thus contributing checkpoint silencing, and progression into anaphase. Given the minus end-directed motility of this motor protein, the prevailing model posits that dynein actively evicts checkpoint proteins (e.g., Mad1, Mad2, etc.) away from properly attached kinetochores by transporting them as cargoes. In this study, we set out to more carefully assess the role of dynein in the eviction of spindle assembly checkpoint effectors from kinetochores. In addition to determining the relative contribution of dynein to checkpoint protein eviction, we find that lateral attachments are indeed sufficient to initiate kinetochore dynein activity, and that checkpoint effectors within the fibrous corona are capable of signaling a checkpoint arrest from a small number of unaligned chromosomes. Our results indicate that the role of kinetochore-associated dynein in the eviction of checkpoint proteins and in checkpoint silencing is markedly reduced in cells in which checkpoint effectors can no longer accumulate at the corona. Thus, dynein's role in checkpoint silencing is likely restricted to evicting checkpoint proteins from the corona rather than from the outer kinetochore.
This work is supported through a grant from the National Science Foundation, NSF1518083SMM and JGD contributed equally to this project*

B191/P1877

Understanding chromosome segregation by drug-mediated tension responses

V. Todi, M. Gupta; Iowa State University, Ames, IA.

Improper chromosome segregation during cell division can be deleterious by causing birth defects and aneuploidy, or even resulting in the development of tumors. Chromosome biorientation is crucial to ensure faithful segregation, wherein kinetochore-microtubule attachment as well as microtubule-generated tension across the attachments of sister chromatids is required to satisfy the Spindle Activation Checkpoint (SAC) and progress into anaphase. It is therefore, essential to understand the pathways leading to activation of the SAC and associated mechanisms that promote chromosome biorientation prior to anaphase. Toward this goal, we developed a novel Taxol-sensitive yeast strain with which we isolated the tension-specific and attachment-mediated responses at kinetochores, which both induce delay in anaphase onset. This revealed discrete functions for SAC proteins; whereas Bub1, Bub3, Mad1, Mad2 and Mad3 are all required for SAC signaling due to unattached kinetochores, Bub1 and Bub3 specifically facilitate a transient delay in response to low tension at attached kinetochores. When microtubule-kinetochore attachments lack sufficient tension, Aurora B kinase (Ipl1 in yeast) phosphorylates kinetochore-associated proteins to promote detachment and subsequent SAC activation. Our Taxol-sensitive yeast model offers a unique opportunity to investigate the functions of microtubule-generated tension and associated proteins in tension-mediated error correction and anaphase onset delay mechanisms which help promote the high-fidelity of chromosome segregation. It will also help elucidate the mode of highly successful cancer therapeutics. Our overall goal is to understand how microtubule-generated tension at kinetochores regulates key aspects of bipolar attachment and chromosome separation. The inclusive impact of the tension- and attachment-based responses will significantly advance our knowledge of how healthy cells ensure genome stability and support the development of therapeutic strategies for states such as cancer and developmental disabilities.

B192/P1878

Special phase separation regulates kinetochore-microtubule attachments

J. McGory; University of Massachusetts, Amherst, MA.

Kinetochores connect chromosomes and spindle microtubules to ensure proper segregation during cell division. Crosstalk between the motor protein dynein and kinetochore-microtubule attachment factors promotes accurate chromosome segregation through a poorly understood pathway. Here we identify a functional linkage between the intrinsically disordered protein (IDP) Spc105 (*Dm*KNL1) and dynein using an opto-genetic assay. Using this assay, we determined that Spc105 has a propensity to phase separate in living cells. We uncovered a minimal phase separating region that is sufficient to link Spc105 to dynein and is necessary for accurate chromosome segregation. Additionally, our work demonstrates that recruitment of dynein targeting factors to Spc105 requires both protein binding motifs and phase separation. Our results identify a previously undiscovered molecular link between an IDP and dynein, and also implicate phase separation in the regulation of microtubule attachment stability.

B193/P1879

Interactions between the central spindle microtubules and the kinetochores promotes homologous chromosome bi-orientation in meiosis.

S. Wu, J. K. Jang, N. Changela, J. Shapiro, J. Joshi, **K. McKim**; Rutgers University, Piscataway, NJ.

The reason for pairing and crossing over between homologous chromosomes is so that they will segregate from each other in the first meiotic division. The two chromosomes of a bivalent, which are connected by at least one chiasma, bi-orient by attaching to microtubules from opposite spindle poles. This process involves a mechanism that regulates the interactions between kinetochores and the microtubules to prevent homologous chromosomes attaching to the same pole. How the error correction/avoidance mechanisms operate in meiosis I is poorly understood. Our previous work has shown that a central spindle Kinesin 6 (Subito) and the chromosome passenger complex (CPC), which includes the Aurora B kinase, are required for meiotic spindle assembly. The CPC is first recruited to the chromosomes, where it promotes recruitment of microtubules. The CPC then promotes spindle assembly, and then moves to the central spindle microtubules in a process that depends on Subito. Within the central spindle, the CPC and Subito are required for accurate bi-orientation of the homologous chromosomes. We are currently investigating how the central spindle complex interacts with the kinetochores to regulate microtubule attachments. Our results support a model in which Aurora B kinase associated with the central spindle microtubules regulates kinetochore-microtubule attachments. One of the targets is SPC105R/ KNL1, which is a hub that integrates several activities at the kinetochore. We have developed a system to study the functions of SPC105R and found It has several separable domains including domains for recruiting most kinetochore components including MIS12 and NDC80, checkpoint proteins such as Mad1 and Mad2, and bi-orientation proteins required for bi-orientation such as MPS1, the RZZ complex, and the CPC. These results show that SPC105R integrates several important genetically separable meiotic functions such as the SAC and error avoidance.

B194/P1880

Control of Chromosome Segregation in *Arabidopsis* Meiosis

F. Böwer[1], K. Lampou[1], S. Komaki[2], Y. Hamamura[1], C. Yang[3], A. Schnittger[1]; [1]Universität Hamburg, Hamburg, GERMANY, [2]Nara Institute of Science and Technology, Ikoma, JAPAN, [3]Huazhong Agricultural University, Wuhan, CHINA.

The spindle assembly checkpoint (SAC) is a safeguarding mechanism preventing anaphase onset (AO) until all kinetochores are properly attached to spindle microtubules. The importance of the SAC in sustaining the vegetative growth and development of *Arabidopsis thaliana* becomes apparent when the microtubule cytoskeleton is challenged. However, not much is known about the function of the SAC in meiosis in plants. Thus, we set out to study chromosome segregation during male meiosis in *Arabidopsis* by utilizing our recently published live cell imaging protocol of *Arabidopsis* anthers. This work led to a cytological framework of SAC action in meiosis in which we could monitor with great temporal resolution the order of SAC assembly and disassembly. Importantly, we find that the SAC is active in meiosis but cannot block meiotic progression for more than 6 hours indicating a weak and permissive SAC. In addition, we find evidence for a striking diversification of the SAC with some core components known from yeast and animals having an adapted function in *Arabidopsis*. Looking then into the regulation of the meiotic SAC, we find that SHUGOSHIN 1 (SGO1) interacts with central players of the SAC. Conversely, SGO1 promotes the timely AO in a SAC-dependent manner. We found that SGO1 in turn is required to assure the correct localization of the phosphatase PP2A, and in *pp2a* mutants, anaphase onset is also significantly delayed in a SAC-dependent manner. Interestingly, we also found with a functional reporter a previously unrecognized dynamic localization pattern of SGO1: the majority of SGO1 was associated with microtubules prior to nuclear envelope breakdown (NEB), followed by a rapid transport to the centromeric regions of chromosomes. Intriguingly, bypassing the microtubule association of SGO1 with a nuclear localization signal also resulted in prolongation of metaphase I. This gives rise to a complex network of highly ordered events of assembly and disassembly of the SAC with an important participation of phospho-control in regulating kinetochore-microtubule attachments.

B195/P1881

Chromosome size affects alignment efficiency in mammalian mitosis

M. K. Chong, S. Dumont; University of California, San Francisco, San Francisco, CA

The kinetochore connects chromosomes to spindle microtubules during cell division. Accurate chromosome segregation requires sister kinetochores to biorient, attaching to opposite spindle poles. To achieve biorientation, the kinetochore destabilizes incorrect attachments and stabilizes correct ones. How it discriminates correct from incorrect attachments is not clear. Here, we test the model that tension serves as the stabilizing cue at correct attachments and how that model affects chromosomes of different sizes. Live imaging of mitotic PtK2 cells reveals long chromosomes align at the metaphase plate more slowly than short chromosomes. Using laser ablation to shorten long chromosome arms—reducing polar ejection forces on them—we show that chromosomes align faster after ablation, indicating chromosome size affects alignment efficiency. Finally, artificially enriching for incorrect attachments using STLC washouts and imaging error correction live, we show that long chromosomes exhibit a delay in correcting errors, rather than simply in attachment formation. We propose a model where increased polar ejection forces on long chromosomes stabilize not only correct but also incorrect

attachments, delaying their biorientation and alignment. As such, long chromosomes may experience more challenges correcting errors and, as a result, higher missegregation rates.

B196/P1882

Inhibitor-2 promotes Protein Phosphatase 1 activity and mitotic progression in the *C. elegans* embryo

N. Varshney, K. Oegema, A. Desai; University of California San Diego, San Diego, CA.

The catalytic subunit of Protein phosphatase 1 (PP1c) is responsible for a major fraction of Ser/Thr dephosphorylation events in the cell. PP1's catalytic activity and specificity is tightly regulated by a conserved group of regulatory/biogenesis factors, including Inhibitor-2 (I2), a widely conserved protein that was discovered based on its binding and inhibition of PP1's catalytic activity. Using the early *Caenorhabditis elegans* embryo as an *in vivo* model system to investigate PP1c and its regulatory network, we find that the two PP1c isoforms, GSP-1 and GSP-2, act redundantly prior to anaphase onset, with their double depletion leading to severe defects. By contrast, the effect of I2 (named SZY-2 in *C. elegans*) inhibition is significantly milder, with the primary defect being delayed anaphase onset. Anaphase onset is controlled by the Anaphase Promoting Complex/Cyclosome (APC/C) activated by its substrate adapter CDC-20. Cdk1/2 phosphorylation of CDC-20 inhibits its binding to and activation of the APC/C; removal of inhibitory phosphorylation on CDC-20 is catalyzed by PP1c docked on the kinetochore scaffold protein KNL-1 as CDC-20 fluxes through the kinetochore. The anaphase onset delay observed in the I2 depletion is similar to the PP1c docking mutant of KNL-1 and is rescued by a non-phosphorylatable form of CDC-20. Depletion of I2 in the PP1c docking mutant of KNL-1 further delayed anaphase onset, suggesting that I2 promotes PP1c-mediated dephosphorylation of CDC-20 in the cytoplasm. Analysis of *in situ* tagged GFP fusions of the two PP1c isoforms revealed a reduction in overall PP1c levels and in kinetochore-localized PP1c following I2 depletion. Collectively, these data indicate that I2 is important for normal levels and activity of PP1c and its loss negatively affects PP1c activity at kinetochores and in the cytoplasm that promotes mitotic progression via CDC-20 dephosphorylation. Thus, unlike its name, I2 acts as a promoter of specific PP1c functions *in vivo*. Using transgene-based replacement of endogenous I2 with engineered mutants, we are currently addressing the contribution of distinct conserved I2 interfaces with PP1c to elucidate the mechanism by which it contributes to PP1c function in mitotic progression.

B197/P1883

Human Artificial Chromosomes as a Tool to Discover New Human CIN Genes

M. Liskovykh[1], E. Chiang[2], K. Cheng[2], H. Masumoto[3], W. Earnshaw[4], N. Kouprina[1], V. Larionov[1]; [1]National Cancer Institute, Bethesda, MD, [2]NCATS, Rockville, MD, [3]Kazusa DNA Research Institute, Chiba, JAPAN, [4]Wellcome Centre for Cell Biology, Edinburgh, UNITED KINGDOM.

Mitosis is a fundamental biological process that still holds many secrets. Among these are the mechanisms preventing chromosome instability (CIN). CIN is a phenomenon driven by problems with chromosome segregation during cell division. Errors in chromosome segregation can cause cell death or lead to the production of abnormal daughter cells that can be dangerous to the organism, e.g., by predisposing cells to cancer progression or being a cause for problems in early neurodevelopment. A comprehensive list of all the genes whose misfunction leads to CIN (CIN genes) would be an important step toward a deeper understanding of mitotic regulation. To date, the study of CIN genes in humans has been fragmented, with genes generally discovered by chance or in screens for abnormal cell cycle,

cell size, and proliferation rate. The incompleteness of the list is demonstrated by the fact that new CIN genes are found every year. There was no systematic search for human CIN genes because of the lack of a suitable experimental approach. Given this need, we decided to build a test-system to check as many human genes as possible for their role in chromosome segregation (i.e., loss of function causes CIN). And we tried to answer the key scientific question - which genes are responsible for precise chromosome segregation during mitosis?To identify CIN genes, we developed a novel high-throughput imaging assay that is based on the use of a synthetic human artificial chromosome (HAC), carrying a dual cassette simultaneously expressing two destabilized versions of the GFP. The HAC, which was assembled from normal and synthetic alpha-satellite repeats, contains a functional centromere that allows its stable inheritance as a nonessential chromosome. The HAC loss rate is roughly 10-fold higher than the native chromosomes. Thus, it is relatively stable, but nonetheless sensitized. This makes the assay more manageable, allowing me to observe a statistically significant number of events in realistic sample size when studying the CIN phenotype in human cells. We successfully applied this HAC-based system, establishing reproducible and sensitive assay performance when testing human protein kinases for their role in proper chromosome transmission. Using this system, we identified a novel set of genes whose knockdown results in increased CIN.After the success of using HAC for the identification of new kinases involved in CIN progression. We applied the same approach even on a bigger scale. Recently we conducted genome-wide siRNA screening of 19000 genes to check their role in CIN. Here we would like to share our recent findings in the attempt to find a comprehensive list of human CIN genes.

B198/P1884

POLO/PLK1 regulates PP1 to ensure timely Spindle Assembly Checkpoint and genome stability

M. Moura[1,2], J. Barbosa[1], S. Cunha-Silva[1,2], M. Silva[1], M. Oswald[1], S. Lemaire[3], J. Nilsson[4], A. Saurin[5], M. Bollen[3], C. Conde[1]; [1]i3S - Institute for Research and Innovation in Health, University of Porto, Porto, Portugal, [2]ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal, [3]Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium, [4]The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark, [5]School of Medicine, University of Dundee, Dundee, United Kingdom

Protein phosphatase 1 (PP1) is a major orchestrator of mitotic exit. PP1 dephosphorylates KNL1 and MPS1 to inactivate the Spindle Assembly Checkpoint (SAC) and ensure timely anaphase onset. However, the mechanisms that prevent PP1 from prematurely driving these events remain ill-defined. Here, we report Polo/PLK1 as a downregulator of PP1 activity in early mitosis. We found that Polo/PLK1 phosphorylates PP1 in its RVxF-binding pocket *in vitro* and in *Drosophila* S2 cells. The phosphorylation is readily detected at unattached/tensionless kinetochores and decreases as chromosomes congress to the metaphase plate. Replacing endogenous PP1 with a phosphomimetic version prevents the inactivation of MPS1 causing a SAC-dependent metaphase delay. Conversely, expression of unphosphorylatable PP1 significantly weakens the SAC response of S2 cells in the presence of colchicine. Notably, tethering phosphomimetic PP1 to kinetochores fails to restore timely SAC inactivation and anaphase onset in asynchronous cultured cells or accelerate mitotic exit when incubated with spindle poisons. These observations suggest that PLK1-mediated phosphorylation of PP1 exerts its inhibitory effect independently of PP1 kinetochore localization. Moreover, biochemical assays indicate that the catalytic efficiency of recombinant PP1 remains unaltered by PLK1 phosphorylation. We are now testing the non-mutually exclusive hypothesis that phosphorylation of PP1 hinders its interaction with SAC substrates (MPS1, KNL1) and/or enhances its binding to inhibitory RIPPOs. Regardless of the underlying

mechanism, the fine-tuning of PP1 activity by PLK1 during mitosis assumes critical relevance in proliferative tissues. *In vivo* analysis shows that, unlike for the wild-type phosphatase, expression of phosphomimetic PP1 transgene fails to restore the fidelity of chromosome segregation and genome stability in larval neuroblasts of *pp1-87b* mutants. These results uncover a new strategy to regulate PP1 activity in order to ensure a timely SAC and avert aneuploidy.

B199/P1885

Clustering of the scaffolding protein CENP-T activates recruitment of Ndc80 complexes to assemble a functionally active outer kinetochore

E. V. Tarasovetc[1], G. B. Sissoko[2,3], A. Maiorov[1], I. M. Cheeseman[2,3], E. L. Grishchuk[1]; [1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, [3]Whitehead Institute for Biomedical Research, Cambridge, MA.

Proper kinetochore assembly requires binding of multiple copies of different proteins in an ordered and localized manner. In particular, the human kinetochore scaffolding protein CENP-T recruits two Ndc80 molecules, which are required for stable interaction with spindle microtubules. However, when the CENP-T-kinetochore localization domain is removed, individual CENP-T molecules do not associate robustly with Ndc80 when present in the cytoplasm, highlighting the importance of the centromere localization of CENP-T. We hypothesized that clustering of CENP-T molecules at centromeres plays a role in Ndc80 recruitment. To test this, we created genetically-encoded 60-subunit clusters of CENP-T's outer kinetochore recruitment domain and expressed them in human cells. Clustered CENP-T, but not monomeric CENP-T^{1-242}, robustly recruited Ndc80 and other outer kinetochore proteins. Moreover, complexes formed by CENP-T clusters and outer kinetochore components isolated from cells bind to microtubules and move processively with dynamic microtubule tips. To investigate the mechanism that leads to assembly of a functionally active outer kinetochore on clustered CENP-T, we tested whether this phenomenon can be recapitulated using recombinant components. We used a real time TIRF fluorescence assay to monitor interactions between GFP-tagged Ndc80 and CENP-T^{1-242} present in either clustered or monomeric forms. Both forms of CENP-T bind to two Ndc80 complexes, but we found that Ndc80 molecules dissociate significantly faster from monomeric compared to clustered CENP-T. In addition, the stability of CENP-T-Ndc80 binding increases over time, indicating a "maturation" process of Ndc80 binding sites on CENP-T. Such maturation was concurrent with the presence of multiple weakly-bound Ndc80 molecules around CENP-T clusters. The formation of such molecular clouds was not observed with monomeric CENP-T molecules, which experienced much slower maturation compared to clustered CENP-T. Thus, the enhancement of Ndc80 recruitment and stabilization of its binding to CENP-T is an intrinsic feature of CENP-T clusters and is associated with different kinetics of Ndc80 binding and maturation. We propose that, in cells, these molecular mechanisms promote efficient and stable binding of Ndc80 and other outer kinetochore components specifically at the centromere loci, while avoiding interactions between the soluble components in the cytoplasm.

B200/P1886

The Astrin-SKAP Complex Lubricates the Kinetochore-Microtubule Attachment

M. Rosas Salvans[1], R. Sutanto[1], p. suresh[2], S. Dumont[1]; [1]University of California, San Francisco, san francisco, CA, [2]Massachusetts Institute of Technology, cambridge, MA.

The kinetochore links chromosomes to spindle microtubules to drive chromosome segregation at cell division. While we know nearly all mammalian kinetochore proteins, how these give rise to the strong yet dynamic microtubule attachments required for function remains poorly understood. Here, we focus on the Astrin-SKAP complex, which localizes to bioriented kinetochores and is essential for chromosome segregation, but whose mechanical role is unclear. Live imaging reveals that SKAP depletion dampens movement and decreases coordination of metaphase sister kinetochores, and increases tension between them. Using laser ablation to isolate kinetochores bound to polymerizing vs depolymerizing microtubules, we show that without SKAP kinetochores move slower on both polymerizing and depolymerizing microtubules, and that more force is needed to rescue microtubules to polymerize. Thus, in contrast to previously described kinetochore proteins that increase grip on microtubules under force, Astrin-SKAP reduces grip, increasing attachment dynamics and force responsiveness and reducing friction. Together, our findings suggest a model where the Astrin-SKAP complex effectively “lubricates” correct, bioriented attachments to help preserve them.

B201/P1887

Microtubule plus end targeting motif of Ska1 regulates kinetochore-microtubule attachment and chromosome alignment in human cells.

R. M. RADHAKRISHNAN[1], S. T. Kizhakkeduth[2], V. M. Nair[1], S. Ayyappan[2], B. R[1], N. Babu[1], A. Prasannajith[1], K. Umeda[3], V. vijayan[2], N. Kodera[3], T. K. Manna[1]; [1]Biology, Indian Inst Science Ed/Res (IISER) - Keral IISER TVM - 20122196, Thiruvananthapuram, INDIA, [2]Chemistry, Indian Inst Science Ed/Res (IISER) - Keral IISER TVM - 20122196, Thiruvananthapuram, INDIA, [3]Bio-AFM Frontier Research Center, Kanazawa University, Kanazawa, JAPAN.

Attachment of the dynamic plus ends of microtubules attachment with the kinetochores of chromosomes is essential for the segregation of chromosomes during mitosis. The mechanisms of how kinetochore attachment is stabilized and its movement are coupled with the dynamic plus ends are poorly understood. In budding yeast, ring structures formed by Dam1 complex proteins on the microtubules are implicated to couple kinetochores to the depolymerizing microtubule by dynamically moving/sliding along the microtubule lattice following microtubule depolymerization. The mechanisms of kinetochore coupling and the organization of the molecular factors involved in the process in metazoans have not been completely identified. The absence of Dam1 complex components in higher eukaryotes necessitates the importance of identifying the regulators in higher eukaryotes for this function. Vertebrate Spindle and kinetochore-associated (Ska 1,2,3) complex proteins have been proposed to have an analogous role to Dam1. Microtubule plus end-binding protein, EB1, plays a critical role in coupling microtubule plus ends to the kinetochore by mediating interaction with the Ska in human cells. However, the mechanism underlying EB1 interaction with Ska is incompletely understood. Here, we show that the interaction of Ska1 N-terminal loop region with microtubule plus end protein EB1 through a conserved motif regulates Ska complex recruitment to the kinetochore and metaphase chromosome alignment. Deletion or mutation of Ska1 motif, SHLP disrupts EB1-Ska1 interaction and delocalizes Ska complex proteins from kinetochores, and induces chromosome alignment defects.

Mutation of the motif, however, does not affect Ska complex assembly. High-speed atomic force microscopy imaging visually demonstrate Ska1 loop mediating contacts with the C-terminal region of EB1 dimer leading to the formation of stable extended structure. NMR analysis showed that the Ska1 SHLP motif interacts with the residues in the EB-homology domain of EB1, which are known to bind to several plus end-associated proteins. Our results demonstrate that the Ska1 loop with its conserved motif regulates EB1-Ska1 interaction and stable kinetochore-microtubule attachment. They also implicate that the SHLP motif-mediated EB1-Ska1 interaction could provide microtubule plus-end attachment specificity for the vertebrate kinetochore.

B202/P1888

Towards the Structural Basis for CENP-V Mediated Regulation of Chromosome Segregation

L. Di Pompeo, A. A. Jeyaprakash, W. C. Earnshaw; Wellcome Trust Centre for Cell Biology, Institute of Cell Biology, University of Edinburgh, Edinburgh, UNITED KINGDOM.

Centromere protein V (CENP-V) is an outer kinetochore protein, originally identified in isolated mitotic chromosomes and very well conserved among vertebrates. A previous study using RNAi to deplete canonical CENP-V in HeLa cells suggested that the protein was involved in the compaction of mitotic chromosomes and completion of cell division. More recently, CENP-V was shown to bind microtubules and to maintain spindle integrity during meiosis of mouse oocytes. How CENP-V achieves these diverse functions is still unknown, and is the subject of my Ph.D. studies. Studies of human CENP-V are complicated by the presence of a canonical CENP-V gene plus three paralogs that may or may not encode functional proteins. Analysis of the protein sequence suggested that CENP-V contains a glutathione-dependent formaldehyde-activating (GFA) domain, reported to be involved in formaldehyde metabolism in bacteria. By using a combination of biochemical and structural characterization and cell biology, my research specifically aims to investigate whether CENP-V does have GFA-like enzymatic activity and whether this might be associated with the metabolism of formaldehyde released in nuclear demethylation reactions. My high-resolution crystal structures confirm that CENP-V has a GFA-like domain that binds reduced glutathione (GSH) via a binding pocket stabilised by a zinc ion and the glutathione itself. These observations support the hypothesis of a catalytic activity similar to the bacterial enzyme. I have also confirmed that human CENP-V binds tightly to microtubules *in vitro* mainly via its unstructured N-terminal region that precedes the GFA domain. My ongoing work aims to use structure-based mutants and conditional knock-out to understand how glutathione and microtubule binding activities of CENP-V are linked to its suggested roles in maintaining chromosome structure and/or spindle integrity.

B203/P1889

A phosphoproteomic approach to investigate the role of the Bub1 kinase domain in chromosome segregation

A. Bunning[1], J. Walley[2], M. Gupta[1]; [1]Genetics, Development, and Cell Biology, Iowa State University, Ames, IA, [2]Plant Pathology and Microbiology, Iowa State University, Ames, IA.

Accurate chromosome segregation is crucial for cell survival and preventing aneuploidy. A bioriented spindle must be formed where each kinetochore is attached to a microtubule emanating from the opposite spindle pole body. These bioriented attachments are reinforced by microtubule-generated tension at kinetochores that creates the pulling force needed to separate sister chromatids during

anaphase. Both microtubule attachments and tension at kinetochores are vital for forming a bioriented spindle needed for proper chromosome segregation. Bub1 and Bub3 are key proteins in the Spindle Assembly Checkpoint (SAC), a signaling cascade that prevents anaphase onset if one or more kinetochores are unattached. Bub1 and Bub3 are recruited to unattached kinetochores, leading to an anaphase onset delay by preventing activation of the APC/C (anaphase promoting complex). Bub1 and Bub3 have been shown to delay anaphase onset in the presence of attached, low tension kinetochores independent of the canonical SAC. The Bub1 kinase domain is not needed for SAC signaling but localization of Sgo1, a protein implicated in tension sensing and localization of error correction machinery. How the Bub1 kinase domain specifically responds to tensionless kinetochores and promotes chromosome segregation remains unclear. To address this, we used quantitative mass spectrometry in budding yeast cells to investigate differences in phosphorylation between nocodazole-treated bub1-Δkinase and wild type cells. This experimental design isolates tension-specific phosphorylations of the Bub1 kinase domain. We identified over 15,000 phosphorylations, and of the total downregulated phosphorylations in the Bub1-Δkinase cells, roughly 350 sites were significant and enriched. Some of these significant sites were found on proteins implicated in anaphase onset timing (Pds1 and Apc1) and cohesion maintenance and establishment. Using a CRISPR-based mutation method, we generated point mutations in the endogenous locus of Apc1 and Pds1, creating phospho-null and phospho-memetic mutants. Surprisingly, these point mutants were not sensitive to benomyl, suggesting that spindle assembly and the SAC are fully functional. We will perform qualitative chromosome loss assays to further explore chromosome segregation fidelity in these mutants. Further analysis from this data set will help to uncover the importance of understanding how the Bub1 kinase domain ensures chromosome segregation through regulating anaphase onset timing and sister chromatid cohesion.

B204/P1890

Kinetochore motor CENP-E drives congression and promotes stepwise end-on conversion of polar chromosomes by opposing Aurora B-mediated phosphorylation at the outer kinetochore

K. Vukusic, I. M. Tolić; Ruder Boskovic Institute, Zagreb, CROATIA.

Congression is a movement of chromosomes toward the spindle equator that, if unsuccessful, results in unaligned polar chromosomes, a phenomenon frequently observed in cancer cells. Interestingly, peripheral polar chromosomes are most susceptible to congression failure, and these chromosomes exclusively require the kinetochore motor CENP-E for congression. Current models state that CENP-E-driven congression is independent of biorientation and involves plus-end-directed activity of the motor domain regulated by Aurora kinases. However, Aurora kinases also oppose stable end-on attachments of the kinetochore to microtubules by phosphorylating other components of the kinetochore, and CENP-E-independent congression of polar chromosomes has been reported. Therefore, it remains unknown why only polar chromosomes require CENP-E-mediated congression and how their congression relates to the activity of Aurora kinases. To systematically explore the relationship between the role of CENP-E during congression and the mechanisms regulating biorientation, we established a library of live-imaged RPE1 cells with unaligned polar chromosomes treated with small-molecule inhibitors of various mitotic regulators. Here, we report that polar chromosomes initiate congression immediately without CENP-E if biorientation is allowed by acute inhibition of Aurora B. Interestingly, initiation of congression required loss of outer kinetochore pool of Aurora B, whereas it did not require loss of centromeric aurora B and chromosome cohesion or activity of chromokinesins and phosphatases. Moreover, congression was not induced by perturbation of activities of Mps1, Aurora A, and dynein. However, by using STED

microscopy, we show that subset of polar kinetochores during CENP-E-independent congression struggled to completely reach metaphase plate due to generation of syntelic or merotelic attachments. Importantly, by tracking markers of kinetochore tension and Mad2 dynamics, we show that a similar type of congression accompanied by biorientation is also observed after restoration of CENP-E activity regardless of aurora B activity, and occasionally in cells without CENP-E, but in both cases without enrichment in erroneous attachments. Lastly, under all conditions, only chromosomes located near the spindle poles required CENP-E to align, whereas the motor was not required for alignment if centrioles were removed from the centrosomes. In conclusion, we unravel the complex coordination between congression and biorientation and propose that CENP-E-mediated opposition to activity of Aurora B kinase close to centrosomes is a crucial first step during the congression of polar chromosomes that promotes their gradual transition from lateral to amphitelic end-on attachments.

B205/P1891

Reconstitution of Kinetochore Motility and Microtubule Dynamics Reveals a Role for a Kinesin-8 in Establishing End-on Attachments

J. R. Torvi, J. Wong, D. Serwas, A. Moayed, D. G. Drubin, G. Barnes; University of California, Berkeley, Berkeley, CA

During mitosis, individual microtubules make attachments to chromosomes via a specialized protein complex called the kinetochore to faithfully segregate the chromosomes to daughter cells. Translocation of kinetochores on the lateral surface of the microtubule has been proposed to contribute to high fidelity chromosome capture and alignment at the mitotic midzone, but has been difficult to observe in vivo because of spatial and temporal constraints. To overcome these barriers, we used total internal reflection fluorescence (TIRF) microscopy to track the interactions between microtubules, kinetochore proteins, and other microtubule-associated proteins in lysates from metaphase-arrested *Saccharomyces cerevisiae*. TIRF microscopy and cryo-correlative light microscopy and electron tomography indicated that we successfully reconstituted interactions between intact kinetochores and microtubules. These kinetochores translocate on the lateral microtubule surface toward the microtubule plus end and transition to end-on attachment, whereupon microtubule depolymerization commences. The directional kinetochore movement is dependent on the highly processive kinesin-8, Kip3. We propose that Kip3 facilitates stable kinetochore attachment to microtubule plus ends through its abilities to move the kinetochore laterally on the surface of the microtubule and to regulate microtubule plus end dynamics.

B206/P1892

Checkpoint-independent control of rapid embryonic mitoses by PLK1-mediated recruitment of CDC20 to kinetochores

J. M. Houston[1], M. Ohta[2], S. Gomez-Cavazos[3], A. Deep[1], K. Corbett[1], K. Oegema[1], P. Lara-Gonzalez[4], T. Kim[5], A. Desai[1]; [1]University of California, San Diego, San Diego, CA, [2]Okinawa Institute of Science And Technology, Onna, JAPAN, [3]Bristol Myers Squibb, San Diego, CA, [4]University of California, Irvine, Irvine, CA, [5]Pusan National University, Busan, KOREA, REPUBLIC OF.

Early embryogenesis in many species is characterized by rapid cell cycles with fast mitoses. In contrast to somatic cell divisions, the spindle assembly checkpoint is weak in early embryos, and the mechanisms governing mitotic timing are not well understood. Mitotic timing is controlled by Cdc20-mediated activation of the Anaphase Promoting Complex/cyclosome (APC/C). Phosphorylation of Cdc20 by Cdk1/2

prevents it from binding and activating the APC/C. Using the early *C. elegans* embryo, we show that mutation of a key inhibitory phosphorylation site on CDC-20 to either increase the persistence of or prevent phosphorylation correspondingly delays or accelerates anaphase onset; these effects of altering CDC-20 phosphorylation on embryonic mitotic duration are independent of the spindle checkpoint. Notably, accelerating mitosis sensitizes embryos to mitotic perturbations, and delaying mitosis leads to embryonic lethality. Thus, CDC-20 phosphoregulation is a spindle checkpoint-independent mechanism for optimizing mitotic duration in rapidly dividing early embryos. We previously showed that kinetochores accelerate anaphase onset by recruiting CDC-20 to kinetochores where it is dephosphorylated by a localized phosphatase pool (Kim, Lara-Gonzalez et al. 2017, *Genes Dev. 31*:1089-1094). CDC-20 is recruited to kinetochore by interaction with an ABBA motif in BUB-1. Surprisingly, mutation of a conserved docking site on BUB-1 for Polo-like Kinase 1 (PLK-1), ~100 aa distal to the ABBA motif, resulted in a mitotic delay identical to that resulting from mutation of BUB-1's ABBA motif. To identify the target(s) of PLK-1 kinase activity, we conducted a clustered mutagenesis screen focused on BUB-1. This effort identified putative PLK-1 sites within and adjacent to the BUB-1's ABBA motif that were required for CDC-20 kinetochore recruitment. In vitro binding assays with purified components showed that BUB-1 binding to CDC-20 is strongly stimulated by PLK-1 phosphorylation and that the putative target sites in the ABBA motif are essential for this stimulation. Collectively, these data indicate that PLK-1 tethered on BUB-1 controls mitotic progression by phosphorylating sites in the BUB-1 ABBA motif to enhance CDC-20 flux through the kinetochore. This enhanced flux accelerates CDC-20's activation by dephosphorylation and ensures timely mitotic exit during embryogenesis.

B207/P1893

A novel mechanism of Spindle Assembly Checkpoint control through CDK1 regulation of outer-kinetochore assembly

I. Manley, U. Gruneberg; The Sir William Dunn School of Pathology, University of Oxford, Oxford, UNITED KINGDOM.

The Spindle Assembly Checkpoint (SAC) is essential for maintaining genomic stability by monitoring attachment of chromosomes to the mitotic spindle and preventing premature chromosome segregation in mitosis. Checkpoint regulation is coordinated by the opposing activities of kinases and phosphatases. CDK1 is the major mitotic kinase required for mitotic entry and activation of the SAC. In particular, CDK1 regulates recruitment of the principle checkpoint kinase MPS1 to kinetochores, a critical event for downstream checkpoint signalling. Here, we demonstrate that circumventing this requirement for CDK1 is insufficient for SAC signalling when CDK1 is inhibited, therefore revealing a crucial, unknown role of CDK1 in SAC signalling. Using high resolution live and fixed cell imaging, we confirm that outer-kinetochore assembly is CDK1 dependent and disassembly of outer-kinetochore proteins is differentially regulated by the CDK1-opposing phosphatases PP1 and PP2A-B55. Critically, we observe that the outer-kinetochore protein and checkpoint scaffold KNL1 is rapidly lost at the metaphase-to-anaphase transition, before other outer-kinetochore proteins. This occurs through a novel CDK1-mediated interaction between the MIS12 complex and KNL1 that is critical for regulating checkpoint function. Therefore, specific phosphatase-mediated disassembly of the outer-kinetochore supports complete checkpoint shutdown through KNL1 loss while leaving microtubule-kinetochore attachments intact through NDC80, thus safeguarding directionality of the metaphase-to-anaphase transition.

B208/P1894

Checkpoint-independent control of rapid embryonic mitoses by PLK1-mediated recruitment of CDC20 to kinetochores

J. M. Houston[1], M. Ohta[2], S. Gomez-Cavazos[3], A. Deep[1], K. Corbett[1], K. Oegema[1], P. Lara-Gonzalez[4], T. Kim[5], A. Desai[1]; [1]University of California, San Diego, San Diego, CA, [2]Okinawa Institute of Science And Technology, Onna, JAPAN, [3]Bristol Myers Squibb, San Diego, CA, [4]University of California, Irvine, Irvine, CA, [5]Pusan National University, Busan, KOREA, REPUBLIC OF.

Early embryogenesis in many species is characterized by rapid cell cycles with fast mitoses. In contrast to somatic cell divisions, the spindle assembly checkpoint is weak in early embryos, and the mechanisms governing mitotic timing are not well understood. Mitotic timing is controlled by Cdc20-mediated activation of the Anaphase Promoting Complex/cyclosome (APC/C). Phosphorylation of Cdc20 by Cdk1/2 prevents it from binding and activating the APC/C. Using the early *C. elegans* embryo, we show that mutation of a key inhibitory phosphorylation site on CDC-20 to either increase the persistence of or prevent phosphorylation correspondingly delays or accelerates anaphase onset; these effects of altering CDC-20 phosphorylation on embryonic mitotic duration are independent of the spindle checkpoint. Notably, accelerating mitosis sensitizes embryos to mitotic perturbations, and delaying mitosis leads to embryonic lethality. Thus, CDC-20 phosphoregulation is a spindle checkpoint-independent mechanism for optimizing mitotic duration in rapidly dividing early embryos. We previously showed that kinetochores accelerate anaphase onset by recruiting CDC-20 to kinetochores where it is dephosphorylated by a localized phosphatase pool (Kim, Lara-Gonzalez et al. 2017, *Genes Dev. 31*:1089-1094). CDC-20 is recruited to kinetochore by interaction with an ABBA motif in BUB-1. Surprisingly, mutation of a conserved docking site on BUB-1 for Polo-like Kinase 1 (PLK-1), ~100 aa distal to the ABBA motif, resulted in a mitotic delay identical to that resulting from mutation of BUB-1's ABBA motif. To identify the target(s) of PLK-1 kinase activity, we conducted a clustered mutagenesis screen focused on BUB-1. This effort identified putative PLK-1 sites within and adjacent to the BUB-1's ABBA motif that were required for CDC-20 kinetochore recruitment. In vitro binding assays with purified components showed that BUB-1 binding to CDC-20 is strongly stimulated by PLK-1 phosphorylation and that the putative target sites in the ABBA motif are essential for this stimulation. Collectively, these data indicate that PLK-1 tethered on BUB-1 controls mitotic progression by phosphorylating sites in the BUB-1 ABBA motif to enhance CDC-20 flux through the kinetochore. This enhanced flux accelerates CDC-20's activation by dephosphorylation and ensures timely mitotic exit during embryogenesis.

B209/P1895

Alignment and segregation of sex trivalents in metaphase I and anaphase I

A. Borseth[1], R. Beebe[1], N. Pedraza-Aguado[1], M. Thomas[1], Z. Zhu[1], T. Müller-Reichert[2], G. Fabig[2], **L. Paliulis**[1]; [1]Bucknell University, Lewisburg, PA, [2]Technische Universität Dresden, Dresden, Germany

Chromosome alignment appears to be critical for proper segregation of a correct quantity of genetic information into daughter cells, yet there are many examples in many taxa of chromosomes that never align prior to segregation. In general, homologous autosomes recombine to form a bivalent in meiosis I. Bivalents then attain bipolar attachments to the spindle by metaphase I, and align in the center of the spindle. Metaphase I alignment precedes separation of homologues to opposite poles in anaphase I. Sex bivalents (e.g. X-Y bivalents) behave like autosomal bivalents, forming bipolar attachments and aligning at the center of the spindle with the autosomal bivalents. The praying mantids *Hierodula membranacea*

and *Tenodera sinensis,* and the cellar spider *Pholcus phalangioides* have X_1, X_2, and Y sex chromosomes, and in males X_1, X_2, and Y chromosomes link together in a sex trivalent. The sex trivalent is placed such that the X_1 and X_2 kinetochores face one spindle pole while the Y kinetochore faces the opposite pole. With one kinetochore-microtubule attachment opposing two kinetochore-microtubule attachments, one might assume that the sex trivalent would not align with the autosomes, but would be closer to the spindle pole associated with the two X chromosomes. We have found that the sex trivalent aligns with all of the autosomes on the metaphase plate in both species of mantids and in the cellar spider. We proposed that one reason for the alignment of the sex trivalent with the autosomes was that the X_1 and X_2 kinetochores are associated with fewer microtubules than the Y kinetochore. We show that the X_1 and X_2 kinetochores were each associated with fewer microtubules than the Y kinetochore, and than any autosomal kinetochore in the cell.

B210/P1896

Kinetochore Structure and Localization is Altered in Yeast Strains with Only 2 Chromosomes

J. Stanton, P. McConnell, K. Bloom, E. Yeh; University of North Carolina - Chapel Hill, NC, CHAPEL HILL, NC

In S. cerevisiae, the haploid genome is distributed into 16 chromosomes. In metaphase, kinetochores cluster into two foci upon biorientation of 16 pairs of sister chromatids.To address the minimal number of kinetochores required to build a network of DNA loops at the pericentromere, essential for the fidelity of chromosome segregation, we examined kinetochores and cohesin in a yeast strain engineered to contain two chromosomes. We tagged kinetochore proteins and cohesin subunits with GFP (Kinetochore Ame1-GFP and Ndc80-GFP, cohesin Scc1-GFP) to visualize their organization. In a 16-chromosome strain, Ndc80 and Ame1 appear as two foci associated with kinetochore microtubule plus-ends. In the 2-chromosome strain, Ame1 appeared as two foci. In contrast Ndc80 was distributed along the spindle axis into ~3-5 foci of varying intensity. Using quantitative intensity measurements to assess protein number, we found Ame1 at 30% the number of Ame1 molecules in the 16-chromosome strain. Based on the number of chromosomes (2 vs. 16) we expected Ame1 to be 12.5% the level of Ame1 in the 16-chromosome strain. Thus, there is about 2.5X more Ame1 than expected based on the number of centromere DNA sequences. The disperse distribution of Ndc80 is further indication of the disruption of kinetochore organization in the engineered yeast strain. Visualization of Scc1-GFP indicates that cohesin is not enriched in the pericentromere in the 2-chromosome strain, as it is in the 16-chromosome strain. These data reveal that the structure of the kinetochore and pericentromere are distinct in the 2-chromosome strain.

B211/P1897

Spatial Map of Polo like kinase-1 (Plk1) in prometaphase

R. X. Norman[1], C. L. Carlsen[2], R. F. Lera[1], M. E. Burkard[1]; [1]Medicine, University of Wisconsin-Madison, Madison, WI, [2]Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI.

Accurate chromosome segregation during mitosis is essential to prevent aneuploidy. The kinetochore is a proteinaceous complex that plays a major role in ensuring faithful segregation by serving as a hub for various events including microtubule binding, kinase signaling, and Spindle Assembly Checkpoint (SAC). Polo-like kinase-1 (Plk1) is known as the master regulator in mitosis and is one of the kinases orchestrating various signaling events during mitosis. Plk1 regulates processes including mitotic entry,

kinetochore-microtubule binding, SAC, and cytokinesis. The Polo Box Domain (PBD) of Plk1 recruits Plk1 to its substrates. We found that Plk1 is also involved in protecting the centromere integrity during mitosis against the microtubule pulling forces. We found that when Plk1 is chemically inhibited, the centromeres unwind, leading to the removal of kinetochore proteins including CENP-A, C, T, and Hec1. Targeting Plk1 activity to the centromere was able to rescue this phenotype. Using delta analysis, we also found that a major pool of Plk1 exists at the centromere away from CENP-U and Bub1, the two major recruiters of Plk1 as described by other studies. In order to identify the Plk1 recruiter at the centromere, we employed a proximity-based biotin labeling approach. We fused Plk1 WT and Plk1 PBD mutant with TurboID, a biotin ligase to biotinylate the Plk1 neighborhood and identify the proteins using mass spectrometry. We also generated TurboID fused to kinetochore proteins such as CENP-U, Bub1, CENP-L, Dsn1, and Kif2c as controls to ensure the biotinylation is restricted to the targeted site. We found that the biotinylation using this system was restricted to the tagged protein neighborhood. We also found that the Plk1 neighborhood was labeled within 10 minutes allowing us to study Plk1 localization in a specific stage of mitosis. Using mass spectrometry, we found that the Plk1 is localized near INCENP (inner-kinetochore), PICH, HELLS(centromere), CENP-C (CCAN), Bub1, BubR1, CENP-E (outer-kinetochore) in prometaphase. Using a modified Expansion Microscopy method (mExM) developed in the lab, we were also able to visualize a pool of Plk1 at the inner centromere.

Cytokinesis 2

B212/P1898

Nanoscale organization of the midbody revealed by expansion microscopy

N. M. Hümpfer[1], G. Russo[2], T. Brill[1], K. Bruckmann[1], S. Donat[3], J. Schmoranzer[3], M. Krauss[2], H. Ewers[1]; [1]Institute for Chemistry and Biochemistry, Freie Universität Berlin, Berlin, GERMANY, [2]Leibniz Institute for Molecular Pharmacology, Berlin, GERMANY, [3]Advanced Medical BIOimaging Core Facility, Charité Universitätsmedizin Berlin, Berlin, GERMANY.

Septins are GTP-binding proteins originally discovered acting in the cell division cycle of budding yeast. Cell division implies cytokinesis as the last step, which is common to all eukaryotic cells. The role of septins in cytokinesis is conserved from yeast to mammals. Their knock down leads to cytokinetic defects, such as multinucleation. To date, septins are considered the fourth component of the cytoskeleton; owing to their ability to form oligomers that assemble into higher order structures such as filaments and rings. Moreover, septins interact with actin and microtubules, and link the cytoskeleton to the plasma membrane.

Both the plasma membrane and the cytoskeleton undergo remodeling during cytokinesis. First, the membrane is constricted at the cleavage furrow. The two emerging cells are connected by a thin tubular structure called the midbody. This remodeling is mirrored by the changes in septin architecture. During cytokinesis, septins populate the membrane of the cleavage furrow, form rings that flank the midbody, attach to midbody microtubules and are found in membrane blebs extruded from the midbody during constriction.

It is elusive how the different septin structures are composed and what distinguishes them in their biological function. We aim to elucidate the roles of septins during cytokinesis using super-resolution microscopy. However, the midbody is densely packed with proteins and therefore resists conventional immunofluorescence staining.

Here, we employ ultrastructure expansion microscopy (U-ExM) to visualize proteins in the midbody,

taking advantage of the decrowding effect inherent to U-ExM. The approximate fourfold expansion of the sample leads to an increase in resolution to 70 nm on a confocal microscope. In contrast to other super-resolution techniques, U-ExM enables multiplexing of targets and collection of volumetric information in a short time. We generate a dataset of unique spatial resolution and cellular context depicting the evolution of septins in cytokinesis.
Our results shed light on the diversity of structures formed by septins during cytokinesis and their tight temporal and functional appearance. In addition, we can show septins in concert with cytokinetic partners, such as anillin and the endosomal sorting complex required for transport (ESCRT). Moreover, we complement our data with lattice light sheet imaging. Live cell recordings of genome-edited cells undergoing cytokinesis allow us to set the U-ExM data in a temporal context. Altogether, we generate a spatio-temporal map of septin reorganization in concert with their partners during cytokinesis, providing the basis for future functional studies.

B213/P1899

Molecular Dynamics Simulations Suggest Anillin-related Mid1 is Adaptive and Multimodal Contractile Ring Anchoring Protein

A. R. Hall, Y. K. Choi, W. Im, D. Vavylonis; Lehigh University, Bethlehem, PA.

The positioning of the cytokinetic ring at the cell equator in animals and fungi depends crucially on the anillin scaffold proteins. During fission yeast interphase and early mitosis, anillin-related Mid1 binds to the plasma membrane and helps establish a broad band of cytokinetic nodes near the cell center. Mid1 consists of a C-terminal globular domain with two potential regions for membrane binding, the PH and C2 domains, both of which prefer PIP2 lipids, and an N-terminal intrinsically disordered region that is strongly regulated by phosphorylation. The PH and C2 domains are joined by a connector domain and the C2 domain contains a predicted flexible region (L3 loop) that is important for membrane binding. Previous studies have shown that both PH and C2 domains can associate with the membrane. However, it's unclear if they can simultaneously bind to the membrane in a way that allows dimerization or oligomerization of Mid1, and if one domain plays a dominant role. In order to elucidate Mid1's membrane binding mechanism, we used the available structural information of the PH, C2, and connector domains in all-atom molecular dynamics simulations of Mid1 near membrane compositionally based on experimental measurements (including PIP2 lipids.) To avoid bias due to initial conditions, we simulated 10 systems starting from different Mid1 orientations with respect to the membrane. We also performed control simulations of a known membrane binding-defective mutant. The results indicate that Mid1 initially binds through the C2's L3 loop, and can further bind through the PH domain and C2's L1 loop. These multiple modes of binding may reflect Mid1's multiple interactions with membranes and other node proteins, and ability to sustain mechanical forces.

B214/P1900

Probing Mechanical and Functional Contributions of F-actin to Microtubule Aster Organization in Cycling *Xenopus* Egg Extract

L. Bai, C. M. Field, T. J. Mitchison; Department of Systems Biology, Harvard Medical School, Boston, MA.

As cells exit mitosis during anaphase, correct positioning of the cleavage plane depends on asters organized by microtubules. In small somatic cells, anaphase asters are positioned by cortical dynein, but in large egg cells where microtubules do not reach the cortex for most of anaphase, the forces are less

understood. A related question is the physical nature of asters. Do they behave as a rigid array of discrete microtubules or a deformable gel? Actomyosin also plays a central role in cell division. In small cells, cortical actomyosin likely dominates cytomechanics, but in large frog eggs, bulk cytoplasmic networks may also be important. To probe microtubule, actin and keratin mechanics in frog eggs, we developed a *Xenopus laevis* egg extract system in which actin-intact, cycling extract was spread under oil. This system allows live imaging over multiple cell cycles and access to mechanical and biochemical perturbation. F-actin was a dominant mechanical player in our extract system. When F-actin was intact, forces exerted by a needle propagated for hundreds of microns and asters behaved as a deformable gel. When F-actin was depolymerized, forces did not propagate over long distances and the aster was easily fragmented by the needle. In the actin-intact system, the interior region of asters was more deformable than the periphery, with implications for force transmission from the periphery to centrosomes during aster movement and centrosome positioning. Functionally, we found that leaving F-actin intact improved segregation of chromosomes and daughter compartments compared to published work where cyclin extract was prepared with actin depolymerized. CPC-enriched zones between sister asters, which determine cleavage planes in intact cells, also formed more robustly when F-actin was intact. The main intermediate filament protein in eggs is keratin. We found that keratin filaments depended on both aster microtubules and F-actin for normal organization. By studying all three cytoskeletal networks in the cell-free system, we are generating novel insights into egg cytomechanics, in particular revealing central roles of bulk cytoplasmic F-actin.

B215/P1901

Dynamics at the nanoscale within the constricting contractile ring in fission yeast

B. Levesque, K. Bellingham-Johnstun, G. Mathew, J. Belmonte, C. Laplante; North Carolina State University, Raleigh, NC.

The contractile ring is a highly complex and dynamic structure. To understand how the contractile ring works, we must determine its molecular organization and the motions of its components. The motions of protein complexes within the contractile ring are governed by their molecular environment and the forces applied to them and therefore contain a wealth of information regarding the function of the contractile ring. Single molecule localization microscopy (SMLM) can resolve protein complexes in dense protein arrays and data acquired in live cells contains dynamic information of their motions at the nanoscale. We developed NanoTrax, an algorithm that tracks clusters of single molecules at the nanoscale, to measure the dynamics of protein complexes within dense cellular structures in data acquired by SMLM in live cells. NanoTrax fits the center of clusters of single molecule emitters over time. NanoTrax ends the tracking when the number of localizations per cluster falls below a minimum threshold of emitters or when an influx of emitters is detected, indicating a collision with another object. In fission yeast, the contractile ring assembles by the coalescence of a band of nodes, complexes of cytokinesis proteins, through actin and myosin interactions. We applied NanoTrax to determine the motions of cytokinesis nodes inside the constricting contractile ring of fission yeast cells. The motions of nodes both in the band and the contractile ring were either diffusive or directed. In both the band and the ring, the majority (~65%) of node motions were diffusive. The high proportion of diffusive nodes within the contractile ring suggests that nodes spend most of their time unbound from the actin filament network. Diffusive nodes had no movement bias within the ring and diffused in all directions while directed nodes primarily moved around the circumference of the ring presumably following the organization of the actin filaments. Interestingly, diffusive nodes never escaped the width of the ring

suggesting the presence of a mechanism that keeps nodes constrained. Our work establishes a new method to examine the dynamics of protein complexes at the nanometer scales and determine how the molecular environment within the contractile ring influences its function.

B216/P1902

Neighbor cells restrain furrowing during epithelial cytokinesis

J. Landino, E. Misterovich, S. Chumki, B. Adhikary, A. L. Miller; University of Michigan, Ann Arbor, MI.

During epithelial cytokinesis, contractile ring ingression challenges tissue homeostasis by generating mechanical force that pulls on neighboring cells *via* cell-cell junctions. How epithelial neighbor cells respond to and regulate cytokinesis is not well understood. Previous work in our lab has shown that apical cell-cell junctions at the furrow are specifically reinforced through the recruitment of the force-sensing protein, Vinculin, and overexpression of a dominant-negative Vinculin mutant increases the rate of furrow closure. This suggests that the cytokinetic contractile array in epithelial cells is subject to resistive forces from non-dividing neighbor cells which regulate furrow closure. To investigate neighbor cell function in epithelial cytokinesis, we examined the localization of contractility factors (active Rho, F-actin, and Myosin II) in neighbor cells of gastrula-stage *Xenopus laevis* embryos and found these factors accumulate specifically at and near junctions neighboring the cytokinetic furrow, indicating that non-dividing neighbors assemble a contractile array in response to furrow ingression. We next asked whether neighbor cell actomyosin could modulate cytokinesis and predicted that increased stiffness or contractility in neighbor cells would slow the rate of furrow ingression. We first tested this by overexpressing the actin cross-linking protein α-actinin in neighbor cells and found that high level α-actinin overexpression in neighbors slowed furrowing. We also activated Rho-mediated contractility in neighbor cells with high spatiotemporal precision using the TULIP optogenetic system. Optogenetic activation of Rho-mediated contractility in neighbors paused furrowing for the duration of optogenetic stimulation. Notably, for cells with two different neighbors (one control and one with increased contractility), the rate of furrowing was only changed on the side of the cell with a contractile neighbor. Additionally, preliminary experiments show that optogenetic stimulation of Rho-mediated contractility in both neighbor cells blocks successful furrow ingression and induces cytokinetic failure. These findings indicate that neighbor cell actomyosin contractility regulates the speed and success of epithelial cytokinesis.

B217/P1903

Post-anaphase microtubule networks and interphase microtubules cooperate to re-center the nucleus after spindle breakdown

K. Bellingham-Johnstun, A. Thorn, J. Belmonte, C. Laplante; NCSU, Raleigh, NC

Centering the nucleus is critical for symmetrical cell division in fission yeast. During mitosis, the spindle pushes the daughter nuclei to the cell tips and the nucleus must reposition to the cell center. We measured that the nucleus remains off center after the disassembly of the spindle until ~40 minutes after septation. Depolymerization of microtubules in cells that recently completed anaphase prevented nuclear repositioning. The presence of the mitotic spindle pole body (mSPB) and post-anaphase array (PAA) microtubules coincided with the timing of nuclear repositioning before septation. We leveraged known mutations in the *mto1* gene, encoding a component of the gamma-tubulin complex, to determine the specific role of the mSPB and PAA microtubules during nuclear repositioning. The mSPB

microtubules are connected to the nuclear envelope at the SPB and their plus ends grew toward the end of the cell. In *mto1(1-1085)* cells, where mSPB microtubules are absent, the daughter nuclei remained at the cell tips. The PAA microtubule network assembled in the plane of the contractile ring with microtubules extending away from the ring toward the nucleus. In *mto1-427* cells, which lack PAA microtubules, the daughter nuclei traveled closer to the division plane. These results suggest that the mSPB microtubules push the nucleus away from the end of the cell while the PAA microtubules limit its movement toward the contractile ring. This push-push mechanism positioned the nucleus at ~1/3 of the distance between the end of the cell and the contractile ring by the end of cytokinesis. In the newborn cell, the nucleus reached the cell center at a velocity of 0.03 µm/min, slower than the repositioning of an artificially displaced nucleus in interphase cells. We used Cytosim simulations constrained by experimentally measured microtubule dynamics to determine the mechanism that influences nuclear displacement velocity. We found that the ratio of the distance between the nucleus and the old cell end (l) and the nucleus and the new cell end (L) governs nuclear displacement velocity. Smaller l/L ratios resulted in faster displacements. This relationship recapitulated our experimentally measured displacement velocities in wild-type, *mto1-427* and *mto1(1-1085)* cells. Together, these microtubule-dependent mechanisms ensure the centering of the nucleus ahead of cytokinesis.

B218/P1904

CUL3 E3 Ligase-Dependent Contractile Ring Ubiquitination Underlies Unilateral Cytokinesis in *Caenorhabditis elegans* Zygotes

C. Hsu[1,2], W. Fan[1,2], K. Sugioka[1,2]; [1]Zoology, University of British Columbia, Vancouver, BC, CANADA, [2]Life Sciences Institute, Vancouver, BC, CANADA.

Cytokinesis is the last step of cell division during which a mother cell is physically separated into two daughter cells via the contractile ring. Eccentric closure of the contractile ring, called unilateral cytokinesis, plays crucial roles in animal morphogenesis. Although previous studies revealed several unilateral cytokinesis regulators such as anillin and adherens junction, the molecular mechanisms regulating asymmetric contractile ring constriction remain elusive. Here we show that CUL-3 E3 ubiquitin ligase regulates asymmetric contractile ring closure. By performing 4D live-imaging, we found that *cul-3(RNAi)* resulted in concentric ring closure. Immunostaining of dividing cells using an anti-poly-ubiquitin antibody revealed that the contractile ring is asymmetrically ubiquitinated. Furthermore, this asymmetric contractile ring ubiquitination is lost in *cul-3(RNAi),* suggesting that ring ubiquitination is CUL-3-dependent. To identify the potential substrate of CUL-3-dependent ubiquitination, we have tested several actomyosin regulators required for unilateral cytokinesis. We found that CYK-1/formin and ANI-1/anillin are not required for contractile ring ubiquitination. However, contractile ring ubiquitination was lost after the knockdown of POD-1/coronin. A previous study reports the physical interaction between POD-1 and an adaptor protein of CUL-3 E3 ligase MEL-26 (Luke-Glaser et al., 2005). Although the study concluded that POD-1 is not the target of ubiquitination based on Western blotting, the subcellular concentration of POD-1 may be controlled by the CUL-3 E3-ubiquitin ligase-dependent degradation. To test this hypothesis, we will quantify POD-1 protein levels with and without CUL-3 activity. Our study will reveal the intricate mechanism of actomyosin regulation during cytokinesis.

B219/P1905

Anillin forms linear structures and facilitates furrow ingression after septin and formin depletion

M. Lebedev[1], **E. Zanin**[1], F. Chan[2], J. Bellessem[3], D. Osório[2], E. Rackles[3], T. Mikeladze-Dvali[3], A. X. Carvalho[2]; [1]Department Biologie, FAU Erlangen, Erlangen, GERMANY, [2]Instituto de Investigação e Inovação em Saúde (i3S), Porto, PORTUGAL, [3]Department Biologie, Ludwig-Maximilians University, München, GERMANY.

In animal cells a contractile ring consisting of formin-nucleated filamentous actin and myosin II assembles at the cell equator at the end of cell division. Contractile ring constriction deforms the plasma membrane and thereby forms the two daughter cells. In the absence of formin activity no unbranched F-actin is generated, no cleavage furrow forms and cell division fails. Surprisingly it was shown that a temperature-sensitive formin (CYK-1) mutant can ingress a cleavage furrow when septins are co-depleted (Jordan et al., 2016). To understand how those embryos ingress a cleavage furrow in the absence of CYK-1-nucleated F-actin, we investigated the dynamics of the contractile ring components anillin (ANI-1) and myosin II (NMY-2) in CYK-1-depleted C. elegans embryos. We found that ANI-1 forms linear structures after CYK-1 depletion, which are decorated by NMY-2. The linear ANI-1 structures circumferentially align around the cell equator similar to F-actin in control embryos. Therefore we tested whether ANI-1 is required for furrow ingression in CYK-1 and septin co-depleted embryos. Indeed we observed that ANI-1 is essential for cleavage furrow ingression in CYK-1 and septin co-depleted embryos. To tests, which region of ANI-1 is responsible for the formation of linear structures, we split ANI-1 into two parts. The N-terminal part comprises the putative myosin II binding domain, actin-binding domain and a large unstructured linker region. The C- terminal part contains the RhoA, membrane (C2) and septin (PH) binding domains. We find that the N-terminal ANI-1 part forms linear and the C-terminal part circular structures. Further, the N-terminal part partially rescues furrow ingression in CYK-1 and septin co-depleted embryos whereas the C- terminal one does not. Together, our results reveal that the N-terminal part of ANI-1 forms linear structures and assists furrow ingression in the absence of septins and formins. Since the N-terminal part is highly disordered and such regions frequently undergo liquid-liquid phase separation, we speculate that a condensation-based mechanism mediates the contraction of the ANI-1 network. Together, we uncover a novel ANI-1 dependent and unbranched F-actin independent mechanism of furrow ingression during cell division.

Jordan, S.N., T. Davies, Y. Zhuravlev, J. Dumont, M. Shirasu-Hiza, and J.C. Canman. 2016. Cortical PAR polarity proteins promote robust cytokinesis during asymmetric cell division. The Journal of Cell Biology. 212:39-49. doi:10.1083/jcb.201510063.

B220/P1906

Two-Color Super-Resolution Imaging Reveals the Spatial Organization of Two Type II Myosins within Contractile Ring in Fission Yeast

C. Bi[1], D. Ma[1], W. Sayyad[2], T. D. Pollard[2], F. Huang[1]; [1]Purdue University, West Lafayette, IN, [2]Yale University, New Haven, CT.

During cytokinesis, myosin-II constricts the contractile ring that separates one cell into two daughter cells. The fission yeast cytokinetic contractile ring contains two types of myosin II, Myo2 and Myp2, which join the ring at different times but confocal imaging and genetics studies showed that both contribute to the constriction and integrity of the ring. Previously, using 2D live cell single molecule localization microscopy, we showed that cytokinetic nodes, the basic building blocks of the ring, persist

after ring assembly and throughout constriction. Confocal and super resolution microscopy suggested the Myp2 is located further from the plasma membrane than Myo2. However, the precise ultrastructural arrangement of the two type II myosins remains in question. Here, we investigated the relative spatial arrangement of Myo2p and Myp2p within contractile ring using two-color super-resolution microscopy based on salvaged fluorescence imaging. Our aim is to determine how the spatial distributions of the two myosin II proteins evolve during contractile ring constriction. We established a robust protocol for fixing and staining fission yeast with nanobodies. We used the *cdc25-22* temperature sensitive strain to synchronize the cells in mitosis. By radiometric detection of spectrally overlapped fluorescence tags, Alexa Fluor 647 and CF660C, we obtained two-color super-resolution images of nanobody labeled Myo2p and Myp2p with minimized wavelength induced misalignment. Quantitative analysis of the nanoscale images should provide useful information for modeling contractile ring assembly and constriction.

B221/P1907

Disruption of Cell Division by P34R Mutated RAC1

E. McBeath[1], L. Cruz[2], A. Herrera Montejano[2], R. Yarmand[1], M. Hofmann[1]; [1]University of Texas MD Anderson Cancer Center, Houston, TX, [2]Monterrey Institute of Technology School of Medicine and Health Sciences, Monterrey Nuevo León, MEXICO.

Background: While investigating the cause of resistance to the drug dabrafenib in PDX.008.CL, an anaplastic thyroid cancer cell line with a dabrafenib sensitive BRAF mutation, we discovered that these cells had a combined amplification and P34R mutation of the *RAC1* gene. RAC1 is a small GTPase involved in cell motility and cytoskeletal reorganization. It is known to shuttle between the cytoplasm (inactive state) and the plasma membrane (active state). RAC1 overexpression and hyperactivity are common in many cancers. However, the mechanisms by which altered RAC1 drives tumor progression are unknown. We hypothesize that the P34R RAC1 mutation drives hyperactivity, in turn dysregulating chromosome segregation and/or cytokinesis during cell division. This leads to an increased level of cell death, random mutations and altered protein expression from which a stable metastatic cell can rapidly arise.
Methods: mNeonGreen-tagged $RAC1^{WT}$ and $RAC1^{P34R}$ proteins were transiently expressed in the papillary thyroid cancer (PTC) cell line MDA-T85, which in addition to the $BRAF^{V600E}$ mutation, contains the normal two copies of $RAC1^{WT}$. Stable transfectants were selected by cell sorting. Cells were visualized by phase contrast, confocal, and time-lapse fluorescence microscopy.
Results and discussion: PDX.008.CL cells were 10x more resistant to dabrafenib compared to several $BRAF^{V600E}$-mutated PTC cell lines. Treatment of PDX.008.CL cells with the RAC1 activity inhibitor EHop-016 restored sensitivity to dabrafenib. This implies that excess RAC1 activity due to the P34R mutation and/or gene amplification is directly involved in resistance to dabrafenib. When transiently transfected with fluorescent $RAC1^{P34R}$ and compared with $RAC1^{WT}$, the PTC cell line MDA-T85 had reduced levels of $RAC1^{P34R}$ in the cytoplasm, distinct cell membrane and nuclear envelope fluorescence, increased cell death, and frequently a clear disruption of cytokinesis and disorganized nuclear structures. This may have led to the polyploidy observed in the metastatic patient sample and derived PDX.008.CL cell line. MDA-T85 cells stably expressing $RAC1^{P34R}$ showed shape and cytoskeletal changes identical to that seen in PDX.008.CL, indicating dedifferentiation. This suggests that eventually, further changes occurred that overcame the cell death caused by the effect of overactive $RAC1^{P34R}$ on cell division.

B222/P1908

Cytoplasmic divisions without nuclei

A. Bakshi, F. Echegaray Iturra, A. Alamban, M. Rosas-Salvans, S. Dumont, **M. G. Aydogan**; University of California, San Francisco, San Francisco, CA.

Cytoplasmic divisions have been commonly considered a sequel to nuclear divisions, even in the absence of DNA replication. Here we found in fruit flies that the cytoplasm can compartmentalize and divide without nuclei. Our targeted screen for potential necessary and sufficient conditions revealed that, although the cytoplasmic compartments are tightly associated with centrosomes, they can form without astral microtubules and divide without centrioles. Although a focal pool of microtubules is *necessary* for maintaining cytoplasmic compartments, this is not sufficient for their initial formation. Actin filaments are similarly an essential component of cytoplasmic compartments; however, their myosin II-based contractility is unexpectedly dispensable for divisions. We show that the myosin II-based contractility is instead involved in regulating the *pace* of cytoplasmic divisions. Importantly, our results revealed that the cytoplasmic divisions without nuclei can occur in a periodic manner autonomously of the Cdk-Cyclin oscillator that normally drives the cell cycle. We demonstrate that such autonomy of cytoplasmic divisions is preserved even in normal development, where it is leveraged to extrude mitotically delayed nuclei from the blastoderm, safeguarding embryos against local delays in mitotic entry prior to morphogenesis. We propose that an active coordination between otherwise autonomous cycles of cytoplasmic and nuclear divisions act as a quality control mechanism for genome integrity and partitioning in development.

B223/P1909

Midbody reorganization precedes ring canal formation during gametogenesis

K. L. Price, L. Cooley; Yale University School of Medicine, New Haven, CT

Animal gametes typically develop through some or all of gametogenesis as syncytia of cells attached by stable intercellular bridges called ring canals. However, how canonical cytokinesis is altered during germ cell division to produce ring canals is poorly understood. Using time-lapse imaging of cytokinesis proteins in dividing germ cells in the Drosophila male germline, we observe that ring canal formation occurs via reorganization of the germline midbody, a structure classically associated with its function in recruiting abscission-regulating proteins in complete cytokinesis. Centralspindlin complex components Pav (MKLP1) and Tum (MgcRacGAP), while initially localized to the central spindle and cleavage furrow, accumulate in a dense focus resembling a canonical midbody core. The midbody-like focus is compositionally similar to the midbodies formed during complete cytokinesis; the centralspindlin-containing midbody core is encircled by a ring of Septin-2, Anillin, and Citron kinase/Sticky proteins, all known components of the midbody ring. However, the germline midbody is short-lived, and rapidly reorganizes to join the proteins in the midbody ring resulting in a ring canal with an open lumen. This reorganization from midbody-to-ring canal is accompanied by a ~5-fold decrease in centralspindlin fluorescence intensity suggesting that degradation or relocalization of centralspindlin protein is required for ring canal formation. We found a remarkably similar midbody-to-ring canal transformation in mouse and Hydra testes, suggesting it is an intrinsic feature of gamete formation. Interestingly, we find a role for Sticky in the midbody-to-ring canal transition. Germ cell-specific knockdown of Sticky in the testis results in the formation of ectopic midbodies, decreased ring canal diameter (as shown by Naim et al., 2004), as well as a decrease in the average fluorescence of Pav in ring canals. Live imaging of Pav in

nos>sti RNAi testes reveals that Sticky is necessary for the timely midbody-to-ring canal transition. Of the ring canals that form, the timing from formation of the central spindle to nascent ring canal is delayed in contrast to controls and some midbodies never reorganize to form ring canals. Furthermore, we find a reduction in fecundity in *nos>sti RNAi* males suggesting an important role for Sticky during incomplete cytokinesis in spermatogenesis.

B224/P1910

Bridging the [CYK-4] GAP: The CYK-4 GAP Domain Activates ECT-2 and Inactivates Rac to Promote Cytokinetic Furrow Ingression

A. Schlientz[1], S. Gómez-Cavazos[1,2], K.-Y. Lee[2], P. Lara-González[3,2], A. Desai[1,2,1], K. Oegema[1,2,1]; [1]University of California, San Diego, La Jolla, CA, [2]Ludwig Institute for Cancer Research, La Jolla, CA, [3]University of California, Irvine, Irvine, CA

During cytokinesis, contractile ring assembly is controlled by the Rho family small GTPase RhoA, whose key activator, the Rho guanine nucleotide exchange factor (GEF) ECT2, is held in an autoinhibited state. Thus, a central question in cytokinesis is understanding how ECT2 autoinhibition is relieved to enable contractile ring assembly. ECT2 is activated by centralspindlin, which is comprised of two molecules of the microtubule motor kinesin-6 and two molecules of CYK-4. Centralspindlin is required for assembly of the central spindle, and the CYK-4 subunit is important to recruit centralspindlin to the equatorial plasma membrane where it interacts with and activates ECT2. CYK-4 has two regions implicated in furrow ingression: an N-terminal region that is phosphorylated by Polo-like Kinase 1 to enhance its binding to the BRCT repeat region of ECT2, and a C-terminal GTPase-activating protein (GAP) domain. Whether the CYK-4 GAP domain directly activates ECT2 or acts indirectly by inactivating the competing Rho family GTPase Rac has been controversial. Here, we address how the GAP domain of CYK-4 promotes furrow ingression in the *C. elegans* embryo. In support of the model that the CYK-4 GAP domain promotes cytokinesis by inactivating Rac, Rac depletion rescued the furrow ingression defect of a CYK-4 GAP domain mutant but not of an N-terminal phosphosite region mutant. To determine if the CYK-4 GAP domain also directly activates ECT-2, we analyzed furrow ingression in embryos lacking the nematode-specific ECT-2 activator NOP-1. Under this condition, simultaneous mutation of the CYK-4 N-terminal phosphosite region and of the C-terminal GAP domain's interface with Rho GTPases prevented furrow ingression. In contrast, CYK-4 constructs in which only the N-terminal phosphosite region or only the C-terminal GAP domain were mutated supported furrow ingression. Essentially identical results were obtained in Rac-depleted embryos. These findings indicate that the C-terminal GAP domain of CYK-4 promotes furrow ingression independently of regulation of Rac and are consistent with a model in which the N-terminal phosphosite and C-terminal GAP domains of CYK-4 act coordinately to fully relieve ECT-2 autoinhibition.

B225/P1911

A similar two-domain strategy is used by IQGAPs to regulate cytokinesis in budding yeast, fission yeast, and mammalian cells

K. Wang[1], H. Okada[1], C. Wloka[1,2], E. Bi[1]; [1]University of Pennsylvania, Philadelphia, PA, [2]University of Groningen, Groningen, Netherlands

IQGAP is a multi-domain protein known to regulate cytokinesis in eukaryotes from yeast to human. However, how the predicted domains function in cytokinesis and whether the sequences between the

domains have evolved during speciation to accommodate organism-specific features of cytokinesis have largely remained a mystery. Here, by making precise deletions of any specific regions at the chromosomal locus of *IQG1*, which encodes the IQGAP in budding yeast, coupled with GFP tagging and quantitative live-cell imaging, we have defined the boundary of the calponin homology domain (CHD) and discovered an unexpected domain, which we designate Myosin Tail Associated domain (MTA). The CHD domain localizes to actin cables and the MTA domain localizes to the division site in a myosin-II dependent manner. The CHD and MTA domains together target to the division site more efficiently and more specifically than either domain alone. We further demonstrated that these two domains function together to enable efficient actomyosin ring (AMR) assembly, constriction, and removal. Next, we tested the corresponding regions of the budding yeast Iqg1's CHD and MTA in Rng2, the IQGAP in fission yeast, and found that the MTA-corresponding region localizes to the division site by binding to both myosin-IIs and the F-BAR protein Cdc15. For this reason, we named the MTA-corresponding region "Cytokinesis Factor (CykF) binding domain". Similar to budding yeast, the CHD-CykF module in Rng2 localizes to the division site during cytokinesis and regulates the AMR assembly and constriction. Finally, we examined this module-like design in IQGAP3 in Hela cells. We found that the CHD does not localize to the division site by itself whereas the MTA-corresponding region in IQGAP3, i.e., IQGAP-specific repeats (IR) domain, localizes to the division site only during late stage of furrowing. Strikingly, the CHD and IR together, localize to the division site robustly and during the entire furrowing process, and this localization depends on anillin. Anillin is known to interact with actin and myosin-II and play an important role in cytokinesis. Taken together, these analyses uncover a novel two-domain strategy used by IQGAPs to control cytokinesis from yeast to human, which reflects the principle of evolution - conservation (indicated by the CHD) and speciation (indicated by the MTA/CykF/IR).

B226/P1912

Bni5 interacts with septins and non-muscle myosin-II via distinct domains to carry out its roles in actin retrograde flow and cytokinesis

H. Okada, K. Wang, J. Marquardt, E. Bi; University of Pennsylvania, Philadelphia, PA

Myo1, the sole non-muscle myosin-II (NM-II) in the budding yeast *Saccharomyces cerevisiae*, is recruited to the septins, a family of cytoskeletal proteins that are organized into a cortical ring at the future division site (i.e., the bud neck), at the beginning of the cell cycle, and then interacts with actin filaments in anaphase to form an actomyosin ring (AMR) to drive cytokinesis. While its role in cytokinesis is similar to that of NM-IIs in other organisms, the mechanism underlying its early recruitment and function at the bud neck remain poorly understood. To address this question, we focused our analysis on Bni5, the sole linker between the Myo1 and the septins before cytokinesis. First, we constructed and found a striking difference between an N-terminally and a C-terminally GFP-tagged Bni5 strains by quantitative live-cell imaging analysis. The C-terminally tagged Bni5 showed ~10 min delay in the initial recruitment due to disruption of a direct association to the septins. Next, we performed a structure-function analysis of Bni5, which is predicted to contain three coiled-coiled (CC1-3) regions and a long-disordered region between CC1 and CC2. We examined the in vivo behaviors of ~30 different fragments of Bni5 by time-lapse microscopy in wild-type and various mutant strains. These analyses have led to the identification of the N-terminal CC1 region as the Myo1-binding domain, the C-terminal CC2 and its immediate upstream ~30 amino-acid extension as the septin-binding domain, and the CC2 + CC3 region as the Elm1 kinase (a septin stabilizer)-binding domain. We also found that deletion of *BNI5* slowed down actin retrograde flow, which maintains yeast longevity before cytokinesis. Furthermore, we observed that

deletion of *BNI5* reduced the amount of Myo1 at the division site by half at the onset of cytokinesis. Importantly, this reduction was sufficient to drive cytokinesis under normal conditions but failed to do so when cells were exposed to various stresses (e.g., actin filament perturbation). Taken together, Bni5 promotes Myo1 recruitment to the division site via distinct interactions to ensure efficient execution of two different but consecutive processes during the cell cycle: (1) actin retrograde flow to maintain longevity before cytokinesis, and (2) AMR assembly for the robustness of cytokinesis against environmental insults.

B227/P1913

An anillin-CIN85 complex regulates the re-organization of septin filaments of distinct compositions to drive intercellular bridge maturation

A. Wilde, T. Panagiotou; University of Toronto, Toronto, ON, Canada

Cytokinesis, the last stage of cell division, orchestrates the physical separation of newly forming daughter cells. After the cleavage furrow ingresses between the segregated chromosomes, the intercellular bridge (ICB) is established that serves as the final connection between daughter cells. This dynamic structure undergoes an ordered maturation process: extending, thinning, and tubulating membrane before it is ultimately abscised. Disruption of any of these intermediates restricts cytokinetic efficiency and success, promoting tumorigenesis among other pathologies. The cytokinetic scaffold anillin and the family of filamentous septins have been identified as key players in ICB establishment and development, where the interaction between anillin's C-terminal PH domain and septins is required to mature the ICB. Septins exist in cells as membrane-associated filaments composed of hexameric and octameric units that are differentiated by the hexamer's omission of septin monomers from the SEPT3-subgroup, where the sole member expressed in HeLa cells is SEPT9. While it is unclear if hexameric and octameric units construct homo- or hetero-typic filaments in vivo, the cellular functions of septins appear invariably attributed to octamers. Intriguingly, while other septins are required to complete early cytokinetic events, SEPT9 is the sole septin required for ICB abscission, indicating that septin hexamers and octamers may perform different functions during cytokinesis. How septin hexamers and octamers function to establish the ICB, and their relation to anillin during this process, remains unclear. Here we report that the novel cytokinetic factor CIN85 is required for ICB assembly and maturation, by simultaneously binding both anillin and septin octamers. CIN85 directly interacts with SEPT9 and the N-terminus of anillin, while the C-terminus of anillin interacts with SEPT11-positive septin units. Strikingly, these SEPT11-positive units lack SEPT9, indicating they are septin hexamers. We find that this network of interactions is required to stabilize septin octamers on the ICB membrane. We propose that octameric and hexameric homotypic septin filaments are connected and reorganized through anillin-CIN85 complexes to actively form and mature the ICB for successful cell division.

G0, G1, S-phase and Cell Proliferation Control

B228/P1914

Revealing b-TrCP activity dynamics in live-cells with a genetically encoded biosensor

D. Paul[1,2], S. C. Kales[1], J. A. Cornwell[1], M. M. Afifi[1], G. Rai[1], A. Zakharov[1], A. Simeonov[1], S. D. Cappell[1]; [1]NIH, Bethesda, MD, [2]Lcbg, NCI, Bethesda, MD.

The F-box protein beta-transducin repeat containing protein (β-TrCP) acts as a substrate adapter for the SCF E3 ubiquitin ligase complex, plays a crucial role in cell physiology, and is often deregulated in many types of cancers. Here, we developed a fluorescent biosensor to quantitatively measure β-TrCP activity in live, single cells in real-time. We found β-TrCP remains constitutively active throughout the cell cycle and functions to maintain discreet steady-state levels of its substrates. We found no correlation between expression levels of β-TrCP and β-TrCP activity, indicating post-transcriptional regulation. A high throughput screen of small-molecules using our reporter identified receptor-tyrosine kinase signaling as a key axis for regulating β-TrCP activity by inhibiting binding between β-TrCP and the core SCF complex. Our study introduces a method to monitor β-TrCP activity in live cells and identified a key signaling network that regulates β-TrCP activity throughout the cell cycle.

B229/P1915

Regulation of cell size and Wee1 by elevated levels of Cdr2

R. A. Berg, J. B. Moseley; Dartmouth College, Hanover, NH.

Many cell cycle regulatory proteins catalyze cell cycle progression in a concentration-dependent manner. In fission yeast *S. pombe*, the protein kinase Cdr2 promotes mitotic entry by organizing cortical oligomeric nodes that lead to inhibition of Wee1, which itself inhibits Cdk1. *cdr2Δ* cells lack nodes and divide at increased size due to overactive Wee1, but it has not been known how increased Cdr2 levels might impact Wee1 and cell size. Using a Tetracycline-inducible expression system, we found that a 6X increase in Cdr2 expression caused hyperphosphorylation of Wee1 and reduction in cell size. This overexpressed Cdr2 formed clusters that sequestered Wee1 adjacent to the nuclear envelope. Cdr2 mutants that disrupt either kinase activity or clustering ability failed to sequester Wee1 and to reduce cell size. We propose that Cdr2 acts as a dosage-dependent regulator of cell size by sequestering its substrate Wee1 away from Cdk1 in the nucleus. This mechanism has implications for other clustered kinases, which may act similarly by sequestering substrates.

B230/P1916

Mechanisms determining where DNA replication initiates in the human genome

T. Noble[1,2], C. G. Sansam[2], B. Majchrzycka[2], K. Wittig[1,2], C. Xu[3], C. L. Sansam[2,1]; [1]Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, [2]Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK, [3]Biostatistics and Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

The selection of replication origins is a defining characteristic of DNA replication in higher eukaryotes, yet its mechanism in humans has not been well-defined. In yeast, origin selection involves replication initiation factor (Sld3-Sld7) recruitment to origins during G1. In this study, we use Cut&Run to examine genomic binding locations for TICRR and MTBP, the Sld3 and Sld7 orthologs. We have constructed two

HCT116 human colorectal cancer cell lines in which the endogenous TICRR or MTBP loci were tagged at their carboxy-termini with mClover. Using these cell lines, we have shown that TICRR and MTBP genomic binding sites can be mapped using Cut&Run with anti-GFP antibody. We mapped TICRR and MTBP binding throughout the cell cycle by performing experiments in asynchronous, G1, or G2-arrested cells. Peaks of TICRR and MTBP binding frequently overlap at Ini-seq replication origins. Additionally, our results show HCT116 TICRR and MTBP peaks overlap with MTBP peaks previously defined (Kumagai et al. Cell Rep. 2020) in a DLD-1 cell line. Interestingly, our data show that TICRR and MTBP binding patterns are less defined in asynchronous cells than G1, possibly due to cell cycle phase-specific recruitment of TICRR-MTBP to replication origins in human cells.

B231/P1917

Fission yeast cells integrate multiple pathways for geometry-based cell size control

K. E. Miller[1], C. A. Vargas-Garcia[2], A. Singh[3], J. B. Moseley[1]; [1]Geisel School of Medicine at Dartmouth, Hanover, NH, [2]Colombian Agricultural Research Institute, Bogotá, COLOMBIA, [3]University of Delaware, Newark, DE.

The size of a cell is critical to its function and physiology. Many studies have established that eukaryotic cells delay cell cycle transitions until a threshold cell size is reached. However, the specific aspect of cell size that is monitored is less well understood in most cases. Fission yeast cells enter mitosis and divide at a specific cell surface area due to regulated activation of Cdk1, in part through surface area-dependent accumulation of the protein kinase Cdr2. Using a panel of genetic mutations that alter cell size, we find that fission yeast cells only divide by surface area under a certain size threshold but shift to volume-based divisions at a larger size. The threshold for changing from surface area to volume-based size control is set by ploidy, consistent with longstanding connections between ploidy and cell size. These results led us to investigate the molecular nature of size control beyond surface area and Cdr2. We tested how the concentration of mitotic activators Cdc13/cyclin and Cdc25, which accumulate with increasing cell size, scale with different parameters of cell geometry. We find that Cdc25 concentration increases specifically with cell volume, as opposed to other aspects of size. In contrast, the concentration of mitotic cyclin Cdc13 does not scale with cell size, but rather accumulates with time regardless of cell size or growth. We combined these molecular pathways measuring volume, surface area, and time into a mathematical model for fission yeast cell cycle progression. Our results lead to an integrated model for cell size control based on multiple signaling pathways that report on distinct aspects of cell size and growth, including cell surface area (Cdr2), cell volume (Cdc25), and time (Cdc13). Such an integrated system provides robust and dynamic properties for size control upon changes to individual pathways or size parameters.

B232/P1918

Phosphorylation of p27 at T197 promotes cell cycle progression through transcriptional coregulation of cJun and STAT3 action

A. Bagheri, S. Razavipour, J. Slingerland; Oncology, Georgetown University, Washington, DC.

p27 is a cyclin dependent kinase (CDK) inhibitor that binds cyclin-CDKs to arrest the cell cycle. p27 undergoes PI3K/AKT-mediated C-terminal phosphorylation that alters its protein-protein interactions and function. Our lab showed C-terminally phosphorylated p27 (p27pT157pT198) promotes its association with cJun. Genomic profiling showed p27 is co-recruited with cJun to over half of cJun

chromatin binding sites to either activate or repress target genes. p27/cJun activated gene targets include TGFB2, and are associated with EMT, and programs that upregulate stem cells and alter cell adhesion and migration. We pursue the novel hypothesis that cyclic changes in C-terminally phosphorylated p27 abundance govern p27-regulated transcriptional activity across the cell cycle, switching p27 from a corepressor of target genes in G0, to a transactivator upon PI3K/AKT activation in mid-G1. In synchronized NIH3T3 cells, we made the novel observation that cJun, phospho-activated cJunpS63, and phosphorylated STAT3 (STAT3pY705) levels periodic across the cell cycle. cJun is minimally expressed in quiescence, and is strongly upregulated in early G1. Phosphorylated cJun demonstrates a different pattern of expression than total cJun. While total cJun expression level remains constant at all time points after T=3 hours, cJunpS63 rises sharply in G1 and then declines with cell cycle progression. Total STAT3 levels are constant across G0 to S phase. The expression pattern for phosphorylated STAT3pY705 is similar to that of cJun pS63, minimal in quiescence, then increases as the cells enter cell cycle.To investigate the binding of p27pT197 to both c-Jun and STAT3 in NIH3T3 cells, we used a phospho-specific antibody against p27pT197 to perform Co-IP. p27pT197 not only co-precipitates with each of c-Jun and STAT3, but also this co-precipitation is periodic during cell cycle progression. The greater co-precipitation of p27pT197 with c-Jun and STAT3 in mid-G1-to S phase of the cell cycle suggests that complex formation is actively regulated. These data raise the intriguing possibility that p27 co-regulation of cJun and STAT3 transcription might be critical for cell cycle progression from quiescence to mid-G1. This is currently under investigation. Ongoing work investigating cell cycle-regulated profiles of p27-STAT3-cJun driven gene expression is underway and will be presented.The potential role of p27-regulated gene expression in the coordination of cell cycle progression has not been explored to date. A better understanding of how p27/cJun/STAT3 transcriptional complexes are regulated in normal tissue and in development might identify new therapeutic targets to be exploited for tissue regeneration and cancer therapy.

B233/P1919

Tissue confinement regulates cell growth and size in epithelia

J. Devany[1], M. J. Falk[1], L. J. Holt[2], A. Murugan[1], M. L. Gardel[1]; [1]Department of Physics, University of Chicago, Chicago, IL, [2]Institute for Systems Genetic, New York University, New York, NY.

Cell proliferation is a central process in tissue development, homeostasis and disease. Yet how proliferation is regulated in the tissue context remains poorly understood. Here, we introduce a quantitative framework to elucidate how tissue growth dynamics regulate cell proliferation. We show that tissue growth causes confinement that suppresses cell growth; however, this confinement does not directly affect the cell cycle. This leads to uncoupling between rates of cell growth and division in epithelia and, thereby, reduces cell size. Division becomes arrested at a minimal cell size, which is consistent across diverse epithelia in vivo. Here, the nucleus approaches a volume limit set by the compacted genome. The loss of Cyclin D1-dependent cell size regulation results in an abnormally high nuclear-to-cytoplasmic volume ratio and DNA damage. Overall, we demonstrate how epithelial proliferation is regulated by the interplay between tissue confinement and cell size regulation.

B234/P1920

Differential APC/C^{Cdh1} activity controls two independent S phase entry pathways

S. Upadhya[1], N. Ratnayeke[2], T. Meyer[1]; [1]Cell & Developmental Biology, Weill Medical College/Cornell University, New York, NY, [2]Chemical & Systems Biology, Stanford University School of Medicine, Stanford, CA.

Cells make the fundamental decision to proliferate in G1 phase by inactivating the E3 ubiquitin ligase APC/C^{Cdh1} and activating the cyclin-dependent kinases CDK2 and CDK1, as well as the kinase CDC7 that promotes DNA replication. APC/C^{Cdh1} degrades numerous proteins that promote cell-cycle progression, and its inactivation allows for the buildup of these proteins. Recent work has shown that CDK1 alone is sufficient to start S phase without CDK2 and CDC7 activity. However, other studies suggested that cells can start S phase without requiring CDK1 activity. We hypothesize that cells utilize two mechanisms to enter S phase, with or without CDK1 activity. Our study makes use of a live-cell reporter system that we developed for non-transformed human MCF10A epithelial cells to measure the inactivation of APC/C^{Cdh1}, activation of CDK2/1, and precise time of S phase entry, all in the same cell. Earlier work shows that premature APC/C^{Cdh1} inactivation accelerates S phase entry and that DBF4 is degraded during G1, thereby suppressing G1 DBF4-CDC7 kinase activity. Surprisingly, we now found that (i) DBF4 is only weakly degraded by APC/C^{Cdh1} and CDC7 kinase is already active in G1, and (ii) cells frequently start S phase before they inactivate APC/C^{Cdh1}. However, Cdh1 knockdown also causes increased proliferation. Together with measurements using selective kinase inhibitors and genetic manipulations of critical regulators, our initial data suggests that APC/C^{Cdh1} regulation is pivotal to control which path cells take to proliferate: To enter S phase, cells either require (1) increased activity of both CDK2 and CDC7, which does not require cells to inactivate APC/C^{Cdh1} and also does not require CDK1 activity, or (2) increased activity of CDK1, which requires that cells first inactivate APC/C^{Cdh1} but does not require CDC7 and CDK2 activity. Thus, our work provides evidence that mammalian cells can use two independent paths to start S phase with one path requiring APC/C^{Cdh1} inactivation and the other not. Our goal is to understand how cells can control these two paths with the ultimate goal of identifying optimal drug combinations that could be used as therapies to regenerate tissues or treat cancer.

B235/P1921

Temperature modulates the period of *in vitro* cell cycle oscillations

F. Tavella[1], E. Flores[2], Q. Yang[1]; [1]Biophysics, University of Michigan, Ann Arbor, MI, [2]University of Texas at San Antonio, San Antonio, TX.

Temperature is known to affect the cell cycle duration in vivo. However, it remains elusive how temperature affects the core signaling network driving cell cycle oscillations and what components are essential for this response. Theoretical studies suggest that positive feedback regulates the period and amplitude tunability of biological oscillators. To this end, we analyze how the period and amplitude of a cell cycle oscillator centered around cyclin-dependent kinase (Cdk1) responds to changes in temperature. We encapsulate Xenopus Laevis cycling egg extract in cell-sized microfluidic droplets and study cell cycle oscillations at different temperatures using a Cdk1 FRET sensor. We study the system between 15 °C and 45 °C and analyze the role positive feedback plays in the response by using Wee1 and Cdc25 inhibitors--two of the core components of Cdk1's positive feedback loop. Both low and high temperatures are shown to increase the oscillator's period with the fastest oscillations occurring between 22 °C and 24 °C. A custom device for the generation of a temperature gradient was generated

for this project and is discussed in detail, as well as the techniques used for droplet formation and long-term imaging. This study opens the door for understanding the connection between the network connectivity of an oscillator and its robustness in response to temperature.

B236/P1922

Uncovering the post-transcriptional regulation of cyclin B in *S. pombe*

J. M. Rogers, **S. G. Chethan**, X. Yao, J. Chen, S. Hauf; Virginia Tech, Blacksburg, VA.

Cyclins are the "engine" of the cell cycle. They derive this name from their cyclic protein abundance, oscillating with the cell cycle. Cyclins activate cyclin-dependent kinases (CDKs), which then phosphorylate a multitude of substrates to execute cell cycle events. Understanding the patterns of cyclin abundance is important to elucidate how cells orchestrate these cell cycle events at the appropriate time and in the proper order. In fission yeast (*Schizosaccharomyces pombe*), the entire cell cycle can be driven by a single cyclin (Cdc13/cyclin B) and a single CDK (CDK1). Cdc13, as its orthologs across other eukaryotes, begins accumulating during DNA replication and peaks in mitosis, when it is targeted for rapid degradation. Using live cell imaging to analyze Cdc13 dynamics in single cells, we show that Cdc13 concentration increases during interphase in a strikingly linear fashion. Such linearity could theoretically be explained if Cdc13 had a long protein half-life so that its steady-state concentration is not reached within one cell cycle. However, our measurements suggest that the Cdc13 half-life is too short to explain this linear pattern. Because we find that the cdc13 mRNA concentration stays constant throughout interphase, we propose that additional post-transcriptional regulation influences Cdc13 abundance. Interestingly, cdc13 and other CDK regulators have some of the longest 5'UTRs in the *S. pombe* genome, raising the possibility that this unique linear pattern is influenced by cell cycle-dependent translational regulation. Our current experiments focus on testing the role of the 5'UTR and other factors which may contribute to this post-transcriptional regulation. Revealing the mechanisms that control Cdc13's accumulation may illuminate new facets of cell cycle regulation and will allow us to determine the importance of Cdc13 accumulation in coordinating cell cycle events.

B237/P1923

Modes of intracellular amino acid sensing in budding yeast

A. Jalihal, J. Palme, M. Springer; Systems Biololgy, Harvard Medical School, Boston, MA.

Unicellular organisms must tightly coordinate nutrient availability with cellular growth. It is known that major regulators of cellular growth perceive intracellular nutrient cues (Conrad, 2014). This design of nutrient concentration sensing is expected to produce an unstable response, as the delay between perception and cellular growth response might occur on different timescales. Furthermore, concentration sensing is sensitive to the sensor levels. We have recently described a novel, alternative hypothesis regarding the mode of nutrient sensing, namely flux sensing (Palme, 2022, In Preparation). Flux sensing can be achieved by a bi-functional enzyme that also possesses signaling activity; such an enzyme can convey information related to the flux through the reaction that it is catalyzing. We explore a potential flux sensing mode of nutrient perception by the growth regulatory kinase TORC1. We hypothesize that the glutamine sensor Pib2 functions as a concentration sensor, and the leucine tRNA synthetase Cdc60 functions as a flux sensor. Our model of flux sensing in nutrient perception provides insight into an important question of cellular growth control.

B238/P1924

Characterization of the nuclear envelope/ER protein Msc1 in budding yeast

P. Gholami Tilko, L. Carson, H. Yu; Florida State University - Tallahassee, FL, tallahassee, FL.

A landmark of cell division in higher eukaryotes is the breakdown of the nuclear envelope, which reforms upon the completion of mitosis. In contrast, in lower eukaryotes such as the budding yeast *S. cerevisiae*, the nuclear envelope remains intact during cell division, a process known as closed mitosis. Although cell division has been studied extensively in yeast, the mechanism by which the nuclear envelope separates during anaphase is not entirely clear. Recently, it has been shown that a localized nuclear envelope breakdown occurs at anaphase in the fission yeast *S. pombe*. This localized nuclear envelope breakdown is regulated by the inner nuclear membrane protein Les1, which constricts nuclear envelope breakdown to the anaphase bridge. In *S. cerevisiae*, the homolog of Les1 is the meiotic sister-chromatid recombination protein Msc1. Using a bimolecular fluorescence complementation assay, we have found that Msc1 localizes to the lumen of the nuclear envelope and ER. Through domain analysis, we show that the N-terminus of Msc1 is both necessary and sufficient for its localization to the ER lumen. To determine Msc1 interacting factors, we performed a targeted genetic screen, which revealed that Msc1 genetically interacts with the Doa10 branch of the ERAD pathway. We speculate that like Les1, Msc1 functions in nuclear envelope remodeling. Uncovering the function of Msc1 will provide insight into the conserved mechanisms between closed and open mitosis.

B239/P1925

YAP and TAZ regulation of the cell cycle controls the number of fat cells produced during adipogenesis

H. Berger[1], S. Sharma[2], M. N. Teruel[2]; [1]1Department of Biochemistry, Drukier Institute of Children's Health, & Weill Center for Metabolic He, Weill Medical College/Cornell University, New York City, NY, [2]Department of Biochemistry, Drukier Institute of Children's Health, & Weill Center for Metabolic He, Weill Medical College/Cornell University, New York City, NY.

Overexpression of YAP in mice leads to a 3-fold increase in fat mass (PMID: 30309656), suggesting that YAP activity strongly promotes adipogenesis. YAP overexpression downregulates TAZ, which is known to inhibit adipogenic gene expression in mature fat cells, leading the authors to conclude that loss of TAZ in these mice leads to increased adipogenesis. However, this conclusion does not take into consideration the well-established role of YAP in most other cell systems which is to promote proliferation. Adipogenesis occurs through a competition between proliferation and differentiation processes (PMID: 32553172). In response to an adipogenic stimulus, progenitor cells first enter a clonal expansion period during which they undergo a variable number of cell divisions, followed by a differentiation period in which they increase expression of PPARγ. If the level of PPARγ reaches a critical threshold, the progenitor cell will exit the cell cycle and commit to terminal differentiation. Here we test whether YAP and TAZ have similar proliferation-promoting roles as in other cell systems and that YAP and TAZ promote proliferation during the clonal expansion phase to increase adipogenesis.
We use single-cell timelapse microscopy to track cell cycle progression, differentiation timing and cell lineage simultaneously, together with dox-auxin constructs which allow us to rapidly increase and decrease the levels of YAP and TAZ in cells. We show that loss of TAZ has no effect on the fraction of differentiating cells, and that overexpression of YAP and TAZ both strongly drive proliferation and increase the fraction of actively proliferating progenitor cells. YAP overexpression leads to increased Cyclin D1, suggesting that YAP activity regulates CDK4/6 activity. Furthermore, loss of both YAP and TAZ

leads to cell cycle exit and early differentiation, suggesting that YAP and TAZ activity regulates the duration clonal expansion of pre-adipocytes. Finally, we show that differentiation stimulus strongly downregulates total TEAD expression levels, while total YAP and TAZ levels do not significantly change. These results can explain why adipocytes can remain post-mitotic, even though TAZ levels increase strongly late in adipogenesis. TEADs, which are well-established transcriptional targets of YAP and TAZ, regulate expression of many cell cycle genes. Downregulation of TEADs likely blocks a cell-cycle role for YAP or TAZ late in adipogenesis.The results of this study will likely enable control of the clonal expansion period during adipogenesis, and thereby enable control of the number of fat cells generated in response to feeding and other adipogenic stimuli.

B240/P1926

Stem cell size homeostasis in mammalian epithelia

S. Xie[1], G. Q. G. de Medeiros[2], P. Liberali[2], J. M. Skotheim[1]; [1]Stanford University, Stanford, CA, [2]Friedrich Miescher Institute for Biomedical Research, Basel, SWITZERLAND.

Cell size impacts cellular biosynthesis and stem cell fitness. How cell size is controlled, especially in a crowded multicellular context, is poorly understood. We analyzed the growth of single epidermal stem cells during in vivo homeostatic turnover. We find the G1/S transition is cell size-dependent, where smaller-born stem cells spend longer and grow more during their G1 phase. We find that in agreement with previous models, the dilution of RB1 (retinoblastoma protein) by cell growth may couple G1/S transition rate to stem cell size. We further find that in intestinal organoids, as intestinal stem cells commit to differentiation, the size-dependence of G1/S transition remains invariant despite faster cell growth rates. Our results point to cell size a major predictor of the timing of stem cell divisions during mammalian tissue homeostasis.

B241/P1927

A nuclear mechano-sensing mechanism activates the aneuploidy response following centromere crisis

S. Herve*[1,2], A. Scelfo*[2], Y. Miroshnikova[1], G. Bersano-Marchisio[2], M. Deygas[2], G. Nader[2], A. Mace[2], M. Gentili[2], N. Manel[2], M. Piel[2], S. Wickstrom[3], D. Fachinetti[2]; [1]NIH/NIDDK, Bethesda, MD, [2]Institut Curie, Paris, FRANCE, [3]Max Planck Institute for Biology of Ageing, Munster, GERMANY.

Errors during cell division lead to an unbalanced karyotype (aneuploidy) that is associated with cellular inflammation and transformation. In response to aneuploidy, cells elicit a surveillance mechanism that stops proliferation and promotes senescence, however the molecular mechanisms that sense aneuploidy are still a matter of debate. Centromeres, unique chromosomal loci built on long tandem DNA repeats and specialized proteins, are critical players of chromosome separation and prevent aneuploidy. By inducing tunable levels of chromosome missegregation with rapid (< 2 hr) removal of key centromeric proteins in multiple human cell models, we identify a novel causal link between centromere crisis and cell cycle arrest, using a combination of imaging and cell mechanical measurements. We find that cells activate a G1 arrest following centromere dysfunction via a mechano-sensing mechanism that responds to nucleus deformation and epigenetic changes, but independently of prolonged mitosis, the DNA damage response or presence of micronuclei. This novel link between changes in the mechanical properties of the nucleus following mitotic errors and the promotion of the aneuploidy response provides a transformative view on the molecular mechanisms behind the genesis of genome instability.

B242/P1928

G2/M synchronization with CDK1 inhibitor suppresses genome-wide mutations and genome rearrangements during genome editing

K. Kondo, N. Fukuda, K. Soga, S. Yoshiba, J. Narushima, N. Shibata, C. Taguchi, K. Sakata, R. Kato; National Institute of Health Sciences, Kawasaki, Japan

(Background) CRISPR-Cas9 is a promising technique and has great potential for medical applications, such as correcting disease-causing mutations. Extensive studies to mitigate off-target effects in the genome have been performed because the off-target activity of the CRISPR-Cas9 system is a potential concern for therapeutic genome editing applications. There are now methods to reduce the off-target effect to some extent. On the other hand, CRISPR-Cas9 system has been reported to induce large deletions and mega-scale truncations using a single gRNA. The mechanism by which small indels occur is well known, however, little is known about the mechanism underlying the development of large deletions. In general, NHEJ can act immediately as the first player in DNA repair and inhibit large deletions. These recent reports raise the question: could cells with such large deletions be the result of escaping proper DNA repair machinery? If so, the cells may have many different types of mutations throughout their genome. (Methods) To answer this significant question, we applied the Thymidine Kinase (TK) assay to reveal the potential activity of CRISPR-Cas9 system. Genome engineered TK6 ($TK^{+/-}$)-derived TSCE5 cells, where the gRNA and I-*Sce*I recognition sequences were introduced into the intron 4 of *TK*1 locus on one allele, were used to detect large deletions (>80 bp) on the *TK1* locus as the on-target site. The cells can survive in the presence of trifluorothymidine due to the TK1-deficient phenotype ($TK^{-/-}$). Using the assay system, genome-wide small and large indels and structural variants initiated by CRISPR-Cas9 or I-*Sce*I were investigated throughout the genome in the normal and G2/M-synchronized conditions using next-generation sequencing. Mutation signatures were also investigated using SigProfilerMatrixGenerator (signature & transition/transversion ratio). (Results) The large deletion (>80 bp) at the *TK1* locus was detected at frequencies of 1×10^{-4}. This was 500 times lower than small deletion in TSCE5. Next, A greater number of genome-wide mutations occurred in the normal conditions, and single gRNA induced large deletions over 25 kb at the on-target sites and complex genome rearrangements. G2/M synchronization using the CDK1 inhibitor RO3306 during genome editing, after double thymidine block/Colcemid treatment, dramatically attenuates the number of genome-wide mutations (total variants, 5 in G2/M vs 859 in normal conditions). Ts/Tr was 1.0 in G2/M, whereas it was 0.05 in normal conditions. Our findings highlight that the cell conditions induced by G2/M synchronization may prevent the development of genome-wide mutations and rearrangements, possibly due to the suppression of replication machinery.

B243/P1929

Cyclin B isoforms coordinate mitotic entry and exit events to ensure the normal pace of embryonic divisions

P. Lara-Gonzalez[1], S. Variyar[2,3], J. Budrewicz[4], A. Schlientz[3,2], J. Houston[3,2], K. Oegema[2,3], A. Desai[2,3]; [1]Developmental and Cell Biology, University of California Irvine, Irvine, CA, [2]Ludwig Institute for Cancer Research, La Jolla, CA, [3]Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA, [4]Oregon Health & Science University, Portland, OR.

Entry into mitosis is promoted by Cdk1, a kinase whose activity depends on its binding partner cyclin B. The cdk1-cyclin B complex phosphorylates multiple cellular targets to promote processes such as spindle

assembly, chromosome segregation, and cytokinesis. How mitotic entry is coordinated with embryonic development and how different cdk1-dependent mitotic events are propely orchestrated are both important open questions. Here we focus on cyclin B, which has three evolutionarily conserved isoforms: B1, B2 and B3. Using *C. elegans*, we found that individual depletion of cyclin B1 or B3, while resulting in embryonic lethality, did not prevent mitotic entry in early embryos. By contrast, co-depletion of cyclin B1 and B3 blocked mitotic entry. Thus, these two cyclin B isoforms function redundantly to activate cdk1 for mitotic entry in early embryos, enabling us to characterize in detail the effects of loss of cyclin B1 versus B3-associated cdk1 kinase activities. This analysis revealed remarkably opposite phenotypes of cyclin B1 and B3 inhibitions. Cyclin B1 depletion led to premature chromosome segregation, while cyclin B3 depletion caused a significant delay in mitotic events such as spindle assembly, chromosome alignment and chromosome segregation. These observations indicate that cyclin B3 is an accelerator of mitotic progression in rapidly dividing early embryos. Strikingly, in cyclin B3-depleted embryos, two key events of mitotic exit are reversed: cytokinesis, the process of partitioning the cell, initiates prior to chromosome segregation. To understand the origin of this reversal, we developed fluorescent biosensors for two enzymes central to mitotic exit: the anaphase-promoting complex/cyclosome (APC/C), a ubiquitin ligase that triggers mitotic exit, and separase, the protease whose activity is specifically required for chromosome segregation. This analysis revealed a delay in separase activation relative to APC/C activation following cyclin B3 depletion, explaining the reversed order of mitotic exit events. Thus, in addition to accelerating mitotic progression, cyclin B3 plays a specific role in ordering mitotic exit events by promoting separase activation. Overall, this work reveals remarkable differences between cyclin B isoforms in controlling cdk1 in the early embryo and identifies cyclin B3 as a promoter of rapid and properly ordered mitotic divisions, a key feature of embryonic development where rapid increase in cell number must be coordinated with processes such as cell fate determination and morphogenesis.

B247/P1930

YAP Promotes an Enhanced Mitogen Signaling State that Supports Cell Proliferation under Quiescence-promoting Cues

K. Ferrick[1], T. Meyer[2]; [1]Stanford University, Stanford, CA, [2]Weill Cornell Medical College, New York, NY

Inactivation of the Hippo kinase cascade promotes YAP/TAZ activation and TEAD-dependent transcription of pro-proliferative and pro-survival genes. The Hippo pathway is critical for regulating periods of organogenesis and wound healing yet can also be disrupted for tumorigenesis and metastatic behavior. Merlin (NF2) is a tumor suppressor that functions as a scaffold protein and is an upstream regulator of the Hippo pathway. However, Merlin has putative Hippo-independent roles in mitogen and migration signaling pathways that may further contribute to its potent tumor suppressor activity. We set out to determine how loss of Merlin maintains cell proliferation following mitogen withdrawal and high cell density, conditions that normally promote quiescence. We used live-cell reporters of ERK and AKT activity and quantitative immunofluorescence to assess changes in single-cell signaling dynamics and protein regulators of cell-cycle progression. In addition to Merlin's anticipated role in classic YAP target gene regulation, we observed that Merlin KO cells gained sensitivity to several mitogens. Inhibition of LATS1/2 kinases with a recently published inhibitor or overexpression of YAP phenocopied this novel and generalized mitogen response. We also observed prolonged ERK signaling in response to mitogen stimulation as well as higher basal AKT activity, suggesting that aberrant YAP activity reprograms the proliferative signaling architecture of cells. Transcriptional analysis of quiescent cells induced with YAP

further supports that pathways sustaining mitogen signaling were rapidly up-regulated. We propose that prolonged YAP activity in Merlin KO cells increases the proliferative signaling potential of cells, even in restrictive, low-growth factor conditions.

B248/P1931

Cdt1 inhibits CMG helicase in early S phase to separate origin licensing from DNA synthesis

N. Ratnayeke[1], Y. Baris[2], M. Chung[1], J. T. P. Yeeles[3], T. Meyer[4]; [1]Stanford University, Stanford, CA, [2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, [3]MRC Laboratory of Molecular, Stanford, CA, [4]Weill Cornell Medicine, New York, NY

Human cells license tens of thousands of origins of replication in G1 and then must stop all licensing before DNA synthesis in S phase to prevent re-replication and genome instability that ensue when an origin is licensed on replicated DNA. However, the E3 ubiquitin ligase $CRL4^{Cdt2}$ only starts to degrade the licensing factor Cdt1 after origin firing, raising the question of how cells prevent re-replication before Cdt1 is fully degraded. Here, using quantitative microscopy and in vitro reconstituted human DNA replication, we show that Cdt1 inhibits DNA synthesis during an overlap period when Cdt1 is still present after origin firing. Cdt1 inhibits DNA synthesis by suppressing CMG helicase progression at replication forks, and DNA synthesis commences once Cdt1 is degraded. Thus, in contrast to the prevailing model that human cells prevent re-replication by strictly separating licensing from firing, licensing and firing overlap, and cells instead separate licensing from DNA synthesis.

B249/P1932

Cell Division-Enabled Leaf System (CDELS), a transiently induced mitotic model for testing cell cycle-dependent protein expression in tobacco leaves

Y.-R. Lee, C. H. Huang, B. Liu; University of California, Davis, Davis, CA

Plant growth is brought about by mitotic cell division. Successful cell division harnesses the input of proteins that function in cell cycle-dependent manners in order to regulate intracellular motility of cytoskeletal reorganization and chromosome segregation. Fluorescence microscopic assays of mitotically active proteins have been dependent on time-consuming and sometimes technically challenging transformation experiments. To facilitate the discovery and observation of cell cycle-dependent localization and dynamics of plant mitotic proteins, we developed a transiently induced cell division system in tobacco (*Nicotiana benthamiana*), namely Cell Division Enabled Leaf System (CDELS). Plasmid constructs that expressed the D-type cyclin and protein(s) of interest tagged with a fluorescent fusion were delivered into the leaves of *N. benthamiana* by agrobacterial infiltration. Ectopic expression of cyclin D induced differentiated leaf epidermal cells to re-enter mitosis and subsequently cytokinesis, resembling what took place in meristematic cells. The dynamic localization of fluorescent fusion protein(s) was observed throughout the course of mitotic cell division by live-cell fluorescence microscopy. This CDELS allows us to not only discover novel mitotically active proteins but also promptly capture their dynamics and relationship with other known and unknown factors during cell division.

B250/P1933

Cell Cycle Dependent Degradation of RB1 Protein is Controlled by Hyperphosphorylation

S. ZHANG, E. Zatulovskiy, L. F. Valenzuela, J. Skotheim; Stanford University, Stanford, CA

RB1 is a key cell cycle regulator that controls cell cycle progression and contributes to controlling cell size. The amount of RB1 protein remains constant during G1 phase and accumulates during S/G2 phase. This results in a declining concentration of RB1 that reflects cell size so that the G1/S transition is size-dependent. However, the mechanism that regulates RB1 protein amounts during cell cycle progression is still unclear. We found that the RB1 mRNA remained at approximately constant concentration across the cell cycle, and its translational efficiency (TE) did not change significantly in different cell cycle phases, suggesting that RB1 protein is post-translationally regulated. By performing live imaging on reporter cell lines, we found that the protein half-life of RB1 was significantly different between G1 and S/G2 phases. RB1 was stabilized following the G1/S transition. Examination of RB1 phospho-site mutants suggested that the hyperphosphorylation of RB1 mediated the stabilization of RB1 protein in S/G2 phases. Mathematical modeling provided further insights into how the cell cycle dependent half-life of RB1 can give rise to its differential scaling behaviors during cell cycle progression. Taken together, our work supports a protein degradation-based mechanism controlling the dynamics of RB1 protein amounts through the cell division cycle.

B251/P1934

Heterogeneity of RNA-Binding Protein DND1 in G0 male germ cells may provide a selection process for spermatogonial stem cell development

K. Friedman[1], T. Hatkevich[2], B. Capel[2]; [1]University of Richmond, Richmond, VA, [2]Duke University, Durham, NC

The ability to reproduce is dependent upon proper development of the germline. In male *Mus musculus*, germline development involves male germ cells (MGCs) differentiating into spermatogonial stem cells (SSCs), the precursors for male gametes. During this differentiation period, MGCs enter a quiescent phase (G0), an essential stage for SSC development. This G0 phase accompanies changes in transcription as well as chromatin accessibility and re-methylation of DNA. Dead-End 1 (DND1) is an RNA binding protein whose expression has been shown to be essential for the entry and maintenance of G0. During G0, MGCs heterogeneously express DND1, resulting in two MGC populations: “DND1-hi” cells (those which highly express DND1) and “DND1-lo” cells. Bulk RNA-seq studies from our lab show that DND1-hi cells have a distinct transcriptome as compared to DND1-lo cells and highly express transcripts that encode for proteins involved in SSC development. Despite two distinct DND1-expressing MGC populations present in G0, the biological significance of DND1 heterogeneous expression on SSC development remains unknown. Considering only a fraction of G0 MGCs differentiate into SSCs, we hypothesize that the G0 DND1-hi cells develop into SSCs, while the DND1-lo cells undergo apoptosis. To investigate if G0 DND1-lo cells are set to apoptose we used whole-mount immunofluorescence to examine expression of cellular and apoptotic markers throughout G0. We observed the presence of LEFTY, a marker for pre-G0 MGCs that undergo apoptosis, in E12.5 (just prior to G0) and E14.5 (the start of G0); however, LEFTY was absent in a later G0 time point (E16.5). Presence of LEFTY expression at E12.5 and E14.5 did not correlate with the DND1-lo or DND1-hi populations. Instead, LEFTY was present in all early G0 MGCs, despite the level of DND1 expression. We also examined the presence of ɣ-H2Ax, a marker for double-strand DNA breaks that marks cells undergoing apoptosis. We saw ɣ-H2Ax in E14.5

and E16.5 MGCs, indicating that apoptosis occurs in these stages but expression of ɣ-H2Ax was anti-correlated with DND1 expression, suggesting that by the time ɣ-H2Ax is detected, MGCs have stopped expressing DND1. We conclude that this marker is too late in the cell death pathway to test the hypothesis that apoptosis is more likely in DND1-lo cells. We will continue to test our hypothesis by looking at additional apoptotic markers, such as AnnexinV, examining if DND1-hi cells are less likely to undergo apoptosis during G0 compared to DND1-lo cells.

Cancer Therapy - Inhibitor Activity 2

B253/P1935

Investigation of Ceramidase Inhibitor Effect in BALB/c Mice with Breast Cancer

M. KUTLU; Eskisehir Technical University, ESKİŞEHİR, TURKEY.

Abstract In recent years, many studies have been carried out to determine the cancer or anticancer effects of drugs that have an effect on sphingolipid metabolism. Thanks to studies with ceramides, new dimensions are added to cancer research. Ceramides are structural molecules found in the membranes of cells and involved in very important metabolic processes such as growth, differentiation and apoptosis. Inhibition of ceramidases is an alternative way to increase apoptosis and ceramide accumulation in cancer cells. The key molecule in this metabolism for cancer treatment is ceramide. Depending on the level of ceramide in the cells, the cells are directed to death or survival can be sustained. Ceramidase enzymes are enzymes that break down the intracellular ceramide, lower the ceramide level in the cells and cause the cells to escape from death. Cancer is a disease that occurs with the uncontrolled division and proliferation of cells and is under the influence of genetic and environmental conditions. There are more than 100 known types of cancer. Breast cancer is a type of cancer that occurs in both men and women. Breast cancer is the most common type of cancer in women after lung cancer and is the second leading cause of cancer-related deaths. With a rate of 24.1%, breast cancer ranks at the top of all cancer cases in women in our country. Developing innovative treatment strategies and understanding the biology of this type of cancer are essential to reduce the death rate from breast cancer. Suppression of ceramidase enzymes as new targets for cancer therapy is shown in the literature. In this study, a breast cancer model was created in BALB/c mice, and then the mice were treated with ceramidase inhibitors D-erythro-MAPP and D-erythro-MAPP KLN. Apoptotic effects of D-erythro-MAPP and D-erythro-MAPP KLN formulation in lung tissue of mice were determined by immunohistochemical marking. It was determined that the staining results of caspase-3 and PARP1 antigens gave statistically significant results according to the H-score values obtained. **Keywords:** D-erythro-MAPP, Breast cancer, Ceramide, Apoptosis.

B254/P1936

Identification of Synergistic Therapeutics for Basal-like Breast Cancer Brain Metastases

A. D. Valentine[1], T. H. Turner[2], N. S. Rashid[3], N. S. Hairr[4], T. J. Leftwich[4], J. C. Harrell[4]; [1]Biochemistry, Pathology, Virginia Commonwealth University, Richmond, VA, [2]Internal Medicine/Neurology, Mayo Clinic, Jacksonville, FL, [3]Biology, University of Richmond, Richmond, VA, [4]Pathology, Virginia Commonwealth University, Richmond, VA.

Each year over 280,000 people in the United States will be diagnosed with invasive breast cancer and over 40,000 people will die from metastatic disease. The most difficult subtype to treat is basal-like

triple negative breast cancer (TNBC), as this subtype is highly metastatic and can become chemotherapy resistant. TNBC lacks estrogen and progesterone receptors, as well as HER2, which are actionable drug targets - making it difficult to treat. Furthermore, most chemotherapeutics designed to target TNBC are unable to treat brain metastases due to their inability to permeate the blood-brain barrier (BBB). Therefore, it is crucial to develop novel BBB-permeable treatments for brain-metastatic TNBCs. A selective inhibitor for nuclear export (SINE) was chosen as the prime chemotherapy candidate due to its indication for inhibiting a nuclear export protein which is highly expressed in basal-like TNBC and is negatively associated with brain metastasis-free survival. To identify BBB-permeable drug candidates for synergism studies with this SINE, 34 BBB-permeable drugs were screened in vitro in four basal-like TNBC patient-derived xenograft (PDX) suspension culture models. Based on observed supra-additive trends, 4 drugs were selected for in vitro testing in combination with our SINE of interest; these drugs target dihydropyridine calcium channels, histone deacetylases, neurotrophic tyrosine receptor kinases, and malaria. Synergism was identified with the 4 BBB-permeable drugs and favorable dose reductions were observed in all four PDX models. Based on these results, ongoing studies are now determining the efficacy of these drugs as single agents or combinations on mammary tumors and brain metastases with luciferase-labeled PDXs.

B255/P1937

Tocotrienol exerts on-target malignant activity against leukemia

M. L. P. Bueno[1], M. Foglio[2], S. T. O. Saad[1], **F. Marconi Roversi**[1]; [1]Hematology and Transfusion Medicine Center-University of Campinas / Hemocentro-UNICAMP, Campinas, São Paulo, Brasil, Campinas, BRAZIL, [2]Faculty of Pharmaceutical Sciences-University of Campinas / Faculdade de Ciência Farmacêutica-UNICAMP, Campinas, São Paulo, Brasil, Campinas, BRAZIL.

Acute Leukemias comprise a group of hematopoietic malignancies with uncontrolled proliferation of immature blood cells that lost the capability of differentiation. Nowadays, the advance on understanding and improving treatment strategies depends on identifying and developing novel potential compounds that are more effective and less toxic than the currently available chemotherapeutics. Tocotrienol, a vitamin E isoform, was reported to be a potential anti-cancerous agent due to its antimetastatic activity in solid neoplasms and anti-inflammatory effects on the immune system. Hence, our aim was to investigate the role and effects of tocotrienol compound (provided by New Max Industrial) in leukemia. A panel of acute leukemia cell lines (U937, OCI-AML3, RS4;11, Jurkat) were treated with tocotrienol in a time -concentration manner. Viability was evaluated by MTT assay, apoptosis by flow cytometry, and protein levels by Western Blot. To mimetize some microenvironment participation in the leukemia biology, a coculture with monocyte and leukemia cells was done. Tocotrienol treatment resulted in a potent antineoplastic activity in a concentration-dependent manner (1.4-14.0μM): reducing 50% cell growth (U937: 9.5μM, OCI-AML3: 4.4μM, RS4;11: 12.8μM, Jurkat: 14.1μM) associated with increased cell death (fold-increase (FI) U937: 0.9-5.8, OCI-AML3: 1.4-8.6, RS4;11: 1.1-5.0, Jurkat: 0.9-5.1). Tocotrienol also increased leukemia cell death cocultured with tocotrienol-programmed monocytes (FI: 32.9; P<.0001). Tocotrienol was able to reduce ROS production (fold decrease (FD) U937: 1.1-6.9, OCI-AML3: 1.0-7.3, RS4;11: 1.1-5.9, Jurkat: 1.0-5.7) in a concentration-dependent manner (1.4-14.0μM) (all P<.05). Tocotrienol reduced AKT and ERK protein phosphorylation, important overexpressed molecules in leukemia and modulated caspase 3 and 9. On the other hand, tocotrienol treatment of primary cells isolated from healthy donors did not modulate cells survival or apoptosis. In conclusion, tocotrienol arises as an effective cytotoxic and antineoplasm agent for

leukemia treatment without altering apoptosis and survival of normal cells (on-target malignant cell killing activity), probably by blocking ROS production and, consequently, interrupting PI3K and MAPK signaling.Funding: FAPESP grants #2017/21801-2, #2019/25247-5, #2021/05320-0

B256/P1938

Effects of ZnO and CuO NPs on Human Thyroid Cancer Cells (ML-1) vs Rat Medullary Thyroid Carcinoma (CA77)

A. Peters, K. Kim, K. Monahan, N. Weaver; Missouri State, Springfeild, MO.

Effects of ZnO and CuO NPs on Human Thyroid Cancer Cells (ML-1) vs Rat Medullary Thyroid Carcinoma (CA77) *Authors: Alyse Peters, Katelyn Monahan, Nakaja Weaver, and Kyoungtae Kim* Nanoparticles (NPs) contribute to the technologies in agriculture and food development, but there is little understanding of how nanoparticles impact human health. To further understand the effects of NPs, this study investigates metal nanoparticles (Me-Nps) on ML-1, CA77 and non-cancerous fibroblast cells to discover their impact. The cytotoxicity of the selected NPs was explored through an XTT viability assay, an superoxide and an apoptosis analysis on cells. XTT results conveyed ZnO and CuO having the greatest decreased viability, therefore CuO and ZnO were selected for a further gene expression study. Similar to the ML-1 cells, CA77 cells also showed a decrease in cell viability quantified by an XTT assay. The production of superoxide in the ML-1 cells when treated with CuO and ZnO was found to be not significantly altered during 24-hour incubation with both nanoparticles; Ca77 cells illustrated a similar trend, but these nanoparticles had a slight decrease in superoxide. The apoptosis assay revealed that ZnO and CuO increased cell death which led to the conclusion that the cause of decreased cell viability comes more from apoptosis rather than superoxide. Ca77 cells show a slightly increased trend though not statistically significant. Consistently, our RNAseq studies illustrated upregulated apoptotic, inflammation, DNA damage response (p53), and xenobiotic metabolism genes with the ZnO treatment. Downregulated genes in ZnO-treated experiments include Golgin family and cytochrome p450 family genes. When treated with CuO NPs, DNA regulation, apoptotic process, cell migration, abiotic stimulus, and DNA repair genes are all upregulated. Cell-cell adhesion, synapse organization and cell proliferation are all downregulated. RNAseq studies were repeated for Ca77 cells. The major pathways upregulated when treated with CuO include inflammation mediated by cytokine and chemokine, interleukin signaling pathway and TGF beta signaling pathway. The major downregulated pathways include angiogenesis, Wnt signaling, and VEGF signaling with CuO treatment. ZnO treatment on Ca77 cells upregulates thyrotropin-releasing hormone signaling receptor pathway, Wnt signaling pathway, integrin signaling pathway, and inflammation mediated by cytokine and chemokines. Downregulated pathways include endothelin signaling pathway, blood coagulation, and angiogenesis. These experiments give powerful insight on potential Me-Nps effects on the human body and animal models. Future experiments include proteomic studies to evaluate the affected proteins in both cell lines when treated with ZnO and CuO.

B257/P1939

Role of the nuclear export protein CRM1 in DNA repair pathway.

Y. Yang, R. Kumar, J. Mendonca, L. Wilson, D. Topiwala, K. Boyapati, N. Thomas, I. Sharma, M. Carducci, S. Kachhap; Johns Hopkins University School of Medicine, Baltimore, MD.

PARP inhibitors have recently been shown to be very successful in treating prostate cancer (PCa) that harbor mutations in DNA repair genes. Since only 20% of prostate cancer patients carry mutation in DNA

repair genes, innovative strategies are needed to expand the clinical usage of PARP inhibitors beyond DNA repair mutant tumors. An innovative strategy would be to create conditions that mimic repair mutants in prostate tumors and then treat them with PARP inhibitors. We found that CRM1, a nuclear export protein that is upregulated in PCa and amplified in neuroendocrine PCa, takes an active part in DNA repair. Our data demonstrates that treatment of PCa cells with Selinexor, a highly selective CRM1 inhibitor with reduced toxicities undergoing several clinical trials, results in BRCA1 and RAD51 downregulation and decreased DNA repair; creating conditions analogous to cells harboring DNA repair mutation. These findings manifest that CRM1 impacts DNA repair and its inhibition results in a defect of DNA repair capacity. We further demonstrate that combination of PARP inhibitor or DNA repair pathway inhibitor and Selinexor significantly decreases growth of PCa cells.

B258/P1940

Co-treatment of gallic acid and cisplatin suppresses LPS-induced cancer properties in MDA-MB 231 breast cancer cells

A. R. Aikins, P. Pasgo, J. T. Quarshie, S. M. Baffoe, P. A. Birikorang, Y. Bediako, W. S. K. Gbewonyo; Biochemistry, Cell & Molecular Biology/West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, GHANA.

Breast cancer remains the most frequently diagnosed cancer in women worldwide. The presence of lipopolysaccharide (LPS) on the cell wall of Gram-negative bacteria identified in some breast cancer tissues have been implicated in inflammatory responses via the TLR4/NF-κB signaling cascade. Inflammation is associated with chemoresistance, which could be suppressed through combination therapy. This study sought to investigate the anticancer effect of combining the phenolic compound, gallic acid (GA) - which has anti-inflammatory properties - with cisplatin on LPS-induced breast cancer progression. Using MDA-MB-231 cells as experimental models, we found that both GA and GA-cisplatin co-treatment reduced cell proliferation in a dose- and time-dependent manner. RT-qPCR analysis and ELISA showed that the GA-cisplatin co-treatment downregulated TLR4 as well as LPS-induced IL-8 expression. Furthermore, GA and GA-cisplatin suppressed LPS-induced cell migration. Inhibiting TLR4 repressed cell proliferation, suggesting a proliferative role for TLR4 in breast cancer and supporting its potential as a therapeutic target. Altogether, we show that GA-cisplatin co-treatment suppresses breast cancer proliferation, LPS-induced inflammation and cell migration via inhibition of TLR4 expression. These findings provide a rationale for the consideration of GA in combination therapy with cisplatin for improved chemotherapy.

B259/P1941

Targeting CYP11A1 as a potential therapeutic approach for renal cell carcinoma

H. T. M. Ong[1], T. H. Kim[2], E. Ates[1], J. C. Pyun[2], M. J. Kang[3]; [1]KIST School, University of Science and Technology, Seoul, Korea, Republic of, [2]Yonsei University, Seoul, Korea, Republic of, [3]Korea Institute of Science and Technology, Seoul, Korea, Republic of

Clear cell renal carcinoma (RCC) is commonly known for its metastasis propensity to outspread to other organs and does not exhibit symptoms in the early stage. Recent studies have shown that deficiencies in CYP11A1 expression can lead to fatal adrenal failure if left untreated and are associated with downstream regulation in various cancer types. However, the molecular mechanisms of CYP11A1 and kidney cancer proliferation remain unclear. In this context, normal and renal carcinoma cell lines

(Hek293 and Caki-1) were transfected with CYP11A1 to stimulate overexpression and examined by immunocytochemistry staining. Levels of oxidative stress were determined by absorbance assay and cell cycle distribution was investigated by flow cytometry. The role of related signaling pathways in the activation of CYP11A1 was tested by western blot. Results observed that CYP11A1 defeated the migration of the cancer cell line along with epithelial-intermediate metastatic (EMT) markers. A significant decrease in the expression of cyclin B1 and phosphorylation of Cdc2 was detected whereas the cyclin-dependent kinases (Cdk2 and Cdk4) were not altered. In addition, CYP11A1 overexpressed resulting in the up-regulation of ATM/ATR while phosphorylation of upstream signals related to G2/M phase arrest and blockaded of C-Raf/ERK pathway. Consistently with these data, CYP11A1 overexpression reversed the EMT process, induced G2 phase arrest, generated ROS, and promoted apoptosis in RCC. This study reveals a promising therapeutic target to suppress kidney cancer proliferation without affecting normal cells, thus improving the survival rate of cancer patients.

B260/P1942

Enhanced the antitumor effects of chimeric antigen receptor T cells in glioblastoma

E. A. Ogunnaike[1], A. Valdivia[1], M. Yazdimamaghani[1], E. Leon[1], H. Du[1], S. Khagi[1], Z. Gu[2], B. Savoldo[1], F. S. Ligler[3], S. Hingtgen[1], G. Dotti[1]; [1]University of North Carolina - Chapel Hill, NC, Chapel Hill, NC, [2]Zhejiang University, Chapel Hill, NC, [3]North Carolina State University and University of North Carolina at Chapel Hill, Raleigh, NC

Limitations in the delivery of biotherapies to treat glioblastoma (GBM), a lethal and hard to- treat cancer of the brain, includes antigen targets, therapeutic delivery, and invasive tumor microenvironment. Failure to sustain the delivery to address T cell exhaustion and proliferation while considering the GBM-tumor microenvironment (TME) adversely affects therapeutic antitumor efficacy in both animal models and humans. We develop a physiological biomaterial, a fibrin-based hydrogel (FBH) encapsulating chimeric antigen receptor (CAR) T cells (F-CAR-T), that can afford treatment combinations to close these critical gaps and lack of knowledge for translation of the CAR-T cell therapy. Regional delivery of chimeric antigen receptor (CAR) T cells in glioblastoma represents a rational therapeutic approach as an alternative to intravenous administration to avoid the blood-brain barrier impediment. Here we developed a fibrin gel that accommodates CAR-T cell loading and promotes their gradual release. We used several bioanalytical tools such as atomic force microscopy, cryogenic scanning electron microscopy, and confocal microscopy to evaluate the porous network capability to accommodate the T cells and the interaction results. We performed immunological and functional assays were used to assess the T cells released from the gel, nutrient exchange, and phenotypical characterization. Using a model of sub-total glioblastoma resection, we demonstrate that the fibrin gel delivery of CAR-T cells within the surgical cavity enables superior antitumor activity compared to CAR-T cells directly inoculated into the tumor resection cavity.

B261/P1943

Gene silencing of LAT1 and ASCT2 Transporters via Short Interfering Nucleic Acid Therapies upon Human Colon Cancer HCT-116 Cell Xenografts

C. Carneiro[1], P. J. Kennedy[2], S. Bessa[1], **P. Soares-da-Silva**[3,1]; [1]Phyzat Biopharmaceuticals, Porto, Portugal, [2]Phyzat Biopharmaeuticals, Porto, Portugal, [3]MedInUP – Center for Drug Discovery and Innovative Medicines, University of Porto, Porto, Portugal

Overexpression of LAT1 (SLC7A5) and ASCT2 (SLC1A5) transporter supports the continuous growth and proliferation of cancer cells and has been suggested to be a marker of colorectal adenocarcinoma prognosis (Pharmacol Ther. 230:107964,2021; Anticancer Res 22:2555-2557,2002; Mol Imaging Biol 19:421-428,2017). We aimed to characterize novel short interfering nucleic acid therapies (siNAT) targeting LAT1 and ASCT2 mRNAs in HCT-116 human colon cancer cells and evaluate its *in vitro* and *in vivo* effect. HCT-116 cells were transfected with 0.1-25 nM of double-stranded Nucleic Acid (dsNA) complexed with a commercial liposomal transfection agent. LAT1 and ASCT2 gene and protein expression were analysed by RT-qPCR and western blot, respectively. LAT1 and ASCT2 transporter activity was quantified by [^{14}C]-L-leucine and [^{14}C]-L-alanine uptake. The effect of LAT1 and ASCT2 downregulation on cell growth and viability was determined using crystal violet assay. Immune deficient female BALB/c nude mice (CAnN.Cg-Foxn1nu/Crl) were used for subcutaneous implantation of HCT-116 cells (1x10^{6}) previously treated with the transfection agent alone or complexed with anti-LAT1 or anti-ASCT2 dsNAs for evaluation of tumour growth. Animal experimental procedures followed the EU Directive 2010/EU/63 and the DGAV (project No. 34-2021). The anti-LAT1 and anti-ASCT2 dsNAs significantly decreased LAT1 and ASCT2 mRNA expression (IC_{50}=1.4 nM and 1.8 nM, respectively) causing 80%-70% knockdown of protein transporter expression and leading to > 50% reduction in substrate uptake. Treatment of HCT-116 cells with an anti-LAT1 and ASCT2 dsNAs significantly reduced cell proliferation (1-25nM), leading to 70 % arrestment in cell proliferation. Treatment of HCT-116 cells with an anti-LAT1 dsNA significantly reduced cell proliferation (>50%), and the combination of dsNA with 5-FU (5 µM) or Oxa (2 µM) had a synergistic effect on proliferation. Tumours derived from cells transfected with anti-LAT1 or anti-ASCT2 dsNAs grew 3X slower than tumours derived from cells treated with empty transfection agent. Altogether these results further support the potential or LAT1 and ASCT2 for antineoplastic treatment and exploit siNAT as tool to target LAT1 and ASCT2 in colon cancer.
Part of this work was developed within the SIRNAC project (33399), co-financed by NORTE2020, PT2020 and the European Union.

Cancer Therapy- Targeting Kinases 1

B262/P1944

Albendazole exerts antiproliferative effects on Hs578 triple-negative breast cancer cells by inhibiting mTOR and akt signaling pathways

R. Koon, **K. Suh**; High Point University, High Point, NC.

Albendazole is an antihelminthic drug used for the treatment of a variety of parasitic worm infestations by targeting microtubules which play an important role in cell division. However, albendazole has been shown to exhibit anti-cancer properties recently. The mammalian target of rapamycin (mTOR) kinase is an important component of PTEN/PI3K/Akt signaling pathway. The mTOR signaling pathway promotes tumor progression and regulates autophagy and apoptotic cell death. Therefore, studies suggest that targeting mTOR signaling pathway could be an effective strategy for the treatment of cancer. In the present study, we investigated the growth inhibitory effect of albendazole and its associated mechanism in Hs578T triple-negative breast cancer cells. When Hs578T cells were treated with albendazole, viability was inhibited, and apoptotic cell death was induced in a dose- and time-dependent manner. Apoptosis was verified by detecting the cleavage of poly(ADP-ribose) polymerase, staining fragmented nuclei using Hoechst dye, and measuring the activity of executioner caspases. Furthermore, we observed increased expression of pro-apoptotic protein Bak which is known to be involved in pore formation on

mitochondrial outer membrane. On the other hand, the level of anti-apoptotic protein Bcl-XL was downregulated upon treatment of Hs578T cells with the drug. In cells treated with albendazole, the activity of mTOR was significantly inhibited which was measured by western blotting, immunocytochemistry, and enzyme-linked immunosorbent assays. The expression of mTOR signaling complex constituents such as Raptor and Rictor was also downregulated. This resulted in the inactivation of mTOR target proteins such as ribosomal protein S6. Finally, we performed RNA sequencing analyses to identify novel targets of albendazole. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses revealed multiple genes that are differentially expressed including the genes that are associated with DNA replication, cell cycle, and VEGF signaling pathways. Taken together, these results indicate that albendazole could be a potent inhibitor of mTOR signaling pathway in triple-negative breast cancer cells and suggest its potential use as chemotherapeutic agent against breast cancer.

B263/P1945

Assistant Professor

J. Hong; Department of Physiology, Daegu Catholic University School of Medicine, Daegu, KOREA, REPUBLIC OF.

Lysosome is the major machinery of RTK proteolysis. Some of endocytosed RTKs return to the cell membrane after the specific ligand stimulation and signal transduction by recycling endosomes. The other endocytosed RTKs are being digested via lysosomal proteolysis after signaling processes. The ratio of recycling and lysosomal proteolysis depends on the specific receptor. Among RTKs, EGFR is majorly being degraded in lysosomes after the ligand stimulation rather than undergoing to the recycling process. However, this proteolysis is downregulated in hypoxic condition such as the central core of solid tumors. The downregulation of EGFR degradation is because of the suppressed lysosomal activity in hypoxic conditions by the suppress of the nuclear translocation of TFEB.Here we demonstrate that, in cultured mammalian cells and mouse models, the enhancement of lysosomal activity leads to the degradation of EGFR in colon cancer cells. We overexpressed V-ATPase components in DLD-1 cells that increased the proteolysis of EGFR in lysosomes. The cells were also xeno-transplanted to immune-suppressed nude mice to induce tumors. When the lysosomal activity was enhanced in DLD-1 colon cancer cells, the growth was significantly decreased. Furthermore, when the mice were treated with a tyrosine kinase inhibitor, Osimertinib, the decrease of tumor growth was much more decreased. From this study, we suggest lysosomal activation can be another therapeutic approach for EGFR-mediated cancers including colon cancers. Furthermore, this can be a new combination therapy together with the previous antibody therapies and TKI therapies.

B264/P1946

Anti-HER2 Therapies Regulate MAF1 Expression in Breast Cancer

G. Olshefsky[1], **L. Schramm**[2]; [1]Garden City High School, Garden City, NY, [2]St. John's University, Queens, NY.

In 2022, the United States anticipates 287,850 new invasive breast cancer diagnoses. TFIIIB amplification and overexpression frequently occur in breast cancer. MAF1 negatively regulates TFIIIB. In HER2-positive breast cancer patients, compared to controls, MAF1 mRNA expression decreases. There is a significant increase in the methylation of the MAF1 promoter ($p = 2.25 \times 10^{-5}$) in HER2-positive breast cancer.

Decreased MAF1 protein expression occurs in HER2-positive breast cancer (p = 4.66 x 10^{-5}). Treatment of HER2-positive breast cancer cell lines with the anti-HER2 agent lapatinib increased MAF1 mRNA expression in BT474 (q = 0.02) and SKBR3 (q = 0.08) cells. BT-474 cells treated with Herceptin (trastuzumab) also increased MAF1 mRNA expression (FDR = 2.60 x 10^{-6}). Next, we examined MAF1 expression in response to anti-HER2 therapy (trastuzumab) in HER2-positive breast cancer patients (n = 564) using relapse-free survival status at five years (n = 1,329) as the clinical output to perform a receiver operating characteristic (ROC) analysis. Increased MAF1 mRNA expression occurs in HER2 - positive breast cancer patients treated with trastuzumab (p = 2.7 x 10^{-6}, AUC = 0.874). These data suggest MAF1 may be a predictive biomarker for anti-HER2 therapy in HER2-positive breast cancer.

B265/P1947

Concomitant inhibition of KRAS and EGFR by miR-1 and miR-155 in colon cancers

J. Kim[1], M. Hwang[1], Y. Kwon[2], W. Kim[2]; [1]Department of Pharmacology, Department of Biomedicine & Health Sciences, The Catholic University of Korea, Seoul, KOREA, REPUBLIC OF, [2]Natural Product Research Center, KIST Gangneung Institute of Natural Products, Gangneung, KOREA, REPUBLIC OF.

Colorectal cancer (CRC) is one of the most common fatal cancers worldwide. EGFR is overexpressed in CRC and has been reported to promote colon cancer growth. Colon cancer can respond to the EGFR-targeting drugs such as cetuximab. However, no effective therapeutic strategy has been developed in targeting KRAS with mutation. Here, we found novel miRNAs, which simultaneously target EGFR and KRAS in colon cancer. Target Scan Database screened 54 miRNAs for targeting EGFR and KRAS simultaneously. Of these candidates, miR-1 and 155 were validated by Western blot. Luciferase assay revealed that these two miRNAs are directly binding to the 3'-UTRs of EGFR and KRAS. Furthermore, cell proliferation assay proved that miR-1 and 155 inhibit the cell proliferation and growth. These findings demonstrate that miR-1 and miR-155 mediate down regulation of EGFR and KRAS, suggesting that targeting EGFR and KRAS with miR-1 and 155 might be useful therapeutic strategies against colon cancer growth.

B266/P1948

Sphingosine Kinase 1 Promotes Lung Cancer Cell Growth

A. B. Nguyen[1], V. Balaji Ragunathrao[2], D. Mehta[2]; [1]Department of Biology, Villanova University, Villanova, PA, [2]Department of Pharmacology and The Center for Lung and Vascular Biology, The University of Illinois College of Medicine, Chicago, IL.

Sphingosine kinase 1 (SPHK1) is a cytosolic enzyme that generates the bioactive lipid, sphingosine-1-phosphate (S1P), from sphingosine. S1P drives the growth, vascularization, and invasiveness of tumors. SPHK1 has been shown to be highly mutated and overexpressed in many types of cancer, such as ovary, skin, breast, or lung cancer. Here we assessed the role of SPHK1 in regulating the proliferation of lung cancer using the Lewis lung carcinoma (LLC) cell line. Differences in the expression levels and localization of SPHK1 were examined in LLC cells after culturing for 24, 48, 72, 96, and 120 hours. We found that SPHK1 mRNA and protein expression increased in LLC, reaching a maximum at 96 hours. Interestingly, while SPHK1 is often characterized in the cytosol, confocal imaging showed nuclear translocation of SPHK1 at 72 hours in a subpopulation of cells, which was sustained till 120 hours. To address the causal role of SPHK1 in regulating LLC proliferation, we targeted SPHK1 using CRISPR, small interfering RNA (siRNA), and a specific small molecular inhibitor of SPHK1, PF-543. Inhibition of SPHK1 reduced LLC

viability after all treatments. CRISPR-Cas9 was more effective than siRNA as it had higher cytotoxicity of over 80%. PF-543 was tested at different concentrations, showing a gradual increase in cytotoxicity as its concentration increased from 1 nM to 100 nM and an IC_{50} of 4.9 nM. In conclusion, our study shows that SPHK1 induces the proliferation of lung cancer cells and therefore SPHK1 is a promising target to inhibit lung cancer growth.

B267/P1949

Examining the role of EGFR family signaling in promoting prostate cancer proliferation in an androgen receptor-low environment

S. Wilkinson[1], A. Ku[1], I. King[1], A. Baj[1], D. Low[1], H. Ye[2], F. Karzai[1], S. A. Harmon[1], N. T. Terrigino[1], J. R. Bright[1], S. Y. Trostel[1], R. T. Lis[1], B. Turkbey[1], W. L. Dahut[1], A. G. Sowalsky[1]; [1]National Cancer Institute, Bethesda, MD, [2]UCLA, Los Angeles, CA.

Prostate cancer (PC) remains the second-leading cause of death among men in the United States. In patients presenting with locally-advanced disease, neoadjuvant androgen deprivation therapy may represent the last chance for a cure, as treatment-resistant micrometastic spread will ultimately drive relapse, and progress to lethal PC. To determine novel mechanisms of treatment resistance, we tested the hypothesis that mechanisms of treatment resistance are present at baseline, which would predict poor therapeutic response. Utilizing tumors from 39 patients with locally-advanced PC and RNAseq to measure differentially expressed genes as a function of posttreatment residual tumor volume, we identified pathway alterations, including EGFR family members, at baseline that promote a poor therapeutic outcome. We found that patient tumors with low androgen receptor (AR) activity at baseline exhibited a poor treatment response, potentially due to increased dependency on alternate pathways. Interestingly, these tumors also consistently exhibited a significant increase in the expression of EGFR family signaling (EGFR, HER2, and HER3) at baseline, which was positively correlated with the volume of posttreatment tumors. To determine a mechanistic role for EGFR in disease progression, PC cell lines were treated with a panel of EGFR family and androgen receptor (AR) inhibitors. Protein and RNA expression, cell viability, migration, and invasion were measured to identify the impact of dual inhibition. We found afatinib (EGFRi), lapatinib (HER2i) and neratinib (EGFR/HER2i) showed the greatest anti-tumor efficacy *in vitro*, as treatment with these agents rapidly and dynamically increased AR expression and activity. We observed synergistic effects on cell viability, proliferation, invasion, and migration by treating PC cell lines with combination therapy of anti-androgen therapy and these inhibitors. Consequently, we found that simultaneously disrupting AR activity and HER2-mediated bypass of AR blockade re-sensitized resistant PC cells to androgen deprivation therapy. These data highlight the role of EGFR and HER2 signaling in bypassing androgen inhibition to promote AR-independent PI3K-mediated proliferation pathways. As these effects were observed in a treatment-naive patient population, they represent an early divergent path in tumorigenesis, where prostate tumors initially develop to have intrinsically lower AR activity, which would contribute to treatment resistance and metastatic spread. Current work is focused on validating these findings using genetic abrogation of EGFR and HER2 in additional clinical samples, organoids, and patient-derived xenograft models.

B268/P1950

Investigating cellular senescence as a novel treatment for glioblastoma

R. Hines[1,2], K. Morris[2], P. Vaughan[2], T. Hinchcliffe[3], P. Traktman[4,5,6,7], K. Vaughan[2,8]; [1]Graduate Division, Indiana University School of Medicine, Indianapolis, IN, [2]Biological Sciences, University of Notre Dame, Notre Dame, IN, [3]Hormel Institute, University of Minnesota, Austin, MN, [4]Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC, [5]Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, [6]Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, [7]Microbiology and Molecular Genetics, Medical College of Wisconsin, Milwaukee, WI, [8]Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN.

The reformation of the nuclear envelope at the end of mitosis is a highly regulated process. We identified a novel pathway involved in nuclear envelope reformation that, when disrupted, induces defects in nuclear lamina assembly. These defects mimic Hutchinson Gilford Progeria Syndrome (HGPS) with blebbed nuclei and uneven accumulation of Lamin A around the nuclear perimeter. As seen in HGPS, nuclear blebbing can lead to cellular senescence through the cGAS-cGAMP-STING pathway. In order to form an intact nucleus, phosphorylation of both Lamin A and Barrier - to - Autointegration (BAF) are required for their interaction at the nuclear envelope. The protein vaccinia-related kinase 1 (VRK1) has previously been shown to phosphorylate BAF. Inhibition of VRK1 through either genetic approaches or drugs in a glioblastoma (GBM) cell line resulted in blebbed and highly micronucleated nuclei. Our results indicate that targeting the reformation of the nuclear envelope could be used as a novel treatment pathway for GBM by avoiding targeting the cell cycle. Inducing nuclear blebbing and, therefore, cellular senescence would help circumvent the problems GBM treatments typically have in highly mutated GBM tumors.

B269/P1951

Loss of PRR14L Sensitizes Cells to Aneuploidy Induction

A. Liu[1], A. Narkar[1], T. Bertomeu[2], J. Zhu[3], M. Tyers[2], R. Li[1]; [1]Johns Hopkins University School of Medicine, Baltimore, MD, [2]Universite de Montreal, Montreal, QC, Canada, [3]National University of Singapore, Singapore, Singapore

One cellular state often observed in cancer is aneuploidy, which describes cells with unbalanced chromosome content. Aneuploidy is associated with a number of negative outcomes in cancer, such as drug resistance and metastasis, thus making it an attractive therapeutic target. Utilizing the MPS1 kinase inhibitor NMS-P715 to induce aneuploidy, we carried out a pooled CRISPR/Cas9 screen to identify target genes that reduce aneuploid cell fitness when lost, and identified the gene PRR14L as a potential novel target. PRR14L is a gene with currently unknown function that has previously been linked to mitosis via both midbody localization and a connection to PP2A. We subsequently generated monoclonal knock-outs of PRR14L and validated that loss of PRR14L sensitizes cells to aneuploidy. Live-cell imaging experiments have revealed that loss of PRR14L increases the frequency of mitotic slippage and consequent tetraploid daughter cells in response to MPS1 inhibitors. Current efforts to further characterize the function of PRR14L have revealed a potential link to Aurora Kinase B, and the results will be discussed.

Cancer Therapy- Drug resistance

B270/P1952

Venetoclax resensitizes cells lacking APC expression to paclitaxel treatment

J. Prosperi; IUSM-South Bend/Notre Dame, South Bend, IN

Patients with triple negative breast cancer (TNBC) are treated with traditional chemotherapy, such as paclitaxel (PTX). Despite the efficacy of taxanes, many tumors will recur due to drug resistance. Therefore, the need to understand the mechanism behind drug resistance is critical to improve patient outcome and survival. Our lab was the first to show that loss of the *Adenomatous Polyposis Coli (APC)* tumor suppressor caused resistance to PTX in the MDA-MB-157 human TNBC cell line. In the absence of APC, apoptosis induction was decreased, as measured through cleaved caspase 3 and annexin/PI staining. To understand the molecular mechanisms behind APC-mediated PTX response, we analyzed the BCL-2 family of proteins and found a robust increase of the pro-survival family member, Bcl-2. The BH3 mimetic, ABT-199 (Venetoclax), which specifically targets Bcl-2, has been used as a single or combination therapy in multiple hematologic malignancies. In addition, ABT-199 has shown promise in multiple subtypes of breast cancer. Therefore, we used ABT-199 in combination with PTX to address the hypothesis that APC-induced Bcl-2 increase is responsible for PTX resistance. Combination treatment in three CRISPR-mediated APC knockout TNBC cell lines (MDA-MB-157, MDA-MB-231, and SUM159) caused changes in cell proliferation and apoptosis. Combined, these data suggest restored sensitivity to PTX using ABT-199. Our studies are the first to show that Bcl-2 inhibition can restore PTX-sensitivity in APC mutant breast cancer cells. These studies are critical to advance better treatment regimens in patients with TNBC.

B271/P1953

Investigating the Efficacy of DPI-503 Against Drug-Resistant mtBRAF-Driven Metastatic Melanoma

S. Kumar, R. K. Mehta, M. Tan, V. Krishnamoorthy, M. Nyati; Department of Radiation Oncology, University of Michigan, Ann Arbor, MI.

Malignant melanoma remains one of the fastest-growing cancers worldwide. About 99,780 new melanomas will be diagnosed in the US in 2022. While primary cutaneous melanoma can be excised via surgery, metastatic melanoma requires advanced therapies to be managed. Approximately 50 percent of metastatic melanoma patients have a mutation in the BRAF gene. The mutation of BRAF causes its persistent activation, leading to subsequent activation of downstream signaling and cancer cell phenotypes. While BRAF antagonizing therapies such as vemurafenib exist, patients acquire resistance to vemurafenib and other therapeutics via various pathways such as mutational mechanisms. Thus, a new therapeutic is required to treat drug-resistant mutant-BRAF driven metastatic melanoma. We hypothesize novel, non-PROTAC, orally bioavailable small molecule DPI-503 causes tumor cytotoxicity via EGFR ablation leading to fewer xCT and SGLT1 nutrient transporters. Consequently, the sudden decrease in essential nutrients along with increased reactive oxidative species and mitochondrial oxygen consumption rate caused by DPI-503 may cause overwhelming tumor cell cytotoxicity faster than the cell is able to adapt. The aim of this study was to investigate the mechanism by which DPI-503-induced loss of EGFR and associated nutrient transporters affect cellular metabolism and cause cytotoxicity. The aim of this study was investigated using in vitro models including several isogenic paired melanoma cell lines that showed resistance to vemurafenib (A375/A375-MEK1, M14/MDA-MB435). Cell culture

and IC50 calculations contributed greatly to preliminary in-vito results.
Preliminary results show DPI-503 having a lower IC50 in A375-MEK than vemurafenib, suggesting that DPI-503 is a more potent inhibitor of BRAF even in metastatic melanoma cell lines. Further research including tumor growth studies and PDX models will be needed to evaluate the efficacy and safety of DPI-503 in-vito.

B272/P1954

Targeting the nuclear envelope as a potent strategy to kill chemoresistant cancer

J. Chen[1], M. Wang[1], R. Huang[1], J. Chen[2,3], B. Vanslembrouck[2,3], M. LeGros[2,3], C. Larabell[2,3], T. Wang[4,5,6], I. Shih[4,5,6], Y. Chen[1,7,8]; [1]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, [2]Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, CA, [3]Department of Anatomy, University of California San Francisco, San Francisco, CA, [4]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medical Institutions, Baltimore, MD, [5]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, [6]Department of Gynecology and Obstetrics, Johns Hopkins Medical Institutions, Baltimore, MD, [7]The Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD, [8]Center for Cell Dynamics, Johns Hopkins University, Baltimore, MD.

Reduced expression of lamin A/C is a feature of metastatic cancer since elevated lamin A/C levels stiffen the nucleus and impede cell migration through confinements. We explored the possibility of exploiting the fact that reduced lamin A/C in metastatic cancer makes the nucleus more susceptible to mechanical damage. We investigate whether paclitaxel (PTX), a commonly used chemotherapeutic drug for many cancer types known to induce nucleus deformity, can further stress the nucleus. We observed PTX alone failed to effectively kill metastatic ovarian cancer despite inducing multi-lobular nuclei. We next examined the co-treatment of PTX and fostamatinib, a recently approved adjuvant by FDA. Co-treatment of PTX and fostamatinib at low dosage caused significant cell death (60%), while when either drug was used alone, negligible cell death was observed. Immunostaining of lamin A/C in fostamatinib-treated cells showed little change compared to control. Examination of nuclei in co-treated cells by soft X-ray tomography revealed that the multiple nuclear lobules are connected, with surface area enlarged by 82%, diluting the nuclear lamina. We also observed that 52% of PTX/fostamatinib co-treated cells have locally depleted lamin A/C, which leads to nuclear DNA leakage into cytoplasm and cell death, likely caused by the synergistic damaging effect of both drugs. Interestingly, we found this drug combination is also effective in treating carcinoma-associated fibroblasts. In summary, we demonstrated co-treatment of PTX induced multilobular nuclei with diluted nuclear lamina, prone to rupture and subsequent cell death. Our result suggests compromising the nuclear envelope integrity with two orthogonal mechanisms, lamin A/C reduction and mechanical stress by deformation, can effectively eliminate cancer cells.

B273/P1955

ZNF217 and NRG1/ErbB3 Signaling Promote Endocrine Therapy Resistance via the Ligand-Independent Activation of ER Alpha

P. Tennakoon[1], Z. Song[1], J. Wu[1], K. Carthy[1], M. Miramontes[1], R. Goyal[2], C. Suarez[1], J. Li[1], L. E. Littlepage[1]; [1]Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN, [2]Biochemistry, University of Texas, Austin, TX.

Around 70% of breast cancer tumors are estrogen receptor positive (ER+), while ~33% of these tumors eventually relapse as recurrent metastatic tumors resistant to endocrine therapies (tamoxifen, fulvestrant). We identified the oncogene and transcription factor ZNF217 (human)/Zfp217 (mouse) as an ER modulating protein that drives tamoxifen and fulvestrant resistance. To study the role of Zfp217 in tamoxifen response *in vivo*, we treated mice with PyMT ± Zfp217 tumors with tamoxifen. Vector-expressing control tumors significantly responded to tamoxifen, while tumors overexpressing Zfp217 did not. Treatment with fulvestrant gave similar results.The objective of this study was to identify the molecular mechanism of how ZNF217 causes endocrine therapy resistance. Ligand independent activation of ER by growth factor signaling pathways is a main mechanism of endocrine therapy resistance. Significantly, ZNF217 directly activates the ErbB3/AKT signaling pathway. We investigated transcriptomic and genomic changes driven by ZNF217 & ErbB3 signaling activated by neuregulin (NRG1) and how they drive tamoxifen resistance. We identified a novel prognostic ZNF217- and NRG1 (ligand of ErbB3)-dependent gene expression signature by RNA-Seq and non-canonical ER binding sites in the genome by ChIP-Seq in MCF7 cells. We also discovered ER- and NRG1-dependent ZNF217 binding sites in the MCF7 cells using CUT&RUN sequencing. These transcriptomic and genomic changes correlated with lower survival in ER+ breast cancer patients receiving tamoxifen and identified key pathways altered in a ZNF217-dependent manner after NRG1 induction. After ChIP-Seq and CUT&RUN data integration, we identified both PCK1 and PHGDH as novel inducers of tamoxifen resistance that rewire cellular metabolism. In MCF7 cells, both genes induced endocrine therapy resistance, while their deficiency made cells more susceptible to endocrine therapy. Significantly, overexpression of Pck1 in murine mammary tumors accelerated tumor burden and endocrine therapy resistance, while Pck1 knockdown made tumors susceptible to endocrine therapy. In summary, NRG1 induces a differential ZNF217-dependent gene expression signature and non-canonical ER genomic binding in ER+ breast cancer cells. Significantly, two targets, PCK1 and PHGDH, promote endocrine therapy resistance in vivo and are themselves potential therapeutic targets.

B274/P1956

Barcode-sequencing screen for modulators of anticancer ruthenium complex sensitivity in*S.cerevisiae*.

A. Chinn, P. Hanson; Furman Univserity, Greenville, SC

Phenotypes uncovered via pharmacogenomic analyses provide a viable tool for understanding the effects of diverse drugs on cellular pathways. Furthermore, these unbiased genome-wide assays can identify genes with unanticipated and/or previously unknown roles in cellular drug responses. In this study, we analyzed pools of barcoded budding yeast (*S.cerevisiae*) deletion strains to identify genes that alter sensitivity to the anticancer ruthenium complex trans-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019). Early experimental assays featured model populations of two strains with differing KP1019 sensitivity. In these trials, we found that the commonly used approach of treating yeast immediately after thawing produced inconsistent directional selection compared to growing yeast for five

generations prior to treatment. An analysis of a full pool of barcoded yeast deletion strains was conducted using next generation sequencing, detecting 3290 unique upstream barcodes representing 63.5% of nonessential deletion strains. While no significant pathway enrichments were found, some deletion strains were far more or far less abundant in KP1019 treatment groups compared to the controls, indicating potential roles in drug sensitivity and resistance respectively. The strain lacking the evolutionarily conserved DNA repair gene *UNGI* experienced the greatest decrease (more than 22-fold) in the presence of drug. This finding is in line with previous research showing increased KP1019 potency in other DNA repair loss-of-function yeast mutants. Additionally, yeast lacking the translation regulator (*STM1*) increased in abundance nearly 10-fold in KP1019 test groups, with the most enriched mutant (*YMR254C* at a just over 10-fold increase) lacking a gene of currently unknown function. In the context of previous studies linking KP1019 treatment to increased ribosomal protein abundance in yeast, our findings provide further evidence for the putative role of ribosomes in the cellular response to the drug. While these results offer support for current models of KP1019 action and validate the screen design, future studies may uncover novel genes and pathways that modulate KP1019 bioactivity. Improved understanding of the genetic factors that alter the efficacy of KP1019 may create opportunities for genetically tailored medicines and combination therapies in the future.

Extracellular Matrix in Pancreatic and Gastric Cancer

B275/P1957

Metaplasia- and cancer-associated fibroblasts promote dysplastic progression of human stomach metaplastic cells

E. W. Contreras-Panta[1], S. Lee[2], D. Gibbs[3], Y. Won[2], J. Min[2], C. Zhang[2], S. Hong[2], J. Roland[2], L. Ferri[4], V. Sangwan[4], J. Ragoussis[4], S. Camilleri-Broët[4], J. Caruso[5], M. Strasser[3], P. Gascard[5], T. Tlsty[5], S. Huang[3], E. Choi[2], J. R. Goldenring[2]; [1]Cell and Developmental Biology, Vanderbilt University, Nashville, TN, [2]Vanderbilt University Medical Center, Nashville, TN, [3]Institute for Systems Biology, Seattle, WA, [4]McGill University, Montreal, QC, CANADA, [5]University of California, San Francisco, San Francisco, CA.

Gastric cancer remains one of the leading causes of cancer-related death in the world. Intestinal type gastric cancer in the human stomach evolves in the setting of well-defined metaplasia intermediates. Spasmolytic Polypeptide-Expressing Metaplasia (SPEM) is considered the first type of metaplasia in the stomach. Importantly, increasing evidence has shown that SPEM can progress into Intestinal Metaplasia, Dysplasia or even cancer. However, the factors promoting this progression remain unknown. Recently, fibroblasts were reported to influence carcinogenesis in different organs. However, the roles of Metaplasia-Associated Fibroblasts (MAFs) and Cancer-Associated Fibroblasts (CAFs) in stomach metaplasia progression towards advanced neoplastic stages have not been well studied. In that regard, our lab has made efforts to establish different fibroblast and gastroid lines from human stomach resections with the objective to determine the effects of MAFs and CAFs in SPEM cells from human stomach gastroids. Using Air Liquid Interface (ALI) technique, we co-cultured metaplastic gastroid cells with either MAFs or CAFs on transwell filters. Culture of gastroid cells only and co-cultured with Normal tissue Associated Fibroblasts (NAFs) were used as control. After 14 days of ALI, we observed that gastroid cells co-cultured with MAFs and CAFs showed an aggressive growth pattern by forming polyp-like structures that protruded up from the transwell filter. In contrast, gastroid cells only or co-cultured with NAFs did not cause an aggressive growth pattern. Through immunostaining, we observed the increased expression of dysplastic markers, including TROP2 and CEACAM5, and decreased expression of

metaplastic markers, including CD44v9 and AQP5, in the gastroid cells co-cultured with MAFs and CAFs. Of special interest, dysplastic markers were prominently expressed in the polyp-like structures. Moreover, after height measurements in cross-sections, we observed significantly higher heights in the MAFs and CAFs co-culture conditions. Finally, Transmission Electron Microscopy confirmed the aggressive growth pattern of epithelial cells co-cultured with MAFs, and especially with CAFs, often showing multiple lumen structures. These findings suggest that MAFs and CAFs could promote metaplasia progression into dysplasia. Further studies will determine whether a subpopulation of MAFs and CAFs are responsible for these changes.

B276/P1958

Fibrosis to Fitness: Examining the Role of Tumor Stiffness in Pancreatic Ductal Adenocarcinoma

J. M. Rosas[1], J. Campanale[2], J. L. Harwood[2], A. A. Pitenis[3], D. J. Montell[1]; [1]Biomolecular Science and Engineering, University of California, Santa Barbara, Santa Barbara, CA, [2]Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA, [3]Materials Engineering, University of California, Santa Barbara, Santa Barbara, CA.

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal forms of cancer, with a mortality rate of ~90%. A hallmark of PDAC disease progression is an oncogenic mutation in a member of the rat sarcoma virus family, K*RAS* (*KRASG12D*), arising in the epithelia of pancreatic ducts. This mutation correlates with increased fibrosis, characterized by deposition, alignment, and maturation of extracellular matrix (ECM) proteins including collagen. ECM remodeling gives rise to stiffened pancreas tissue and compromised duct structure. Prior atomic force microscopy measurements of pancreas explants report average elastic modulus values from E=1kPa (normal) to 4kPa (PDAC). However, the relationship between tissue stiffness and duct structure during disease progression remains unclear. This work employs a hydrogel platform to study lumen formation of tissue-derived pancreas organoids in 3D. This *in vitro* platform is composed of polyacrylamide hydrogels with tunable bulk mechanical properties (elastic modulus, 500 Pa<E<100kPa). Hydrogels are processed into small particles to create microgels (100±10µm diameter) with tunable rheological properties (storage modulus, 5Pa<G'<100kPa) that control the viscoelastic behavior of the microenvironment. Microgel-based environments incorporating Matrigel™ (25, 50, and 100 vol.%) enable investigation of pancreas organoid lumen formation. After 48h in 100 vol.% Matrigel™ (G'=90±5Pa), organoids derived from healthy (Normal) and KRASG12D (Tumor) genotyped mice formed lumens with similar diameters: 130±60µm and 140±90µm, respectively. In contrast, Normal organoids form lumen with decreasing diameters in microenvironments with increasing storage modulus: soft (50 vol.% Matrigel™, G'=40±10kPa, diam.: 110±50µm); stiff (25 vol.% Matrigel™, G'=65±3 kPa, diam.: 90±40µm), suggesting lumen formation is sensitive to environmental stiffness. Tumor-derived organoids do not show similar dependence on microenvironmental stiffness and form lumens with comparable diameters across 100 vol.% Matrigel™ (150±90 µm), soft (140±80µm), and stiff (150±80µm) hydrogel environments. This work demonstrates stiffness-dependence in pancreatic ductal lumen formation specific to organoids derived from healthy tissues and an insensitivity of tumor organoids to similar mechanical stresses. These results may assist in the fundamental understanding of tumor cell fitness in stiffening microenvironments.

B277/P1959

Pathophysiological Role of Golgi Apparatus in the gastric Cancer

J. Kim[1], C. Shrestha[1], S. Cho[2], M. Hwang[1], S. Kang[1], W. Kim[2]; [1]Department of Pharmacology, Department of Biomedicine & Health Sciences, The Catholic University of Korea, Seoul, KOREA, REPUBLIC OF, [2]Natural Product Research Center, KIST Gangneung Institute of Natural Products, Gangneung, KOREA, REPUBLIC OF.

Gastric cancer (GC) has a high incidence and mortality rate with a dismal 5-year survival rate due to poor prognosis in early stages. As reported, epithelial-mesenchymal transition (EMT) activation leads epithelial cells to lose its intercellular adhesions and develops migratory fibroblastic properties and promotes cell invasion, metastasis and chemo-resistance properties; however the molecular mechanisms are still unknown. Here, we show the structural and functional differences of Golgi Apparatus (the GA) between diffuse and intestinal type GC cell lines. Diffuse GC cell lines associated with EMT-subtype. Of interest, the diffuse type GC cell lines had a more compacted the GA, while the intestinal type GC cell lines had dispersed the GA. In addition, cell invasion, migration assays showed that diffuse type GC had higher migration and invasion. These results demonstrated that compactness of Golgi structure associates with metastasis, invasion and cell growth in GC.

B278/P1960

A novel role for the GEF DOCK8 as a key regulator of lysosome-mediated pancreatic cancer invasion

O. L. Gutierrez Ruiz[1], K. M. Johnson[2], E. Krueger[2], R. E. Nooren[1], N. Cruz-Reyes[1], C. J. Holtz Heppelman[3], M. Fernandez-Zapico[4], M. McNiven[2], G. Razidlo[2]; [1]Biochemistry and Molecular Biology, Mayo Clinic Graduate School of Biomedical Sciences, Rochester, MN, [2]Division of Gastroenterology & Hepatology, Mayo Clinic, Rochester, MN, [3]Mayo Clinic, Rochester, MN, [4]Division of Oncology Research, Mayo Clinic, Rochester, MN.

Metabolic reprograming and increased lysosome activity are features of pancreatic ductal adenocarcinoma (PDAC) orchestrated by the oncogene KRAS. Lysosomes serve as a signaling platform for nutrient homeostasis and confer pro-invasive abilities during metastasis. Yet, how oncogenic KRAS regulates lysosome function to potentiate tumor cell invasion remains unclear. Using comparative proteomic analysis of isolated lysosomes, we identified that oncogenic KRAS enhances the aberrant expression of DOCK8 and induces its novel recruitment to lysosomes in a subset of PDAC cells, as confirmed by fluorescence microscopy and immunoblotting. DOCK8 is a guanine nucleotide exchange factor (GEF) primarily expressed in immune cells where it mediates cell migration by activating the Rho GTPases Rac1 and Cdc42. Upon its ectopic expression in pancreatic cancer cells, we identified DOCK8 as a novel pro-invasive factor as depletion of this GEF by siRNA or knockout significantly decreases tumor cell invasion, and conversely, overexpression of DOCK8 increases invasion in a GEF dependent manner. We sought to determine how this lysosome-associated GEF contributes to invasive migration. While DOCK8 does not regulate global lysosome function, it does selectively impact lysosome size, motility, and specific proteolytic capacity. Interestingly, we observed that DOCK8 is required to stimulate the actin nucleation machinery at the lysosome surface where it drives actin-mediated lysosomal propulsion, thereby regulating lysosomal trafficking velocity. Furthermore, we found that depletion of DOCK8 markedly reduces lysosome-dependent degradation of the extracellular matrix due to significantly reduced levels and activity of the lysosome-associated protease Cathepsin B, which has been described to support tumor cell invasion. Importantly, this degradation defect can be restored by

re-expression of Cathepsin B. In summary, we have identified the GEF DOCK8 as a key pro-invasive factor that is aberrantly expressed in a subset of pancreatic cancers and describe a novel mechanism by which DOCK8 regulates actin polymerization at lysosome membranes to promote their motility and proteolytic capacity to mediate KRAS-driven pancreatic cancer invasion.

B279/P1961

Bioinformatic analyses reveal a novel role for Ang-Tie2 signaling in pancreatic cancer-associated fibroblasts

J. C. Gardiner, E. Cukierman; Fox Chase Cancer Center, Philadelphia, PA

Pancreatic cancer (PC) is a devastating disease with a five-year survival rate of ~11%. Its aggressiveness is largely mediated by the expansive tumor microenvironment that includes cancer-associated fibroblasts (CAFs) and the dense extracellular matrix (ECM) they produce. Recent work from our lab indicated that the synaptic neuronal protein, Netrin G1 (NetG1), is pathologically upregulated in pancreatic CAFs and its loss alters the fibroblasts' ability to perform tumor supportive functions (*e.g.* inflammatory cytokine secretion). Additionally, tissue microarray analysis showed that PC patients expressing higher levels of NetG1 in fibroblasts had poorer overall survival than patients with lower NetG1 expression, suggesting that NetG1 may demarcate tumor-supporting CAFs. Thus, the goal of this project is to determine whether comparisons of NetG1 high and low expressing CAFs could elucidate other novel regulators of CAF pro-tumor function. Using publicly available transcriptomics data of PC patient-matched normal fibroblasts and CAFs, CAFs were first stratified into NetG1^{low} or NetG1^{high} expressing cohorts to identify differentially expressed genes (DEG). Network and computational analysis revealed a core signature comprising 67 genes represented in 16 clusters from the initial 530 DEG input list. Interestingly, the largest cluster was enriched for "Tie2 Signaling", which is typically associated with angiogenesis and endothelial cell function but not with PC due to the blood vessel collapse that arises from the dense ECM. Western blot analysis demonstrated that our patient-derived pancreatic CAFs amply express Tie2 whose activity (denoted by phosphorylation) increases upon stimulation with the angiopoietin (Ang) ligands. Moreover, Ang stimulation of Tie2 decreased CAF interleukin-6 and interleukin-8 secretion while small molecule inhibition increased cytokine secretion. Collectively, these data suggest that Ang/Tie2-mediated signaling can influence CAF pro-tumorigenic functions and may provide a novel target to normalize the tumor-enabling microenvironment in patients.

B280/P1962

The effect of Hippo pathway dysregulation on *Helicobacter pylori*-mediated gastric cancer

B. Yu[1], S. Lee[1], B. Hwang[1], H. Jeong[2], K. Nam[2], Y. Lee[1]; [1]Department of Internal Medicine, Yonsei University College of Medicine, seoul, KOREA, REPUBLIC OF, [2]Severance Biomedical Science Institute and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, seoul, KOREA, REPUBLIC OF.

BACKGROUND: *Helicobacter pylori* is known to activate chronic pre-inflammatory signal pathways in gastric epithelial cells, may participate in gastric carcinogenesis. Hippo pathway dysregulation is known to play an important role in the prognosis of cancer progression and metastasis. However, the role of YAP/TAZ, a major gene in the Hippo pathway in *H. pylori*-mediated gastric cancer is not well understood. The purpose of this study was to investigate the role of Hippo pathway in the gastric carcinogenesis following chronic infection with *H.pylori* based on clinical/molecular/histological observations. **Materials**

and Methods: Clinically derived gastric organoids, human gastric epithelial cell lines (AGS, MKN74, GES-1), *H. pylori* strains Hp 60190 (CagA (+) and CagA (-)) were used. We evaluated the effects of YAP/TAZ on inflammatory response, EMT, invasion and gastric cancer-associated tumorigenic properties using not only *H. pylori*-infected gastric epithelial cell lines, but also human gastric organoids with excellent physiological similarity. Organoids were cultured long-term in media containing various growth factors. Gastric cancer mice were established, and EMT gene analysis was performed using WB, PCR and IHC. Electron microscopy images and TEER were measured in infected 2D organoids. **RESULTS:** Gastric organoids contained various types of normal gastrointestinal cells and showed major characteristics of gastric mucosa such as MUC5AC, MUC6, and LGR5. Mucosal surfaces secreted mucus that could overcome a few bacteria. *H. pylori* infection caused inflammation lasting more than 72 hours, and IL-8 , an inflammatory mediator, was overexpressed in CagA positive strains. It was confirmed that *H. pylori* infection induces overexpression and nucleus translocation of YAP/TAZ, which converts gastric epithelial cells into mesenchymal cells. YAP/TAZ overexpression was significantly correlated with the junction protein ZO-1, suggesting an important role in gastric cancer progression. *H. pylori* attachment reduced expression of the junctional proteins (E-cadherin, Zo-1). We confirmed that *H. pylori* preferentially attaches to the gap between cells in order to reach the parietal cell located at the base of the upper gland via EM image. **CONCLUSION:** *H. pylori* infection accelerates gastric carcinogenesis by inducing persistent inflammation, loss of junction protein, through Hippo pathway dysregulation. This disrupts the normal morphology of cells and induces migration, invasion and EMT processes. Our findings help to understand the regulatory circuits of inflammation, defense, and gastric carcinogenesis in the interactions between *H. pylori* and the epithelial surface.

B281/P1963

Hyaluronic acid and glycosylation regulate pancreatic cancer cells glycocalyx/actin cortex architectures and mechanical properties

A. Massey; National Institutes of Health, Bethesda, MD

Pancreatic cancer is currently one of the most lethal malignancies worldwide; this is principally due to difficulty with early detection and poor response rates to conventional chemotherapeutics. One of the reasons behind this is the glycocalyx - a dense, brush-like layer covering cells. Normally, this structure normally acts as a protective barrier. In cancer however, various components of the glycocalyx are either upregulated or aberrantly glycosylated which can enhance tumor progression through factors such as immune evasion, metastasis, and drug resistance. Although these effects are understood biochemically, less is known about how the glycocalyx affects cancer cells at a biophysical level. In this study, we investigated the biophysical effects of glycocalyx architectural modulation in pancreatic cancer cells via atomic force microscopy and confocal imaging. Our preliminary results show a distinct architectural change in the glycocalyx with hyaluronic acid perturbations via hyaluronidase and aggrecan treatments, as well as with a perturbation of the glycosylation status with PNGase- and neuraminidase-induced enzymatic degradation. Both types of perturbations led to significant changes in the stiffness of normal and cancerous cells, with a differential trend noted between normal and cancerous cells. Changes to both the glycocalyx and actin cytoskeleton structure were observed with these treatments, suggesting that modulations to the glycocalyx directly impact the underlying actin cortex. Future studies will examine how the modulation of different transmembrane proteins affect cellular mechanics and the overall structure of the glycocalyx.

B282/P1964

Synergistic effects of TPP1 promoter mutations and TERT promoter mutations on telomere maintenance and immortalization in melanoma

P. Chun-on[1,2,3], A. M. Hinchie[2], H. C. Beale[4], A. A. G. Silva[2], E. Rush[5], C. Sander[5], C. J. Connelly[6], B. K. N. Seynnaeve[5,7], J. M. Kirkwood[5], O. M. Vaske[8,9], C. w. Greider[9,6], J. K. Alder[2]; [1]Environmental and Occupational Health, School of Public Health University of Pittsburgh, Pittsburgh, PA, [2]Dorothy P. and Richard P. Simmons Center for Interstitial Lung Disease, Division of Pulmonary, Allergy, and Critical Care Medicine, Pittsburgh, PA, [3]Faculty of Medicine and Public Health, HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy, Bangkok, THAILAND, [4]Environmental and Occupational Health, UC Santa Cruz, Genomics Institute, University of California, Santa Cruz, CA, [5]University of Pittsburgh Medical Center, Hillman Cancer Center, Pittsburgh, PA, [6]Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, [7]Department of Pediatrics, Division of Pediatric Hematology/Oncology, University of Pittsburgh School of Medicine, Pittsburgh, PA, [8]UC Santa Cruz, Genomics Institute, University of California, Santa Cruz, CA, [9]Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA.

Limitless replicative potential is a defining capability of cancer. Telomere maintenance in cancer cells typically depends on reactivation of telomerase expression to allow somatic cells to bypass replicative senescence. *TERT,* the reverse transcriptase component of telomerase, promoter mutations have been found ~75% of melanoma and create *de novo* E-twenty six (ETS) transcription factor binding motifs which function to increase *TERT* expression. *TERT* promoter mutation acquisition alone extends the proliferative capacity of cells but cannot prevent bulk telomere shortening and additional somatic changes are therefore required for full immortalization. Here, we identified a cluster of variants in 5' region of *ACD* gene encoding TPP1. TPP1 is a component of the shelterin complex and functions in both telomerase recruitment and repeat addition processivity. The two most common re-occurring variants in *TPP1* promoter create or modify ETS binding sites and increase *TPP1* expression similar to *TERT* promoter mutations. TPP1 over-expression has a synergistic effect with increased TERT expression on telomere lengthening in primary BJ fibroblasts. We engineered the two most frequent *TPP1* promoter variants into human melanoma cell lines using CRISPR/Cas9-mediated genome editing. Introduction of the *TPP1* promoter variants at the endogenous locus led to increased *TPP1* gene expression compared to the parental cell lines. Analysis of whole genome sequencing of melanoma suggests that ~5% of cutaneous melanoma have both *TERT* promoter mutations and *TPP1* promoter variants. Our findings suggest that *TPP1* promoter variants cooperate with *TERT* promoter mutations to enhance telomere maintenance and immortalization in melanoma.

B283/P1965

ARID1A loss leads to enhancement of genomic instability by epigenetic dysregulation in aggressive Osteosarcoma

K. Fatema[1], Y. Wang[2], S. Plyler[1], A. Pavek[1], K. Jones[3], J. Barrott[1]; [1]Department of Biomedical and Pharmaceutical Sciences, Idaho State University, Pocatello, ID, [2]Huntsman Cancer Institute, University of Utah, SLC, UT, [3]Huntsman Cancer institute, University of Utah, SLC, UT.

Osteosarcoma is the most common pediatric malignant bone tumor in the USA. Genetically it is the most complex cancer and is associated with quick progression towards metastasis, for which there are limited targeted therapies. Genetic mutations to use as biomarkers are muddied by the complex genomic instability existing at baseline in most osteosarcomas. Moreover, a significant drop in survival occurs because of our lack of differentiating between responders and non-responders to conventional chemotherapies early in the treatment regimen.With the purpose of understanding the molecular mechanisms associated with chemoresistance and metastasis, we identified an epigenetic modifier, ARID1A, to be responsible for a more aggressive osteosarcomagenesis in a forward genetic screen. Arid1a is a member of an epigenetic chromatin remodeling complex, SWI/SNF, and has been implicated in the progression and worse prognosis of other cancers. We studied Arid1a deletion in genetically engineered mouse models as well as human osteosarcoma cell lines to evaluate its impact. Furthermore, upon loss of Arid1a, genomic instability was enhanced, which we predict can serve as a biomarker for chemosensitivity. Gene expression analysis and ATAC-seq data further implicate disruption of DNA repair pathways upon deletion of Arid1a. Arid1a resulted in a general trend of chromatin compaction, which we posit results in the negative regulation of important tumor suppressor genes. Using ClaraT bioinformatics tools to highlight the involvement of specific hallmarks of cancer, it was determined that genomic instability and EMT-related pathways were enhanced in the Arid1a knockout models. This is consistent with our gross phenotypic analysis of our mice that exhibited more tumors per mouse and more metastatic tumors in the Arid1a knockout mice. Not only that, but the most recent implementation of Nanopore sequencing also provided convincing evidence toward genomic instability to be a precursor to aggressiveness in osteosarcoma.In conclusion, the loss of Arid1a in osteosarcoma enhances the aggressive and metastatic phenotype, correlating to decreased chemosensitivity. Efforts to better understand the intersection between genomic instability and epigenetic dysregulation can lead to earlier decision-making to pursue alternative and sometimes experimental therapies.

B284/P1966

Non-secretory alpha1-antitrypsin isoform produced from _SERPINA1_ long transcripts

A. A. Maslakova[1], S. A. Golyshev[2], D. M. Potashnikova[1], A. M. Moisenovich[1], I. V. Orlovsky[3], M. A. Rubtsov[1], O. V. Smirnova[1]; [1]Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, RUSSIAN FEDERATION, [2]A.N. Belozersky Institute of Physical and Chemical biology, M.V. Lomonosov Moscow State University, Moscow, RUSSIAN FEDERATION, [3]Unaffiliated, Moscow, RUSSIAN FEDERATION.

SERPINA1 gene encodes an archetypical serpin (serine protease inhibitor) - alpha1-antitrypsin (AAT). There are a number of *SERPINA1* long transcripts containing *SERPINA1* main ORF, which are believed to give rise to the same protein of 418 a.a.r. After processing by a secretory machinery, glycosylated mature 394 a.a.r. AAT exits the cell to protect the body from excessive proteolysis. Besides its

extracellular activity, it is now established that AAT may act inside the cells, either exogenously added or endogenously produced. In the first case, it inhibits non-target proteases such as caspases protecting lung endothelial cells from apoptosis [Petrache I. et al., 2006]. In the latter case, an intracellular AAT protects cancer cells from autophagic cell death [Shapira M.G. et al., 2014]. Here we hypothesized that intracellular AAT can be produced by alternative translation from long *SERPINA1* transcripts. We defined DU145 (prostate)- and HepG2 (liver)-specific *SERPINA1* long transcripts by 5'-RACE and individually probed their translation in living cells by fusing with eGFP. Western blot analysis of cell lysates and secretomes showed that a non-secretory isoform is translated from individual long transcript alongside with the secretory antitrypsin. The rate of the main ORF translation (a secretory AAT production) inversely depends on the length of the transcript's 5'-UTR, which confirms the earlier data on the predicted negative regulation of translation by uORFs [Corley M., 2017]. Unlike the "main" protein product, non-secretory isoform is produced at the nearly same rate from all long transcripts. Although intracellular AAT would be advantageous for tumor cells, it is unclear whether alternative *SERPINA1* translation is a phenomenon of cellular transformation or a normal cellular process. We believe that alternative splicing of *SERPINA1* pre-mRNA is the cellular mechanism to regulate the balance between secretory and intracellular antitrypsin. This work was supported by RFBR project (№16-34-01095 mol_a), Scientific Project of the State Order of the Government of Russian Federation to Lomonosov Moscow State University (№121032300075-6) and the Interdisciplinary Scientific and Educational School of Moscow University «Molecular Technologies of the Living Systems and Synthetic Biology». Part of this study was performed using equipment obtained under MSU Program of Development (BD FACSAria SORP cell sorter).

B285/P1967

The RNA binding domains 3 and 4 of Nucleolin are predicted to be drivers of nucleolin -miRNA interactions

A. San[1,2], D. Palmieri[3], A. Saxena[1,2,4], **S. Singh**[1,2,4]; [1]Biology, Brooklyn College, CUNY, Brooklyn, NY, [2]The Biochemistry PhD Program, The Graduate Center of the City University of New York, New York, NY, [3]Department of Cancer Biology and Genetics, The Ohio State University Wexner Medical Center, Columbus, OH, [4]The Biology PhD program, The Graduate Center of the City University of New York, New York, NY.

RNA binding proteins (RBPs) regulate many important cellular processes through their interactions with RNA molecules. RBPs are critical for posttranscriptional mechanisms keeping gene regulation in a fine equilibrium, the dysregulation of which is an established hallmark of tumorigenesis. Human nucleolin (NCL) is a multifunctional RBP that interacts with different types of RNA molecules, in part through its four RNA binding domains (RBDs). Particularly, NCL interacts directly with microRNAs (miRNAs) and is involved in their aberrant processing linked with many cancers, including breast cancer. However, the molecular details of the NCL-miRNA interaction remain obscure. In this study, we used an *in silico* approach to characterize the molecular details as to how NCL targets miRNAs. Here, we present structural models of NCL-RBDs and miRNAs, as well as predict scenarios of NCL-miRNA interactions generated using docking algorithms. Our study suggests a predominant role of NCL RBDs 3 and 4 (RBD3-4) in miRNA binding. We provide detailed analyses of specific motifs/residues at the NCL-substrate interface in both these RBDs and miRNAs. Finally, we propose that the evolutionary emergence of more than two RBDs in NCL in higher organisms coincides with its additional role/s in miRNA processing. Our study shows that RBD3-4 display sequence/structural determinants to specifically recognize miRNA

precursor molecules. The insights from this study can ultimately support the design of novel antineoplastic drugs aimed at regulating NCL-dependent biological pathways with a causal role in tumorigenesis.

B286/P1968

A genome-wide screen for USP28-dependent control of cell proliferation reveals genes important for mitotic progression and p53 regulation

B. E. Mierzwa[1,2], F. Meitinger[1,2,3], A. Desai[1,2], K. Oegema[1,2]; [1]Ludwig Institute for Cancer Research, La Jolla, CA, [2]University of California San Diego, La Jolla, CA, [3]Okinawa Institute of Science and Technology, Onna, JAPAN.

The deubiquitinase USP28 is a tumor suppressor and an essential component of a recently identified pathway called the mitotic stopwatch that monitors mitotic duration to suppress the proliferation of cells produced after prolonged mitosis. When mitotic duration surpasses ~3X its normal length, the stopwatch stabilizes p53 in the resulting daughter cells, leading to cell cycle arrest or death. To better understand the pathways that control mitotic duration, how USP28 controls daughter cell progression through G1, and whether USP28 interfaces with other cellular pathways, we performed a genome-wide CRISPR/Cas9 screen in the p53-positive cell line RPE1 to identify genes whose knockout leads to an acute differential effect on proliferation in the presence or absence of USP28. In this screen, USP28 WT and USP28(-/-) RPE1 cells were transduced with a pooled lentiviral CRISPR sgRNA library, Cas9 expression was induced, and samples were taken at timepoints between 0 and 14 days after induction to monitor the dropout kinetics of gRNAs corresponding to each gene. Genes whose gRNAs dropped out with different kinetics in USP28(-/-) and USP28 WT were identified. Genes required for mitotic progression were expected to drop out more slowly in USP28(-/-) cells, which lack a functional mitotic stopwatch. Indeed, in a ranked list of essential genes whose knockouts dropped out with slower kinetics in USP28(-/-) compared to USP28 WT, 55 of the top 130 genes had been previously implicated in mitotic pathways, such as spindle assembly, kinetochore and centrosome function, and promotion of mitotic exit. In a live imaging-based secondary screen, we have so far directly monitored mitotic timing for 72 of the top 100 genes, of which 46 exhibited mitotic delays. Notably, a number of the high-confidence essential genes that dropped out more slowly in USP28(-/-) did not regulate mitotic progression. Among these is a group of about 10 genes that appear to be negative regulators of the p53 pathway. We are currently investigating how modest p53 elevation could differentially suppress the growth of USP28 WT cells. Motivated by the frequent loss of USP28 in cancer, we are also characterizing genes whose gRNAs drop out more rapidly in USP28(-/-) compared to USP28 WT cells, which is leading us to identify pathways whose inhibition is synthetically lethal with loss of USP28.

B287/P1969

Uncovering Dynamics of p53 Using Cryo-EM Models

M. J. Solares[1], G. Jonaid[1], D. F. Kelly[2]; [1]Huck Institutes of Life Sciences, Penn State University, University Park, PA, [2]Biomedical Engineering, Penn State University, University Park, PA.

In the war against cancer, we have discovered the most deregulated protein in all cancer: tumor suppressor TP53 (p53). Found implicated in half of all cancer cases, plays a pivotal role in cell pathways ranging from DNA repair, cell cycle arrest, and apoptosis. Unfortunately, little structural information is available. The N-terminal domain (NTD) and C-terminal domain (CTD) are unable to be resolved due to

their flexible and disordered nature. These regions hold key regulatory roles and the source of many conflicting theories behind the mechanisms of action. There is an immense need for full, length p53 structures from cancer cells. We have overcome limitations to traditional cryo-electron microscopy (EM) techniques by adapting material science and biochemical knowledge to resolve the first full-length p53 monomer and dimer. First, we extracted and enriched p53 oligomers from a glioblastoma cells (U87MG) to preserve disease-related modifications. Second, we have replaced conventional copper grids with silicon nitride-based microprocessor chips (microchips) which are known for their pristine flat surfaces and reliable ability to perform well under extreme conditions. Microchips were functionalized with an added layer of nickel-NTA to increase capture of p53 in appropriate ice thickness. Finally, we have adapted new flexible model fitting techniques with ISOLDE and PHENIX software packages to create biologically *and* energetically relevant ribbon structures to verify our reconstructions. Our full length model of p53 monomers helped inform the dimer reconstruction (~4.2 Å). Interestingly, there was no density available that would correspond to DNA leading us to believe that we had resolved an "inactive state" dimer. By observing the interface of inactive p53 dimer and comparing it to an active or DNA-bound dimer, we were able to identify the residues that could mediate the protein-protein interaction. There are potential residues that may be ubiquitinated to block MDM2 binding, a well-known regulatory partner, to sequester p53 in the cell or acetylated to initiate apoptotic pathways. Further, several "hot spot" mutations fall within or are adjacent to this interface region, which may be a key therapeutic target in the future. With these insights, we were able to reconstruct a possible model to explain the conformational changes p53 dimers follow as they transition from an inactive to an active DNA-binding state. This wealth of information can help aid in deciphering several mysteries behind p53. We have allowed previously incomplete models to guide diagnosis and treatments, which has allowed the emergence of competing theories behind a mechanism of action. By starting to pinpoint real mechanisms of action of p53, we can help shape the future of oncological research.

B288/P1970

Identifying Golgi glycosyltransferase *B4GALT1* as a p53-responsive gene in lung cancer

S. Bui, D. Stark, A. Houmani, Y. Wang; Molecular Cell and Developmental Biology, University of Michigan, Ann Arbor, MI.

Aberrant glycosylation, a well-recognized cancer hallmark, plays an essential role in tumor initiation and progression via cell adhesion, cell-cell recognition, intracellular signaling, and ECM interactions. Although cancer-associated glycan antigens have been identified across cancer glycomes, a group of antigens was recently reported as a result of incomplete galactose-capping on GlcNAc residues. Galactosylation is performed by a set of β-1,4-galactosyltransferases (B4GALTs), with B4GALT1 as the major isoenzyme for this reaction. However, the potential regulation network of the B4GALT1 and its significance in cancer remains to be determined. Expression of B4GALT1 in lung cancer was identified in Human Protein Atlas and Oncomine. Survival analyses were performed with Kaplan-Meier plotter. Human lung cancer cell lines of varying tumor protein p53 (p53) statuses were treated with cisplatin and cycloheximide then characterized through qPCR and Western blot. Lectin-binding was used to detect cell surface galactosylation using high resolution microscopy. Functional assays to assess migration and cell adhesion/spreading were conducted in WT & p53 knock-out A549. After bioinformatic analysis of putative p53-binding domains in the B4GALT1 promoter, these regions were cloned into a Luciferase reporter and tested. Bioinformatic analysis identified a significant correlation with reduced expression of B4GALT1 and p53; interestingly, this was associated with poor prognosis in lung cancer patients.

Depleting p53 markedly reduced B4GALT1 at both the mRNA and protein levels. Meanwhile, pharmacological induction of p53 stimulates expression of the Golgi enzyme, and augments the level of β1,4-galactosylation. Systematic screening of 13 alternative promoters in the BGALT1 locus and the canonical promoter upstream of the transcription start site, revealed four possible p53-consensus binding sites. Furthermore, p53-mediated down-regulation of B4GALT1 reduced galactosylation levels of intracellular and cell surface proteins, and consequently decreased cell adhesion/spreading and enhanced cell migration. Restoring B4GALT1 in p53-deficient cells phenocopied p53 wildtype cells in functional assays. The positive correlation between p53 and B4GALT1 was confirmed in 10 different cancer cell lines, suggesting a clinically relevant role for this protein in tumorigenesis. Thus, our study reveals B4GALT1 is a target of p53 and provides a genetic and biochemical mechanism leading to glycosylation aberrations potentially related to carcinogenesis. Together these data suggests an exciting avenue for developing new therapeutic strategies in cancers with p53-deficiency which may leverage cell surface glycosylation and specifically use B4GALT1 as a biomarker.

B289/P1971

Cell cycle arrest mediated by endoplasmic reticulum-resident transcription factor OASIS suppresses glioblastoma development.

A. Saito, Y. Kamikawa, T. Ito, K. Imaizumi; Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima City, Japan

Cells survey the internal and external conditions to determine to progress or stop cell cycle for the maintenance of the DNA accuracy. DNA damages caused by the various insults such as double strand break and incomplete replication induce cell cycle arrest and its repair. Old Astrocyte Specifically Induced Substance (OASIS) is an endoplasmic reticulum (ER)-resident transmembrane transcription factor. OASIS is cleaved in response to ER stress by regulated intramembrane proteolysis (RIP), subsequently generating the N-terminal fragments containing a basic leucine zipper (bZIP)-type DNA binding domain, which act as a transcription factor. Our previous studies have demonstrated that OASIS is preferentially expressed in specific cells including astrocytes and osteoblasts to regulate their differentiations. Additionally, OASIS is upregulated in long-term-cultured astrocytes undergoing cell cycle arrest due to loss of DNA integrity by repeated replication. However, the roles of OASIS in cell cycle have not been explored. We found that OASIS arrests cell cycle of astrocytes at G2/M phase after DNA damage via direct induction of p21. Although p21 is known as a direct target of p53, the cell cycle arrest by OASIS-21 axis does not have any crosstalk with p53-p21 signaling pathway. In a brain injury model, *Oasis*-/- reactive astrocytes surrounding the lesion core showed the sustained growth and the inhibited cell cycle arrest, resulting in prolonged gliosis. Intriguingly, a definite number of glioma and glioblastoma patients was included in the category featuring quite low expression level of OASIS due to DNA methylation in its promoter. The several number of glioblastoma cell lines also showed the low expression level of OASIS accompanied by hypermethylation of its promoter. Specific removal of this hypermethylation in glioblastoma transplanted to nude mice by epigenomic engineering suppressed its tumorigenesis. Taken together, OASIS is novel cell cycle inhibitor of astrocytes, and has potential to act as tumor suppressor for glioblastoma. The discovery of key molecule linking with the glioblastoma suppression contributes to elucidate the precise mechanisms for the glioblastoma development, that may lead to the novel therapeutic approaches targeting OASIS.

B290/P1972

Penta-O-galloyl-β-D-glucose (PGG) targets aggressive androgen independent prostate cancer by ROR1 inhibition

V. Sivaganesh, A. Iskander, T. Ta, B. Peethambaran; Saint Joseph's University, Philadelphia, PA

Late stage prostate cancer is a debilitating form of cancer due to its ability to progress aggressively despite androgen deprivation therapy (ADT). Though prostate cancer initially responds to ADT, late stage prostate cancer can survive by activating a myriad of oncogenic signaling cascades that are independent of the androgen signaling axis. Therefore, we must continue to explore prostate cancer associated markers that have potential for targeted therapy, such as the Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1). Furthermore, plant-derived compounds may serve as an abundant resource for targeted therapeutics. In this study, we explore the mechanism of ROR1 inhibition by a plant-derived gallotannin Penta-O-galloyl-β-D-glucose (PGG), which mitigates oncogenic signaling cascades and suppresses aggressive prostate cancer phenotypes. Results suggest that ROR1 was highly expressed in the androgen receptor negative, androgen-independent (ARneg-AI) PC3 cell line. Hence, PGG exhibited greater cytotoxic and apoptotic activity in PC3 (IC50 of 31.64 μM) in comparison to normal prostate epithelium RWPE-1 (IC50 of 74.55 μM). PGG inhibited ROR1 and downstream oncogenic signaling, which was corroborated by diminished migration and invasion of PC3 cells upon PGG treatment. PGG was also able to suppress migration and invasion of ARneg-AI DU145, which exhibited moderate levels of ROR1 in comparison to PC3. Throughout the study, PGG minimally affected RWPE-1. These results suggest that PGG, via the inhibition of ROR1, enables the selective targeting of aggressive ROR1-expressing prostate cancers. Furthermore, this study provides insight into how ROR1 drives oncogenesis in ARneg-AI prostate cancer.

B291/P1973

Reassessing cobalamin requirements for cell culture

D. J. Elzi, R. Jacob, V. I. Rebel; bioAffinity Technologies, San Antonio, TX

Cobalamin (Vitamin B12) is an essential co-factor for the synthesis of components required for cell growth and maintenance. Cobalamin is important for one-carbon metabolism which is involved with genome methylation and nucleotide synthesis. Cancer cells have increased metabolism, and we hypothesized that their proliferation would be negatively affected if grown in cobalamin-deficient medium. To test this, we grew cells in defined serum-free medium (SFM) with or without cobalamin. The cobalamin concentration used in these cultures was 1.65×10^{-11} M, similar to that contained in a 10% FBS solution. The serum-free media was based on either DMEM (DMEM-SFM) or MEM (MEM-SFM), to which defined growth factors, amino acids, and lipids were added. Neither DMEM-SFM or MEM-SFM had detectable cobalamin as measured by ELISA, which had a detection limit of 5.38×10^{-11} M cobalamin. Reagents required to propagate cells other than the SFM all tested negative for cobalamin by the same ELSA. We adapted cancer cell lines from lung (H460), breast (MDA-MB-231), prostate (PC3, DU145), and brain (U251) tumors to DMEM-SFM, and two Burkitt lymphoma cell lines (NAMALWA and RAJI) to MEM-SFM. We found that except for PC3 and the Burkitt lymphoma cell lines, all other cell lines required an extracellular matrix when cultured under serum-free conditions. We were unable to identify a cobalamin-free source of extracellular matrix (they contained at least 7.37×10^{-11} M cobalamin) and focused therefore on those cells which did not require it. PC3 cells grew similarly in DMEM-SFM with or without cobalamin, as measured by cell confluency. Nine-week growth curves of NAMALWA cells, as

calculated by population doubling, were also similar in both media, while RAJI cells formed tight clusters in both media which we were unfortunately unable to reliably quantify. These findings suggest that cancer cells may require far less cobalamin (10^{-11} M or less) for cell growth than currently recommended (10^{-9} M or higher). Studies aimed at understanding the physiological role of cobalamin in cellular functions should therefore reconsider the routine usage of cobalamin supplementation if cell cultures are used.

B292/P1974

Mad1 upregulation in breast cancer: causes and consequences

S. E. Copeland, J. Wan, A. Roopra, B. A. Weaver; UW-Madison, Madison, WI

Mitotic arrest deficient-1 (Mad1), an essential component of the mitotic checkpoint, is upregulated in 20% of breast cancer patients. Upregulation of Mad1 causes destabilization of the p53 tumor suppressor as well as chromosome missegregation during mitosis. Patients with high levels of Mad1 mRNA expression have a poorer prognosis than patients with intermediate or low levels of Mad1, and Mad1 upregulation is sufficient for transformation and promotion of orthotopic tumor growth in immunocompromised animals. Unlike many kinetochore proteins, Mad1 is not transcriptionally regulated by the transcription factor FoxM1. Thus, the mechanism for upregulating Mad1 in cancer, as well as the functional consequences in the context of an intact immune system, remain unknown. Bioinformatics analysis identified Histone Deacetylase 1 (HDAC1) as a likely cofactor involved in Mad1 transcriptional regulation. Consistent with this, HDAC inhibition with Trichostatin A (TSA) or Valproic Acid (VPA) increases Mad1 mRNA and protein levels 5- to 10-fold in multiple breast cancer cell lines. HDAC inhibition also induces Mad1 nuclear puncta, a localization pattern seen in primary breast cancer and breast cancer cell lines following Mad1 upregulation. To identify which transcription factor targets HDAC1 to the Mad1 promoter, we developed a Mad1 promoter reporter assay, which has identified a repressive region of the Mad1 promoter. Ongoing work will identify the transcription factor(s) responsible for negative regulation of Mad1. To determine the consequences of Mad1 upregulation, we have generated a tetracycline (tet)-inducible Mad1 mouse model by inserting a tet responsive promoter and HA tag before the first coding exon of the Mad1 gene. Mice containing one allele of tet-inducible Mad1 and two alleles of a ubiquitously expressed reverse tet-transactivator (rtTA-M2) show inducible expression of HA-Mad1 following one week or one month of exposure to the tet analog doxycycline. HA-Mad1 expression results in decreased p53 levels and increased mitotic defects in colon. We are currently testing whether Mad1 upregulation promotes colon tumor initiation or progression in the context of inflammation. To determine whether Mad1 upregulation plays a causal role in breast cancer, we are generating HA-Mad1 mice that express the tet-transactivator under control of a mammary-specific promoter (MMTV-tTA). Ongoing experiments will determine whether upregulation of Mad1 is sufficient to induce tumorigenesis in the presence of an intact immune system.

B293/P1975

Investigating the chromatin architecture of telomeres in high-risk neuroblastoma

C. Gilmore; St. Jude Children's Research Hospital, Memphis, TN

Neuroblastoma is a devastating pediatric solid tumor affecting about 800 children in the US each year. It has been established that risk-stratification of neuroblastoma can be distinguished based on telomere maintenance mechanisms. Typically, telomeres shorten with every cell division until they reach a

threshold length that causes a cell to undergo p53-mediated senescence. Cancer cells, including those in high-risk neuroblastoma, bypass senescence by lengthening their telomeres to enable replicative immortality. There are two known ways in which cancer cells can lengthen their telomeres: reactivation of telomerase, which is transcriptionally silenced in somatic cells, or through a homologous recombination-based method, alternative lengthening of telomeres (ALT). Genetic aberrations in two genes associated with telomere maintenance mechanisms are indicators of high-risk disease in neuroblastoma patients, and interestingly these two mutations are never seen in the same tumor. Mutations in the chromatin remodeling protein ATRX are associated with ALT while *MYCN* amplification, the number one indicator of high-risk disease, is associated with the upregulation of telomerase. However, the role of *ATRX* mutations in ALT remains poorly understood. We hypothesize that ALT+ tumors have an altered chromatin architecture at telomeres that allow for a cell to be permissive to homologous recombination at telomeres. To investigate the chromatin architecture at individual telomeres, we have used lattice lightsheet microscopy to determine the association of individual telomeres with chromatin marks HP1 and H3.3. Further, we have used correlative light electron microscopy to determine the compaction of chromatin at telomeres in the high resolution to determine if telomeres in ALT+ versus telomerase+ cells localize differently to euchromatin or heterochromatin. These methods will provide individual telomere resolution of chromatin architecture, which has been highly debated in normal cells and cells using telomerase or ALT. ALT is extremely heterogenous, varying drastically from cell-to-cell and telomere-to-telomere and thus the ability to interrogate the nuclear organization of telomeres at the single telomere level will provide insight not achievable with most standard methods. Telomere maintenance is an important yet not fully understood aspect of tumor biology and clearly plays a role in high-risk neuroblastomas. These mechanisms could be a target for therapeutics because of their unique and essential role in cancer cells. These studies will ultimately help address why certain cells undergo one telomere maintenance mechanism over the other and help elucidate the role of mutations in chromatin remodeling protein ATRX in high-risk ALT+ tumors.

Epigenetics and Chromatin Remodeling

B295/P1976

Examining roles for the Mi-2 chromatin re-modeler in cell cycle exit

E. Buchert, Y. Ma, L. Buttitta; University of Michigan, Ann Arbor, MI.

Precise coordination of the cell cycle and differentiation is crucial to proper organ development. Upon terminal differentiation, many cells will exit from the cell cycle altogether and transition to a postmitotic state. We have investigated how cell cycle genes are robustly silenced at cell cycle exit using *Drosophila melanogaster* and have uncovered changes in chromatin accessibility at enhancers of specific cell cycle genes that are rate-limiting for cell cycle progression. Importantly, we find that the closing of these enhancers is developmentally controlled and not influenced directly by the cell cycle, suggesting the differentiation process acts in part through chromatin remodeling to coordinate cell cycle exit with terminal differentiation. We hypothesize that closing of chromatin at rate-limiting cell cycle genes contributes to the robustness of cell cycle exit in terminally differentiated cells. To identify the remodelers involved, we performed a genetic screen and identified Mi-2, a member of the NuRD complex, as a candidate involved in chromatin closing at cell cycle exit. To investigate the localization of Mi-2, we developed a modified CUT&RUN protocol, that works on terminally differentiated *Drosophila* tissues, which are often encased in cuticle. We have confirmed that this protocol can also be used with

ATAC-seq, to examine chromatin accessibility changes in cuticle-bound tissues. In addition, we have validated a functional, endogenously tagged Mi-2 that we will use to study Mi-2 localization on the genome during terminal differentiation. These custom tools and protocols provide us with the opportunity to study how Mi-2 function affects chromatin accessibility during terminal differentiation and determine where Mi-2 is binding in the genome at cell cycle exit.

B296/P1977

Regulation of the chromatin landscape and gene expression through the microbiota

L. A. Gates[1], B. S. Reis[2], P. J. Lund[3], M. Leboeuf[1], M. Paul[4], T. S. Carroll[4], B. A. Garcia[3], D. Mucida[2], C. Allis[1]; [1]Laboratory of Chromatin Biology & Epigenetics, The Rockefeller University, New York, NY, [2]Laboratory of Mucosal Immunology, The Rockefeller University, New York, NY, [3]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO, [4]Bioinformatics Resource Center, The Rockefeller University, New York, NY.

While the microbiota has key roles in physiology and human health, understanding the mechanistic basis of how the microbiota regulates cellular processes in the context of a complex tissue system remains a major challenge. The commensal microbiota in the mammalian intestine generates diverse metabolites that act on neighboring host cells and impact physiology. One such class of microbial metabolites are short chain fatty acids (SCFAs), which accumulate to high concentrations in the intestinal lumen through the fermentation of dietary fiber. SCFAs impact signaling, cellular metabolism, and the chromatin landscape. One way that SCFAs impact chromatin are through being deposited onto histones as chemical modifications, called histone acyl marks. Certain histone acyl marks have been reported to positively regulate gene expression, including the well-studied histone acetylation. However, the functional roles of other less-characterized histone acyl marks and especially their physiological roles *in vivo* are largely unknown. We hypothesized that SCFAs can be written onto chromatin as histone acyl marks in the intestine, which then function in mediating key transcriptional programs in response to environmental changes. Using the mouse intestinal tract as a model, we demonstrate that select acyl modifications on histones are dependent on the presence of microbes, which occurs in a tissue and cell-specific manner. We have furthermore characterized several specific acyl marks that were previously unstudied and found that they are associated with active gene regulatory elements in primary intestinal epithelial cells. These histone acyl marks are regulated by the presence of specific microbial metabolites, which induce the expression of select metabolic gene programs. Together, these studies demonstrate that the microbiota, microbial metabolites, and chromatin are coupled in the intestine. Our studies have thus explored the fate of SCFAs that are deposited onto chromatin and revealed roles of understudied histone modifications. In addition, these findings will help elucidate mechanistic roles of microbial metabolites and their function in gene regulation.

B297/P1978

Cell cycle defects in zebrafish hepatocytes with epigenetic damage due to *uhrf1* loss

B. Madakashira Pranesh, K. C. Sadler; Biology, New York University Abu Dhabi, Abu Dhabi, UNITED ARAB EMIRATES.

Cancer is characterized by aberrant changes to the epigenetic landscape, with the global loss of DNA methylation as an early feature in most types of cancer. We discovered that the ubiquitin like,

containing PHD and RING Finger Domains 1 (Uhrf1), a key component of the DNA maintenance methylation machinery that promotes heterochromatin, is an oncogene when over expressed and its absence causes cell cycle arrest and cell death in zebrafish livers. Mutation of *uhrf1* or its partner, DNA methyl transferase 1 (*dnmt1*), have global DNA hypomethylation and display a similar phenotype characterized by failure of hepatic outgrowth. Surprisingly, *uhrf1* mutant livers display a unique cell cycle arrest characterized by prolonged DNA replication and hepatocyte cell death whereas hepatocytes in *dnmt1* mutants withdraw from the cell cycle. Uhrf1 directs Dnmt1 for degradation and therefore Dnmt1 is elevated in *uhrf1* mutants. *dnmt1* knockdown in *uhrf1* mutants rescued the DNA replication defect, suggesting that the *uhrf1* mutant phenotype could be partly attributed to elevated *dnmt1* levels. *uhrf1* loss in the liver also disrupts normal hepatocyte nuclear morphology and nuclear rim organization. We found that nearly all *uhrf1* deficient hepatocytes were devoid of heterochromatin marked by DAPI staining, H3K9me3 and that lamin B2 staining was either missing or in the nucleoplasm. The fact that loss of *dnmt1* phenocopies nuclear rim phenotype (loss of lamin B2) in *uhrf1* mutant points to DNA methylation directing this effect. Blocking S-phase entry with the cdk 4/6 inhibitor and chemotherapeutic, Palbociclib, rescued the liver size, cell cycle defect, cell death and also nuclear morphology significantly in *uhrf1* mutants. These studies show that DNA methylation is essential for genome organization, dictating hepatocyte nuclear shape and morphology. In contrast, *uhrf1* and *dnmt1* function uniquely during cell cycle, with *uhrf1* playing an active role in S-phase timing and cell cycle progression, along with keeping Dnmt1 levels in check. Taken together, these results indicate a novel cell cycle promoting function of *uhrf1* during liver development that could be largely independent of its role in DNA methylation.

B298/P1979

Exploiting epigenetic sensitivities of cells lacking the retinoblastoma tumor suppressor pRB

G. Zamalloa[1], M. Pruitt[1], N. Hermance[1], H. Gali[2], R. Flynn[2], A. Manning[1]; [1]Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA, [2]Boston University, Boston, MA.

The retinoblastoma tumor suppressor (pRB) has multifaceted roles in the regulation of gene expression, chromatin structure, and genome integrity. Prior work from our group and others have demonstrated that loss of pRB activity leaves cells sensitive to replication stress and the acquisition of replication-dependent DNA damage. To test whether these features of pRB deficient cells may represent an exploitable weakness, we performed an imaging-based screen to identify epigenetic regulators that, when inhibited, cooperate with depletion of pRB to promote DNA damage and compromise cell viability. From the 96 drugs tested, we found that inhibition of Poly ADP-Ribose Polymerase 1 (PARP1) differentially promotes DNA damage in pRB-deficient cells compared to isogeneic control cells. My data demonstrates that pRB-deficient cells treated with PARP inhibitors exhibit replication stress in the form of single-stranded DNA gaps, such that many cells fail to progress to mitosis, while those that do progress exhibit late-replicating regions that become susceptible to DNA damage upon mitotic onset. This sequence of assaults to genome integrity compromise continued cell proliferation and increased cell death. Importantly, we have recapitulated this sensitivity to PARP1 inhibition in RB1-null cancer cells, suggesting that PARP1 is a clinically relevant therapeutic target in pRB-deficient cancers.

B299/P1980

Bisphenol A Increases BRF2 Expression in Y79 Human Retinoblastoma Cells

A. O'Connor[1], W. Johnson[1,2], L. Schramm[1,3]; [1]Department of Biological Sciences, St Johns University, Queens, NY, [2]Department of Biology, University of Virginia, Charlottesville, VA, [3]Environmental Studies Program, St Johns University, Queens, NY.

Accurate RNA polymerase III transcription requires TFIIIB. Deregulation of TFIIIB activity occurs in various human cancers, including lung, breast, liver, prostate, and blood cancers. In general, oncogenes, tumor suppressors, and MAF1 regulate TFIIIB activity. TFIIIIB activity is regulated by polyphenols, including the flavonoids epigallocatechin gallate (EGCG) and daidzein, in the context of cervical and breast cancer, respectively. This study aims to determine if the polyphenol bisphenol A (BPA), classified as a xenoestrogenic environmental pollutant, regulates TFIIIB activity in vitro. We performed differential gene expression analyses of publicly available RNA sequencing datasets. TFIIB-related factor 2 (BRF2) expression is significantly increased in Y79 retinoblastoma cells treated with 40 mM BPA for 48 hours. MAF1 expression is significantly increased in two of three differential gene expression analyses performed. However, we did not observe consistent and significant changes in the expression of the TFIIIB subunits B-related factor 1 (BRF1) or B double prime 1 (BDP1). BPA has been classified as an endocrine disruptor and demonstrated to have both agonist and antagonist effects through nuclear steroid hormone receptors. Specifically, BPA has been demonstrated to bind to the androgen receptor (AR), estrogen receptor alpha (ESR1), and beta (ESR2) receptors. To determine if BPA regulates BRF2 and MAF1 expression through androgen or estrogen receptors, we analyzed TFIIIB and MAF1 promoters to identify putative androgen and estrogen binding sites. We identified putative AR, ESR1, and ESR2 binding sites in both BRF2 and MAF1 promoters. Interestingly, the BDP1 promoter has no putative ESR2 binding sites and the BRF1 promoter binding sites but does not contain AR binding sites. Our data suggest BPA may modulate BRF2 and MAF1 expression in BPA-treated Y79 cells through a combination of nuclear hormone receptor cross-talk.

B300/P1981

The Synthetic Opioid Fentanyl Negatively Regulates RNA Polymerase III Subunits POLR3A and POLR3B Expression in Hepatocarcinoma Cells

W. Johnson[1,2], A. O'Connor[1], L. Schramm[1,3]; [1]Department of Biological Sciences, St. John's University, Queens, NY, [2]Department of Biology, University of Virginia, Charlottesville, VA, [3]Environmental Studies Program, St. John's University, Queens, NY.

The increase in consumption and pharmaceutical availability of opioids, synthetic opioids, and fentanyl derivatives, has led to an increased rate of opioid-related dependencies and deaths within the United States spurring a national opioid epidemic. From 1999-2020, over 564,000 Americans have died from opioid overdoses. The current synthetic opioid fentanyl epidemic began in 2013. Opioids induce acute toxic leukoencephalopathy (ATL) and delayed post-hypoxic leukoencephalopathy (DPHL). Specific core RNA polymerase III subunits are deregulated in leukodystrophy and leukoencephalopathy. The aim of this study is to determine if the synthetic opioid fentanyl regulates core RNA polymerase III subunit expression *in vitro*. We performed transcriptomic differential gene expression analyses of publicly available RNA sequencing datasets of HepG2 cells exposed to 100 ng/ml fentanyl or untreated. Fentanyl treatment of HepG2 cells significantly, and specifically, decreased RNA polymerase III subunit A (POLR3A) and RNA polymerase III subunit B (POLR3B) expression. We observed no change in RNA

polymerase III subunit K (POLR3K), TFIIIB, and MAF1 expression in response to fentanyl. To identify a potential mechanism for the observed fentanyl-induced decrease in POLR3A and POLR3B expression, we analyzed select core RNA pol III subunit promoters for putative transcription factor binding sites previously demonstrated to be regulated by fentanyl. Neuronal Differentiation 1 (NEUROD1), a basic-loop-helix transcription factor, known to be regulated by opioids and has been implicated in morphine tolerance was of interest in this study. Strikingly, we identified four putative NEUROD1 binding sites in the POLR3A promoter and five NEUROD1 binding sites in the POLR3B promoter. No NEUROD1 binding sites were identified in the RNA polymerase III subunit H (POLR3H) promoter and two putative NEUROD1 binding sites in the POLR3K promoter. Neither POLR3H nor POLR3K gene expression was altered in response to fentanyl. We identified no neuronal differentiation 2 (NEUROD2) or neurogenin 2 (NEUROG2) binding sites downstream of the transcription start sites in POLR3A, POLR3B, POLR3H, or POLR3K promoters. Together, these data suggest that fentanyl regulates POLR3A and POLR3B expression in HepG2 cells, and the regulation may occur through the activity of NEUROD1.

B301/P1982

Rrp5 knockdown alleviates growth suppression in a *saccharomyces cerevisiae* ALS/FTD FUS model

S. Cobos[1,2], S. A. Bennett[1,2], E. Son[2], R. Segal[2], M. Kozlov[2], M. P. Torrente[3,2]; [1]The Graduate Center, New York, NY, [2]Brooklyn College, Brooklyn, NY, [3]The Graduate Center, New York City, NY.

Amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) comprise a neurodegenerative disease spectrum, affecting thousands of people around the world. Symptoms typically manifest after age 60, when breakdown of different neuronal types in distinct locations of the nervous system occurs. The exact mechanism behind ALS/FTD progression remains elusive. Mutations in the *fused in sarcoma* (*FUS*) gene lead to misfolding and aggregation of the FUS protein, a common hallmark of ALS/FTD pathology. As genetics alone fails to explain the role that FUS aggregates play in ALS/FTD pathology, we seek to explore the epigenetic channels linked to protein aggregation. A *Saccharomyces cerevisiae* FUS overexpression model revealed decreases in levels of H3K9ac, H3K14ac, and H3S10ph. Treatment with the HDAC inhibitor Trichostatin A led to improved cell viability and restoration of H3K9ac and H3K14ac levels in the same model. Here, we shed light on how the epigenetic landscape connects to FUS proteinopathy. Co-immunoprecipitation paired with tandem mass spectrometry revealed 39 putative FUS binding partners, including the RNA binding protein Rrp5. Rrp5 is a conserved rRNA biogenesis protein found in both humans and yeast that is essential for the generation of mature 18S rRNA. Here, we show that an Rrp5 knockdown strain is resistant to growth suppression elicited by FUS overexpression. These findings reveal mechanistic details tying FUS aggregation to the epigenome, expanding our knowledge of how FUS aggregates lead to toxicity in ALS/FTD.

B302/P1983

A High-Throughput RNA Imaging CRISPR Screen Identifies the Cohesin Complex as a Regulator of Interferon Stimulated Genes

L. Ozbun[1], A. Carcamo[1], L. Rinaldi[2], G. Fettweis[2], S. Kim[2], G. Hager[2], **G. Pegoraro**[1]; [1]High-Throughput Imaging Facility, NCI/NIH, National Cancer Institute/NIH, MD, [2]Hormone Action and Oncogenesis Unit, NCI/NIH, National Cancer Institute/NIH, MD.

Automated and multiplexed visualization of RNA species *in situ* can be harnessed as a novel class of assays for chemical or functional genomics screens to discover cellular processes regulating gene

expression programs. To this end, we miniaturized Hybridization Chain Reaction amplified RNA FISH (HCR RNA FISH) in a high-throughput format. We first optimized all the HCR-RNA FISH staining, imaging, and quantification steps using automated liquid handling, high-throughput microscopy, and high-content image analysis in a 384-well format. As a proof of principle for the applicability of high-throughput HCR-RNA FISH, we then used this technique to measure changes in the *IFIT3* mRNA levels in an arrayed CRISPR-Knock Out (KO) screen of 860 human epigenetics genes to systematically identify cellular pathways involved in the transcriptional response of Interferon Stimulated Genes (ISG) to interferon-γ. The screen and additional validation experiments revealed that knock-out of several components of the Cohesin complex, or of the Cohesin loading factors NIPBL and MAU2, leads to a substantial increase in the upregulation for several different ISG induced by interferon-γ. Knock-out of several subunits of the NuA4 acetylase complex, and of the Cyclin Kinase sub-Module (CKM) of Mediator had a similar effect. These results were further confirmed in qRT-PCR experiments, which also showed that knock-out of NIPBL leads to an increase the basal level of expression of ISGs in the absence of interferons. Interestingly, in these experimental conditions the upregulation of ISG does not seem to depend on the DNA Damage Response (DDR), nor on activation of the cGAS-STING-IRF3 axis. Finally, differential RNA-seq experiments in a cell line expressing a chemically degradable version of the Cohesin subunit RAD21 showed strong upregulation of a wide range of ISG 24 hrs post RAD21 depletion. Altogether, these results show the utility of high-throughput HCR RNA FISH for CRISPR functional genomics screens, identify multiple protein complexes whose depletion results in upregulation of ISG in the presence of interferon-γ, and point to a strong, yet not fully characterized, mechanistic role for the Cohesin complex in the regulation of this transcriptional program.

B303/P1984

Genomewide Methylation in Serum Exosomes of Pregnant Women Experiencing Preeclampsia

U. S. Shinde[1], T. Madan[2], D. Das[3], N. H. Balasinor[4]; [1]Neuroendocrinology, ICMR- National Institute for Research in Reproductive Health, Mumbai, Mumbai, INDIA, [2]Innate Immunity, ICMR- National institute for research in reproductive health, Mumbai, Mumbai, INDIA, [3]Stem Cell Biology, ICMR- National institute for research in reproductive health, Mumbai, Mumbai, INDIA, [4]Neuroendocrinology, ICMR-National institute for research in reproductive health, Mumbai, Mumbai, INDIA.

Preeclampsia (PE) is a pregnancy-related disorder, characterized by new onset hypertension with multi-system involvement occurring after the 20th week of gestation. One of the factors causing PE is defective placental development. Currently, available serum biomarkers are unable to detect PE in the early stages. Exosomes are extracellular vesicles secreted by the trophoblast during pregnancy by the 6th week of gestation. Exosomal concentrations are altered in placental disorders. Due to the inaccessibility of placental tissue, exosomal factors could act as a better predictor of PE. Several studies have reported preeclampsia-specific DNA methylation changes in the placenta. Early epigenetic changes during pregnancy can have an impact on trophoblast function, contributing to PE as evidenced by the strong association between altered gene expression and methylation in pre-term PE placentas and first trimester trophoblasts. The objective of this study was to analyze the serum exosomal DNA methylome signatures by Whole genome bisulfide sequencing (WGBS) from 3rd-trimester pregnant women experiencing early preeclampsia (EOPE) and normative pregnant women. Serum was collected from 3rd-trimester control and EOPE women. Exosomes were isolated and the characterization of exosomes was done using Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA), and expression of exosomal protein markers like CD63, CD81, and TSG101 by western blotting. The results of

TEM and NTA showed that exosomes were in the desired size range (50-150nm). DNA was extracted from serum exosomes and pooled exosomal DNA (N=5) samples were subjected to WGBS sequencing. With the cut-off threshold set at <5% methylation difference, we obtained a total of 355 differentially methylated CpGs (DMCs), of which, the majority were of mitochondrial origin. A growing body of research has reported that oxidative stress, decreased trophoblast differentiation, and invasion, both of which have been linked to preeclampsia pathogenesis, are related to mitochondrial dysfunction. Therefore, DMCs of the mitochondrial genes like MT-TL1, MT-CO2, MT-ND2, MT-RNR2, MT-CYB, MT-TF, MT-ND6, MT-CO3, MT-ATP8, MT-NDL4, MT-ND5 were selected for validation by pyrosequencing in the study population. Overall, this study suggests that the DNA methylome of exosomes in the serum of women with preeclampsia could be further investigated for potential diagnostic biomarkers.

B304/P1985

Integrated epigenetic and transcriptome analysis in multiple sclerosis

Q. Ma, S. J. Caillier, S. Muzic, M. R. Wilson, R. G. Henry, B. A. C. Cree, S. L. Hauser, A. Didonna, J. R. Oksenberg; UCSF, San Francisco, CA.

The most accepted model of multiple sclerosis (MS) pathogenesis involves multi-layered interactions between complex genetic and environmental influences. Genome-wide association studies have identified 233 genome-wide significant loci associated with susceptibility, collectively highlighting the importance of adaptive and innate immune responses in driving risk. Despite this progress, the current MS genetic map only partially accounts for the estimated disease heritability. Like other complex genetic traits, epigenetic effects are a reasonable explanation for the missing heritability in MS. Epigenetic modifications can regulate gene expression in a heritable fashion without altering the DNA sequence. Moreover, environmental signals may influence epigenetic modifications contributing to the phenotype, possibly linking environmental risk factors to genetic susceptibility. Bisulfite sequencing (BS-seq) remains the gold standard method to profile genome-wide DNA methylation at a single nucleotide resolution. To gain additional insights into immune cell-type specific epigenetic regulations in MS, we applied BS-seq to conduct a genome-wide analysis of DNA methylation patterns in CD4+ and CD8+ T cells, CD14+ monocytes, and CD19+ B cells isolated from 29 untreated, recently diagnosed MS patients and 24 healthy controls. We show that CD19+ B cells from new-onset untreated cases display more significant methylation changes, primarily DNA hypomethylation, compared to the other cell types. Altogether, 4,933 MS-associated differentially methylated regions were identified in B cells. This epigenetic signature underlies specific genetic programs involved in B cell differentiation and activation. Integration of the methylome with changes in gene expression and susceptibility-associated loci further confirms that hypomethylated regions are linked to the upregulation of cell activation transcriptional programs. Potential therapeutic targets, including SLAMF7, were identified. We further apply a trimodal single-cell assay, named TEA-seq that simultaneously measures gene expression, epigenetic modification, and cell surface markers on paired cerebrospinal fluid and blood mononuclear cells, with the goal of generating a multi-cellular, multi-omics atlas associated with MS. Altogether, our results pinpoint the role of aberrant DNA methylation in connecting defects in the periphery with central nervous system autoimmunity and corroborates the key role of B cells in the initial stages of MS.

B305/P1986

C9orf72 Dipeptide Repeat Proteinopathy in Yeast is Accompanied by Alterations in Histone H3 and H4 Post-Translational Modifications

C. Janani[1,2], S. N. Cobos[1,3], A. Grebe[4,5], M. P. Torrente[1,6]; [1]Department of Chemistry, Brooklyn College, Brooklyn, NY, [2]Scholars Program, Brooklyn College, Brooklyn, NY, [3]PhD. Program in Chemistry, CUNY Graduate Center, New York, NY, [4]PhD. Program in Biochemistry, CUNY Graduate Center, New York, NY, [5]Department of Biology, City College of New York, New York, NY, [6]PhD. Programs in Chemistry, Biochemistry, and Biology, CUNY Graduate Center, New York, NY.

Amyotrophic Lateral Sclerosis (ALS) is an incurable neurodegenerative disorder characterized by progressive paralysis. While most cases have no known genetic component, the most common genetic cause for the disease is an accumulation of hexanucleotide repeat expansions in the C9orf72 gene which lead to the production of dipeptide repeat proteins. Among these, a proline and arginine (PR) repeat protein leads to cytotoxicity. To better understand how these dipeptide repeat proteins interact with the epigenome, specifically histone post-translational modifications (PTMs), we characterized histone PTM levels on various lysine residues on Histone H3 and H4 in the context of a yeast PR_{50} overexpression model. We find significant increases in the levels of H3K9ac, H3K14ac and H3K27ac, as well as an increase in the levels of H4K16ac. Acetylation levels on other sites remain unaffected. In addition, we found significant increases in the levels of H3K4me1, H3K4me2, H3K36me2, H3K36me3 and H3K79me3. We hypothesize that increased activity of the histone acetyltransferase GCN5 is responsible for enhanced acetylation of H3K9ac, H3K14ac and H3K27ac. Furthermore, the increase in H3K4me1 may be impacting GCN5 acetylation site preferences. Overall, these changes show that PR_{50} proteinopathy in yeast is connected to the epigenome. A better understanding of the epigenetic mechanisms at play in ALS may illuminate potential therapeutic targets for ALS and other neurodegenerative diseases.

B306/P1987

Discovering mechanisms regulating centromeric boundary maintenance and genome stability

P. Sidhwani, A. Straight; Stanford University, Stanford, CA.

As cells divide, they must precisely distribute their chromosomes to daughter cells for development to proceed normally. This process, known as “chromosome segregation,” is facilitated by specialized regions of the chromosome: the core centromere, which serves as a point of attachment of the chromosome to the mitotic spindle, and the pericentromeres, which flank the core centromere to facilitate proper sister chromatid cohesion and alignment in metaphase. In addition to being functionally distinct, the core- and peri-centromeric regions are also structurally distinct. The core-centromere contains nucleosomes with the histone H3 variant CENP-A whereas the pericentromere is highly condensed by the constitutive heterochromatic mark H3K9me3. In fission yeast, tRNA transcription maintains the boundary between these regions, preventing heterochromatin spread into the core centromere. However, the mechanisms regulating centromeric boundaries in humans remain largely unknown. In our work, we describe a novel assay to discover regulators of centromeric boundary maintenance in humans using CRISPR screening. Our studies promise to provide new insights into the regulation of constitutive heterochromatin and boundary maintenance in humans, with broader implications in identifying genetic risk factors for diseases like cancer.

B307/P1988

TDP-43 represses cryptic exons in transcription machinery to regulate DNA demethylation at neuronal enhancers

S. Hill[1], S. Seddighi[1], Y. Qi[1], W. Wu[1], D. Ramos[1], J. Reyes[1], D. Wang[1], E. Callen[1], C. Esnault[1], R. Dale[1], S. Coon[1], S. Coon[1], M. Prudencio[2], M. Prudencio[2], L. Petrucelli[2], J. Lippincott-Schwartz[3], A. Nussenzweig[1], M. Ward[1]; [1]National Institutes of Health, Bethesda, MD, [2]Mayo Clinic, Jacksonville, FL, [3]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA

Epigenetic features, such as DNA methylation, are regulated to control chromatin structure and gene expression. In neurons, altered DNA methylation has been linked to age-related neurodegenerative diseases including Alzheimer's disease and related dementias (ADRD), yet the relationship between DNA methylation and neurodegeneration remains unclear. Unlike dividing cells that can passively demethylate DNA during replication, neurons use active DNA demethylation, which involves excision of methylated cytosines and DNA repair. We recently developed a new method, Synthesis-Associated Repair sequencing (SAR-seq), to capture and sequence sites of recurrent DNA synthesis or repair. Using this method, we discovered ~30,000 sites of DNA synthesis across the neuronal genome at enhancers, likely playing key roles regulating active DNA demethylation. To investigate the relationship between methylation and neurodegenerative disease-associated proteins, we used human induced pluripotent stem cell-derived neurons (i^3Neurons) and CRISPR interference (CRISPRi) to knock down TDP-43, a hallmark of ALS, FTD and AD. In TDP43-knockdown (KD) i^3Neurons, we observed substantial reduction of SAR-seq intensity. TDP-43 is an RNA-binding protein and splicing repressor that prevents intronic sequences, called cryptic exons, from being included into mature RNA transcripts. We performed RNA-seq in TDP-43 KD i^3Neurons and found 100s of destabilized transcripts with cryptic exons. We determined that two of the destabilized genes are also essential for neuron survival and belong to the TFIIH complex, which regulates transcription initiation. We found that TFIIH components localize to SAR-seq sites and TFIIH inhibition reduces SAR-seq intensity, suggesting that TFIIH acts upstream of active demethylation. Furthermore, we found that TDP-43 KD i^3Neurons display reduced transcription, and that rescue of TFIIH components in these neurons restored transcriptional activity. These data are consistent with a model where TDP-43 splices TFIIH components, which are required for transcription, which then acts upstream of active DNA demethylation, and suggest a novel mechanism through which TDP-43 may regulate transcription and DNA demethylation.

B308/P1989

Formation and unique chromatin architecture of biomolecular condensates by the YAP1-binding transcription factor TEAD1 in renal cell carcinomas

J. Demmerle, B. He, D. Cai; Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

The chromatin adaptor protein YAP1 is an important regulator of transcriptional activation and chromatin architecture in response to Hippo pathway signaling. YAP1 binds TEAD-family transcription factors and forms phase-separated biomolecular condensates, specifically transcriptional hubs, in response to hyperosmolarity and other cellular and environmental cues. In Renal Cell Carcinoma (RCC), a particularly lethal form of kidney cancer with poor treatment options, YAP1 and its transcription factor partner TEAD1 are often up-regulated and indicate poor prognosis. We have found that in certain patient-derived RCC cell lines with high levels of YAP1 and TEAD1 expression, TEAD1 forms atypical large nuclear foci, distinct from normal kidney cells and other subtypes of RCC. These few large TEAD1 foci are

co-occupant with the repressive histone mark H3K9me3 and are generally located near the nuclear periphery or nucleolus. This is in contrast to TEAD1 patterns both in normal cells and within other regions of the same nuclei, where TEAD1 is distributed into many sub-resolution foci and associates with markers of active chromatin. As YAP1-TEAD complexes are important mediators of chromatin architecture and genome organization, these findings provide insight into how those mechanisms can be hijacked in cancer. We also investigate the roles of small-molecule YAP1-TEAD inhibitors as tools to manipulate chromatin landscapes. We have used a combination of high-resolution microscopy, ChIP-seq, and biochemical methods to identify the unique chromatin architecture of these cancer-associated large TEAD1 foci, and propose that they represent a novel subtype of biomolecular condensate where normally activating transcription factors are recruited into repressive heterochromatin.

B309/P1990

Comparison of the transcriptional and epigenetic landscape in CDK4/6 inhibitor and doxorubicin mediated therapy induced senescence

J. Yeung[1], J. Rendleman[1], N. Pagane[2], I. Duba[1], A. Scortea[1], L. Anderson[1], R. Hosuru[3], M. Klein[4], A. Koff[4], V. Risca[1]; [1]Rockefeller University, New York, NY, [2]Massachusetts Institute of Technology, Cambridge, MA, [3]University of Pittsburgh, Pittsburgh, PA, [4]Memorial Sloan Kettering Cancer Center, New York, NY

Liposarcomas are rare cancers that often undergo amplification of the 12q13-15 locus, which encodes for cyclin-dependent kinase CDK4; CDK4/6 inhibitors (CDK4/6i) therefore offer a promising targeted therapy for these tumors, in which therapeutic and surgical options are scarce. CDK4/6i can lead to either reversible or irreversible exit from the cell cycle (quiescence or senescence, respectively), but the underlying mechanisms are not well understood, as most types of senescence are thought to involve some DNA damage signaling as the trigger. We used ATAC-seq and RNA-seq in a liposarcoma cell line model to determine the shared and distinct transcriptional and chromatin regulatory signatures associated with cell cycle arrest and senescence caused by palbociclib, a CDK4/6i and doxorubicin, an inhibitor of topoisomerase II that induces cell death or senescence through DNA damage. ATAC-seq analysis shows dynamic patterns of accessibility as cells transition from quiescence to senescence over 28 days of CDK4/6i treatment. NF-kB motifs in promoter-proximal regions displayed increased accessibility as cells commit to the senescence state. Furthermore, we observe differences in accessibility and gene expression between CDK4/6i and doxorubicin induced senescence around chr6 q21-22 locus that contains the genes, *FRK and FYN*. These genes encode Src tyrosine kinases which negatively regulate cell proliferation and has been implicated as a tumor suppressor through upregulation of p21, an endogenous CDK4/6 inhibitor. Interestingly, FRK and FYN's expression levels are significantly higher in CDK4/6i than doxorubicin. Hierarchical clustering of RNA-seq data identify a shared cell cycle arrest signature in both types of therapies characterized by genes enriched in TNFA via NFKB signaling pathway. To further the changes in cellular inflammatory and immune responses, we compared senescence associated secretory phenotype (SASP) genes' expression between the two conditions. Highest-expressed SASP genes in doxorubicin-induced senescence take part in interleukin signaling, whereas CDK4/6i specific SASP genes are characterized by roles in extracellular matrix remodeling and regulation of insulin-like growth factor. These findings provide further evidence of shared and unique epigenetic and transcriptional changes in different types of therapy-induced senescence.

B310/P1991

Identification and characterization of conserved methylation-dependent/independent DNA regulatory elements across mammalian SLC9C1 genes

J. A. Abele, C. C. Gardner, T. Winkler, P. F. James, S. Anas, C. Reckers; Miami University, Oxford, OH.

Sodium Hydrogen Exchangers (NHEs) are an important family of proteins involved in regulating intracellular pH. In sperm, NHEs have been shown to be essential for cell motility and fertilization. The SLC9C1 gene, which encodes for the NHE10 protein and is exclusively expressed in the testis/sperm, has been shown to be critical for male fertility. SLC9C1 knockout male mice are infertile due to immotile sperm. Recently, an infertile human male with a frame-shift deletion in the SLC9C1 gene resulting in loss of exon 22 was identified. A separate study found that men with decreased sperm motility have significantly decreased levels of NHE10 protein in their sperm cells. Studies have also found that aberrant DNA methylation of promoter regions of genes involved in sperm motility are associated with low sperm motility. We decided to study if SLC9C1 gene expression is regulated by DNA methylation and if this is epigenetic mechanism is conserved across mammalian species. To do this we first performed bioinformatic analyses on mouse, rat, and human SLC9C1 and found that there is a conserved CpG Island (CpGI) just upstream of the promoter. Bisulfite sequencing analysis on DNA isolated from lung (a somatic tissue) and testis uncovered differential methylation of the CpGIs and the promoters between these tissues. In testis, SLC9C1 is hypomethylated, while in lung it is hypermethylated. The potential regulatory elements were then cloned into luciferase expression vectors and transfected into either HEK293 cells (a somatic cell line) or GC-1 SPG cells (a spermatogonial cell line) and dual luciferase assays were performed to test the DNA regulatory activity of the promoter and CpGI. Both the CpGIs and promoter regions exhibit promoter activity and these regulatory elements are methylation sensitive. In addition, the CpGI exerts regulatory effects on the native as well as exogenous promoters. Our work has shown that the SLC9C1 promoter and the conserved (with respect to relative location) CpGI upstream of the promoter perform similar regulatory functions across mammalian species and are methylation sensitive. This work lays the groundwork for future experiments to perform targeted DNA methylation or demethylation of the SLC9C1 gene to identify specific CpGs in these regions necessary for the methylation-sensitive regulatory activity.

B311/P1992

Functions of the chromatin regulator Set4 in highly regulated stress response gene expression programs

M. Negesse, Y. Jethmalani, M. Ramos, D. Park, E. Green; University of Maryland, Baltimore County, Baltimore, MD

Chromatin regulation is a dynamic process and an essential aspect of cell growth and survival. It further allows cells to adapt to changing environmental conditions, by regulating the expression of genes essential for survival during stress. One essential class of chromatin regulators are methyltransferases, a class of enzymes that methylate histones to promote differential gene expression. The yeast chromatin regulator Set4 is a member of the Set3 subfamily of SET-domain containing methyltransferases that appear to have inactive catalytic domains. In budding yeast, this family consists of Set3 and Set4, and there are orthologs in worms, flies, mice, and humans, such as MLL5 and SETD5. In addition to the conserved SET domains, the majority of these factors include PHD fingers that bind to H3K4 methylation and facilitate their recruitment to chromatin. Interestingly, the Set4 PHD finger is not known to bind

histones in standard assays and the mechanism by which it is recruited to chromatin is unknown. We have previously shown that Set4 is important for promoting cell survival in oxidative stress and recent work has indicated a role for Set4 in promoting survival and cell wall integrity during hypoxia. This appears to be largely due to the regulation of hypoxia-induced gene expression programs, including a large subset of genes encoded within subtelomeres. We have determined that Set4 plays a clear role in chromatin regulation within these regions, including regulating the association of histone deacetylases and the levels of histone acetylation. This appears to be through the regulated recruitment of Set4 to chromatin. Here, we will present our current model for understanding the regulation of stress response genes by Set4 under different conditions and possible models for how Set4 might be regulated at chromatin. Altogether, this work provides new insights into a poorly understood chromatin regulator which plays critical roles during stress responses.

B312/P1993

Regulation of chromatin remodeling enzymes during quiescence entry and exit

C. Cucinotta; Fred Hutchinson Cancer Research Center, Seattle, WA

Most cells on earth are nondividing, yet studies of chromatin regulation have often focused on proliferating cells. We uncovered specialized functions of chromatin remodelers in the nondividing cellular state of quiescence. Quiescence is a reversible G0 essential for long-term survival, wound healing, stem cell differentiation, and immune cell activation. During quiescence entry, histones are stripped of acetylation marks, and transcription is shut down. Despite this repressive chromatin architecture, we demonstrated that Pol II reactivation occurs within minutes of exit. By mapping each chromatin remodeling enzyme in quiescent cells, we identified quiescence-specific binding profiles, suggesting specialized roles for these remodelers in quiescence. We uncovered new functions for one such chromatin remodeler in slowing quiescence escape. We previously reported that the RSC chromatin remodeler binds and poises the genome for rapid activation during cell cycle re-entry. We have since identified separate RSC subcomplexes managing quiescence entry and exit. Together, these results describe new roles for conserved chromatin remodelers in regulating global chromatin reactivation from a highly repressed state.

B313/P1994

Towards a single-molecule perspective of lineage specification in the early mammalian embryo

S. Basu; University of Cambridge, Cambridge, United Kingdom

Revealing how pluripotent cells generate distinct cell lineages in the early mammalian embryo is central to uncovering the mechanisms that drive mammalian development and for unlocking the potential of pluripotent cells for regenerative medicine. The objective of my lab is to provide single-cell and single-molecule insights into nuclear protein complexes that influence the ability of pluripotent cells to differentiate. Although recent studies have characterised the enzymatic activities of some of these protein complexes at the nucleosome-scale, little is known about their intra-nuclear dynamics or how they influence spatiotemporal 3D enhancer-promoter relationships. To investigate these dynamics, we have established tools for live-cell 3D tracking of single HaloTag-tagged nuclear proteins and inactive dCas9-labelled genes. We have also developed machine-learning algorithms to infer properties about the resulting trajectories, properties that allow us to determine the chromatin binding kinetics of key nuclear protein complexes and also to determine how they control chromatin movement at specific

enhancers/promoters. Using these approaches, we reveal the chromatin binding kinetics of two major protein complexes that regulate differentiation - the chromatin remodeller NuRD and the histone methyltransferase KMT2B. Furthermore, we show that both NuRD and KMT2B influence the chromatin binding of specific transcription factors and the range that genes explore within the nucleus. We then use single-cell transcriptomics (scRNAseq) and chromosome conformation capture (scHi-C) to show that these changes in chromatin movement are linked to the length-scale over which enhancers activate transcription at nearby genes. We propose a model in which the movement of enhancers influence gene activation during pluripotent cell differentiation. Our results highlight the importance of making dynamic measurements at single-cell and single-molecule resolution to provide insight into transcriptional control during pluripotent cell differentiation. We are currently establishing these technologies within live mouse embryos and we believe this will help the field understand the underlying molecular mechanisms of cell fate decisions *in vivo*.

Post-transcriptional Gene Regulation

B314/P1995

Post-transcriptional regulation of the copper transporter ATP7A via the 3' untranslated region

N. J. Ferguson, T. J. Whitlow, Y. Zhang, K. E. Vest; Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, Cincinnati, OH.

Copper (Cu) is an important trace nutrient that requires tight regulation due to its potential for toxicity. Impaired Cu regulation leads to a variety of disease states. Regulation of Cu is accomplished through a series of Cu transporter proteins connected through an intricate network of protein and small molecule chaperones. ATP7A is an Cu transporter that functions as the primary Cu detoxification pathway for most mammalian cells. This highly conserved P-type ATPase is a dual function transporter that incorporates Cu into secreted cuproproteins and also directly exports Cu from the cell when levels are high. Although ATP7A regulation is well studied at the transcriptional and post-translational level, post-transcriptional regulation remains understudied. Post-transcriptional regulation of gene expression is critical for normal tissue development and regeneration, so this lack of understanding represents a major gap in knowledge. The objective of our study is to elucidate the mechanisms mediating regulation of ATP7A using skeletal muscle cell differentiation as a model system. Skeletal muscle stores about a quarter of total systemic Cu and has a well characterized regenerative pathway that can be used to model dynamic metabolic states including fluctuating Cu needs. We previously discovered that muscle cell differentiation requires Cu and ATP7A. Using this model we found that Atp7a mRNA is readily degraded in proliferating myoblasts and is stabilized as these cells differentiate into myotubes. Closer examination of the Atp7a transcript revealed a long 3' untranslated region (UTR) with multiple conserved regulatory elements. We discovered that the 3' UTR mediates differentiation-dependent regulation in the context of a luciferase reporter. Through literature review and CLIP-seq data, we have identified the RNA-binding protein HuR as a candidate for regulating Atp7a mRNA via the 3' UTR. Our RNA-IP data suggests HuR binds Atp7a transcript in proliferating myoblasts but not in myotubes. Additionally, siRNA knockdown of HuR leads to an increase in Atp7a mRNA and altered Cu metabolism in myoblasts.These results suggest that HuR is involved in the dynamic, post-transcriptional regulation of Atp7a and provide the foundation for future studies to decipher the integrated regulation of ATP7A at all levels.

B315/P1996

Long Read Analysis Pipeline Predicts RBPs with Isoform-specific Functions

S. H. Park[1,2], M. Schertzer[2,3], D. A. Knowles[2,3,4]; [1]Department of Biomedical Engineering, Columbia University, New York, NY, [2]New York Genome Center, New York, NY, [3]Department of Computer Science, Columbia University, New York, NY, [4]Department of Systems Biology, Columbia University, New York, NY.

Alternative splicing (AS) plays a critical role in gene regulation and protein expression by generating different messenger RNA (mRNA) isoforms from the same pre-mRNA. AS is tightly associated with RNA binding proteins (RBPs), as RBPs bind to genetic sequences and regulate RNA processing. Importantly, RBPs often regulate AS of their own transcript and the pre-mRNA of other RBPs, so that the majority of RBPs are expressed as more than one isoform. Because AS typically results in different protein products, it is very likely to affect the downstream function of RBPs, but studies of isoform-specific RBP functions are limited. Here, we aim to identify annotated and novel RBP isoforms across different tissues, predict downstream functional effects based on amino acid sequence, and test them in human cells. To identify high-confidence isoforms, we analyzed 96 PacBio long-read RNA-seq datasets from the ENCODE consortium using a customized FLAIR pipeline. First, similar to the standard FLAIR pipeline, we aligned and corrected reads, then grouped them by their splice junctions to return credible isoforms. However, our analysis differs in that we filtered reads more stringently and ignored the 5' and 3' untranslated regions (UTRs). Next, we used NCBI's ORFfinder to identify the amino acid sequence of each isoform. We identified a total of 22,723 annotated and novel RBP isoforms, with an average of 14 isoforms per RBP. For example, we identified 5 annotated and 40 novel isoforms of RBFOX2, which is a well-studied splicing factor. Approximately two-thirds of the RBFOX2 isoforms lacked both the N and C terminal nuclear localization signals, which we predict will affect protein localization, and thus have downstream consequences for genes with RBFOX2-dependent splicing. Additionally, two RBFOX2 isoforms differed in the highly conserved RNA binding domain, which we expect to affect RNA recognition and affinity. To test downstream functions of these isoforms, we will express six FLAG-tagged RBFOX2 isoforms in human stem cells. We will use a FLAG antibody to perform isoform-specific RNA-immunoprecipitation, IP-mass spectrometry, and western blot to investigate differences in RNA binding, co-factor binding, and protein localization, respectively. Overall, our method provides a reliable strategy to identify and mechanistically investigate isoform-specific functions of RBPs.

B316/P1997

Identification of exon definitive regions for *MPC1* microexon splicing and its usage for splicing-mediated gene correction

E. Koh, D. Shin, K. Kim; Yonsei University, Seoul, KOREA, REPUBLIC OF.

Alternative splicing of microexons (3-30 bp) is involved in important biological processes in brain development and human cancers. Although there are many non-3x bp (not in-frame) microexons, understanding their splicing process is only the beginning. Based on our studies with endogenous mRNA analysis and minigene constructs, we showed that a 4 bp microexon of *mitochondrial pyruvate carrier1* (*MPC1*) is constitutively included in mRNA despite the short proximity between the 3' splice site (3'ss) and 5' splice site (5'ss) across the microexon. We also elucidated that there is a strong exon definition region in the proximal flanking introns of *MPC1* microexon, which is sufficient to facilitate the inclusion of *MPC1* microexons as well as artificially reconstituted microexons residing in different genomic

contexts. Ultimately, we defined a nucleotide fragment from the upstream 67 bp of *MPC1* microexon to the downstream 6 bp, as a core exon island, which has a strong exon definition to concatenate its microexon and neighboring exons by splicing. Furthermore, we showed that insertion of the core exon island into a target exon or intron induced to skip the target exon or to enhance the splicing of an adjacent exon, respectively. Collectively, we suggest that the exon island derived from *MPC1* microexon modify genuine splicing patterns depending on its position, thereby providing insights on strategies for splicing-mediated gene correction.

B317/P1998

A Kinetic Proofreading Model of Splice Site Selection

B. Donovan, D. Larson; National Cancer Institute, NIH, Bethesda, MD.

The U2AF heterodimer is an essential splicing factor that binds tightly to 3' splice sites to nucleate formation of the spliceosome E-complex. However, recent work from the Larson Lab establishes that an intron may contain dozens of U2AF binding sites, each with some probability of promoting spliceosome assembly at that specific location. Considering this pervasive U2AF binding, we seek to understand the characteristics of binding events that support spliceosome assembly. We approached this problem using single-molecule imaging of U2AF binding both *in vitro* and *in vivo.* In live-cell single-molecule tracking (SMT) experiments, we observe a broad distribution of U2AF dwell times that best follows a power-law distribution with timescales ranging from 1 s to > 100s. In contrast, when measuring purified recombinant U2AF binding to surface-tethered RNAs containing a consensus 3' splice site, the dwell time distribution is exponential, with a dominant characteristic dwell time of 18 ± 2 s. Importantly, we observe essentially no events longer than 100 seconds in this *in vitro* assay, suggesting that the long-lived U2AF dwell-times observed *in vivo* may be involved in splicing. To test this hypothesis, we monitored U2AF colocalization to an endogenous pre-mRNA (visualized using MS2-stem loops integrated ~4000 nt upstream of the 3' SS within the intron) in live cells using 3D orbital tracking spectroscopy. Indeed, we observe that U2AF binds for 100s of seconds to a pre-mRNA undergoing the splicing reaction. Taken together, this data supports a kinetic proofreading model where the vast majority of U2AF binding events are unproductive and transient. However, in certain circumstances (likely in coordination with other early-spliceosome associated proteins) stable association of U2AF to its pre-mRNA substrate dramatically increases the probability of spliceosome assembly.

B318/P1999

Paired mutational analyses of RNA-binding proteins-interactome and overall survival in cancer patients

E. Zheng[1], A. Saxena[2]; [1]Biology, Brooklyn Technical High School and Brookly College, The City University of New York, Brooklyn, New York, NY, [2]Biology, Brookly College, The City University of New York, Brooklyn, New York, NY.

Cancer is a disease of many altered molecular traits. The cancer disparity amongst ethnic groups is further distinguished using the -omics (genomics, transcriptomics, and proteomics) data. The database suggests that RNA binding proteins (RBPs) are key players in the process of tumorigenesis. Nucleolin (NCL) is one of the most abundant nucleolar proteins that is over-expressed in cancer. NCL plays a pivotal role in a vast variety of cellular processes. There are, however, limited data on how RBP-interactome provides cellular homeostasis during stress responses. In this project, we focused on

understanding the interplay between NCL and 11 other RBPs it interacts with (NPM1, PABPC1, TARBP2, FMR1, YBX1, HNRNPD, HNRNPU, PARP1, ELAVL1, ZFP36, & HNRNPA1). We decipher mutational analyses in these selective sets of RBPs and their impact on the patients' overall survival. We used "The Cancer Genome Atlas" PanCancer studies and cBioPortal to perform paired mutational analyses of NCL with its RBP-interactors. Samples with mutations in both NCL and 10 other RBP-interactors (excluding ZFP36) indicated an overall better prognosis. Subsequently, all samples in the pairs with both genes mutated showed a higher tumor mutational burden. Most of these mutations were also in the RNA-binding domains of the genes. This suggests that mapping mutations in the RNA-binding domains of genes can provide an advantage for a patient's survival. These paired mutational analyses can help inform the pathways affected during the progress of tumorigenesis and assist in identifying potential prognostic biomarkers.

B319/P2000

Polyadenosine promotes complex formation between LARP4a and PABPC1

L. Pitman[1], E. Valkov[1], R. Maraia[2]; [1]National Cancer Institute, NIH, Frederick, MD, [2]Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD

La-related protein 4a (LARP4) is an RNA-binding protein involved in regulating the stability and translatability of messenger RNAs (mRNAs) at the transcriptome level. LARP4 regulates stabilization and protection of the polyadenosine [poly(A)] tail at the 3' ends of mRNAs; however, how LARP4 mechanistically contributes to poly(A) tail regulation is not well understood. Previous studies indicate that LARP4-mediated poly(A) tail regulation involves binding both to the poly(A) substrate and cytoplasmic polyadenosine-binding protein 1 (PABPC1). Using in vitro pull-down assays, we show that recombinant full-length LARP4 most stably interacts with PABPC1 only in the presence of a poly(A) substrate. Mass photometry data further validate the results of pull-down assays, suggesting that stable interaction between LARP4 and PABPC1 is mediated by a poly(A) substrate. Using our established in vitro biochemical system, we will map the exact interacting interface between LARP4 and PABPC1 and subsequently characterize the LARP4/PABPC1/poly(A) complex using cryo-electron microscopy.

B320/P2001

Studying the impact of Diamond-Blackfan anemia associated mutations on Ribosome Structure and Function

S. Parveen, A. Rai Chowdhury, D. Girodat; University of Arkansas, Fayetteville, Fayetteville, AR

An essential process of gene expression that is poorly understood is how protein and RNA constituents assemble to form a ribosome, the catalytic component responsible for synthesizing proteins in all cells. In eukaryotes, the ribosome is comprised of four ribosomal RNA segments and seventy-nine ribosomal proteins all of which need to come together during ribosome biogenesis. Ribosomal proteins are essential for proper ribosome structure and function. Variation of ribosomal proteins by site specific mutations results in human diseases known as ribosomopathies. Ribosomopathy variant ribosomes are hallmarked by defects in ribosome assembly and translational output. The resulting heterogenous pool of ribosomopathy variant ribosome structures is unclear. Additionally, to understand these diseases it is imperative to identify which genes are actively translated into proteins by these ribosomes. Diamond-Blackfan anemia (DBA) is among the most well-studied ribosomopathy, making it a model disease to study the pathologies. To explore the impact of DBA causing variant ribosome structure and

translational output, we generated ribosomal protein variants in *Saccharomyces cerevisiae*. Using cryo-electron microscopy, mass spectrometry and next-generation sequencing we are performing structure-function analysis of 10 variants of the ribosomal protein, L5 which is located at the central protuberance of the large ribosomal subunit, a core functional region of the ribosome. We expect that these variants will perturb ribosome structure through deviations in ribosomal protein abundance. Furthermore, these studies will allow us to investigate if DBA ribosomal protein variants affect protein synthesis and gene expression. These experiments will be critical towards understanding the pathology of DBA and ribosomopathies.

B321/P2002

Post-transcriptional control of mating-type genes in*Saccharomyces cerevisiae* spores

R. Yeager, M. McMurray; University of Colorado Sch Med, Aurora, CO

Abstract: Gametogenesis in diploid cells of the budding yeast *Saccharomyces cerevisiae* produces four haploid meiotic products called spores. Spores are dormant until nutrients trigger germination, when they bud asexually or mate to return to the diploid state. Each sporulating diploid produces a mix of spores of two haploid mating types, **a** and **α**. In asexually dividing haploids, the mating types result from distinct, mutually exclusive gene expression programs responsible for production of mating pheromones and the receptors, all of which are silent in diploids. It was assumed that spores only transcribe haploid- and mating-type-specific genes upon germination. Using RNA-seq on spore populations of each mating-type, we find that dormant spores harbor transcripts representing all these genes regardless of their mating-type, with the exception of Mata1, which we found enriched in Mat**a** spores. Of particular interest to our group are the mating pheromones and receptors, due to their cell-type specific roles in mating. Seemingly produced in an unbiased fashion during meiosis, and found equally in spore populations of both mating-types, these transcripts appear to be subject to multiple levels of translational regulation leading to high fidelity expression in only the appropriate mating-type upon germination. The protein products of these transcripts are not seen until germination, and are also regulated such that upon germination, each receptor protein is only present in spores of the appropriate mating-type. We find that antisense transcripts to the Ste3 mating-type receptor are present in all spores and may be responsible for the translational silencing of the Ste3 transcripts found inappropriately in Mat**a** cells. We also find evidence of 5'UTRs in meiotic Ste2 and Ste3 transcripts containing multiple uORFs that may translationally regulate these transcripts. Further, we note that a cryptic poly-A signal leading to short Ste2 transcripts may explain the presence and subsequent translational regulation of the Ste2 transcripts found inappropriately in Mat**α** spore populations. These findings add to the growing number of examples of post-transcriptional regulation of gene expression during gametogenesis and explain tightly regulated expression of key mating-type related proteins, despite a broad transcriptome found present in the early spore life.

Nuclear Morphology and Positioning

B323/P2003

A mechanical signal on the prophase nucleus contributes to mitotic entry

M. Dantas, A. Oliveira, P. Aguiar, H. Maiato, **J. Ferreira**; i3S - UP, Porto, PORTUGAL.

As cell prepare to divide, they must ensure that enough space is available to assemble the mitotic machinery without perturbing tissue homeostasis. To do so, cells undergo a series of biochemical reactions regulated by cyclin B1-CDK1 that trigger a reorganization of the actomyosin cytoskeleton and ensure the coordination of cytoplasmic and nuclear events.
Along with the biochemical events that control mitotic entry, mechanical forces have recently emerged as important players in the regulation of cell cycle events. However, the exact link between mechanical forces and the biochemical pathways that control mitotic progression remains to be established.
Here, using a combination of micromanipulation techniques with high resolution live cell imaging, we identify a mechanical signal on the nucleus that helps set the time for nuclear envelope permeabilization (NEP) and mitotic entry. This signal relies on actomyosin contractility, which leads to nuclear unfolding during the G2-M transition, activating the stretch-sensitive cPLA2 on the nuclear envelope and contributing to the spatiotemporal translocation of cyclin B1 to the nucleus. Our data demonstrate how nuclear mechanics during the G2-M transition contribute to timely and efficient mitotic spindle assembly and prevents chromosomal instability.

B324/P2004

The nuclear cargo adaptor KASH protein UNC-83 regulates the choice of dynein vs. kinesin-1 motor activity to move nuclei in opposite directions during C. elegans development

S. Gumusderelioglu[1], K. Chiba[2], E. Gregory[1], N. Sahabandu[1], S. Niwa[2], G. Luxton[1], R. McKenney[1], D. Starr[1]; [1]University California-Davis, Davis, CA, [2]Tohoku University, Sendai, JAPAN.

Nuclear movements driven by dynein and kinesin are important for developmental events including pronuclear migration during fertilization and vertebrate central nervous system development. Like most cargo, nuclei are simultaneously bound to both dynein and kinesin, resulting in their bi-directional movement along a microtubule with a net directionality towards one end. However, how nuclei bias their direction is poorly understood. We address this problem by studying bidirectional nuclear movement during C. elegans development. In this model, the outer nuclear membrane KASH protein UNC-83 serves as a cargo adaptor to recruit both dynein and kinesin-1 to nuclei. While dynein is the major force producer for larval P-cell nuclear migration, kinesin-1 drives nuclear migration in embryonic hypodermal precursors. It is unknown how UNC-83 regulates the choice of motors at different times in development. We hypothesize that the net directionality of nuclear movement in C. elegans is determined by the developmentally regulated expression of alternative isoforms of UNC-83. Using C. elegans genetics, we showed that mutations affecting the long UNC-83a/b isoform disrupts dynein-dependent movements, while disrupting all isoforms affects both nuclear migrations. Residues 58-233 specific to the long UNC-83a/b isoform are necessary for dynein-dependent nuclear migration. Moreover, we found that a line expressing the long isoform under control of the short isoform's promoter disrupted nuclear migration in embryonic hypodermal precursors, indicating that the UNC-83a/b-specific domain was sufficient to disrupt kinesin-1 mediated nuclear migration. We tested if the short isoform UNC-83c directly activates kinesin-1 motor activity using single molecule TIRF microscopy

assays. The presence of UNC-83c increased kinesin-1 binding to microtubules and stimulated processive movements. In contrast, UNC-83a/b did neither. These results support our current model where UNC-83c directly activates kinesin-1 to move nuclei in embryonic hypodermal precursors, while UNC-83a/b counteracts UNC-83c's ability to activate kinesin-1, causing dynein to become the dominant motor during larval P-cell nuclear migration. This work is an important step towards understanding how nuclei coordinate bidirectional microtubule-dependent transport during development.

B325/P2005

Control of nuclear size by osmotic forces in*Schizosaccharomyces pombe*

J. LEMIÈRE[1], P. REAL-CALDERON[1], F. CHANG[1], L. J. HOLT[2], T. G. FAI[3]; [1]Cell & Tissue Biology, University of California, San Francisco, San Francisco, CA, [2]Institute for Systems Genetics, New York University Langone Health, New York, NY, [3]Mathematics and Volen Center for Complex Systems, Brandeis University, Waltham, MA.

The size of the nucleus scales consistently with cell size so that the nuclear-to-cell volume ratio (N/C ratio) is maintained during cell growth in many cell types. Despite this simple and longstanding observation, the mechanism responsible for this scaling is still mysterious. The N/C ratio is not determined merely by DNA amount, but is influenced by factors such as properties of the nuclear envelope and nuclear transport. Here, we developed a physical-based model for nuclear size control based upon osmotic pressure. The nuclear envelope can be regarded as a semi-permeable barrier that allows water and small ions to pass, but is relatively impermeable to large macromolecules. These macromolecules generate colloid osmotic pressure differences between the compartments that inflate the nucleus. Nuclear size may arise as a passive outcome of the numbers of osmotically-active macromolecules in the nucleus and cytoplasm. We analyze N/C ratio regulation in fission yeast using quantitative tools and find that the fission yeast nucleus, in contrast to the more complex mammalian one, behaves as an ideal osmometer, whose volume is determined in a linear relationship by its osmotic environment following the Van't Hoff law. We also propose a theoretical model to explain homeostasis behavior that maintains both the N/C ratio during cell growth and the behavior by which cells born with an aberrant N/C ratio correct it over time. We show that the N/C ratio correction rate is the additive inverse of cell's growth rates. These studies support a quantitative model explaining how nuclear size is determined by physical mechanisms that integrates osmotic forces and biosynthesis of macromolecules. Importantly, this model does not call for a complex feedback regulatory mechanism to explain N/C ratio robustness. Abnormal nuclear size and shape are a hallmark of many cancer cells and has been used as a phenomenological diagnostic criterion; our results begin to shed light onto mechanisms for how N/C ratio changes during diseases and development.

B326/P2006

Branched actin CDC-42 networks function with a LINC and dynein pathway to move nuclei through constricted spaces in *C. elegans* development

J. Ho[1], L. Ma[1], L. Gurerro[1], D. Libuda[2], D. Starr[1]; [1]University California, Davis, CA, [2]University Oregon, Eugene, OR.

Cell migration through narrow spaces is critical for many developmental and disease processes, including immune cell intravasation and cancer metastasis. Some cells can squeeze through openings about 5% the resting diameter of the nucleus. Nuclear deformability appears to be the limiting step for

cell migration through constrictions. However, the mechanisms that facilitate nuclear movement through narrow spaces are unclear. We established a model in larval C. elegans using P-cell nuclei which migrate through a narrow constriction as a normal part of development. First, the linker of the nucleoskeleton and cytoskeleton (LINC) pathway recruits microtubule motors, primarily dynein, to the nuclear envelope. Knockdown of dynein heavy chain leads to a P-cell nuclear migration defect. Second, we identified components of a branched actin network using a forward genetics screen, including CGEF-1, a predicted CDC-42 GEF, that enhanced the nuclear migration defect in LINC complex mutants. Knockdown of CDC-42, ARP2/3, and non-muscle myosin-1 using the auxin-inducible degradation system leads to a P-cell nuclear migration defect, indicating that branched actin networks are necessary for nuclear migration. In our branched actin model, CGEF-1 activates CDC-42, which goes on to activate branched actin nucleators such as ARP2/3. Myosin-1 is also required to organize the actin networks. In our working model, dynein is recruited to the surface of nuclei to provide a pulling force on the nucleus while branched actin helps to deform the nucleus. Myosin-1 could act by providing a pushing force on the nucleus from behind to help move the nucleus through the constriction.

B327/P2007

Deforming safely: nuclear envelop folds define two regimes of nuclear volume, pressure and tension

A. Williart[1], R. Rollin[2], G. P. F. Nader[3], J. Joanny[2], P. Sens[2], N. Manel[4], M. Piel[5]; [1]UMR144, Institut Curie, Paris, FRANCE, [2]UMR168, Institut Curie, Paris, FRANCE, [3]Division of Cell Pathology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, [4]U932, Institut Curie, Paris, FRANCE, [5]Cancer Cell Biology Department, Institut Curie, Paris, FRANCE.

In the body, cells are often subjected to mechanical constraints and can undergo large deformations. This can occur when cells move through dense tissues or capillaries, or often during tumor development. When the cell deforms, its nucleus, as its largest and stiffest organelle, is also subjected to deformation, up until loss of nuclear envelope integrity and rupture. Using a 2D confinement device, we can *in vitro* impose a range of controlled heights to the cells, alter their mechanical state and that of their nucleus, and study the consequences of deformations. The mechanical deformation of the nucleus is associated with changes in the state of the nuclear envelope. Non-adhered, non-confined cells have nuclei which display large folds that gradually open upon confinement. This is associated with a decrease of NE fluctuations, which we interpret as an increase in NE tension (Lomakin et al., 2020). We show in three different cell lines that the nucleus follows two regimes depending on the deformation magnitude. Under mild confinement, the nucleus deforms at constant volume, with a low NE tension and a low internal pressure. In this regime, nuclear deformation is fully translated into NE unfolding. Because the NE contains a stiff lamina layer, when deformation reaches a threshold of full NE unfolding, the NE abruptly tenses, defining a precise deformation threshold and a switch to a deformation regime with volume loss up to 50%, high NE tension and high internal nuclear pressure, leading to the formation of nuclear blebs. We propose that the first regime defines a “safe” deformation range with a sharp threshold, defined by the amount of NE folds, below which a regime begins with altered volume, chromatin density and NE integrity. Combining a physical model for the nuclear volume and for the nuclear envelope mechanics, and using mechanical measures obtained with a wedged cantilever, and detailed quantitative imaging, we explain the origin of nuclear envelope folds, the mechanical response of the nucleus to deformation, with the two different regimes and the sharp transition, the onset of NE tension, nuclear internal pressure and appearance of nuclear blebs. We believe that our work provides a physically sound explanation for these phenomena which have recently proven to be responsible for

specific cellular responses to mechanical constraints. p { line-height: 115%; margin-bottom: 0.25cm; background: transparent }

B328/P2008

Nuclear deformation and dispersion pathways cooperate to drive epithelial plasticity

R. Budhathoki, N. de Leeuw, J. T. Blankenship, D. Loerke; UNIVERSITY OF DENVER, DEPARTMENT OF BIOLOGICAL SCIENCES, Denver, CO.

Planar polarized cell intercalation is a local event that brings tissue-scale changes for convergent extension movements during embryonic development in *Drosophila*. The nucleus, being the largest organelle in a cell, may pose a significant mechanical barrier to cells attempting to adopt extreme shapes and intercalate to achieve optimal body-axis elongation. How this largest organelle allows cells to remodel for better packing, a crucial step for this developmental process, has been largely unstudied. Here, we examine how the nucleus responds to mechanical forces imposed by the contractile forces during the rapid phase of germband extension in *Drosophila*. We find that two central mechanisms allow nuclei to respond to these stresses: 1) nuclear deformation, and 2) the dispersion of nuclei to alternate planes along the apical-basal axis. First, we tested whether cortical cell deforming forces are necessary drivers for the shaping of nuclear dimensions, as well as whether nuclear redistribution along the apical-basal axis is responsive to these actomyosin forces. Disrupting cortical force generating machinery showed that nuclei no longer deformed and had a limited redistribution along the apical-basal axis. Interestingly, however, nuclei lost their ability to re-center themselves in cells. We next tested the effects of restricting nuclear deformability through the use of kugelkern disruptions. Embryos with “non-deformable” nuclei displayed a major shift towards the second mechanism, i.e., a more dynamic redistribution in the apical-basal axis. Although this redistribution allowed cells to change shape and still participate in cell intercalation, epithelial regularity was deeply compromised. We next tested embryos that possessed “non-dispersible” nuclei by inhibiting microtubule polymerization. Interestingly, this caused a jamming phenotype in which nuclei became locked in the same apical-basal plane and cell intercalation was completely halted. Lastly, we asked what would happen in epithelia in which nuclei are both non-deformable and non-dispersible. In these circumstances, nuclei engaged in a tug-of-war to occupy the same z-plane and cell intercalation was completely disrupted. This suggests the existence of an active force-sensing process that keeps nuclei in the same plane unless internuclear tensions engage the re-distribution mechanism that regulates apical-basal nuclear densities. Altogether, our results uncover a critical role for the nucleus in epithelial remodeling that demonstrates how local level effects on nuclear positioning and deformability are necessary to achieve tissue-level elongation.

B329/P2009

Nuclear F-actin and Lamin A antagonistically regulate nuclear shape

S. Mishra, D. Levy; University of Wyoming, Laramie, WY.

Nuclear shape influences various cellular processes such as gene expression, cell cycle progression, and cell migration. Abnormal nuclear shape is a hallmark of diseased states like cancer and laminopathies. Two important regulators of nuclear shape in mammalian cells are the nuclear lamina and perinuclear cytoplasmic actin cap. Although cytoplasmic F-actin is known to regulate nuclear shape, open questions remain about the role of nuclear F-actin in regulating nuclear shape as well as possible interactions between nuclear F-actin and Lamin A/C. To address these questions, we assembled nuclei in F-actin

intact *Xenopus* egg extract, a simplified cell-free system lacking endogenous Lamin A/C. While nuclei were roughly round when F-actin was depolymerized, nuclei were bilobed in shape in the presence of F-actin, exhibiting nucleoplasmic and nuclear rim-localized actin filaments. This result suggested a role for nuclear F-actin in regulating nuclear shape. The composition of the two lobes was heterogeneous, with the larger lobe having more F-actin but less DNA, nuclear pores, and lamin B3. Interestingly, addition of recombinant Lamin A partially rescued the bilobed nuclear morphology, suggesting that Lamin A and F-actin have opposing roles in regulating nuclear shape. This hypothesis was further tested in mammalian cells. When nuclear-targeted actin was overexpressed in Lamin A knockdown HeLa cells, the nuclei were misshapen with some of the nuclei becoming multilobed. In some cells, nucleoplasmic and rim-localized F-actin were observed, coinciding with nuclear domains having less DNA, Lamin B1, and nuclear pores, similar to *Xenopus* extract. Nuclear-targeted polymerization-defective actin did not cause nuclear lobulation, showing that dynamic nuclear F-actin is responsible for altering nuclear shape as opposed to nuclear G-actin. Inducing nuclear export of actin by overexpression of exportin-6 rescued the shape defect caused by Lamin A knockdown, further supporting the idea that Lamin A/C and nuclear F-actin antagonistically modulate nuclear shape. Using small molecule inhibitors in *Xenopus* extracts and HeLa cells, we found that formins are responsible for inducing nuclear lobulation and not myosin or Arp2/3. Future studies will focus on how formins and nuclear actin dynamics regulate nuclear shape in laminopathies and cancers.

B330/P2010

SUN1-mediated nucleus-cytoskeletal tension determines progerin-induced nuclear deformation

J. Jo, **D. Kim**; KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul, KOREA, REPUBLIC OF.

Hutchinson-Gilford progeria syndrome (HGPS) caused by mutation in the LMNA, leading to increased production of truncated prelamin A, progerin. As the HGPS cells display accumulated progerin at nuclear envelope that results in nuclear deformation. Accumulation of progerin induces the increased level of the inner nuclear membrane protein SUN1, which alters nucleus-cytoskeletal connections. While SUN1 overexpression in HGPS cells correlated with nuclear defects, however, how the molecular mechanism of progerin-induced SUN1 accumulation deforms the nuclear morphology remains unclear. Here, we introduce doxycycline-inducible progerin expression system in HeLa cells to precisely control the progerin expression-induced nuclear deformation. To generate stable cell lines for doxycycline-inducible progerin expression, PiggyBac transposon containing blasticidin resistant gene was co-transfected with the plasmids (Δ50 LMNA, mCherry-Δ50 LMNA) into HeLa cells. We showed that doxycycline-induced progerin expression reduced the nuclear tension by SUN1-dependent molecular connections with LMNA, which in turn resulted in NE wrinkling. As determined by quantitative analysis of immunofluorescence microscopy, progerin expression accumulates the phosphorylated myosin along with the F-actins, which dictates that progerin expression could enhance the cytoskeletal tension. Moreover, the specific inhibition of myosin activity without sacrificing F-actin organization restored the nuclear tension and ultimately, reduced the NE wrinkling. We demonstrated that progerin-induced nuclear deformation by progerin expression was mediated by the reduced nuclear tension, which was accompanied by SUN1 overexpression-dependent myosin tension rather than F-actin connectivity through SUN1-nesprin 2 bridging. Reconfirmed by siRNA-mediated SUN1-depleted Tet-On HeLa cells, we concluded that progerin-induced nuclear deformation was determined by SUN1 dependent nuclear tension. This work unveils the mechanism of nuclear deformation by progerin expression, which could

fill in the missing link between myosin activity-dependent cytoskeletal tension and SUN1-mediated nuclear tension. We further expect that progerin-inducible Tet-On system will provide a new approach to investigate how the progerin expression alter the nucleus-cytoskeletal connection, which is critical to find important role of nuclear lamina mediating mechanotransduction in biological aging process.

B331/P2011

Emerin dysregulation compromises nuclear integrity during metastatic transformation

E. Hansen, J. M. Holaska; Biomedical Sciences, Cooper Medical School At Rowan, Camden, NJ.

Breast cancer metastasis causes a majority of breast cancer-related deaths. In metastasis, cancerous tumor cells spread through the body by moving into and out of nearby blood vessels by squeezing through gaps in the endothelium. Nuclei in cancer cells are often smaller and more malleable, allowing for easier metastasis. We showed both invasive breast cancer cell lines and patient samples have less emerin expression, misshapen nuclei, and higher metastasis rates than non-cancerous controls. We hypothesize emerin reduction causes compromised nuclear integrity during metastatic transformation. The mechanism by which emerin modulates nuclear stiffness is not well understood. Increasing stiffness of the tumor microenvironment (TME) was shown to increase nuclear softening, suggesting emerin may respond to changes in TME. Interestingly, this increased stiffness in the TME and subsequent nuclear softening correlates with increased metastasis. Thus, we predict that increased extracellular matrix (ECM) stiffening decreases emerin protein expression to cause nuclear softening. Supporting this hypothesis, we show ECM stiffening decreased emerin expression and altered nuclear integrity. We posit that emerin is acting as a mechanosensor that responds to extracellular stimuli through the linker of nucleoskeleton and cytoskeleton (LINC) complex by modulating structural and/or transcriptional outcomes to cause nuclear softening and drive metastasis in triple negative breast cancer (TNBC). How emerin transduces these signals and how the loss of emerin causes dysfunctional mechanotransduction during cancer progression has yet to be elucidated. Previous work showed substrate stiffness causes LINC-dependent changes in nuclear F-actin and altered the transcriptional activity of the pro-migratory protein MKL1, both of which are affected by emerin. We overexpressed nuclear actin and actin polymerization mutants in TNBC cells and showed that increasing nuclear actin polymers rescued nuclear structure. We also tested whether transcriptional activity of MKL1 contributes to nuclear softening upon ECM stiffening. How emerin senses ECM changes via the LINC complex to modulate nuclear actin polymerization and/or transcription is also being tested. This analysis will provide key mechanistic insight into how changes in ECM stiffness cause nuclear softening during metastatic transformation.

B332/P2012

Measurement of Mechanical Strain on Lamin A/C and Vimentin Intermediate Filaments using FRET-force Biosensors

B. E. Danielsson[1], B. G. Abraham[2], E. Mäntylä[2], D. E. Conway[3], T. O. Ihalainen[2]; [1]Virginia Commonwealth University, Richmond, VA, [2]Tampere University, Tampere, FINLAND, [3]Ohio State University, Columbus, OH.

Intermediate filament proteins provide mechanical rigidity both in the form of cytoskeletal networks as well as the nuclear lamina. However, to date there is not yet a technical approach to directly measure mechanical forces on intermediate filament proteins at the protein level. To further study these

structures, we developed two FRET based force biosensors that can be used to observe the mechanical state of either Lamin A or vimentin network. A novel strategy of indirectly tagging the target protein using nanobodies was used to avoid disruption of filamentous lamin or vimentin. Using the lamin A biosensor in MDCK epithelial cells, we were able to show that the nuclear lamina is subjected to significant force. These forces are dependent on nuclear volume, actomyosin contractility, functional LINC complex, chromatin condensation state, cell cycle, and EMT. Interestingly, large forces were also present on nucleoplasmic lamins. Additionally, using the vimentin biosensor in NIH3T3 fibroblasts we observed that the vimentin network experiences significant strain. Vimentin strain is dependent on actomyosin contractility and the LINC complex. Overall we demonstrate that nanobody-based biosensors can be utilized to study the mechanobiology of complex protein networks, including intermediate filaments.

B333/P2013

Linker Cell-type Death in C. elegans: a Physiological Model for Nuclear and Chromatin Aberrations

O. Yarychkivska, Y. Lu, S. Shaham; Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

Changes in nuclear morphology and chromatin organization are commonly observed in physiological and pathological conditions such as developmental cell death, aging, progeria, and aggressive cancers. However, the precise consequences, implications and molecular nature of these changes for disease progression are not well understood. We focus on Linker Cell-type Death (LCD) as a physiological model to understand the contributions of nuclear and chromatin changes to developmental cell death. LCD refers to the programmed death of the *C. elegans* linker cell (LC), which dies using a caspase-independent, non-apoptotic, morphologically-conserved process. The LC is a sex-specific migrating leader cell that transitions from having an oval nucleus to one with a nuclear envelope (NE) bearing striking indentations as LCD takes place. We have discovered dynamic changes in nuclear morphology and chromatin in our *in vivo* LCD setting over time. We used endogenously-tagged fluorescent lamin to show that the nuclear lamina acquires unusual ring formations that precede onset of visible NE deformations. The LC then undergoes stereotypic events of competitive phagocytosis, splitting (small cytoplasmic fragment buds off), and engulfment resulting in a refractile cell morphology. Using animals staged at different times, as well as long-term live imaging of individual animals, we discovered that the nuclear lamina dissociates as the LC splits. Simultaneously, linker histone H1 and nucleosome-associated histone H2B staining transitions from nuclear puncta to a cell-wide diffuse distribution. Surprisingly, following LC budding, the lamina fully reassembles with the unusual ring formations resembling metastatic cancer cells, and histones are re-incorporated into chromatin puncta. EM studies demonstrate that as LCD progresses, LC acquires various NE defects and forms novel nuclear/cytoplasmic vesicles. To decipher the molecular mechanism behind these unusual nuclear dynamics, we are conducting a whole-genome LC-specific RNAi screen for animals exhibiting defects in lamina dynamics. We are specifically interested in identifying genes involved in nuclear disassembly and re-formation. Because nuclear changes are prominent in a host of pathological settings in humans, we believe that our studies may identify factors relevant for these conditions as well.

B334/P2014

The Nucleus Bypasses Obstacles by Deforming Like a Drop with Surface Tension Mediated by Lamin A/C

A. Katiyar[1], J. Zhang[2], J. Antani[1], Y. Yu[3], K. Scott[4], P. P. Lele[1], C. Reinhart-King[5], N. Sniadecki[6], K. Roux[7], R. Dickinson[3], T. P. Lele[1]; [1]Texas A&M University, College Station, TX, [2]University of Arkansas, Fayetteville, AR, [3]University of Florida, Gainesville, FL, [4]Sanford research, Sioux Falls, SD, [5]Vanderbilt University, Nashville, TN, [6]University of Washington, Seattle, WA, [7]Sanford Research, Sioux Falls, SD.

Migrating cells must deform their stiff cell nucleus to move through pores and fibers in tissue. Lamin A/C is known to hinder cell migration by limiting nuclear deformation and passage through confining channels, but its role in nuclear deformation and passage through fibrous environments is less clear. Here we studied cell and nuclear migration through discrete, closely spaced, slender obstacles which mimic the mechanical properties of collagen fibers. Nuclei bypassed slender obstacles while preserving their overall morphology by deforming around them with deep local invaginations of little resisting force. The obstacles did not impede the nuclear trajectory and did not rupture the nuclear envelope. Nuclei likewise deformed around single collagen fibers in cells migrating in 3D collagen gels. In contrast to its limiting role in nuclear passage through confining channels, lamin A/C facilitated nuclear deformation and passage through fibrous environments; nuclei in lamin-null (*Lmna−/−*) cells lost their overall morphology and became entangled on the obstacles. Analogous to surface tension-mediated deformation of a liquid drop, lamin A/C imparts a surface tension on the nucleus that allows nuclear invaginations with little mechanical resistance, preventing nuclear entanglement and allowing nuclear passage through fibrous environments.

B335/P2015

The farnesyltransferase inhibitor lonafarnib improves nuclear morphology in ZMPSTE24-deficient patient cells with the progeroid disorder MAD-B.

K. Odinammadu, K. Shilagardi, S. Michaelis; The Johns Hopkins School of Medicine, Baltimore, MD

Several related progeroid disorders are due to defective post-translational processing of prelamin A, the precursor of the nuclear scaffold protein lamin A, encoded by *LMNA*. Prelamin A is first farnesylated at its C-terminus and subsequently this farnesylated C-terminal segment is cleaved off by the zinc metalloprotease ZMPSTE24. The premature aging disorder Hutchinson Gilford progeria syndrome (HGPS) and a related progeroid disease, mandibuloacral dysplasia (MAD-B), are due to mutations in *LMNA* and *ZMPSTE24*, respectively, that result in failure to process and accumulate permanently farnesylated prelamin A. The farnesyl transferase inhibitor (FTI) lonafarnib is known to correct the aberrant nuclear morphology of HGPS patient cells and improves the health-span and life-span in children with HGPS. Importantly, and in contrast to a previous report, we show here that FTI treatment also improves the aberrant nuclear phenotypes in MAD-B patient cells with a mutation in *ZMPSTE24*. We also present evidence that, as expected, patient cells with *LMNA* mutations that alter residues at a distance from the ZMPSTE24 cleavage site are proficient in prelamin A processing, and their nuclear morphology defects do not improve with FTI treatment. Additionally, we examine for the first time, prelamin A processing in fibroblasts from two patients with the laminopathy mutation *LMNA-R644C*. Despite the proximity of residue R644 to the prelamin A cleavage site between Y646 and L647, neither patient cell line shows a prelamin A processing defect, and both have normal nuclear morphology. This work clarifies the processing status in a variety of laminopathy patient cells and supports the hypothesis

that MAD-B patients that are prelamin A processing-deficient due to a *ZMPSTE24* mutation may benefit from FTI treatment.

B336/P2016

Nuclear growth links chromatin state to lamina organization in micronuclei

M. Zych[1,2], A. Mammel[1], E. Hatch[1]; [1]Fred Hutchinson Cancer Research Center, Seattle, WA, [2]University of Washington, Seattle, WA

Micronuclei form when missegregated chromosomes recruit their own nuclear envelope and have been commonly used as a marker of chromosome instability. Micronuclei frequently rupture, which results in genome instability, upregulation of metastatic genes, and increased immune signaling. Our lab found that gaps in the lamin meshwork are correlated with micronucleus rupture frequency but how lamina gaps form in micronuclei remains a critical, unanswered question. We found that micronuclei containing gene dense chromosomes are less likely to contain lamina gaps and are more stable. For individual chromosomes we see a correlation between micronucleus size and lamina gap number, suggesting that nuclear growth drives gap formation and that growth is delayed in gene dense micronuclei. Live-cell imaging supports a link between growth and lamina gap formation. Nuclear import is a key determinant of nucleus expansion, and we find a correlation between decreased Ran and the Ran GEF RCC1 and slower post-mitotic expansion in micronuclei compared to the main nucleus. Further supporting this model of lamina gap formation, we find that RCC1 is especially depleted from gene dense micronuclei, consistent with previous data that RCC1 is enriched on heterochromatin. We find that increasing histone methylation on gene dense micronuclei with methylstat increases lamina gaps, RCC1 recruitment, and micronucleus rupture. We also find that increasing histone acetylation on gene poor micronuclei decreases rupture. These treatments have significantly stronger effects than disrupting transcription, support the importance of histone modifications in micronucleus stability. We are currently working to further define the role of RCC1 in this connection between chromatin state and lamina gaps to outline how nucleus growth is regulated in micronuclei. Ultimately this work will enable new insight into how nucleus size is regulated and identify a new function for chromatin in this regulation.

Nuclear Pore Complexes

B337/P2017

The role of O-GlcNAcylation in mRNA nuclear export studied by single-molecule super-resolution microscopy

S. L. Junod, W. Yang; Temple University, Philadelphia, PA

Characterizing the relationship between the nuclear export of mRNA and the O-GlcNAcylation of nuclear pore proteins is a critical process for understanding the dynamic effects of post-translational modifications on gene expression. Specifically, it was reported that the concentration of O-GlcNAcylated nucleoporins (Nups) in the nuclear pore complexes (NPCs) could be significantly altered by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) inhibitor drugs. Combining two single-molecule super-resolution microscopy methods, STORM and SPEED microscopy, we found that the amount of messenger ribonucleoprotein particles (mRNPs) that successfully export through the NPC is approximately doubled when increasing the O-GlcNAcylation in the NPC. Reversely, five-fold fewer mRNPs can exit the nucleus to reach the cytoplasm with the decreased O-GlcNAcylation of Nups.

Moreover, a virtual 3D imaging by SPEED microscopy revealed that the spatial distributions of these altered O-GlcNAcylated Nups colocalized well with the locations of Nups that are rich in phenylalanine-glycine (FG) repeats, suggesting the passage of mRNPs through the selective permeability barrier in the NPC is regulated by the amount of O-GlcNAcylated FG-Nups.

B338/P2018

The linker-scaffold architecture enables dilation and constriction of the nuclear pore complex

S. Petrovic, A. Hoelz; California Institute of Technology, Pasadena, CA.

Eukaryotic cells selectively transport macromolecules in and out of the nucleus through the nuclear pore complex (NPC). In humans, the NPC is a ~110 MDa assembly of ~1,000 copies of 34 different proteins, collectively termed nucleoporins. NPCs are built on an evolutionarily conserved core that presents eightfold and pseudo-twofold rotational symmetry along the nucleocytoplasmic axis and axes coplanar with the nuclear envelope, respectively. This symmetric core consists of two opposing outer rings anchored atop each of the two nuclear membranes and an interposed inner ring that encircles the central transport channel. Within it, natively unfolded nucleoporin regions rich in phenylalanine-glycine motifs emanate from the inner ring to form the diffusion barrier. Outer rings are decorated by cytoplasmic filament and nuclear basket nucleoporins that break the pseudo-twofold symmetry.Though the arrangement of most “scaffold” folded nucleoporins in the NPC’s symmetric core was previously determined, the topology and molecular details of the multivalent natively unfolded “linker” nucleoporins that hold the scaffolds together remained uncharted. Combining biochemical reconstitution, mutational analysis, crystal and single-particle cryo-electron microscopy structure determination, docking into cryo-ET maps, and cell-based assay validation, we elucidated the molecular architecture and evolutionary conservation of the linker-scaffold network that mediates the rubber band-like cohesion of the NPC’s building blocks. Linkers insert short defined motifs into scaffold surface pockets, with flanking regions reinforcing the binding through disperse fuzzy interactions.The mechanical tethering of the nucleus to the cytoskeleton imparts membrane tension that dilates nuclear pores by at least ~20 nm. We show how the linker-scaffold coalesces the inner ring protomers into eight flexibly connected rigid spokes that translate along with the dilating pore to produce lateral gaps for the transport of membrane proteins, without disrupting the NPC’s structural integrity. Moreover, our near-atomic composite structures of the yeast and human NPCs in dilated and constricted states are roadmaps for the mechanistic dissection of NPC (dis)assembly, NPC-associated diseases, the directional anchoring of asymmetric nucleoporins, and the role of the NPC’s mechanosensation in nucleocytoplasmic transport.

B339/P2019

TorsinA is essential for the timing and localization of neuronal nuclear pore biogenesis

S. Kim[1], S. Phan[2], D. Boassa[2], T. R. Shaw[3], S. S. Pappas[4], M. H. Ellisman[2], S. L. Veatch[3], S. J. Barmada[5], W. T. Dauer[4]; [1]Cellular and Molecular Biology Graduate Program, University of Michigan Medical School, Ann Arbor, MI, [2]National Center for Microscopy and Imaging Research, Department of Neurosciences, University of California San Diego, San Diego, CA, [3]Department of Biophysics, University of Michigan, Ann Arbor, MI, [4]Peter O’Donnell Jr. Brain Institute, UT Southwestern, Dallas, TX, [5]Department of Neurology, University of Michigan Medical School, Ann Arbor, MI.

Nuclear pore complexes (NPCs) are large protein complexes composed of hundreds of nucleoporins that mediate nucleocytoplasmic transport of protein and RNA. NPC abnormalities are implicated in DYT1 dystonia, a neurodevelopmental movement disorder caused by a loss-of-function mutation in the gene encoding torsinA, a AAA+ protein localized to the endoplasmic reticular (ER)/nuclear envelope (NE) endomembrane space. Despite increasing evidence implicating NPC dysfunction in DYT1 and other neurological diseases, NPC biogenesis in neurons remains poorly understood. Furthermore, the biological function of torsinA and the molecular defects underlying DYT1 dystonia remain largely unknown. In mouse primary neurons, we observed a striking upregulation in NPC biogenesis during neuronal maturation. While NPCs are uniformly distributed in wild-type (WT) neurons, torsinA-knockout (KO) neurons develop severe clusters of NPCs. Despite abnormal localization, NPC density is normal in torsinA-KO neurons, suggesting that torsinA is essential for NPC spatial organization, but not number. TorsinA-KO neurons develop abnormal evaginations of the inner nuclear membrane (NE blebs) that coincide with the formation of abnormal NPC clusters. Using scanning transmission electron microscopy tomography, we find that clusters of NE blebs connect to the inner nuclear membrane via NPC-like pore structures. This finding establishes a spatial link between NPC clusters and NE blebs and is consistent with a model in which loss of torsinA causes halted NPC assembly and NE bleb formation at sites of new NPCs. However, while NPC clusters persist, NE blebs resolve as neurons continue to mature, allowing NPC assembly to proceed albeit with a delay compared to WT NPC formation. These data demonstrate that torsinA is dispensable for interphase NPC assembly and that its loss causes delayed, rather than permanently halted, NPC biogenesis. Taken together, our data suggest that torsinA is essential for proper spatial and temporal organization of NPCs during a key period of neuronal development and implicate delayed NPC biogenesis in the pathogenesis of DYT1 dystonia.

B340/P2020

Nucleoporins are degraded via upregulation of nuclear ESCRT-III/Vps4 complex in *Drosophila* models of C9-ALS/FTD

S. DUBEY, K. Maulding, H. Sung, T. Lloyd; Johns Hopkins University School of Medicine, Baltimore, MD

Disruption of nucleocytoplasmic transport (NCT) has been implicated in Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The mechanisms by which expansion of GGGGCC in C9orf72 disrupts NCT is poorly defined. In this study, we find that the expression of GGGGCC repeat induces proteasome-mediated degradation of select nucleoporins of the NPC in *Drosophila* adult brain. In addition, we show that Vps4 ATPase and the endosomal-sorting complex required for transport complex-III (ESCRT-III) are required for degradation of nucleoporins through proteasomal degradation pathway. Knockdown of ESCRT-III/Vps4 complex in GGGGCC expressing neuron restores nucleoporin levels. Reducing ESCRT-III/Vps4 complex also rescues nucleocytoplasmic transport and suppresses repeat toxicity. GGGGCC expression upregulates nuclear ESCRT-III/Vps4 expression, and expansion microscopy demonstrates that the nucleoporins are translocated into the cytoplasm before undergoing proteasome-mediated degradation. These findings demonstrate a mechanism for nucleoporin degradation and NPC dysfunction in neurodegenerative disease.

ER and Golgi Transport

B342/P2021

SURF4-induced tubular ERGIC selectively accelerates ER-to-Golgi transport kinetics

R. Yan[1,2], K. Chen[2], B. Wang[2], K. Xu[2]; [1]Department of Genetics, Harvard Medical School, Boston, MA, [2]Department of Chemistry, University of California, Berkeley, Berkeley, CA.

The endoplasmic reticulum (ER)-to-Golgi transport is the first and a critical step of protein secretion and intracellular sorting. Although the protein components of this pathway have been characterized in detail, we know relatively little about the cell biological basis of ER-to-Golgi transport, such as how morphologically diverse cargo carriers are shaped and their structure-function relationships. Combining fast multiplexed live imaging, super-resolution microscopy, and genetic perturbations, we identified a highly elongated tubular ER-Golgi intermediate compartment (t-ERGIC) that selectively expedites the ER-to-Golgi transport for soluble cargoes of the receptor SURF4. Lacking the canonical ERGIC marker ERGIC-53 yet positive for the small GTPases Rab1A/B, the t-ERGIC is further marked by its extraordinarily elongated and thinned shape. With its large surface-to-volume ratio, high intracellular traveling speeds, and ER-Golgi recycling capabilities, the t-ERGIC selectively accelerates the trafficking of SURF4-bound cargoes. The biogenesis and cargo selectivity of t-ERGIC both depend on SURF4, which recognizes the N-terminus of soluble cargoes and co-clusters with the selected cargoes to expand the ER-exit site. Knockdown of SURF4 precludes the formation of t-ERGIC, yet intriguingly, its cargoes can still be transported at a significantly slower rate, indicating a role of the SURF4/t-ERGIC axis in modulating cargo transport kinetics. Furthermore, we showed that in the steady state, the t-ERGIC-mediated fast ER-to-Golgi transport is antagonized by the KDEL-mediated ER retrieval, which may be harnessed to enrich specific proteins to the ERGIC. Together, our results suggest that specific cargo-receptor interactions give rise to distinct transport carriers that regulate the trafficking kinetics. Given the early embryonic lethality of SURF4 knockout in mice and the emerging results of SURF4 modulation in metabolic diseases, we speculate that the t-ERGIC-dependent cargo transport plays an essential role in embryonic development and tissue homeostasis through its regulation of cargo trafficking kinetics and thus the spatiotemporal dynamics of the secretome.

B343/P2022

Molecular mechanisms of SURF4-mediated protein secretion

J. Maldutyte, N. Gomez-Navarro, S. Peak-Chew, E. A. Miller; Cell Biology, MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

Protein secretion is an essential process that drives organelle biogenesis, cell growth and communication. Once synthesised and processed in the Endoplasmic Reticulum (ER), secretory proteins are incorporated into transport carriers that are generated by the COPII coat. Efficiency and accuracy of cargo incorporation into ER-derived carriers is considerably enhanced by transmembrane cargo receptors. ER export receptors are especially important for secretion of soluble cargo as they provide a transmembrane bridge to the cytosolic COPII coat. In this study, we focus on SURF4, a receptor for soluble secreted and lysosomal cargo. We characterised the spectrum of SURF4 clients in HEK-293TREx and HuH7 cell lineages using mass spectrometry. Amongst the top hits, we identified many oligomeric Ca^{2+}-binding proteins, including Cab45 and NUCB1. Using *in vitro* translation and site-specific photo-crosslinking, we show direct co-translational SURF4 engagement with the cargo via the N-terminal ER-

ESCAPE motif. This result supports a fast-export mechanism for preventing cargo oligomerization in an unwanted organelle and for the first time shows a cargo receptor is able to start interacting with an unfolded and not fully translated client. Additionally, with the aid of structural prediction-guided mutagenesis and site-specific cross-linking, we map a putative cargo interaction surface on SURF4 which comprises acidic residues in SURF4 luminal loops and a hydrophobic pocket. Altogether, these data define the SURF4 cargo repertoire and describe the biochemical basis for cargo recruitment into COPII vesicles.

B344/P2023

Cell cycle-dependent regulation of a COPII subunit by post-translational modification

G. Georgiou[1], B. Bisnett[2], B. Condon[3], S. Rodriguez[4], J. Maiwald[5], M. Boyce[1]; [1]Biochemistry, Duke University, Durham, NC, [2]Boston Consulting Group, Minneapolis, MN, [3]Drexel University, Philadelphia, PA, [4]Duke University, Durham, NC, [5]Haverford College, Haverford, PA.

Coat protein II (COPII) vesicles are responsible for faithfully packaging and trafficking one-third of the human proteome from the ER towards its final intra- or extracellular destinations. The coat is composed of a minimum of five subunits, including Sec24C, one of four human Sec24 paralogs, which forms heterodimers with Sec23 to assemble the COPII inner coat. Despite the biological significance of intracellular trafficking, the mechanisms by which COPII subunits, such as Sec24C, adapt to cellular and environmental cues that promote changes in ER behavior and protein flux remain mostly unknown. Previous work from our lab identified that Sec24C is post-translationally modified by the addition and removal of O-linked β-N-acetylglucosamine (O-GlcNAc), an intracellular form of glycosylation, on at least seven discrete residues. Though the purpose of these modifications on Sec24C remains incompletely understood, O-GlcNAcylation has previously been demonstrated to regulate several cellular processes, including cell death, cell cycle progression, and cytoskeleton assembly. Here, we examined the role of Sec24C glycosylation in the regulation of COPII trafficking through different stages of the cell cycle because trafficking burdens and COPII assembly have been shown to vary dramatically by cell cycle phase. We found that Sec24C directly interacts with Aurora A, a mitotic kinase, and phosphorylation of Sec24C by Aurora A may influence its glycosylation, and vice-versa. Sec24C demonstrates both increased glycosylation and phosphorylation in G_2/M-phase, relative to G_1. Additionally, preliminary data indicate that inhibition of Aurora A by the small molecule alisertib leads to an increase in Sec24C glycosylation. Therefore, cross-talk between O-GlcNAcylation and O-phosphorylation by Aurora A and/or other kinases may regulate Sec24C and COPII function during mitosis. Ongoing work is focused on identifying site-specific changes to Sec24C phosphorylation and glycosylation and their functions throughout the cell cycle by mass spectrometry and trafficking assays.

B345/P2024

Two O-GlcNAcylation Sites in the Disordered Region of Sec24D are Critical for Collagen Trafficking*in vivo*

T. Hirata[1], D. Choudhary[2], B. Bisnett[1], N. Linhart[1], E. Knapik[2], M. Boyce[1]; [1]Duke University, Durham, NC, [2]Vanderbilt University Medical Center, Nashville, TN.

Protein transport from the endoplasmic reticulum (ER) to the ER-Golgi intermediate compartment (ERGIC) or the Golgi is achieved by coat protein II (COPII)-coated vesicles. The COPII coat is comprised of inner and outer coat protein complexes and assembled at ER exit sites (ERES). The inner COPII coat is

composed of Sec23-Sec24 heterodimers, and Sec13-Sec31 heterotetramers form the outer coat. COPII-dependent transport of proteins is known to be regulated by post-translational modifications, such as phosphorylation and O-GlcNAcylation. O-GlcNAcylation is a reversible form of glycosylation found on many nuclear and cytosolic proteins in many organisms. OGT transfers GlcNAc residues on the Ser/Thr residues of substrate proteins, whereas OGA removes GlcNAc from the substrate proteins. Recently, we identified multiple O-GlcNAcylated sites on several COPII components, including Sec24D, one of the four Sec24 isoforms in vertebrates. However, how O-GlcNAcylation regulates the functions of COPII is largely unknown.In this study, we aimed to uncover the function of O-GlcNAcylation on Sec24D. We mutated two glycosites on human Sec24D (T9 and S13) and analyzed their function by taking advantage of the Sec24D-mutant zebrafish line, *bulldog*, which accumulates intracellular collagen due to trafficking defects. We first examined Sec24D O-GlcNAc levels in human cells and confirmed significant reductions in glycosylation in both the T9A and S13A mutants. Next, we expressed either human Sec24D wild type or mutants in *bulldog* zebrafish embryos and examined the intracellular accumulation of collagen in chondrocytes. Expression of wild type human Sec24D in *bulldog* zebrafish restored normal collagen trafficking, whereas the T9A and S13A mutants did not, indicating that O-GlcNAcylation on these sites is critical for collagen trafficking. To better understand the molecular mechanisms behind these observations, we purified proteins interacting with wild type or mutant Sec24D, with or without OGA inhibition. Preliminary data indicate that some proteins interact with mutant, but not wild type, Sec24D, and these interactions are reduced by OGA inhibition, suggesting that O-GlcNAcylation on these sites may prevent protein binding. Therefore, O-GlcNAcylation on human Sec24D may facilitate COPII-dependent collagen trafficking by regulating protein-protein interactions.

B346/P2025

Non-degradative Ubiquitin Signals are the Key Regulators of COPI-mediated ER-Golgi Homeostasis

S. S. Date, 1, T. R. Graham; Vanderbilt University, Nashville, TN

The ER and the Golgi play a central role in protein processing and sorting. As proteins move constantly between these organelles and Golgi compartments, proper retrieval, sorting, and localization of resident proteins, chaperones, and coat components becomes increasingly important to maintain functional ER-Golgi dynamics. Perturbations in retrieval, sorting and localization of ER-Golgi proteins are associated with neurodegenerative diseases, autoimmune disorders, and congenital diseases. COPI plays a critical role in retrieval and retrograde trafficking of proteins between the Golgi compartments and from Golgi to ER. The prevailing notion in the trafficking field is that COPI recognition of dilysine motifs is the primary mechanism for COPI to recognize and retrieve cargo. However, this notion does not explain the dependence of cargoes that do not have dilysine motifs on COPI, or why cells grow normally when both dilysine-binding sites in COPI are mutated. These observations indicate additional mechanisms for COPI to recognize, bind, and sort cargoes. We previously demonstrated that the WD repeat domains of alpha and beta'-COP bind polyubiquitin chains. Several COPI subunits, cargos, and ArfGAP Glo3 are ubiquitinated under physiological conditions with non-degradative ubiquitin linkages. Detailed analysis of COPI-dependent cargo localization and coat-cargo interactions using live-cell imaging, SILAC mass spectrometric analysis, and biochemical studies provide compelling evidence that the ability of COPI to bind ubiquitin, but not dilysine motifs, is critical for the proper localization of several important COPI cargoes such as the SNAREs Snc1, Gos1, Bet1, and Sec22. Additionally, we also show the dependence of some key Golgi-ER chaperones on the ability of COPI to recognize ubiquitin. Altogether our results help

redefine the fundamental mechanisms associated with cargo recognition by COPI and provide novel insights into how non-degradative ubiquitination plays a key role in ER-Golgi homeostasis.

B347/P2026

Acute COG inactivation unveiled its immediate impact on Golgi & illuminated the nature of intra-Golgi recycling vesicles

F. Sumya, I. Pokrovskaya, Z. D'Souza, V. Lupashin; UAMS, Little Rock, AR.

Conserved Oligomeric Golgi (COG) complex orchestrates intra-Golgi retrograde trafficking and glycosylation of macromolecules, but the detailed mechanism of COG action is unknown. The auxin-inducible degradation system was employed to investigate initial defects resulting from COG dysfunction. This approach allows a speedy and efficient depletion of COG4, which provides the ability to accumulate COG complex-dependent (CCD) vesicles and investigate primary cellular defects associated with the acute depletion of COG. The results revealed that upon COG4 depletion, both lobe A and lobe B COG subcomplexes were mislocated from Golgi. As predicted, v-SNAREs (GS15, GS28) were relocalized into CCD vesicles, while t-SNAREs (STX5, YKT6) remained attached to the Golgi membrane. Coiled-coil tethers also demonstrated differential response: t-tethers GM130 and P115 remained associated with Golgi, while the v-tethers (giantin and golgin-84) show off-Golgi patterns in COG4 depleted cells. Surprisingly, the majority of COG complex-interacting Golgi Rab-GTPases (Rab2a, Rab6a, Rab30a) did not relocate to CCD vesicles after acute COG4 depletion. These results indicate that COG interacting Rabs are primarily acting from the acceptor Golgi membrane during the intra-Golgi vesicle recycling process. Importantly, all tested Golgi enzymes (B4GalT1, MGAT1, GalNT2, FUT 8) and Golgi resident proteins (GPP130, TMEM165, SDF4, TGN46) were significantly relocalized from Golgi into CCD vesicles. Accumulated CCD vesicles were relatively stable - partial degradation of vesicle content was observed only after prolonged (24-48 hours) COG depletion. Superresolution microscopy analysis of CCD vesicles indicated that medial and trans-Golgi enzymes MGAT1 and B4GalT1 recycle in different populations of CCD vesicles. Native IP was used to separate and characterize different classes of CCD vesicular carriers. Moreover, acute COG depletion significantly affected three Golgi-based vesicular coats- COPI, AP1, and GGA, suggesting that COG uniquely orchestrates tethering of multiple types of intra-Golgi CCD vesicles produced by different types of coat machinery. In summary, the study provided a first detailed view of primary cellular defects associated with COG complex dysfunction and uncovered heterogeneous nature of vesicular intermediates that recycle Golgi enzymes and other resident Golgi proteins in human cells.

B348/P2027

Two Model Secretory Cargoes Show Different Kinetics of Golgi Export

F. M. Valbuena, B. S. Glick; The University of Chicago, Chicago, IL

The Golgi apparatus processes and sorts biosynthetic cargo proteins, but there are still questions about the basic pathways of cargo transit through the Golgi. *Saccharomyces cerevisiae* contains non-stacked Golgi cisternae, and cargoes are continuously present within these cisternae, which mature over time by means of retrograde vesicles that recycle resident Golgi proteins. A similar cisternal maturation process is thought to operate in mammalian cells, in which Golgi stacks are laterally connected to form a Golgi ribbon. The implication is that cisternae progress through each Golgi stack in a conveyor belt-like fashion while carrying the cargoes forward. However, the existence of cisternal maturation in mammalian cells

has been challenged based on two findings. First, the kinetics of cargo export from the Golgi were reportedly exponential-like, in disagreement with the expectation that a conveyor belt-like mechanism would yield linear kinetics. Second, cargoes reportedly exchanged between Golgi ribbons in fused cells, in disagreement with the expectation that cargoes would remain within the cisternae. We have now revisited both issues using inverse fluorescence recovery after photobleaching (iFRAP). Our analysis took advantage of "mini-ribbons", which are small Golgi elements separate from the main ribbon. Mini-ribbons receive and export secretory cargoes, and they exchange resident Golgi proteins with the main ribbon. One set of experiments employed the transmembrane secretory cargo VSVG. We find that VSVG departs from Golgi mini-ribbons with linear kinetics. These results support the cisternal maturation model. A second set of experiments employed the soluble secretory cargo ESCargo. Interestingly, when iFRAP is performed using the main Golgi ribbon, ESCargo departs with exponential-like kinetics, but when iFRAP is performed using mini-ribbons, ESCargo departs with linear kinetics. These results can be explained by the observation that ESCargo moves between Golgi stacks, as detected by exchange between the main ribbon and mini-ribbons. We postulate that ESCargo recycles at some frequency within the Golgi, probably by partitioning into retrograde vesicular carriers. Our data point toward a unified picture of intra-Golgi transport in diverse eukaryotes.

B349/P2028

Filling in the GAPs- Spatiotemporal analysis of Arf1 regulation at the Golgi

K. Manzer, C. Fromme; Cornell University, Ithaca, NY.

The Golgi serves as the cellular hub for intracellular membrane trafficking. Golgi trafficking is orchestrated by the small GTPase Arf1, which recruits several effector proteins to the Golgi membrane at precise points in cisternal maturation to facilitate vesicle formation. To achieve this, Arf1 is tightly controlled by GEFs and GAPs, which reside on specific compartments to activate and inactivate Arf1, respectively. The differential localization of GEFs and GAPs is essential for maintaining proper Arf1 function. While the spatiotemporal dynamics of the GEFs are well-characterized, they were unknown for the Arf GAPs. Through the use of live-cell time-lapse fluorescence microscopy, we established the timing of each of the yeast Golgi-localized Arf GAPs relative to the major Golgi trafficking pathways. We found that a distinct GAP is present at each stage of Golgi maturation, with Glo3 functioning at the early Golgi, Gcs1 at the medial Golgi, and Age2 at the late Golgi. To further understand how the differential localization of the GAPs is achieved, we are investigating the mechanism of localization of the particularly unstudied Arf GAP, Age2. We identified a highly conserved sequence in Age2 that may comprise an amphipathic helix and is essential for membrane binding. Interestingly, we also found that the interaction between the catalytic GAP domain and its substrate, Arf1, is required for Age2 recruitment. Lastly, despite the role of Age2 in clathrin-dependent trafficking, we did not observe a role for clathrin or its adaptors in Age2 localization. This ongoing work establishes a timeline for Arf1 regulation at the Golgi and provides mechanistic insight into how this timeline is achieved.

B350/P2029

SARS-CoV-2 Envelope hijacks an ARFRP1-AP1-AP1AR *trans*-Golgi network-to-endolysosome trafficking pathway to drive lysosomal deacidification

G. Pearson, M. Broncel, A. Snijders, J. Carlton; Francis Crick Institute, London, United Kingdom

The SARS-CoV-2 structural proteins Membrane(M), Envelope (E), Nucleocapsid (N), and Spike (S), assemble into nascentvirions at the ER-Golgi-Intermediate Compartment (ERGIC) and bud into thesecretory pathway lumen to allow viral egress. Recent work has described anatypical secretion route for coronaviruses involving delivery to deacidifiedlysosomes and subsequent release through lysosome-plasma membrane fusion. Here,by developing inert tagging strategies for SARS-COV-2 E, we identifytrafficking motifs and host cell interaction partners required for itsER-export, its delivery to the ERGIC and its forward traffic from the *trans*-GolgiNetwork (TGN) to endolysosomes. We show that SARS-CoV-2 is oligomeric inendolysosomes and contributes to pH neutralisation in these organelles. ACRISPR-knockout screen based on our TurboID proximity-ome reveals roles for theARF1-related GTPase, ARFRP1, in controlling Golgi-to-endolysosome traffickingof SARS-CoV-2 E. We identify an interaction between SARS-CoV-2 E's cytoplasmicdomain and the beta-adaptin subunit of AP1 and show that both GGA1 and AP1 andthe microtubule adaptor for AP1 cargo, AP1AR, are necessary for delivery ofSARS-CoV-2 E from the Golgi to endolysosomes. Given the recent evidence thatAP1 is an essential host-factor for for SARS-CoV-2 replication, we suggest thatE-driven lysosomal deacidification may contribute to a favourable replicationenvironment for this virus.

B351/P2030

Altered secretory trafficking and processing of amyloid precursor protein variants associated with familial Alzheimer's disease

J. Wang, L. Fourriere, P. A. Gleeson; Bio21 Institute, University of Melbourne, Melbourne, Australia

Alzheimer's disease (AD) is characterized by high levels of neurotoxic amyloid beta peptides (Aβ) which are generated by the cleavage of amyloid precursor protein (APP) by proteolytic secretases. The intracellular processing of APP, and secretion of Aβ, is regulated by the trafficking itineraries of APP and its secretases. However, the relative contribution of APP processing in the secretory and the endocytic pathways in AD is poorly defined. Here, we employ the Retention Using Selective Hooks (RUSH) technique to track the anterograde trafficking and processing of newly synthesized APP. We define the anterograde trafficking, and temporal and spatial regulation of cleavage events, of wild-type APP (APPwt), as well as the disease-causing Swedish APP variant (APPswe), and the disease-protective Icelandic APP variant (APPice). Interestingly, we find that both APP variants displayed altered trafficking and processing along the secretory pathway compared with APPwt. We show that newly synthesized APPswe is transported through the Golgi less efficiently compared with APPwt, and is associated with enhanced amyloidogenic APP processing and Aβ production. In contrast, APPice is transported more rapidly through the Golgi apparatus to the endosomal compartments, and is associated with lower Aβ production through the secretory pathway. These data reveal that APP and the familial APP variants differ in the kinetics of transport through the Golgi to the endocytic pathway, findings relevant for understanding the molecular mechanisms associated with AD susceptibility of the familial APP mutations, and the design of therapeutics to reduce Aβ production.

B352/P2031

***TRAPPC6B* Biallelic Variants are Associated with TRAPP II-Specific Defects and Cause Neurodevelopmental Disorder and Microcephaly in Humans**

H. Almousa[1], S. Lewis[2], R. Maroofian[3], M. Kreuer[2], M. Sacher[1]; [1]Department of Biology, University Concordia, Montreal, QC, CANADA, [2]Department of Child Health and Neurogenetics, University of Arizona, Arizona, AZ, [3]Department of Neuromuscular Disorders, Queen Square Institute of Neurology, London, UNITED KINGDOM.

Background The transport protein particle (TRAPP) complexes play essential roles in vesicular trafficking pathways that are crucial for proper cell functioning. Defects in some TRAPP subunits are associated with neurodevelopmental disorders in humans. **Methods** Through Genematcher, we gathered multiple patients with biallelic mutations in the *TRAPPC6B* gene and conducted detailed clinical and neuroradiologic assessments. We then performed functional studies to assess the effect of the identified variants on Golgi integrity and membrane trafficking. **Results** We identified 7 homozygous nonsense (n=12 patients) and 7 canonical splice site mutations (n=13 patients). In addition, we identified a single patient with a compound heterozygous mutation (one nonsense allele and one amino acid substitution allele) with a milder phenotype. All individuals suffer from microcephalic neurodevelopmental disorder, speech impairment and movement disorder. MRI imaging revealed reductions in several brain regions (cortex, cerebellum, and corpus callosum) with frequent white matter hyperintensity. *Drosophila* locomotor studies supported a role of the *TRAPPC6B* ortholog in neuromotor function. We focused our functional studies on two individuals with the following homozygous variants: c.454C>T, p.Q152* (individuals S1 and S2) and c.149+2T>A, predicted splice variant (individuals S3 and S4). Molecular studies revealed a weakened interaction between mutant TRAPPC6B (c.454C>T, p.Q152*) and its TRAPP binding partner TRAPPC3. Patient-derived fibroblasts from S1 and S2 have reduced levels of TRAPPC6B as well as TRAPP II complex-specific members (TRAPPC2L, TRAPPC9 and TRAPPC10) Interestingly, the levels of the TRAPPC6B homologue TRAPPC6A were found to be elevated. The reduction in TRAPPC6B affects Golgi integrity and trafficking into the Golgi in both S1 and S2, as well as in S3 and S4-derived fibroblasts. These defects were rescued by wild type TRAPPC6B. Interestingly, treatment of S2 fibroblasts with Ataluren, a small molecule that reads through premature stop codons, also rescued the trafficking defect, suggesting a possible therapeutic approach. **Conclusion** Our data provide additional support for TRAPPC6B biallelic variants association with neurodevelopmental disorder, microcephaly, language impairments and movement disorder in humans. These phenotypes overlap with alterations in other TRAPP subunits, mainly components of the TRAPP II complex. This suggest that TRAPPC6B is essential for brain development through its role in the TRAPP II complex.

B353/P2032

Steady-state Regulation of Secretory Cargo Export by Inositol Trisphosphate Receptors and Penta EF Hand Proteins

A. J. Held[1], J. R. Sargeant[1], J. Hojanazarova[1], C. Madreiter-Sokolowski[2], R. Malli[2], W. F. Graier[2], J. C. Hay[1]; [1]University of Montana, Missoula, MT, [2]Medical University of Graz, Graz, AUSTRIA.

Ca^{2+} release by inositol trisphosphate receptors (IP3Rs) regulates diverse physiological processes in many cell types. Notably, agonist-stimulated Ca^{2+} signaling involving IP3Rs can modulate ER export rates through activation of the Ca^{2+}-dependent COPII coat adaptor, ALG-2. It is unknown, however, whether

IP3Rs and ALG-2 regulate ER export rates at steady state. Here we report that partial depletion of IP3Rs from normal rat kidney (NRK) epithelial cells caused a marked increase of ER export of the transmembrane cargo VSV-G. Depletion of IP3R-3, the major IP3R isoform in NRK cells, had a larger impact on secretion than the other extant isoform, IP3R-1. Under standard growth conditions, spontaneous cytosolic Ca^{2+} oscillations usually occurred simultaneously in successive groups of contiguous cells, generating intercellular Ca^{2+} waves (ICWs) that moved across the monolayer. Unexpectedly, IP3R-3-depleted cells exhibited increased cell participation in this spontaneous signaling. IP3R-3-depleted cells also exhibited increased agonist-dependent Ca^{2+} signaling, suggesting a general potentiation of the remaining IP3R-1 channels. Increased spontaneous signaling was accompanied by increased ALG-2 and decreased peflin at ER exit sites (ERES), resulting in increased targeting of Sec31 to ERES. Furthermore, the effects of IP3R depletion on the secretion rate required ALG-2. Kinetic colocalization analysis found that in IP3R-3 depleted cells, VSV-G was sequestered more quickly into ERES, suggesting that an early step in ER export, perhaps cargo sorting, was affected. In addition, while IP3R-3 depletion caused increased export of the COPII client cargo VSV-G, it caused decreased export of the bulk flow cargo construct GFP-F_M4-hGH, once again pointing to cargo sequestration or sorting as the functional locus of Ca^{2+} and ALG-2. We conclude that in NRK cells, IP3Rs regulate spontaneous Ca^{2+} signaling that helps determine the basal secretion rate by modulating early COPII-dependent steps through ALG-2. Furthermore, the IP3R channel density and/or isoform composition significantly impacts this phenomenon.

B354/P2033

An ERAD-independent role for rhomboid pseudoprotease Dfm1 in mediating sphingolipid homeostasis.

S. Bhaduri, S. E. Neal; UNIVERSITY OF CALIFORNIA San Diego, San Diego, CA.

Nearly a third of nascent proteins are targeted to the endoplasmic reticulum (ER) where they are correctly folded and assembled before being delivered to their final cellular destinations. To prevent the accumulation of toxic misfolded membrane proteins, ER-associated-degradation (ERAD) removes these clients from the ER membrane to the cytosol in a process known as retrotranslocation. Recent work from our lab demonstrated that rhomboid pseudoprotease Dfm1 is involved in the retrotranslocation of ubiquitinated integral membrane ERAD substrates. To survey for potential interaction partners of Dfm1 we performed protein-proximity labeling by BioID (proximity-dependent biotin identification) followed by mass spectrometry and identified several interacting proteins known to play a role in the sphingolipid biosynthesis pathway. Specifically we found that Dfm1 physically interacts with the SPOTS complex which is composed of serine palmitoyltransferase (SPT) enzymes and accessory components, critical for catalyzing the first rate-limiting step of the sphingolipid biosynthesis pathway. We demonstrate for the first time that Dfm1 has a role in ER-Golgi export, a function that is independent of Dfm1s canonical ERAD retrotranslocation function. Specifically, we show that loss of Dfm1 results in the accumulation of phosphorylated Orm2 (a negative regulator of SPT) at the ER, suggesting a novel role for Dfm1 in trafficking Orm2 from the ER and its subsequent degradation by EGAD (Endosome and Golgi associated degradation). Moreover recruitment of AAA ATPase Cdc48 by Dfm1 which is critical for its role in ERAD retrotranslocation is dispensable for Dfm1s role in ER export. Given that the accumulation of human Orm2 homologs ORMDLs are associated with many maladies our study serves as a molecular foothold for understanding how dysregulation of sphingolipid metabolism leads to various diseases.

B355/P2034

Topological Regulation of a Transmembrane Protein by Luminal-to-Cytosolic Retrotranslocation of Glycosylated Sequence

J. Wang*, S. Han*, J. Ye; Department of Molecular Genetics, UT Southwestern Medical Center, Dallas, TX.

* Co-first authors.

Transmembrane proteins must adopt proper topology to perform their functions. The topology of a membrane protein, which depicts the orientation of their soluble segments relative to the plane of membranes, are thought to be fixed and determined by their primary sequence. This assumption has been challenged by recent observations demonstrating that some transmembrane proteins can adopt more than one topology, and topological alteration has emerged as a mechanism to regulate transmembrane proteins.

We recently reported that in mammalian cells, ceramide can alter the topology of TM4SF20 (transmembrane 4 L6 family 20). In cells cultured under normal conditions, TM4SF20 primarily exists with the C-terminus in the lumen. This form of TM4SF20 inhibits proteolytic activation of CREB3L1 (cAMP response element binding protein 3-like 1). In cells treated with doxorubicin, a chemotherapeutic reagent, the accumulation of ceramide results in production another form of TM4SF20 with a cytosolic C-terminus. This form of the protein stimulates proteolytic activation of CREB3L1, allowing the transcription factor to active expression of genes that inhibit cancer cell proliferation. Thus, the topology of TM4SF20 is regulated by ceramide level, but the mechanism for topological regulation of TM4SF20 was obscure.

In the current study, unexpectedly, we uncovered a retrotranslocation process that plays an important role in the topology regulation. We demonstrated that TM4SF20 is synthesized in the endoplasmic reticulum (ER) with a cytosolic C-terminus and a luminal loop before the last transmembrane helix where N132, N148 and N163 are glycosylated. In the absence of ceramide, trafficking of TM4SF20 from ER to Golgi allows complete retrotranslocation of the sequence surrounding glycosylated N163 but not N132 from lumen to cytosol. Accompanying this retrotranslocation, the C-terminus of the protein is relocated from cytosol to lumen. Ceramide inhibits the retrotranslocation process by delaying the ER-to-Golgi traffic of TM4SF20, thereby causing accumulation of the ER form of the protein. The retrotranslocation event was revealed by a novel method we developed to determine the localization of N-linked glycosylation sites by PNGase F treatment on sealed microsomes: N-Glycans in the cytosol are trimmed without detergent, while those in the lumen require detergent to be accessible by PNGase F. Our observations suggest that topology of a transmembrane protein may not be fixed once the protein is synthesized, and it can be altered through retrotranslocation. Thus, in addition to ER-associated degradation (ERAD) of transmembrane proteins, retrotranslocation may also play a critical role in regulating the topology of these proteins.

B356/P2035

Endoplasmic reticulum is involved in cell membrane repair

Y. Hirose[1], R. Yamanaka[2], **K. Miyake**[3]; [1]Sch. Med., Int. Univ. Health & Welfare, Narita, JAPAN, [2]Sch. Health Sci. Narita, Int. Univ. Health & Welfare, Narita, JAPAN, [3]Cent. Basic Med. Res. Narita, Int. Univ. Health & Welfare, Narita, JAPAN.

Plasma membrane disruption is repaired by mechanism involving Ca^{2+}-regulated exocytosis, but the origin of these vesicles is unknown. Using the super-resolution system Zeiss Airyscan (Airyscan), we have successfully imaged the dynamics of Sec61βGFP-expressing ER during the repair of two-photon laser-induced membrane damage in BS-C-1 cells. The tubular endoplasmic reticulum collapsed instantly, and numerous ER vesicles were formed near the damaged membrane site. However, in the presence of extracellular calcium, these segmented vesicles did not fuse to the plasma membrane and slowly fused each other to form the original tubular ER. On the other hand, staining of unfixed damaged membranes with calnexin antibody resulted in staining, strongly supporting the fusion of the ER with the damaged area. Furthermore, Airyscan observation with Brefeldin A (BFA), which inhibits retrograde transport from the ER to the Golgi sac, revealed tubes extending from the ER to the plasma membrane during membrane repair and subsequent vesicle columns. Those segmented vesicles were observed to slowly fuse into the damaged membrane like a meteor shower. This suggests that the BFA is putting a brake on the fusion of vesicles that would normally fuse to the damaged membrane at high speed. Finally, a plasma membrane repair assay labeled for ATP leakage showed that BFA clearly inhibits plasma membrane repair. This suggests that plasma membrane repair requires calcium influx and that the necessary speed at which vesicles fuse to the damaged membrane is not captured by our conventional microscopy techniques. In contrast, we did not observe BFA-induced membrane fusion of Sec61β-GFP in skeletal muscle fibers, nor did we observe any effect of Sec61β-GFP on membrane repair.

B357/P2036

IP6K1- a novel regulator of gastric chief cell secretion in mammalian digestion physiology

J. Sen, R. Bhandari; Laboratory of Cell Signalling, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, INDIA.

Inositol hexakisphosphate kinases (IP6Ks) catalyse the synthesis of the inositol pyrophosphate 5-IP7 (5-diphosphoinositol pentakisphosphate) from IP6 (inositol hexakisphosphate). Mice lacking the IP6K1 enzyme show different physiological defects including male infertility, delay in blood clotting, reduced insulin levels, and lower body weight compared to wild type mice. The expression of IP6K1 is conserved throughout the gastrointestinal tract epithelium, including stomach, ileum, colon, and rectum. We have observed that IP6K1 is highly expressed in isolated mouse gastric glands. Chief cells of the stomach secrete enzymes like pepsin and gastric lipase that help in digestion of protein and lipid ingested in food. Immunofluorescence studies show that IP6K1 is localized to chief cells in the gastric gland. Pyloric ligation of the stomach followed by carbachol treatment revealed that gastric secretion from IP6K1 knockout mice has lower levels of pepsin and gastric lipase compared with wild type mice. IP6K1 knockout mice also display a reduced number of chief cells in their gastric glands. Chief cells lacking IP6K1 show a reduction in the number of secretory granules containing pepsinogen C and gastric lipase, and also show defective Golgi morphology. In the AGS gastric cancer cell line, CRISPR mediated knockout of IP6K1 led to reduced granular intensity of pepsinogen C, correlating with the phenotype observed in mouse gastric glands. Using mass spectrometry analysis, we have identified IP6K1 binding proteins in

AGS cells that are involved in vesicle formation and exocytosis. We propose that IP6K1 expression in gastric chief cells plays an important role in the regulation of secretory granule formation and digestion physiology in mammals.

B358/P2037

Jagn1 isoforms are tissue specific critical regulators of zebrafish development

H. M. Rosengrant, P. E. Vanwinkle, E. Sztul, E. J. Lee; Department of Cell, Developmental and Integrative Biology, University of Alabama At Birmingham, Birmingham, AL.

The Jagunal homolog 1 (Jagn1) protein is a transmembrane endoplasmic reticulum (ER) component that cycles at the ER-Golgi interface. Jagn1 is highly evolutionarily conserved from humans to plants, suggesting that it is essential to support one or more fundamental cellular process(es). Jagn1 was first identified in the fruit fly species D.melanogaster as an essential component for egg development, possibly playing a role in the trafficking of yolk proteins. Subsequent studies showed that humans with homozygous mutations in the Jagn1 gene exhibit Severe Congenital Neutropenia caused by impaired neutrophil maturation. Neutrophils obtained from these patients contain decreased numbers of secretory granules and enlarged ERs, suggesting a possible ER to Golgi traffic defect.The sequence of Jagn1 does not have any similarities to any other protein, but Jagn1 is a tetraspanin, and therefore belongs to a group of proteins known to “chaperone” cargo proteins during their trafficking between compartments. Consistent with that function, Jagn1 contains sorting motifs that may implicate it in trafficking cargo proteins in the anterograde and retrograde directions between the ER and the Golgi. To study the organismal functions of Jagn1, we have a developed a zebrafish D.rerio model system. Zebrafish have two JAGN genes: Jagn1a (expressed almost exclusively in the brain) and Jagn1b (expressed ubiquitously). We utilized the CRISPR/Cas9 system to generate fish deleted of either JAGN. We uncovered that the Jagn1a knockout zebrafish larvae display no obvious phenotypes and grow to adulthood, but exhibit a neuronal defect reflected in altered circadian rhythm. In contrast, Jagn1b knockout larvae die early in development, with none reaching adulthood. We are currently probing the physiological basis of larval death.To understand the molecular role that Jagn1 performs in cells, we aim to elucidate what other proteins Jagn1 interacts with in cells. We have generated Jagn1 constructs tagged with the BirA* biotin ligase at either the N or the C-terminus; that when supplemented with biotin will biotinylate proteins neighboring Jagn1. Such biotinylated proteins are then purified on streptavidin beads and identified via mass spectrometry. A number of putative interactors have been identified and will be tested for direct binding. Jagn1 has also been implicated in a cellular response to ER stress, and we are also investigating if and/or how the Jagn1 interactome changes during cellular stress. Together, our studies provide a fundamental new insight into the role of Jagn1 in cellular and organismal homeostasis.

B359/P2038

Tmed10 regulates the secretion of insulin like growth factor 2 for myoblast differentiation

F. Yang[1], **T. LI**[1], Y. Heng[1], S. Zhou[1], G. Wang[1], J. Wang[2], X. Chen[1], Z.-P. Yao[2], Z. Wu[1], Y. Guo[1]; [1]Hong Kong University Sci & Technol, Hong Kong, China, [2]The Hong Kong Polytechnic University, Hong Kong, China

The insulin-like growth factor 2 (IGF2) plays critical roles in cell proliferation, migration, differentiation, and survival. Despite its importance, the molecular mechanisms mediating the secretion of IGF2 remain unclear. Here we utilized a Retention Using Selective Hook (RUSH) system to analyze molecular

mechanisms that regulate the secretion of IGF2. We found that a type I transmembrane protein, TMED10, is essential for the secretion of IGF2 and for differentiation of mouse myoblast C2C12 cells. Further analyses indicate that the residues 112-140 in IGF2 are important for the secretion of IGF2 and these residues directly interact with the GOLD domain of TMED10. We then reconstituted the release of IGF2 into COPII vesicles. This assay suggests that TMED10 regulates the packaging of IGF2 into COPII vesicles to be efficiently delivered to the Golgi. Moreover, TMED10 also regulates ER export of newly synthesized TGN-localized cargo receptor, sortilin, which subsequently regulates TGN export of IGF2. These analyses indicate that TMED10 is critical for IGF2 secretion by directly regulating ER export and indirectly regulating TGN export of IGF2, providing novel insights into the trafficking of IGF2 for myoblast differentiation.

B360/P2039

An in vitro vesicle formation assay to reveal novel cargo proteins that depends on AP-1 or AP-4 to be exported out of the trans Golgi network

Z. PENG, J. Fan, Y. GUO; Hong Kong University of Science and Technology, Hong Kong, China

The *trans*-Golgi network (TGN) is an essential station of protein sorting in the secretory transport pathway. The fidelity of protein transport depends on package of specific cargo proteins into transport vesicles, followed by delivery to their destinations. Adaptor protein (AP) complexes are key players that mediate protein sorting at the TGN. Although significant progresses have been achieved in understanding the underlying molecular mechanisms, the spectrum of cargo proteins that depends on a specific AP complex to be incorporated into transport vesicles remains largely unclear. Here, we performed an in vitro vesicle formation assay using wild type HeLa cells or HeLa cells knockout (KO) of the gamma subunit of AP-1 or the epsilon subunit of AP-4. Through this approach, we reconstituted the requirement of AP-1 or AP-4 to package their clients, Vangl2 and ATG9A, into transport vesicles. We then performed label-free quantitative mass spectrometry analyses of the isolated vesicles from wild type cell or KO cells. This quantitative analyses revealed specific clients of AP-1 and AP-4. Further analyses indicate that one of these identified cargo proteins, 45 kDa calcium-binding protein (Cab45), depends on AP-1 to be enriched into TGN-derived vesicles. Our study identified novel AP-1 and AP-4 clients and indicates that the vesicle formation assay is a powerful approach to reveal novel cargo clients of specific cargo adaptors.

B361/P2040

Characterization of the Distribution and Kinetics of the Yeast Golgi Glycosylation Machinery

C. F. Lee-Smith, B. S. Glick; University of Chicago, Chicago, IL

A core function of the Golgi apparatus is the post-translational modification of secretory cargoes, primarily through N- and O-linked glycosylation. There is a long-standing assumption that Golgi glycosylation enzymes are arranged in a polarized distribution that matches the order of oligosaccharide processing. However, the distribution of yeast glycosylation enzymes has not been rigorously evaluated in the context of Golgi maturation. Here, we describe an optimized method for the fluorescent tagging and visualization of yeast glycosylation enzymes, and we outline steps for achieving a precise mechanistic understanding of the intra-Golgi distribution of the glycosylation machinery.
Functional tagging of yeast glycosylation enzymes is challenging. These enzymes are type II integral membrane proteins. The short N-termini are critical for Golgi localization, and the C-termini are exposed

to an oxidizing luminal environment that can cause misfolding of fluorescent tags. C-terminal tagging of certain glycosylation enzymes such as Och1, Kre2, and Mnn1 with an oxidation-resistant GFP variant results in missorting of the proteins to the vacuole, presumably due to recognition by a quality control system. We found that an internal truncation of the vacuolar hydrolase receptor Vps10 improves the localization of Golgi enzymes and reduces their missorting to the vacuole. The additional deletion of a Vps10 homolog, which we term Vth3, further enhances the Golgi localization of Och1.
This strain background will be critical for assessing the arrival and departure kinetics of yeast glycosylation enzymes. We will perform kinetic analyses of Och1 and Mnn1, which are the initiating and terminating enzymes for N-linked glycosylation, respectively, and also of Kre2 and other enzymes involved in glycosylation. Kinetic data will enable us to determine when each enzyme is present during Golgi maturation, and which membrane traffic pathway each enzyme follows to recycle within the Golgi. The results will provide the first comprehensive map of Golgi glycosylation enzyme distribution in any organism.

B362/P2041

Auto-inhibitory interactions of Sec7, master regulator of the Golgi complex

B. Brownfield, J. Fromme; Cornell University, ITHACA, NY

In eukaryotes, protein and lipid secretory trafficking is orchestrated by the Golgi apparatus where proteins are modified and sorted in a cascade of functionally distinct compartments. The composition of these compartments is maintained primarily by vesicular traffic facilitated by Arf and Rab GTPases. These GTPases are regulated by a GTP/GDP cycle facilitated by Guanine Nucleotide Exchange factors (GEFs) and GTPase Activating Proteins (GAPs) to induce GDP/GTP exchange and GTP hydrolysis respectively. Arf1 and its close paralogs are required for virtually all vesicle biogenesis events at the Golgi complex. At the final compartment, the *trans*-Golgi network, these vesiculation events are tightly regulated to balance membrane flux through endosomal and secretory pathways. In budding yeast, Arf1 is activated at the *trans*-Golgi network by the Arf GEF Sec7, the homolog of the human ARFGEF/BIG proteins. Previous work has determined that Sec7 is regulated by autoinhibition, positive feedback, and GTPase crosstalk, yet the mechanistic basis for these regulatory features is unknown. To understand how Sec7 is regulated, I have determined the structure of the flexible Sec7 dimer by cryoEM at 4.9 Å resolution. Focused refinements on individual monomer subunits improved resolution to 3.8 Å, sufficient for molecular model building. This structure reveals the mechanism for Sec7 autoinhibition: an interaction between the catalytic GEF domain and the HDS domains occlude the catalytic surface. AlphaFold modeling predicts the putative active conformation, with the GEF domain positioned near the putative membrane binding site. Functional studies are currently underway to determine the physiological relevance of our structural findings.

B363/P2042

Selective ER-Golgi traffic of G protein-coupled receptors: role of the ER-localized C1orf27-UFSP2 complex

X. Xu, H. Li, G. Wu; Augusta University, Augusta, GA

G protein-coupled receptors (GPCRs) regulate a wide variety of cell functions under physiological and pathological conditions; however the molecular mechanisms underlying their anterograde surface transport after synthesis in the endoplasmic reticulum (ER) are not well defined. In *C. elegans*, ER-

localized odr-4 and its partner protein odr-8 have been implicated in the delivery of olfactory GPCRs to the cilia of chemosensory neurons, and we have recently shown that C1orf27, human homolog of odr-4, controls the ER export of GPCRs in mammalian cells. Here, we demonstrate that C1orf27 forms a stable complex with Ufm1-specific protease 2 (UFSP2), human homolog of odr-8, leading to the recruitment of UFSP2 to the ER membrane in mammalian cells. We also show that, similar to C1orf27, UFSP2 knockout significantly impedes the ER-to-Golgi export kinetics of a group of GPCRs, without affecting the transport of non-GPCR plasma membrane proteins. More intriguingly, simultaneous depletion of C1orf27 and UFSP2 does not cause more deleterious effects on receptor transport, suggesting that they act functionally as one unit. Furthermore, C1orf27, but not UFSP2, directly associates with some GPCRs the complex regulates, such as α_2-adrenergic receptors. Taken together, these data demonstrate an important role for the C1orf27-UFSP2 complex formed at the ER membrane in the ER-Golgi traffic of nascent GPCRs by virtue of its ability to interact directly.

B364/P2043

Exploring alternative secretion pathways of proteins involved in *C. elegans* eggshell formation

L. M. Kulber, S. Olson; Molecular Biology, Pomona College, Claremont, CA.

In eukaryotes, protein trafficking is a ubiquitous process in which proteins are distributed to organelles throughout the cell and secreted to the extracellular space. The vast majority of secreted proteins encode signal peptides which direct them through a canonical secretory pathway mediated by COPII-coated vesicles. In metazoans, certain proteins are secreted to form an extracellular matrix around oocytes and embryos (known as the eggshell in *Caenorhabditis elegans*). Previously, we identified three eggshell proteins, PERM-2, PERM-4, and CBD-1, whose secretion was unaffected by the RNAi depletion of SEC-16A.1, an essential protein of the COPII coat. This was a surprising finding since all structural proteins of the *C. elegans* eggshell contain signal peptides that should direct them through the canonical secretory pathway. We are exploring three hypotheses that could explain this finding. (1) SEC-16A.2 (a homolog of SEC-16A.1) compensates for SEC-16A.1 depletion. We deleted *sec-16A.2* via CRISPR-Cas9. PERM-2, PERM-4, or CBD-1 secretion was unaffected in these strains. (2) COPII coat formation is only partially dependent on SEC-16A.1. We knocked down other COPII coat proteins such as SEC-23. Previous *sec-23(RNAi)* experiments led to the deterioration of the gonad, resulting in ambiguous data. Next steps include *sec-24a(RNAi)* and *sec-23* feeding RNAi which should result in a less severe phenotype. (3) PERM-2, PERM-4, and CBD-1 are secreted via unconventional protein secretion (UPS). If the first two hypotheses fail to explain the differential secretion phenotypes, we will conduct a high-throughput RNAi screen to identify proteins implicated in unconventional secretion. With over 30% of human genes encoding a signal peptide sequence, it is important to comprehensively understand the mechanisms by which proteins are variably trafficked and secreted in a cargo-specific manner.

Extracellular Vesicles and Unconventional Secretion

B365/P2044

The Exocyst Complex Directs the Exocytic Trafficking of Multivesicular Endosomes for Exosome Secretion

D. Liu[1], K. Tao[2], B. Wu[1], M. Szczpaniak[2], M. Rames[3], C. Yang[1], T. Svitkina[1], Y. Zhu[1,4], Z. Yu[1], X. Nan[2,3], W. Guo[1]; [1]Department of Biology, School of Arts & Sciences, University of Pennsylvania, Philadelphia, PA, [2]Program in Quantitative and Systems Biology, Department of Biomedical Engineering, Oregon Health and Science University, Portland, OR, [3]Cancer Early Detection Advanced Research Center, Knight Cancer Institute, Oregon Health and Science University, Portland, OR, [4]Department of Pathology & Laboratory Medicine, Children's Hospital of Philadelphia Research Institute, Philadelphia, PA.

Introduction: Exosomes are secreted to the extracellular milieu when multivesicular endosomes (MVEs) dock and fuse with the plasma membrane. However, MVEs can also fuse with lysosomes for degradation. How MVEs are directed to the plasma membrane rather than to lysosomes is unclear. Here we aim to study whether the exocyst, an octameric protein complex consisting of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84, mediates MVE trafficking to the plasma membrane for exosome secretion. **Methods**: shRNAs that target members in the exocyst complex were used to construct the stable exocyst subunit knockdown cell lines. Exosome secretion from these cell lines was measured by Nanoparticle Tracking Analysis (NTA). Exosomes were then collected by ultracentrifugation and examined for the levels of exosomal markers such as CD63 and Tsg101. MVEs along with exocyst subunits were analyzed using the above cell lines by live-cell microscopic imaging, electron microscopy, and DNA-PAINT super resolution imaging. Moreover, ectopic expression of Sec3 on mitochondria membrane was used to monitor whether MVEs are targeted to mitochondria rather than the plasma membrane. **Results**: Knockdown of subunits the exocyst complex led to intracellular accumulation of MVEs and reduced the secretion of exosomes into the culture media. Chemical inhibition of the exocyst subunit Exo70 also led to defects in exosome secretion and MVE trafficking. Exocyst partially co-localize with MVEs in cells. Ectopic targeting of the exocyst subunit Sec3 to mitochondria directed members of the exocyst and MVEs to the mitochondria rather than the plasma membrane. **Summary/Conclusion**: The exocyst complex mediates the targeting of the MVEs to the plasma membrane for exosome secretion. **Funding**: NIH grants R35 GM141832 to W.G.

B366/P2045

Biogenesis of neuronal exosomes via ESCRT machinery

E. C. Dresselhaus, M. Rozencwaig, R. Soslowsky, M. F. Pescosolido, B. Ermanoska, A. A. Rodal; Brandeis University, Waltham, MA.

Exosomes arise from fusion of endosomal multivesicular bodies (MVB) with the plasma membrane, releasing their intraluminal vesicles (ILVs) into the extracellular space. These vesicles can serve degradative or signaling functions in the brain and are implicated in the spread and clearance of neuropathological proteins. At present, the mechanisms by which cells determine whether an MVB fuses with the plasma membrane, rather than canonical fusion with lysosomes, are poorly understood. This is particularly true in neurons, where these organelles form and mature differently in the somatodendritic compartment compared to synapses. The *Drosophila* larval NM serves as a powerful model synapse for the study of the trafficking and function of exosome cargoes. Here, we show for the

first time that exosome release at this synapse requires a canonical pathway for ILV formation mediated by the Endosomal Sorting Complex Required for Transport (ESCRT) proteins. Presynaptic knockdown of the ESCRT components *tsg101*, *shrub,* and *vps4* leads to a postsynaptic loss of a variety of exosome cargoes as well as a presynaptic buildup of these cargoes within intracellular compartments. This finding provides the opportunity to investigate how ILV formation controls the trafficking and function of neuronal exosome cargoes. We found by live imaging that neuronal loss of *tsg101* also impacts axonal trafficking of APP, leading to accumulation along the axon and impaired retrograde trafficking, without affecting trafficking of mitochondria. Remarkably, the signaling activities of the exosome cargoes Synaptotagmin-4 and Evi/Wingless are preserved upon loss of ESCRT machinery, despite being trapped in presynaptic terminals, suggesting that these cargoes function cell autonomously in the neuron, and do not require transsynaptic transfer. Our results indicate that exosome release depends on ESCRT machinery and may serve primarily as a proteostatic rather than intercellular signaling mechanism for some cargoes at this synapse.

B367/P2046

Alpha-Synuclein Regulates the Release of Extracellular Vesicles in Human Cutaneous Melanoma Cells

N. Aloy[1], M. W. Graner[2], C. Coughlan[2], S. N. Witt[1]; [1]Biochemistry & Molecular Biology, Louisiana State University Hlth Sci Ctr, Shreveport, LA, [2]University of Colorado Anschutz Medical Campus, Denver, CO.

Alpha-Synuclein **(**α-syn) is a small, intrinsically disordered protein of the synuclein family, which has multiple functions in cells. In neuronal cells, α-syn localizes to the nucleus, synaptic terminal, binds to the plasma membrane and has a role in both endocytosis and exocytosis. α-syn has a known role in promotion of Parkinson's disease (PD), and emerging evidence suggests that α-syn is one of the key proteins that is correlated to an increased incidence of melanoma in PD patients. The role of α-syn in melanoma progression is poorly understood. α-Syn was reported to colocalize to the multivesicular bodies (MVBs) and plasma membrane in both melanoma and neuronal cells but the physiological function of α-syn in melanoma cells remains a mystery. α-Syn is found in exosomes of PD patients and in the serum of melanoma patients. α-syn is also involved in the assembly of SNARE proteins and dilation of fusion pore in neuronal cells. We hypothesized that α-syn regulates the trafficking and secretion of modulators of melanoma progression through extracellular vesicles. We found a significant decrease in the release of extracellular vesicles (EVs) in *SNCA*-knock out (*SNCA*-KO) cells compared to control SK-MEL-28 melanoma cells, and EV release was restored upon re-expression of α-syn using lentivirus (in one of the two knock-out clones). One of the knock-in (KI) clones did not rescue the EV-release and work is going on to validate that. We further found that the level of the protein CD81, one of the most common EV markers, was significantly decreased in *SNCA*-KO clones, and the expression of CD81 was rescued upon lentiviral re-expression of α-syn. Interestingly, the level of the protein CD63 was increased in *SNCA*-KO cells compared to the control and *SNCA*-KI SK-MEL-28 cells. Based on these findings, we suggest that α-syn functions in the pathway that releases EVs, perhaps by modulating the levels of CD63 and CD81.

B368/P2047

Ubiquitination of FMR1 by Trim25 determines miRNA selectivity

K. E. King, P. Ghosh, **A. L. Wozniak**; University of Kansas Medical Center, Kansas City, KS.

Background: The mechanisms by which exosomal cargo loading occurs are not fully understood. We previously showed that inflammasome activation results in the exosomal loading of the RNA binding protein FMR1 and its associated microRNA, mir-155-5p. The mechanism by which this occurs is dependent on cleavage of RILP (Rab interacting lysosomal protein). RILP is a Rab7 effector that directs trafficking through the endo-lysosomal pathway. It undergoes caspase-1 dependent cleavage during inflammation, resulting in altered trafficking of intracellular vesicles including the multivesicular body, the site of exosome biogenesis. Interestingly, the cleavage status of RILP directly influences which cargo are put into the exosome suggesting the existence of distinct RILP-mediated sorting mechanisms. The **aim** of this study is to determine the mechanism by which FMR1 and cleaved RILP (cRILP) cooperate to selectively load specific exosomal cargo. **Results**: The Endosomal Sorting Complex Required for Transport (ESCRT) consists of four distinct complexes and exosome cargo sorting involves a ubiquitinated protein being recognized by the ESCRT-0 complex. To determine if FMR1 loading is ESCRT-dependent, we first examined its ubiquitination status. We found that after inflammasome activation, FMR1 is K63-ubiquitinated and expression of the cRILP alone is sufficient to promote this ubiquitination. Expression of a non-cleavable RILP did not promote the K63-ubiquitination of FMR1. We further found that the E3 ligase TRIM25 is responsible for not only ubiquitinating FMR1 but also inducing its loading into the exosome by promoting an interaction with the Hrs component of the ESCRT-0 complex. Interactions between FMR1 and Hrs are lost when TRIM25 is knocked down. The loss of TRIM25 prevents the exosomal enrichment of FMR1 as well as its associated miRNA, mir-155-5p without affecting the ability of FMR1 to bind miR-155-5p. **Conclusion**: Altogether, the results suggest that inflammation-mediated loading of FMR1 and its associated microRNAs are dependent on cRILP-mediated recruitment of TRIM25 and eventual recognition by the exosome biogenesis machinery. This study provides insight into the mechanism behind inflammation-mediated selective exosome loading and enhances our general understanding of exosome cargo loading.

B369/P2048

Secretion of DNA and nuclear envelope-derived exosomes from the back of actively migrating neutrophils

S. B. Arya[1,2], S. P. Collie[3], C. A. Parent[1,2,4]; [1]Department of Pharmacology, University of Michigan, Ann Arbor, MI, [2]Life Sciences Institute, Ann Arbor, MI, [3]Cellular and Molecular Biology Graduate Program, University of Michigan, Ann Arbor, MI, [4]Department of Cell and Developmental Biology, Ann Arbor, MI.

Neutrophils are the first line of defense in response to injury or infection. Once neutrophils reach affected sites, they contain the area by phagocytosing pathogens and releasing powerful proteases from cytoplasmic granules. In addition, neutrophils release their genomic DNA, studded with toxic enzymes, which ensnare and kill microbes in neutrophil extracellular traps (NETs). Excessive NET release aggravates inflammation, delays resolution, and subsequently leads to host tissue injury during various autoimmune diseases, and infections. Neutrophils migrating towards shallow chemoattractant gradients emanating from injured/infected tissues amplify their recruitment range by releasing the secondary chemoattractant leukotriene B_4 (LTB_4). LTB_4 is synthesized from arachidonic acid through the action of the cytosolic enzyme 5-lipoxygenase (5-LO), the endoplasmic reticulum/nuclear envelope-resident

protein 5-lipoxygenase activating protein (FLAP), and leukotriene A_4 hydrolase. The LTB_4 synthesizing enzymes and LTB_4 are packaged in and released from extracellular vesicles called exosomes. We recently showed that the biogenesis of LTB_4-containing exosomes originates at the nuclear envelope (NE) of activated neutrophils. We found that the neutral Sphingomyelinase 1 (nSMase1)-dependent generation of ceramide-rich lipid-ordered membrane microdomains is required for FLAP and 5-LO clustering at the NE, and subsequent NE-budding. With nano-scale microscopic resolution achieved by 4x isotopic expansion of samples, we found 5-LO-positive intraluminal vesicles within NE-resident Lamin B Receptor (LBR)-positive limiting membranes, generating NE-derived multivesicular bodies (NE-MVBs). Interestingly, we discovered that both NE-derived MVBs and exosomes are distinct from conventional plasma membrane-derived CD63-positive MVB/exosomes. We also observed the presence of DNA in the lumen of the NE-MVBs. Using SYTOXgreen, a membrane-impermeable DNA-binding dye, we visualized in real time the secretion of DNA from the back of chemotaxing neutrophils - a process that was dependent on nSMase1 activity and on the presence of LBR, which is known to bind DNA. We further determined that decondensed chromatin is present in the lumen of NE-MVBs and found that increasing chromatin decondensation, by inhibiting histone deacetylases (HDAC), increases NET secretion. Finally, we determined that hexadecenal, the end metabolite of the sphingomyelin pathway and a known inhibitor of HDAC, promotes DNA packaging in NE-MVBs, thereby providing a physiological pathway in the regulation of NET release. Taken together, our study provides novel mechanistic insights into an inside-out pathway of DNA and exosome secretion originating from the NE of activated neutrophils.

B370/P2049

Spatiotemporal Imaging Tools for Studying The Role of Exosomes in Migrating Cancer Cells

B. Sung, A. Weaver; Vanderbilt University, Nashville, TN.

Exosomes are a type of small extracellular vesicle (EV) that are actively secreted from cells and originate from multivesicular endosomes. Recently, the mechanisms by which exosomes mediate cell-cell communication and regulate cellular physiology and behavior have been investigated using live imaging techniques visualizing the spatial and temporal dynamics of exosome secretion and interactions with cells. Here, we introduce several spatiotemporal imaging tools for studying the function and secretion of exosomes, and demonstrate their utility in visualization of secreted exosomes in 3D culture and in vivo. Using a pH-sensitive, stable live cell reporter of exosome secretion, pHluorin_M153R-CD63, we identify a key role for exosomes in promoting leader-follower behavior during cancer cell migration in 2D and 3D environments. These findings are complemented by experiments showing that exosome secretion drives directionally persistent migration of cancer cells in a topologically defined electrospun fiber environment. Finally, using a combination of pH-sensitive and non-pH-sensitive fluorescent tags, we visualize multiple steps of exosome secretion and uptake in migrating cancer cells. Overall, we find that dynamic exosome secretion drives directionally persistent migration and quorum sensing behavior.

B371/P2050

Annexin A6 mediates calcium-dependent exocytosis of multivesicular bodies in intact and streptolysin O-permeabilized cells

J. M. Ngo, J. K. Williams, R. Schekman; Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

Exosomes are an extracellular vesicle (EV) subtype that is exported to the extracellular milieu upon fusion of multivesicular bodies (MVBs) with the plasma membrane. Exosomes have elicited broad interest due to their utility as disease biomarkers and potential role in intercellular communication. Despite this interest, the molecular mechanisms underpinning exosome secretion are incompletely understood. To assess the molecular determinants underlying exosome secretion, we developed sensitive and robust cellular and *in vitro* biochemical assays to measure exosome secretion. Our results demonstrate that exosome secretion is coupled to Ca^{2+}-dependent plasma membrane repair elicited by treatment with either a bacterial pore-forming toxin or mechanical stress. Using a targeted proteomics approach, we identify Annexin A6 (ANXA6), a well-known plasma membrane repair protein, as a MVB binding protein and demonstrate that it is required for Ca^{2+}-dependent exosome secretion both in cells and *in vitro*. Overall, our results suggest that cells employ MVBs as a membrane source for plasma membrane lesion repair during physiological mechanical stress or bacterial infection, and that this repair process results in the coincident egress of exosomes into the extracellular space. We propose that this unconventional secretion process may contribute significantly to the heterogeneity of EVs in biological fluids.

B372/P2051

Endolysosomal fusion attenuates extracellular vesicle biogenesis

G. V. Shelke, C. D. Williamson, M. Jarnik, J. S. Bonifacino; NIH, Bethesda, MD

Intercellular crosstalk is critical for the maintenance of cellular homeostasis and it is partly mediated by secreted factors including extracellular vesicles (EVs). EVs are membrane-bound, nano-sized particles that carry bioactive molecules such as proteins, lipids and nucleic acids. EVs are widely used as biomarkers and potential therapeutic agents. A subset of small EVs (sEVs) known as exosomes are released from the cell by fusion of multivesicular bodies (MVBs) with the plasma membrane. This process is alternative to the more extensively characterized fusion of MVBs with lysosomes. However, the conditions and protein machinery that control the fusion of MVBs with the plasma membrane versus lysosomes are poorly understood. Our work addresses the role of endolysosomal fusion and positioning in the regulation of exosome biogenesis and release. The BLOC-1-related complex (BORC) and Arf-like GTPase-8 (ARL8) are two components of the machinery that regulates both endolysosomal fusion through recruitment of the homotypic fusion and vacuole protein sorting (HOPS) complex, and endolysosomal positioning through recruitment of the kinesins KIF1B and KIF5B. We used various biochemical and microscopic approaches to examine exosome biogenesis and release in cells with knock out (KO) or knock down (KD) of BORC, ARL8, HOPS or KIF1B-KIF5B. We found that BORC-, ARL8-, and HOPS-KO cells displayed enhanced exosome secretion. In contrast, exosome secretion was unaffected by KIF1B-KIF5B KO under basal conditions. These findings indicated that HOPS-mediated fusion of MVBs with lysosomes decreases the pool of exosomes available for extracellular release. In contrast, anterograde transport of MVBs toward the cell periphery did not influence exosome release. Further analyses by transmission electron microscopy showed that BORC- and ARL8-KO cells loaded with

horseradish peroxidase exhibited enlarged MVBs full of intraluminal vesicles (precursors of exosomes). Additionally, BORC- and ARL8-KO cells exhibited reduced transport of internalized EGF, EGF-receptor and dextran, as well as newly-synthesized cathepsins, to lysosomes, all consistent with a block in endolysosomal fusion. In summary, our studies have identified a key regulatory step for exosome biogenesis, by demonstrating that HOPS-mediated fusion of MVBs with lysosomes attenuates exosome formation and ensuing release

B373/P2052
Identification of autophagy-related genes regulating unconventional secretion of amyloid-β
J. Kim, T. Kim, S. Lee; INHA UNIVERSITY, Incheon, Korea, Republic of

It has recently been reported that unconventional protein secretion of leaderless cytosolic proteins is important for various cellular functions such as inflammation, cell signaling and neurodegeneration. Some inflammatory cytokines, such as IL-1β, IL-18 and HMGB1, are known to be secreted by unconventional protein secretion called secretory autophagy. Latest studies have shown that neurotoxic proteins, such as α-synuclein, DJ-1 and amyloid-β, are secreted by unconventional protein secretion. Extracellular accumulation of amyloid-β is a prominent pathological hallmark of AD, but the secretion of amyloid-β has not been studied much. While the canonical degradative autophagy pathway is well established, it is unclear as to whether the players in degradative autophagy pathway also participate in the secretory autophagy pathway. In this study, we investigated the role of secretory autophagy machinery in secretion of neurotoxic proteins, such as amyloid-β and DJ-1. We found secretion of neurotoxic proteins was reduced in cells lacking a specific set of autophagy-related genes. A detailed mechanism will be shown in the presentation.

Rab GTPases

B374/P2053
An InR-Vps34/PI3P-Kinesin-2 axis accelerates the anterograde axonal transport of a subset of Rab4-associated vesicles in*Drosophila*
K. Singh, A. Sarkar, S. Das, K. Ray; Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, INDIA.

Rab4 GTPase organizes endosomal sorting essential for maintaining the balance between recycling and degradative pathways. Elevated Rab4 levels in the CNS have been associated with synaptic atrophy and neurodegeneration in *Drosophila* and humans. Insulin signaling, which activates Rab4-associated membrane trafficking in adipocytes, regulates synaptic plasticity in the CNS. In addition, a reduction in insulin signaling can ameliorate symptoms associated with neurodegeneration and aging. However, the underlying mechanism(s) remain unclear. Using *in vivo* time-lapse imaging of *Drosophila* larvae, we show that insulin signaling in neurons increases the anterograde speed of a subset of Rab4 vesicles through a novel InR-Vps34/PI3P-Kinesin-2 axis, which elevates the overall anterograde flux of these vesicles in the segmental-nerve axons. Further, we find that synaptic influx of Rab4 in CNS of *Drosophila* larvae is periodically altered during development, and is inversely correlated with the synaptic density in this region. Together, these observations delineate a potentially unique role of insulin signaling in stimulating the movement of a subset of Rab4-associated vesicles through the ESCRT complex in axons and its impact on synaptic stability.

B375/P2054

DENND2B functions as an activator of Rab35 to regulate cytokinesis

R. Kumar, V. Francis, M. Ioannou, G. Kulasekaran, E. Banks, A. Córdova, M. Khan, P. McPherson; Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, QC, CANADA.

Cytokinesis is the final stage of cell division. Successful cytokinesis depends on coordinated membrane trafficking pathways regulated by small GTPases including Rabs, molecular switches activated by guanine nucleotide exchange factors (GEFs). Cytokinesis requires the formation of an intercellular cytokinetic bridge (ICB) connecting the two daughter cells that then undergoes abscission, a step that relies on depolymerization of actin. Rab35 recruits MICAL1 to oxidate and depolymerize actin filaments but the activation and recruitment of Rab35 for cytokinesis is not understood. Here we report that DENND2B, a protein previously implicated in cancer and a multiple congenital disorder is required to recruit and activate Rab35 at the ICB for successful abscission. Knockdown (KD) of DENND2B delays abscission timing and results in increased multinucleated cells. Biochemical analysis unexpectedly reveals that the DENN domain of DENND2B interacts with an inactive mutant of Rab35 and functions as a Rab35 GEF whereas the N-terminus of DENND2B interacts with an active mutant of Rab35, suggesting an effector relationship. Functionally, loss of DENND2B causes significant reduction of Rab35 enrichment and overaccumulation of F-actin at the ICB and leads to lagging chromatin at the ICB region. The presence of lagging chromatin is known to activate the NoCut/abscission checkpoint delaying abscission and we discover that DENND2B regulates the abscission checkpoint. Our work thus identifies DENND2B as a crucial player in cytokinetic abscission and provides insight into the multisystem disorder associated with DENND2B mutation.

B376/P2055

The small GTPase *RAB35* coordinates epithelial polarization and cell survival in a human model of epiblast formation

A. Rengarajan, K. Taniguchi; Medical College of Wisconsin, Wauwatosa, WI

The formation of a central lumen in the developing human epiblast is a critical step that occurs during implantation. However, there is a significant knowledge gap in the mechanistic understanding by which epiblast cells initiate polarization and form a central lumen. To uncover key molecular players involved in this process, we recently employed a spatial proteomics approach to profile apical and basolateral membrane territory proteomes of a previously established human pluripotent stem cell-derived model of epiblast formation (hPSC-epiblast model), and identified a cohort of proteins enriched in the polarized membrane territories (Wang *et al.*, *Science Advances* 2021). In particular, using loss- and gain-of function analyses, we revealed a role of RAB35 in regulating apical membrane morphogenesis of developing hPSC-epiblast model. This present study investigates the molecular and cellular mechanisms by which RAB35 controls lumenogenesis of the human epiblast model. Detailed characterizations of the *RAB35*-KO epiblast models showed additional novel defects: *RAB35*-KO epiblast models 1) form ectopic satellite lumens, and 2) are significantly smaller in size. These defects can be rescued through an inducible expression of a wild-type RAB35-GFP fusion construct throughout hPSC-epiblast model development (for 4 days (D4)). Interestingly, a similar rescue is observed when RAB35-GFP is induced during the initial 48 hours (D0-D2), but not when RAB35-GFP is induced at later time points. In controls,

by D1, hPSC form an apicosome, an apically charged membrane bound structure, and, once the apicosomes are formed, cells initiate radial organization, and the apicosomes fuse at the radial center to give rise to a lumen by D2. Intriguingly, *RAB35*-KO hPSC display formation of multiple apicosomes, instead of a singular apicosome as seen in controls. Furthermore, while a central lumen is formed, *RAB35*-KO epiblast models at D2 display an increased number of unfused apicosomes and satellite lumens, suggesting that apicosome fusion event is delayed in the absence of RAB35. Surprisingly, while proliferation rate in *RAB35*-KO epiblast model is comparable to controls, apoptosis rate is significantly higher in the KO background, a likely cause of the reduced size in the *RAB35*-KO epiblast models. Finally, an ectopic expression of a wild-type form of CDC42 Rho GTPase, a known downstream RAB35 effector, leads to a partial restoration of apical membrane defects as well as the increased apoptosis. Taken together, these results reveal previously unrecognized roles of RAB35 in apical membrane formation likely through CDC42, as well as in promoting cell survival.

B377/P2056

Structural basis for specific activation of the Rab11 GTPase by the TRAPPII complex

S. Bagde, J. Fromme; Cornell University, Ithaca, NY

Secretion and endocytic recycling are essential eukaryotic processes that involve transport of protein cargoes to the plasma membrane in membrane-bound vesicles. The formation of these vesicles is regulated by activation of the Rab GTPase Rab11. The activation of GTPases at membrane compartments is dependent on the localization of their specific guanosine exchange factors (GEFs), which catalyze the exchange of GDP with GTP and stabilize the active GTPase on the membrane. The multisubunit TRAPPII complex acts as Rab11 GEF and recruits it to the *trans*-Golgi network (TGN) and recycling endosome (RE) membranes. More than 60 different Rabs are present in mammals and the budding yeast model has 11 different Rabs. Specific localization of each of these Rabs to different membrane compartments is important for determining organelle identity and membrane organization of these compartments. While multiple GTPases and GEFs localize at the TGN, the mechanistic details of how TRAPPII specifically activates Rab11 are unknown. Moreover, another related GEF, the TRAPPIII complex, shares the same catalytic subunits with TRAPPII and yet activates another GTPase, Rab1. A steric gating mechanism has been proposed in which TRAPPII selects against Rab1 based on the length of the C-terminal hypervariable domain. We determined the cryo-EM structure of yeast TRAPPII bound to its substrate Rab11 at an overall resolution of 3.7Å. Analysis of the atomic structure revealed specific interactions between Rab11 and the TRAPPII complex, and how TRAPPII interacts with the membrane. We tested the physiological relevance of these observed interactions using *in vivo* functional studies. We determined that the TRAPPII subunit Trs130 provides a ‘leg’ that lifts the TRAPP catalytic site above the membrane, thereby preventing access to the catalytic site by Rab1. We find that the TRAPPIII complex selects against Rab11 based on repulsive interactions with the catalytic subunits. TRAPPII surmounts these repulsive interactions using subunit Trs120 as a ‘lid’ to enclose the active site. We also observed an alternative conformation of the TRAPPII complex which may facilitate access of Rab11 to the TRAPP catalytic site. Taken together, these experiments reveal the mechanism of specific activation of Rab11 on the membrane by TRAPPII, a key step for initiation of secretory vesicle formation. As Rab11 and the TRAPPII complex are conserved throughout eukaryotes, this mechanism may be widely conserved across all eukaryotic secretory systems.

B378/P2057

Structures of full-length LRRK1 and mechanisms of autoinhibition

R. G. Abrisch[1], J. M. Reimer[1], A. Dickey[1], Y. Lin[1], S. Mathea[2], D. Chatterjee[2], D. Alessi[3], S. Knapp[2], M. Daugherty[1], A. E. Leschziner[1], S. Reck-Peterson[1]; [1]University of California San Diego, San Diego, CA, [2]Goethe-Universität, Frankfurt, Germany, [3]University of Dundee, Dundee, United Kingdom

Leucine Rich Repeat Kinase 1 and 2 (LRRK1 and LRRK2) are homologs in the ROCO family of proteins in humans. Despite their shared domain architecture and involvement in intracellular trafficking through phosphorylation of Rab proteins, their disease associations are strikingly different: mutations in LRRK2 are linked to Parkinson's Disease (PD), while those in LRRK1 are linked to osteopetrosis and osteosclerotic metaphyseal dysplasia. Furthermore, all PD-linked mutations in LRRK2 increase kinase activity and are autosomal dominant gain-of-function, while disease-linked mutations in LRRK1 lead to loss of kinase activity and are autosomal recessive loss-of-function. To understand these differences, we solved cryo-EM structures of LRRK1 in its monomeric and dimeric forms. Both differ from the corresponding LRRK2 structures, and the dimer is particularly dissimilar; unlike LRRK2, which is sterically autoinhibited as a monomer, LRRK1 is sterically autoinhibited in a dimer-dependent manner, a state stabilized by two disordered regions of the structure. Strikingly, there is a second level of autoinhibition that only occurs in LRRK1, where the DYG motif in its kinase is prevented from assuming an active conformation. Indeed, LRRK1 phosphorylation of Rab7a in human cells is markedly increased by mutations in LRRK1 that disrupt either autoinhibition mode and further enhanced by disrupting both modes. Our work suggests that LRRK1 has added layers of regulation that are not present in LRRK2. We also discuss our results in the context of the evolution of the LRRK family of proteins.

B379/P2058

Role of Rab4A on intracellular iron transport in MDA-MB-231 breast cancer cells

P. Pampa Mandal, I. Crosbourne, R. Abini-Agbomson, J. Barra, **M. Barroso**; Albany Medical College, Albany, NY

Iron is an essential element for several cell functions and tumor cells demand high amounts of iron for support their cell growth and proliferation. Iron-bound transferrin (Tf) binds to the transferrin receptor (TfR) at the plasma membrane and TfR-Tf complexes are internalized into endosomes, where iron dissociates and is transported into mitochondria for use in heme synthesis and iron-sulfur cluster (Fe-S) biogenesis. Rab4, a marker of early endosome, is responsible for proper sorting and recycling of Tf-TfR complex to plasma membrane. To understand the role of Rab4A isoform in intracellular iron transport in MDA-MB-231 breast cancer cells, we generated stable cells displaying Rab4A knockdown using shRNA or Rab4A knock-out cells using CRISPR. The level of knockdown was assessed by qPCR and immunoblotting and showed significant decrease in Rab4A mRNA and protein expression, respectively. As expected, Rab4A knockdown slows down Tf-TfR recycling. The mitochondrial iron biosensor, RDA, red-fluorescence undergoes rapid quenching upon iron translocation into the mitochondria. Interestingly, Rab4A knockdown in MDA-MB-231 led to increased RDA quenching levels, suggesting elevated mitochondrial iron translocation. Next, we assessed the effect of Rab4A on labile iron pool by conducting Ferro-orange imaging assay. The result demonstrates that Rab4A knockdown lowers the labile iron pool (LIP) in MDAMB231 cells. Therefore, our results indicate that Rab4A knockdown leads to the accumulation of mitochondrial iron and reduced LIP levels. Altered iron acquisition and utilization lead to metabolic

changes which are a hallmark of cancer. Therefore, elucidating the role of Rab4A in intracellular iron transport should further our understanding of the role of mitochondrial iron in breast cancer.

B380/P2059

Dynamics of the Rab11-Rabin8-Rab8 cascade in membrane tubulation and ciliogenesis

I. Saha, Q. Lu, C. J. Westlake; National Cancer Institute, NIH, Frederick, MD

Rabs, a family of GTPases, which function as molecular switches by alternating between a GTP-bound active state and GDP-bound inactive state, play a major role in membrane trafficking that coordinate biogenesis, transport, tethering and fusion of membrane associated structures in cells. Guanine nucleotide exchange factors (GEF) promote GTP binding while GTPase-activating proteins (GAP) stimulate GTP hydrolysis to the GDP bound state. Several Rab cascades have been described wherein a GTP bound Rab recruits a GEF to activate another Rab needed to organize cellular membrane compartments. The Rab8 GEF Rabin8 functions in a Rab11-Rab8 cascade important for primary cilium assembly and neurite outgrowth. However, the precise mechanism of this cascade is poorly understood. In this study, we set out to characterize the membrane dynamics of Rab11-Rabin8-Rab8 cascade. To show Rab8 activation on Rab11-Rabin8 membranes we performed live cell imaging and observed accumulation of newly translated tRFP-Rab8, following doxycycline inducible expression, on SNAP-Rab11a/GFP-Rabin8 vesicles. We thereby demonstrate that the Rab11-Rabin8-Rab8 cascade is required for Rab8 membrane tubule formation stimulated by Cytochalasin D treatment in RPE-1 cells. Moreover, we find that other trafficking regulators linked to this Rab ciliogenesis cascade are important for Rab8 tubulation in these cells suggesting a conservation in function for these membrane assembly processes. Strikingly, by live cell imaging with a super resolution Elyra7 microscope we could visualize the interplay of GFP-Rab11a and tRFP-Rab8a during membrane tubule assembly. Subsequently, we evaluate Rab11-Rab8 membrane dynamics during ciliogenesis. Notably we show that GFP-Rab11 is observed in the cilium more prominently with tRFP-Rab8a expression. Furthermore, tRFP-Rab8a ciliary transport is enhanced by Rab11 and/or Rabin8 as determined by FRAP studies. Together these studies indicate Rab8 is activated on Rab11-Rabin8 membranes during membrane tubulation and ciliary membrane assembly. Ongoing work will be described aimed at determining where Rab8 activation occurs during ciliary membrane assembly and the association of the Rab11-Rabin8-Rab8 cascade with the ciliary pocket membrane.

B381/P2060

Identification of paralog-specific Rab27 GTPase effectors

I. M. Lehman, J. M. Ngo, J. K. Williams, R. W. Schekman; Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

Extracellular vesicles (EVs) are membrane-bound compartments that are exported to the extracellular environment after fusion of multivesicular bodies with the plasma membrane. Exosomes are an EV subpopulation that have garnered particular interest in the scientific community due to recent studies suggesting a role for exosomes in intercellular communication in both normal and disease states. Despite broad interest in exosomes, little is known about how their release is regulated. Rab27a and Rab27b are two Rab GTPase paralogs (71% amino-acid identity) that regulate different steps of the exosome secretion pathway. To address how these proteins control separate steps of exosome secretion, we utilized both biochemical and cellular approaches to identify Rab27 paralog-specific

interactors and visualize their subcellular localization. We purified recombinant Halo-tagged Rab27a and Rab27b and utilized them as bait for SILAC-based quantitative proteomics. We then determined that TBC1D2B and CCDC186 are unique interactors of Rab27b, whereas Melanophilin preferentially binds to Rab27a. Next, we demonstrated that these three interactors bind specifically to the GTP-bound state of their respective Rab27 paralog, indicating that they are indeed Rab27 effector proteins. Finally, we generated stable cell lines expressing fluorescent Rab27a and Rab27b fusion proteins under the control of the low expression L30 promoter, and show that they localize to peripheral vesicles and the Golgi apparatus, respectively. Overall, we have identified unique and preferential effectors of Rab27b and Rab27a, respectively. Future work will focus on delineating the relationship between these unique effectors on Rab27 subcellular localization and exosome secretion.

B382/P2061

Targeting of the Tail-Anchored Rab GAP (GTPase Accelerating Protein) Gyp8 to Peroxisomes Is Regulated by the AAA ATPase Msp1

D. P. Nickerson, M. A. Quinn, A. Lin, C. Vuong, A. Mora, S. Connolly, J. T. Maddox; Dept of Biology, California State University San Bernardino, San Bernardino, CA.

Rab GTPase signaling proteins (Rabs) are key regulators of vesicular transport, controlling where and when membranes dock and fuse, ensuring lipid and protein cargoes are delivered to appropriate destinations. Rabs depend upon GTPase accelerating proteins (GAPs) to trigger GTP hydrolysis and return the signaling active, GTP-bound Rab to its inactive, GDP-bound state. Rab GAPs contribute to the efficiency and fidelity of transport pathways, and defective Rab GAPs are implicated in a variety of human diseases. Rab GAPs tend to be cytosolic proteins that transiently localize at membranes to survey for their client Rab. Since its initial discovery, the cellular localization and functions of the yeast (*S. cerevisiae*) Rab GAP Gyp8 have remained essentially uncharacterized. We report that Gyp8 is an atypical transmembrane (TM) GAP whose association with peroxisomes and mitochondria is regulated by the AAA ATPase Msp1, a chaperone that functions to remove tail-anchored proteins from peroxisomes and mitochondria. Computational analysis of Gyp8 predicted a single-pass TM domain near the carboxy terminus, characteristic of a tail-anchored (TA) protein. Subcellular fractionation demonstrated that Gyp8 localized exclusively to a membrane fraction and was resistant to chemical membrane extraction. Fluorescence microscopy indicated that GFP-tagged Gyp8 co-localized with peroxisome, endoplasmic reticulum (ER) and mitochondrion markers in wild type cells. In the absence of peroxisomes, GFP-Gyp8 redistributed to the ER. Loss of machineries regulating membrane insertion or extraction of TA proteins resulted in redistribution of GFP-Gyp8 to mitochondrial membranes. Truncation analyses of Gyp8 indicated that the TM and luminal domains are necessary and sufficient to direct localization to peroxisomes. Growth experiments of cells lacking specific Rab GAPs indicate a peroxisome-related carbon utilization defect in cells lacking Gyp8. Many Rab GAPs enforce spatiotemporal boundraries for activities of their cognate Rab GTPases. We report that Gyp8 spatially restricts localization of the secretory Rab Ypt1. Our results demonstrate regulatory mechanisms to control localization of a transmembrane Rab GAP at multiple organelles, a novel alternative to strategies of transient membrane association and dissociation used by soluble Rab GAPs.

B383/P2062

Structural basis for Rab6 GTPase activation by the Ric1/Rgp1 complex

R. Feathers; Cornell University, Ithaca, NY

Membrane trafficking is a highly coordinated process in eukaryotes essential for maintaining lipid homeostasis and sorting proteins to the proper location inside cells. The Golgi complex serves as the hub of this dynamic set of pathways where it directs cargo sorting through vesicle mediated transport. Vesicles of varying lipid composition loaded with different protein cargo are in constant flux through the Golgi and precise spatial and temporal regulation of these processes requires communication between compartments. Rab GTPases are small signaling proteins that act as molecular switches to regulate vesicular trafficking. Rab6 functions at the Golgi where it is important for endosome to Golgi retrograde transport in both yeast and metazoans and for anterograde exit from the TGN in metazoans. The Ric1/Rgp1 protein complex is a conserved guanine nucleotide exchange factor (GEF) required for activation of Rab6. In mammalian cells Ric1/Rgp1 is known to be regulated by the Rab33 GTPase, but it remains unresolved how the activity of the complex is regulated and the mechanism by which it catalyzes Rab6 nucleotide exchange. Furthermore, little is known about the structure and domain organization of Ric1/Rgp1. We have used cryo-EM to determine the structure of the yeast Ric1/Rgp1-Rab6(Ypt6) complex, representing the key intermediate of the nucleotide exchange reaction. This structure reveals the overall architecture of the complex and has enabled us to identify the specific interactions that govern the recognition and activation of Rab6. Ongoing studies aim to test the physiological significance of these interactions as well as a proposed mechanism for localization of the complex on the Golgi membrane surface.

Polarity in Development

B385/P2063

Understanding the role of Dishevelled in establishing epidermal planar cell polarity

P. Sil, D. Devenport; Molecular Biology, Princeton University, Princeton, NJ.

Planar cell polarity (PCP) pathway directs the coordinated alignment of cell polarity across the plane of a tissue and guides morphogenesis in a range of organisms from planaria to mammals. A hallmark of the PCP pathway in epithelia is the asymmetric distribution of its components along cell-cell junctions, with the seven-pass transmembrane cadherin Celsr1 partnering with atypical GPCR Frizzled (Fz) and cytoplasmic Dishevelled (Dvl) on one side and four-pass transmembrane Vangl and cytoplasmic Prickle(Pk) on the opposite side of the cell. The cytoplasmic adaptors, Dvl and Pk, are essential for the asymmetric distribution of their transmembrane counterparts, but the molecular mechanisms by which they help to organize asymmetric PCP complexes are poorly understood. PCP proteins exhibit signalosome-like properties, where they assemble into relatively stable, asymmetric clusters along cell junctions. We hypothesize that Dvl promotes the formation of junctional PCP clusters, given the well-documented ability of Dvl proteins to oligomerize. In this study, we have determined the distribution and function of Dvl2 and Dvl3 in the mouse embryonic epidermis and tested if and how Dvl organizes and stabilizes Fz-containing complexes along cell junctions to asymmetrically partition PCP components. Additionally, using a structure-function approach in primary mouse keratinocytes in vitro we have investigated how Dvl-Fz interactions impact the enrichment, dynamics and organization of Fz along cell junctions.

B386/P2064

Kinase Signaling Regulates Hair Cell Planar Polarity and Establishes a Line of Polarity Reversal in the Mouse Inner Ear

S. Jia, E. M. Ratzan, R. Abrar, E. J. Goodrich, L. Heiland, **M. R. Deans**; University of Utah School of Medicine, Salt Lake City, UT.

Planar polarity is the polarized organization of cells and cellular structures within the plane of an epithelium and underlies organ and tissue function. Core planar cell polarity (PCP) signaling coordinates the orientation of cells along a common axis, however it remains unclear is how PCP signaling is interpreted by individual cells during differentiation. We are addressing this using the utricle and saccule of the mouse inner ear which contain the sensory receptor hair cells that detect motion and gravity. Hair cells function via the deflection of a planar-polarized bundle of stereocilia located on the apical cell surface, and the orientation of the bundle determines the direction of motion that an individual hair cell can detect. Although the planar polarity of vestibular hair cells resembles *Drosophila* wing hairs, they also exhibit planar bipolarity because they are divided between two groups with oppositely oriented bundles. The two groups are adjacent and meet at a cellular boundary called the Line of Polarity Reversal (LPR), and due to the orientation of their bundles, these hair cells detect and respond to motion in opposite directions. We identified a serine-threonine kinase that functions as a planar polarity effector within one group of hair cells in the utricle or saccule, and therefore is required to establish planar bipolarity and position the LPR. Following gene deletion in mice, the intrinsic polarity of the bundle is no longer aligned with the polarized distribution of core PCP proteins. Kinase expression is restricted to hair cells on one side of the LPR in utricles and saccules, and bundle orientation in these regions is randomized in knockouts. This pattern of expression is complementary to the transcription factor Emx2 which is expressed on the other side of the LPR and is also required for its formation. We find that Emx2 represses kinase gene transcription, but kinase overexpression is sufficient to reorient stereociliary bundles when delivered to Emx2-expressing hair cells using AAV. We propose a model in which planar bipolarity of the utricle and saccule is established by parallel functions Emx2 and kinase signaling, each acting in hair cells located on opposite sides of the LPR. In this model the position of the LPR is determined by the boundary of Emx2 since it actively represses the alternative kinase-mediated polarity pathway.

B387/P2065

Identifying mechanisms underlying Orb2-dependent regulation of neural stem cell centrosome asymmetry

J. Buehler, D. A. Lerit; Department of Cell Biology, Emory University School of Medicine, Atlanta, GA.

Neural stem cells (NSCs) orchestrate repeated rounds of asymmetric cell division designed to pattern the developing brain with neurons and glia. During each round, the centrosomes facilitate the segregation cell fate determinants that predispose each daughter cell to a different fate. Drosophila Orb2 is a cytoplasmic polyadenylation element binding protein (CPEB) ortholog involved in the post-transcriptional regulation of diverse mRNAs across multiple tissues. Our lab recently reported that loss of orb2 results in NSC centrosome maturation defects and microcephaly, but the mechanisms and cell types behind these phenotypes are unknown. To begin to dissect the role of Orb2 in neurodevelopment, we are testing whether Orb2 regulates centrosome activity or brain volume via its conserved RNA-

binding domain. Our preliminary data suggest a functional Orb2 RNA-binding domain is required for centrosome asymmetry. However, localization experiments revealed that Orb2-GFP does not localize to centrosomes, but rather is diffusely distributed within the NSC cytoplasm. Taken together, these data suggest Orb2 functions indirectly, likely through one or more mRNA targets, to instruct centrosome activity. Consistent with this idea, an in-silico analysis of crosslinking immunoprecipitation and sequencing data (CLIP-seq) of Orb2 targets within Drosophila S2 cells identified an enrichment of RNAs with centrosome ontologies. To identify mRNA targets involved in centrosome activity control, we are presently conducting CLIP RT-PCR to identify RNAs bound to Orb2 within the third instar larval brain. This work reinforces a model whereby Orb2 contributes to the posttranscriptional regulation of centrosome genes critical for NSC asymmetric centrosome maturation, guiding proper neurodevelopment.

B388/P2066

Soluble factors produced by the epithelial cell induce macrophages differentiation

A. Siqueira Berti, H. Carvalho; University of Estadual De Campinas-UNICAMP, Campinas, Brazil

Androgens are important for prostate gland development and homeostasis. Androgen deprivation leads to the influx of immune cells, in particular, of different populations of macrophages. We have shown that prostatic epithelial cells cultured in the absence of androgenic stimulus induced to the production of soluble factors that influence macrophage polarization and motility. Furthermore, LNCaP conditioned medium affects macrophages RAW 264.7 cells promoted prostate epithelial cell death (J Cell Physiol 2019).O objetivo deste trabalho foi analisar os fatores solúveis produzidos pela LNCaP ausência de andrógenos que induzem a diferenciação de macrófagos. O meio condicionado LNCaP foi analisado por espectrometria de massa para identificar todas as proteínas. A linhagem celular de macrófagos Raw 264,7 foi cultivada em meio completo, suplementado com 50% de meio condicionado LNCAP, na presença ou ausência de R1881 e recebeu tratamento com 1 µM de Granulina e após 24h imunocoloração para INOs. O meio condicionado LNCaP foi analisado por espectrometria de massa e a Granulina foi identificada após esta análise. Observamos que as células LNCaP liberam granulina e os macrófagos tratados com granulina aumentaram a expressão de INOs. Apesar da ausência de andrógenos nos macrófagos e cocultura da linha de células tumorais, resulta em uma frequência aumentada de Tunel (fragmentação de DNA) e células duplamente positivas de caspase 9 clivadas. Essas observações sugerem que a granulina liberada pelo LNCAP atua na diferenciação de macrófagos que leva à morte das células epiteliais. Este trabalho apoiado pela CAPES.

B389/P2067

Crawling under your skin: Understanding how planar cell polarity generates polarized cell movements during hair follicle morphogenesis

R. Sharan[1], L. Leybova[1], X. Du[2], M. Shelley[2], D. Devenport[1]; [1]Princeton University, Princeton, NJ, [2]Flatiron Institute, New York, NY

Breaking symmetry is essential for the development of complex structures at almost every scale, from macromolecular to cellular to organismal. One such symmetry breaking process is establishment of planar cell polarity (PCP), the coordinated orientation and alignment of cells within the plane of a tissue. This is through the asymmetric localization of the three transmembrane proteins, Frizzled-6, Vangl2, and Celsr1. PCP is required for collective cell behaviors such as alignment of single-cell protrusions of the

Drosophila wing to multicellular structures such as the mammalian hair follicle. However, while the components of the PCP pathway have largely been identified, how they regulate downstream cytoskeletal dynamics to generate collective cell movements in multicellular structures is not well understood. Our previous work has shown that PCP and downstream myosin is required for counter-rotational cell flows that drive polarized hair follicle morphogenesis. These cell flows are further required for organizing the cell fates of the hair placode by repositioning centrally-located to the anterior and sweeping peripheral cells to the posterior. Given these previous data, we hypothesized that PCP might polarize myosin or myosin activity at intercellular junctions to bias cell rearrangements. However, visualization of several myosin reporters in both live and fixed embryonic epidermal tissues, we did not find evidence of planar polarized myosin at junctions, nor did we observe a strong correlation between myosin and PCP protein localization. Rather, through 3D reconstruction of the hair follicle epithelium, we found that the basal surface of placode cells forms anterior-facing protrusions and that the basal surface precedes the apical surface, suggesting PCP may direct polarized cell crawling. In support of a mechanism involving crawling, Rac1 inhibition (NSC 23766) of embryonic skin explants lead to disrupted hair follicle polarization. Further, a continuum mechanics model *in silico* supported the idea that forces applied at the basal surface can drive movements at the apical surface.

B390/P2068

Biogenesis of lysosome-related organelles complex-1 (BLOC-1) Required for Planar Cell Polarity in the Inner Ear

C. Tower-Gilchrist; Emory University, School of Nursing, Atlanta, GA

Planar cell polarity (PCP) plays a critical role in embryonic development and in functioning of tissues. PCP is described as the distinct cell polarity exhibited among neighboring cells along an axis parallel to the plane of the sheet. More importantly, dysregulation of PCP results in neural tube closure defects including craniorachischisis, misorientation of stereociliary hair bundles in the cochlea, and deafness. One conserved feature of PCP is the asymmetric localization of core PCP proteins, Vangl2 and Frizzled, to opposing cell boundaries. We have previously characterized the role of Adaptor Protein Complex -3 (AP-3) in PCP and regulating the intracellular trafficking of Vangl2. Although studies have demonstrated the importance of protein trafficking in the asymmetrical sorting of PCP transmembrane proteins, the molecular mechanisms still remain poorly understood. One way to provide insight into these mechanisms is to identify additional Vangl2 binding partners. Previous studies have shown that BLOC-1 interacts with AP-3. In this study we use mouse cochlea to examine the role of BLOC-1 in planar cell polarity and Vangl2 localization. We first examined the localization of Vangl2 in the organ of Corti of the cochlea and posterior cristae obtained from loss of function BLOC-1 sandy mice ($Dtnbp1^{sdy/sdy}$) and pallid ($Pldn^{pa/pa}$). Within the organ of Corti of pallid ($Pldn^{pa/pa}$) mice, there were notable reduction in Vangl2 membrane localization. Furthermore, we observed misorientation of sterociliary hair cell bundles along with extra hair cell rows in the cochlea of sandy ($Dtnbp1^{sdy/sdy}$) mice. These findings suggest a new role for BLOC-1 in PCP and that it is important for the membrane targeting of Vangl2. Future studies will determine if BLOC-1 directly interacts with Vangl2.

Neuronal Degeneration and Regeneration

B391/P3111

Neurologic responses of the developing nervous system to arsenic exposure

T. H. Adebambo, A. R. Bharadwaj, D. A. Lerit; Emory University, Atlanta, GA

Neurodegenerative disorders including Alzheimer's disease (AD) and Parkinson's disease (PD) have been linked to environmental exposure to arsenic and other neurotoxins. The blood-brain barrier (BBB) is known to be permeable to arsenic, and there has recently been an increase in research into the potential health hazards of chronic exposure. Arsenic neurotoxic course in the central nervous system, however, is still poorly understood. Understanding how arsenic contributes to neurodevelopmental and neurodegenerative disorders may lend insight into novel therapeutics. We report here a chronic toxicity study of arsenic as well as the neurotoxic action on neurodevelopment in developing *Drosophila*. Age-matched newly emerged *wild-type* first instar larva were exposed to different concentration groups (50 µM, 100 µM, 250 µM, 500 µM and 1000 µM), and a control group fed an equal amount of yeast paste and bromophenol blue mixed in *Drosophila* meal. A dose-dependent relationship was evaluated by monitoring development through first instar larval stage, pupation, and fly eclosion. The effect of arsenic on *Drosophila* neurodevelopment was determined by measuring age-matched brain volumes from randomized images. Our preliminary data indicate arsenic administration results in a dose-dependent delay in larval pupation and adult eclosion relative to the mock-treated control. There was also a marked decrease in the number of pupa and eclosed arsenic-exposed flies, consistent with the idea that chronic exposure to elevated concentrations of arsenic is lethal. Unexpectedly, we noted brain volume was significantly increased in treated third instar larva compared the control group. In addition to laying precedence to the understanding of the mechanism of arsenic neurotoxicity in the *Drosophila* central nervous system, our findings suggest that arsenic affects fly eclosion by slowing down the rate of eclosion and reducing the number of eclosed flies.

B392/P2069

Pro- Inflammatory Factor and Beta- Amyloid Accumulation Pathology: Parkinson's Disease

S. E. Vendrell- Gonzalez[1], J. Roldan- Khan[2], A. De Freitas- Suarez[1], G. Jimenez-Jordan[1], F. Reyes-Ortiz[1], J. Alves[1], M. Inyushin[1]; [1]Universidad Central Del Caribe, Bayamon, PR, [2]University of Puerto Rico Medical Sciences Campus, Bayamon, PR

Neuroinflammation, more specifically the pro-inflammatory effect caused primarily by microglia activation, are crucial in understanding Parkinson's Disease (PD) progression. Studies show an expression of cytokines involved in neuroprotection and neurotoxicity throughout PD progression. Levels of neuroprotective cytokines, like TNF-a, IL-6, IL-1b and glutamate are seen increase throughout. Our study focuses on analyzing pro-inflammatory factors and AB accumulation to better understand the progression of PD and how that affects the neurovascular unit. To accomplish this*, Sprague Dawley* rats were injected with 6-Hydoxydopamine (6-OHDA) to induce dopaminergic neuron cell death and promote a similar inflammatory response in the CNS as seen in PD patients. Throughout the experiment, affected and unaffected brain tissue samples were compared using ELISA, Western Blot, Specific Cytokine Signaling and fluorescent Microscopy. Preliminary immunoassayed data showed AB 1-40 peptide accumulation inside the blood vessels and affected areas. Using this data as a base, Immunoassayed tissue sections were used to analyze the concentration of dopaminergic neurons,

showing a correlation between dopaminergic neurons reduction and AB accumulation. Secondly, a cytokine profile study was performed to compare and confirm pro-inflammatory cytokine commonly seen in PD patients. Protein quantification assays such as Western Blot and ELISA were used to corroborate cytokine and AB concentration in brain tissue samples. Western Blot Assay confirmed increased pro-inflammatory cytokine concentration for tissues treated with 6-OHDA. Meanwhile, ELISA assay confirmed AB accumulation was significantly greater in affected brain samples. Preliminary ELISA data was also used previously to confirm the immunoassayed AB accumulation. Improving our understanding of the effect pro-inflammatory factors have in the CNS and in the progression of PD puts us closer to be able to delay or reverse dementia symptoms so commonly seen in PD patients.

B393/P2070

LIF-mediated cell proliferation in rat fetus astrocyte and neuronal cell lines after exposure to HIV-gp120

F. Reyes-Ortiz[1], R. Vega-Román[1], S. E. Vendrell- Gonzalez[1], A. De Freitas-Suarez[1], G. Jiménez-Jordán[1], M. Inyushin[1], J. Alves[1], R. Noel, Jr.[2]; [1]University of Central Del Caribe, Bayamón, PR, [2]Ponce Health Sciences University, Ponce, PR

HIV-induced neurotoxicity remains an important, yet unmanageable, manifestation of the disease, despite effective pharmacological therapy in the control of viral replication. HIV-gp120 is associated to induce HIV-associated neurocognitive disorders (HANDs), affecting 20% to 50% of infected individuals, increasing with age. Hence, novel approaches are explored, necessary to prevent or alleviate CNS damage after HIV infection. Leukemia Inhibitory Factor (LIF) is a cytokine known for its protection of glial cells, inducing GFAP astrocyte differentiation, as well as increasing self-renewal of neuronal stem cells to promote proliferation and survival of neurons. Efficacy of LIF is evaluated, as it promotes neuronal cell survival and differentiation, by assessing inflammation and cell proliferation factors within an in vitro model of rat embryonic hippocampal astrocytes and cerebral cortex neurons exposed to HIV-gp120. Astrocytic and neuronal cells are extracted and later treated with LIF in a primary co-culture. Neuronal markers, MAP2 and NeuN, are used to determine neuronal differentiation of mature and young neurons, respectively. Confocal Microscopy preliminary results show an increase in the expression of NeuN and MAP2 in the presence of LIF, compared to cells treated with HIV-gp120, suggesting that LIF is promoting neuronal development. Changes in the expression of these proteins are determined by immunoblotting. GFAP levels show presence of inflammation in cells treated with HIV-gp120. Further studies are explored using BrdU and TUNEL assays as apoptosis and inflammation markers to determine the role of LIF in promoting cell proliferation, when exposed to HIV-gp120 and its associated neuronal and astrocyte cell death. BrdU immunoassays detect high levels of stained proliferated cells responding to LIF, resulting in low levels of inflammation and apoptosis, compared with cells exposed to HIV-gp120. TUNEL reagents are used to detect late stage apoptotic cells during inflammation when exposed HIV-gp120. Better understanding of these mechanisms require the replication of independent confocal microscopy experimentation, and evaluation of the expression of pro-inflammatory cytokines in neuronal cell death. Evidencing the neuroprotective function of LIF on HIV, in our model, has the potential to contribute as a future direction against neurodegenerative disorders underlying HIV-induced CNS damage.

Acknowledgements: Supported by Universidad Central Del Caribe and the Associate Dean for Research and Graduate Studies office, Alliance-NIMHD-NIH, Expanding Undergraduate Students Education,

Opportunities and Options in Clinical and Translational Research, the US Department of Education: Title V Grant Award #P031S160068 and MAC-FRED Program 2018.

B394/P2071

HIV-induced tension provoked by the homeostatic effects between neurotoxicity and the neuroprotection provided by LIF

J. Alves[1], A. De Freitas-Suarez[1], J. Roldán-Kalil[2], R. Vega-Román[1], S. Vendrell-Gonzalez[1], F. Reyes-Ortiz[1], G. Jimenez-Jordan[1], M. Inyushin[1]; [1]University of Central Del Caribe, Bayamon, PR, [2]University of Puerto Rico Medical Sciences Campus, San Juan, PR

Despite modern HIV therapies, it has not been sufficient to combat its neurotoxic proteins which are detrimental to the neurocognition of its subjects resulting in HIV-Associated Neurocognitive Disorders (HAND). Proteins such as HIV-gp120 and HIV-nef are responsible for the development of HANDs. Our project aims to study another approach to prevent or alleviate central nervous system damage after HIV infection by evaluating the protective effects of the Leukemia Inhibitor Factor (LIF). In addition, we seek to diminish or prevent acute HIV infection via immunization. For our first interest, neuronal and astrocytic cells are extracted from our embryonic rat model and later treated with LIF, a neuroprotective factor, in a primary co-culture. Neuronal markers are used to detect neuronal differentiation of mature and young neurons. Our preliminary results show an increase in the expression of NeuN and MAP2 in the presence of LIF indicating neuronal growth, compared to its counterparts treated with HIV-gp120. In order to analyze apoptosis and inflammation, we performed studies using BrdU and TUNEL assays. TUNEL reagents are applied to detect late-stage apoptotic cells during inflammation when exposed to HIV-gp120. BrdU immunoassay resulted in low levels of inflammation and apoptosis, compared with cells exposed to HIV-gp120. Another interesting approach is developing an effective HIV vaccine to diminish infection. By targeting dendritic cells (DC) we pre-activate in-vivo DC before HIV immunization using certain adjuvants that interact with DC in order to achieve rapid enhancement of immune response. To properly observe the effect of DC stimulation on the effectiveness of a Gag DNA vaccine in-vivo Imiquimod was employed as an adjuvant in concentrations of 25, 50, and 100nM. Low concentrations of Imiquimod were found to effectively stimulate Gag production by up to 25% as well as T-cells response.On the contrary, higher concentrations of Imiquimod reduced immunization effects by up to 55%. The aim of this study is to discuss the neuroprotective properties of LIF in order to develop a treatment for the affected population of HANDs. In addition, we discuss a possible alternative for an effective HIV vaccine as an agent to combat this unrelenting disease. These different goals each carry the weight of ensuring a better quality of life for the vulnerable population. **Acknowledgments:** Supported by Universidad Central Del Caribe (UCC) and the office of the Associate Dean for Research and Graduate Studies, The Alliance-NIMHD-NIH, Expanding Undergraduate Students Education, Opportunities and Options in Clinical and Translational Research Supported by the US Department of Education: Title V Grant Award #P031S160068 and MAC-FRED Program 2018.

B395/P2072

Tau secretion by VAMP8 impacts its intra- and extracellular cleavage

J. Pilliod[1], M. Gélinas-Faucher[2], **N. Leclerc**[2]; [1]CRCHUM, Montréal, QC, Canada, [2]University of Montreal, Montréal, QC, Canada

In Alzheimer's disease, Tau, a microtubule-associated protein, becomes hyperphosphorylated, detaches from microtubules, and accumulates in the somato-dendritic compartment where it forms insoluble aggregates. Tau also accumulates in the CSF of patients indicating that it is released by neurons. Consistent with this, several laboratories including ours have shown that Tau is secreted by neurons through unconventional secretory pathways. Recently, we reported that VAMP8, an R-SNARE found on late endosomes, increased Tau secretion and that secreted Tau was cleaved at the C-terminal. In the present study, we examined whether the increase of Tau secretion by VAMP8 affected its intra- and extracellular cleavage. Upon VAMP8 overexpression, an increase of Tau cleaved by caspase-3 in the cell lysate and medium was observed. This was correlated to an increase of active caspase-3 in the cell lysate and medium. Using a Tau mutant not cleavable by caspase-3, we demonstrated that Tau cleavage by caspase-3 was not necessary for its secretion upon VAMP8 overexpression. By adding recombinant Tau to the culture medium, we demonstrated that extracellular Tau cleavage by caspase-3 could occur because of the release of active caspase-3, which was the highest when VAMP8 was overexpressed. When cleavage of Tau by caspase-3 was prevented by using a non-cleavable mutant, secreted Tau was still cleaved at the C-terminal, the asparagine N410 contributing to it. Lastly, we demonstrated that N-terminal of Tau regulated the secretion pattern of a Tau fragment containing the microtubule-binding domain and the C-terminal of Tau upon VAMP8 overexpression. Collectively, the above observations indicate that VAMP8 affects the intra- and extracellular cleavage pattern of Tau.

B396/P2073

Dysregulated epidermal keratinocyte dynamics contribute to chronic damage signaling and impaired healing of thermal burn injury

A. Horn, A. Fister, J. Squirrell, A. Huttenlocher; University of Wisconsin, Madison, WI.

Repair of epithelial injury requires the coordinated activity of keratinocytes, the primary constituent of the epidermis. Directed migration of keratinocytes facilitates immediate wound closure, while production of signaling molecules such as reactive oxygen species (ROS) promotes long-term healing. While epithelial tissue is well-adapted to repair from mechanical trauma, thermal burn injuries heal poorly. To investigate whether dysregulated keratinocyte behavior contributes to wound healing defects in burned tissue, we used the larval zebrafish, which allows for real-time visualization of keratinocyte dynamics during tissue repair. In contrast to injury by mechanical tailfin transection, we find that burn injury induces excessive keratinocyte movement, reminiscent of collective migration. Disorganized keratinocyte movement immediately following injury results in accumulation of epithelial damage over time. Inhibiting keratinocyte movement by injuring in the presence of isotonic medium prevents long-term epithelial damage and suggests that keratinocytes respond to osmotic cues in the burn environment. While it is known that epithelial repair requires transient and wound-localized ROS production, we find that burn injury causes chronically-elevated ROS across the tissue-scale. However, blocking keratinocyte movement spatially restricts ROS production to the site of injury, suggesting regulated keratinocyte migration is a mechanism for localizing repair signaling. To determine whether excessive keratinocyte movement contributes to poor burn wound healing, we assessed the function of

peripheral sensory neurons. Clinical observations highlight that patients suffer from loss of sensory perception in burned tissue, and chronic ROS increase is linked to neurodegenerative disease. We find sensory neuron regeneration and function is compromised following burn injury compared to tailfin transection; however, preventing excessive keratinocyte movement and subsequent tissue damage through treatment with isotonic medium, or reducing ROS by antioxidant treatment, is sufficient to rescue sensory neuron function. These results demonstrate the requirement of tightly regulating keratinocyte dynamics following tissue injury. In the context of thermal burn injury, dysregulated keratinocyte function contributes to neuronal damage and loss of sensory perception in wounded tissue.

B397/P2074

Keratinocyte-dependent oxidative stress impairs sensory neuron regeneration after tissue injury

A. Fister, A. Horn, J. Squirrell, A. Huttenlocher; Pediatrics, University of Wisconsin, Madison, WI.

Damage to the skin results in a cascade of wound healing and regenerative responses that mediate tissue repair. Sensory neuron function is also impaired by tissue damage, and reactive oxygen species (ROS) signaling is required for their recovery after mechanical injury. Clinical observations reveal that while sensory function is recovered after mechanical injury, thermal burn injury results in either chronic loss of touch sensation or pain and the underlying mechanisms of this defect remain unclear. To understand why this occurs, we took advantage of the optical transparency and simple epithelial structure of larval zebrafish to visualize the mechanical (transection) and burn wound environment *in vivo*. After transection, sensory neurons regenerate well and maintain sensory perception. After burn, sensory neurons regenerate poorly, and larvae display a prolonged loss of sensation in the wound area. Live imaging reveals that the early burn response, but not the mechanical response, is characterized by the uncoordinated and excessive movement of intertwined keratinocytes and sensory neurons. Real time analysis of neuronal damage and calcium flux shows prolonged neuronal damage in thermal injury that is not a direct result of tissue movement. In association with this this damage, there is dysregulated reactive oxygen species signaling that is restored after a tail transection following the burn. Reduced ROS induced by the tail transection or treatment with the antioxidant N-Acetyl Cysteine (NAC) rescued the defect in sensory neuron regeneration and function after thermal injury. Taken together, the findings suggest that a balance and spatiotemporal regulation of ROS signaling is necessary for sensory neuron regeneration and repair.

B398/P2075

CHIP ameliorates neuronal damage in H_2O_2-induced oxidative stress in HT22 cells and gerbil ischemia

I. Hwang[1], K. Hahn[2], H. Kwon[3], Y. Yoon[1], D. Kim[4]; [1]Seoul National University College of Veterinary Medicine, and Research Institute for Veterinary Science, Seoul, KOREA, REPUBLIC OF, [2]Seoul National University College of Veterinary Medicine, Seoul, KOREA, REPUBLIC OF, [3]Gangneung-Wonju National University College of Dentistry, Gangneung, KOREA, REPUBLIC OF, [4]Gangneung-Wonju National University, Research Institute of Oral Sciences, College of Dentistry, Gangneung, KOREA, REPUBLIC OF.

Carboxyl terminus of Hsc70-interacting protein (CHIP) is highly conserved and is linked to the connection between molecular chaperones and proteasomes to degrade chaperone-bound proteins. In this study, we synthesized the transactivator of transcription (Tat)-CHIP fusion protein for effective delivery into the brain and examined the effects of CHIP against oxidative stress in HT22 cells induced by hydrogen

peroxide (H_2O_2) treatment and ischemic damage in gerbils by 5 min of occlusion of both common carotid arteries, to elucidate the possibility of using Tat-CHIP as a therapeutic agent against ischemic damage. Tat-CHIP was effectively delivered to HT22 hippocampal cells in a concentration- and time-dependent manner, and protein degradation was confirmed in HT22 cells. In addition, Tat-CHIP significantly ameliorated the oxidative damage induced by 200 μM H_2O_2 and decreased DNA fragmentation and reactive oxygen species formation. In addition, Tat-CHIP showed neuroprotective effects against ischemic damage in a dose-dependent manner and significant ameliorative effects against ischemia-induced glial activation, oxidative stress (hydroperoxide and malondialdehyde), pro-inflammatory cytokines (interleukin-1β, interleukin-6, and tumor necrosis factor-α) release, and glutathione and its redox enzymes (glutathione peroxidase and glutathione reductase) in the hippocampus. These results suggest that Tat-CHIP could be a therapeutic agent that can protect neurons from ischemic damage.

B399/P2076

Neuronal activity regulates matrin-3 in a calcium and calmodulin dependent manner

J. Wu, A. Malik, C. Gillies, Q. Doctrove, S. Barmada; University of Michigan, Ann Arbor, MI.

RNA binding protein (RBP) dysfunction and abnormal neuronal excitability are hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Even so, it remains unclear if and how activity dependent processes regulate RBPs. In a subset of familial ALS cases, the mutations in the gene encoding the DNA-RNA binding protein matrin-3 (MATR3) causes familial ALS and FTD. MATR3 pathology, including cytoplasmic mislocalization and increased nuclear staining, also occurs in sporadic cases of ALS, suggesting a potentially important role for MATR3 in disease pathogenesis. Consistent with this, we demonstrated that both knockdown and overexpression of MATR3 results in neurotoxicity, indicating proper MATR3 homeostasis is essential for neuronal survival. Here, we show that neuronal activity regulates MATR3 abundance through a calcium-dependent mechanism, providing a crucial link between neuronal excitability and disease pathogenesis in ALS and FTD. Stimulation of neuronal activity with the glutamate receptor agonist NMDA leads to the rapid degradation of MATR3 in mature primary neurons. Both calcium and calpains—a family of calcium-sensitive cysteine proteases—are required for MATR3 degradation in response to NMDA. Furthermore, treatment with W-7, an inhibitor of the calcium signal transduction protein calmodulin (CaM), blocks MATR3 turnover. Previous studies hinted at a direct interaction between CaM and the RNA binding domain of MATR3, and we found that NMDA treatment rapidly inhibits MATR3's ability to bind its RNA targets. These observations suggest a model in which NMDA receptor activation results in calcium influx, CaM binding to MATR3 and release of its RNA substrates, and subsequent MATR3 degradation via calpains. This model may also help explain the toxicity of a newly-identified *MATR3* mutation affecting a conserved residue (F488L) adjacent to MATR3's RNA binding domains. Collectively, our data connect two conserved phenomena in ALS and FTD—neuronal hyperactivity and RNA binding protein pathology—and may have direct implications for the regulation of RBPs as well as disease pathogenesis.

B400/P2077

Genetic Interaction between Protein Quality Control System and GSK3β Signaling Pathway in ALS Disease Models

H. Choi[1,2], J. Lee[3], K. Kim[4]; [1]Soonchunhyang Institute of Medi-bio Science, Soonchunhyang University, Cheonan, KOREA, REPUBLIC OF, [2]Department of Integrated Biomedical Science, Soonchunhyang University, Cheonan, KOREA, REPUBLIC OF, [3]Department of Medical Biotechnology, Soonchunhyang

University, Asan, KOREA, REPUBLIC OF, [4]Department of Medical Science, Soonchunhyang University, Asan, KOREA, REPUBLIC OF.

Fused in sarcoma (FUS) and TATA-box binding protein associated factor 15 (TAF15) belong to the FET family of structurally similar DNA/RNA-binding proteins involved in DNA repair and RNA processing. FUS/TAF15 are associated with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Normally FUS/TAF15 resides primarily in neuronal nuclei whereas cytoplasmic mislocalized FUS/TAF15 accumulates, causing neuronal toxicity. However, the molecular mechanisms underlying FUS/TAF15 -mediated ALS pathogenesis are largely unknown. Here, we found that *Shaggy*, which is a *Drosophila* homolog of *GSK3β* is a potential modulator of FUS/TAF15 -induced neuronal toxicity. Moreover, shaggy enzyme activity was increased in the FUS/TAF15-induced ALS model. Knockdown of *Shaggy* in fly neurons attenuates neurodegenerative phenotypes, including motor neuron degeneration, and mitochondrial dysfunction. In addition, we demonstrated F-box protein Slimb controls the FUS/TAF15 protein levels, and SCF^{slimb} is a critical regulator for shaggy-mediated suppression of FUS/TAF15-induced toxicity in *Drosophila*. These findings revealed a novel mechanism of neuronal protective effect through SCF^{Slimb}-mediated FUS/TAF15 degradation via GSK3β inhibition.

B401/P2078

PFOS exposure disrupts glutamate-dependent sensory behavior in C. elegans

K. Kessel, E. Luth; SIMMONS University, Boston, MA.

Perfluorooctanesulfonic acid (PFOS) is part of the polyfluoroalkyl substance (PFAS) family that were used industry and consumer products like cookware and clothing. The accumulation of PFAS in the brain has been proposed to lead to behavioral and cognitive disorders. However, the specific mechanisms underlying the effects of PFAS-induced neurotoxicity still need to be explored. Previous studies in the roundworm C. elegans have reported that PFOS affects the dopaminergic system, but its effects of glutamate signaling have not been explicitly explored. Here, we explore the effects of PFOS on glutamate-dependent sensory behavior in C. elegans. To test this we looked at the optogenetic activation of ASH sensory neurons in channelrhodopsin-expressing transgenic worms. This has been shown to trigger glutamate-dependent locomotor reversals. We exposed synchronized L1 worms to PFOS concentrations ranging from 0.001 ppm-10 ppm (0.0017 uM-17 uM) as well as a methanol control. We found that exposure to as little as 0.1 ppm PFOS significantly reduced the light-induced reversal response rate compared to the methanol control. Importantly, we only observed growth defects at concentrations above 10 ppm, suggesting that the impaired glutamate-dependent reversal response was independent of gross developmental delay. Our preliminary data indicated that channelrhodopsin::YFP expression in ASH neurons is not altered upon exposure to 1 ppm PFOS. This implies that the capacity for ASH depolarization is not affected by PFOS exposure and the source of the behavioral defect is downstream of sensory neuron activation. We conclude that 0.1-10 ppm PFOS impairs the function of glutamate neurons in C. elegans. Our results provide a benchmark for testing low concentrations of other PFAS on glutamate neurons. This will help us in the future identify how PFOS impacts the central nervous system, possibly affecting memory, cognition, mood and motor skills.

B402/P2079

Role of Y-family polymerases in neuronal genome maintenance and aging

M. Abdelmageed[1], S. Paul[2], **A. Paul**[1]; [1]Neural Behavioral Sciences, Penn State College of Medicine, Hershey, PA, [2]Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey, PA.

Genomic instability increases with age due to an accumulation of DNA damage and distinct DNA damage response pathways have evolved with a host of distinct polymerases that act via different pathways such as nucleotide excision repair, base excision repair to mend DNA lesions. In contrast, the relatively newly discovered Y-family DNA polymerases can bypass damaged DNA in dividing cells, however, their functions in enduring post-mitotic cell types like neurons are largely unknown, where the non-homologous end joining (NHEJ) pathway has been shown to repair double-stranded DNA breaks. To understand the biological role of Y-family polymerases in maintaining the central nervous system (CNS) genome we studied its expression as function of aging and neurodegeneration. We discovered that most Y-family polymerases are expressed extensively in the brain and its expression in sub-cellular compartments are significantly altered as a function of chronological age. We identify that such alterations in neurons are non-uniform across different brain areas perhaps reflecting metabolic demand and demonstrate interacting partners of one member of Y-family polymerase, Polymerase kappa (PolK) to be associated with a NHEJ pathway protein. We further identify the subcellular compartments of PolK sites within the neurons and explore the potential relationship of PolK with cytoplasmic DNA and immune activation.

B403/P2080

Synaptojanin 2-mediated transport of mRNA maintains mitophagy and mitochondrial function in axons and synapses

W. S. Gibbs, PhD, T. L. Schwarz, PhD; Boston Children's Hospital, Boston, MA.

In neurons, maintaining healthy mitochondria is complicated by the need for delivery and clearance in long axons. Mitochondrial proteins require rejuvenation despite their distance from the nucleus. Moreover, proteins such as PINK1 have extremely short half-lives, suggesting the need for local translation. PINK1, a serine-threonine protein kinase, regulates mitochondrial function and quality control pathways, including mitophagy. Mutations in PINK1 cause autosomal recessive Parkinson's disease.We are studying the role of mRNA transport in preserving mitochondrial function and turnover. Harbauer et al, 2022 demonstrated the association and co-transport of PINK1 mRNA with axonal and dendritic mitochondria. Synaptojanin 2 (SYNJ2) tethers PINK1 mRNA to the outer mitochondrial membrane (OMM) through its interaction with OMP25. SYNJ2 has a predicted RNA Recognition Motif (RRM) that had not previously been studied, but is critical for PINK1 mRNA localization to mitochondria. This neuron-specific mechanism works in concert with the translation of the PINK1 transcript.We have now used UV-crosslinking of FLAG-tagged SYNJ2 expressed in rat cortical neurons for gel shift assays and demonstrated that SYNJ2 binds neuronal RNA and that this binding is prevented by introducing three alanine mutations in the SYNJ2 RRM ($SYNJ2^{AAA}$). We have also used CRISPR to introduce the AAA mutation into the endogenous SYNJ2 locus of mice and of human iPSCs. The mice are homozygous, viable and fertile and the iPSCS can be differentiated into iNeurons. We have used these systems to determine the functional significance of SYNJ2 RRM. In axons of $SYNJ2^{AAA}$ iNeurons, depolarization of mitochondria failed to induce the accumulation of PINK1 protein that occurred in isogenic control axons. Similarly, the induction of phospho-ubiquitination, a direct product of the PINK1/Parkin dependent

mitophagy pathway, was prevented in SYNJ2^{AAA} mouse primary cortical neurons. In addition, mitochondrial bioenergetics was impacted by the mutation of the RRM: under stress conditions, ATP levels were reduced in the mutants compared to WT mouse primary neurons. Our work demonstrates the functional importance of SYNJ2 in mitochondrial mRNA transport and translation for regulating mitochondrial health and will further our understanding of how distal mitochondrial mechanisms are compromised in neurodegeneration.

B404/P2081

Evidence that autophagy plays roles in alzheimer's disease

M. Ranjbar[1], E. L. Bearer[2]; [1]University of New Mexico, Albuquerque, NM, [2]Department of Pathology, University of New Mexico Health Sciences Center, Albuquerque, NM.

Intro: Neurodegenerative diseases are tightly related to the process of Aging in the cells. Autophagy as a critical mechanism for the turnover of cell contents can be a key pathway in order to regulating aging. By the way, the whole genomic pathway of autophagy is not known clearly. With a holistic knowledge of genetic alterations in the autophagy genetic map, we may be able to have a more profound picture of the potential roles of genetic variations in autophagy genes and their relationship with neurodegenerative diseases such as Alzheimer's Disease (AD).Method: A comprehensive list of potential autophagy genes was prepared, using 5 different sources. As one of the references, a machine learning (ML) method was used to find so-called autophagy dark genes, which are genes that can be related to the enormous autophagy machine but are currently ignored by the autophagy database. After that, these genes were checked with previous studies on the genomics of AD to find their presence in the pathologic pathway of the disease. Single nucleotide polymorphisms (SNPs) of the discovered genes were observed from the previously collected sequencing data.Results and Discussion: More than 9800 gene names were found in the comprehensive list. Additionally, a list of the top 20 autophagy dark genes was prepared. Our further exploration showed strong connections of those genes to the autophagy system and AD. It suggests the potential roles of autophagy in neurodegenerative diseases such as AD. This project is supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences of the National Institutes of Health through Grant Number UL1TR001449 and by NIH Common Fund U24 CA224370.

B405/P2082

Microscoop, a discovery-based image-guided proteomics technology, reveals novel factors on amyloid-beta aggregates in differentiated SH-SY5Y cells

C. Huang, **W. Chong**, C. Huang, Y. Chen, C. Chung, H. Chang, J. Liao; Syncell Inc., Taipei, TAIWAN.

Aggregation of amyloid-β peptides (Aβ) is a hallmark of Alzheimer’s disease (AD). Subcellular distribution of Aβ is well recognized under a microscope, but its pathological and physiological functions remain unclear, partially due to our limited understanding of interacting proteins and corresponding signaling pathways associated with Aβ. Existing spatial proteomics technologies focus on mapping of known proteins using antibody panels/arrays, hindering hypothesis-free proteome discovery. In this study, we used Microscoop^{TM}, a fully-automated microscopy-guided subcellular photolabeling with a machine learning-based precision recognition to enable discovery-based image-guided proteomics. We applied Aβ1-42 deposition in human neuroblastoma, SH-SY5Y, differentiation cells as an AD model. Multiple images of Aβ1-42 aggregates were applied to segment to locations of specific Aβ1-42 aggregates of

interest using convolutional neural networks (CNN)-based deep learning. Microscoop was used to illuminate these segmented regions to induce photochemical reactions of proprietary photosensitive probes and trigger spatial covalent labeling of proteins adjacent to Aβ1-42 aggregates. This spatial-specific photochemical labeling process was repeated automatically on thousands of microscopic fields of view to accumulate enough Aβ1-42-associated proteins for LC/MS-MS-based proteome identification. A series of novel factors were discovered to be associated with Aβ aggregation in SH-SY5Y differentiated cells. We further validated these newly identified proteins using antibody staining and found that these proteins indeed colocalized with Aβ1-42. The finding of these novel factors opens a door to reveal associated signaling pathways related to AD. They may also serve as new diagnostics biomarkers or new AD drug target. Our study not only reveals the Aβ-associated spatial proteome, but also demonstrates its possible broad applications on discovery-based spatial proteomics in neuroscience.

B406/P2083

Intricate viral proteins: A deathly hallow to affect the brain

D. Tiwari, H. C. Jha; Department of Biosciences and Biomedical Engineering, Indian Institute of Technology Indore, Madhya Pradesh, Indore, INDIA.

Historically, virus infections have been linked to neurodegenerative disorders. Members of the *Herpesviridae* family are the most well-known culprits for causing damage to the neurological milieu. Among them, Epstein-Barr virus (EBV) is one pathogen with high population penetrance. It infects >95% of the global population asymptomatically and establishes lifelong latency. In some cases, EBV infection could give rise to fatal consequences such as gastric cancer, blood cancer, and neurodegenerative disorders (NDs). However, what triggers the initiation of such grievous outcomes is still not clearly understood. Although, the clinical presence of EBV in CSF and tissue samples of patients suffering from NDs, along with its recently established neurotropic potential, strongly suggests its role in neurodegeneration. Further, various processes closely linked with NDs like Alzheimer's (AD) and Parkinson's disease (PD), such as neuroinflammation, cell-cycle disruption, and Reactive Oxygen Species (ROS) homeostasis, are shown to be affected by EBV. We hypothesize that EBV has the potential to modulate neuronal cellular processes, which are crucial for the development of neurodegenerative pathology, like in AD.The present study attempts to understand the mechanism underlying virus-mediated neurodegeneration that might lead to AD via malfunctioning protein metabolism. The formation of amyloid beta ($A\beta_{42}$) aggregates is a characteristic feature of AD, and previous reports have linked the members of the *Herpesviridae* family with proteinaceous aggregate formation. Therefore, in this study, we tried to determine the aggregate forming tendency of EBV proteins. We performed the *in-silico* analysis of various EBV proteins to evaluate their aggregation proclivity and found that a 12 amino acid long peptide derived from EBV glycoprotein-M (146-157) has a high aggregation tendency. Further, *in-vitro* analysis of the EBV-$gM_{146-157}$ peptide established the formation of aggregates in the solution by a process that followed time- and concentration-dependent kinetics. The viral protein aggregates formed were observed to have a secondary structure similar to $A\beta_{42}$ and were highly cytotoxic to neurons (IMR-32) even at a low concentration of 37μM. We have also observed the increased amyloid-beta aggregate seeding capability of the viral peptide with increasing concentration, corroborating the possibility of viral peptide-mediated aggregation cascade initiation. Our findings demonstrated a positive correlation between amyloid aggregate deposition and viral infection. The proposed hypothesis provides a possible mechanistic explanation of EBV's role in developing AD pathophysiology by initiating the proteinaceous aggregate deposition cascade that has been elusive to date.

B407/P2084

TPPP Forms Liquid Condensates and Aggregates in Multiple Systems Atrophy

H. S. Richardson, S. Kemal, J. Nowacki, A. Kopach, M.-m. Fu; National Institute of Neurological Disease and Stroke, Bethesda, MD

Oligodendrocytes are glial cells that make myelin sheaths to increase the speed of neuronal electrical signals. They use radial and lamellar microtubules to seek out and wrap axons. An important protein in this process is tubulin polymerization promoting protein (TPPP), which our lab showed nucleates microtubules outside the cell body from satellite organelles called Golgi outposts. Previous papers noted that TPPP accumulates in oligodendrocytes in Multiple Systems Atrophy (MSA), a neurodegenerative disease characterized by aberrant aggregation of the protein alpha-synuclein (a-syn) in oligodendrocytes. However, the link between the function of TPPP in oligodendrocyte cell biology and its role in disease has yet to be uncovered. We now find that TPPP is a dynamic protein that forms liquid condensates in order to nucleate new microtubules. When using fluorescence recovery after photobleaching (FRAP) on oligodendrocyte cells, TPPP fluorescence recovery happens within a minute. However, TPPP is prone to aberrant aggregation and shaking for 1-2 days can induce recombinant TPPP to form long fibrils and chunks several μm in size. By FRAP, similar aggregates of TPPP can be seen on oligodendrocyte microtubules when TPPP is overexpressed. To see if these accumulations also occur in human samples, immunofluorescent staining was used on human MSA and control brains. When co-stained with myelin basic protein (MBP), fibrillar TPPP accumulations are colocalized with MBP sheaths. Yet, in MSA cases there were fibrillar TPPP accumulations without MBP, showing a possible breakdown of the myelin sheath. There was also an increased amount of perinuclear accumulations in the MSA cases. In MSA brains co-stained with a-syn and TPPP, many perinuclear aggregates had TPPP only (55%) while only 35% were colocalized and 10% were a-syn only. These results indicate that TPPP fibrillar accumulations may represent a precursor stage that begins in the myelin sheath, where Golgi outposts are normally found and where non-pathological TPPP performs important cellular functions. First, the TPPP may cause the myelin sheath to begin degrading. Then, the overabundant TPPP may find its way into the cell body and continue to accumulate. Finally, TPPP and a-syn co-aggregate in oligodendrocyte cell bodies. Ongoing studies will look at the effects of TPPP phosphorylation on its potential to form liquid condensates and aggregate in cells and disease.

B408/P2085

Genetic validation of novel drug targets to prevent alpha-synuclein-induced dopaminergic degeneration

E. S. Luth[1], **R. Kienle**[1], E. San Andres[1], C. Beauvais[1], U. Dettmer[2], N. Ramalingam[2]; [1]Biology, Simmons University, Boston, MA, [2]Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Parkinson’s disease (PD) is a neurodegenerative disease that causes a progressive loss of movement. Hallmarks of PD are the degeneration of dopamine-producing neurons in the substantia nigra and the formation of alpha-synuclein (aSyn) aggregates called Lewy bodies. Currently, there are only treatments for PD symptoms, none actually stop neurodegeneration. We recently identified the cytoskeleton-associated proteins myosin-V and spastin as potential modifiers of the toxicity induced by aSyn in a drug screen with neuroblastoma cells. To validate these potential therapeutic targets in an animal model that

undergoes age-related aSyn-induced neurodegeneration, we used transgenic *C. elegans* that overexpress aSyn and have fluorescently tagged dopamine neurons, allowing for visualization of dopaminergic degeneration. We crossed worms with deletions in either *hum-2* or *spas-1*, *C. elegans* homologs for myosin-V and spastin, respectively, into this transgenic strain. Using fluorescence microscopy, we found that 7-day adult mutant worms overexpressing aSyn had more visible head dopaminergic neurons than transgenic worms with wildtype copies of *hum-2* or *spas-1*. Also, a greater percentage of mutant worms had a complete set of anterior dopaminergic neurons compared to worms with only the aSyn transgene. Overall, our data suggest that the knockout of *hum-2* or *spas-1* prevents dopaminergic neuron degeneration caused by aSyn overexpression. These results support efforts to pursue myosin-V and spastin as novel drug targets to stop the dopaminergic neurodegeneration in PD. In the future, we plan to look at dopamine-mediated behavior in aSyn-overexpressing worms with *hum-2* or *spas-1* mutations to see if, in addition to protecting against their overt loss, disrupting *hum-2* or *spas-1* preserves dopaminergic neuron function.

B409/P2086

TMBIM6 prevents the apoptosis on in vitro and in vivomodels of Parkinson´s Disease

P. Ahumada-Montalva[1], W. Gómez[2], M. Calegaro-Nassif[2], M. Sanhueza[3], R. Vidal[4], D. Rojas-Rivera[1]; [1]Cell Death and Biomedicine Laboratory, Universidad Mayor, Santiago, CHILE, [2]2Autophagy and Neurodegeneration laboratory, Universidad Mayor, Santiago, CHILE, [3]Centro de resilencia, adaptación y mitigación, Universidad Mayor, Temuco, CHILE, [4]Center for Integrative Biology, Universidad Mayor, Santiago, CHILE.

Introduction: Parkinson´s disease (PD) is a common neurodegenerative syndrome characterized by dopaminergic (DAergic) neurons death, and consequent extrapyramidal movement disorder. Unfortunately, PD has no cure. Tmbim6 is an anti-apoptotic protein that integrates several key cellular processes. Tmbim6 arises as protective factor in other disorders, but its possible neuroprotective role in PD is unknown. Here, we hypothesize that Tmbim6 plays a neuroprotective role in PD, avoiding DAergic neurons death. **Methods:** We inhibited Tmbim6 on primary cortical neuron cells (CXNs) using small molecules and siRNA. Then, Additionally, we decreased Tmbim6 expression on whole brain- and DAergic neurons- of drosophila using siRNA and assessment locomotor and survival. Then, we injected small molecules inhibitor of Tmbim6 on substantia nigra of mouse and the motor skills and DAergic neurons was assessment. Also, we overexpressed Tmbim6 on CXNs genetic technique and then cell death was assessment by real-time cell death assays, meanwhile cleaved caspase 3 levels was assessment by western blotting. **Results:** In brief, we demonstrated *in vivo* that the loss of Tmbim6 in pan- and DAergic-neurons causes a rapid and significant deficit in locomotor ability on flies exposed to rotenone, in contrast to the control. Also, we had shown inhibition of Tmbim6 on substantia nigra causes a rapid and significant deficit in locomotor skills. Finally, we shown that overexpression of Tmbim6 decrease both cell death and levels of cleaved caspase 3 induced by 6-OHDA and rotenone on CXNs. **Discussion:** For first time, we demonstrated *in vitro* and *in viv*o the neuroprotective role of Tmbim6 in sporadic PD models. We demonstrated that inhibition of Tmbim6 on primary cortical neurons triggers the cell death, also we observed that lack of Tmbim6 on neurons cause decreased in life spam and locomotor ability in drosophila, principal features of PD-like neurodegeneration. We concluded that Tmbim6 is critical to neuronal survival. For last, the overexpression of human Tmbim6 result in decreased cell death caused by 6-OHDA and rotenone on cortical neurons. We conclude that overexpression of Tmbim6 is

neuroprotector on PD. Taken together, our work is the first to strongly suggest a possible neuroprotective role of Tmbim6 in PD.

B410/P2087

A novel neuroprotective role of Reelin signaling in a cellular model of NPC disease

C. Alarcon-Godoy, A. Pacheco, N. Sommer, M. Benitez, M. Marzolo; Dept. Cell and Molecular Biology, Pontificia University Catolica Chile, Santiago, CHILE.

Niemann-Pick type C (NPC) disease is a rare lipid-storage disorder mainly caused by mutations in the NPC1 transporter, which regulates the export of unesterified cholesterol from lysosomes. The accumulation of cholesterol and glycosphingolipids in endo-lysosomal compartments impairs lysosomal degradation functions and trafficking. In neurons, lysosomal impairment evokes oxidative stress and neuronal cell death, driving severe neurological symptoms in NPC patients. Reelin is a secreted glycoprotein with several functions in neurodevelopment and the adult brain that triggers a complex signaling pathway upon binding to ApoER2 or to VLDL, receptors belonging to the LDLR family. Reelin has protective effects in neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. To evaluate if Reelin has a neuroprotective effect in an NPC condition, we induced an NPC1-like phenotype using the U18666A inhibitor, which accumulates cholesterol in the lysosomes. Scholl's analyses showed that the addition of Reelin in primary hippocampal neurons treated with U18666A partially recovered dendritic arborization. Moreover, Reelin increased the survival of neurons treated with U18666A by MTT assay and reduced apoptotic nuclei. Interestingly, reelin effects were possibly associated with a reduction of the NPC1 phenotype induced by U18666A by decreasing the accumulation of cholesterol (determined by the intensity of filipin staining) compared to mock and causing a re-distribution of those endo-lysosomes to the cell periphery. The bioavailability of Reelin and its signaling components could modulate the pathology's essential traits, allowing some functional recovery of damaged organelles.

B411/P2088

Blockade of endo-lysosomal pathway promotes progranulin-deficient microglial toxicity via proinflammatory lipids

K. Hashimoto, E. J. Huang; University of California San Francisco, San Francisco, CA

While microglia are key cells to maintain brain functions by preventing damage, it is becoming clear that aging changes microglial functions toward to having neuronal aggressive character. However, it remains unclear the details how aged microglia influences neurodegeneration.Dominant mutations in the human Progranulin (*GRN*) gene dramatically reduce Progranulin levels in the brain and are causally connected to frontotemporal dementia. To investigate how Progranulin deficiency promotes neurodegeneration, we have shown that Grndeficient ($Grn^{-/-}$) mice develop hyperactivation of microglia, which affects cytoplasmic TDP-43 aggregation and neuronal loss via excessive complement production. Another phenotype in $Grn^{-/-}$ mice is the abnormal lipidosis in aged brain, however, the mechanisms leading to these lipid phenotypes and how the lipid metabolic defect impact neurodegeneration in $Grn^{-/-}$ mouse brain are not revealed. Here, we combined sucrose density gradient-based subcellular fractionation of $Grn^{+/+}$ and $Grn^{-/-}$ aged mouse brains with proteomic and lipidomic analyses to show that Progranulin deficiency caused proteomic changes that affected the endo-lysosomal pathway and altered lipidomic profiles in $Grn^{-/-}$ brain early endosomes and lysosomes. Interestingly, confocal microscopic analyses

showed that drastic accumulation of lipids was occurred in *Grn*$^{-/-}$ microglia and neurons, not in astrocytes and oligodendrocytes. To determine the detail mechanisms how *Grn*$^{-/-}$ microglia show lipid metabolic defect, we performed lipidomic profiling in primary microglia and microglia conditional media from *Grn*$^{+/+}$ and *Grn*$^{-/-}$ mice and analyzed subcellular localization of lipids in *Grn*$^{+/+}$ and *Grn*$^{-/-}$ primary microglia. These data indicated that lipidomic alteration in *Grn*$^{-/-}$ microglia were caused by the failure to engage lipids to lysosome-mediated degradation, leading to increase in lipid storage and secretion. Using microglia-neuron co-culture system, we showed that the lipid-enriched *Grn*$^{-/-}$ microglia promote dendritic degeneration and cell death in *Grn*$^{-/-}$ neurons, which can be neutralized by monoclonal antibody E06, a functional blocker of oxidized phospholipids. These results propose novel insights how endo-lysosomal block in *Grn*$^{-/-}$ microglia promote disrupt microglia-neuron homeostasis, thereby promoting neurodegeneration by the accumulation and secretion of proinflammatory lipids.

B412/P2089

Autolysosomal Exocytosis of Lipids Protect Neurons from Ferroptosis

I. Ralhan[1], J. Chang[1], M. J. Moulton[2], L. D. Goodman[2], N. Y. J. Lee[1], G. Plummer[1], H. Pasolli[3], D. Matthies[4], H. J. Bellen[2], M. S. Ioannou[1]; [1]University of Alberta, Edmonton, AB, CANADA, [2]Baylor College of Medicine, Houston, TX, [3]The Rockefeller University, New York, NY, [4]National Institutes of Health, Bethesda, MD.

During oxidative stress neurons release lipids that are internalized by glia and stored in lipid droplets. This process is essential to maintain the health and function of the nervous system. Defects in this coordinated process play an important role in several neurodegenerative diseases. Yet, the mechanisms of lipid release and its consequences on neuronal health are unclear. Here, we demonstrate that lipid-protein particle release by autolysosome exocytosis protects neurons from ferroptosis, a biochemically distinct form of cell death driven by lipid peroxidation. During oxidative stress in primary cell culture or fly retina, neuronal lipid release depends on the lysosomal exocytic machinery; VAMP7 and syntaxin 4. We show that these lipids are released through exocytosis of autolysosomes. We observe membrane-bound lipid-protein particles by transmission electron microscopy and demonstrate these particles are released from neurons using cryo-electron microscopy. Failure to release these lipid-protein particles causes lipid hydroperoxide accumulation and cell death by ferroptosis. Our results reveal how neurons use autolysosomal exocytosis to rid themselves of peroxidated lipids generated during oxidative stress. Given the number of brain pathologies that involve ferroptosis, defects in this pathway likely play a key role in the pathophysiology of neurodegenerative disease.

B413/P2090

Aberrant neuronal connectivity between the ventral hippocampus and medial prefrontal cortex leads to social impairments in *Kansl1*-deficient mice

B. Jeon, T. Ford; Department of Biological Sciences, Kent State University, Kent, OH.

Koolen-de Vries syndrome (KdVS), also known as 17q21.31 microdeletion syndrome, is a neurodevelopmental disorder and displays social behavior deficits. While haploinsufficiency of the *KAT8 regulatory NSL complex complex subunit 1* (*KANSL1*) gene is associated with KdVS, the cellular basis of *KANSL1* haploinsufficiency regarding social impairments is unknown. Here, using *Kansl1* heterozygous knockout mice, we report that the brain circuit between the ventral hippocampus (vHi) and medial prefrontal cortex (mPFC) is disrupted in the *Kansl1* mutant mouse. Stereotaxic injections of

fluorescence-tagged AAVs and dyes revealed an increase in the vHi-mPFC connection in the *Kansl1* haploinsufficient mouse compared to the wild type control. Accordingly, the cellular activity marked by c-Fos expression is altered in the mPFC of the *Kansl1* mutant mouse. This change of the vHi-mPFC circuit appears to involve parvalbumin interneurons in the mPFC. We further show that the abnormal connection in the *Kansl1* mutants is functionally relevant to social deficits. *Kansl1* haploinsufficient mice display social exploration and recognition deficits. DREADD-mediated chemogenetic inhibition of the vHi-mPFC circuit improved the social phenotypes in the mutant mice. Our results highlight the critical status of the vHi-mPFC brain circuit as a potential pathogenic factor for KdVS.

B414/P2091

A Brain Permeable Bioluminescent Substrate for NanoLuc based Reporters

J. R. Walker[1], Y. Su[2], X. Wu[2], K. Casey[2], G. Hong[2], **T. A. Kirkland**[3], M. Z. Lin[2]; [1]Amgen CORPORATION, Thousand Oaks, CA, [2]Stanford University, Palo Alto, CA, [3]PROMEGA CORPORATION, Madison, WI

Here we report thediscovery of a new brain-permeant NanoLuciferase (NLuc) substrate which we arecalling cephalofurimazine (CFz). CFz is a fluorinated analog of furimazine, andwas discovered through extensive SAR studies looking for NLuc substrates thatshowed good penetration into the CNS and brain distribution. We show the signal from this substrate in thebrain of transgenic mice expressing NLuc fused to an orange fluorescent proteinCyOFP. This fusion was linked with two differentproteins, CamkIIa and Vglut2, that show known expression throughout the brain,and we demonstrate that the signal obtained is consistent with the known expressionpattern of those proteins in the mouse brain. We then demonstrate that the CFz/NLuc-CyOFP system is sufficient toimaging brain activity in freely moving mice using a head mounted camera. Finally, aided by the facility by which NLucbased biosensors can be constructed, we pair CFz with a calcium sensor based onNLuc and show calcium dependent bioluminescence in the brain in response tohind limb stimulation.

B415/P2092

Exposure to the non-phthalate plasticizer, acetyl tributyl citrate (ATBC) results in cell cycle arrest in Neuro 2a cells

L. Carnell, K. A. Sease, A. K. Binder; Central Washington University, Ellensburg, WA

Phthalate plasticizers are noncovalently bound compounds used to make plastics malleable in a variety of household products such as plastic food wrap, personal care products, toys, and medical tubing. Phthalates have been shown to leach out of these plastic products and compromise organismal health by disrupting the nervous and reproductive systems. As a result of these detrimental effects, non-phthalates plasticizers have been developed and are being used as alternatives, but the effects of phthalate-free plasticizers on cellular health have not been extensively studied. Our goal was to examine the effects of one alternative plasticizer, acetyl tributyl citrate (ATBC), on neurons using the mouse neuroblastoma (Neuro2a) cell line as a model. Previous studies in our lab using Neuro2a cells demonstrated that ATBC at concentrations of 100µM or greater resulted in a significant decrease in the number of cells 72 hours after treatment. However, this decrease was not due to increased cell death as measured by propidium iodine staining. To further examine the mechanism for the decrease in cell number in ATBC-treated Neuro 2a cells, we utilized flow cytometry and quantitative reverse-transcriptase PCR (qRT-PCR) to measure cell cycle arrest and identify proteins involved in these pathways. We determined that 100 µM ATBC-treatment resulted in a decrease of cells in G2 by 14%

compared to vehicle-treated controls (P = .01109; N=2). Using qRT-PCR, we measured 24 hours after ATBC treatment a 1.9 fold increase (P = .01943; N=6) in expression of *p53*. Together these results suggest that ATBC is causing cell cycle arrest in dividing Neuro 2a cells. Further studies will examine whether these cells are undergoing senescence or continuing onto apoptosis. The data suggest that the phthalate-free plasticizer ATBC is also detrimental to cell proliferation and may not be a safer alternative for phthalates.

Synaptic Biology: Glucose Metabolism

B416/P2093

The role of mitochondrial Sirtuin 3 in the metabolic plasticity of synaptic transmission

A. Tiwari[1], A. Hashemiaghdam[1], M. Laramie[1], C. Bergom[2], G. Ashrafi[1]; [1]Cell Biology and Physiology, Washington University in St. Louis, St. Louis, MO, [2]Department of Radiation Oncology, Washington University in St. Louis, St. Louis, MO.

Neurons spend 70-80% of the brain's total ATP budget and much of this energy is directed to nerve terminals to support synaptic transmission. Neurons predominantly utilize glucose as their main energy source, however, they can also utilize alternative fuels like lactate when glucose becomes scarce during intense electrical activity or prolonged fasting. These alternative fuels are primarily oxidative and require mitochondrial oxidative phosphorylation (OXPHOS) to produce ATP. Metabolic plasticity in fuel usage is critical to maintain neuronal function in the face of glucose shortage, yet we know relatively little about when and how neurons utilize alternative fuels, or what the functional consequences might be. Here, we uncover a neuronal signaling pathway that is activated during glucose deprivation and enables nerve terminals to upregulate mitochondrial ATP synthesis and maintain synaptic transmission. To delineate neuronal metabolic response to fuel restriction, we first performed RNA sequencing analysis of cultured cortical neurons deprived of glucose. We uncovered the activation of the CREB pathway including induction of the transcription factor PGC1-a, a master regulator of mitochondrial function, and one of its known targets Sirtuin 3 (Sirt3). We similarly showed an induction of Sirt3 expression *in vivo* in the hippocampi of mice kept on intermittent fasting diet as compared to mice fed ad-libitum. Consistent with the upregulation of Sirt3 expression, we observed a reduction in the acetylation of mitochondrial proteins in glucose-deprived cultured neurons. The mitochondrial deacetylase Sirt3 has been shown to regulate ATP synthesis in non-neuronal cells through post-translational modification of OXPHOS enzymes. We examined how Sirt3 regulates ATP metabolism in neurons in absence of glucose. We focused on nerve terminals as they are the site of synaptic transmission and a locus of high ATP demand in neurons. Using the optical ATP sensor Syn-ATP, we determined that Sirt3 is required for maintenance of baseline ATP level in nerve terminals supplied with lactate. Through a combination of proteomic and optical methods, we revealed that Sirt3 regulates mitochondrial pyruvate uptake through deacetylation of the mitochondrial pyruvate carrier (MPC), and showed that an acetyl-mimetic form of the MPC subunit 1 was defective in pyruvate uptake. Furthermore, we demonstrated that Sirt3 is essential for metabolic support of synaptic vesicle retrieval after release in nerve terminals supplied with lactate. In summary, our study uncovers that the upregulation of Sirt3 during glucose deprivation ensures neuronal metabolic fitness and sustains neurotransmission by regulating mitochondrial pyruvate uptake.

B417/P2094

Differential localization of specific isoforms of the glycolytic protein GPI-1 reveals subcellular organization of glycolysis in neurons

I. Gonzalez[1], A. Wolfe[1], R. Goodman[2], D. Colón-Ramos[1]; [1]Departments of Neuroscience and Cell Biology, Yale University, New Haven, CT, [2]Vollum Institute, Oregon Health & Science University, Portland, OR.

How glycolysis, a multi-step enzymatic pathway for glucose metabolism, is subcellularly organized is not well understood. Our lab has shown that glycolytic enzymes such as Phosphofructokinase (PFK-1.1) and Aldolase are capable of reversibly localizing to condensates near neuronal presynaptic sites upon transient energy stress (Jang et al., 2016; Jang et al., 2021). Here we demonstrate that glucose-6-phosphate isomerase (GPI-1), a glycolytic protein that catalyzes the second step of glycolysis, also shows presynaptic subcellular localization, and examine the implications of this localization for neuronal metabolism and synaptic function. The *gpi-1* gene encodes two isoforms (GPI-1A and GPI-1B) that differ in 35 amino acids in the N-terminus which is predicted to form a coiled-coil domain. We observe GPI-1A, which lacks the coiled-coil domain, displays diffuse localization throughout neurites, while the domain-containing GPI-1B isoform displays presynaptic enrichment. Fusion of the N-terminal domain of 35 amino acids to GFP is sufficient to drive presynaptic localization, while disruptions of predicted interaction residues in the coiled-coil domain eliminates presynaptic enrichment of GPI-1B. To examine the role of this localization in neuronal metabolism, we collaborated in adapting HYLight, a new sensor for the glycolytic metabolite FBP, for use *in vivo* in *C. elegans*. We determined that coiled-coil mutants with impaired GPI-1B localization display altered FBP accumulation, suggestive of abnormal rates of glycolysis as compared to wild type. *gpi-1* mutants also demonstrate impaired capacity for PFK-1.1 to form condensates near presynaptic sites during transient energy stress, revealing a role for GPI-1 in the formation of glycolytic condensates near synapses. Our *in vivo* studies uncover mechanisms of subcellular organization that depend on the cell-specific expression of specific isoforms of glycolytic proteins, and allow us to now examine how the tissue-specific subcellular organization of glycolytic proteins regulate metabolic and neuronal function *in vivo*.

B418/P2095

Neuronal activity-driven O-GlcNAcylation promotes mitochondrial plasticity

S. B. Yu[1], R. Sánchez[1], Z. Papich[1], T. Whisenant[2], **G. Pekkurnaz**[1]; [1]Neurobiology, UC San Diego, La Jolla, CA, [2]UC San Diego, La Jolla, CA.

Neuronal activity consists of a series of molecular events, from action potential firing to synaptic vesicle recycling, which require large expenditures of energy. Continuous fulfillment of this energy demand relies on fuel availability and mitochondrial ATP synthesis. However, the molecular mechanisms regulating the activity-driven stimulation of ATP production are largely unknown. Here, we describe a novel signaling pathway capable of regulating mitochondrial bioenergetics, mediated via the enzyme O-GlcNAc transferase (OGT) and O-GlcNAcylation. We used *in vivo* and *in vitro* approaches to demonstrate that neuronal activity upregulates O-GlcNAcylation in neuronal processes. We identified mitochondria as the loci of this modification. This mechanism is activated by an increase in glucose influx and consequent O-GlcNAcylation, which allows neuronal mitochondria to sense fuel availability and promotes mitochondrial bioenergetics to compensate for high energy expenditure. To determine which mitochondrial proteins are responsible for these adjustments, we mapped the mitochondrial O-GlcNAcome of neurons. A majority of identified proteins participate in oxidative phosphorylation and

ATP production. Finally, by measuring activity-dependent on demand ATP synthesis, we demonstrate that when mitochondrial O-GlcNAcylation is blocked, neurons fail to replenish ATP after neuronal stimulation. Our findings suggests that O-GlcNAc cycling provides fuel-dependent feedback control in neurons to optimize mitochondrial performance based on neuronal activity and energy demand. This mechanism thereby couples glucose metabolism to mitochondrial bioenergetics and plays a key role in sustaining neuronal energy homeostasis.

B419/P2096

The Role of Ketone Body Metabolism in Neuronal Function

M. I. Stunault, S. G. Clemens, M. Laramie, G. Ashrafi; Washington University in St. Louis, Saint Louis, MO.

Glucose is the main fuel resource for brain activity. However, when glucose availability is limited, neurons can switch to using ketone bodies (KB) in order to sustain neuronal activity. Three KB are synthetized in the liver by breaking down fatty acids: acetate, aceto-acetate and β-hydroxybutyrate. KB enter the blood circulation and peripheral organs, including the brain, where they are broken down by mitochondria to produce ATP. Ketogenic diets are effective treatment for several pathologies from heart failure to drug-resistant epilepsies. However in excess, KB can be harmful as for example in diabetic ketoacidosis. Therefore, a better understanding of the metabolic effects of KB on neuronal function is of great interest for the development of effective dietary interventions.Thus, the aim of this study is to determine the metabolic changes mediated by ketone body utilization in nerve terminals. We first investigated how supplementation of cultured cortical or hippocampal neurons with KB impacts their viability. In contrast to non-neuronal cell lines, KB improved neuronal viability in a dose-dependent manner, as compared to glucose-deprived conditions.

We have previously shown that neurons can utilize lactate and pyruvate to sustain synaptic activity, and the mitochondrial pyruvate carrier MPC1 was proposed to mediate neuronal KB utilization. Using an shRNA, we demonstrate here that MPC1 is not required for neuronal survival with KB, highlighting the distinction between ketone body and pyruvate metabolism.

To determine if neuronal metabolic plasticity in KB utilization is mediated by transcriptional reprogramming, we examined how the expression of key ketolytic genes were effected in the hippocampi and cortices of mice fed a ketogenic diet (KD). In particular, we aimed to determine whether neurons displayed a transcriptional signature that was consistent with elevated KB levels in the serum of KD-fed mice. To model neuronal KB utilization *in vitro*, we also examined the transcription changes induced by KB in cultured cortical neurons and compared these changes to KD-fed mice. We then investigated the metabolic effects of KB on neuronal function by determining whether mitochondrial oxidation of KB in neurons produces sufficient ATP for synaptic transmission. Using a live imaging technique, we quantified ATP levels in nerve terminals following electric stimulations in neurons supplied with either glucose, lactate and pyruvate, or ketone bodies. Together, our studies demonstrate the metabolic specialization of neurons in utilizing KB as an alternative fuel source. As the mammalian nervous system is composed of many cell types including glia, immune cells, and many neuronal subtypes, it is important to characterize the KB metabolism in a cell-type specific manner.

Establishing and Maintaining Organelle Structure

B421/P2097

Calcium dependence and kinetics of repair small membrane lesions in electropermeabilized cells

O. Pakhomova, S. Bhattacharya, U. Mangalanathan, M. Silkunas, G. Silkuniene, E. Gudvangen, A. Pakhomov; Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA.

Electric stress, in particular with short high-power pulses, offers a unique opportunity to generate small and very small (1-10 nm) membrane lesions without the use of toxins or other chemicals. These lesions are highly permeable to ambient Ca^{2+}, which could expectedly initiate membrane repairs. At present, the involvement of active repair mechanisms and the role of Ca^{2+} in the restoration of electropermeabilized membranes have not been understood. We explored the impact of Ca^{2+} on the kinetics of membrane resealing in mammalian cells after injury with high-power electric pulses (EP). Membrane permeabilization and resealing were monitored by the uptake of YO-PRO-1 (YP) dye, which becomes brightly fluorescent upon entry into electropermeabilized cells and binding to nucleic acids. Bovine pulmonary arterial endothelial cells (BPAE) or human epithelial kidney cells (HEK-293) were grown in a monolayer on glass coverslips, which were positioned on a fluorescent microscope stage equipped with electrodes for EP delivery. Nano- or microsecond duration EP were delivered to selected cells on a coverslip. Ca^{2+} was removed or added after the EP by a fast-step perfusion system. The YP fluorescence intensity of the individual cells was recorded before and after EP application and quantified with MetaMorph software. The time constant τ for membrane recovery was calculated using exponential functions applied to fit YP uptake data. Kinetic analyses of YP dye entry into BPAE cells electroporated with nanosecond EP suggested the involvement of three repair processes: (1) fast, Ca^{2+}-independent repairs with τ of ~15 s; (2) slow, Ca^{2+}-dependent repairs with τ of ~60 s; and (3) still slower Ca^{2+}- independent repairs with τ of ~115 s. The time constants were not affected by the severity of membrane damage, indicating that the repairs were accomplished by recruiting the same repair machinery proportionally to the damage. Future work will attempt to link these processes to the specific cellular machinery.

B422/P2098

Signaling responses that mediate sarcolemmal membrane repair

N. Weisleder; Physiology and Cell Biology, Ohio State Universtiy, Columbus, OH.

Plasma membrane repair occurs in many different cell types as a necessary process to maintain the integrity of the lipid bilayer to provide the essential barrier function of membrane. The specific cellular mechanisms at work in membrane repair are known to involve multiple processes, including exocytosis of intracellular vesicles to the injury site to create a membrane repair patch, endocytosis of lined pores into vesicles to remove them from the membrane, and rearrangement of the cytoskeleton to facilitate membrane repair. Compromised membrane repair responses can lead to, or exacerbate, pathology in multiple human diseases, including muscular dystrophy, cardiovascular disease and neurodegenerative disorders. This is particularly true in the skeletal muscles where repeated contraction cycles and the resultant mechanical stress on the membrane causes disruptions in bilayer in the course of normal cell function. Influx of Ca2+ from the extracellular space through these disruptions and into the cytosol can damage multiple cellular components and result in cell death. Thus, this influx of Ca2+ in skeletal muscle triggers the exocytosis of intracellular vesicles to the injury site where they fuse to form a sarcolemma

repair patch that restores the barrier function of the membrane and allows cell survival. While these elements of this process are known there are several questions that remain about the molecular machinery that facilitates this process. Our work shows that conserved signaling cascades are essential for the induction of membrane repair in multiple cells types. Membrane repair assays on skeletal muscle and non-muscle cell lines demonstrated that this process is dependent on activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling axis through the downstream target Akt1. One mechanism found to increase membrane repair following PI3K/Akt1 activation is elevated exocytotic and endocytotic activity. Further studies indicate that the PI3K/Akt1 pathway is relevant to membrane repair in isolated mouse muscle bundles injured with multi-photon microscopy as PI3K or Akt1 inhibition prevents membrane resealing. Stimulation of this repair response through activation of PI3K/Akt1 signaling can increase membrane repair capacity in transfected muscles from dystrophic mice, indicating that increased PI3K/Akt1 may have potential as a therapeutic treatment for muscular dystrophy.

B423/P2099

Sources of variation of nuclear shape dynamics ingrowing hiPSCs

J. Cass[1], .. Allen Institute for Cell Science[1], J. Theriot[2], S. Rafelski[1]; [1]Allen Institute for Cell Science, Seattle, WA, [2]Biology and Howard Hughes Medical Institute, Seattle, WA.

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. We focus first on the nucleus, asking how hiPSCs control robust growth and dynamic shape changes of this key cellular structure during colony growth. We took advantage of a mEGFP-tagged laminB1 line from the Allen Cell Collection of endogenous tagged hiPSC lines to capture nuclear dynamics during colony growth (allencell.org). We imaged live, growing hiPSC colonies in 3D over two days at five minute intervals and then used deep learning image transformation and segmentation methods to segment and track the individual nuclei in these colonies at high resolution. In a previous study we developed a framework to analyze the mean and variability of locations of cellular structures. Likewise, we now focus on investigating mean nuclear shape dynamics and their variability. We explore the mean trajectory of individual nuclei throughout nuclear shape space, and analyze the variations observed around this mean shape. Analysis of the timescale of these variations, relationship between individual and population variations, and links between growth rate and shape behaviors enable us to pursue the biological causes of observed nuclear shape dynamics. For example, we explore the role of the colony context in nuclear shape, characterizing the relationship between a nucleus' position within the colony, its mobility through that local environment and its path through shape space. We find that the growth rate of nuclei and their rate of shape change are correlated, indicating a link between how quickly nuclei grow and how rapidly they explore the available shape space. We also looked at how nuclear shape dynamics vary by initial colony size, and change in response to disruptions to key nuclear processes such as DNA synthesis and import. We found these perturbations to have different impacts on distinct aspects of shape. This general framework for analyzing growth and shape of cellular structures will be extended to each of the key intracellular structures in an integrative fashion.

B424/P2100

Integrated intracellular organization and its variations in human iPS cells

M. Viana[1], Allen Institute for Cell Science[1], J. Theriot[2], S. M. Rafelski[1]; [1]Allen Institute for Cell Science, Seattle, WA, [2]Department of Biology and Howard Hughes Medical Institute, University of Washington, Seattle, WA.

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain cellular organization, and how they transition between states during differentiation and disease. As an initial step towards this goal, we generated the "hiPSC Single-Cell Image Dataset" with over 200,000 live cells in 3D spanning 25 major cellular structures. We used these images to develop a computational framework that embraces the vast cell-to-cell variability observed within a normal population and permits quantitative analyses of distinct, separable aspects of organization within and across different cell populations. The framework provides a systematic way of measuring the average locations both of individual structures and all pairs of structures (average location phenotype and average structure similarities), as well as the variability in these locations (stereotypy and concordance). We found cellular organization was very robust across the wide range of cell shapes in the normal interphase population. We also used our framework to perform a comparative study of cellular organization in two cell subpopulations that stood out morphologically in the dataset: colony edge cells and mitotic cells. We found that several structures showed a polarized location towards the colony edge but no change in the wiring (variability and relationships among structures). In contrast, our second subpopulation comparison focused on early mitotic cells confirmed that they undergo a dramatic intracellular reorganization, in which not only the average locations of structures, but also their wiring, changed substantially. A "meta-analysis" of our findings revealed a possible hierarchy of dependencies as cells reorganize. The conceptual aspect of our framework is generalizable, extensible and can be used to perform robust statistical analyses on cell shape and intracellular spatial locations and their variability across cell types and conditions. The experimental and algorithmic implementations of this analysis framework are modular. The choice of which to use is dependent on the specific biological question and this will dictate specific inputs required such as how many cells or cellular structures are needed, what kind of precision is possible, or what kinds of segmentation and data analysis algorithms should be used.

B425/P2101

Role of VRK1 in Nuclear Size Regulation in *Xenopus laevis*

H. Prabha, D. L. Levy; Molecular Biology, University of Wyoming, Laramie, WY.

Acquisition of the characteristic size of intracellular organelle is a fundamental question in cell biology. It is important to gain a better understanding of the mechanisms that control nuclear size in humans because nuclear size and shape are known to change in cancer. Vaccinia-Related Kinase (VRK1) is a serine-threonine protein kinase belonging to the casein kinase-1 family. Its expression is increased in actively dividing cells in testis, thymus, fetal liver and carcinomas with poor prognosis. VRK1 regulates entry into the cell cycle, chromatin condensation in G2/M, Golgi fragmentation, and Cajal body dynamics. VRK1 also plays important roles in the assembly and disassembly of the nuclear envelope, and a recent screen for nuclear morphology effectors identified VRK1 as a putative regulator of nuclear size. We are investigating the function of VRK1 in nuclear size regulation using *Xenopus laevis* egg extracts that support de novo nuclear assembly and growth. Immunofluorescence confirmed the presence of

VRK1 in egg extracts, revealing a punctate intranuclear staining pattern. In order to understand the role of VRK1 in regulating nuclear size we supplemented extract with a small molecule inhibitor of VRK1 either during or after nuclear assembly. We find that VRK1 inhibition results in a significant reduction in nuclear size concomitant with the appearance of nuclear membrane invaginations and nuclei remain import-competent and intact. This effect was more prominent when VRK1 was inhibited post-nuclear assembly. We also observed a redistribution of chromatin upon VRK1 inhibition. Future work will focus on identifying the VRK1 substrate relevant to the regulation of nuclear size.

B426/P2102

Role of Golgins in the cisternal stacking of Golgi apparatus

R. Dahara[1,2], **D. Bhattacharyya**[3,4]; [1]Cell and Tumor Biology, ACTREC, Tata Memorial Center, Navi Mumbai, INDIA, [2]Homi Bhabha National Institute,, Mumbai, INDIA, [3]Internal Medicine, University of Michigan, Ann Arbor, MI, [4]Homi Bhabha National Institute, Mumbai, INDIA.

Regulation of the exotic morphology of the Golgi apparatus is still an enigma. The core of the argument is the cisternal stacking problem, an elusive regulatory mechanism that holds the Golgi cisterna together. GRASP family of proteins was initially implicated in cisternal stacking; however, several groups recently demonstrably argued against such a role (Grond et al., 2020; Zhang and Seemann, 2021). The lack of functional GRASP proteins in model systems harboring stacked Golgi(Levi et al., 2010) also supports such a conclusion and rationalizes the existence of some other universal factors for cisternal stacking. A critical error is ignoring the Golgi apparatus's dynamic nature while framing the problem of cisternal stacking. In light of the cisternal maturation model, Golgi cisternal stacking needs to be a reversible mechanism— it needs to be initiated at the 'cis' side of the cisternal stack and be un-initiated at the 'trans' side. At the end of each such cycle, an unstacked TGN should be separated from the cisternal stack resulting in the form of 'TGN peeling.' 'TGN peeling' is direct experimental evidence of reversible cisternal stacking and has been microscopically validated (Jain et al., 2019; Mogelsvang et al., 2003). For the first time, we documented the role of the GRIP domain Golgin in the reversible cisternal stacking(Jain et al., 2019). Using budding yeast *Pichia pastoris*, we demonstrated how the Arl3-Arl1 GTPase cascade switch regulates GRIP domain Golgin *Pp*Imh1 to mediate reversible cisternal stacking between medial and trans cisterna. Since GRIP domain proteins are conserved in plants, animals, and fungi, it is plausible that this reversible cisternal stacking mechanism is evolutionarily conserved. In the present study, we have explored the role of other Golgins in regulating the cisternal stacking. Using double deletion analysis and chimera studies, we have analyzed the rate of TGN peeling effect and overall Golgi morphology in various conditions using live and electron microscopy. Our study indicates that PpImh1 and other Golgins function synergistically in mediating the reversible cisternal stacking. Our model and data reinforce our previous model and provide further details in Golgin mediated cisternal stacking of the Golgi apparatus.

B427/P2103

Multivesicular body (MVB) maturation involves immobilization on a subset of microtubules by septin GTPases

B. P. Robinson, N. R. Bass, E. T. Spiliotis; Department of Biology, Drexel University, Philadelphia, PA.

Multivesicular bodies (MVBs) are endocytic organelles which sort ubiquitinated proteins into intraluminal vesicles (ILVs) and target them for lysosomal degradation or extracellular release in

exosomes. The intracellular positioning of MVBs is critical for these cargo outcomes, but it is poorly understood how MVBs mature and traffic along the microtubule (MT) cytoskeleton. Previous work has shown that septin GTPases are essential for MVB maturation and ILV formation, but the underlying function(s) and mechanism(s) are unknown. Here, we have discovered that in MDCK epithelia, ~70% of MVBs, which contain the tetraspanin marker protein CD63, the ESCRT-I protein TSG-101 and the intraluminal vesicle lipid lysobisphosphatidic acid (LBPA), associate with a subset of microtubules which are coated with septin GTPases. In contrast to MVBs, a smaller percentage (~35%) of EEA1-containing early endosomes localize to septin-coated microtubules. Quantitative analysis of CD63-positive MVBs showed that immature EEA1-positive/LBPA-negative MVBs were more preferentially bound to septin (SEPT7)-coated microtubules than mature EEA1-negative/LBPA-positive MVBs. Quantitative analysis of CD63-positive MVBs showed that immature EEA1-positive/LBPA-negative MVBs were more preferentially bound to septin (SEPT7)-coated microtubules than mature EEA1-negative/LBPA-positive MVBs. Time-lapse microscopy showed that CD63-containing MVBs were more immotile and continuously bound to septin-coated microtubules, while EEA1-containing early endosomes were overall more mobile and less associated with septin-bound microtubules. To test whether septins slow down MVB motility on microtubules, we reconstituted in vitro the motility of CD63-GFP-positive MVBs, which were isolated from MDCK cells. Strikingly, MVBs were largely immotile on microtubules that were decorated with recombinant SEPT2/6/7/9 complexes, while they moved processively in the absence of septins. Taken together, these data indicate that MVB biogenesis requires septin-mediated immobilization of maturing MVBs on a subset of microtubules, which may allow for protein sorting, acidification, and cargo exchange with endosomes prior to trafficking and fusion with the lysosome or the plasma membrane.

B428/P2104

Transmembrane mucins regulate the morphology of the endoplasmic reticulum through negative curvature generation.

M. C. Goudge[1], J. C. H. Kuo[2], M. J. Paszek[2]; [1]Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, [2]Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY.

The endoplasmic reticulum (ER) is a large, highly dynamic membrane-bound organelle that is essential for many basic cellular functions. Necessary for its proper function are the diverse set of morphologies that the ER membrane exhibits. While an increasingly large body of work has centered around proteins responsible for curvature generation through insertion into the cytoplasmic face of the ER membrane, relatively little is known about protein curvature generators acting on the lumenal face of the ER membrane. Here, we show that transmembrane mucins, a family of membrane anchored biopolymers, generate membrane curvature through entropic crowding forces on the lumenal face of the ER membrane. Using a genetically-encoded Muc1 expression system in combination with live-cell imaging, we find that a surprisingly large fraction of cellular mucin is localized to the ER. We show that with increasing concentrations of intracellular mucin, the prominence of sheets increases in the ER concomitant to a loss in tubules. This morphological transition occurs rapidly and reversibly, with sheets forming and disappearing on the order of hours upon mucin synthesis and translational inhibition, respectively. By applying expansion microscopy, we characterized these mucin-induced sheets at high resolution. Notably, these sheets are organized as cisternae lying perpendicular to the radial direction of the cell. Finally, we characterized mitochondrial morphology and spatial organization in response to the

mucin-induced ER restructuring. Mitochondria in Muc1-expressing cells were found to be less tubulated, smaller, and less homogenously distributed throughout the cell than in their non-Muc1 expressing counterparts. Together, these results suggest a novel role for the large transmembrane mucins to regulate the morphology of the ER and its interactions with other organelles.

B429/P2105

Vimentin intermediate filaments scaffold Endoplasmic Reticulum (ER) tubule matrices and pattern global ER mass distribution via Nesprin3/Plectin

A. S. Moore, J. Lippincott-Schwartz; Howard Hughes Medical Institute, Ashburn, VA.

The endoplasmic reticulum (ER) is a continuous membrane-bound organelle found in all eukaryotes. In cultured cells, the ER adopts an elaborate net-like organization characterized by densely stacked sheets in the perinuclear region, sparsely interconnected tubules in the cell periphery, and a complex mixture of both sheets and tubule matrices therebetween. These local ER geometries are maintained by membrane shaping proteins, which tune nanoscale ER curvature to support either planar or highly curved ER organizations. Whilst the factors controlling local ER membrane morphology have been extensively investigated, the mechanisms by which these geometries are spatially compartmentalized within different regions of the cytoplasm are not well understood. Here, using advanced light and electron microscopy techniques, we explore the cytoskeletal control of ER mass distribution. We find that the vimentin intermediate filaments (VIFs) make extensive contacts with the ER and, in concert with microtubules, control the global distribution of ER mass throughout the cytoplasm. In multiple cell types, we observe VIF densities termed “knots” assemble on and stabilize highly concentrated matrices of peripheral ER tubules. Tubules within vimentin-scaffolded ER matrices are immobilized and constricted in diameter, with reduced luminal content and a higher surface area-to-volume ratio than surrounding ER. Vimentin assembly on the ER is coordinated by the LINC complex protein Nesprin3, which recruits cytoplasmic intermediate filaments to the surface of the ER via the giant cytolinker protein plectin. Vimentin-scaffolded ER matrices entrap other cytoplasmic organelles including mitochondria and lysosomes, facilitating extensive and prolonged organelle-organelle contact. Depletion of vimentin eliminates ER matrices and results in an increased abundance of planar ER in the cell periphery. Together, these data provide the first evidence that the vimentin cytoskeleton plays important roles in the stabilization and positioning of tubular ER.

B430/P2106

The sorting factor VPS13A is pivotal for quality control of mitochondria and endoplasmic reticulum

G. Rodriguez[1], E. Tank[1], R. Fuller[2], A. Tidball[1], J. Parent[1], S. Barmada[1]; [1]Neurology, University of Michigan, Ann Arbor, MI, [2]Biological Chemistry, University of Michigan, Ann Arbor, MI.

Chorea acanthocytosis (ChAc) is an autosomal-recessive neurodegenerative disorder caused by mutations in the gene encoding vacuolar protein sorting factor 13A (VPS13A). The yeast orthologue, VPS13, aids in the transport of lipids between organelles, and the N-terminal region shows strong sequence homology to the evolutionarily conserved autophagy-related genes (*Atg*) protein family. Although these studies suggest a function for VPS13 proteins in lipid transfer and autophagy, the physiological function and regulation of VPS13A in mammalians cells remains unknown.Here I utilized HEK293T cells in which the native VPS13A has been labeled with mNeonGreen, to identify VPS13A interactors by mass spectroscopy and unbiased proteomics. I also used VPS13A-KO HEK293T cells to

determine how functional loss of VPS13A affects critical pathways relevant to ChAc. These studies, as well as published work on yeast VPS13, led me to investigate specialized branches of autophagy that may be regulated by VPS13A. Loss of VPS13A affects the level of receptors involved in mitochondria-specific autophagy (mitophagy) and endoplasmic reticulum-limited autophagy (ER-phagy). These findings highlight a central function for VPS13A in ER-phagy and mitophagy, a hypothesis I am now exploring through dynamic studies of ER-phagy and mitophagy in HEK293T cells.Future studies will examine the contribution of VPS13A to protein clearance and cellular survival, in human neurons differentiated from induced pluripotent stem cells donated by individuals with ChAc. Collectively, these investigations will help define a function for VPS13A in neurons, outline disease mechanisms, and highlight pathways that may be targeted to prevent neuron loss in this disorder.

B431/P2107

In situ architecture of the lipid transport protein VPS13C at membrane contacts

S. Cai[1,2,3,4], Y. Wu[1,2,3,4], A. Guillén-Samander[1,2,3,4], W. Hancock-Cerutti[1,2,3,4], J. Liu[5], P. DeCamilli[1,2,3,4]; [1]Departments of Neuroscience and Cell biology, Yale University School of Medicine, New Haven, CT, [2]Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, [3]Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT, [4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, [5]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT.

VPS13 is a eukaryotic lipid transport protein localized at membrane contact sites. Previous studies had suggested that it may transfer lipids between adjacent bilayers by a bridge-like mechanism. Direct evidence for this hypothesis from a full-length structure and from electron microscopy (EM) studies in situ was still missing, however. Recently, by using cryo-focused ion beam (cryo-FIB) milling and cryo-electron tomography (cryo-ET), we revealed the presence of rod-like densities bridging two adjacent membranes at human VPS13C-mediated membrane contacts in HeLa cells (see Abstract #392 2021 ASCB meeting). We have now applied two approaches to verify that these rod-like densities represent VPS13C. First, we examined a VPS13C truncated mutant, which is predicted to be ~5nm shorter compared with full-length VPS13C by AlphaFold. We found that both the length of rod-like density and the intermembrane space were ~5 nm shorter (Cai, Wu, Guillen-Samander, Hancock-Cerutti, Liu, & De Camilli, pmid: 35858323). Second, subtomogram averaging of rod-like densities in cells expressing full-length VPS13C revealed a twisting rod-like structure, which is overall consistent with the AlphaFold predicted structure showing a twisted β-sheet running along the entire length of the rod. One intriguing finding from subtomogram averaging analysis is that the density is less continuous with the ER membrane than with the endo/lysosome membrane (pmid: 35858323). Ongoing work further investigates transport mechanism of VPS13C by examining its conformational heterogeneity in situ. Taken together, our study provides in situ evidence for a bridge model of VPS13 in lipid transport. (This work was supported in part by a postdoctoral fellowship from the Parkinson Foundation to SC)

B432/P2108

Establishing a role for ATG2-mediated Lipid Transport at the Lipid Droplet

J. L. Korfhage, N. Wan, K. M. Reinisch, T. J. Melia; Cell Biology, Yale University, New Haven, CT.

ATG2 is a member of the VPS13-family of rod-shaped proteins thought to facilitate bulk lipid transport between membranes. ATG2 has primarily been studied in the context of autophagy wherein it is thought

to serve as the primary route of lipid flow into the growing phagophore. ATG2 can bind approximately 20 lipids, and its ability to transport lipids between liposomes has been well established *in vitro*. Although its role has primarily been defined in the context of autophagy, ATG2 localizes overwhelmingly to lipid droplets (LDs), and double knockout of ATG2A and B (ATG2 DKO) leads to marked growth and accumulation of LDs as well as defects in fatty acid transport from LDs to mitochondria. Importantly, both of these phenotypes are independent of autophagy. Furthermore, ATG2 intrinsically supports two functions: tethering of two membranes in support of contact site maintenance as well as biochemical facilitation of lipid transport. Thus, how ATG2 supports LD homeostasis and specifically whether its lipid transport function is necessary has not been tested.
To test whether lipid transport is necessary for any phenotype of an VPS13-family protein, lipid transport *per se* must be blocked without disrupting the protein's capacity to bridge membranes. Here we **1)** develop a lipid transport dead ATG2 mutant (TD-ATG2A) which folds properly and localizes to LDs. TD-ATG2A maintains the overall tertiary structure of WT ATG2A while the N-terminal region of the hydrophobic groove is occluded by the mutation of 10 hydrophobic residues to hydrophilic residues. **2)** Using *in vitro* lipid transport assays based on FRET dequenching, we show that TD-ATG2A is incapable of facilitating liposome to liposome lipid exchange. **3)** Expression of WT ATG2A but not TD-ATG2A in an ATG2 DKO background rescues LD accumulation observed in ATG2 DKO cells, thereby establishing ATG2 lipid transport activity as necessary for the maintenance of LD homeostasis in cells.

B433/P2109

ATG9 vesicles are incorporated into nascent autophagosome membranes

T. J. Olivas[1], S. Yu[1], Y. Wu[1,2,3], L. Luan[1], P. Choi[1], S. Nag[1], P. De Camilli[1,2,3], K. Gupta[1,4], T. J. Melia[1]; [1]Cell Biology, Yale University, New Haven, CT, [2]Neuroscience, Yale University, New Haven, CT, [3]Howard Hughes Medical Institute, New Haven, CT, [4]Nanobiology Institute, Yale University, West Haven, CT.

The autophagosome is a double-membrane organelle that traps cytoplasmic cargo and traffics it to the lysosome for degradation. How the autophagosome forms is uncertain, but a prevailing model suggests lipids are moved from the ER through the lipid transporter ATG2 to ATG9 vesicles, which then expand to comprise the growing autophagosomal membrane. However, incorporation of ATG9 into the expanding phagophore is not readily observed; detection of this putative autophagosome-resident protein is made challenging both because the majority of ATG9 vesicles in the cell are not involved in the biogenesis at any given time and because the dilution of one or a few vesicle membranes by potentially millions of transported lipids would result in a very low density of ATG9 on the mature autophagosome. Here we develop approaches to address each of these limitations. We show that in genetic knockouts of ATG2, ATG9 vesicles accumulate at sites of aborted autophagosome formation. Focused-ion beam scanning electron microscopy reveals that without ATG2, these putative autophagosome seed vesicles do not expand, but instead accumulate within a large vesicle cluster surrounded by ER that we have termed the pre-ATG2 compartment. By fluorescence microscopy, we also detect downstream modifiers of the autophagosome membrane in this compartment, including the lipid-anchored form of the LC3 proteins, which suggests that a biochemically competent seed membrane is present. To establish whether ATG9A is found on the same membrane as LC3B, we use styrene maleic acid (SMA) copolymer nanodiscs to isolate nanoscale spans of autophagosome membrane away from all other potential contaminants. Through rigorous combinations of isolation and purification, we reveal that ATG9A and LC3B are co-resident within the same 10 nm diameter membrane segment and likely engage in a protein-protein

complex on both autophagosome precursor membranes from the pre-ATG2 compartment and WT autophagosomes. Thus, we assert that the ATG9 vesicle is the seed membrane for the autophagosome.

B434/P2110

The NTPase activity of the Double FYVE Domain Containing Protein 1 (DFCP1) regulates lipid droplet metabolism.

V. A. Ismail, T. Naismith, D. J. Kast; Washington University in St. Louis, Saint Louis, MO.

Lipid droplets (LDs) are dynamic lipid storage depots that play a central role in cellular metabolism by providing lipids for the repair and biogenesis of membranous organelles, and serving as a source of energy during times of nutrient stress. However, the molecular mechanisms that regulate the growth and degradation of LDs are poorly understood. It has emerged that proteins that makes contact between LDs and the ER play a critical role in regulating LD metabolism. Recently, the autophagy related protein, Double FYVE Domain Containing Protein 1 (DFCP1) was shown to reside at the interface of the ER and LDs, however little is known about DFCP1's roles in autophagy and LD metabolism. Using a combination of cell biology and biochemical approaches, we show that DFCP1 is a novel NTPase that regulates FFA metabolism by modulating the catabolism of LDs. Specifically, we show via Seahorse assays and TLC that DFCP1 inhibits fatty acid-dependent cellular metabolism and that DFCP1 KD increases overall processing of triacylglycerides. By expressing strategic truncations in cells and then purifiying LDs, we show that DFCP1 accumulation on LDs is independent of PI3P-binding, but requires a combination of the ER-binding domain and a unique NTPase domain. Using size-exclusion chromatography and antibody-based NTPase assays, we show that this novel NTPase domain has the ability to dimerize and can hydrolyze ATP and GTP. Furthermore, mutations in the DFCP1 that impact nucleotide hydrolysis or dimerization result in changes in the accumulation of DFCP1 on LDs, LD density and size, and targeting of LDs by autophagosomes. Importantly, the magnitude of these changes depends on the nutritional status of the cell. Collectively, our findings indicate that DFCP1 is a nucleotide-dependent metabolic switch that modulates the cellular storage of free fatty acids.

B435/P2111

Yeast Lunapark inhibits atlastin-mediated ER fusion by interfering with formation of *trans*-atlastin complexes.

E. Jang[,1], Y. Jun[,1]; GIST, Gwangju, Korea, Republic of

The overall structure of the endoplasmic reticulum (ER) is composed of sheets and networks. The ER network is a dynamic structure, constantly undergoing fusion and fission. In *Saccharomyces cerevisiae*, ER membrane fusion is mediated by the dynamin like GTPase Sey1p. The ER network is maintained by the membrane proteins; reticulon/Rtn1p, DP1/Yop1p and Lnp1p, a member of the conserved Lunapark family, stabilizes three-way junctions. Although there are evidences which suggest that Lnp1p acts as antagonist of Sey1p to balance polygonal network formation, the underlying mechanism remains unclear. Here, we designed an in vitro assay to quantify trans-, cis- Sey1p complex formation using isolated yeast microsomes. Using this assay, we identified increase of *trans*-Sey1p complex formation in the absence of Lnp1p. Moreover, the N-terminus deleted (NTΔ) Lnp1p lost its inhibitory function in Sey1p mediated membrane fusion. Similar to Lunapark in higher eukaryotes, Lnp1p is also attaches to the membrane using N-terminus, but without myristoylation site. Through the N-terminus of Lnp1p attached to the membrane, NTD (GTPase-Helix bundle) of Sey1p attaches to the membrane. Membrane-

attached Sey1p by N-terminus of Lnp1p is limited in formation of trans- Sey1p complex for ER membrane fusion. Collectively, our data suggest that Lnp1p acts as an antagonist of Sey1p before ER membrane fusion by inhibiting the formation of trans-sey1p complex through its N-terminus prior to initiation stages of ER membrane fusion.

B436/P2112

Membrane fusion by *Drosophila* atlastin does not require GTP hydrolysis

D. Crosby, T. H. Lee; Carnegie Mellon University, Pittsburgh, PA

Atlastin (ATL) GTPases undergo trans dimerization and a power stroke-like crossover conformational rearrangement to drive endoplasmic reticulum membrane fusion. Fusion depends on GTP, but the role of nucleotide hydrolysis has remained controversial. For instance, nonhydrolyzable GTP analogs block fusion altogether, suggesting a requirement for GTP hydrolysis in ATL dimerization and crossover, but this leaves unanswered the question of how the ATL dimer is disassembled after fusion. We recently used the truncated cytoplasmic domain of wild-type *Drosophila* ATL (*D*ATL) and a novel hydrolysis-deficient D127N variant in single turnover assays to reveal that dimerization and crossover consistently precede GTP hydrolysis, with hydrolysis coinciding more closely with dimer disassembly. Moreover, while nonhydrolyzable analogs can bind the *D*ATL G domain, they fail to fully recapitulate the GTP-bound state. This predicted that nucleotide hydrolysis would be dispensable for fusion. Here we report that the D127N variant of full-length *D*ATL drives both outer and inner leaflet membrane fusion with little to no detectable hydrolysis of GTP. However, the trans dimer fails to disassemble and subsequent rounds of fusion fail to occur. Our findings confirm that ATL mediated fusion is driven in the GTP-bound state, with nucleotide hydrolysis serving to reset the fusion machinery for recycling.

B437/P2113

Single-particle cryo-EM analysis of the purinosome

S. J. Calise, J. Molfino, M. S. Dickinson, J. Quispe, J. M. Kollman; University of Washington, Seattle, WA

The purinosome is a membraneless organelle assembled from the six enzymes of the *de novo* purine nucleotide biosynthesis pathway, which dynamically reorganize in the cytosol to increase metabolic flux when cellular purine demand is high. Recent studies have used cell-based assays to demonstrate liquid-like properties of the purinosome, despite the lack of intrinsically disordered or low-complexity domains in pathway enzymes. We aim to reconstitute the purinosome structure and biochemical activity of the pathway *in vitro* to define the molecular mechanisms of assembly and potential allosteric regulation among purinosome enzymes. Here, we employed single-particle cryogenic electron microscopy (cryo-EM) to determine structures of purified human purinosome components in solution. We determined a 4.1 Å resolution cryo-EM structure of the bifunctional phosphoribosylaminoimidazole carboxylase/succinocarboxamide synthetase (PAICS) apoenzyme in its octamer state, which catalyzes the sixth and seventh steps in purine synthesis and is thought to be a hub for purinosome assembly. Although PAICS interacts with all other pathway enzymes in cells, it did not make binary or ternary interactions with different combinations of trifunctional purine biosynthetic protein adenosine-3 (GART), phosphoribosylformylglycinamidine synthase (PFAS), adenylosuccinate lyase (ADSL), or aminoimidazole carboxamide ribonucleotide transformylase/inosine monophosphate cyclohydrolase (ATIC) in our *in vitro* assays, suggesting assembly requires more complex multimeric mixtures or a missing cellular cofactor yet to be identified. Crystal structures exist for most pathway enzymes, but no

previous structures of human PFAS had been determined, likely owing to its insolubility when expressed in *E. coli*. To get around solubility issues, we optimized purification of PFAS from human Expi293F cells. Twin-Strep-tagged PFAS was initially purified by affinity capture, prior to removal of the tag with tobacco etch virus protease and subsequent purification by size-exclusion chromatography. We then determined high-resolution cryo-EM structures of PFAS in its apoenzyme state (3.2 Å), in complex with substrate analogues (4.4 Å, preliminary), and in complex with products (4.2 Å, preliminary), establishing a structural basis for human PFAS function. Additional cryo-EM structures of remaining pathway enzymes will be critical for future *in vitro* reconstitution of the purinosome.

B438/P2114

Reconstitution of membrane fusion by human atlastin-3 reveals differential regulation of ATL paralogs

S. Bryce, D. Crosby, T. Yang, M. Stolzer, D. Durand, T. H. Lee; Biological Science, Carnegie Mellon University, Pittsburgh, PA.

Atlastin (ATL) GTPases maintain endoplasmic reticulum (ER) network structure through GTP-dependent membrane fusion. Among the three differentially expressed ATL paralogs (1-3) in humans, ATL1 and ATL2 catalyze membrane fusion in vitro, are sufficient to rescue ER defects in ATL1/2/3 knockout (KO) cells and are autoinhibited by their respective C-termini. Although ATL3 shares sequence and structural conservation with ATL1/2, whether it has a similar function has been questioned for several reasons. First, its fusion activity has not been reconstituted; second, there are conflicting reports as to whether ATL3 is sufficient to maintain ER network structure; and third it has lower GTPase activity. Consequently, it has been hypothesized that ATL3 does not primarily act as a fusogen.
Here we demonstrate that ATL3 catalyzes efficient GTP-dependent membrane fusion in vitro. Though the ATL3 G domain dimer affinity is lower than ATL1/2, this is offset by its higher abundance in cells, and ATL3 is sufficient to rescue ER defects in ATL1/2/3 KO cells. Surprisingly, human ATL3 lacks detectable C-terminal autoinhibition prompting us to investigate the evolution of ATL autoinhibition. Phylogenetic analysis, as well as the functional diversity of autoinhibitory C-termini in ATL1/2 splice isoforms suggests that C-terminal autoinhibition is a recent evolutionary innovation that may help fine tune ER structure under diverse conditions.

B439/P2115

Structures of Vac8-containing protein complexes reveal the underlying mechanism by which Vac8 coordinates multiple cellular processes

N. KO[1,2], H. Kim[3,2], J. PARK[1,2], H. Kim[3,2]; [1]GIST, GWANGJU, Korea, Republic of, [2]Cell Logistics Research Center, GIST, Gwangju, Korea, Republic of, [3]UNIST, ULSAN, Korea, Republic of

Vac8, a yeast vacuolar protein with armadillo repeats, mediates various cellular processes by changing its binding partners; however, the mechanism by which Vac8 orchestrates these processes remains poorly understood. Vac8 interacts with Nvj1 to form the nuclear-vacuole junction (NVJ) and with Atg13 to mediate cytoplasm-to-vacuole targeting (Cvt), a selective autophagy-like pathway that delivers cytoplasmic aminopeptidase I directly to the vacuole. In addition, Vac8 associates with Myo2, a yeast class V myosin, through its interaction with Vac17 for vacuolar inheritance from the mother cell to the emerging daughter cell during cell divisions. Here, we determined the X-ray crystal structure of the Vac8-Vac17 complex and found that its interaction interfaces are bipartite, unlike those of the Vac8-Nvj1 and Vac8-Atg13 complexes. When the key amino acids present in the interface between Vac8 and

Vac17 were mutated, vacuole inheritance was severely impaired in vivo. Furthermore, binding of Vac17 to Vac8 prevented dimerization of Vac8, which is required for its interactions with Nvj1 and Atg13, by clamping the H1 helix to the ARM1 domain of Vac8 and thereby preventing exposure of the binding interface for Vac8 dimerization. Consistently, the binding affinity of Vac17-bound Vac8 for Nvj1 or Atg13 was markedly lower than that of free Vac8. Likewise, free Vac17 had no affinity for the Vac8- Nvj1 and Vac8-Atg13 complexes. These results explain how vacuole inheritance and other Vac8-mediated processes, such as NVJ formation and Cvt, occur independently of one another.

B440/P2116

GM130 scaffolds the Golgi ribbon by associating with RNA

Y. Zhang, J. Seemann; UT Southwestern Medical Center, Dallas, TX

The Golgi apparatus is essential for processing and sorting of membrane proteins. This secretory function of the Golgi is highly conserved and is performed by stacks of flattened cisternae. Vertebrate cells contain about 100 stacks that are interconnected into a single continues ribbon that resides next to the centrosomes. This distinct polarized localization of the Golgi ribbon facilitates directional protein sorting and is indispensable for cell polarization. Golgi ribbon fragmentation is commonly observed in a broad range of diseases, including neurodegenerative diseases and muscular dystrophies. Under physiological conditions, the ribbon structure is altered in response to cellular stress and during mitosis. However, the molecular mechanism that dynamically remodel the ribbon structure remain unclear. Previous data suggested that the ribbon is organized by the Golgi membrane proteins GRASP55 and/or GRASP65. However, our data showed the two proteins only indirectly maintain the ribbon integrity together with GM130 and Golgin-45. In efforts to define their co-factors, we identified a complex of RNA with GRASP65 and GM130 and confirmed that it is GM130 that directly binds RNA and further recruits RNA binding proteins. Acute degradation of RNA or GM130 disrupted the lateral linking of Golgi ribbon and caused Golgi fragmentation. We further found that GM130 together with RNA undergoes liquid-liquid phase separation to form RNA-dependent condensates in cells. We conclude that the GM130-RNA association is essential to dynamically organize the Golgi ribbon and support the integrity of the Golgi structure.

B441/P2117

Role Of *Atlastin* In Organising Axonal ER And CNS Lipid Droplets In *Drosophila*

E. Gonzaga, A. Riaz, L. Zhao, E. Zlámalová, T. Doherty, F. Gallo, K. Müller, C. O'Kane; University of Cambridge, Cambridge, United Kingdom

Hereditary Spastic Paraplegias (HSPs) are a rare group of inherited diseases characterised by progressive weakness and spasticity of the lower limbs. HSP patients endure physical impairments, bladder dysfunction, neuropathic pain and, in complicated cases, seizures. Over 73 causative Spastic Paraplegia genes (SPGs) are attributed to this clinically and genetically diverse disease. SPG3A, caused by *atlastin1* (*ATL1*) mutations, is the second most common autosomal dominant SPG, and affect a protein that appears instrumental in fusing endoplasmic (ER) tubules to form 3-way junctions and a continuous network. To find links between this function and axonal maintenance, we use *Drosophila* to understand the role of its single Atlastin protein in modelling axonal ER.
Homozygous *atl* mutant *Drosophila* larvae exhibit severe locomotion defects. Using fluorescence confocal microscopy, we see that *Drosophila atl* loss-of-function mutant larvae have increased levels of

ER in motor axons compared to controls, and serial EM shows an increased number of ER tubules. Consistent with the molecular role of *atlastin* in ER tubule fusion, fluorescence recovery after photobleaching (FRAP) experiments show reduced continuity of ER in mutant axons compared to controls. The elevated levels of ER and reduced continuity can both be rescued by a wildtype *atlastin* construct. Additionally, BODIPY staining shows that lipid droplet numbers are elevated in *atl* mutant larval nerves compared to controls. Our findings suggest that ATL1/SPG3A mutations may contribute to distal axonopathy via impaired ER continuity, and support the importance of neuronal ER in HSP pathology.

B442/P2118

The Mammalian Rhomboid-Like Derlins Regulate Endoplasmic Reticulum Morphology

N. A. Scott, S. E. Neal; University of California, San Diego, La Jolla, CA

The endoplasmic reticulum (ER) forms a continuous intracellular membrane system, consisting of the nuclear envelope, flat sheets, and an elaborate network of tubules throughout the cytoplasm. The ER plays a role in several essential cellular processes such as the synthesis of transmembrane and secreted proteins, post-translational modification of proteins, lipid synthesis, and Ca^{2+} homeostasis. Moreover, the ER is susceptible to protein misfolding. To survive the constant threat of protein misfolding, cells are equipped with ER protein quality control systems that detect and degrade these aberrant molecules. Previously, we and others have demonstrated that rhomboid-like derlins are major mediators of ER protein quality control where they are involved in the retrotranslocation of ubiquitinated misfolded substrates from the ER for cytosolic degradation by the proteasome. To survey for potential substrates of derlins, we employed liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics in derlin knockout (KO) HEK293 cells. To our surprise, ATL-3 was significantly enriched in the derlin KOs, indicating that ATL-3 is a potential substrate of derlins. ATL-3 belongs to a family of proteins known as atlastins (ATL), which is comprised of three mammalian isoforms: ATL-1, ATL-2, and ATL-3. ATLs are membrane anchored GTPases in which their major function is to shape the ER via fusion of ER membrane tubules. Although the generation of different ER morphologies is well known, how the ER shape is regulated is not well characterized. In this study, we biochemically validated that derlins regulate the level of ATL proteins. Furthermore, we demonstrate that derlins KO affects the shape and distribution of the ER. Taken together, our studies suggest a novel role for derlins in modulating ER shape and morphology. Because mutations in ATLs are associated with hereditary spastic paraplegia, our findings will aid in the understanding of how rhomboid-like derlins contribute to this pressing malady.

Organelle Contact Sites

B443/P2119

Topological constraints in ER-vacuolar network enables damping during ultra fast cellular contractions

R. Chang, M. Prakash; Department of Bioengineering, Stanford University, Stanford, CA.

Cellular systems are known to exhibit some of the fastest movements in the biological world - but little is known as to how single cells can dissipate this energy rapidly and adapt to such large accelerations without sub-cellular damage. To study intracellular adaptations under extreme forces - we investigate Spirostomum ambiguum - a giant cell (1-4mm in length) well known to exhibit ultrafast contractions (50% of body length) within 5 msec with a peak acceleration of 15g. Utilizing transmitted electron

microscopy (TEM) and confocal imaging, we discover a novel association of rough endoplasmic reticulum (RER) and vacuoles throughout the cell - forming a contiguous fenestrated cubic membrane architecture that topologically entangles these two organelles. A nearly uniform inter-organelle spacing of 60nm is observed between RER and vacuoles, closely packing the entire cell. Using an overdamped molecular dynamics simulation, with parameters matching all relevant mechanical properties of vacuoles and RER, we demonstrated that the topological constraints created by RER can enhance the ability of vacuoles to resist boundary forces and preserve spatial relationships between organelles. Because this dynamics arises primarily from entanglement of two networks incurring jamming transition at a subcritical volume fraction - we term this phenomena "topological damping". Our findings suggest a new mechanical role of RER-vacuolar meshwork as a metamaterial capable of dissipating energy in an ultra-fast contraction event.

B444/P2120

Scs2 modulates mitochondria-ER contact sites through Num1

J. C. Casler, H. L. Anderson, L. L. Lackner; Northwestern University, Evanston, IL.

Membrane contact sites (MCSs) are sites of close membrane apposition that tether organelles to organize their intracellular distribution and facilitate the exchange of biological materials. In yeast, the mitochondria-ER cortex anchor (MECA) is a tripartite MCS between the plasma membrane (PM), the ER, and mitochondria. The core component of MECA, Num1, is required for the cortical distribution of the mitochondrial network and also functions in nuclear inheritance by serving as an anchor for dynein. Previous work has shown that Num1 interacts with the PM and mitochondria via two distinct lipid binding domains; however, the molecular mechanism by which Num1 interacts with the ER is unknown. Here we demonstrate that Num1 contains a two phenylalanines in an acidic tract (FFAT) motif in its C-terminus that interacts with the integral ER membrane protein Scs2. Deletion of the FFAT motif disrupts Num1-ER localization but is dispensable for mitochondrial tethering and dynein anchoring. Interestingly, ectopically localizing Num1 is sufficient to modulate the extent of organelle contacts with the ER in a manner that depends on the Num1 FFAT motif. This work defines the molecular mechanism by which a tripartite MCS is formed and demonstrates how cells can modulate these interactions to alter the extent of organelle contacts. We hypothesize that dynamic regulation of these Num1-organelle interactions allows MECA to serve as a central organizational hub that facilitates the exchange of lipids or metabolites between organelles in response to cellular needs.

B445/P2121

In Situ Cryo-CLEM of a Tethering Complex at the ER-Mito Contact Sites

R. Paraan[1], K. Nakamura[2], S. Aoyama[2], T. Nagao[2], Y. Hirabayashi[2], F. Polleux[3], B. Carragher[1], C. Potter[1]; [1]NYSBC-SEMC, New York, NY, [2]Department of Chemistry and Biotechnology, University of Tokyo, Tokyo, JAPAN, [3]Zuckerman Institute, Columbia Univeristy, New York, NY.

The eukaryotic organelles such as the endoplasmic reticulum (ER), the nuclear envelope, mitochondria, and the endosomes can come to a close vicinity of each other and engage in dynamic ultrastructures known as contact sites. These contact sites are regulated by various tethering complexes most of which are not fully known specially in mammalian cells. The ER-mitochondria contact sites carry out diverse physiological functions such as calcium ion and lipid transport, hence their possible involvement in the early pathophysiology of neurodegenerative diseases such as Alzheimer's. Despite their important role

in cell health and disease, the molecular landscape of these contact sites are poorly understood. In this study we have identified an ER-resident protein PDZD8 and its mitochondrial binding partner and we have shown that they form tethering complexes at ER-mito contact sites of mammalian cells. First, a list of candidates for the mitochondrial binding partners of PDZD8 was obtained using the TurboID method. The interaction between PDZD8 and one of the candidates was confirmed by Co-ImmunoPrecipitation. We showed by fluorescence microscopy that the two proteins co-localize at the contact sites of mammalian cells and that the overexpression of the mitochondrial protein up-regulated PDZD8. We used cryo-correlative light and electron microscopy to capture this molecular interaction in close-to-native conditions. Using focused ion-beam milling, 35 lamellae or thin sections were prepared for cryo-electron tomography from HeLa cells overexpressing the mitochondrial partner tagged with a fluorophore. The overexpression increased the abundance of the contact sites most of which were occupied by the overexpressed protein as confirmed by correlative fluorescence imaging of the lamellae. A few thousand tethering complexes were identified in the tomograms in total with some heterogeneity in terms of the length of the tethers. We are using sub-tomogram averaging to generate a map of the different domains of these complexes. Coupled with the cryo-EM single particle analysis of a truncated complex, we aim to characterize the tethers in their native environment.

B446/P2122

Regulation of lipid droplet biogenesis and maintenance at endoplasmic reticulum-mitochondria contact sites

V. F. Monteiro-Cardoso[1], V. Guyard[1], M. Omrane[2], C. Boulogne[3], N. El Khallouki[1], R. Le Bars[3], O. Faklaris[4], N. Vitale[5], A. R. Thiam[2], **F. Giordano**[1]; [1]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Inserm U1280, Université Paris-Saclay, Gif-sur-Yvette, FRANCE, [2]Laboratoire de Physique de l'École Normale Supérieure, ENS, Université PSL, CNRS, Sorbonne Université, Université de Paris, Paris, FRANCE, [3]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, FRANCE, [4]MRI, BioCampus Montpellier, CRBM, Univ. Montpellier, CNRS, Montpellier, FRANCE, [5]Centre National de la Recherche Scientifique, Université de Strasbourg, Institut des Neurosciences Cellulaires et Intégratives, Strasbourg, FRANCE.

Lipid droplets (LDs) are intracellular organelles that play a central role in lipid metabolism and regulate cellular energy balance. In their hydrophobic core, surrounded by a phospholipid monolayer, LDs store energy in the form of neutral lipids and prevent their toxic accumulation in the cell. LDs originates from the endoplasmic reticulum (ER) membranes and alterations in their biogenesis are associated with many metabolic diseases such as diabetes and heart disease or viral infections. The ER-protein seipin, localizing at ER-LD junctions, controls LDs nucleation and growth. However, the molecular mechanisms that spatially and temporally regulate LD biogenesis in the cell remain largely unknown. By using a combination of biochemical and cell biology approaches, including live cell imaging, proximity ligation assays and electron microscopy, we have found that the ER subdomains closely associated to mitochondria, also called Mitochondria-Associated ER Membrane (MAMs), are hotspots for LD biogenesis. We have started to reveal the morphological and molecular features of these subdomains, identifying key proteins localized at the ER-mitochondria interface that are involved in the regulation of LD formation. Among those, we have shown that the lipid transfer proteins ORP5 and ORP8 relocate to MAM subdomains where LD originate at earlier steps of LD biogenesis. We have also shown that ORP5/8 regulate seipin recruitment to MAM-LD contacts, and their loss of function impairs LD biogenesis. Importantly, the integrity of ER-mitochondria contact sites is crucial for ORP5/8 function in regulating

seipin-mediated LD biogenesis. Our study uncovers a new role for ORP5/8 in orchestrating LD biogenesis and maturation at MAMs and brings novel insights into the metabolic crosstalk between mitochondria, ER, and LDs at membrane contact sites.

B447/P2123

Investigating the Role of ER-Mitochondria Junctions on Store-Operated Ca^{2+} Entry

Y. Lin[1], F. Tsai[1,2]; [1]Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, TAIWAN, [2]Department of Internal Medicine, National Taiwan University Hospital, Taipei, TAIWAN.

Store-operated Ca^{2+} entry (SOCE) is the major Ca^{2+} influx that regulates essential cellular functions. SOCE is mediated by the ER-Ca^{2+} sensor STIM1 and plasma membrane protein ORAI1. Numerous studies revealed that mitochondria are also regulators of SOCE. However, the exact mechanism is still unclear. The percentage of mitochondria that contact with ER is around 5-20%. Many substances can be transferred at the contact sites between ER and mitochondria, including Ca^{2+}. This ER-mitochondria connection and the ability of ER to spatially and temporally coordinate with plasma membrane may allow mitochondria to regulate SOCE activity. To elucidate the role of mitochondria-associated membranes in SOCE regulation, we disrupted ER-mitochondrial junctions (EMJs) by knocking down MFN2 and PDZD8, which are known ER-mitochondrial tethering proteins. Our preliminary data revealed that EMJs were required for SOCE activation and maintenance. We noticed that mitochondria were re-distributed to STIM1-ORAI1 regions upon SOCE activation, which supported our hypothesis. To elucidate how EMJs affect SOCE, we further examined STIM1 dynamics in EMJs-depleted cells. STIM1 oligomerization was suppressed in MFN2- and PDZD8-knockdowns cells. Moreover, STIM1 puncta were unstable in these EMJs-depleted cells. We also noticed a similar increase of puncta decomposition in cells expressing mutant STIM1 that could not bind to EB1. Furthermore, knocking down RHOT1 and TRAK1, which coordinately transport mitochondria along microtubules, also suppressed SOCE, supporting an important role of microtubules in EMJ-regulated SOCE. We are now working on the mechanisms of how ER-mitochondria and microtubules are coordinated, providing the cells with stable SOCE for important cellular functions.

B448/P2124

MIGA2 is a lipid transporter and its lipid transfer ability is essential for its role in mitochondrial and lipid droplet biology

Z. Hong, J. Adlakha, N. Wan, E. Guinn, F. Giska, K. Gupta, T. J. Melia, K. Reinisch; Yale University, New Haven, CT.

Lipid transport proteins at membrane contact sites, where organelles are closely apposed, are critical in redistributing lipids from the endoplasmic reticulum (ER), where they are made, to other cellular membranes. Such protein mediated transfer is especially important for maintaining organelles disconnected from secretory pathways, like mitochondria. Here we identify mitoguardin-2, a mitochondrial protein at contacts with the ER and/or lipid droplets (LDs), as a lipid transporter. An X-ray structure shows that the C-terminal domain of mitoguardin-2 has a hydrophobic cavity that binds lipids. Mass spectrometry analysis reveals that both glycerophospholipids and free fatty acids co-purify with mitoguardin-2 from cells, and that each mitoguardin-2 can accommodate up to two lipids. Mitoguardin-2 transfers glycerophospholipids between liposomes as well as between lipid droplets and liposomes in vitro, and this transport ability is required for roles both in mitochondrial and LD biology. While it is not

established that protein-mediated transfer at contacts plays a role in LD metabolism, our findings raise the possibility that mitoguardin-2 functions in transporting fatty acids and glycerophospholipids at mitochondria-LD contacts.

B449/P2125

PLIN5 interacts with FATP4 at membrane contact sites to promote lipid droplet-to-mitochondria fatty acid transport

G. E. Miner, C. M. So, W. Edwards, S. Cohen; University of North Carolina Chapel Hill, Chapel Hill, NC.

Cells adjust their metabolism by remodeling membrane contact sites that channel metabolites to different fates. Lipid droplet (LD)-mitochondria contacts change in response to fasting, cold exposure, and exercise. However, their function and mechanism of formation have remained controversial. We focused on perilipin 5 (PLIN5), an LD protein that tethers mitochondria, to probe the function and regulation of LD-mitochondria contacts. We demonstrate that efficient LD-to-mitochondria fatty acid (FA) trafficking and ß-oxidation during starvation of myoblasts requires both phosphorylation of PLIN5 and an intact PLIN5 mitochondrial tethering domain. We further identified the acyl-CoA synthetase, FATP4 (ACSVL4) as a novel mitochondrial interactor of PLIN5. The C-terminal domains of PLIN5 and FATP4 constitute a minimal protein interaction capable of inducing organelle contacts. Our work suggests that starvation leads to phosphorylation of PLIN5, lipolysis, and subsequent channeling of FAs from LDs to FATP4 on mitochondria for conversion to fatty-acyl-CoAs and subsequent oxidation.

B450/P2126

A generalizable tool platform for visualizing lipid droplet-organelle contact sites

X. Li[1], L. Climer[1], C. Lindow[1], A. G. Tebo[2], **C. Chang**[1]; [1]St. Jude Children's Research Hospital, Memphis, TN, [2]HHMI - Janelia Research Campus, Ashburn, VA.

Lipid droplets are dynamic fatty acid storage depots essential for cellular lipid and energy homeostasis. Lipid droplets dynamically stockpile surpluses of fatty acids from the endoplasmic reticulum (ER), and transport them to mitochondria, peroxisomes, and endo-lysosomal system, where fatty acids are utilized in lipid synthesis and ATP production to meet metabolic demands. Targeted transport of fatty acids relies on lipid droplet-organelle contact sites, dynamic apposing nanodomains between organelles that allow directed and efficient material flux. Defects in the formation of lipid-organelle contact sites are associated with developmental, metabolic, and neurological disorders. However, many facets of contact site biology remain unclear, primarily because detection of these dynamic, minute loci in living cells is technically challenging. To overcome this difficulty, we engineered a tool platform based on Flurogen-Activated Bimolecular Complementation at CONtact sites, FABCCON, using the reversible splitFAST system to visualize and control the formation of organelle contacts sites. We engineered the C-terminal fragment of splitFAST onto the cytosolic surface of lipid droplets by fusing it with tandem repeats of Spastin's hairpin motif known to insert into lipid droplets. The N terminal half of splitFAST was targeted to other organelles of interest by using corresponding localization sequences. After optimizing the affinity of FABCCON and cognate fluorogen, we successfully visualized lipid droplet-ER, -mitochondria, -peroxisomes, and -lysosomes contact sites in living cells, shortly after fluorogen addition. Complementation of splitFAST at contact sites is completely reversible as the signal disappeared shortly following fluorogen washout indicating minimal perturbation to the contact sites. Our data also showed that FABCCON can provide higher spatial-temporal precision of contact site detection compared to

conventional organelle colocalization analysis. In addition, FABCCON can quantitively enhance contact site formation with higher concentration of fluorogen, providing the possibility to rescue the deficiency of contact sites caused by genetic alteration. Notably, FABCCON is applicable to contact sites between any organelles provided with specific targeting information. We have applied the FABCCON platform to detect mitochondria-peroxisome and -lysosome contact sites where active fatty acid trafficking occurs. Altogether, FABCCON is a generalizable platform for quantitative detection and enhancing of organelle contact sites with increased spatial-temporal precision.

B451/P2127

Employing inducible mitochondrial tethers to characterize membrane contact site function

C. Harper, L. Lackner; Northwestern University, Evanston, IL.

Organelles perform distinct, essential functions in the cell, and while once thought to operate independently, it is increasingly clear that organelle membrane contact sites (MCSs) are critical for organelle function and overall cellular homeostasis. The contact site between mitochondria, the ER, and the plasma membrane in *S. cerevisiae*, known as the mitochondria-ER-cortex-anchor (MECA), is important for mitochondrial distribution, nuclear inheritance, mitochondrial function, and homeostasis of other contact sites. We hypothesize that MECA also establishes mitochondrial membrane tension required for proper mitochondrial division and health. Characteristic of MCSs, MECA formation is driven by a tether protein, called Num1. To investigate the role of Num1 in facilitating contact site functions, we engineered inducible organelle tethers using native MCS proteins and well-characterized organelle-interaction domains coupled with dimerization systems, such as rapamycin-induced dimerization. By controlling the formation and dissociation of the engineered tethers, we can assess the kinetics of contact site formation and functions. We find that although many synthetic tethers between mitochondria and the plasma membrane replicate wild type mitochondrial distribution, only those that include specific domains of Num1 rescue all contact site functions. Thus, Num1 establishes a specific environment at MECA, perhaps to facilitate molecular transport, that is critical for the myriad of functions of this contact site. Deciphering the role Num1 plays in each function of MECA will inform our understanding of the processes facilitated and coordinated by MCSs and how organelles interact for optimal cellular function.

B452/P2128

Peroxisomes maintain mitochondrial redox homeostasis through a novel contact site

L. DiGiovanni[1,2], M. Boutry[1], P. Khroud[1,2], P. Kim[1,2]; [1]The Hospital for Sick Children, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada

Robust reduction of mitochondrial derived reactive oxygen species (ROS) is essential to maintain mitochondrial and cellular health. Peroxisomes, a metabolic organelle with high redox capacity, are suspected to contribute to the redox homeostasis of mitochondria. Loss of peroxisome biogenesis or peroxisomal antioxidant function results in elevated mitochondrial oxidative stress, suggesting that peroxisomes may function as a sink to quench mitochondrial derived ROS. However, the mechanism of this role, and specifically how exchange of ROS between two organelles could be spatially and temporally achieved, is not understood. Here, we show for the first-time direct evidence of peroxisomes functioning to reduce mitochondrial derived ROS through contact of peroxisomes with mitochondria. We define a novel contact site mediated by peroxisomal membrane protein Acyl-CoA Binding Domain

Containing 5 (ACBD5), that facilitates direct contact between peroxisomes and mitochondria. Contact deficient cells had measurable mitochondrial dysfunction through multiple metrics. Mitochondria from ACBD5 deficient cells had an elevated sensitivity to oxidative stress, as measured using mitochondrially targeted redox probe roGFP, and could be rescued using an artificial peroxisome-mitochondria tether. We further showed that the peroxisome redox environment was sensitive to increases in mitochondrial ROS production, dependent on ACBD5 mediated peroxisome-mitochondria contact, providing evidence for the exchange of ROS between mitochondria and peroxisomes at sites of contact. Peroxisome-mitochondria contact increased in response to elevated mitochondrial oxidative stress, suggesting peroxisome contact is an adaptive cellular response to mitochondrial stress. This study demonstrates the novel and critical role peroxisome contact plays in mitochondrial antioxidant defense.

B453/P2129

Lipid transfer activity of ORP3 at ER-plasma membrane contacts maintains exocytic sites by stabilizing membrane association of LL5β

C. J. Bott, J. Y. Lim, J. E. Casanova; Cell Biology, University of Virginia, Charlottesville, VA.

ER-Membrane Contact Sites (ER-MCS) are hubs for a large number of lipid transfer proteins, which facilitate non-vesicular transport of lipids between adjacent membrane domains, and are thus major determinants of local membrane composition and functional identity. We have previously shown that the lipid transfer protein ORP3, in a complex with the Arf-GEF IQsec1, extracts plasma membrane PI4P in exchange for ER phosphatidylcholine at MCS immediately adjacent to focal adhesions. Using mass spectroscopy, we found that ORP3 and IQSEC1 co-immunoprecipitated multiple members of the Cortical Microtubule Stabilizing Complex (CMSC), including KANK2, liprin, LL5β, CLASP2, and ELKS, which was confirmed via western blot. ELKS facilitates the targeting of post-Golgi RAB6+ exocytic carriers to specific plasma membrane sites that, like ORP3-containing MCSs, are clustered adjacent to focal adhesions. ELKS is anchored at the plasma membrane through its interaction with the PIP3-binding protein LL5β, and previous studies have shown that the CMSC, anchored by LL5β, marks 'exocytic hotspots'. The interaction between ORP3 and the CMSC suggested that ORP3 could affect CMSC localization or function. In support of this hypothesis, we found that lentiviral knockdown of ORP3 disrupted the targeting of microtubule tips to the plasma membrane. While ORP3 depletion did not obviously change the steady-state localization of ELKS or LL5β via TIRF microscopy, it did reduce the interaction of ELKS with LL5β, and with Rab6, by co-immunoprecipitation. We posit that ORP3, via its lipid exchange activity, modulates the stability of the LL5β/ELKS/RAB6 complex at the plasma membrane. We have previously shown that the ORP3/IQSec1 complex is recruited to ER/PM contact sites in response to STIM/ORAI-mediated calcium influx. To determine how ORP3 affects LL5β/ELKS/RAB6 complex dynamics, we performed time-lapse imaging via TIRF microscopy. Thapsigargin, which induces Store Operated Calcium Entry (SOCE), triggers rapid recruitment of ORP3 to ER/PM contacts and triggers its lipid exchange activity. We found that the amount of GFP-LL5β in plasma membrane puncta remained relatively constant in response to calcium influx. In contrast, ~30% of LL5β dissociated from the PM after 20 minutes of thapsigargin treatment in ORP3-depleted cells. This loss could be rescued with WT-ORP3, but not by an ORP3 mutant that is deficient in lipid transfer. Taken together, these data suggest that ORP3-mediated lipid transfer stabilizes the CMSC at the plasma membrane during Ca2+ influx. Future directions include monitoring the dynamics of other CMSC components, its interaction with Rab6+ vesicles, and quantifying the effects of ORP3 dysfunction on secretion.

B454/P2130

PDZD8 and its mitochondrial binding partner tether the ER and mitochondria

K. Nakamura[1], **S. Aoyama-Ishiwatari**[1], T. Nagao[1], R. Paraan[2], Y. Sakurai[1], Y. Du[1], S. Suga[1], M. Tsuboi[1], M. Nakakido[1], K. Tsumoto[1,1,1], Y. Kishi[1], C. Kwak[3], H.-W. Rhee[3,3], H. Kosako[4], B. M. Humbel[5,6], B. Carragher[2], C. Potter[2], F. Polleux[7,7,7], Y. Hirabayashi[1,1]; [1]The University of Tokyo, Tokyo, Japan, [2]New York Structural Biology Center, New York, NY, [3]Seoul National University, Seoul, Korea, Republic of, [4]Tokushima University, Tokushima, Japan, [5]University of Lausanne, Lausanne, Switzerland, [6]Okinawa Institute of Science and Technology, Onna-son, Okinawa, Japan, [7]Columbia University, New York, NY

Mitochondria and endoplasmic reticulum (ER) form membrane contact sites (MERCSs), where the organelle membranes are closely apposed within the range of 10 to 30 nm but not fused. MERCs play critical roles in cellular functions such as lipid biogenesis and regulation of Ca^{2+} dynamics. We have identified Pdzd8, an ER-resident protein, as an important ER-mitochondria tethering protein (Hirabayashi et al. Science 2017) but its binding partner on the outer mitochondrial membrane (OMM) was still unknown. Using a combination of CRISPR-Cas9 knockin cell lines and (1) unbiased mass spectrometry screens following immunoprecipitation of endogenous PDZD8 and (2) proximity labeling by PDZD8-TurboID, we identified proteins in complex with PDZD8 and localizing in the proximity of PDZD8, respectively. Analyses using recombinant proteins showed that one of the mitochondrial proteins identified in our proteomic screens interacts directly with PDZD8 and immunofluorescence analysis combined with high resolution microscopy reveal that Pdzd8 and this OMM protein are juxtaposed at MERCS. Electron microscopy analysis revealed that this protein is required for tethering the ER and mitochondrial membranes. Knocking out both proteins reveal no additive effects compared to individual knockout cells demonstrating that these two proteins form not only a structural complex but function in the same complex. Finally, we are implementing Cryo-EM tomography to visualize this new tethering complex in cells. These findings suggest that the PDZD8 protein complex is essential for the formation of the MERCS ultrastructure and investigation of this protein complex will uncover the physiological functions of this new tethering complex at MERCs in various cell types including neurons.

B455/P2131

Motion of single molecular tethers reveals dynamic subdomains at ER-mitochondria contact sites

C. J. Obara[1], J. Nixon-Abell[2], A. S. Moore[1], C. Blackstone[3], J. Lippincott-Schwartz[1]; [1]HHMI, Ashburn, VA, [2]University of Cambridge, Cambridge, United Kingdom, [3]Massachusetts General Hospital and Harvard Medical School, Charlestown/Boston, MA

To coordinate cellular physiology, eukaryotic cells rely on the inter-organelle transfer of molecules at specialized organelle-organelle contact sites. Endoplasmic reticulum-mitochondria contact sites (ERMCSs) are particularly vital communication hubs, playing key roles in the exchange of signaling molecules, lipids, and metabolites. ERMCSs are maintained by interactions between complementary tethering molecules on the surface of each organelle. However, due to the extreme sensitivity of these membrane interfaces to tether disruption, a clear understanding of their fine structure and regulation is still lacking. Here, we combine 3D electron microscopy with high-speed molecular tracking of a model organelle tether, VAPB, to map the structure and diffusion landscape of ERMCSs. From EM reconstructions, we identified subdomains within the contact site where ER membranes dramatically deform to match local mitochondrial curvature. In parallel live experiments, we observed that the VAPB tethers that mediate this interface were not statically enriched, but rather highly dynamic, entering and

leaving the site in seconds. These subdomains enlarged during nutrient stress, indicating ERMCSs can readily remodel under different physiological conditions. A disease-associated mutation in VAPB altered the normal fluidity of contact sites, likely perturbing effective communication across the contact site and preventing remodeling. These results establish high speed single molecule imaging as a new tool for mapping the structure of contact site interfaces and suggest that the diffusion landscape of VAPB is a crucial component of ER-mitochondria contact site homeostasis.

B456/P2132

Elucidating the identity and function of mitochondrial membrane contact site tethers in budding yeast gametogenesis

C. Ruediger[1], E. Sawyer[2], G. King[1], D. Jorgens[3], E. Ünal[1]; [1]University of California, Berkeley, Berkeley, CA, [2]University of Colorado, Boulder, Boulder, CO, [3]Electron Microscope Lab, University of California, Berkeley, Berkeley, CA

Mitochondria are essential organelles containing their own genome. As such, mitochondrial segregation is a crucial aspect of gametogenesis necessary to produce viable gametes. However, how mitochondria are inherited into gametes remain unknown. Budding yeast gametogenesis provides a powerful model to uncover and dissect mechanisms of mitochondrial inheritance. During budding yeast gametogenesis, the mitochondrial network detaches from the cell periphery upon regulated degradation of a mitochondrial-plasma membrane tethering complex (MECA) and forms extensive membrane contacts with the nuclear envelope in meiosis II as chromosomes are segregated into newly forming gametes. Subsequently, portions of the network are pinched off and re-establish mitochondrial-plasma membrane associations within the nascent gametes. I examined the role of previously known and recently discovered mitochondrial tethers, including ERMES, *LAM6*, and *CNM1*, during gametogenesis. These analyses revealed the individual contributions of these tethers to be largely dispensable for both mito-nuclear contacts and mitochondrial inheritance, suggesting the existence of redundancy between known pathways and/or novel regulators. Finally, I found that ectopic tethering of mitochondria to the cell periphery with an engineered tether increased the production of respiratory-incompetent gametes, suggesting remodeling of mitochondrial contact sites is critical to ensure gamete health.

B457/P2133

Regulation of mitochondria and endoplasmic reticulum dynamics via O-GlcNAcylation

R. G. Sanchez, 1[1], W. Paul, 1[2], G. Pekkurnaz, 1[1]; [1]UCSD/SDSC, NIST, La Jolla, CA, [2]Pomona College, Pomona, CA

Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Arial",sans-serif;} The endoplasmic reticulum (ER) is a vast network of tubules and sheets that span from the nucleus to the plasma membrane. The ER plays a major role in protein secretion, Ca^{2+} homeostasis and lipid synthesis, the latter two functioning synergistically with mitochondria. It is known that the mitochondria and the ER can form mitochondrial-ER contact sites (MERCS) that facilitate lipid, and Ca^{2+} homeostasis. However, the mechanism behind the formation and dynamics of MERCS is not well understood. Here we propose

that the post-translational modification O-GlcNAcylation, a major nutrient sensor that couples metabolism with cellular function and signaling, is altering mitochondrial and ER dynamics to favor MERCS. We show that increased O-GlcNAcylation decreases mitochondrial and ER motility in COS7 cells. Both the mitochondria and ER demonstrate a perinuclear clustering when O-GlcNAc is increased. In addition, ER analysis reveals decreased tubular length and width primarily affecting the perinuclear region of the cell. Using Split-green fluorescent Protein based Contact site. Sensor (SPLICS) associated with the ER and mitochondrial outer membranes we identified MERCS motility and localization differences influenced by O-GlcNAc. We propose that co-regulation of ER and mitochondria via MERCS is essential for cellular morphological alterations in response to cellular stress.

B458/P2134

Organelle Membrane Contacts Toolbox: Dimerization-dependent Fluorescent Probes to Visualize Lipid Droplet-Organelle Contact Sites

J. V. Ragusa, G. E. Miner, S. Y. Smith, W. Showalter, C. M. So, S. Cohen; University of North Carolina At Chapel Hill, Chapel Hill, NC

Eukaryotic cells contain membrane-bound organelles that compartmentalize various molecules and biochemical processes. Although many studies have identified unique attributes of each organelle, there is increasing interest in visualizing and evaluating the functional consequences of organelle interactions. Lipid droplets are dynamic organelles that store lipids which can be readily mobilized for membrane synthesis, signal transduction, or energy. Unlike other organelles, lipid droplets are characterized by a single phospholipid monolayer that renders them incompatible with vesicular trafficking. Thus, lipid droplets must form physical contacts, called membrane contact sites, with other organelles for the exchange of molecules. Membrane contact sites are defined as sites where two organelles are within 10-70 nm. They are traditionally visualized by electron microscopy to achieve high resolution, but it is costly, low throughput, and not amenable to live cell imaging. Epifluorescence and confocal microscopy reconcile these limitations but cannot distinguish organelle proximity from true membrane contacts due to the diffraction limit of 200 nm. Therefore, we generated a toolbox of genetically encoded dimerization-dependent fluorescent probes to accurately visualize membrane contact sites within live cells. Using minimal targeting sequences, we developed probes for lipid droplets, mitochondria, endoplasmic reticulum, peroxisomes, lysosomes, and the plasma membrane. Organelles are labeled with quenched monomers of a fluorescent heterodimer that fluoresce brightly upon dimerization when two organelles are within 10-70 nm. We show that the probes accurately localize to targeted organelles and fluoresce only at membrane contact sites. Titration of plasmid concentration is crucial for using these probes without driving membrane contact site formation. Because these probes dimerize reversibly, we also demonstrate the ability of these probes to visualize the dynamics of membrane contact sites over time. This toolbox will be a valuable resource for cell biologists studying organelle-organelle contacts.

Mitochondria in Pathology

B459/P2135

Endosomal removal and disposal of dysfunctional, immunostimulatory mitochondrial DNA

L. E. Newman[1], N. Tadepalle[1], S. Weiser Novak[1], C. R. Schiavon[1], G. R. Rojas[1], J. A. Chevez[2], I. Lemersal[3], M. Medina[4], S. Rocha[1], C. G. Towers[1], D. A. Grotjahn[4], U. Manor[1], G. S. Shadel[1]; [1]Salk Institute for Biological Studies, La Jolla, CA, [2]University of California, San Diego, La Jolla, CA, [3]La Jolla Institute for Immunology, La Jolla, CA, [4]The Scripps Research Institute, La Jolla, CA

Maternally inherited mitochondrial DNA (mtDNA) encodes essential subunits of the mitochondrial oxidative phosphorylation system, but is also a major damage-associated molecular pattern (DAMP) that engages innate immune sensors when released into the cytoplasm, outside of cells or into circulation. Release of mtDNA during infection contributes to antiviral resistance in response to a growing number of viruses, but unfortunately also causes pathogenic inflammation in many disease contexts. Cells experiencing mtDNA stress due to depletion of the mtDNA-packaging protein, Transcription Factor A, Mitochondrial (TFAM), or Herpes Simplex Virus 1 (HSV-1) infection exhibit elongated mitochondria, mtDNA depletion, enlargement of nucleoids (mtDNA-protein complexes), and activation of cGAS/STING innate immune signaling via mtDNA released into the cytoplasm. However, the relationships between enlarged nucleoids, altered mitochondrial dynamics, and mtDNA-mediated activation of the cGAS-STING pathway remain unclear. Here, we show that entire enlarged nucleoids are released from mitochondria, remain bound to TFAM, and colocalize with the cytosolic innate immune sensor cGAS. These nucleoids arise at sites of mtDNA replication due to a block in mitochondrial fission at a stage when endoplasmic reticulum (ER) actin polymerization would normally commence, which we propose is a checkpoint to ensure that mtDNA has completed replication and is competent for segregation into daughter mitochondria through fission. After release, enlarged nucleoids colocalize with the late endosomal marker RAB7, suggesting that enlarged nucleoids traffic through endosomes. Blocking the activation of RAB5, which mediates the early steps in the endosomal pathway, prevents escape of enlarged nucleoids from mitochondria, indicating that endosomes extract faulty nucleoids. Endosomal rupture then allows for mtDNA escape into the cytosol and cGAS activation. Thus, we propose that defects in mtDNA replication and/or segregation enact a late mitochondrial fission checkpoint that, if persistent, leads to selective removal of dysfunctional nucleoids by a mitochondrial-endosomal pathway. Immunostimulatory mtDNA is ultimately degraded by late endosomes to prevent excessive innate immune signaling, but overload of this pathway causes mtDNA escape from endosomes and cGAS/STING signaling. This mtDNA quality control pathway might represent a therapeutic target within the context of mtDNA-driven antiviral defenses, as well as chronic inflammatory diseases where mtDNA has been implicated.

B460/P2136

Metatranscriptomic analysis uncovers prevalent mitovirus sequences compatible with mitochondrial translation

A. Begeman[1], A. Babaian[2], S. C. Lewis[1]; [1]University of California, Berkeley, Berkeley, CA, [2]Independent researcher, Vancouver, BC, CANADA.

Despite RNA viruses being ubiquitous components of the biosphere, relatively little is known about their general biodiversity or how they may adapt replication strategies to exploit the biology of their host. A

hallmark of (+)ssRNA viruses is the ability to remodel host endomembranes for their own replication; however, the interplay between viruses and host organelles that already harbor native gene expression systems, such as mitochondria, remains poorly characterized. Here we search thousands of publicly available RNA sequencing runs for novel viral species and report the discovery of 763 new virus sequences belonging to the family Mitoviridae, a family of (+)ssRNA viruses that have previously been suggested to interact with the mitochondria. The identified sequences fill in existing gaps in known mitovirus diversity, and allow us to further identify previously uncharacterized mitovirus clades, and potentially a new virus class all together. Using this new expansion of diversity, we were able to annotate new mitovirus specific protein motifs, and identify hallmarks of mitochondrial translation such as mitochondrial specific codons and adapted mitochondrial codon usage. Together, this data expands the known diversity of mitochondrial viruses, and provides strong evidence for their evolutionary adaptation to co-opt mitochondrial biology for their survival.

B461/P2137

Damaged mitochondria recruit the effector NEMO to activate NF-κB

O. Harding, E. L. F. Holzbaur; Department of Physiology, University of Pennsylvania, Philadelphia, PA.

Mitochondrial damage presents an immediate danger to cellular homeostasis. Dysfunctional mitochondra fail to produce the energy required to maintain cellular function and can release reactive oxygen species or initiate other damaged-induced signaling pathways that promote inflammatory responses. Mitophagy is a quality control pathway by which damaged mitochondria are isolated and cleared via the activation of the ubiquitin ligase Parkin and subsequently, mitophagy receptors. Mutations that impair mitophagy or environmental perturbations to the clearance pathway are associated with neurodegenerative diseases including Parkinson's disease and ALS. Here we demonstrate a novel link from mitochondrial dysfunction to the activation of NF-κB, the principal transcription factor that regulates immune activation. Following mitochondrial damage, the NF-κB essential regulator NEMO/IKKɣ is recruited to mitochondria in a Parkin- and p62/SQSTM1-dependent manner. Although NEMO and the well-studied mitophagy receptor Optineurin (OPTN) are structurally similar, NEMO and OPTN maintain separate domains on the outer mitochondrial membrane. Instead, NEMO colocalizes extensively with p62, suggesting that NEMO and p62 together form phase-condensates that can activate the NF-κB inflammation pathway via the kinase IKKβ. Indeed, active phospho-IKKβ colocalizes with NEMO on damaged mitochondria, and cells with mitochondrial damage exhibit upregulation of inflammatory cytokines. These findings demonstrate that damaged mitochondria serve as an intracellular platform for innate immune signaling by promoting the formation of activated IKK complexes in a Parkin-dependent manner. Finally, mitochondria that recruit NEMO are less likely to be engulfed by clearance autophagosomes. Thus, we propose that mitophagy and NF-κB signaling are competing pathways regulating the response to cellular stress.

B462/P2138

Enhanced Mitochondrial Biogenesis Promotes Cell Survival in Human Stem Cell Derived Retinal Ganglion Cells of the Central Nervous System

M. Surma, K. Anbarasu, K. Huang, J. S. Meyer, **A. Das**; Indiana University, Indianapolis, IN.

Mitochondrial dysfunction has been the primary suspect for central nervous system (CNS) disorders such as glaucoma, parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). Mitochondrial

quality control (MQC) is a complex process which includes degradation of damaged mitochondria (mitophagy), biogenesis of healthy mitochondria and fission/fusion dynamics. It is still unclear the mechanism of improving mitochondrial homeostasis that will confer neuroprotection to the CNS neurons. Among CNS neurons, retinal ganglion cells (RGCs) are the most sensitive towards mitochondrial dysfunction. As such, inherited optic neuropathies such as Leber hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA) are diagnosed by the presence of mutations in the mitochondrial electron transport subunits or fusion gene OPA1 respectively. Among glaucoma patients with normal eye pressure, ~17% patients showed Optineurin (OPTN-E50K) mutation, OPTN is a critical player for mitophagy. E50K forms insoluble aggregates with its regulator Tank-binding kinase1 (TBK1) which also found mutated among several glaucoma and ALS patients. Thus, RGCs provide a unique CNS neuron type for the investigation of improving MQC mechanisms for developing neuroprotection strategy.Here, we used patient derived induced pluripotent stem cells (iPSCs) and CRISPR edited human embryonic stem cells (H7-ESC, WiCell) with E50K mutation for developing RGC protection mechanism. We used a robust well-characterized human stem cell differentiated RGCs (hRGCs) for this work. Our data shows hRGCs promote mitobiogenesis under acute mitochondrial stress by CCCP as a compensatory mechanism for survival. TBK1 negatively regulates mito-biogenesis by suppressing AMPK mediated PGC1α activation. Remarkably, we found TBK1 inhibition by a potent drug BX795 led to E50K aggregate dissolution, activation of the mitobiogenesis pathway and reduced mitochondrial swelling in E50K hRGCs by biochemical, confocal and electron microscopy experiments. Furthermore, we observed BX795 increased respiratory capacity and cell survival for both the wild-type and E50K hRGCs as an indication for neuroprotection. Our study for the first time identified mechanisms of improving mitochondrial homeostasis that conferred glaucoma neuroprotection which may be applicable for other CNS disorders where mitochondrial homeostasis is compromised.

B463/P2139

Neuronal specific *CASK* deletion in mice is lethal and produces aberrant cortical mitochondrial and neuronal activities

H. S. Bhonsle[1], K. Mukherjee[1], H. Goodkin[2], S. Srivastava[1,3]; [1]Fralin Biomedical Research Institute at Virginia Tech Carilion, Roanoke, 24016, VA, [2]Department of Neurology, University of Virginia School of Medicine, Charlottesville, 22908, VA, [3]Department of Internal Medicine, Virginia Tech Carilion School of Medicine, Roanoke 24016, VA.

CASK is an X-linked gene in mammals, encoding for calcium/calmodulin-dependent serine protein kinase that is ubiquitously expressed in all tissues. Constitutive *CASK* deletion in mice causes neonatal lethality, and CASK deletion in human males is incompatible with life suggesting its essential role for survival. Pathogenic mutations in *CASK* exhibit a diverse clinical spectrum including intellectual disability, autism spectrum disorder, microcephaly with pontine and cerebellar hypoplasia, epileptic encephalopathy (EE), and growth retardation. Notably, *CASK* heterozygous mutations in females causes MICPCH, whereas *CASK* hemizygous mutations in males are linked to EE. To better understand the function of CASK and underlying etiopathogenic mechanisms, we generated a neuronal-specific *CASK* knockout ($CASK^{NKO}$) mouse by using the Cre-LoxP system. Interestingly, we found that the $CASK^{NKO}$ mice exhibit severe growth retardation and infantile-onset recurrent tonic spasms and myoclonus beginning postnatal day 8 (P8) which progressively worsens with age, and die before adulthood (i.e. P25). The overall brain size of $CASK^{NKO}$ mice is ~35-40% small along with a disproportionately small cerebellum and an isometrically small cortex compared to the age-and-sex-matched $CASK^{wildtype}$ littermate control mice. Electron

microscopy analysis revealed reduced number of mitochondria in the cortex of *CASK*^{NKO} mice compared to the control mice. A significant decrease in brain mitochondrial respiration was also observed in the *CASK*^{NKO} mice compared to the control mice. Strikingly, an unbiased RNA sequencing analysis revealed significant changes in the levels ~228 mitochondrial transcripts encoded by both nuclear and mitochondrial genomes in the brain of *CASK*^{NKO} mice compared to the control mice indicating a severe impairment in mitochondrial gene expression. Finally, we determined alterations in neuronal morphology, pathology, and activity by performing the immunohistochemical staining of cortical brain sections using confocal microscopy analysis. Although we observed no changes in brain lamination and synaptic density, surprisingly, we found that cortical layer-specific markers (i.e. CUX1 and TBR1) were mis-expressed in the *CASK*^{NKO} mice, cortex width was also reduced associated with an increase in neuronal density, and c-fos staining was decreased compared to the control mice. Additionally, astrocyte-specific GFAP immunostaining was markedly increased in the cortex of *CASK*^{NKO} mice compared to the control mice suggesting increased reactive astrogliosis and proliferation. Altogether, our findings suggest that neuronal CASK is essential for postnatal brain development and plays critical roles in regulating both mitochondrial and neuronal functions.

B464/P2140

Endolysosome iron dyshomeostasis is mechanistically linked to endocytosed HERV-K envelope protein-induced mitochondrial depolarization and neurotoxicity

P. W. Halcrow[1], N. Kumar[1], D. Quansah[1], J. Steiner[2], A. Nath[2], J. D. Geiger[1]; [1]Biomedical Sciences, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND, [2]Section for Infections of the Nervous System, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD.

Human endogenous retroviruses (HERVs) are associated with the pathogenesis of amyotrophic lateral sclerosis (ALS); a disease characterized by motor neuron degeneration and cell death. The HERV-K subtype HML-2 envelope protein (HERV-K Env) is activated in brain of people living with ALS and through CD98HC-linked interactions causes motor neuron degeneration, mitochondrial dysfunction, and cell death. HERV-K Env-induced increases in oxidative stress are implicated in the pathogenesis of ALS, and ferrous iron (Fe^{2+}) generates reactive oxygen species (ROS). Endolysosome stores of Fe^{2+} are central to iron trafficking and endolysosome de-acidification releases Fe^{2+} into the cytoplasm. Because HERV-K Env is an arginine-rich protein that is likely endocytosed and arginine is a pH-elevating amino acid, it was important to determine HERV-K Env effects on endolysosome pH and whether HERV-K Env-induced neurotoxicity is downstream of Fe^{2+} released from endolysosomes. Here, we showed using SH-SY5Y neuroblastoma cells that HERV-K Env (1) is endocytosed via CD98 receptors, (2) concentration-dependently de-acidified endolysosomes, (3) decreased endolysosome Fe^{2+} levels, (4) increased cytosolic and mitochondrial Fe2+ and ROS levels, (5) depolarized mitochondrial membrane potential, and (6) induced cell death; effects blocked by an antibody against the CD98 receptor and deferoxamine an endolysosome-iron chelator. Thus, HERV-K Env-induced increases in cytosolic and mitochondrial Fe^{2+} and ROS as well as cell death appear to be mechanistically caused by HERV-K Env endocytosis, endolysosome de-acidification, and endolysosome Fe^{2+} efflux into the cytoplasm.

B465/P2141

Mitochondrial Morphology and MICOS Complex Changes Across Aging in Cardiac Muscle of C56Bl/6 Male Mice

A. G. Marshall[1], Z. Vue[1], E. Garza-Lopez[2], K. Neikirk[1], L. Vang[1], H. K. Beasley[1], S. Murray[3], A. Hinton, Jr.[1]; [1]Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, [2]Internal Medicine, University of Iowa, Iowa City, IA, [3]Department of Cell Biology, The University of Pittsburgh School of Medicine, Pittsburgh, PA.

Introduction Cardiovascular diseases (CVD) remain a major cause of death globally and new therapies are sorely needed. The heart is an energetically demanding tissue, with mitochondria estimated to comprise 1/3 of adult cardiomyocytes, representing an interesting target for novel therapeutics. Key factors regulating mitochondrial morphology, such as the mitochondrial contact site and cristae organizing system (MICOS), have not been well explored in cardiac tissues. **Materials and Methods** We used transmission electron microscopy (TEM) and serial block facing-scanning electron microscopy (SBF-SEM) to quantitatively analyze the 3D networks in cardiac muscle samples of mice through advanced aging. **Results** Across aging, we observed breakdown of mitochondria: decreased area, increased number, fragmentation, and loss of cristae morphology. Knockdown of key MICOS proteins in fibroblasts mimicked what was observed in aged cardiac tissue. **Conclusion.** We have identified the MICOS complex as potential targets for intervention in cardiac health.

B466/P2142

3D Reconstruction of Mouse Gastrocnemius Muscle Show Decrease in the MICOS Complex and Altered Mitochondrial Morphology Across Age

K. Neikirk[1], Z. Vue[1], L. Vang[1], E. Garza-Lopez[2], A. Hinton, Jr.[1]; [1]Vanderbilt University, Nashville, TN, [2]University of Iowa, Iowa City, IA.

Mitochondria are active organelles, undergoing harmonized cycles of fission and fusion, driven by 'mitochondrial dynamics,' to retain their morphology, distribution, and size. Mitochondrial dynamics has emerged as a critical process in maintaining cellular homeostasis. Interestingly, it has been long respected that a decrease in mitochondrial function escorts aging in specific tissues. For example, skeletal muscle gradually loses mass, strength, endurance, and oxidative capacity during aging. This decrease might, in turn, contribute to the observed age-dependent decline in organ function by mutations or alterations in mitochondrial fusion and fission proteins associated with several diseases. However, new players in regulating mitochondrial shape and cristae shape have been recently studied, such as the mitochondrial contact site and cristae organizing system (MICOS) complex. The MICOS complex is a multi-protein interaction hub that helps define cristate and mitochondria architecture. However, the MICOS complex has not been implicated in regulating organelle structure changes during aging. Thus, we hypothesized that loss of the MICOS complex during aging may increase mitochondrial fragmentation, decrease nanotunnels, and alter cristae morphology. To do this, we examined the three-dimensional morphology of mitochondria networks in young (3-month) and aged (2-year) murine gastrocnemius muscle via serial block face-scanning electron microscopy and the Amira program for segmentation, analysis, and quantification. We found differences in mitochondrial network configuration, nanotunneling, size, shape, number, contact sites, and MICOS gene expression in skeletal muscle during aging. We also found an association between OPA-1 and the MICOS complex in the gastrocnemius with mitochondrial aging. Furthermore, the loss of the MICOS complex was linked with

decreased oxidative capacity and altered mitochondrial metabolism. Potentially, this suggests a novel relationship between the MICOS complex and aging in skeletal muscle.

B467/P2143

3D Reconstruction of Aged Kidney Shows Unique and Diverse Mitochondrial Phenotypes in the Tubular Epithelium

T. Barongan[1], Z. Vue[2], L. Vang[2], K. Neikirk[1], J. McBride[3], A. O. Hinton, Jr.[2]; [1]University of Hawaii At Hilo, Hilo, HI, [2]Vanderbilt University Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, [3]Vanderbilt Institute of Nanoscale Science and Engineering, Vanderbilt University, Nashville, TN.

The kidney is a vital organ that demands an astonishing amount of energy, in terms of mitochondrial count and oxygen consumption, to maintain its functions, such as metabolism, hormone secretion, nutrients reabsorption, and filtration of blood. In recent years, cristae (mitochondrial inner membrane folds) have been shown to be important for mitochondrial function and has been associated with human diseases due to aberrant cristae morphologies, such as renal sclerosis and acute glomerulonephritis. We hypothesized that 3D reconstruction would show mitochondrial dysfunction marked by fragmentation across aging in kidney. To confirm this hypothesis, we used serial block face-scanning electron microscopy to observe mitochondrial 3D reconstruction of the kidney tubule epithelium in 3-month (young adults) and 2-year (elderly) mice. In all samples, we observed round mitochondria next to the nucleus, whereas further away from the nucleus, the mitochondria display unique structures, such as large, fragmented, elongated, compact, nanotunnels and toroid. Overall, we observed more fragmentation in the 2-year compared to the 3-month mice, marked by a decrease in area and volume. From these studies, we conclude that fragmentation occurs in aged kidney samples, which may contribute to decreased performance.

B468/P2144

Changes in Mitochondrial Cristae Morphology that Occur with Aging in Brown Adipose Tissue

A. Crabtree[1], Z. Vue[1], D. Stephens[1], A. Marshall[1], H. K. Beasley[1], S. Damo[2,1], A. Hinton, Jr.[1]; [1]Vanderbilt University, Nashville, TN, [2]Fisk University, Nashville, TN

Mitochondria are required for energy production and give brown adipose tissue (BAT) its characteristic color due to their high iron content. The physiological function and bioenergetic capacity of mitochondria are connected to the structure, folding, and organization of its inner-membrane cristae. During the aging process, mitochondrial dysfunction is observed, and the regulatory balance of mitochondrial dynamics is often disrupted, leading to increased mitochondrial fragmentation in aging cells. Dysregulation of mitochondrial dynamics is thought to play a role in age-related disorders and higher susceptibility of cells to various stress conditions during progressive aging. Therefore, we hypothesize that the morphological changes observed in aging BAT mitochondria and cristae can imply alterations to energy dynamics. To test this hypothesis, we developed a quantitative three-dimensional approach to map cristae network organization in mouse BAT at electron microscopy resolution. Using this methodology, we investigated the three-dimensional morphology of mitochondrial cristae in young (3-month) and aged (2-year) murine BAT tissue via serial block face-scanning electron microscopy and the Amira program for segmentation, analysis, and quantification. Upon analysis, we found that there were changes in mitochondrial volume, cristae volume, tubular ratios, maximum length/ minimum

length ratios, and cristae surface/ mitochondrial volume ratios during aging. Additionally, we observed changes in mitochondria morphology and lipid droplet appearance, structural integrity, decreased cristae surface area, and mitochondrial interactions with lipids in 2-year aged BAT murine samples compared to 3-month murine samples. Overall, these data define the nature of the mitochondrial structure in BAT, quantify young and old mitochondrial morphology, lipid droplet, and cristae morphology differences, and suggest that changes in structure may lead to alterations in functionality and bioactivity.

B469/P2145

Cardiomyocytes treated with particulate matter and palmitate increase apoptosis through mitochondrial fission

Y.-L. CHEN; College of Medicine, National Taiwan University, Taipei, Taiwan

There is growing evidence that exposure to fine particulate matter (PM) air pollution increases the risk of cardiovascular disease morbidity and mortality. According to previous studies, PM in the air enters the respiratory tract, contacts the alveoli, and enters the blood circulation, leading to the progression of cardiovascular disease. Who is most vulnerable is uncertain. Individuals with cardiometabolic disease, including hyperlipidemia and obesity, may be at greater risk. PM pollution may also lead to cardiometabolic disturbances, increasing the risk of cardiovascular disease. The effect of PM on cardiac function and mitochondrial damage under a high-lipid treatment is currently unknown. We used rat cardiomyocytes (H9c2) as an in vitro cell model using palmitic acid (PA) and PM to simulate hyperlipidemia and an air pollution environment. These results demonstrated that the apoptosis-related factor PUMA, a regulator of apoptosis upregulated by p53, was increased in cardiomyocytes treated with PA or PM alone, whereas PUMA expression was significantly increased in cells co-treated with PA and PM. Apoptosis was aggravated in cardiomyocytes treated with PA and PM, as measured by TUNEL assay and Annexin V/PI. Western blot results showed that under the combined treatment of PA+PM, CASPASE3 were significantly increased, and BCL2 (B-cell lymphoid 2) was significantly decreased. Simultaneous exposure to PA and PM increases mitochondrial reactive oxygen species (ROS) production by MitoSOX Red staining. Furthermore, using Mitotracker staining, PA+PM treatment significantly shortened mitochondrial length, indicating mitochondrial fission. The expression of mitochondrial fission-related proteins p-DRP1 (phosphodynamics-related protein 1) and FIS1 (mitochondrial fission 1 protein) was significantly increased compared to the PA or PM-treated groups alone. According to these results, combined exposure of PA and PM worsens mitochondrial function and leads to cardiomyocyte apoptosis. In conclusion, co-exposure of PA and PM increased oxidative stress and mitochondrial fission, and further exacerbated cardiomyocyte apoptosis

B470/P2146

COVID-19 treatment drug Remdesivir increases mtDNA copy number, but only causes mild changes to mitochondrial function.

N. DeFoor[1], J. L. Browning[1], E. K. G. Basso[1], V. Stevenson[2], S. Li[3], T. Ge[3], A. M. Pickrell[1]; [1]Virginia Tech, Blacksburg, VA, [2]Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, [3]Medical University of South Carolina, Charleston, SC

SARS-CoV-2 causes the severe respiratory disease COVID-19 which has led to illness in an estimated 615 million people worldwide. Remdesivir (RDV) was the first fast-tracked FDA approved treatment drug for

COVID-19. RDV acts as an antiviral ribonucleoside (adenosine) analogue that becomes active once it has been metabolized in the liver. It then diffuses into the host cell and terminates viral RNA transcription due to its ability to evade proofreading exoribonucleases. Previous studies have shown that certain nucleoside analogues unintentionally inhibit mitochondrial RNA or DNA polymerases or cause mutational changes to mitochondrial DNA (mtDNA) itself. For instance, the human immunodeficiency virus (HIV) treatment drug Zidovudine (AZT) works as a thymidine analogue to halt viral DNA transcription during reverse transcription. However, patients treated with AZT years later displayed myopathies, which was later discovered to be due to off-target mitochondrial damage from long-term usage. Similarly, Fialuridine (FIAU) was tested for the treatment against hepatitis B virus (HBV), but did not pass Phase II clinical trials due to unforeseen mitochondrial damage. These past findings on the mitochondrial toxicity of ribonucleoside analogues motivated the study to investigate what effects RDV may have on mitochondrial function. Using in vitro and in vivo models treated with RDV, we characterized changes to mtDNA copy number and mitochondrial protein expression. We found that a 2.5uM treatment of RDV increases mtDNA copy number in Mv1Lu (mink lung) cells over 72 hours with concomitant increases in mtDNA-encoded proteins. However, these changes do not appear to affect enzymatic activity. When testing young adult CD-1 mice given a 10 day course of RDV, liver tissue also displayed increased mtDNA levels 30 days post-treatment. However, mitochondrial protein expression and liver pathology appeared mostly unaffected. When examining skeletal muscle, lung, and cardiac tissue, mtDNA and mtDNA-encoded proteins appeared mostly unaffected. Pathology performed on lung tissue displayed normal cellular morphology and no gross changes upon examination. Future studies will determine how RDV increases mtDNA copy number during acute treatment periods and if aged animals display a heightened sensitivity to RDV treatment paradigms.

B471/P2147

IL-1β-induced Inflammation Impairs Immunometabolic Response in Corneal Epithelial Cells

J. Sanches[1], N. Mussi[1], H. Baniasadi[1], D. M. Robertson[2]; [1]UT Southwestern Medical Center, Dallas, TX, [2]UT Southwestern Medical Center, White, TX

Purpose: As a mucosal surface, the corneal epithelium plays an important role in mediating innate immune responses across the front surface of the eye. Pathogens, changes in tear film osmolarity, and tissue damage can all trigger inflammation, leading to tissue damage. In the present study, we used the pro-inflammatory cytokine, IL-1β, to investigate the effects of inflammation on mitochondrial and metabolic homeostasis in corneal epithelial cells.**Methods:** Telomerase-immortalized human corneal epithelial cells were cultured in serum-free keratinocyte basal media. To establish an inflammatory environment, cells were treated with 50 ng/mL or 100 ng/mL of IL-1β for 72 hours. Metabolism was measured in real time using a Seahorse metabolic flux analyzer. Total cell number per well was used for normalization. Protein expression for metabolic, mitochondrial, and mitophagy-related proteins was assessed using western blot. Confocal microscopy was used to evaluate intracellular reactive oxygen species (ROS) production. Cytokines were measured by ELISA and liquid chromatography mass spectrometry analysis was used for targeted metabolomics.**Results:** IL-1β-induced inflammation promoted a fuel switch indicating metabolic adaptation between 50 ng/mL and 100 ng/mL. This was associated with an increase in spare and maximum respiratory capacity (p=0.043 and p=0.001, respectively). The higher concentration of IL-1β increased the ECAR response (p=0.03) and non-mitochondrial oxygen consumption (p=0.002). Long-term treatment with IL-1β-induced inflammation downregulated mitochondrial and mitophagy proteins in a mTOR independent manner.IL-1β-induced

inflammation increased IL-8 and IL-6 levels, as well as intracellular ROS production. Targeted metabolomics analyzed 69 metabolites ($p<0.05$). Glucose-6-phosphate and fructose-6-phosphate were more prominent in the control group. Oxidative enzymes such as NADH and NADPH were prominent in IL-1β treated cells, as well as coenzyme A, fumarate, nicotinamide, and phosphoenolpyruvate. IL-1β also enhanced levels of several amino acids, including as glutamate, alanine, and tryptophan. **Conclusion:** These data confirm that inflammation triggers metabolic and mitochondrial changes in corneal epithelial cells. The increase in spare respiratory capacity enables the cell to better respond to stress and promote cell survival.

B472/P2148

Relocation of the mitochondrial protein HADHA to the cell surface under starvation and hypoxic stress conditions.

F. Veloso-Bahamondes[1], C. Retamal[1], A. González[1,2,3], **C. Oyanadel**[1]; [1]Universidad San Sebastián, Santiago, Chile, [2]P. Universidad Católica de Chile, Santiago, Chile, [3]Centro Científico y Tecnológico de Excelencia Ciencia & Vida, Santiago, Chile

Most of mitochondrial proteins are synthesized in the cytosol as precursors bearing an N-terminal targeting signal. Beta-oxidation mitochondrial-trifunctional-protein (MTP) is a mitochondrial matrix protein but its alpha-subunit, hydroxyacyl-CoA deshydrogenase (HADHA), has also been found at the cell surface, corresponding to the otherwise called Gastrin Binding Protein (GBP). GBP binds gastrin and mediates autocrine mitogenic effects in normal gastric and colon carcinoma cells. The mechanism addressing HADHA/GBP from the cytosol to either the mitochondria or the cell surface remains unknown. Interestingly, cytosolic HADHA has recently been described to interact with LC3, a protein crucial in autophagy processes. Autophagy increases during starvation, removes cytosolic components and also mediates unconventional secretion of cytosolic cargoes, which is an alternative trafficking route of integral membrane proteins to the plasma membrane. In this work we study whether HADHA presequence has sorting information to target the protein to the cell surface in a regulated manner, especially under stress conditions usually found in tumoral microenvironments, such as starving and hypoxic conditions. We used GFP coupled to HADHA presequence as reporter and performed surface biotinylation assays to assess the cell surface distribution and confocal imaging to visualize mitochondria dynamics. We show that HADHA/GBP contains sorting information to target both mitochondria and plasma membrane. Interestingly, starvation and chemical hypoxia with cobalt chloride both increased GBP/HADHA at the cell surface of tumoral cells, along with the described mitochondrial fragmentation and LC3-II expression reflecting autophagy under this treatment. As mitochondrial fragmentation and autophagy imply metabolic changes, these results suggest that tumor cells under stress conditions can increase the targeting of HADHA/GBP to cell surface as the result of metabolic adaptative process. The functional consequences of such protein relocation remains unknown. Acknowledgements ANID/FONDECYT 11181015 and Centro de Envejecimiento y Regeneración, ACE210009, and Centro Ciencia & Vida, Basal FB 210008

B473/P2149

Mitochondrial calcium overload in alzheimer's disease

P. Jadiya[1], D. Tomar[1], J. Elrod[2]; [1]Wake Forest School of Medicine - Winston-Salem, NC, Winston-Salem, NC, [2]Temple University, Philadelphia, PA

Background. We recently reported that compromised mitochondrial calcium ($_mCa^{2+}$) efflux (mitochondrial Na^+/Ca^{2+} exchanger or NCLX-dependent) causes $_mCa^{2+}$ overload in Alzheimer's Disease (AD). Genetic deletion of neuronal NCLX (the primary route for $_mCa^{2+}$ efflux) in the 3xTg-AD mouse model accelerated memory deficits and AD progression. Furthermore, increased neuronal $_mCa^{2+}$ efflux using NCLX overexpression in the 3xTg-AD mouse model reduced AD-phenotype. Our previous study suggests that $_mCa^{2+}$overload is a primary contributor to AD pathology by promoting metabolic dysfunction and neuronal cell death. However, whether $_mCa^{2+}$ overload alone is sufficient to cause neuronal pathology independent of amyloidosis or tau pathology remains unidentified. **Methods.** To test this, we generated a neuronal-restricted knockout of NCLX, its loss predisposes to $_mCa^{2+}$ overload, in wild-type mice (Nclx-nKO), and evaluated age-associated changes in cognitive functions and neuropathology. **Results.** We observed that neuronal-specific loss of $_mCa^{2+}$ efflux in wild-type mice increased amyloid deposition with an age-dependent decline in spatial memory, suggesting that $_mCa^{2+}$ overload is sufficient to promote an AD-like phenotype.

B474/P2150

Chemical Genetics Screen to Identify Functional Effects of a Quinazoline Derivative, EVP4593, in Budding Yeast

L. Hiestand[1], Y. S. Shen[1], W. Sloan[2], D. Lashley[2], O. Kerscher[3]; [1]Biology, The College of William and Mary, Williamsburg, VA, [2]Chemistry, The College of William and Mary, Williamsburg, VA, [3]Biology, William & Mary, Williamsburg, VA.

Mitochondria are essential eukaryotic organelles. Dysfunction of mitochondria can lead to mitochondrial myopathies and may contribute to neurodegenerative diseases, cancer, and diabetes. EVP4593 is a 6-aminoquinazoline derivative that has been shown to inhibit complex I of the mitochondrial electron transport chain, causing the release of reactive oxygen species (ROS). EVP4593 inhibits respiration of isolated mitochondria in the nanomolar range (IC50 = 14-25 nM). In contrast, we established that wildtype budding yeast cells can survive up to ~100µM EVP4593 when grown on a non-fermentable carbon source. To better understand the cellular pathways and processes affected by EVP4593, we conducted a genome-wide chemical genetics screen of the yeast knockout collection. The objective was to identify yeast gene deletion strains that exhibit growth defects when subjected to a sublethal concentration of EVP4593 [15µM]. We screened ~5100 yeast mutants and identified 20 yeast genes that are required for resistance to 15µM EVP4593 in glycerol-containing media. In summary, the genes identified in our screen are functionally involved in 1) regulating mitochondrial metabolism, 2) vacuolar targeting, 3) DNA replication, and 4) stress tolerance. Additionally, we investigated cellular phenotypes associated with the exposure to EVP4593, such as changes in mitochondrial structure and the accumulation of oxygen radicals. In conclusion, our study leverages the powerful yeast models system to identify the genetic pathways involved in EVP4593 sensitivity and the mechanisms the cell uses to protect its mitochondria from damage.

Signaling Receptors (RTKs and GPCRs)

B476/P2151

Irisin acts through its integrin receptor in a novel two-step process

M. A[1,2], T. E. Wales[3], H. Zhou[4], M. J. Mittenbühler[1,2], C. R. Kim[1,2], S. Draga-Coletă[5,6], C. Gorgulla[1,7,8], K. A. Blackmore[1,2], Z. Wang[1,8], M. P. Jedrychowski[1,2], H. Seo[1,8], K. Song[1,8], A. Z. Xu[1,8], L. Sebastian[1,8], D. Bogoslavski[1,2], S. P. Gygi[2], H. Arthanari[1,8], S. Dhe-Paganon[1,8], P. R. Griffin[9,10], J. R. Engen[3], B. M. Spiegelman[1,2]; [1]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, [2]Department of Cell Biology, Harvard Medical School, Boston, MA, [3]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA, [4]Department of Biological Chemistry and Molecular Pharmacology, Blavatnik Institute, Harvard Medical School, Boston, MA, [5]Virtual Discovery, Inc, Chestnut Hill, MA, [6]Non-Governmental Research Organization Biologic, Bucharest, ROMANIA, [7]Department of Physics, Harvard University, Cambridge, MA, [8]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, [9]UF Scripps Biomedical Research, Jupiter, FL, [10]Scripps Research, Jupiter, FL.

Exercise benefits the human body in many ways. Irisin is secreted by muscle, increased with exercise, and conveys many physiological benefits, including thermogenesis in adipose tissues, bone remodeling, and improved cognition and resistance to neurodegeneration. Irisin acts via αV integrins; however, a mechanistic understanding of how small polypeptides like irisin can signal through integrins is poorly understood. Using mass spectrometry and cryo-EM, we demonstrate that the extracellular heat-shock protein 90α (eHsp90α) is secreted by muscle with exercise and acts, without involving its chaperone activity, as a required cofactor that “opens” the integrin αVβ5 structure to allow for high affinity irisin binding and signaling through an eHsp90α/αV/β5 complex. The extracellular Hsp90α-mediated integrin activation is independent of any of the known intracellular activation mechanisms. By including hydrogen/deuterium exchange data, we generate and experimentally validate a 2.98 Å RMSD irisin/αVβ5 complex docking model. Irisin binds very tightly to an alternative interface on αVβ5 distinct from that involved in its interaction with known ligands. These data together elucidate a non-canonical mechanism by which a small polypeptide hormone like irisin can function through integrins.

B477/P2152

Compartment-Specific Activation of Proton-Sensor GPR65 is Uncoupled from Receptor Trafficking

L. M. Morales-Rodriguez, M. Puthenveedu; Pharmacology, University of Michigan, Ann Arbor, MI.

Proton-sensing G protein-coupled receptors (GPCRs) allow cells to sense and respond to changes in physiological pH. This family of GPCRs are unique in that, upon internalization and trafficking to acidic intracellular compartments, they are exposed to environments that are more likely to activate the receptor. However, little is known about whether and how proton-sensing receptors signal from intracellular compartments, and how proton-sensing GPCR signaling is regulated. Here we use proton-sensing receptor GPR65 as a prototype to study how receptor location at intracellular compartments influences proton-sensing receptor signaling. We use live imaging of GPR65-expressing cells to show that GPR65 internalizes from the plasma membrane and localizes to early and late endosomes at both neutral and acidic pH. Further, GPR65 internalization is required for a full cAMP response after exposure to acidic extracellular pH. Together, our findings show that GPR65 dynamically traffics to and signals

from multiple cellular compartments. Importantly, our results suggest that, unlike for most known GPCRs, activation of GPR65 is uncoupled from receptor trafficking.

B478/P2153

The Disulfide Bonds in the C-terminal Domains of the Insulin Receptor Regulate Insulin-mediated Receptor Endocytosis

S. H. Park, E. Choi; Department of Pathology and Cell Biology, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY.

Insulin receptor (IR) endocytosis plays a key role in insulin homeostasis. IR, a subfamily of receptor tyrosine kinases, is a dimer consisting of two protomers connected by disulfide bonds. The IR has an inverted V-shape in the absence of insulin. Upon insulin binding, IR undergoes a large conformational change and forms a T-shape which allows the intracellular tyrosine kinase domains of each protomer to get closer. The insulin activated IR triggers activation of its downstream pathways and initiates insulin internalization through the IR endocytosis. Disulfide bonds of the cysteine triplet at the C-terminus of the a-subunit of IR (a-CT), connect two IR protomers. As insulin binding to IR induces a large distance relocation of the a-CT, certain structural flexibility of the a-CT is required for the active IR conformation and IR signaling. Here, we examined how altering the disulfide bonds to break the link affects the IR endocytosis. We generated stable cell lines of HepG2 stably expressing IR-GFP wild-type (WT) and a mutant expressing IR with a shorter linker by deleting five amino acids from the linker (IR-del5). The levels of ectopically expressed IR-GFP WT and mutants were higher than that of the endogenous IR. Then we observed IR internalization across the time point after insulin treatments. Cells were fixed and the amount IR on the plasma membrane was analyzed through immunofluorescence assay. As expected, 100 nM of insulin treatment induces IR-GFP WT internalization. However, HepG2 cells expressing IR-del5 failed to carry out insulin-mediated endocytosis in comparison to IR-WT, likely due to the lack of structural flexibility. This result further suggests that the structural flexibility of disulfide linked a-CTs is a key factor for the conformational transformation and activation of downstream pathways.

B479/P2154

Gs-independent Activation of AC9 by the CAP1-Rap1 Complex in Mammalian Cells

X. Zhang[1], **D. Altschuler**[1], A. Pizzoni[1], C. Qi[2], V. Korkhov[2]; [1]University of Pittsburgh, Pittsburgh, PA, [2]Institute of Molecular Biology and Biophysics, Paul Scherrer Institute, Zurich, SWITZERLAND.

Gs-coupled GPCRs (GsPCRs) are involved in many biological functions due to their role on cAMP production. While cAMP was classically considered to originate solely from the plasma membrane, recent results demonstrated that GsPCRs can sustain cAMP signaling from an internalization-dependent endocytic compartment. However, the molecular mechanisms involved in sustained signaling are still unknown. Cyclase-Associated Protein 1 (CAP1), highly conserved in evolution, was originally isolated in yeasts as a bifunctional protein required for Ras-mediated cyclase activation and F-actin dynamics regulation. However, its adenylyl cyclase (AC)-associated function was thought to be lost in mammals. We have reported that mammalian CAP1, via its C-terminal domain interacts with Rap1 in a geranylgeranyl-specific manner. Moreover, CAP1 in complex with Rap1 binds and activates mammalian cyclase *in vitro* and modulates cAMP dynamics in mammalian cells. We will discuss new data showing that only AC9, a Golgi/TGN localized AC isoform, manifested CAP1-Rap1 sensitivity. Utilizing purified reagents, we showed that while CAP1's N-terminal coiled-coil domain (CC) interacts with AC9's C1a loop,

Rap1, like GαS-GTP, binds AC9's C2 catalytic loop and activates AC9 in a GTP and effector domain-dependent manner. Moreover, Rap1-GTP competes with GαS-GTP for AC9 binding and, like GαS, Rap1-GTP communicates allosterically to the forskolin binding site. Furthermore, CAP1-Rap1 action on AC9 can be recapitulated in GαS-deficient cells. Our experimental results are consistent with a model in which CAP1-Rap1 wraps around the C1-C2 domains of AC9; the CC domain of CAP1 binds AC9-C1 and Rap1-GTP interacts with AC9-C2, leading to a GαS-independent AC activation. Thus, like CAP/srv2-Ras2 in yeast, CAP1-Rap1 plays a role in cAMP dynamics in mammalian cells via AC9 activation. Since Rap1 is a bona fide downstream effector of cAMP, we propose that AC9-CAP1-Rap1 represents a new positive feedback loop working as a regulatory unit and a signal amplifier responsible for the intracellular GsPCR's sustained signaling.

B480/P2155

Investigating the relation between G protein regulation and spermathecal contraction

M. Raisa Amin, E. J. Cram; Biology, Northeastern University, Boston, MA.

Cell contractility is vital for the maintenance and regulation of several biological systems, including the circulatory, respiratory, and digestive systems, etc. To study cell contraction, we use the C. elegans spermatheca, a contractile tube composed of smooth muscle-like cells, that stores sperm and is the site of oocyte fertilization. Regulated spermathecal contraction is needed to push the embryo into the uterus. Spermathecal cell contraction is regulated by actin and myosin, and is activated, in part, by the phospholipase, PLC-1. Previous studies have shown that the heterotrimeric G-protein alpha subunit, GSA-1/Gαs, and protein kinase KIN-1/PKA-C are involved in ovulation and Ca2+ release. Hence, we conducted a candidate RNAi screen on genes implicated in smooth muscle disorders; to identify candidates with defects in oocyte transit through the spermatheca. We identified several candidates that increased oocyte trapping in the spermatheca, including ftt-2. Interestingly, some of these genes may be involved in G protein regulation. Our preliminary results suggest that the study of spermathecal contractility may reveal conserved role of genes in human smooth muscle disorders.

B481/P2156

Control of skin stem cell homeostasis by G-alpha-s signaling

K. Lund, R. Iglesias-Bartolome; Laboratory of Cellular and Molecular Biology, National Cancer Institute, NIH, Bethesda, MD.

G-alpha-s (Gs) is a heterotrimeric G protein activated downstream of G-protein-coupled receptors (GPCRs) and is involved in numerous physiological and pathological processes. Gs signals by increasing cyclic AMP (cAMP) levels, activating the kinase PKA and the exchange factor EPAC. Previous work in our lab has shown that the knockout of Gs in mouse skin keratinocytes leads to rapid stem cell expansion by the inactivation of PKA. However, Gs-activating and cAMP-inducing agents are commonly used to increase keratinocyte proliferation in cell culture, and Gs activation can lead to hair follicle stem cell proliferation in the skin. This project aims to understand how specific pathway components contribute to this dual role of Gs signaling in keratinocytes and, ultimately, identify targets to modulate skin stem cell activity.

We first sought to identify the pathways that Gs uses to increase keratinocyte proliferation in isolated keratinocytes. As previously reported, the GPCR-Gs signaling activator epinephrine increased keratinocyte proliferation in 2D and 3D organotypic cultures. On the other hand, downregulation of Gs

by small interference RNA (siRNA) resulted in reduced keratinocyte proliferation. This reduction in proliferation could be partially rescued by the cAMP-inducing agent forskolin, indicating that Gs and cAMP are necessary for keratinocyte proliferation in vitro.
To investigate if PKA mediates the role of Gs, we activated this pathway by expressing a constitutive-active mutant form of PKA. As a complementary experiment, we used a PKA inhibitor peptide (PKI) to evaluate the effects of reduced PKA activity. Remarkably, no difference in proliferation or 3D organotypic culture thickness was observed in either overactive or inhibited PKA conditions.
Since PKA mediates the effect of Gs inactivation in vivo, these results indicate that Gs can function through different mechanisms to regulate keratinocyte proliferation. We are currently in the process of characterizing the pathway downstream of Gs that leads to keratinocyte proliferation. Interestingly, we found that EPAC knockdown does not affect keratinocyte proliferation, revealing that a non-canonical cAMP-regulated event might mediate the effect of Gs.

B482/P2157

Hyperactivation of Insulin-like growth factor signaling in Adipose-derived stem cells promotes early onset of metabolic syndrome in prenatally arsenic exposed mice

K. Koshta[1,2], A. Chauhan[1,2], V. Srivastava[1,2]; [1]Developmental Toxicology Division, CSIR- Indian Institute of Toxicology Research, Lucknow, INDIA, [2]Academy of Scientific & Innovative Research, Ghaziabad, INDIA.

Metabolic Syndrome (MetS) is the cluster of multiple pathological conditions including glucose intolerance, obesity, increased serum triglycerides, reduced HDL-C and hypertension which increases the risk for cardiovascular diseases, type 2 diabetes and various cancerous outcomes. Adipose dysfunction is one of the mechanisms for progression of MetS, particularly upon exposure to endocrine disrupting chemicals such as arsenic. In our animal experiments, gestational exposure to arsenic (0.04mg/kg) lead to early onset of metabolic syndrome in offspring which was associated with adipose dysfunction. The role of arsenic in gestational programming of adipose-derived stem cells (AdSCs) and its contribution to MetS needs to be assessed. We isolated the AdSCs from epididymal white adipose tissue (EWAT) of male offspring to determine the effects of gestational arsenic conditioning on their proliferation and differentiation potential. The functional assays such as insulin stimulated glucose uptake (ISGU) and adipokines secretion were evaluated after differentiation and underlying cellular mechanisms were identified. We observed accelerated proliferation and differentiation potential in arsenic exposed AdSCs. The expression of adipogenic markers such as Perilipin, C/EBPα, PPARγ and Stearoyl-CoA Desaturase 1 (SCD1) were significantly increased. Excess lipid accumulation, verified through lipophilic Nile red staining, resulted in adipocyte hypertrophy. Several pro-inflammatory adipokines were elevated in arsenic exposed AdSCs including Leptin and TNF-α along with Resistin which mediates insulin resistance. Reduced ISGU suggested impaired insulin response in arsenic exposed AdSCs. Insulin-like growth factor 1 (IGF1) was found to be highly expressed in arsenic exposed AdSCs. It was accompanied by hyperactivation of its receptor-IGF1R through phosphorylation and enhanced lipid accumulation. The levels of downstream phospho-Akt & ERK1/2 were elevated leading to accelerated proliferation and differentiation. Our findings suggest that prenatal arsenic exposure results in enhanced adipogenic potential of AdSCs leading to adipocyte hypertrophy to accommodate excess lipid. Hyperactivation of IGF1-MAPK signaling cascade is found to be primarily responsible for this accelerated adipogenesis. Adipocytes trigger insulin resistance as a defensive conduct to prevent further lipid deposition which could be further contributing to ectopic lipid deposition in non-adipose tissues such as kidney, liver, pancreas and muscle fibers leading to early onset of metabolic syndrome.

B483/P2158

FGFR mediates TGFβR signaling to switch fibroblast differentiation between adipocytes and myofibroblasts

T. Hong[1], T. Lu[1], E. Yen[1], F. Tsai[1,2]; [1]Department of Pharmacology, National Taiwan University College of Medicine, Taipei, TAIWAN, [2]Department of Internal Medicine, National Taiwan University Hospital, Taipei, TAIWAN.

Thyroid eye disease (TED) is a severe orbitopathy that frequently occurs in patients with Grave's disease. TED patients suffer from accumulation of adipose and fibrous tissue within the orbital cavity, indicating aberrant activities of growth hormone signaling. Fibroblast growth factors (FGF) are a growth hormone that plays a crucial role in regulating fibroblast proliferation and differentiation into adipocytes. Our recent work revealed that serum levels of FGF1 and FGF2 were elevated in patients with TED, and that FGF1 transformed orbital fibroblasts into pre-adipocytes while FGFR inhibitors transformed them into myofibroblasts. Since FGFR signaling regulates TGFβR signaling through microRNAs let-7 and miR-20a, we examined if FGF effects on orbital fibroblasts were also through TGFβR modulation. Using immunoblots, we confirmed that TGFβ increased the expression of α-SMA, the marker of myofibroblast activation and that FGF and FGFR inhibitors reduced and increased α-SMA, respectively, compatible with our previous results of FGF effects on orbital fibroblasts. Interestingly, TGFβ inhibition blocked α-SMA, supporting the pivotal role of TGFβR on FGFR-mediated α-SMA. Surprisingly, immunoblots showed that FGFR inhibitors enhanced TGFβ effects on α-SMA, but knockdown of FGFR inhibitors enhanced TGFβ effects on α-SMA, implying the complex nature of FGFR signaling on TGFβR modulation. We are currently elucidating their underlying mechanisms, with the ultimate goal to develop valid FGFR- TGFβ-based therapy for TED.

B484/P2159

The Role and Regulation of mTORC2 in Lung Cancer Cell Migration

A. N. Werner, A. I. Kumar, P. G. Charest; University of Arizona, Tucson, AZ.

The mechanistic Target of Rapamycin Complex 2 (mTORC2) is a signaling complex playing a key role in promoting cell migration and has been implicated in the migration of cancer cells. Cancer cell migration is a key step in the metastasis of cancer, which is responsible for 90% of cancer-related deaths. However, the regulation and role of mTORC2 signaling in cancer cell migration is incompletely understood. Recent evidence suggest that the Ras oncogene promotes mTORC2 activation in cancer. Here, we investigated the role of Ras and other signaling pathways in regulating mTORC2 activation and function in the migration of Non-Small Cell Lung Cancer (NSCLC) cells, using A549 cells as a model. We observed that activation of the chemokine receptor, CXCR4, and growth factor receptor, EGFR, activate mTORC2, both individually and in a cooperative manner, in a way that suggests receptor crosstalk. We also find that activation of CXCR4 and EGFR lead to cell migration that is mediated by mTORC2. Interestingly, we observed that increased mTORC2 activation by CXCR4 and EGFR does not increase cell migration. In addition, we find that Ras is not involved in mTORC2-mediated cell migration in A549 cells. These cells express an oncogenic constitutively activating mutation in the K-Ras isoform, which we also found to not play a role in mTORC2-mediated cell migration in A549 cells. Instead, our results suggest a signaling pathway that utilizes PI3K in mTORC2 activation by both CXCR4 and EGFR. Current work investigates the subcellular location of mTORC2 activation by EGFR and CXCR4, as well as other potential

players in the involved signaling pathways. A better understanding of the signaling pathways mediating lung cancer cell migration could lead to the identification of potential targets to prevent the metastatic spread of lung tumors.

B485/P2160

D-Galactose induces Toll-like Receptor-2 gene expression and Mitochondrial dysfunction in Neonate Skin Fibroblast Cultures

G. Dhaunsi, M. Al-Essa; Kuwait University, Kuwait, KUWAIT.

Objective: Galactosemia is an autosomal recessive disorder with a wide range of clinical abnormalities ranging from liver cirrhosis, cataracts, neurological defects and an increased risk of infections, and its underlying pathogenic mechanisms are still being investigated. Mitochondrial energy production is essential for cell survival and Toll-like receptor-2 (TLR-2) expression is linked with apoptotic cell death, however their role in pathogenesis of Classical Galactosemia has remained unexplored. This ex vivo study was carried out to examine mitochondrial β-oxidation of fatty acids and gene expression of Toll-like receptor-2 under conditions of hyper-galactosemia and/ high glucose. *Methods*: Fibroblasts were grown from post-circumcision foreskin of 3-8 days old healthy neonates and cultured to confluency in RPMI growth medium. Confluent cell cultures were treated with varying concentrations (0-20mM) of galactose (Gal) and/or high glucose (25 mM. HG) for 24 -72 hrs. Following treatment of cell cultures with experimental agents, cells were harvested and homogenized in 50mM Tris-HCl buffer, pH 7.4 for assay of palmitoyl-CoA oxidase and cytochrome c oxidase enzyme activities. Gene expression of Carnitine palmitoyl-CoA transferase (CPT-I) and TLR-2 was examined using RT-PCR and Western blot analysis. *Results:* Treatment of cell cultures with high glucose (HG) and 10 -20 mM D-Galactose (D-Gal) for 72 hrs. significantly ($p < 0.05$) decreased the palmitoyl CoA oxidase activity when compared with that in cells cultured under normal glycemic conditions (control). Both HG and D-Gal markedly decreased the gene expression of CPT-1 after 24 hrs. of treatment, however the reduction in levels of CPT-1 was statistically significant ($p < 0.01$) in cells co-treated with HG and D-Gal when compared with control cells or those treated with HG or D-Gal alone. D-Gal significantly decreased ($p < 0.01$) the Cytochrome c oxidase activity whereas HG did not have any marked effect of its own but further enhanced the D-Gal-induced reduction in cytochrome c oxidation. RT-PCR and Western blot analyses revealed significantly increased ($p <0.01$) gene expression of TLR-2 in cells treated with 10 -20 mM D-Gal which was found to be markedly enhanced by co-treatment with HG. *Conclusion*: This study demonstrates that D-Gal treatment impairs activities of key tow mitochondrial enzymes, CPT-1 and Palmitoyl CoA oxidase, that are involved in β-oxidation of fatty acids and induces gene expression of TLR-2. Co-treatment with HG enhanced the D-Gal-induced mitochondrial dysfunction and TLR-2 gene expression suggesting that high glucose potentially worsens pathogenic events of Galactosemia.

B486/P2161

Mechanism of the Class A GPCR Pathway Underlying Osteogenic Differentiation of Mesenchymal Stromal Cells and Hard Tissue Regeneration

S. Park, E. Choi, J. Jung, J. Kim; School of Dentistry, Seoul National University, Seoul, KOREA, REPUBLIC OF.

Mesenchymal stromal cells (MSCs) have high proliferation potential with multi-lineage differentiation capacity and is a key cellular component for regeneration of hard tissue such as teeth and bone.

Previous studies have attempted to apply MSCs to induce hard tissue regeneration, but the trial remains a challenge. G protein-coupled receptor (GPCR), a membrane protein that play an important role in various pathological and physiological controls, is regarded as the main targets in the pharmaceutical market. However, the development of a drug targeting GPCRs has not been established for regeneration of damaged hard tissues. Here we present a targeting strategy of class A GPCRs for hard tissue regeneration by promoting the differentiation of endogenous MSCs into osteoblastic and odontogenic progenitor cells. Through *in vitro* screening designed to target class A GPCRs, we discovered six target receptors (LPAR1, F2R, F2RL1, F2RL2, S1PR1, ADORA2A) and candidate drugs with potent effect on biomineralization. When the discovered candidates were treated to cultured human MSCs, alizarin red and osteogenic marker gene responses were induced. Through bulk RNA sequencing, transcriptional changes related to phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT) signaling pathways were identified in common. In particular, the expression of p53 protein, a downstream signaling component of the PI3K-AKT pathway, was increased upon treatment with core candidate drugs. In the regenerative process, p53 acts as a key transcription activator for genes that modulate osteogenic differentiation. Moreover, the therapeutic potential of class A GPCR-targeting drugs was demonstrated in tooth pulpotomy and calvaria defect models. In this study, we discovered a key candidate group of class A GPCRs and targeted inhibitors associated with regeneration of hard tissue. Collectively, the findings of this study provide a theoretical background for a new regenerative strategy of damaged hard tissues.

B487/P2162

Phase Separation of Epidermal Growth Factor Receptor (EGFR) Directs Spatiotemporal Adaptor Protein Organization

M. Ullo, L. Case; Biology, Massachusetts Institute of Technology, Cambridge, MA.

Receptors at the plasma membrane receive and transmit environmental cues to guide a cell's behavior. The Epidermal Growth Factor Receptor (EGFR) family of receptors are critical in regulating cellular growth and survival by coordinating localization of cytosolic adaptor and signaling molecules to direct activation of MAPK and PI3K pathways. Unfortunately, EGFR and its family members are commonly hyperactivated in several cancers including non-small-cell lung cancer and breast carcinoma. This leads to uncontrolled receptor clustering and dysregulated protein recruitment to hyper-activate cell proliferation and survival—necessary processes for tumorigenesis. However, the spatiotemporal recruitment and organization of downstream adaptor molecules to these EGFR signaling clusters during both physiological and oncogenic signaling remains poorly understood. Recently, it has been demonstrated that many transmembrane receptors, including EGFR, can undergo phase separation to form micron-sized biomolecular condensates on the plasma membrane. These condensates concentrate adaptor proteins and signaling molecules and can alter signaling dynamics. Whether phase separation influences signaling downstream of EGFR-family receptor condensate formation remains unclear. Therefore, we hypothesize unique combinations of EGFRs and adaptor proteins GAB1 and GRB2 yield distinct variation in phase separated condensate kinetics and characteristics. To systematically determine how specific adaptor proteins influence EGFR condensate kinetics and characteristics, we use *in vitro* biochemical reconstitution and TIRF microscopy. We purified and fluorescently labelled recombinant proteins. We attached the intracellular domain of EGFR to a supported lipid bilayer and image with TIRF microscopy and induced clustering and phase separation by adding cytosolic adaptor proteins. We predict that adding different concentrations of GRB2 and GAB1 can induce EGRF clustering and phase separation. Experiments are ongoing to quantify the kinetics of condensate formation, the

material properties of condensates, and the composition of condensates. From these experiments, we will determine if EGFR phase separation, and potentially downstream signaling, can be modulated by the recruitment of different adaptor proteins.

B488/P2163

Structural insights into extracellular TAM receptor tyrosine kinase organization

C. Starbird, K. Ferguson; Yale University, New Haven, CT, CT.

TAM receptors are a clinically important and mechanistically under-studied receptor tyrosine kinase (RTK) subfamily. These receptors have a vital role in maintaining cellular homeostasis through the clearance of apoptotic cells and control of inflammatory and immune responses. Linked to their important regulatory roles, dysregulation of TAM receptors is implicated in numerous disease states including cardiovascular disease, hereditary blindness, infertility, autoimmune disorders, chronic inflammation and cancer. In addition, TAM receptor mediated signaling pathways can be hijacked by viruses to gain entry into host cells. While there is growing interest in TAM receptors as therapeutic targets, their multiple roles in homeostatic processes create challenges for developing therapeutic strategies. Understanding TAM receptor activation mechanisms is important for further investigation of the potential development of targeted therapies. While these receptors are commonly believed to be activated through classical receptor-induced dimerization, there are no current studies that confirm this activation mechanism applies for TAM receptors and preliminary work suggests alternative mechanisms may be possible. My work utilizes a combination of structural, biophysical and biochemical approaches to investigate TAM oligomerization and cross-talk with other receptors, and here I will present early structural analysis of TAM receptors and intriguing similarities to cell-cell adhesion molecules and receptors that are involved in higher order oligomerization or clustering. Furthermore, my presentation will introduce some of the future goals of my new lab, which officially opens in February of 2023.

B489/P2164

Expression of non-receptor tyrosine kinase RNAs in the mature sea star egg.

D. A. Alcázar[1], A. Whetzel[2], D. J. Carroll[2,3]; [1]College of Graduate Studies, Midwestern University, Glendale, AZ, [2]Department of Biochemistry and Molecular Genetics, Midwestern University, Glendale, AZ, [3]Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ.

In Patiria miniata, the increase of calcium at fertilization activates the egg resulting in the permanent block to polyspermy and the re-initiation of the cell cycle. In some animals, Src-family kinases (SFK3, SFK1) play a role in this signal transduction pathway by activating PLCγ via tyrosine phosphorylation. Since non-receptor tyrosine kinases (NRTK) play a major role in multiple signal transduction pathways that are regulated by tyrosine phosphorylation events, the objective of this project is to identify other NRTK mRNAs that are expressed in the sea star egg by examining orthologs of known NRTKs in the P. miniata mature egg transcriptome (NCBI BioProject PRJNA398668). Common NRTK families include Src, Csk, Syk, Tec, Jak, Fak, Abl, Fes, Frk, and Ack. The human orthologues for each NRTK were used as target sequences to identify sea star RNAs encoding putative NRTK proteins utilizing the National Center for Biotechnology Information (NCBI) tblastn software. The putative proteins were predicted using ORFinder followed by blastp against the NCBI non-redundant protein database. Sea star orthologues were identified in 9 out of 10 NRTK families queried, with varying degrees of similarity. The percent identity between putative sea star protein and human orthologue can be as high as 68.52% as seen in

Alb1 and Abl2, which are known to play a key role in cellular growth and survival. There were multiple NRTK open-reading frames discovered to share 100% identity with the putative sea star proteins searched, such as Src and Abl1/2. Other NRTK open-reading frames were seen to maintain a high percentage of identity as well. No orthologue was identified for the vertebrate Frk protein. Expression of each sea star RNAs will be confirmed using RT-PCR on newly isolated sea star egg mRNA. Future experiments will be focused on determining if any of these NRTK proteins play a role at fertilization, including confirming the expression of these NRTK proteins in the mature sea star egg via western blotting.

Signaling from the PM/cytoplasm to the Nucleus

B490/P2165

Activating the cAMP pathway promotes NF-κB and TNF-α expression in LPS-treated Schwann Cells

C. Henry, P. Kimmel, M. Wilcox, A. Asirvatham; Misericordia University, Dallas, PA.

Schwann cells have been found to play a critical role in neuron repair following peripheral nerve injury. During nerve injury, the myelin sheath becomes damaged, stimulating Schwann cells to secrete cytokines and initiate an inflammatory response. This recruits immune cells to the site of injury so that the myelin debris can be cleared. Neuronal growth is then facilitated by heregulin and an unknown growth factor that stimulates the cAMP pathway. Although it is clear Schwann cells play a role in nerve repair, there is still yet to be known regarding the exact mechanisms by which they do so. One potential mechanism is via the nuclear factor kappa B (NF-κB) pathway. Lipopolysaccharide (LPS), a cell wall immunostimulatory component of Gram-negative bacteria, can be used to activate the NF-κB pathway and stimulate the production of inflammatory mediators, like tumor necrosis factor alpha (TNF-α). Preliminary studies on Schwann cells treated with different concentrations of LPS at various time points revealed that lower doses promoted cell growth compared to higher concentrations. To further explore the effects of LPS on cell proliferation, the role of NF-κB and TNF-α secretion were examined. It was hypothesized that cells treated with LPS and growth factors will express less NF-κB and TNF-α than cells treated with LPS only. Cells from the immortalized S16 cell line were treated with or without 1 µg/mL of LPS for 3 hours without growth factors (control media, N_2), 12.5 ng/mL heregulin (H), 2 µM forskolin (F), or H+F. Using immunoblotting, NF-κB expression, as measured by densitometry analysis and expressed as a percent control, was higher in LPS-treated cells than in unstimulated cells. Surprisingly, NF-κB expression was higher in LPS-treated cells with H, F, or H+F (1500%, 1243%, and 1557%, respectively) than without growth factors (526%). The increase in NF-κB expression for the LPS-treated cells was accompanied by an increase in TNF-α expression. As expected, TNF-α expression was highest in LPS-treated cells without growth factors (444%) and lowest in untreated cells with F or H+F (34% and 70%, respectively). In summary, it appears as though 1 ug/mL of LPS and growth factors may act synergistically to upregulate NF-κB while downregulating TNF-α expression. These findings suggest that, during nerve injury, there may be some crosstalk between the cAMP and NF-κB pathways in Schwann cells. Therefore, a better understanding of these pathways may reveal a therapeutic target for the treatment of nerve injury and inflammation.

B491/P2166

Creatine treatment upregulates AKAP149 and creatine kinase expression in S16 Schwann cells.

P. Kimmel, C. Henry, M. Wilcox, A. Asirvatham; Misericordia University, Dallas, PA.

Creatine is an important component of the high-energy phosphate transfer, and is primarily involved in cellular metabolism to regulate levels of ATP. This high-energy phosphate transfer from creatine is facilitated by creatine kinase in the mitochondria. The functions of creatine supplementation are well studied in the muscular and skeletal system; however, the role of creatine in stimulating Schwann cell growth is relatively unknown. Schwann cell growth in vitro is facilitated by heregulin, a neuronal growth factor, and forskolin, a pharmacological agent that activates the cyclic AMP/protein kinase A (PKA) pathway. Anchoring of the cAMP dependent PKA in the mitochondria is accomplished by AKAP149, a scaffolding protein that coordinates multiple signaling proteins. Preliminary studies have shown that incubation of Schwann cells with creatine at a concentration of 2µM, or 20µM stimulated significant proliferation. To explore the expression of AKAP149 and creatine kinase in Schwann cells, it was hypothesized that these proteins would be up-regulated when cells are treated with increasing concentrations of creatine. Furthermore, the expression of AKAP149 and creatine kinase will be increased when the cAMP pathway is stimulated. Immortalized S16 Schwann cells were treated with no growth factors (control media N2), heregulin (12.5ng/mL), forskolin (2µM), heregulin plus forskolin (H+F) and various doses of creatine at 2 or 20 µM, for 1, 3, or 6 hours. Densitometry data generated from Western blots revealed that 2µM creatine treatment for 6 hours combined with forskolin increased both AKAP 149 (214.3%) and CRK (716.9%) expression in comparison to control and other treatments. Furthermore, cells treated with forskolin and 20µM creatine exhibited a synergistic expression of CRK (5448.7%) in contrast to control (143.2%) and mitogen-stimulated cells (H: 89.7%, H+F: 15.4%). Surprisingly, cells stimulated with 20µM creatine under control conditions revealed an increase in AKAP149 expression (278.29%) in comparison to unstimulated cells (H: 30.67%, H+F: 71.77%). These results suggest that creatine treatment augments AKAP149 and CRK expression in the process of Schwann cell growth. Furthermore, AKAP149 as a scaffolding protein may play a role in coordinating creatine kinase with other signaling proteins to regulate cellular concentrations of ATP.

B492/P2167

Creatine attenuates the effect of LPS treatment in S16 Schwann cells

M. Wilcox, C. Henry, P. Kimmel, A. Asirvatham; Misericordia University, Dallas, PA.

Schwann cells are found in the peripheral nervous system and form the myelin sheath that wraps around axons to protect the nerves. Schwann cell growth is regulated by heregulin, a neuronal growth factor, and an unknown mitogen that activates the cAMP pathway. When neurons are injured, inflammation ensues and Schwann cells change their phenotype to begin dividing, which allows remyelination of the neuron. However, the pathways regulating remyelination are not well known. In vitro, the exposure of Schwann cells to lipopolysaccharide (LPS), a bacterial endotoxin, simulates an inflammatory environment. Preliminary observations have shown that creatine, a compound involved in storage of cellular energy, stimulates Schwann cell proliferation, whereas LPS at high doses inhibits cell growth. Based on these observations, it was hypothesized that the addition of creatine to Schwann cells treated with LPS will have higher rates of growth in comparison to cells incubated without creatine. Schwann cells were incubated at a density of 35,000 cells/well in a 96 well plate and treated with control media (N2), heregulin (H), forskolin (F), a pharmacological activator of cAMP, or H+F. The

mitogen-stimulated cells were then incubated with creatine at a concentration of either 2µM or 20µM and LPS at a dose of 500ng, 1µg, or 10µg for 6 hours. Using the MTT viability assay, cell proliferation was calculated by measuring the optical density and then expressed as percent control. The addition of 20µM creatine to the mitogen-stimulated cells elicited an increased cell growth for H (222.8187±44.3188%), and H+F (243.2414±27.4556%) but not F (232.3024±26.7549%) when compared to cells incubated without creatine (H: 116.5053±11.5595%, H+F: 143.8382±25.9536%, F: 252.7071±101.4468%,). Meanwhile, Schwann cells treated with 10µg LPS and growth factors revealed a decrease in proliferation (H: 108.7881±4.9824%, F: 125.3875±3.3548%, H+F: 115.3533±11.9785%). However, addition of 20µM creatine to LPS-treated cells revealed an increase in proliferation for H (256.5137±47.6774%), F (151.7791±20.4877%) and H+F (213.8792±39.8517%). Based on these observations, it was concluded that a dose of 20µM creatine stimulated cell proliferation, treatment with LPS caused a dose-dependent decrease in cell growth, and Schwann cell recovery caused by the addition of creatine after LPS treatment was dependent on the concentration of creatine. Overall, creatine appears to help Schwann cell viability by providing sufficient energy for cells to recover after LPS treatment.

B493/P2168

Protein Interactions Identification of Myocardin-Related Transcription Factors A and B Mechanosensitive Transcriptional Cofactors in Prolonged Cultured Hepatocytes

A. Jiménez-Escobar, G. Vázquez-Victorio; Micro and Nanotechnology Laboratory, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City, MEXICO.

The liver is a fundamental organ for the human homeostasis due to the continuous performance of some vital processes mainly made by hepatocytes. They have a unique polarity generated by intracellular lumen (canaliculi) and sinusoidal extracellular matrix (ECM) interactions. Intercellular forces and ECM properties change in some pathologies such as fibrosis where the stiffness and collagen I deposits increase substantially. Stiffness and loss of polarity are associated with the loss of the hepatocytes phenotype in vitro together with alterations in their metabolic functions, as a consequence. Myocardin-related transcription factors a and b (MRTF-A/B) are transcriptional cofactors involved in cytoskeleton dynamics because of the downstream activity of their target genes. They are also controlled by the cytoskeleton as well. Specifically, they are negatively regulated by the actin polymerization as a result of their RPEL domain interaction with G-actin. Also, these cofactors have other protein domains that allow them to interact with other proteins like ERK and FHL2, which affect MRTF-A/B activity positively and negatively, respectively.

It has been shown that MRTF-A/B are physiologically active in the liver and that they are also involved in liver fibrosis, mostly due to their role in hepatic stellate cells activation. However, it is unknown their role in hepatocytes as well as its regulation by their different interactions. Therefore, to elucidate this problem, the aim of this work is to detect protein interactions of MRTF/A-B of primary fresh hepatocytes and on long-term culture.

We have found a noticeable MRTF-A/B upregulation on stiff and long term cultured hepatocytes. There is a nuclear accumulation of MRTF-A/B in primary hepatocytes in a time dependent manner, however MRTF-B was slightly more cytoplasmic retained even at 72 h of culture. Similarly, stiffness also caused the increase in the MRTF-A/B nuclear accumulation, and it was more accentuated for MRTF-A even. It probably reflects differences in their regulation, as a consequence of modifications in their protein interactions. To clarify this, immunoprecipitations of MRTF-A/B were performed in order to characterize

known interactions, like SRF and actin, in order to detect novel interactions with MS/MS. The results obtained in this project highlights the activity of MRTF-A/B in hepatocytes and points up the relevance of searching the regulatory mechanisms of MRTF-A/B in hepatocytes.

B494/P2169

ER stress promotes REDD1-dependent NF-kB activation and increased inflammatory cytokine expression in the heart

M. K. Gonzalez Aguiar, S. A. Stevens, S. Sunilkumar, A. L. Toro, M. D. Dennis; Penn State College of Medicine, Hershey, PA.

Heart failure is the leading cause of mortality and morbidity in patients with diabetes and obesity. Endoplasmic reticulum (ER) stress and inflammation are hallmarks of myocardial impairment, and interplay between these stress response pathways contributes to disease progression. Here, we investigated a role for the stress response protein regulated in development and DNA damage response 1 (REDD1, also known as DDIT4/RTP801) as a molecular link between ER stress and pro-inflammatory signaling in cardiomyocytes. In the hearts of obese mice fed a pro-diabetogenic diet (42% kcal fat, 34% sucrose by weight), REDD1 mRNA and protein expression was increased as compared to littermates fed a control chow. In coordination with the increase in REDD1 expression in heart of obese mice, markers of ER stress and expression of the pro-inflammatory cytokine IL-1β were upregulated. When human AC16 cardiomyocytes were exposed to thapsagargin to promote ER stress, expression of REDD1 and IL-1β were increased. ER stress was also sufficient to enhance activity of the transcription factor NF-κB in wild-type HEK293 cell cultures, whereas REDD1 deletion prevented the effect. REDD1 deletion also reduced IL-1β expression and macrophage infiltration in the hearts of obese $REDD1^{-/-}$ mice fed a pro-diabetogenic diet, as compared to $REDD1^{+/+}$mice. Overall, the results support a model wherein ER stress contributes to the development of chronic inflammatory response in cardiomyocytes by promoting REDD1-dependent NF-κB activation.

B495/P2170

GLI phase separation for Sonic Hedgehog pathway regulation

S. Row[1], D. Stewart[1], P. A. Umberger[2], S. Ogden[1]; [1]Cell and Molecular Biology, St Jude Children's Research Hospital, Memphis, TN, [2]CMB and Integrated Biomedical Sciences Program, University of Tennessee Health Science Center, Memphis, TN.

The GLI family of transcription factors (GLI1, 2, and 3) are effectors of the Hedgehog (HH) signaling pathway, which directs tissue morphogenesis and homeostasis through induction of precise transcriptional programs. Dysregulation of HH signaling can lead to developmental disorders and cancers including basal cell carcinoma, medulloblastoma, and rhabdomyosarcoma. In the absence of the HH ligand, the transcriptional effectors GLI2/3 are targeted for proteolytic cleavage into truncated repressor forms. Binding of HH to the receptor Patched (PTCH) activates the pathway by inducing PTCH internalization and degradation, thus allowing the signal transducer Smoothened (SMO) to signal to stabilize GLI2/3 in their full-length active forms. Active GLI2/3 translocate to the nucleus and initiate transcription of target genes including *Ptch* and *Gli1*, which drive negative and positive feedback loops, respectively. The E3 ubiquitin ligase substrate adaptor protein Speckle Type BTB/POZ Protein (SPOP) targets GLI2/3 in the nucleus for ubiquitination and subsequent degradation, effectively limiting the transcriptional response. Although the fundamental steps of how HH signal transduction regulates GLI2

in the cytoplasm are established, the details regarding organization and regulation of GLI2 activity in the nucleus have yet to be resolved. GLI proteins have a conserved and structured central zinc finger (ZF) motif flanked by amino- and carboxyl- terminal unstructured domains. The presence of intrinsically disordered regions (IDRs) is sufficient to drive phase transition of proteins in cells. We previously reported that GLI3 enters into SPOP-containing nuclear biomolecular assemblies with Liquid-liquid phase separation (LLPS) characteristics. Herein, we examine LLPS behavior of GLI2 and show that it demonstrates distinct assembly behaviors in the absence and presence of SPOP.

B496/P2171

New View on Information Processing with pH Dynamics Regulating Transcription Factor-DNA Binding Selectivity

K. Kisor, D. L. Barber; University of California, San Francisco, San Francisco, CA

Intracellular pH (pHi) dynamics regulates myriad cell behaviors, including proliferation, migration, cytoskeleton and adhesion remodeling, stem cell differentiation, and transformation, which makes it integral in information processing. We previously identified pH sensitive proteins mediating many of these cell behaviors with pH regulated kinase activity, protein-protein and protein-phospholipid binding. Whether pH regulates protein-nucleotide binding for established effects of pHi dynamics on gene expression, however, remains unknown. We identified at least 65 transcription factors in diverse families that have a conserved histidine, which in available structures forms hydrogen bonds with DNA nucleotides. With the ability of histidine to titrate in the cellular pH range, electrostatic principles of protein-nucleotide hydrogen bonds, and nuclear and cytoplasmic pH being similar, we predicted that transcription factors with histidine-nucleotide hydrogen bonds could have pH regulated DNA binding selectivity. All FOX family transcription factors contain a conserved histidine in their DNA-binding domain (DBD) that in available structures forms hydrogen bonds with nucleotides**.** We confirmed pH regulates DNA binding of FOXM1 and FOXC2 DBDs using fluorescence anisotropy and a canonical FkhP DNA motif for binding to a thymine, with higher affinity at pH 7 compared with 7.5. This finding is consistent with thymine being a hydrogen bond acceptor and binding a protonated histidine. We confirmed the significance of histidine with a mutant FOXC2-His122K, with lysine mimicking a protonated residue with pKa > 12, that had high affinity but pH independent binding and a mutant FOXC2-H122N recurring in cancers that had low affinity pH independent binding. We also confirmed pHi regulates FOXC2 activity in cells. Using MDA-MB-436 cancer cells with a high pHi of 7.7 and a luciferase assay we found increased transcriptional activity for an FkhP motif when pHi was lowered to 7.4 with EIPA, an inhibitor of the proton-extruding NHE1. Our data identify pH dynamics as a previously unrecognized regulator of DNA-binding selectivity for FOXM1 and FOXC2. This regulatory mode has broad significance because histidine-DNA nucleotide binding is also reported for most transcription factors in the SOX family, all members of the MITF/Myc family, and selective members of STAT and ETS families.

B497/P2172

Mechanistic study for mTORC1-dependent regulation of the lysosomal and autophagic transcription factor TFEB

Z. Cui[1], G. Napolitano[2], M. E. G. de Araujo[3], L. A. Huber[3], A. Ballabio[2,4], J. H. Hurley[1]; [1]University of California, Berkeley, Berkeley, CA, [2]Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy, [3]Medical University of Innsbruck, Innsbruck, Austria, [4]Baylor College of Medicine, Houston, TX

The transcription factor TFEB is a master regulator of lysosomal biogenesis and autophagy. The phosphorylation of TFEB by the mechanistic target of rapamycin complex 1 (mTORC1) is unique in its mTORC1 substrate recruitment mechanism, which is strictly dependent on the amino-acid-mediated activation of the RagC GAP FLCN. TFEB lacks the TOR signaling (TOS) motif responsible for the recruitment of other mTORC1 substrates. We used cryo-electron microscopy (cryo-EM) to determine the structure of TFEB as presented to mTORC1 for phosphorylation. Two full Rag-Ragulator complexes present each molecule of TFEB to the mTOR active site. One Rag-Ragulator complex is bound to Raptor in the canonical mode seen previously in the absence of TFEB. A second Rag-Ragulator complex (non-canonical) docks onto the first via a RagC GDP-dependent contact with the second Ragulator complex. The non-canonical Rag dimer binds the first helix of TFEB in a RagCGDP-dependent aspartate clamp in the cleft between the Rag G domains. Mutation of the clamp drives TFEB constitutively into the nucleus whilst having no effect on mTORC1 localization. The remainder of the 108-amino acid TFEB docking domain winds around Raptor and then back to RagA. This structure presents the phosphorylatable Ser residues of TFEB to the mTORC1 active site in a suitable geometry for their phosphorylation. The double use of RagC GDP contacts in both Rag dimers explains the strong dependence of TFEB phosphorylation on FLCN and the RagC GDP state.

B498/P2173

The ubiquitin ligase HUWE1 potentiates WNT/β-catenin signaling through a mechanism mediated by APC, AXIN1 and GSK3A/B that is independent from the control of CTNNB1 stability

J. K. McKenna, Y. Wu, P. Sonkusre, **A. M. Lebensohn**; National Cancer Institute, National Institutes of Health, Bethesda, MD

The WNT pathway is a cell signaling system involved in patterning and morphogenesis during embryonic development, and in maintenance and regeneration of adult tissues in all animals. Defects in WNT signaling lead to many severe developmental abnormalities and adult-onset diseases, including many types of cancer. The WNT/β-catenin pathway is a branch of WNT signaling mediated by the transcriptional co-activator β-catenin (CTNNB1). During WNT/β-catenin signaling, the regulated accumulation and nuclear translocation of CTNNB1 drives the expression of WNT target genes, which execute diverse cellular programs. In the absence of WNT ligands, phosphorylation of CTNNB1 by the so-called 'destruction complex' (DC), a multi-protein assembly composed of several scaffolds and kinases, targets it for ubiquitination and proteasomal degradation. Binding of WNT ligands to their co-receptors disrupts the activity of the DC, preventing further CTNNB1 degradation. CTNNB1 accumulates in the cytoplasm and is translocated into the nucleus, where it drives transcription of WNT target genes. Through comparative genetic screens in haploid human cells, we previously identified HUWE1 as a potent positive regulator of the WNT/β-catenin pathway. HUWE1 is a ubiquitin ligase that regulates dozens of cellular processes. Surprisingly, the contribution of HUWE1 to WNT/β-catenin signaling was only evident when the pathway was hyperactivated by loss of the DC kinase casein kinase 1α (CSNK1A1), but not by loss of the DC scaffold adenomatous polyposis coli (APC). These results were striking because CSNK1A1 and APC have a shared function in the DC controlling CTNNB1 protein stability, and therefore loss of either CSNK1A1 or APC would be expected to have the same effect on signaling. However, the differential contribution of HUWE1 to WNT/β-catenin signaling in cells lacking CSNK1A1 or APC suggested additional, unique functions for CSNK1A1 or APC. Genetic interaction analyses and a novel 'suppressor of suppressor' genetic screen revealed the reason for these paradoxical results: a subset of

DC components, including AXIN1, APC and glycogen synthase kinase 3α or β (GSK3A/B), but excluding CSNK1A1 and AXIN2, are required for HUWE1 to potentiate WNT/β-catenin signaling. Furthermore, HUWE1 promotes WNT/β-catenin signaling through at least two distinct mechanisms, one that increases CTNNB1 protein abundance, and another that is independent from the control of CTNNB1 stability. These results reveal a new role for AXIN1, APC and GSK3A/B in mediating WNT/β-catenin signaling through HUWE1, distinct from their established activity controlling CTNNB1 stability as part of the DC. The implications of these findings for the many cellular processes regulated by WNT/β-catenin signaling remain to be elucidated.

B499/P2174

Soluble Cyclase-mediated Nuclear cAMP Synthesis is Sufficient for Cell Proliferation

A. Pizzoni, X. Zhang, N. Naim, D. Altschuler; University of Pittsburgh, Pittsburgh, PA

G-protein coupled receptors (GPCRs) are the largest family of mammalian receptors accounting for one-third of the targets of current market drugs. Gs-coupled GPCRs that signal through cAMP, representing half of these GPCR-targeted drugs, are involved in many physiological and pathophysiological conditions. While cAMP was classically considered to originate solely from the plasma membrane (PM), this view was recently challenged by results demonstrating that GsPCRs can sustain cAMP signaling from an internalization-dependent intracellular compartment. Importantly, this second cAMP wave was strictly associated with nuclear PKA translocation, cAMP diffusion into the nucleus, and activation of nuclear transcriptional events responsible for triggering specific biological responses. We will discuss new data showing that GsPCRs trigger an internalization-dependent accumulation of nuclear cAMP unexpectedly mediated solely by local soluble adenylyl cyclase (sAC) activation, rather than cAMP diffusion from the cytosol. Both pharmacological and genetic sAC inhibition, which did not affect the cytosolic cAMP levels, completely blunted nuclear cAMP accumulation, PKA activation, and cell proliferation, while an increase in sAC nuclear expression significantly enhanced cell proliferation. Utilizing novel compartment-specific optogenetic actuators we showed that light-dependent nuclear cAMP synthesis stimulates PKA and CREB triggering cell proliferation. Our results indicate that neither PKA translocation nor cAMP diffusion, but rather nuclear sAC activation represents the rate-limiting step for nuclear PKA activation and downstream events leading to cAMP-dependent proliferation. Thus, sAC-mediated nuclear cAMP accumulation is not only necessary but sufficient for cell proliferation. In the context of these and new unpublished data, we will discuss a new three-wave model of cAMP signaling. Our model proposes that after GPCR-mediated adenylyl cyclase (AC) activation at the PM (first cAMP wave) and the internalization of the signaling complex that continues to signal from intracellular membranes (second cAMP wave), an intermediate factor is synthesized or mobilized. Unlike cytosolic cAMP, this factor can enter the nucleus and activates the local sAC which generates a third cAMP wave that controls cell proliferation.

B500/P2175

Analysis of the mechanism of filopodia-based WNT1 Transport

A. M. Villegas-Parra, L. Gallli, L. Burrus; San Francisco State Univeristy, San Francisco, CA.

Wnt gradients regulate cell proliferation and differentiation during embryonic development and adult homeostasis. Uncontrolled production and transport of Wnt proteins can disrupt the Wnt signaling pathway, particularly in cancerous cells. Though much is known about the downstream mechanism of

how Wnt signals are received, less is understood about Wnt gradient production and transport. The production of Wnt ligands requires lipid modification by the acyltransferase Porcupine (PORCN) followed by the binding of Wntless (WLS), a cargo transporter that facilitates Wnt transport from the endoplasmic reticulum to the plasma membrane. Proposed models that explain how lipid-modified Wnts are transported from the producing cell to the receiving cell include diffusion, exosomes, and filopodia. Data from our lab showed that the incubation of Wnt-producing cells with Wnt/β-catenin reporter cells induced over 100-fold more signaling than did the Wnt-conditioned media, suggesting that cell contact is critical for Wnt signaling. Our observation that co-expression of WNT1 with WLS, increases signaling activity, relocalizes WNT1 to the cell membrane, and induces new filopodia projections, further suggests that Wnts could be transported by filopodia, which then contact target cells. As IRSp53, Cdc42, and Ror2 have been implicated in filopodia formation, **I hypothesize that the inhibition of either of these proteins in cells overexpressing WNT1 and WLS will cause a reduction in Wnt signaling.** To test this hypothesis, I created and expressed IRSp53_4k, Cdc42_T17N, and DN Ror2 mutant constructs along with WNT1 and WLS in HEK293T cells. I then measured Wnt signaling activity using a TopFlash luciferase-based assay. I found that none of the inhibitory proteins significantly decreased WNT1 signaling activity. While doing these experiments, we found that WLS promotes the signaling activity of WNT1, but not its so-called functionally redundant sister, WNT3A. Following this differential result, I am assessing differences in the binding of WNT1 and WNT3A to WLS using computational and experimental methods. My findings suggest that neither IRSp53, Cdc42, nor Ror2 appears to be required for WNT1 signaling. Ongoing research includes identifying proteins involved in filopodia induction that are required for Wnt signaling and understanding potential differences in the interaction between WLS and different Wnts.

B501/P2176

Why is glucose-stimulated insulin secretion so glucose specific?

F. Firdos[1], T. Pramanik[1], A. Mittal[1,2]; [1]Indian Institute of Technology Delhi, Delhi, India, [2]Indian Institute of Technology, Delhi, Delhi, India

Inability to produce and/or secrete insulin by pancreatic β-cells results in disorders such as Type 1 diabetes in animals. Despite ~50 years of literature on Type 1 diabetes and establishment of Mouse Insulinoma 6 (MIN6) cells (~32 years ago) as an *in vitro* model system for studying glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells, a careful dissection of the roles of glucose molecules distinctly serving as nutrients and as ligands in GSIS is missing. Prevalent views relate ATP generated from glucose metabolism to GSIS; however, this logic fails in absence of GSIS-like regulation of insulin secretion by ATP from other intracellular sources. Interestingly, glucose transport (outside to inside cells) is reported as 10 to 100-fold higher than intracellular phosphorylation of glucose in pancreatic β-cells. There is a surprising absence of literature on the internalized, but non-phosphorylated, glucose. This indicates that glucose not only works as an extracellular stimulator and nutrient but also must have some specific, yet undiscovered, intracellular role(s) in modulating GSIS. In this work, we analyzed differential gene expression in MIN6 cells, exposed to low and high extracellular-glucose-concentrations (EGC), from whole transcriptome data (55467 distinct transcripts of which 14427 were identified as successfully expressed genes). Other than *Gapdh* (downregulated) and *Atp6v0a4* & *Cox20* (upregulated), expression of 154 genes involved in Glycolysis, TCA cycle, and electron transport chain were found to be unaffected by EGC - indicating that glucose consumption as a nutrient is independent of EGC in GSIS. Overall, we found 75 upregulated and 78 downregulated genes at high EGC. Of these, only 3

upregulated genes (*Atp6v0a4, Cacnb4, Kif11*) were identified to have any association with cellular secretion. ATP6V0A4 is required to acidify secretory vesicles, CACNB4 is involved in β-cell insulin secretion, and, KIF11 is a motor protein involved in vesicular transport. Identification of these 3 molecules, in the specific context of GSIS, allowed for directly testing a hypothesis of intracellular glucose molecules serving as a ligand for them. We report strong evidence for glucose binding ability of ATP6V0A4, CACNB4 and KIF11, thereby providing a possibly remarkable discovery of a direct role of intracellular glucose molecules in GSIS for the first time.

B502/P2177

Molecular mechanisms of ADAM17 regulation by iRhom2 in cell signalling

F. Lu, B. Sieber, M. Freeman; University of Oxford, Oxford, United Kingdom

The rhomboid-like protein iRhom2 is an essential regulator of ADAM17, a metalloprotease which cleaves and releases vital signalling molecules and therefore is significantly implicated in cancer and inflammatory diseases. However, the links between iRhom2 and cancer and the molecular mechanisms of ADAM17 activation by iRhom2, are unclear. Here, using a xenograft model, we have established a central role for iRhom proteins in lung tumour cell growth. I also performed a systematic characterisation of a series of cancer-causing mutations (tylosis and oesophageal cancer, TOC) in iRhom2 and found that TOC mutations in iRhom2: 1) potentiate oncogenic KRAS induced ADAM17 activity, 2) confer a growth advantage in KRAS-driven A549 lung cancer cells, and 3) mediate a positive feedback loop in the KRAS signalling pathway. To further investigate the molecular mechanism, combining a biochemical, cell biology and mutagenesis approach, I have identified residues in iRhom2 which are key to its regulation of ADAM17 and I propose a novel model of this regulation. Overall, these findings shed light on the fundamental mechanism of how iRhom2 regulates ADAM17 and its contribution to cancer signalling.

B503/P2178

MALT1 localizes to an aggresome-like structure and sustains NF-κB activation, with limited turnover in T-lymphocytes.

I. Hussain[1], M. Traver[2], L. Campanello[3], C. Huaman[1], S. Paul[4], H. Shriff[2], W. Losert[3], B. Schaefer[1]; [1]Uniformed Services University, Bethesda, MD, [2]National Institutes of Health, Bethesda, MD, [3]University of Maryland College Park, College Park, MD, [4]The Johns Hopkins University, Baltimore, MD

MALT1 and BCL10 are both critical molecular adaptors for T cell receptor (TCR)-dependent NF-κB activation. Shortly after TCR-ligation, BCL10-MALT1 heterodimers cluster to form filament-like structures called POLKADOTS, cytosolic complexes that recruit and activate NF-κB signaling intermediates. Previous work demonstrated that in TCR-activated effector T cells, BCL10 is degraded following POLKADOTS formation, whereas MALT1 levels remain stable over time. Using confocal and super-resolution microscopy, we show that, in activated effector T cells, multiple diffused MALT1 clusters are transported to a perinuclear aggresome-like structure, which is enclosed by a characteristic vimentin cage and near the microtubule-organizing center (MTOC). MALT1 clustering at the aggresome-like structure peaks at 60-90 minutes post-TCR activation and remains stable for at least another hour before beginning to dissociate. Using pharmacological disruption, we demonstrate that the transport and focal accumulation of MALT1 clusters is dependent on microtubules and dynein. By employing a genetic approach, we show that trafficking of MALT1 clusters to the aggresome-like structure is dependent on the autophagy

adapters p62 and NBR1. Surprisingly, however, this focal accumulation of MALT1 is not dependent on expression of HDAC6, suggesting that this structure is not a canonical aggresome. Finally, the trafficking and focal accumulation of MALT1 clusters is necessary to continue signaling to NF-κB by enhancing MALT1 paracaspase activity and promoting persistence of coalesced phospho-IKK complexes. This work highlights two novel findings: 1) MALT1-IKK clusters play a role in extended maintenance of TCR-dependent NF-κB activation and 2) rather than serving as a depot of protein awaiting degradation, the MALT1 aggresome-like structure forms in an HDAC6-independent manner and actively transduces TCR signals.

B504/P2179

An in silico investigation of the novel therapeutic targets and cellular pathways against brefeldin a and tunicamycin c in colon cancer

V. Naidoo[1], J. Kandhavelu[2], R. Hull[3], S. Mirza[1], C. Penny[1]; [1]University of Witwatersrand, Johannesburg, South Africa, [2]Georgetown University, Washington, WA, [3]University of Pretoria, Johannesburg, South Africa

BackgroundColorectal cancer (CRC) is a prevalent cancer in South Africa, with a high mortality rate. Glycosylation is an important post-translational modification that plays a role in the development and progression of CRC. The nucleoside antibiotic Tunicamycin C (TunC), inhibits glycoprotein synthesis, reduces proliferation, migration, and invasion of colon cancer cells; while Brefeldin A (BFA) a lactone antiviral, inhibits protein transport from the endoplasmic reticulum to the Golgi complex.**Objective**As both drugs have potential in cancer therapy, using computational approaches, this study assesses the role of these glycosylation inhibitors in cancer signalling pathways and identifying key proteins associated with CRC.**Methods**Using the in silico HTDocking program (www.ebi.ac.uk/chebi/; Chemical Entities of Biological Interest), potential targets were mapped for TunC and BFA. Following this, GO was used for gene ontogeny searches, while both Wiki and KEGG were used for pathway searches to identify their molecular biological role/s in CRC.**Results**With a docking stringency score of 7, this analysis revealed 234 and 168 targets for BFA and TunC, respectively. Pathway analysis showed that Tunicamycin may regulate important signalling pathways, such as gastrin signalling (CDC42, PAK1, SRC, KIT, PPARG, FYN, RAC1, IL2, EGFR), AGE/RAGE signalling (CASP9, CDC42, SRC, RAC1, IRAK4, MMP9, EGFR), tyrosine kinase and leptin signalling (CDC42, SRC, PDE3B, FYN, RAC1, ESR1), Gastrin signalling is known to stimulate the proliferation, invasion, and migration of cancer cells, while AGE-RAGE signalling is involved with cell death machinery during tumorigenesis. Both BFA and TunC were shown to be involved in regulating pathways involved in other cancers and infection pathways. Currently using 3D modelling, we are evaluating binding interactions of selected key target proteins, SRC, CASP9, RAC1, c-Kit and Nuclear receptor co-repressor 2 (NCOR2), with TunC and BFA, respectively. **Conclusion**In summary, the pathways and targets implicated in the anticancer activity of these drugs may in the future lead to the use of glycosylation inhibitors as therapies for colon cancer

Rho-family GTPases

B505/P2180

Lis1 suppresses RhoC by inhibiting LNX1 mediated non-degrading ubiquitination

S. Kholmanskikh, S. Singh, M. Ross; Weill Cornell Medicine, New York, NY.

Non-degrading ubiquitination (NDU) is widespread and plays important functions in the regulation of Ras superfamily proteins. In this study, we tested whether NDU affects Rho proteins. We found that RhoC, but not RhoA, is subject to NDU mediated by LNX1 and LNX2 E3 ligases. The affected lysine residues are homologous to those targeted by NDU in H-Ras and K-Ras, where this modification impairs binding to a GAP and increases activity of those GTPases. We found that RhoC NDU also leads to an increase in activity, but via a different mechanism, namely inhibition of RhoC-RhoGDI interaction. The LNX1-dependent RhoC NDU is physiologically relevant as shown by increased actin stress fiber formation upon LNX1 overexpression, which is inhibited by RhoC knockdown. Furthermore, we found that Lis1 (aka PAFAH1B1) inhibits LNX1-dependent RhoC activation via direct binding to LNX1, thus providing a long sought molecular link between level of Lis1 and activity of Rho GTPases. RhoC NDU is indeed increased in the brains of *Lis1fl/-* animals. Furthermore, knockdown of RhoC, but not RhoA, rescues the cell spreading defect displayed *in vitro* by *Lis1fl/-* astrocytes. In conclusion, we report a novel mechanism of Rho protein regulation that links Lis1 to the activity of Rho GTPases and showed specificity of this novel pathway toward RhoC. The latter has important evolutionary and therapeutic implications. Both LNX1 and RhoC are vertebrate specific, while Lis1 is present in all eukaryotes. This suggests that Lis1 regulation of Rho GTPases is an evolutionarily new function of an ancient protein. Hyperactivity in the Rho signaling pathway is associated with multiple human diseases including cardiovascular, cancer, and neurodegenerative disorders. Yet, indiscriminate targeting of Rho pathway has been disappointing due to its intolerable side effects. Discovery of a RhoC selective upstream regulatory mechanism provides an opportunity for design of a more targeted way of manipulating Rho signaling to therapeutic benefit.

B506/P2181

Spatiotemporal crosstalk of Rap1A-RhoA GTPases regulates breast cancer cell motility

C. Sanchez, A. Muesch, L. Hodgson; Albert Einstein College of Medicine, Bronx, NY.

During cancer dissemination and metastasis, cells undergo an epithelial-mesenchymal transition (EMT), which induces cell adhesion remodeling and migration that are regulated by p21 family of small GTPases. Rap1A, a member of the Ras subfamily, is aberrantly expressed in a number of breast cancer cell types but a specific role for this isoform in controlling the dynamic balance of cell adhesions and motility has not been elucidated. Here, we identified a pathway regulating the Rap1A-RhoA antagonism, important for breast cancer cell adhesion dynamics and migration. The spatiotemporal coordination and modulation of adhesion structures play essential roles in cancer. The loss of intercellular cadherin contacts drives signals that induce integrin-based focal adhesion and actin cytoskeletal rearrangement leading to cell motility. Since Rap1A and RhoA appear to have an antagonistic role in controlling cell adhesion and motility, elucidating how these two GTPases are coordinating the cell adhesion dynamics and the balance of regulatory cues to produce transient, localized effects is critically important, yet clearly difficult using conventional approaches. Here, we designed new Förster resonance energy transfer (FRET)-based single-chain biosensors, including near-infrared (NIR) FRET biosensor for RhoA and a cyan-yellow FRET biosensor for Rap1A. Importantly, the NIR miRFP670nano1-miRFP720 fluorescent

protein FRET pair allows direct multiplex imaging with our new CFP-YFP Rap1A biosensor with practically zero spectral overlap, enabling simultaneous imaging. The new multiplex FRET imaging approach allowed us to directly visualize and quantify the Rap1A-RhoA coordination during cell motility and adhesion remodeling. Our data show that there is a localized, antagonistic balance, both in space and time, of the activity of Rap1A versus RhoA within both cell-cell and cell-extracellular matrix adhesion structures. Furthermore, the assembly and disassembly of focal adhesion and cell-cell adhesion components have differential, dynamic-dependence on the spatiotemporal regulation of Rap1A activity. Moreover, upstream regulation of Rap1A activity by C3G and SIPA1, Rap1A-GEF and -GAP respectively, has a direct impact on the activity of RhoA at cell adhesion structures and on cell motility. Importantly, we show here that one of the primary functions of Rap1A at these adhesion structures is to recruit and to control the regulators of cytoskeletal RhoA activity to the sites of adhesion remodeling, which in turn, induce actin cytoskeleton rearrangement and cell motility. Our results point to an important molecular pathway of antagonistic crosstalk between Rap1A and RhoA, controlling EMT and cell motility, which is found to be upregulated during early tumor dissemination.

B507/P2182

ARHGAP17 regulates the spatiotemporal activity of Cdc42 at invadopodia

G. Kreider-Letterman[1], A. Castillo[1], E. Mahlandt[2], J. Goedhart[2], A. Rabino[1], S. Goicoechea[1], R. Garcia-Mata[1]; [1]University of Toledo, Toledo, OH, [2]University of Amsterdam, Amsterdam, NETHERLANDS.

Cancer cells form actin-rich protrusions called invadopodia that can degrade the extracellular matrix and facilitate tumor invasion and intravasation. Invadopodia, like podosomes, are typically organized with a core region comprised of actin, actin nucleators, and scaffolding proteins, and a surrounding ring comprised of adhesion and adapter proteins. The formation of invadopodia is tightly regulated by the Rho family of GTPases. Rho GTPases function as molecular switches that are turned on by guanine-nucleotide exchange factors (RhoGEFs) and turned off by GTPase-activating proteins (RhoGAPs). However, the molecular mechanisms of RhoGEF and RhoGAP signaling at invadopodia are poorly understood. In particular, almost nothing is known about the role of RhoGAPs at invadopodia. Here, we have identified ARHGAP17, a RhoGAP specific for Cdc42, as a key regulator of invadopodia in breast cancer cells. Using a wide range of fixed and live microscopy approaches, including TIRF, STORM, and Cdc42 sensors, we dissected a novel ARHGAP17-mediated signaling pathway that regulates the spatiotemporal activity of Cdc42 at invadopodia. Our results show that during invadopodia assembly, ARHGAP17 localizes to the invadopodia ring and restricts the activity of Cdc42 to the invadopodia core, where it promotes invadopodia growth. Invadopodia disassembly starts when ARHGAP17 translocates from the invadopodia ring to the core, in a process that is mediated by its interaction with the Cdc42 effector CIP4. Once at the core, ARHGAP17 inactivates Cdc42 to promote invadopodia disassembly. Our results in invadopodia provide new insights into the coordinated transition between the activation and inactivation of Rho GTPases.

B508/P2183

Rhob gtpase function during breast cancer invasion

S. Pagano, L. Hodgson; Albert Einstein College of Medicine, Bronx, NY.

RhoGTPases are molecular switches that cycle between active (GTP-bound) and inactive (GDP-bound) states. RhoGTPases regulate many biological processes, including cell motility and adhesion, and are

essential in both normal and disease physiologies, including cancer progression. RhoB is a close paralog of the canonical RhoA and is an important yet poorly characterized GTPase involved in the endosomal recycling and targeting of cell surface receptors, including integrins and growth factor receptors that are important for tumor invasion and dissemination. Moreover, very few studies to date have highlighted the role of RhoB in breast cancer invasion.Our work focuses on the role of RhoB in cell migration and invasion in breast cancer. We address a hypothesis that RhoB may play a key role in these processes by modulating integrins and membrane receptors at the cell surface. We show that the genetic depletion of RhoB impacts invadopodia functions in mammary adenocarcinoma cells and this appears to be modulated through localized, RhoB-targeting of vesicular structures at invadopodia. Reduction in the functional phenotype of invadopodia upon RhoB depletion did not alter cell invasion through Matrigel coated filters, pointing to a mechanism in which a balance between the extracellular matrix degradation and the cell motility may be regulated by RhoB. We are investigating whether changes in cell motility in response to RhoB depletion could compensate for the reduced matrix degradation at invadopodia. The balance between motility and degradation regulated by RhoB through controlling the endosomal recycling requires activation/deactivation of RhoB to properly target the endosomes. To probe this dynamic of RhoB regulation, we have developed and optimized a FRET based RhoB biosensor to visualize the RhoB activity status in living cells.Our results show an involvement of RhoB in invadopodia regulation and its implication in metastatic dissemination through fine-tuning the balance of matrix degradation and cell motility. It is likely that RhoB may coordinate together with other canonical members of the RhoGTPases to regulate this dynamic balance. Ultimately, our investigations will focus into this dynamic coordination of Rho family GTPases in regulating the invasion and dissemination of tumor cells during metastasis, in which RhoB may play an important yet unidentified role.

B509/P2184

Regulation of cell wall integrity in mating *Saccharomyces cerevisiae*

E. Curtis, C. Robertson, D. Lew; Duke, Durham, NC.

To survive in a hypoosmotic environment, yeast cells are surrounded by a cell wall. The wall is remodeled to allow cell growth, which can lead to dangerous wall thinning with potential for catastrophic lysis (Clark-Cotton, Jacobs, and Lew 2022). However, a “Cell Wall Integrity” (CWI) stress response pathway is thought to ensure that the wall remains intact in the face of environmental conditions that threaten wall integrity (Levin 2011; Levin 2005). Nevertheless, when two yeast cells mate, they are able to degrade the intervening cell walls, suggesting that CWI protections are either turned off or over-ridden (Philips and Herskowitz 1997). This study aims to identify whether and how the CWI pathway is regulated during mating in *Saccharomyces cerevisiae*. A key component of the CWI is the kinase Pkc1p, which is activated upon binding to Rho1-GTP at the cell surface (Kamada et al. 1996). Using surface localization of Pkc1 as a reporter of CWI activation, we examined pathway activity during pheromone exposure and mating. We found that Pkc1 was recruited to the polarity site (shmoo tip) in cells exposed to high levels of synthetic pheromone. However, in mating cells Pkc1 was often absent from the polarity site. We observed short visits to the vicinity of the polarity site but Pkc1 localization was much less sustained than it was in shmoos. Expression of an activated Pkc1 mutant delayed the degradation of intervening cell wall during mating. Our results suggest that the CWI pathway is to some degree inactivated in mating cells, presumably to allow cell wall degradation.

B510/P2185

MLK3-dependent Membrane Repair against Cholesterol-Dependent Cytolysins is not Activated by Rho-family small GTPases.

V. G. Kayejo, P. A. Keyel; Biological Sciences, Texas Tech University, Lubbock, TX.

Necrotizing soft tissue infections (NSTIs) exhibit a rapid disease progression characterized by substantial local tissue loss, which results in significant morbidity and mortality rates despite optimal treatment. The causative agents of NSTIs are *Streptococcus pyogenes* and *Clostridium perfringens,* which produce the cholesterol-dependent cytolysins (CDCs) streptolysin O (SLO) and perfringolysin O (PFO), respectively. These CDCs are resisted by mammalian membrane repair activated by Mixed-Lineage Kinase 3 (MLK3) and Mitogen-activated protein kinase kinase (MEK) signaling. However, the upstream activator of MLK3 remains unknown, as is the redundancy of MEK1/2 in membrane repair. Here, using selective GTPase inhibitors and siRNA, we tested the role of Rho-family small GTPases in membrane repair to CDCs and MEK redundancy. Inhibition of cdc42, Rac, or ROCK1/2 in HeLa cells did not increase cell sensitivity to SLO and PFO, nor block the downstream phosphorylation of MLK3 or MEK, as confirmed by western blotting. Using siRNA knockdown in HeLa cells, we tested the redundancy between MEK1 and MEK2 in repair against CDCs. We found that MEK2 siRNA, but not MEK1, increased cell sensitivity about 2-fold to SLO and PFO. We conclude that MEK2 is the critical downstream kinase activated for HeLa cell survival against CDCs, while the upstream activator of the MLK3-MEK2 repair signaling pathway to CDCs is unlikely to be a Rho-family small GTPase.

B511/P2186

C9orf72/SMCR-8 negatively regulates *C. elegans* EGFR signaling via the Arf6 GTPase.

A. Sabbir, A. Sobhani, S. Deng, K. Gauthier, C. Rocheleau; Research Institute of the McGill University Health Centre, Montreal, QC, Canada

An expansion of hexanucleotide repeats in the first intron of C9orf72 is the most prevalentgenetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). RNA andprotein aggregates resulting from the hexanucleotide repeats and C9orf72 haploinsufficiencymay cause disease. Thus, knowing the normal biological functions of the C9orf72 protein isimportant to understand how loss of C9orf72 might contribute to disease progression. C9orf72forms an obligate complex with SMCR8 and they have been implicated in several aspects ofendolysosomal and autolysosomal function. Recent structural and biochemical data indicate thatC9orf72 and SMCR8 function as a Rab and Arf GTPase Activating Protein (GAP) *in vitro*. In *C.elegans*, C9orf72 and SMCR8 protein complex has been proposed to affect the lysosomalhomeostasis. However, the *in vivo* targets of C9orf72/SMCR8 have not been identified.We previously demonstrated in *C. elegans* that the ARF-1 and RAB-7 GTPases are strong negativeregulators of Epidermal Growth Factor Receptor (EGFR)-mediated vulva cell fate specification.We find that C9orf72 and SMCR-8 are also strong negative regulators of EGFR-mediated vulvainduction. RAB-7 promotes EGFR trafficking to the lysosomes and loss of RAB-7 results in EGFRaccumulating in endosomes. ARF-1 targets EGFR trafficking to the apical membrane of the vulvaprecursor cells; away from the EGF ligand localized at basolateral membrane. Loss of ARF-1results in an increased trafficking of EGFR to the basolateral membrane. We found that C9orf72is unlikely to function with either RAB-7 or ARF-1 as loss of C9orf72 does not result in EGFRaccumulating in either endosomes or the basolateral membrane. Instead, we found *C9orf72*mutants have increased level of EGFR on both the apical and basolateral membranes suggestingthat C9orf72 and SMCR8 are involved in

EGFR sorting into the recycling and degradativepathways.ARF-6 promotes endosomal recycling. We found that *arf-6* gain-of-function mutation antagonizesEGFR-mediated vulva induction, and that ARF-6 is required for C9orf72 to negatively regulateEGFR signaling. Our data consistent with C9orf72/SMCR8 functioning as an ARF-6 GAP to inhibitEGFR recycling to the plasma membrane. In a broader perspective, the increased ARF-6 activityand increased cargo delivery to the plasma membrane because of human C9orf72 loss couldcontribute to ALS and FTD development.

B512/P2187

BAG6 regulates stress fiber formation via controlling RhoA ubiquitination

M. Miyauchi, H. Kawahara; Biological Sciences, Tokyo Metropolitan University, Tokyo, JAPAN.

Dynamics of the actin cytoskeletal architecture are regulated at the level of actin polymerization. The Rho family of small GTPases are key regulators of distinct steps of cytoskeletal actin polymerization, which control cell shape, adhesion, migration, and division. They act as molecular switches controlled by GEFs/GAPs, which stimulate GTPase cycling between active (GTP-bound) and inactive (GDP-bound) form. Rho GTPases also associate with cytosolic chaperone proteins known as guanine-nucleotide dissociation inhibitors (GDIs), which maintain the GTPase in its inactive conformation. Binding of GTP induces a conformational change, which allows to interact and activate effector proteins.
Recently, posttranslational modifications such as ubiquitination were also reported to regulate the activity and stability of Rho GTPases. However, regulatory mechanisms how ubiquitination of Rho-family proteins are controlled remains elucidate to date. Previous study suggested that BAG6 interacts with GDP-associated Rab GTPases and recruits it to ubiquitin-dependent degradation (Takahashi et al., 2019). BAG6 is a chaperone/holdase that interacts with hydrophobic polypeptides. BAG6 prevents the excess accumulation of inactive Rab family proteins, whose accumulation impairs intracellular membrane trafficking. However, it is unknown whether BAG6 targets other family of small GTPases. The hydrophobic residues of the SwitchI region of Rab8a are essential for its interaction with BAG6. When we compared the amino acid sequence of SwitchI region of small GTPases, we found that Rho proteins are the most similar to Rab8a. In addition, BAG6 knockdown induced change in cell shape, we hypothesized that BAG6 might regulate Rho proteins.
Our finding in this study was that deficiency of BAG6 resulted in destabilization of cell endogenous RhoA protein that is rapidly degraded by the proteasome, leading to abrogation of stress fiber formation. Strikingly, restoration of RhoA expression through a transient overexpression rescued defects in stress fiber formation induced by BAG6 depletion, bypassing the requirement of BAG6. BAG6 is also necessary for appropriate assembly of focal adhesions as well as cell migration events. These findings reveal a crucial role of BAG6 in maintaining the integrity of actin fiber polymerization by preventing ubiquitination of RhoA small GTPases.

Mechanotransduction

B514/P2188

Regulation of membrane homeostasis by TMC1mechanoelectrical transduction channels

A. Ballesteros, K. J. Swartz; NINDS-NIH, Bethesda, MD.

The mechanoelectrical transduction (MET) channel in auditory hair cells converts sound into electrical signals,enabling hearing. Transmembrane-like channel 1 and 2 (TMC1 and TMC2) are implicated in

forming the pore ofthe MET channel. Here, we demonstrate that inhibition of MET channels, breakage of the tip links required forMET, or buffering of intracellular Ca... induces pronounced phosphatidylserine externalization, membraneblebbing, and ectosome release at the hair cell sensory organelle, culminating in the loss of TMC1. Membranehomeostasis triggered by MET channel inhibition requires Tmc1 but not Tmc2, and three deafness-causing mutationsin Tmc1 cause constitutive phosphatidylserine externalization that correlates with deafness phenotype. Ourresults suggest that, in addition to forming the pore of the MET channel, TMC1 is a critical regulator of membranehomeostasis in hair cells, and that Tmc1-related hearing loss may involve alterations in membrane homeostasis.

B515/P2189

Nanoscale substrate deformation triggers fast Ca^{2+}transient via mechanosensitive cation channels

H. Peussa[1], C. Fedele[2], H. Tran[1], E. Mäntylä[1], A. Priimägi[2], S. Nymark[1], **T. O. Ihalainen**[1]; [1]Faculty of Medicine and Health Technology, Tampere University, Tampere, FINLAND, [2]Faculty of Engineering and Natural Sciences, Tampere University, Tampere, FINLAND.

Cells in our bodies are constantly subjected to mechanical stimuli such as stretch, compression, osmotic stress, or shear. The mechanical information cells receive from their physical environment co-regulates their form and functions, thus allowing cells to adapt to their niche. The physical cues rising from the environment are often locally sensed by specific and highly dynamic protein assemblies at the cell membrane, e.g., in integrin-rich focal adhesions or by large mechanosensitive (MS) ion channel complexes.Calcium-mediating MS ion channels like Piezo1 are particularly interesting for local mechanosensing due to the dual function of calcium. In addition to affecting the electrical potential difference of the cell membrane, calcium also acts as a universal second messenger, participating in numerous cellular signaling pathways, such as contraction, proliferation, secretion, vesicle trafficking, protein synthesis, and apoptosis. However, studies of these fast cellular mechanosensitive responses have proven to be difficult, partly due to the lack of proper tools to locally manipulate the cells. Light responsive materials offer an interesting approach to study how local mechanical perturbation affects cells. Azobenzene-containing light-controllable materials have been recently proposed as smart biointerfaces, as their topography can be precisely manipulated via visible light. Photopatterning such materials with different sinusoidal microtopographies has shown to be very effective in driving cellular morphology and migration.Herein, we exploit the dynamical properties of a light responsive azobenzene-based molecular glass (disperse red 1 molecular glass – DR1-glass) as a dynamic cell culturing substrate for the mechanically activated calcium dynamics in epithelial monolayer. We first optimized the light-induced formation of topographical features by tuning irradiation parameters of a common confocal microscope. We show that local nanoscale material deformation and deformation dynamics cause strong calcium transient in the cells. Based on pharmaceutical experiments, we propose that Piezo1 channels are involved in sensing this deformation. Finally, the flexibility of this approach helped reveal that cells respond distinctively to direct mechanical stimuli and to signals rising from neighboring cells, thus highlighting the role of cell-to-cell communication in the tissues' complex mechanoresponses.

B516/P2190

Contractility and the LINC complex mediate unlocking of the Neurectoderm Lineage Gene Sox1 During Early Differentiation in Mouse Embryonic Stem Cells

M. S. Hamouda[1,2], C. Labouesse[2], G. Wylde[2], Y. Miroshnikova[3], S. Basu[2], K. Chalut[2], C. Waterman[1]; [1]Cell and Tissue Morphodynamics, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, [2]Wellcome-MRC Cambridge Stem Cell Institute, Cambridge, UNITED KINGDOM, [3]Section on Nuclear Mechanotransduction and Cell Fate Dynamics, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD.

During development, cell differentiation often involves morphogenic transformations that shape and organize tissues. Emerging evidence suggests that the LINC complex may transduce the actomyosin-mediated cellular forces of morphogenesis through the nuclear envelope to chromatin. This nuclear mechanoresponse can effect gene expression and epigenetic changes during differentiation, thereby influencing or instructing cell fate determination. We studied the role of the LINC complex and actomyosin activity in neurectoderm lineage specification in mouse embryonic stem cells (mESCs) in an *in vitro* model of exit from naïve pluripotency. This models early embryogenesis, where disorganized cells of the pre-implantation epiblast undergoes a polarization morphogenesis to form a cup-like epithelium in the post-implantation epiblast. We found that mESCs induced to exit naïve pluripotency *in vitro* recapitulated some features the apical constriction morphogenesis that occurs *in vivo. In vitro* colonies formed apical actin rings containing phosphorylated myosin light chain, indicating contractile activity, and radially taught actin cables that severely deformed nuclei. To determine the role of LINC and contractility in lineage specification, we utilized inducible expression of dominant negative LINC and pharmacological perturbation of myosin II. We found that the timely downstream expression of the master neurectoderm lineage transcription factor, SOX1, required an intact LINC complex or myosin II activity during the apical constriction time window. 3D DNA FISH revealed the *Sox1* locus residing at the apical nuclear envelope during this time window. Notably, pluripotency transition was unaffected by LINC decoupling, suggesting an epigenetic basis of *Sox1*'s sensitivity to LINC function. Supporting this, we observed LINC-dependent changes in the levels and nuclear distributions of histone modifications known to be associated with *Sox1* transcriptional regulation and epigenetic poising. CHIP-qPCR revealed that gain of H3K27me3 on the *Sox1* promoter required an intact LINC complex during the apical constriction time window. Together these data suggest that morphogenesis-associated cytoskeletal contractility signals the LINC complex to instruct appropriate chromatin changes that poise *Sox1* for timely downstream expression, effecting cell fate determination during early development.

B517/P2191

Investigating Nuclear Mechanotransduction by Mapping Changes in Genome-Wide Transcription and Chromatin Accessibility After Mechanical Stretch

J. T. Long[1,2], J. Morival[1,2], J. T. Lis[3], J. Lammerding[1,2]; [1]Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, [2]Weill Institute of Cell and Molecular Biology, Cornell University, Ithaca, NY, [3]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Cells can mediate tissue maintenance and growth through mechanotransduction, i.e. the translation of mechanical forces into biochemical signals. Whereas multiple mechanotransduction pathways have been identified at the plasma membrane and cytoplasm, the role of the cell nucleus in mechanotransduction remains unclear. Proteins at the nuclear envelope such as nuclear lamins and the

LINC complex connect cytoplasmic forces directly to the genome with the potential to influence genome organization and transcriptional regulation. It is not yet known whether mechanical forces could therefore result in the opening of genomic regions to influence the transcription of mechanoresponsive genes. Here, we seek to address this gap by developing a system to induce mechanical stress in fibroblasts and map genome-wide changes in transcription and chromatin accessibility temporally and at high resolution. To examine differential gene expression, we performed precision nuclear run-on sequencing (PRO-seq). Unlike RNA-seq which measures the quantity of total cellular RNA, PRO-seq captures nascent RNA for genome-wide temporal observation of early transcription events at base-pair resolution with high signal to noise ratio. We performed PRO-seq to detect early differential gene expression after subjecting human fibroblasts to cyclic stretch. To probe the effect of short-term mechanical stress on genome organization, we also performed the Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) on stretched fibroblasts. PRO-seq experiments detected a rapid increase in polymerase activity within the gene body of several genes encoding for transcription factors associated with cell proliferation, survival, and stress response after mechanical stretch as early as 10 minutes. Cross-referencing PRO-seq data with ATAC-seq revealed that the promoter regions of mechanically induced genes were already accessible before mechanical stretching was induced, and genome-wide chromatin accessibility did not increase with mechanical stretch. These findings suggest that genomic regions associated with mechanoresponsive genes are primed for gene activation to trigger polymerase pause release and elongation. Future work will investigate the role of nuclear lamins and LINC complex proteins in this phenomena and how disruption of these proteins perturbs the cellular mechanotransduction pathways.

B518/P2192

Elevated Extracellular Viscosity Redistributes Mitochondria and Microtubules in Cells

D. Yan[1,2,3], M. Pittman[1,2,3], H. Qureshi[1,2,4], Y. Chen[1,2,3]; [1]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, [2]The Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD, [3]Center for Cell Dynamics, Johns Hopkins University, Baltimore, MD, [4]Department of Classics, Johns Hopkins University, Baltimore, MD.

A large proportion of the human tissue is covered by extracellular fluids that are viscous in nature, such as mucus in the airway and the GI tract. Yet little is known regarding how cells behave at various viscosities. We previously found that adherent cells with active ruffling lamellipodia can sense extracellular viscosity levels. At increased viscosity, single cells with ruffles flatten, and focal adhesion turnover increases, counterintuitively resulting in faster migration. However, how cells at high viscosity migrate in the context of wound healing, where cells collectively migrate to close wounds, remains unknown. Wound healing requires persistency in migration direction, which is regulated by microtubules. Wound healing also requires energy from mitochondria. On one hand, active proliferation and extracellular matrix repair are required, both of which are energy-consuming processes mainly localized in the cell's center. On the other hand, traction forces generated by cells increase at high viscosity, implying higher energy demands at the cell's leading edge. Mitochondria traverse along microtubules. To gain insights into how microtubules facilitate mitochondrial re-distribution to satisfy the growing energy demands at both the cell edge and center, we employed live-cell fluorescence confocal microscopy to study the dynamics of mitochondria and microtubules at various viscosity values. Upon the medium viscosity changing from low to high, we observed a minority of microtubules extending to the cell edge, following the protruding actin cortex, while most microtubules stalled at the

lamella circumference. We simultaneously observed a small subpopulation of mitochondria treading along the long extending microtubules to the cell edge. Furthermore, we found that the flattened nucleus at high viscosity was in closer contact with the ventral side of the cell membrane, while the membrane tension decreased significantly. Our results suggest that at high viscosity microtubules redistribute and guide mitochondria to the cell edge, albeit a small proportion. This redistribution might correlate with decreased membrane tension, allowing microtubules to push far onto the cell edge, as well as repositioning of the nucleus, reorganizing the spatial distribution of perinuclear organelles.

B519/P2193

Altered patterns of cell migration during wound healing at abnormal viscosity

H. Qureshi[1], M. Pittman[2], Y. Chen[2]; [1]Department of Classics, Johns Hopkins University, Baltimore, MD, [2]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD.

Wound healing involves rapid cell migration to the site of injury, relying on fibroblasts and epithelial cells to replenish the extracellular matrix and restore damaged tissue. The speed and directionality of cell migration can be altered in response to various chemical and physical cues, including chemoattractant gradients, substrate stiffness, and extracellular fluid (ECF) viscosity. Yet the mechanical effects of ECF viscosity on healthy and aberrant wound healing are underexplored. A recent finding from our research group demonstrated that single cells with actively ruffling lamellipodia can sense and respond to elevated ECF viscosity and migrate up to twice as fast in viscous medium. However, wound healing involves collective migration within the epithelium, and the effect of viscosity on collective cell motility has not been closely examined. I aim to address this unexplored question and extend our knowledge to how viscosity affects interactions between cell types in the context of wound healing. A 2-D co-culture system consisting of human GM00637 fibroblasts and HaCaT keratinocytes was constructed to model skin wound healing. After incubating co-culture wells overnight, the wells were scratched down the middle, and the cells were imaged as they migrated into the wound site. I found that the two cell types displayed starkly contrasting behaviors when immersed in viscous medium (0.4 Pa·S). In particular, collective migration and proliferation were both decreased in keratinocytes, while fibroblasts exhibited no significant changes in their migration or proliferation. This resulted in predominantly fibroblasts occupying the wound site. Since coordinated arrival of these cells at the wound site is necessary for effective re-epithelialization, disruptions to the relative timing of this migration can lead to fibrosis. In summary, these findings provide insights into the etiology of fibrosis during wound healing and demonstrate the important role of mechanosignaling in pathology.

B520/P2194

DNA repair in soft and stiff contexts

M. Locatelli[1], A. Hinds[2], J. Holmes[2], G. Chang[3], J. Liu[3], Q. Song[2], **P. Vidi**[4]; [1]UNC, Chapel Hill, NC, [2]Wake Forest University School of Medicine, Winston-Salem, NC, [3]IUPUI, Indianapolis, IN, [4]Institut de Cancérologie de l'Ouest, Angers, FRANCE.

Cells feel and respond to the physical properties of their microenvironment. We asked if the stiffness of the extracellular matrix (ECM) regulates DNA repair. Using breast organoid models and mammary epithelial cells cultured on polyacrylamide gels of defined stiffness, we found that the response to DNA damaging drugs and ionizing radiation (IR) is amplified in stiff contexts. This effect could not be explained by cell cycle imbalance. IR induced similar levels of DNA breaks in soft and stiff conditions,

pointing to mechanogenomic regulation of the DNA damage response. Mechanistically, this effect was dependent on the structural continuum spanning integrins at the plasma membrane and the LINK complex at the nuclear envelope. ECM stiffness affected DNA repair by homologous recombination and non-homologous end joining. Cell survival to DNA breaks was also influenced by ECM stiffness. These findings may partly explain elevated breast cancer risk associated with high mammographic density (MD), which reflects high fibrillar collagen and stiff breast tissue. Using public transcriptomic data of normal breast tissue, we found a two-fold higher mutation burden in women with high MD compared to women with low MD. We propose that a mechano-stimulated DNA damage response in stiff tissue promotes survival of cells with DNA lesions at the expense of apoptosis, leading to the propagation of somatic mutations.

B521/P2195

Optogenetic activation of microtubule acetylation elucidates its role in actomyosin dynamics and directional cell migration

A. Deb Roy, C. Saez Gonzalez, E. Yadav, F. Shahid, X. Wang, T. Inoue; Johns Hopkins University, Baltimore, MD

Acetylation of α-tubulin at lysine-40, solely catalysed by α-TAT1, is implicated cell migration and mechanosensing. However, the precise role of microtubule acetylation in these events is not well understood, partly due to a limited understanding of α-TAT1 regulation and lack of tools to specifically actuate microtubule acetylation. Here we report that cytoplasmic localization of α-TAT1 is critical for its function, and that such localization is mediated by its C-terminal region through a combination of nuclear export, phospho-inhibited nuclear import and 14-3-3 mediated cytoplasmic retention. Based on this model of spatial regulation of α-TAT1 function, we developed an optogenetic tool to control microtubule acetylation in live cells to probe its relationship with actomyosin contractility. We modified the previously described Light-inducible nuclear export system (LEXY) to reduce its dark-state activity and tethered the catalytic domain of α-TAT1 to it. This tool, named optoATAT1, was predominantly nuclear in dark and was rapidly shuttled to the cytoplasm upon blue light stimulation in a reversible manner. Hela cells expressing optoATAT1 exposed to blue light showed a robust increase in microtubule acetylation compared to those kept in dark, validating the functionality of the tool. Mouse embryonic fibroblast (MEF) cells from α-TAT1 knock-out (KO) mice showed severe defects in chemotaxis, although they are highly motile compared to wild-type (WT) MEF cells. In addition, the KO MEFs showed defects in cell polarity, actin cytoskeleton and Myosin-II activity. Using a FRET-based Vinculin Tension-sensor, we further show that compared to WT, α-TAT1 KO MEF cells exert little mechanical forces on focal adhesions. Stimulation of optoATAT1 induced rapid activation of actomyosin contractility as evidenced by increased actin stress fibers, Myosin-Light chain accumulation and maturation of focal adhesions. These effects of optoATAT1 on the actin cytoskeleton were dependent on its catalytic activity as well as ROCK signaling, thus establishing a causal relationship between microtubule acetylation and ROCK-mediated actomyosin contractility. To summarize, we have identified a previously unknown regulatory function of the α-TAT1 C-terminal region and based on our finding of spatial regulation of α-TAT1 function, we have developed optoATAT1, a powerful optogenetic tool to control microtubule acetylation that will provide new insights in the role of microtubule acetylation in mechanotransduction and directional cell migration.

B522/P2196

Linking cell mechanics and metabolism in endothelia

N. Cronin; Biochemistry and Molecular Biology, University of Iowa, Iowa City, IA.

Mechanisms for protecting or restoring endothelial function have been the subject of intense scrutiny. These efforts have revealed that fluid shear stress, from proper blood flow, activates signal transduction pathways that allow the cell to reorganize its actin cytoskeleton to withstand tension. Other groups have identified metabolic changes in response to shear and disturbances in regions of disrupted blood flow. These cytoskeletal and metabolic changes are often viewed as being disparate functions - an understanding for how the mechanical properties and behavior of cells to shear stress (i.e. mechanics) is linked to metabolism is incomplete. Here I provide evidence that cell-cell adhesion proteins located at the adherens junctions are directly responsible for sensing fluid shear stress and signal for the activation of AMP-activated Protein Kinase (AMPK), a master regulator of cell metabolism. I determine that the activation of AMPK shifts the metabolic state of these cells to promote energy production, which is necessary to sustain the cellular mechanics needed to withstand fluid shear stress. Furthermore, disruption of this signaling pathway leads to aberrant endothelial function, cell-cell junction stability, and cellular alignment to fluid shear stress. These data confirm that cell mechanics and metabolism are linked in endothelia and may provide insights into endothelial regulation and cardiovascular health and disease.

B523/P2197

Endothelial Glycocalyx in High- and Low-Flow Regions in the Trabecular Outflow Pathway of Bovine Eyes

N. Liu, S. Sosnowik, H. Li, H. Gong; Boston University, Boston, MA.

Intraocular pressure (IOP) plays an important role in the function of the eye, and is maintained through a dynamic balance of production and drainage of aqueous humor. Elevated IOP due to increased resistance in the outflow pathway is a primary risk factor for glaucoma, a leading cause of blindness. The trabecular outflow pathway is the primary pathway for aqueous outflow, and consists of the trabecular meshwork (TM), Schlemm's canal (aqueous plexus (AP) in bovine eyes), collector channels (CCs), and episcleral veins (ESVs). Flow along the pathway is segmental, with areas of varying resistance, resulting in high- and low-flow regions. The pressure gradient across the TM to the AP results in the formation of giant vacuoles (GVs) along the endothelium of the AP. Larger GVs form pores, which serve as pathways for aqueous humor to enter the AP. Glycocalyx is a hair-like structure that can be found covering the endothelium of the outflow pathway and filling some pores, however its role in contributing to or regulating outflow resistance has not been well studied. This study investigated the morphological differences of the glycocalyx in different flow regions along the trabecular outflow pathway in normal bovine eyes.

Six enucleated bovine eyes were perfused at 15 mm Hg for 30 minutes to measure baseline outflow facility, perfused with fluorescein to identify high- and low-flow regions of the outflow pathway, then perfusion- and immersion-fixed with Alcian Blue 8GX to label glycocalyx. Radial wedges of high- and low-flow regions of the anterior chamber angles were dissected and embedded. Ultra-thin sections (70nm) were cut and imaged with a transmission electron microscope. Coverage and thickness of glycocalyx was measured using ImageJ. The thickness and percentage coverage of glycocalyx, and the percentage of pores completely filled with glycocalyx was compared in high- and low-flow regions.

Glycocalyx thickness increased proximally to distally from the TM to ESVs, with glycocalyx covering ESVs being significantly thicker than that on the surface of the TM (P=0.01) and AP (P=0.02) in high-flow regions alone. No significant differences in glycocalyx coverage, glycocalyx thickness and percentage of pores filled with glycocalyx were found between high- and low-flow regions. Glycocalyx was also observed lining the inside of the majority of GVs with pores but not the inside of the majority of GVs without pores. Our results suggest that variations in shear stress play a role in determining glycocalyx morphology in different outflow pathway locations. Further studies are warranted to understand the role of glycocalyx in regulating or restricting aqueous outflow.
Supported by BU Summer 2022 UROP, NIH EY028674 and EY022634, The Massachusetts Lions Eye Research Fund.

B524/P2198

Adaptation of peristaltic pumps allows for cellular behavior analysis in response to flow forces *in vitro*

J. Abello[1], S. Raghavan[2], Y. Y. Yien[3], A. Stratman[1]; [1]Cell Biology and Physiology, Washington University in St. Louis, St. Louis, MO, [2]Department of Biomedical Engineering, Texas A&M University, College Station, TX, [3]Pittsburgh Heart, Lung and Blood Vascular Medicine Institute and Department of Medicine, University of Pittsburgh, Pittsburgh, PA.

Adaptation of peristaltic pumps allows for cellular behavior analysis in response to flow forces *in vitro*

Javier Abello[1], Shreya Raghavan[2], Yvette Y. Yien[3], and Amber N. Stratman[1]
1.Department of Cell Biology and Physiology, Washington University in St. Louis School of Medicine, St. Louis, MO 631102.Department of Biomedical Engineering, Texas A&M University, College Station TX 778433.Pittsburgh Heart, Lung and Blood Vascular Medicine Institute and Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

The vascular system is subjected to mechanical forces that influence its function and architecture. Blood flow forces differ depending on the type of vessel, vessel size, and proximity to the heart (i.e. laminar or pulsatile flow). Studying the effect of mechanical forces on endothelial cells *in vitro* is important to understanding the molecular pathways that regulate the interactions between the different components of the vascular system. However, *in vitro* studies require the implementation of sophisticated equipment to generate flow that effectively resembles the natural blood flow rates and patterns. These kinds of equipment are expensive and can present a challenge for many laboratories that must adhere to a budget. Here we present our inexpensive adaptation of a conventional peristaltic pump to produce both laminar and pulsatile flow. To corroborate the effectiveness of our design we acquired timelapse videos of cell cultures under different flow conditions and compared cellular motility, alignment, and persistence. We further evaluated the differences in VE-Cadherin expression and assessed actin filament orientation and assembly under flow patterns by immunostaining. This study introduces an easy-to-use, cost-effective, and reproducible platform for the study of flow forces *in vitro*, which will allow us to mechanistically dissect the role of mechanical forces on the architecture and functionality of the vascular system.

B525/P2199

Piezo1-mediated Mechanochemical Signaling Regulates Centriole Number in Multiciliated Cells

V. Narayanan, V. G. Rao, S. Kulkarni; Department of Cell Biology, University of Virginia, Charlottesville, VA.

How cells regulate organelle number is an outstanding question in cell biology. Unlike cycling cells that restrict centrioles to two copies per cell, highly specialized multiciliated cells (MCCs) deviate from the strict regulation as a part of the developmental program. MCCs that line the airway epithelium undergo massive amplification of basal bodies (specialized centrioles) to assemble an apical array of motile cilia and promote mucociliary clearance. Mutations that affect the number of centrioles/cilia are associated with impaired MCC function and can result in respiratory diseases, demonstrating that synthesizing the "right" number of centrioles/cilia is critical. Yet, how MCCs count and control centriole number remains a key gap in our understanding. Recent studies have shown that centriole number scales with MCC apical area. By artificially stretching the multiciliated epidermis, we demonstrated that mechanical forces calibrate MCC apical area and trigger centriole amplification via a mechanosensitive (MS) cation channel, Piezo1. However, how stretch-induced Piezo1 activation translates into more centrioles remains unresolved. To address this question, first, we demonstrate that Piezo1 acts a MS Ca^{2+} channel to regulate MCC centriole amplification. Inhibition of Piezo1-mediated Ca^{2+} signaling via Gd^{3+}, a potent inhibitor of stretch-activated Ca^{2+} channels, or intracellular Ca^{2+} chelation using BAPTA-AM, blocked centriole amplification in MCCs. Moreover, we show that Piezo1-mediated Ca^{2+} influx acts through protein kinase C (PKC) to activate Erk1/2 signaling and control MCC centriole number. Erk1/2 signaling, in turn, leads to the nuclear accumulation of a mechanosensitive transcriptional co-activator, Yap1, in MCCs. Strikingly, depleting Yap1 reduces the expression of Foxj1, a master regulator of motile ciliogenesis, suggesting Foxj1 is a target of Yap1 to regulate centriole number. Indeed, Foxj1 depletion affects centriole amplification in a tension-dependent manner. Interestingly, Yap1 and Foxj1 also control the expression of Piezo1, demonstrating a feedforward mechanism to control centriole number in MCCs. Together, our work indicates that Piezo1-mediated Ca^{2+} signaling regulates MCC centriole amplification in a tension-dependent manner via the Erk1/2 - Yap1 - Foxj1 signaling axis. Collectively, our work addresses a significant knowledge gap in mucociliary physiology and uncovers the central role of mechanochemical signaling in regulating organelle numbers to maintain tissue homeostasis.

B526/P2200

All models are wrong: *Xenopus* embryonic epithelial sheets under strain transmit tension perpendicular to apical junctions with little transmitted along junctions.

J. Yang[1], L. A. Davidson[2]; [1]Bioengineering Department, University of Pittsburgh, Pittsburgh, PA, [2]Bioengineering Department, Developmental Biology Department, Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA.

Contemporary mechanical models and force inference methods of epithelial morphogenesis assume transmission of stress and strain occurs primarily along cell-cell junctions and that the mechanics of epithelial sheets is dominated by tension carried along the lengths of spring-like apical junctional complexes. In these models, contributions of tension across the medio-apical cortex are commonly lumped into an isotropically-actin pressure term. Here we test those assumptions with a unique tissue stretcher that combines high resolution live-cell imaging with the application of morphogenesis-scale strain rates. *Xenopus* embryos establish a mature apical junctional complexes replete with tight-,

adherens-, and desmosomal-junctions by blastula stage and develop high transepithelial electrical resistance reminiscent of simple epithelia in organs such as those found in the gastrointestinal tract. To track these structures in *Xenopus* we express cell membrane and apical junctional reporters together with a vinculin tension sensor widely used as a reporter of tension at the cadherin/catenin adhesion complex. Reporter expressing organotypic explants of gastrula stage ectoderm are cultured on a fibronectin-coated polydimethylsiloxane (PDMS) substrate attached to an interchangeable custom cassette. The stretcher is a multipiece 3D-printed microscope stage insert with two motorized actuators that operate on the cassette to achieve > 100% uniaxial strain at the level of the apical junctional complex. The small size and low mass of the stretcher enable the use of piezo- and galvo-based z-focus to track individual cell- and junction-strain and biophysics of apical junctional complexes. Surprisingly, we observed few cell rearrangement events; rather, we observed heterogeneous cell-to-cell deformation. Segmenting junctions around the perimeter of each cell allowed us to analyze the tension between cell vertices and assess how well these correlate with junction length changes. We quantified vinculin intensity along individual cell junctions in these samples, where we expected to see vinculin recruited as the tissue responses to the high strain. Surprisingly, we found no positive correlation between vinculin recruitment and apical junction length increase. We next sought to evaluate the level of tension across junctions. We were also surprised to observe no vinculin recruitment to sites that were parallel to the uniaxial stretch axis, but a significant increased vinculin recruitment to junctions perpendicular to the stretch axis. These findings suggest current vertex-based models and force inference methods may need significant revision if they are to be used as interpretive models of epithelial morphogenesis in tissues with substantial medio-apical junctions.

B527/P2201

A New Computational Model for Mechanosensing and Mechanotransduction of Neuronal Growth Cones

L. Pulido[1], A. Athamneh[1], Y. Efremov[2], A. Raman[2], T. Kim[3], D. M. Suter[1]; [1]Biological Sciences, Purdue University, West Lafayette, IN, [2]School of Mechanical Engineering, Purdue University, West Lafayette, IN, [3]School of Biomedical Engineering, Purdue University, West Lafayette, IN.

Neuronal growth cones are highly motile structures at the tip of the axons and dendrites, which sense a variety of cues including chemical and mechanical to establish functional connections during nervous system development. Substrate-cytoskeletal coupling is an established model for adhesion-mediated growth cone advance through the application of traction force. However, the detailed molecular and biophysical mechanisms underlying the mechanosensing and mechanotransduction process remain to be elucidated. For this reason, we developed a new computational model to better understand the changes in cytoskeletal dynamics, traction force, and substrate deformation when the growth cone interacts with adhesion substrates of different stiffnesses. To achieve this, we have modified an existing motor-clutch model by including both motor and clutch reinforcement with increasing substrate stiffness. Furthermore, we have added an actin flow threshold that indicates when the growth cone is strongly coupled to the substrate. Our modeling results are in good agreement with experimental data from *Aplysia* growth cones with respect to substrate deformation behavior. Modeling also shows that strong coupling is achieved faster on soft substrates suggesting that growth cones prefer softer substrates when given the choice.

B528/P2202

Mechanical cues tune the differential localization of LIM domain proteins

S. Seetharaman[1], T. Chmiel[1], J. Devany[1], J. Winkelman[1], Y. Fang[2], M. Gardel[1]; [1]James Franck Institute, Department of Physics, University of Chicago, Chicago, IL, [2]Department of Medicine, Biological Sciences Division, University of Chicago, Chicago, IL.

The actomyosin network is coupled to mechanosensitive adhesion components such as focal adhesions and adherens junctions, and can generate forces to maintain overall tissue homeostasis. A ubiquitous family of LIM (Lin11, Isl- 1, and Mec-3) domain proteins is associated with stress fibers and adhesions; however, the precise mechanisms of their localization and force-sensing remain unknown. Here, we demonstrate tunability of LIM domain protein localization by altering various intracellular and extracellular mechanical cues in endothelial cells. We show that actomyosin contractility, an intrinsic mechanical stimulus, triggers the shuttling of several members of the LIM family including Zyxin, Paxillin, FHL2 and LIMD1 to stress fibers, focal adhesions, or the nucleus. Specifically, local tension in stress fibers recruits the LIM regions of 18 different LIM proteins to these strain sites. We also show that different external mechanical stimuli can tune LIM protein localization in cells. Interestingly, Zyxin, which predominantly localizes to stress fibers and focal adhesions, can be redirected to adherens junctions and tricellular vertices by altering matrix rigidity-induced cell-cell adhesion signaling. In addition, stretching and shear stresses experienced by vascular endothelial cells drive the differential localization of Zyxin and FHL2. Using RNAseq, we identified that FHL2 is enriched in cells experiencing disturbed shear stresses mimicking atherosclerosis. Under these conditions, FHL2 strongly accumulates at stress fibers and focal adherens junctions, but not linear adherens junctions. Altogether, we have identified and described the intracellular and extracellular mechanical cues that trigger the differential localization of LIM domain proteins. This force-dependent localization can be exploited to study the specific functions and mechanotransduction pathways downstream of LIM domain proteins.

B529/P2203

Differential roles of extracellular matrix on primary and metastatic pancreatic cancer cells in 2d and 3d systems

O. Usman[1], S. Kumar[1], R. Walker III[1], G. Xie[1], L. J. Dooling[2], Y. Wang[1], J. Irianto[1]; [1]Florida State University, Tallahassee, FL, [2]University of Pennsylvania, Philadelphia, PA.

Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis, and it is one of the leading causes of cancer death, with less than five percent 5-year survival rate. PDAC is characterized by high stromal deposition of different extracellular matrix (ECM) proteins which contribute to its resistance to both chemo- and radiotherapies. The ECM being a meshwork of different proteins, elucidating the role of the individual proteins in the ECM is important to the understanding of the disease. Here, by using multiple PDAC lines derived from both primary and metastatic tumors, we investigate the role of several ECMs commonly found in PDAC tumors: collagen I, fibronectin, vitronectin, and laminin, in both 2D and 3D culture systems. Surprisingly, when cells were cultured on a 2D system, only the PDAC lines derived from the primary tumors show differential growth and cellular morphology between the different ECM proteins. Both fibronectin and vitronectin increase cell proliferation rate by 3 folds and promote cell spreading. Transcriptome analysis of one of the primary tumor PDAC lines shows a significant differential expression of genes involved in cell-cell and cell-matrix adhesion. When the cells were cultured in the polyethylene glycol 3D culture system, all of the PDAC lines grow at a similar rate when

exposed to the four different ECMs. However, when focal adhesion kinase (FAK) was experimentally overexpressed in one of the primary tumor PDAC lines, it rescues the sensitivity to the different ECMs in the 3D culture system. In summary, these findings suggest differential ECM response for PDAC lines derived from primary and metastatic tumors, and for cells cultured in 2D and 3D culture systems.

B530/P2204

Multiplexed mechanical mapping reveals macrophage mediated remodeling of brain in vivo

W. So[1], B. Johnson[1], P. B. Gordon[1], G. Scarcelli[2], K. Tanner[1]; [1]National Cancer Institute (NIH), Bethesda, MD, [2]University of Maryland - College Park, College Park, MD

Colony stimulating factor 1 receptor (CSF1R) plays a major role in central nervous system development especially for microglia formation as CSF1R loss in mammalian systems halts macrophage and microglia development. In humans, variants in CSF1R may also cause contribute to developmental disorders associated with brain morphological abnormalities and pathologies involving neurodegeneration. In mice, Csf1r deficiency results in embryonic or perinatal death. Thus, examination of the role of CSF1R in brain homology is challenging. Due to high homology with zebrafish, we specifically looked at a role of csf1ra (a paralogue of the mammalian CSF1R) on the regulation of brain mechanical homeostasis of larval zebrafish *in vivo*. RNA-Seq data analysis revealed alterations in pathways associated with angiogenesis and development of the innate immune system. Using intravital imaging, we observed abnormal brain morphology with an increase in fluid filled ventricles and altered neuron morphology in mutant fish. We then asked if mechanical properties are impaired as tissue mechanics play a crucial role in tissue development and neural stem cell behavior. Using two different microscale mechanical mapping techniques, we quantified the mechanical heterogeneities of different brain regions: fore-, mid-, and hind- brain. In wild type fish, the fore brain is stiffer than that of the mid and hind brain, As the brain develops, all regions become stiffer where the greatest increase in stiffness is measured in the hind brain. In comparison, the brain of the mutant zebrafish showed more homogeneous stiffness across all brain regions. Similar to the mammalian systems, csf1ra depletion in zebrafish will have delay or deficiency in microglia/macrophages within the brain. We then perturbed microglia/macrophage in wild type fish to further delineate the role of macrophage in mechanical homeostasis during brain development. We employed two methods of macrophage depletion using clodronate liposomes and tissue specific ablation using a genetic background NTR coupled to macrophages. In both alterations, the zebrafish brains were softer after treatment compared to the control siblings. We observed altered neuronal and acetylated tubulin structure of the mutant fish following in these treated fish. Our data demonstrates the importance of macrophage remodeling during brain development. It may also be relevant to the possibility that microglia contribute to synaptic plasticity and therefore function. Moreover, our data shows that mechanical properties can be used to identify phenotypes that result due to macrophage mediated tissue remodeling in brain morphogenesis.

B531/P2205

Stiff substrates localize myosin regulatory light chain in nucleus to suppress apoptosis

O. Katsuya[1], I. Seiichiro[1], T. Masayuki[1], A. Enomoto[2], H. Haga[1]; [1]Hokkaido University, Sapporo, Japan, [2]Nagoya University, Nagoya, Japan

Cells respond to physical stimuli, such as substrate stiffness. However, the response mechanism is not well known. Previous research showed that stiff substrates promote di-phosphorylation of myosin

regulatory light chain (MRLC) and as a result increase cellular contraction. It is also reported di-phosphorylated MRLC (2P-MRLC) performs transcriptional regulation in nucleus. These results indicate that MRLC mediates cellular responses to substrate stiffness via transcriptional activation itself. In this study, we aim to clarify the mechanism by which the cells that sense stiffness trigger cellular responses via MRLC. Previous research showed that 2P-MRLC localizes not only to the nucleus but also to the cytoplasm. Thus, we investigated whether substrate stiffness regulates the nuclear localization of 2P-MRLC. First, we prepared soft and stiff substrates of polyacrylamide gels, which are 0.4 kPa and 271 kPa, respectively. Then, human cervical cancer cells (HeLa cells) were cultured on each substrate and MRLC localization was observed. As a result, it was found that 2P-MRLC localized to nucleus in Hela cells on stiff substrates, whereas 2P-MRLC was localized to cytoplasm in the cells on soft substrates. This result revealed that substrate stiffness promoted nuclear localization of 2P-MRLC. Next, we aimed to identify the genes that regulate the nuclear-localized 2P-MRLC in response to substrate stiffness. Specifically, we performed qPCR screening and explored the genes which are up- or down-regulated by both a stiff substrate and MRLC. We found that MafB expression is downregulated by a stiff substrate and MRLC expression. Previous research showed MafB promotes apoptosis in limb morphogenesis. Thus, we hypothesized that suppression of MafB expression, which is induced by the nuclear localization of 2P-MRLC in response to substrate stiffness, restricts apoptosis. We examined the level of cleaved-caspase3, an apoptosis marker, in negative control cells, MRLC-knockdown (KD) cells and MRLC and MafB double-KD cells. MRLC-KD cells exhibit promoted cleaved-caspase3 levels, whereas knock down of MafB rescued the phenomena in MRLC-KD cells. These results indicate that the nuclear localization of 2P-MRLC, in response to substrate stiffness suppresses MafB expression to restrict apoptosis. These findings may provide new insight into how cells respond to substrate stiffness.

B532/P2206

Investigating Roles of Cytoskeletal Genes in *C. elegans* Sex Myoblast Development

M. A. Fitian[1], M. Razi[1], S. A. Shehzad[1], O. B. Ahmed[2], T. L. McKnight[1], A. Fang[2], T. V. Gibney[3], A. M. Pani[3], D. Q. Matus[2], R. C. Adikes[1]; [1]Siena College, Loudonville, NY, [2]Stony Brook University, Stony Brook, NY, [3]University of Virginia, Charlottesville, NY

The cytoskeleton plays integral mechanical roles in cell migration, division, and morphogenesis during animal development. However, our understanding of how cytoskeletal behaviors are regulated *in vivo* remains limited compared to our knowledge of mechanisms *in cellulo*. Here, we set out to characterize developmental roles of candidate genes associated with the cytoskeleton using an *in vivo* model system. To do this, we investigated development of the sex myoblast (SM) cells in *C. elegans*. The SM cells are descendants of the M mesoblast cell and are born in the posterior region of the L1 larva. SMs then travel anteriorly until they reach the gonad at the center of the worm. Upon arrival, the SM cells re-enter the cell cycle, divide three times and differentiate into the uterine and vulval muscle cells, which are required for egg laying. Mutations that impact SM cell migration, division, and/or differentiation can lead to lower egg counts that can be quantified at the plate level. Cytoskeletal regulatory proteins are likely to be important for multiple aspects of SM development including migration, division, and regulation of membrane protrusions that appear after migration is complete. To investigate functions for candidate cytoskeletal regulators in SM development, we are performing a targeted RNAi screen of ~120 genes associated with the cytoskeleton using M-lineage specific RNAi to bypass essential and/or pleiotropic functions for key genes. *C. elegans* were grown on RNAi plates and scored as young adults for phenotypes including sterility, larval growth slow, protruded vulva, uncoordinated, lethal, dumpy,

and bag of worms. We used brood size as a proxy for egg-laying ability with the hypothesis that reduced brood sizes likely indicate defects in SM development. Preliminary data show depletion of *cdc-42*, *ced-10* (*Rac*), *unc-73* (*RhoGEF-kinase*), *arx-2* (*Arp-2/3*), *wsp-1* (*WASP*), *unc-60* (*Cofilin*), and *ani-2, ani-3* (*Anillin*) impact development and brood size. These data suggest that key actin regulators are required for proper SM cell development. Future experiments will investigate the types of defects underlying these phenotypes through further RNAi experiments and imaging. In conclusion, we can take advantage of the unique attributes of the SM cell lineage to dissect how the cytoskeleton is required for development *in vivo*.

B533/P2207

Plectin linkages are mechanosensitive and facilitate nuclear translocation during cell migration through 3D matrices

B. R. Hewitt; Drexel University, Philadelphia, PA

Migrating cells have the remarkable capability of sensing and responding to their environment by switching their mode of migration. Cells migrating in a linearly elastic 3D environment concentrate actomyosin contractility in front of the nucleus to pull the nucleus forward, which increases the interior pressure at the front of the cell. High pressure in front of the nucleus creates cylindrical protrusions called lobopodia. This mode of migration, known as the nuclear piston, relies on contractility and adhesion where actomyosin filaments transmit force onto vimentin intermediate filaments that act as molecular tow cables for the nucleus by docking into the nuclear envelope protein nesprin 3. While best characterized in human primary fibroblasts, the nuclear piston has also been observed in primary human chondrocytes, human fibrosarcoma, and human breast adenocarcinoma. It is unclear how actomyosin filaments connect to vimentin filaments to transmit force to the nucleus to help pull it forward in 3D matrices. We hypothesized that the cytoskeletal cross-linking protein plectin couples actomyosin and vimentin intermediate filaments. Proximity ligation assays (PLA) on 2D glass and FRET imaging in 3D matrices showed that vimentin and actin interact in both a plectin dependent and ROCK-NMII dependent manner. Additionally, plectin interacts with vimentin in response to NMII contractility and substrate stiffness, suggesting that the association of vimentin and plectin complex is mechanosensitive. We found that plectin expression slows migration on 2D glass but is critical for both nuclear piston migration in 3D cell-derived matrix (CDM) and lamellipodial migration in 3D collagen. Finally, we found that plectin is required for the polarization of both NMII and vimentin in front of the nucleus. Taken together, our findings suggest that matrix stiffness and NMII activity govern assembly of vimentin, plectin, and actin. Once assembled, this interconnected cytoskeletal machinery can be activated in 3D matrices to help pull the nucleus forward to generate compartmentalized pressure and lobopodial protrusions.

B534/P2208

Effect of Mechanical Dose on Characteristics of Cartilaginous Matrix Production

S. Jang[1,2], J. Jeong[1], T. Oh[3], B. Song[2], E. Lee[4,2]; [1]Graduate School, Kyung Hee University, Seoul, Korea, Republic of, [2]Akrocell Biosciences, Inc., Seoul, Korea, Republic of, [3]School of Medicine, Kyung Hee University, Seoul, Korea, Republic of, [4]Kyung Hee University, Seoul, Korea, Republic of

For the reconstruction of functional fibrocartilage tissue, induction of ECM alignment and cartilaginous matrix production is essential. Although tensile stimulation is the most effective approach to induce

tissue alignment, recent studies showed that mechano-transduction interferes with the upstream signaling pathway of chondrogenesis. To overcome the anti-chondrogenic effect exerted by mechanical stimulation, we established an optimized fibrocartilage formation protocol by investigating the effect of tensile stimulus mechanical dose on meniscal chondrocytes (MCs). To induce tissue alignment, cable-form tissues constructed with MCs were subjected to 10% static tension for various duration. The state of mechano-transduction was confirmed by YAP nuclear translocation status. We confirmed that mechanical memory in MCs, both induced by surface stiffness and tensile stimulation, did not show irreversibility and lasted less than 24 hours based on YAP localization status. The established uni-axial tissue anisotropy and its retention was confirmed with alignment of actin cytoskeleton and type I collagen. The mechanical stimulation applied for more than 7 days resulted in the maintenance of tissue anisotropy up to 5 days after the tension release. Applying tensile stimulation only for initial 7 days of 3-week chondrogenesis resulted in production of fibrous matrix as well as GAGs matrix, while continuous static tension compromised cartilaginous matrix production. In conclusion, these findings suggest that the optimization of tensile dose can modulate the characteristics of cartilaginous matrix production, which can lead to the successful reconstruction of fibrocartilage in its similarity to natural tissues.

B535/P2209

Phosphoinositide Metabolism by Actin-Regulatory Protein in Cancer

A. Orenberg[1], D. Gau[1], L. Ohayon[1], M. Ricci[1], Y. Bae[2], G. Hammond[1], P. Roy[1]; [1]University of Pittsburgh, Pittsburgh, PA, [2]University of Buffalo, Buffalo, NY

Profilin1 (Pfn1) is an actin polymerization regulatory protein that conventionally promotes cell migration in physiological contexts. Yet, in breast cancer and several other invasive adenocarcinomas, Pfn1 downregulation leads to increased migration and invasion of cancer cells. We previously discovered that increased accumulation of plasma membrane phosphoinositide (PPI) $PI(3,4)P_2$ (a PPI generated downstream of activated PI3-kinase) at the leading edge and subsequent recruitment of $PI(3,4)P_2$-interacting pro-migratory protein complexes is responsible for the hyper-motile phenotype of breast cancer cells during Pfn1 depletion. The aim of this study is to gain fundamental molecular insights into the Pfn1-dependent regulation of PPI metabolism in cells. We combined biochemical techniques, immunofluorescence, and live-cell imaging of PPI reporters to investigate the effect of perturbation of Pfn1 on $PI(3,4)P_2$ dynamics and upstream PPIs including PIP_3 and $PI(4,5)P_2$. Our knockdown and overexpression studies collectively demonstrated that Pfn1 positively and negatively regulates the cellular levels of $PI(4,5)P_2$ and PIP_3, respectively, but does not affect phospholipase C (PLC)-mediated hydrolysis of $PI(4,5)P_2$ in cells. Therefore, the elevation of PI3K-derived PPIs upon Pfn1 depletion is not a secondary consequence of alteration to $PI(4,5)P_2$ hydrolysis. Live imaging of a $PI(3,4)P_2$-specific fluorescent reporter demonstrated that Pfn1 depletion enhances $PI(3,4)P_2$ synthesis in cells. We are currently exploring whether Pfn1 plays a role in modifying the activity of lipid-modifying enzymes to enrich our understanding of the unconventional functions of Pfn1 beyond its direct regulation of actin cytoskeletal rearrangement.

B536/P2210

From Single-Protein to Single-Cell: a unique platform that combines optical tweezers and fluorescence microscopy for the study of Cytoskeletal Processes and Cell Mechanics

S. Dutta[1], T. Simpson[1], B. Groen[2]; [1]Lumicks B.V., Waltham, MA, [2]Lumicks B.V., Amsterdam, Netherlands

Cells and protein filaments such as microtubules, actin and intermediate filaments are highly dynamic structures that constantly interact with each other and their surroundings. The forces governing these interactions play fundamental roles in essential biological processes like cell division, motor transport, protein signaling or cell migration. Therefore, being able to study the mechano-chemical and mechano-biological pathways governing these interactions is essential to better understanding their nature. Force spectroscopy on a single-molecule and single-cell level permits exploring and manipulating these complex interactions. Our force-spectroscopy platform, the C-Trap, integrates optical tweezers, fluorescence microscopy, and an advanced microfluidics system in a truly correlated manner. It enables live, simultaneous, and correlative visualization and manipulation of molecular interactions for a wide range of forces (from picoNewtons to a nanoNewton) and with high temporal resolution (microseconds). Here we present our experiments visualizing and quantifying the elastic properties of protein filaments, the motility of cytoskeletal molecular motors, the characterization of forces exerted by filopodia, or the force-triggered activation of membrane receptors using the C-Trap system. These experiments show that the technological advances in hybrid single-molecule and single-cell methods can be turned into an easy-to-use and stable instrument that opens new venues in many research areas.

B537/P2211

Comparative Cell-Generating Tractional and Intracellular Force Analyses of Acrylamide-Based And Methacrylated Gelatin-Based Techniques for Understanding the Mechanotransduction

S. Hur[1], J. Jeong[1], K. Noh[1], J. Park[2], J. Chung[2], Y. Hwang[1]; [1]Soonchunghyang Institute of Medi-Bio Science (SIMS), Soonchunhyang University, Cheonan, Korea, Republic of, [2]Soonchunhyang University Cheonan Hospital, Cheonan, Korea, Republic of

Cell-generating tractional and intracellular forces within physiological and pathological microenvironments and their transduction to regulate various cell behaviors have been of great interest. Although these mechanical forces exerted cells have been traditionally analyzed by utilizing polyacrylamide (PAAm) hydrogel-based traction force microscopy (TFM) due to its good linear elastic and transparent optical properties, PAAm hydrogel was failed to support three-dimensional (3D) cell encapsulation to understand the cellular forces within 3D microenvironment. Here, we aim to develop a novel method to assess cell-matrix (traction) and intracellular (and intercellular) stresses using an alternative candidate, methacrylated gelatin (GelMA), to overcome the current limitations. Our preliminary results demonstrated that viscoelastic and elastic properties of both PAAm and GelMA-based hydrogels were successfully matched, which were confirmed by measuring Young's modulus, dynamic modulus (G', G'', and tanδ), stress relaxation, and strain revery. Next, we evaluated various cell-generatiing forces of either tonsil-derived stem cells in a single cell level or conjunctiva-derived epithelial cells as a monolayered cell sheet using GelMA-based traction force microscopy (TFM), intracellular force microscopy (IFM), and monolayer stress microscopy (MSM), where the results were also compared and validated using PAAm-based methods. Our initial findings indicate that our GelMA-based TFM, IFM, and MSM methods exhibited cell-generating forces and mechanotransduction in a spatio-temporal manner. Taken together, these results suggest GelMA can be a good candidate for the cell-ECM and intracellular

stress measurement platform and this technique can offer insight into the important roles of dynamic cell-matrix and intracellular stresses in regulating stem cell lineage commitment and disease progression within 3D microenvironments.

B538/P2212

Septin characterization in neonatal cardiomyocytes

A. Vallopra[1], S. Balasubramanian[1], E. T. Spiliotis[2], A. V. Kwiatkowski[1]; [1]University of Pittsburgh, Pittsburgh, PA, [2]Drexel University, Philadelphia, PA

Cardiomyocyte function requires precise, dynamic, and carefully regulated organization of the proteins and organelles within them. The non-contractile cytoskeleton in cardiomyocytes - including cortical actin, microtubules, and intermediate filaments - helps establish and maintain this complex organization. In developing cardiomyocytes, the septin cytoskeletal family of proteins has also been implicated in regulating cellular organization. Septins function in multiple biological processes, including cell division, cell polarity, and membrane remodeling. Septins are expressed in the developing and adult heart; however, their role in cardiomyocyte organization is not well understood. Here we describe our efforts to characterize septins (SEPT)-2,6,7,9 and 11 expression and localization in cultured cardiomyocytes. Since changes in substrate stiffness can impact cardiomyocyte organization, we cultured neonatal cardiomyocytes on PDMS (polydimethylsiloxane) gels of varying stiffnesses (soft to stiff) and coated with either fibronectin or collagen I to create a range of microenvironments. Cardiomyocytes were cultured for 72 hours, fixed, and stained using specific anti-septin antibodies. SEPT7 and SEPT6 localized to discreet areas along the membrane, whereas SEPT11 was largely cytoplasmic. Notably, SEPT6 was present in the nuclei of most, but not all, cardiomyocytes and the only septin that showed nuclear localization. On the collagen I substrates, we observed similar septin localization patterns across all tested stiffnesses. Our results show that multiple septins are expressed in neonatal cardiomyocytes with unique and specific localization patterns that are largely unaffected by substrate stiffness. We speculate that septin membrane and nuclear localization reflects multiple roles in cardiomyocyte organization.

B539/P2213

Plasma Membrane Damage Contributes to Syncytia Formation after Laser Ablation

J. Hua, J. T. O'Connor, A. D. Pumford, L. Nassar, J. White, M. Hutson, A. Page-McCaw; Vanderbilt University, Nashville, TN

Restoration of tissue integrity after injury is essential to the survival of all organisms. We use *Drosophila* pupal notum damaged by laser ablation as a model system to study the wound healing mechanisms. Starting about 10 minutes after laser ablation, mononuclear cells of the pupal epidermis fuse and form multinucleated syncytia to assist with re-epithelialization. The objective of this study is to investigate the mechanisms of wound-induced syncytia formation.

All trauma wounds generate a mixture of damage, but pulsed laser ablation creates a highly reproducible pattern of cellular damage and response. An expanding cavitation bubble is generated within microseconds after wounding, exerting shear mechanical force on cells under its footprint, damaging their plasma membranes and allowing an influx of extracellular calcium within milliseconds after ablation. Concentric to the zone of plasma membrane damage is the area of nuclear membrane damage and cell lysis. Interestingly, syncytia form only after this type of ablation, called single-shot

ablation, during which the notum is hit once by a high-power pulsed laser; syncytia are absent after scanning ablation, when cells are destroyed by a low power laser scanning multiple times over a selected area of cells. Using Ca^{2+} entry as a proxy for plasma membrane damage in single-shot ablation, we reveal that approximately 96% of fused cell borders co-localize with traces of Ca^{2+} captured as early as 30 ms after wounding, and all fused cell borders are surrounded by elevated level of Ca^{2+} within 60 ms of single-shot ablation. In contrast, scanning ablation does not induce plasma membrane damage, as demonstrated by the voltage indicator ArcLight. Surprisingly, overexpression of the TMEM16F scramblase does not enhance wound-induced syncytia formation, suggesting that altered phosphatidylserine exposure pattern on the outer cell leaflet is not contributing to syncytia formation. We conclude that plasma membrane damage primes wound-induced cell fusion.

Dynamics of Focal Adhesions and Invadosomes

B540/P2214

Regulating mechanical properties of focal adhesion through protein liquid liquid phase separation

X. Cheng, L. Case; Biology, Massachusetts Institute of Technology, Cambridge, MA.

Integrin based focal adhesions (FA) are supramolecular complexes at the cell surface that are important for cell adhesion. They anchor the cell to the extra cellular matrix through transmembrane integrin receptors. Talin, vinculin and tensin are present at mature focal adhesions and have important function for linking integrin to the actin cytoskeleton. Despite past discoveries on the genetic and biochemical level, how the protein composition of focal adhesions contribute to the physical property of focal adhesion is not well understood. Recent studies have described certain proteins possess the innate property of going through liquid-liquid phase separation in the cytoplasm, creating mesoscale biomolecular condensates. These condensates form chemical compartments that gives rise to many membraneless organelles. Studies from my lab have describe that several nascent focal adhesion proteins phase separate. With the correct protein composition, we can in vitro reconstitute minimal nascent FA condensates that can cluster integrin on supported lipid bilayers. The multivalency of vinculin binding sites in talin, as well as the large intrinsic disordered region in tensin make them great candidates for phase separation. Overexpression of constitutively active vinculin or tensin lead to cytoplasmic condensates formation, confirming their ability to go through phase separation. These condensates contain focal adhesion kinase and paxillin, both are FA proteins essential to the formation of minimal nascent FA condensates. We hypothesize that the recruitment of talin, vinculin and tensin to the focal adhesion increase the physical strength of the FA condensate, making it able to bear stronger force. To study the mechanical property changes of adhesion condensates, I will introduce these proteins to reconstituted nascent FA condensates to form compositional mature FA condensates. This technique is a significant technical advancement that allows us to precisely control the amount and timing for recruitment, also enables me to use biophysical approaches such as microrheology to analyze the material property of these condensates. I will confirm my results by expressing talin, vinculin and tensin variants used for reconstitution in live cells, and measure FA number, size, protein dynamics and cell migration changes. These are important experiments linking in vitro observations to in vivo physiological events. Together, my results will shed light on how the physical properties of protein compartments lead to specific cellular phenotype.

B541/P2215

Substrate Stiffness Regulates Focal Adhesion Kinase Alternative Splicing in Breast Cancer

M. Millet, E. Pic, C. Luthold, G. Bourriquen, G. Khelifi, B. Trudel, S. Hussein, F. Bordeleau; Université Laval, Québec, QC, CANADA.

Altered alternative splicing is considered as a hallmark of cancer. In fact, alternatively splice protein variants can differentially influence cellular processes such as cell migration and invasion. We have shown previously that increased matrix stiffness in tumors regulates alternative splicing events through increased cell contractility. Interestingly, the focal adhesion kinase (FAK), a protein central to matrix stiffness mechanosensing in focal adhesion, has several isoforms that promote tumor progression. In this context, we hypothesized stiffness-mediated alternative splicing could regulate the expression of FAK splice variants and influence tumor progression. We first investigated FAK mRNA splicing as a function of matrix stiffness using RNAseq. Our data identified stiffness-mediated alternative splicing events in FAK exon 4 exclusion (FAK^{4-}). PCR data from *in vitro* experiments confirmed FAK^{4-} expression was modulated by substrate stiffness, reaching a maximum at around 1, 5 and 10 kPa in MCF10a, MCF7 and MDA-MB-231 cells respectively. Of note, FAK^{4-} expression at these stiffnesses was reduced upon contractility inhibition. To investigate FAK^{4-} clinical relevance, we characterized FAK^{4-} expression as function of the tumor stage as a proxy of tumor stiffness in patients (TCGA). Notably, we found that patients with higher proportions of FAK^{4-} had significantly worst survival odds than patient with lower FAK^{4-} expression. Finally, preliminary data suggest FAK^{4-} expression is regulated by tumor stiffness. Taken together, our data revealed FAK alternative splicing is regulated by matrix stiffness both in patients and *in vitro*. Moreover, the stiffness at which the maximum FAK^{4-} expression is observed is higher in the more invasive MDA-MB-231 cells. Overall, our results show FAK splicing is modulated by tumor mechanics, raising the possibility of an existing interplay between stiffness-driven alternative splicing and FAK-mediated mechanoregulation. Importantly, our data suggest FAK^{4-} could have an important clinical significance.

B542/P2216

A hard path to follow: The LKB1-AMPK axis in mechanosensing and durotaxis

A. K. Howe, J. B. Patterson; Pharmacology, University of Vermont, Burlington, VT.

Cellular metabolism is regulated at the scale of subcellular space and this regulation can couple to structures and events involved in cell migration. Migration is a complex process and is directionally guided by various types of gradients - concentration gradients of diffusible factors drive chemotactic migration while concentration gradients of immobilized extracellular matrix cues drive haptotactic migration. Previously, our laboratory demonstrated that mitochondria are recruited to the leading edge of migrating cells in manner that couples lamellipodial dynamics to local energy status and that is dependent on AMPK, a master sensor and regulator of metabolism. In addition, silencing the expression of LKB1, the major upstream activator of AMPK, decreases the directionality of migrating cells. Recently, we've have investigated whether metabolism also contributes to mechanosensing and mechanically-gated cell migration, known as durotaxis. We've found that mitochondria are recruited towards acute durotactic stretch. Moreover, LKB1 is locally activated in response directional increases in cell-ECM tension. Finally, silencing LKB1 expression disrupts Golgi reorientation and polarization in cells plated on substrates with graded rigidity and inhibits directional, durotactic cell migration. Thus, LKB1, a nutrient-sensing signaling hub, also senses mechanical cues and is required for proper durotaxis. These

observations provide an impetus and a foundation to further explore the molecular mechanisms linking metabolism, migration, and mechanobiology.

B543/P2217

T cells switch between integrin-dependent and integrin-independent migration modes to migrate in complex environments

A. Caillier[1], D. Oleksyn[2], J. F. Miller[2], P. W. Oakes[1]; [1]Cell & Molecular Physiology, Loyola University Chicago, Maywood, IL, [2]Program for Advanced Immune Bioimaging, University of Rochester Medical Center, Rochester, NY.

Lymphocyte migration is essential to ensure a quick and efficient immune response. As they screen the body for antigen presenting cells, they are exposed to different complex environments composed of various extracellular matrix (ECM) components, architectures, and density. While previous work in vitro has shown that immune cells can migrate without integrin-dependent adhesion, other studies in vivo have shown that integrin-dependent adhesion is required to induce the migration of lymphocytes. This suggests that lymphocytes have multiple mechanisms of migration and can adapt to their environment, fluctuating between integrin-independent to integrin-dependent migration as needs of the environment demand.

Using primary t cells, we find that confinement is necessary to induce rapid and robust migration, independent of ECM composition. The presence of ECM proteins, however, does lead to a subtle but significant difference in migration speed and persistence. We also find in the presence of ECM proteins, T cells form focal adhesions that include clusters of integrins and adaptor proteins such as vinculin. When T cells are confined between two deformable surfaces, we can see that focal adhesions coincide with regions of traction stress as measured using Traction Force Microscopy. We find that traction stresses are exerted on both surfaces, though on occasion cells will pull only on one side, while pushing the other surface out of the way. Finally, using micropatterned surfaces we find that cells can switch between integrin-independent to integrin-dependent migration modes depending on whether the ECM coating is present.

Together these data illustrate that t cells are able to effortlessly switch between integrin-dependent and integrin-independent migration to maintain efficient migration, navigate a wide range of complex environments, and facilitate effective an immune response.

B544/P2218

Elucidating Ca^{2+} effects on cell migration by resolving Ca^{2+}-adhesion interactions

H. Chen; National Taiwan University, Taipei, TAIWAN.

<META NAME="author" CONTENT="厚任 陳">Ca^{2+} is important for cell migration. However, whether Ca^{2+} augments or suppresses cell migration has remained controversial. One possibility is that Ca^{2+} help focal adhesion (FA) dynamics by improving both formation and degradation of FA complexes. Altering Ca^{2+} changes the balance between formation and degradation of FA, resulting in the increase or decrease of cell migration depending on basal adhesion status within the cell. Based on this hypothesis, we developed a mathematical model to predict cell migration using its levels and activities of Ca^{2+} and adhesion proteins. Then we used several cell lines to verify this model by perturbing their Ca2+ and adhesion molecules. Our preliminary data supported the validity of this model in head and neck cancer SAS cells, and in ovarian cancer JHOC-9 and OVTOKO cells, under genetic and pharmacological

perturbations of store-operated Ca^{2+} entry, ROCK and paxillin. We are now working on the examination of FA dynamics in these cell lines under various perturbations of Ca^{2+} and adhesion, with the ultimate goal to build an unified model explaining Ca^{2+} effects on cell migration.

B545/P2219

Tks5 promotes lamellipodia and invadopodia formation to drive cancer metastasis

H. JUI-YU; National Taiwan University, Taipei, TAIWAN.

The malignant tumor is notorious for the abnormal growth and invasion into nearby tissue of cancer cells. It has been reported that Tks5, a critical scaffold protein of invadopodia, is highly expressed in many types of cancer cells. Tks5 binds to PI(3,4)P2 and interacts with many proteins responsible actin polymerization, leading to the speculation that Tks5 may have functions other than promote invadopodia formation, critical for cancer cell growth and metastasis.In this study, we found that Tks5 localizes not only to invadopodia, but also at lamellipodia, which is a branched actin-rich structure and is important for cell migration. To decipher Tks5 function in cancer cells, firstly we knockdown Tks5 in HT1080 and found a decrease of invadopodia and lamellipodia area. Similar effects were observed in cells stablely expressing or overexpression of Tks5 SH3A and SH3E domain in HT1080. Moreover, we demonstrate that the stably expressing or treatment of SH3 penetrating peptides can reduce migration velocity and extracellular matrix degradations well as their invasion ability. Our preliminary result also show that Tks5 is required for anchorage-independent growth of cancer cells. Taking together, we anticipate that Tks5 individual domain can compete against endogenous Tks5 for their binding partners and disturb the branch actin polymerization in both lamellipodia and invadopodia, and that makes treating cancer cell with Tks5 individual domain a potential therapeutic way to deal with invasive cancer cells.

B546/P2220

Pressure and stiffness sensing together regulate vascular smooth muscle cell phenotype switching

P. Swiatlowska[1], B. Sit[1], Z. Feng[2], X. Zhou[3], Q. Xiao[3], C. Shanahan[4], C. Yu[2], **T. Iskratsch**[1]; [1]School of Engineering and Materials Science, Queen Mary University of London, London, UNITED KINGDOM, [2]Hong Kong University, Hong Kong, HONG KONG, [3]Queen Mary University of London, London, UNITED KINGDOM, [4]King's College London, London, UNITED KINGDOM.

Atherosclerosis is considered the major underlying cause of cardiovascular diseases. Vascular smooth muscle cells (VSMCs) play a central role in the onset and progression of atherosclerosis. In pre-atherosclerotic lesions, VSMCs switch from a contractile (healthy) to a synthetic (disease) phenotype and subsequently remodel the microenvironment, leading to further disease progression. Several factors have been investigated that regulate this process, but the impact of the mechanical forces acting on the VSMCs has not been studied closely. However, especially, ageing and associated mechanical changes of the extracellular matrix - as well as hypertension are major risk factors for atherosclerosis. Consequently, we sought here to systematically study the impact of mechanical forces on VSMC phenotypic switching, by modulating individually and in combination the stiffness and hydrodynamic pressure. Phenotypic changes of primary human and bovine VSMCs and the A7r5 VSMC line were evaluated using quantitative proteomics, cell morphological and functional analysis (e.g. extracellular matrix degradation), as well as cell mechanical testing. Additionally, we confirmed our findings, using a mouse neointima model (after complete ligation of the left common carotid artery (LCCA)), where we

compared disease state, local tissue stiffness and protein expression and activity pattern.
Thereby, we surprisingly find that hemodynamic pressure and matrix compliance individually affect the VSMCs. However, only the combination of hypertensive pressure and matrix compliance result in a full phenotypic switch that leads to extensive cytoskeletal changes, podosome formation, extracellular matrix remodelling, and overall included 123 differently regulated proteins related to atherosclerosis, cytoskeletal organisation and podosome formation.
We further analyse the molecular mechanism in stiffness and pressure sensing and identify a regulation through different pathways, both converging on the same effector protein, cofilin. Downstream of stiffness sensing, the RhoA-ROCK-LIMK signalling pathway leads to increased cofilin activity, while on the other hand, hydrodynamic pressure induces podosome formation through an increase in intracellular Ca^{2+} concentration that regulates slingshot-dependent cofilin phosphorylation. Using knock down, small molecule inhibitor and activator treatment, we further find that the mechanosensitive Ca2+ influx depends on Piezo1 and activation of Piezo1 directly affects the phenotypic switching.
Altogether, our findings show that pressure and stiffness sensing regulate the VSMC phenotype through different pathways that converge on cofilin, ultimately promoting a switch to a synthetic phenotype in diseased conditions.

B547/P2221

Lipid-driven 2D phase separation of focal adhesion proteins

T. Litschel[1,2], C. Kelley[2], P. Schwille[2]; [1]Harvard University, Cambridge, MA, [2]Max Planck Institute of Biochemistry, Martinsried, Germany

Focal adhesions form liquid-like assemblies around activated integrin receptors at the plasma membrane. Made up of hundreds of proteins, focal adhesions are dynamic structures which can assemble and disassemble quickly, withstand strong actomyosin-applied forces, and form highly stable complexes. How they achieve these flexible characteristics is not well understood. Here, we use recombinant focal adhesion proteins to reconstitute the core structural machinery in vitro, with the goal of understanding the underlying protein dynamics and interactions. We observe liquid-liquid phase separation of the core focal adhesion proteins talin and vinculin for a spectrum of conditions and in combination with several interaction partners. Intriguingly, we show that membrane binding triggers phase separation of these proteins adjacent to the membrane, which in turn induces the enrichment of integrin in the clusters. We also introduce a novel experimental setup to probe talin-membrane interactions down to the single protein level. Our results suggest that membrane composition triggers condensate assembly at the membrane, a regulatory mechanism which could widely apply to membrane-localized biomolecular condensates and provide a pathway of how spatial organization of lipids within the membrane can couple into the cytosol.

B548/P2222

TIRF FRET Imaging of Adhesion ERK Activity

A. Shepherd, M. Mendoza; University of Utah, Salt Lake City, UT

Cell migration is a fundamental cellular process essential for development and wound healing. Unregulated cell migration also drives cancer metastasis. Most epithelial tumor cells move via mesenchymal model cell migration. Mesenchymal-mode migration begins with the advancement of the lamellipodia, where actin polymerization pushes against the cell edge and adhesions transmit traction

against the substrate as membrane tension increases. Extracellular signal-regulated kinase (ERK) has been found to control actin assembly and adhesion turnover, the key mechanisms of cell migration. Our objective in this project is to understand how fluctuations in ERK activity control adhesion dynamics. To do this, we developed a targeted FRET biosensor to detect ERK activity within nascent and focal adhesions. We have shown that the novel EKAREV-FAT biosensor localizes to adhesions. We found that EKAREV-FAT can be used to measure ERK FRET activity in adhesions in live cells using TIRF imaging. The probe responds to ERK activation and inhibition. We have determined the biosensor detects ERK activity on a timescale relevant to adhesion assembly/disassembly and protrusion/retraction of the lamellipodia. We have tracked and quantitatively analyzed adhesions, allowing us to classify adhesion size, turnover, orientation, and proximity to the edge while also measuring the FRET signal from each adhesion. By probing the spatially localized kinase activity signal, we will better understand how different signals to the actin cytoskeleton and adhesions are integrated during cell migration.

Cadherins and Cell-cell Interactions

B550/P2223

Junctional endothelial F-actin bundles regulate size-selective permeability of the vascular barrier

M. Gronloh, W. VanderMeer, A. VanStalborch, J. VanRijssel, **J. Van Buul**; Amsterdam UMC, Amsterdam, NETHERLANDS.

At the interface of the circulation and the underlying tissue, the vascular endothelium forms a semi-permeable barrier that allows the regulated passage of small solutes, macromolecules, and immune cells. However, many cardiovascular and inflammatory diseases are characterized by reduced vascular integrity, demonstrated by reduced barrier function, leading to organ failure and ultimately death. To restore vascular integrity, intervention is directed to promote endothelial cell-cell junctions, although it is not clear how junction stability is regulated. Here we show that junction stability of endothelial cells is regulated by tensile junctional F-actin bundles, resulting in linear junction phenotype and increased barrier function. This increase in the barrier is independent of VE-cadherin. Mechanistically, we show that tight junction proteins Claudin-5 and ZO-1 are recruited by the guanine nucleotide exchange factor (GEF) Trio in a Rac1 and RhoG-dependent manner, to stabilize cell-cell junctions and regulate the passage of small molecules specifically. We propose that by bringing opposing membranes in close contact, these tensile actin-rich bundles support tight junctional pore assembly and the formation of a semi-permeable, size-restricted vascular barrier.

B551/P2224

Podoplanin (PDPN) Enables Transformed Cells to Migrate and Escape Growth Control Mediated by Cadherin Junctions with Adjacent Nontransformed Cells

E. P. Retzbach[1], S. A. Sheehan[1], H. Krishnan[2], Y. Shen[1], J. P. Trama[3], H. Zheng[4], **G. S. Goldberg**[1]; [1]Rowan University, Stratford, NJ, [2]Stony Brook University, Stony Brook, NY, [3]Medical Diagnostic Laboratories, Hamilton, NJ, [4]Rutgers University, Piscataway, NJ.

Oncogenic Src tyrosine kinase activity induces PDPN expression to promote cell migration. However, effects of Src in the absence of PDPN expression has remained unexplored. We analyzed the effects of an inducible Src kinase construct on migration and anchorage independent growth of cells with and without PDPN expression. These data indicate that Src promotes anchorage independent cell growth in

the absence of PDPN expression. However, Src is not able to promote cell migration in the absence of PDPN expression. Phosphoproteomic analysis identified 28 proteins that are phosphorylated in Src transformed cells in a PDPN dependent manner. In addition, continued Src kinase activity is required for cells to assume a transformed morphology since cells reverted to a nontransformed morphology upon cessation of Src kinase activity. Nontransformed cells can also normalize the growth and morphology of neighboring Src transformed cells. Transformed cells must escape this process, called "contact normalization", to become invasive and malignant. However, junctions responsible for this process have not been defined. We found that N-cadherin (N-Cdh) can mediate contact normalization. Cells that express N-Cdh inhibit the growth of neighboring transformed cells in culture, while cadherin deficient cells do not inhibit the growth of these cells. Results from RNA-seq analysis indicate that about 10% of the transcripts affected by contact normalization rely on cadherin mediated communication, and this set of genes includes PDPN. In contrast, cadherin deficient cells do not inhibit PDPN expression or normalize the growth of adjacent transformed cells. These data indicate that nontransformed cells form heterocellular cadherin junctions to inhibit PDPN expression in adjacent transformed cells. Moreover, cadherin competent cells fail to normalize the growth of transformed cells expressing PDPN under a constitutively active exogenous promoter. Therefore, PDPN enables transformed cells to override contact normalization in the face of continued N-Cdh expression. Taken together, these results indicate that nontransformed cells form cadherin junctions with adjacent transformed cells to decrease PDPN expression in order to inhibit tumor cell proliferation, and that PDPN enables transformed cells to migrate and overcome growth inhibition by contact normalization.

B552/P2225

Translational Regulation by Desmosomes

D. Hlavaty[1], K. Badu-Nkansah[1], G. Bjerke[2], R. Yi[3], T. Lechler[1]; [1]Duke University, Durham, NC, [2]University of Colorado Boulder, Boulder, CO, [3]Northwestern University, Evanston, IL.

Desmosomes are cell-cell adhesion complexes that provide tissues with mechanical integrity. Desmosomal disruption results in severe blistering in the epidermis as well as cardiomyopathies. In addition to their canonical adhesive function, desmosomes have many emerging non-canonical functions such as regulating diverse signaling pathways and integrating cytoskeletal networks in keratinocytes. Recent work from our lab revealed a surprising recruitment of both translational machinery and the RNA-induced silencing complex (RISC) to the cell cortex by desmosomes. Using two distinct techniques, we observed that the cell cortex is a site of active translation in keratinocytes and that this localized translation requires desmosomes. We found that mRNAs for translational machinery were also enriched at the cell cortex. These transcripts share a 5'TOP motif, which can be bound by LARP1, a novel desmosome component also identified by our proteomics. We additionally identified the RISC-associated mRNAs using CLEAR-CLIP analysis which showed an enrichment for genes involved in wound healing and cell adhesion. Further, both in vitro and in vivo wound healing assays reveal that the RISC becomes depleted from the cell cortex of keratinocytes near the site of injury. Our results define a new function for desmosomes in translational regulation and suggest that they may act as sensors of adhesion integrity which can directly influence the cell's translatome to quickly restore homeostasis when it is disrupted.

B553/P2226

Epidermal Stratification Requires Retromer-Mediated Desmoglein-1 Recycling

M. Hegazy[1], J. L. Koetsier[1], A. L. Huffine[1], J. A. Broussard[1], B. M. Godsel[1], E. Cohen-Barak[2], E. Sprecher[3], L. M. Godsel[1], K. J. Green[1]; [1]Northwestern University, Chicago, IL, [2]Emek Medical Center, Afula, ISRAEL, [3]Tel Aviv Medical Center and Tel Aviv University, Tel Aviv, ISRAEL.

Sorting transmembrane cargo is essential for tissue development and homeostasis. However, mechanisms of transmembrane sorting and trafficking during the development and regeneration of the stratified epidermis are not well characterized. Here, we describe a novel role for the endosomal trafficking complex, the retromer, in regulating epidermal differentiation and stratification. Through a BioID screen, we identified an interaction between the retromer component, VPS35, and the desmosomal cadherin, Desmoglein-1 (Dsg1), and validated this interaction through proximity ligation assay and co-immunoprecipitation. Dsg1 is first expressed in basal keratinocytes as they commit to differentiate and transit into the superficial layers of the epidermis. We previously showed that when properly localized on the plasma membrane of basal keratinocytes, Dsg1 promotes stratification. Here we show that VPS35 promotes recycling of Dsg1 from the endo-lysosomal system to the plasma membrane to promote basal keratinocyte transit into the next superficial layer. Increasing retromer stability and function with a small molecule chaperone, R55, enhanced retromer association with and plasma membrane localization of Dsg1 and a trafficking-deficient Dsg1 mutant that results in a systemic inflammatory condition called Severe dermatitis, multiple Allergies, and Metabolic wasting (SAM) syndrome. R55 also enhanced the ability of SAM-Dsg1 to induce stratification. Using a Dsg1 knockout mouse and SAM patient skin, we found Dsg1 disruption is associated with increased glucose transporter 1 (GLUT1) plasma membrane localization and retromer association. GLUT1 is a well known retromer cargo that regulates proliferation in basal keratinocytes and is upregulated in the inflammatory skin disease, psoriasis. Dsg1 overexpression results in the cytoplasmic accumulation of GLUT1 raising the possibility that the onset of Dsg1 expression during epidermal differentiation may initiate a switch in retromer cargo trafficking to decrease GLUT1 activity. Our work provides the first evidence for retromer function in epidermal regeneration, identifying it as a potential therapeutic target for epidermal diseases.

B554/P2227

Novel Mechanosensitive Junction Interactor Gish is Required for Apical Constriction and Epithelial Folding

R. Koran, M. Weng; University of Nevada Las Vegas, Las Vegas, NV.

Adherens junctions, the Cadherin-based cell-cell junctions that resist physical tension, often mediate mechanosensitivity during morphogenesis. During the internalization of Drosophila mesoderm, contractile actomyosin on the apical cortex drives apical constriction and epithelial folding. Strong adherens junctions are required in this process to connect actomyosin in individual cells into a supracellular network as well as protect tissue integrity. Our previous studies show that adherens junctions not only connect to but also are strengthened by actomyosin contraction, through growing in packing density and size. However, the molecular mechanism that mediates this junction strengthening is unknown. We have identified Drosophila casein kinase I gamma, Gilgamesh (Gish), as a potential mechanosensitive junction interactor. We found that in the resting state, Gish uniformly localizes to cell membrane. However, upon apical myosin activation in mesoderm cells, Gish appears to be recruited to

junction clusters. Myosin knockdown abolishes such cluster recruitment of Gish, while ectopic myosin activation is sufficient to recapitulate the recruitment. Gish cluster localization also depends on adherens junctions, since Gish can no longer be recruited into clusters in mutant embryos that lose core junction components such as alpha-catenin. Importantly, in Gish mutants, adherens junctions in the mesoderm cannot form large clusters in response to apical myosin contraction and appear to be diffuse on the apical surface. Additionally, large quantities of membrane tethers and blebs accumulate on the apical surfaces of these mesoderm cells. At the tissue levels, apical constriction cannot occur efficiently, leading to failure of epithelial folding. These data suggest Gish is a mechanosensitive junction interactor essential for epithelial tissue folding.

B555/P2228

Investigating the Interaction of Inflammation-Associated Fibroblasts (IAFs) with Colon Epithelial Cells in Inflammatory Bowel Disease (IBD)

Y. Dong, B. Johnson, T. Larman, R. Li; Johns Hopkins University School of Medicine, Baltimore, MD.

Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease that affects the epithelial lining of the gastrointestinal tract. Recent single cell RNA-sequencing (scRNA-seq) studies suggested that inflammation-associated fibroblasts (IAFs) play important roles in the disease pathogenesis and drug resistance. To elucidate IAF's role in IBD, we isolated fibroblasts from patients with IBD and profiled their responses to a panel of pro-inflammatory cytokines. We found a cocktail of the cytokines can induce IAF marker genes in the patient-derived fibroblasts. These cytokine-stimulated fibroblasts recapitulate many characteristics of IAFs, including increased ECM and chemokine secretion. Furthermore, when co-cultured with colon organoids, the cytokine-stimulated fibroblasts induce phenotypic changes in epithelial cells that mimic disease-related features of IBD. We are currently investigating the mechanisms underlying the observed IAF-epithelial cell interaction and will further validate the in vitro observations using biospecimens obtained from IBD patients.

B556/P2229

Mechanosensitive recruitment of Vinculin maintains junction integrity and barrier function at tricellular junctions in vertebrate epithelia

L. Van Den Goor, J. Iseler, A. L. Miller; Molecular, Cellular, and Developmental Biology, University of Michigan, University of Michigan, MI.

Epithelial tissues are critical for generating compartmentalized barriers to ensure proper organ function and prevent pathogen invasion. Apical cell-cell junctions, including adherens junctions (AJs), and tight junctions (TJs), must adhere cells to one another and regulate selective permeability at both bicellular junctions (BCJs) and tricellular junctions (TCJs). Although several specialized proteins are known to localize at TCJs, it remains unclear how actomyosin-mediated tension transmission at TCJs contributes to maintenance of junction integrity and barrier function at these sites. Here, we show that at baseline tension in the *Xenopus laevis* embryonic epithelium, Vinculin is localized in three "spots" surrounding the TCJ. When tension is increased, Vinculin is mechanosensitively recruited to TCJs, and the spots expand to form three elongated "spokes" around the TCJ. Vinculin is known to reinforce the connection between AJs and actomyosin under high tension. As TCJs are sites of locally increased tension in epithelial sheets, we predicted that Vinculin's mechanosensitive recruitment at TCJs anchors actomyosin bundles, and this organization might be important for maintaining junction integrity at TCJs. We first

investigated this idea using fluorescence recovery after photobleaching (FRAP) of mNeon-Vinculin. At baseline tension, Vinculin is more stable at TCJs than BCJs. Under increased tension, Vinculin's stability at BCJs increases such that it is similar to Vinculin's stability at TCJs. Furthermore, in Vinculin knockdown (KD) embryos both junctional and medioapical actomyosin are decreased and disorganized at TCJs. Additionally, Angulin, a key protein for regulating barrier function at TCJs, exhibits reduced stability by FRAP in Vinculin KD embryos compared with controls, suggesting that Vinculin helps stabilize the TCJ and that barrier function could be affected when Vinculin is depleted. To assay Vinculin's role in epithelial barrier function, we used ZnUMBA, a live imaging barrier assay that detects barrier leaks with high spatiotemporal resolution. Our preliminary data reveals increased barrier leaks at TCJs in Vinculin KD embryos compared to controls. Together, these experiments indicate that mechanosensitive recruitment of Vinculin to TCJs is essential for maintaining junction integrity and barrier function at TCJs.

B557/P2230

On the Formation of Ordered Protein Assemblies in Cell-cell Interfaces

N. Boni[1], G. Wiseglass[1], L. Shapiro[2,3], B. Honig[2,3,4,5], Y. Wu[6], R. Rubinstein[1,7]; [1]School of Neurobiology, Biochemistry and Biophysics, Tel Aviv University, Tel Aviv, ISRAEL, [2]Zuckerman Mind, Brain and Behavior Institute, Columbia University, New York, NY, [3]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, [4]Department of Systems Biology, Columbia University, New York, NY, [5]Department of Medicine, Division of Nephrology, Columbia University, New York, NY, [6]Department of Systems and Computational Biology, Albert Einstein College of Medicine, Bronx, NY, [7]Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, ISRAEL.

During neural development, billions of neurons generate a complex network of interactions. Self-avoidance is one of the underlying mechanisms that support this process. Self-avoidance allows each neuron to self-recognize, thereby driving neurons to only interact with non-self neurons. In vertebrates, clustered protocadherins (cPcdhs) are a family of transmembrane proteins composed of approximately 60 different isoforms that mediate the self-avoidance process. Each neuron expresses a random set of approximately 10 isoforms. It is unclear how such a small set of isoforms allows self-recognition and non-self discrimination between billions of neurons. Recent studies suggest that an alternated homophilic interaction between cells (*trans*) and promiscuous interactions on the same cell (*cis*) results in the formation of one-dimensional zipper-like arrays of cPcdhs in the cell-cell interface. In this model, when both membranes express the same set of isoforms they form long one-dimensional zipper-like arrays. In contrast, a mismatch between those sets of isoforms prevents long zipper formation by chain termination. The objective of my research is to understand if these proposed zipper assemblies provide a platform with sufficient diversity required for neuronal self-avoidance. To accomplish this, I use a mesoscopic computer simulation that allows quick testing of numerous conditions. Our results indicate that the combination of *cis* and *trans* interactions leads to the formation of long zipper assemblies. Surprisingly, we found that zipper assemblies spontaneously stack into two-dimensional arrays. Such ordered organizations of proteins have been observed in previous studies that used cryo-tomography and our current results suggest that those two-dimensional arrays form without additional interaction sites. Since zipper-like assemblies have been observed in many other cell-adhesion proteins, these results might be of general relevance.

B558/P2231

SGEF, a RhoG specific GEF, regulates E-cadherin stability, turnover, and transcription in epithelial cells

A. F. Rabino, R. Garcia-Mata; University of Toledo, Toledo, OH.

The canonical Scribble polarity complex, comprised by Scribble, Dlg1 and Lgl, plays a key role in the regulation of epithelial junctions and apical-basal polarity. However, little evidence is available regarding the molecular mechanisms that control its function. We have recently shown that SGEF, a RhoG specific GEF, forms a ternary complex with Scribble and Dlg1, and functions in the regulation of epithelial permeability and adherens junction (AJ) formation. Interestingly, when we silenced SGEF expression in MDCK cells, we observed a striking reduction of E-cadherin protein levels. Here, we characterized the molecular mechanisms that control the SGEF-mediated regulation of E-cadherin expression. Our results show that the exchange activity of SGEF is essential for the regulation of E-cadherin levels. In addition, we demonstrate that the activity of SGEF needs to be targeted to the basolateral membrane to efficiently regulate E-cadherin expression. Additionally, we observe a Src-dependent increase in p120 catenin phosphorylation in the absence of SGEF, which has been previously associated with increased internalization of E-cadherin. Supporting these results, we show that E-cadherin protein levels can be rescued by inhibiting Clathrin mediated endocytosis in SGEF KD cells. E-cadherin downregulation in SGEF KD cells appears to be mediated in part by proteasomal degradation, as inhibiting the proteasome partially rescued this phenotype. However, degradation did not completely account for the severity of E-cadherin downregulation. Besides proteasomal degradation, E-cadherin transcription is drastically reduced SGEF KD cells and correlates with an upregulation of its transcriptional repressor Slug. Taken together, our results suggest that the interaction with the Scribble complex, targets SGEF to the basolateral membrane in epithelial cells, where it mediates the localized activation of RhoG. This in turn regulates the stability, endocytosis, and transcriptional regulation of E-cadherin, and thus the formation and stability of AJ.

B559/P2232

***Ctnnd2*controls layer-specific astrocyte morphogenesis by astrocyte-neuron cadherin interactions**

C. X. Tan[1], D. S. Bindu[1], N. Allen[2], P. Arlotta[3], C. Eroglu[1]; [1]Duke University, Durham, NC, [2]Salk Institute for Biological Studies, La Jolla, CA, [3]Broad Institute of MIT and Harvard, Cambridge, MA.

The brain is often perceived as a large network of interconnected neurons. A chemo-affinity code of cell adhesion molecules and diffusible cues specify which neurons synapse together to form circuits. However, the brain is composed of more than just neurons. Astrocytes are the most abundant glial cells of the brain. Astrocytes dramatically increase their morphological complexity during a critical window of cortical development overlapping with a period of peak circuit formation. A morphologically complex astrocyte has thousands of fine processes that can directly contact neurons and dynamically provide instructive cues for circuit formation, maturation, and function. However, little is known about the mechanisms underlying how astrocytes identify their specific neuronal partners. By conducting a candidate-based reverse genetic screen, we found that *Ctnnd2* (protein: δ-catenin) is expressed abundantly by astrocytes and is robustly required for neuron contact- dependent astrocyte morphological maturation. Prior to this, δ-catenin was widely assumed to be a neuron-specific protein. Knockdown of δ-catenin specifically in astrocytes results in a dramatic loss of astrocyte processes both *in vitro* and *in vivo*. Introduction of *CTNND2* autism mutations in rescue experiments implicated cadherin family of cell adhesion proteins to work upstream of δ-catenin. Cadherins are critical for circuit assembly

because they provide specificity information which enables correct matching of pre- and post-synaptic neurons. Our ongoing work is indicating that astrocytes utilize the neuronal cadherin code to establish astrocyte-neuron *trans*-interactions. These interactions are necessary for astrocyte morphogenesis in a cortical layer-specific manner. This study answers vital knowledge gaps in astrocyte biology by giving insight into the molecular mechanisms underlying astrocyte morphological complexity during development and how astrocyte-neuron interaction functionally specifies cortical heterogeneity. It also sheds light into the causes of synaptic defects and cognitive impairments observed in *CTNND2* autism mutations.

B560/P2233

Topographical analysis of immune-cell contacts reveals the segregation of mechanical activities across immunity

M. De Jesus[1], A. Settle[1], D. Vorselen[2], M. Galiano[3], E. Santosa[1], P. Shah[4], J. Theriot[2], M. Huse[1]; [1]Department of Immunology, Sloan Kettering Institute, New York, NY, [2]Department of Biology, University of Washington, Seattle, WA, [3]Department of Developmental Biology, Sloan Kettering Institute, New York, NY, [4]Department of Cell & Developmental Biology, University of California, Los Angeles, Los Angeles, CA.

Autonomously acting immune cells execute their protective functions across vastly different tissue architectures in the body. We hypothesized that this challenge is met in part by the segregation of efferent immune-mechanical activities into discrete mechanotypes that correlate with distinct immune functions. To this end, we developed a novel experimental system to facilitate three-dimensional mechanical profiling of immune-cell targets: cells interacting with biomimetic hydrogel spheres are imaged by super-resolution microscopy, and the topographies induced by cells on these deformable targets are analyzed. We developed analysis approaches that: (1) identify mechanical features of interest for morphometry, (2) generate an average “radial mechanical profile” of a cell population, (3) cluster single-cell mechanical patterns by spatial frequency (Fourier) analysis, or (4) determine mechanical pattern complexity. This battery of biophysical analyses was applied to compare ontogenically and functionally distant leukocytes (i.e., phagocytes vs. synapse-formers), ontogenically close but functionally distinct leukocytes (i.e., cytotoxic vs. helper T cells), as well as a single functional subset of leukocytes across its developmental states (i.e., CD8+ cells throughout an infection to terminal exhaustion). By radial profiling, phagocytosis and synapsis could be classified as mechanically opposite patterns for the first time. Furthermore, different T-cell synapses exhibited different degrees of protrusive activity, with cytotoxic CD8+ T cells at the peak of infection exerting the most numerous and strongest pockets of concavity on their targets. Interestingly, the actin nucleation-promoting factor *WASp* appeared to differentiate lytic from non-lytic T-cell synapses, indicated by *WASp*-less CD8+ topographies clustering with CD4+ topographies. Lastly, different T-cell subsets and developmental states could be arranged along hierarchies of strength and pattern complexity, implying that dynamic mechanical complexity could help maximize perforin-mediated cytolysis. We found in each of these comparative cases differences in strength and mechanical patterning, suggesting that immune lineages also develop their own idiosyncratic mechanotypes. Together, these findings imply that distinct efferent mechanical activities contribute to overall immune protection.

B561/P2234

Cellular Crowd Control: internal collective cell mechanics compete with external migration cues

G. Shim[1], I. Breinyn[1], A. Wolf[1], T. Zajdel[2], D. J. Cohen[3]; [1]Princeton University, Princeton, NJ, [2]Electrical and Computer Engineering, Carnegie Mellon University, Pittsburgh, PA, [3]Princeton Bioengineering Institute; Mechanical Engineering, Princeton University, Princeton, NJ.

Coordinated cellular migration, emerging from the interplay of cell-substrate and cell-cell forces, is as fundamental to multicellular life as it is beautiful, and underpins foundational processes spanning embryonic development, regeneration, and disease invasion. Given this importance, tools to program or 'herd' large-scale cell migration and tissue growth over thousands of cells in living tissues and organisms have great value. Our group is working on several strategies using external cues to reprogram and 'herd' collective cell migration. For instance, we routinely harness the 'electrotaxis'—directed cell migration along naturally occurring ionic gradients—to program large-scale cell migration using our SCHEEPDOG bioreactor. By manipulating electric fields similar to those found *in vivo (*1 V/cm), we can program both the speed and direction of collective cell migration. Here, we discuss how the internal, collective mechanobiology of the tissue appears to create a tug-of-war with the external 'command'. Our key observation was that tissues became more difficult to control with increasing cell-cell adhesion mediated by E-cadherin. Low levels of E-cadherin enabled smooth and stable migration control, while tissue with high levels of E-cadherin dramatically ripped themselves apart with high levels of cell death. Using inhibitory antibodies against E-cadherin, we proved that briefly disrupting E-cadherin coupling shortly before applying electrical stimulation rescued the tissue response, enabling strong migratory control with no cytotoxicity or mechanical damage and stable growth and expansion post-stimulation. Using this strategy, we were able to demonstrate accelerated healing of a scratch assay in a mature primary mouse skin monolayer. Further evidence of mechanobiological tug-of-wars between external and internal migration cues came when we evaluated both the spatiotemporal and relaxation responses of large tissues undergoing electrotaxis. Where cells are in a tissue determines how well they respond to an electrotactic command, with the greatest issues arising in regions known to possess high traction stresses. Moreover, we found that the stimulated tissue continued to move in the direction of the electric field, albeit more weakly, for hours after the field had been removed, indicating a large-scale reprogramming of the mechanical state of the tissue. While this work was performed using electrotaxis, there are numerous instances in natural systems where achieving appropriate or desired group behaviors requires dynamically tuning mechanical coupling amongst individuals, whether humans in a crowd or cells in a tissue.

B562/P2235

Classical cadherins expressed in the central nervous system show higher evolutionary constraints and negative selection in primates as compared to non-neuronal cadherins

M. Petersen, F. Reyes-Vigil, **J. Bruses**; Mercy College, Dobbs Ferry, NY

Classical cadherins (CDH) comprise a family of single-pass transmembrane proteins that participate in tissue morphogenesis by regulating cell-cell adhesion, cytoskeletal dynamics, and gene expression. Their extracellular domain (ED) forms high-affinity homo and hetero trans-dimers between apposed cell membranes, and the cytoplasmic domain (CD) regulates the actin cytoskeleton and cell signaling through the binding to p120- and beta-catenin. CDHs are grouped into type I (CDH 1, 2, 3, 4 and 15) and type II (CDH 5, 6, 7, 8, 9, 10, 11, 12, 18, 20, 22, and 24) based on differences in ED folding. *CDHs* are

exclusively found in metazoans and their evolutionary origin and expansion coincide with the emergence of multicellularity and vertebrates respectively. This study examined the evolutionary rates of amino acid and nucleotide substitutions of *CDH* orthologs in fourteen species of primates representing tarsiers, old and new world monkeys, lesser and great apes, and humans covering ~60 million years of evolution. All *CDHs* have been under negative (purifying) selection; however, analysis of the ratio of non-synonymous nucleotide substitutions per non-synonymous sites (dN) over synonymous substitutions per synonymous sites (dS) show that the EDs of *CDH2* and *CDH4* have been under significantly higher negative selective pressure than *CDH1* (a type I prototype). The EDs of *CDH5*, *CDH19*, and *CDH24* have been under lower negative selection as compared to *CDH11* (a type II prototype), while the EDs of *CDH7*, *CDH8* and *CDH10* have been under higher negative selection. In contrast, the CDs of *CDH3*, *CDH6*, *CDH12*, *CDH18*, *CDH19*, *CDH22*, and *CDH24* have been under lower negative selection suggesting that some substitutions have been beneficial. These results show that trans-dimerization and regulation of cytoskeletal dynamics have been under independent selective pressures. Substitution rates in type I *CDHs* introns show no significant differences among *CDHs*, indicating that differences in selective pressure observed in protein coding sequences are not due to gene location. A Spearman's correlation analysis shows no significant correlation between gene essentiality and dN/dS ratios. In contrast, *CDHs* expression levels in various human and mouse tissues show a significant negative correlation between dN/dS ratios and *CDH's* expression in the central nervous system. These results suggest that the differences in negative selective pressure observed among classical cadherins is primarily driven by their role in neural development and synapse formation and their significance for the fitness of the organism.

B563/P2236

Determining the molecular interactions of the asymmetric planar cell polarity bridge

A. Goh, D. Devenport; Princeton University, Princeton, NJ

Planar cell polarity (PCP) is an evolutionarily conserved phenomenon in which cells collectively align their polarities across great distances along a single plane of a tissue during development. A defining feature of PCP is the asymmetric localization of its core components, where Frizzled localizes to one side of the cell and Vangl localizes to the other. A transmembrane cadherin, Celsr, localizes to both sides and engages in homophilic adhesion to stabilize Frizzled and Vangl between cells. In order to understand how the polarity is generated within a cell and subsequently propagated to millions of cells, the molecular interactions of PCP components must be characterized. To monitor the intracellular and intercellular protein-protein interactions necessary for the formation of the asymmetric PCP bridge, a junctional recruitment assay was utilized to dissect the mechanism of PCP protein localization, where primary mouse keratinocytes are transfected with PCP protein(s) tagged with a fluorescent protein to observe their localization at cell junctions between transfected neighboring cells after forming an epithelial sheet. In this system, overexpression of Celsr selectively recruits the Celsr-Frizzled PCP subcomplex to the cell-cell border in the adjacent cell, suggesting that unpaired Celsr is in a configuration that has higher affinity for Celsr-Frizzled than Celsr-Vangl.

B564/P2237

The desmoglein 2 interactome in primary cardiomyocytes

Y. Li, **A. P. Campbell**, X. Zeng, N. A. Yates, A. V. Kwiatkowski; University of Pittsburgh, Pittsburgh, PA

Mechanical coupling and chemical communication between cardiomyocytes are accomplished through a specialized adhesive structure called the intercalated disc (ICD). The ICD is formed from three junctional complexes: adherens junctions (AJs) and desmosomes, both of which physically link apposing cardiomyocytes, and gap junctions that electrically couple cells. AJs and desmosomes connect the actin and intermediate filament (IF) cytoskeletons, respectively, of adjoining cells at the ICD and provide structural integrity and mechanical strength. The cardiomyocyte adhesive system is formed from a complex arrangement of molecular components with unique mechanical and signaling properties. How this system is organized at the molecular and cellular level to maintain adhesive homeostasis remains unclear. Previously, we used proximity proteomics to define the N-cadherin (CDH2) interactome in primary neonatal cardiomyocytes and identified adaptor and adhesion proteins that promote AJ specialization. Building from this work, we describe our efforts to define the desmosome interactome in cultured cardiomyocytes. We first measured protein dynamics using fluorescent recovery after photobleaching (FRAP) and show that desmosomal proteins are stable with dynamic properties similar to their AJ counterparts. We then combined quantitative mass spectrometry and proximity labeling to identify proteins associated with desmosomal cadherin desmoglein 2 (DSG2). We found over 300 proteins in the DSG2 interactome, and over half are shared with the CDH2 interactome. Shared hits include two proteins that associate with AJs and desmosomes, plakoglobin and plakophilin 2. Notably, plakoglobin and plakophilin 2 are among the most abundant proteins in both DSG2 and CDH2 interactomes, suggesting that the two proteins may coordinate adhesive complex formation along the ICD. Proteins unique to the DSG2 interactome include the gap junction protein connexin 43 as well as members of the plakin family of cytolinker proteins - desmoplakin, periplakin, and plectin. In contrast to the CDH2 interactome in neonatal cardiomyocytes, the DSG2 interactome is largely devoid of unique secondary scaffolding, adaptor, and/or cytoskeletal proteins that might assist in mechanical adhesion. We suggest that the desmosome core - desmosomal cadherins, plakophilin 2, plakoglobin, and desmoplakin - is sufficient for desmosome function and ICD organization in neonatal cardiomyocytes. In summary, our analysis of the DSG2 interactome adds a critical new dimension to the proteomic atlas of molecular complexes that regulate cardiomyocyte adhesion.

B565/P2238

Exploring the function of Canoe's intrinsically disordered region in linking cell junctions to the cytoskeleton during morphogenesis

N. J. Gurley; University of North Carolina At Chapel Hill, Chapel Hill, NC

Noah J. Gurley[1], Rachel A. Szymanski[1], Kia Perez-Vale[2], Kristi Yow[1], Amber Butcher[1], Emily McParland[1], and Mark Peifer[1,3]Department of Biology[1], Curriculum in Genetics and Molecular Biology[2], Lineberger Comprehensive Cancer Center[3], University of North Carolina at Chapel Hill, North Carolina 27599-3280, USA

The remarkable ability of cells to change shape and move without disrupting tissue integrity is a hallmark of embryonic development. To do so, cells must link the contractile actomyosin cytoskeleton to cell-cell and cell-matrix junctions, and this linkage must be robust and dynamic. We have focused on the multidomain scaffolding protein Canoe (Cno), homologue of mammalian Afadin, which links the

cadherin-catenin complex to actin. It includes two N-terminal Ras-association (RA) domains – which bind the small GTPase Rap1 – followed by Forkhead-associated (FHA) , Dilute (DIL) and PSD95-Disc large-ZO-1 (PDZ) domains. These are separated from the C-terminal F-actin binding domain by a long intrinsically disordered region (IDR). IDRs are now recognized as important players in the multivalent interactions that assemble multiprotein complexes, including those that form phase-separated biomolecular condensates. One of our tasks is to define Canoe's mechanistic role, by taking it apart as a machine. We began by exploring the roles of its PDZ and F-actin binding domains, thinking they provided the direct linkage between cadherin and actin. However, deleting each domain had only modest effects on protein function. In contrast, the Rap1-binding RA domains were critical for Cno function. We now have returned to this analysis, exploring a surprising result from earlier work—protein null alleles of *canoe* have a milder zygotic phenotype than some "canonical" *canoe* alleles, whose defects in dorsal closure gave the gene its name. To explore this, we have sequenced a series of 22 EMS-induced *canoe* alleles; most result from premature stop codons arrayed across the coding sequence. Intriguingly, the strongest "canonical" alleles carry a stop codon early in the IDR. We suspect these alleles may encode a truncated protein that act in a dominant negative fashion, interfering with maternally contributed, wildtype Canoe protein. To test this, we are now exploring the function of this diverse set of Canoe proteins, examining both zygotic and maternal/zygotic mutants. In parallel, we are creating a new series of CRISPR-based mutants at the *cno* locus, including independent deletions of the IDR and DIL domains. Together, these approaches will help define the role of Canoe's IDR in protein function.

B566/P2239

Defining alpha-T-catenin interactions with plakophilin 2

E. Porter, J. Heier, A. Kwiatkowski; University of Pittsburgh, Pittsburgh, PA

Plakophilin 2 (PKP2) is a core component of the desmosome, a junctional complex that connects the intermediate filament cytoskeletons of neighboring cells. In cardiomyocytes, PKP2 is abundant at the intercalated disc (ICD), a specialized junction formed from desmosomes, adherens junctions, and gap junctions that permits adhesion and electrical signaling between cardiomyocytes. PKP2 mutations in humans are linked to arrhythmogenic ventricular cardiomyopathy (AVC), but relatively little is known about the function, the associations with other ICD proteins, and the pathogenesis of heart degradation in AVC. Alpha-T-catenin is an actin-binding protein that connects the cadherin-catenin complex to the actin cytoskeleton to form the adherens junction. Similar to PKP2, mutations in alpha-T-catenin are linked to AVC in humans. Past work has shown that alpha-T-catenin binds directly to PKP2. It is believed that alpha-T-catenin recruits PKP2 to the cadherin-catenin complex to create a unique hybrid junction. We hypothesize that the interaction between alpha-T-catenin and PKP2 functions to coordinate and strengthen intercellular adhesion in cardiomyocytes. To define the binding interface between alpha-T-catenin and PKP2, we constructed, expressed, and purified various fragments of the PKP2 in order to test their binding interactions with alpha-T-catenin using size exclusion chromatography (SEC) and pulldown experiments. Our results revealed that the binding interface in PKP2 lies within amino acid (aa) 73-229, in the unstructured N-terminus. In parallel, we expressed various fragments of PKP2 fused to EGFP in cardiomyocytes and assessed localization. Notably, full-length EGFP-PKP2 and endogenous PKP2 protein were not restricted to the desmosome in cardiomyocytes. Instead, this showed a broad distribution at cell-cell contacts and colocalized with adherens junction proteins. The PKP2 N-terminus (aa73-348) was required and sufficient for recruitment to cell-cell junctions whereas the armadillo region (aa341-837) was not. We speculate that PKP2 is recruited to the adherens junction through

alpha-T-catenin at cardiomyocyte cell-cell contacts to coordinate junctional and cytoskeletal organization.

Bioengineering of Cell-matrix Interactions

B567/P2240

Biophysical programming and phenotypic switching of fibroblasts in tissue remodeling

H. Xiao[1], K. Sylla[1], B. Wilkowski[1], E. Mintah[1], D. Zhang[1], A. Zheng[1], X. Gong[1], E. Herzog[2], M. Mak[1]; [1]Department of Biomedical Engineering, Yale University, New Haven, CT, [2]Department of Medicine (Pulmonary, Critical Care and Sleep), Yale University, New Haven, CT.

Fibroblasts are versatile master regulators that can assemble, degrade, and remodel the extracellular matrix (ECM) in connective tissues. Although excessive tissue repair is associated with most fibrotic diseases, tissue destruction is observed in many end-stage lung diseases including idiopathic pulmonary fibrosis (IPF), termed honeycombing. However, it is not well understood how fibroblasts can be systematically programmed and tuned to transition between tissue maintenance and tissue destruction phenotypes. Our objective is to demonstrate that the biophysical states of fibroblasts are directly programmable and reversible, leading to tunable macroscopic tissue behaviors. We encapsulated fibroblasts in collagen and used molecular screens and gel compaction assay to dissect cell features including invadopodia, protrusions, and contractility. We further determined the impact of matrix properties including boundary conditions, collagen concentration, and crosslinking. Finally, we demonstrated clinical relevance via bioinformatic analyses of healthy vs. IPF patients. Our results showed that lung fibroblasts from both healthy and diseased individuals had the ability to produce holes and this process was upregulated by cytokines such as TGF-β. With high-resolution imaging, we showed that fibroblasts migrated through, densified, and ruptured collagen gels. We used correlation analyses and showed that MMPs were required for hole formation, and cellular contractility mediated hole enlargement. Denser, ribose-crosslinked, or non-anchored gels led to a reduction in hole sizes. We demonstrated that wound healing (hole closure) could be initiated by MMP inhibition and required myosin and ROCK. Finally, we leveraged bioinformatics and showed that many MMPs and invadopodia-related genes could be clinically relevant factors in IPF patients. In conclusion, our study details the phenotypic versatility and switching between tissue destruction and repair by fibroblasts and highlights remodeling events with implications for lung fibrotic disease. Our results suggest that fibroblast activities--the actomyosin machinery and MMP-related degradation--can be tuned to modulate fibrotic diseases and potentially restore tissue homeostasis.

B568/P2241

Mechanical Memory Acquisition of Oral Squamous Cell Carcinoma (OSCC) and Biological Pathways Involved in the Process

S. Moon[1], P. S. Campos[2], B. F. Matte[2], J. K. Placone[3], V. G. Zanella[2], M. D. Martins[2], M. L. Lamers[2], A. J. Engler[1]; [1]University of California San Diego, La Jolla, CA, [2]Federal University of Rio Grande do Sul, Porto Alegre, BRAZIL, [3]West Chester University, West Chester, PA.

Oral cancer and oropharyngeal cancers' 5-year survival rate is 85% according to American Cancer Society, but it significantly decreases with regional or systemic metastasis. Oral squamous cell carcinoma (OSCC) accounts for 95% of all oral cancers. To improve prognosis of this cancer type, it is important to

study OSCC cell's metastatic features throughout the invasion process. In the previous study, we have shown that even non-invasive OSCC cell lines - FaDu and Cal27 - can demonstrate epithelial-to-mesenchymal transition (EMT)-like responses after being conditioned on stiff matrix (20 kPa polyacrylamide gel). To metastasize to the secondary tumor site, tumor cells must disseminate from stiffened tumor tissue and invade through surrounding softer parenchyma. Accordingly, we hypothesized that OSCC cells can acquire "mechanical memory" and the invasive features they have learned from stiffness can be maintained as EMT in the new niche. Cal27 cells were conditioned either by matrix stiffness or by contractility modulation with contractile agonists to acquire EMT markers, and then moved to a new niche (0.48 kPa PA gel) to mimic tumor cells facing softer tissue as they disseminate. Both stiff matrix and enhanced cell contractility allowed cells to have less localization of E-cadherin (epithelial marker) at the edge of the cell, higher expression of non-muscle myosin II A (NMMIIA), and higher migration speed than those without mechanical memory either due to absence of learning source or due to contractility inhibition by Blebbistatin. Phospho-kinase array indicated the possibility of AKT and FAK signaling pathways being involved in "learning" and "recovering" mechanical memory respectively, and inhibition of the pathways supported such possibility. Transcriptomic difference between cell populations with and without "mechanical memory" was identified by RNA sequencing. 822 deferentially expressed genes (DEGs) were identified, and 6 most significant up-/down-regulated genes (SDC4, ICAM1, FN1, ITGAV are up-regulated in the cells with memory, and RhoD and FSCN1 are down-regulated) were listed based on their expression rate. Stratifying TCGA patient data according to their genetic profile of 6 given genes, we could see that patient group whose genetic profile is closer to OSCC cells with mechanical memory has lower progression free interval and overall survival rate.

B569/P2242

Molecular Regulation of Hyaluronan Degradation in the Human Vocal Fold Fibroblasts under Stress

K. Basu, L. Mongeau; McGill University, Montreal, QC, CANADA.

Objective: Vocal fold (VF) scarring alters viscoelastic properties of the VF, and causes dysphonia. Hyaluronic acid (HA), a key polysaccharide is present in the lamina propria and regulates VF viscoelasticity, stiffness, and osmosis to initiate and maintain phonation. Thus, HA-based injectable biomaterials and hydrogels are in demand for vocal fold repair. However, one major drawback to using HA in composite biomaterials is its short residence time within VF. HA degradation releases low molecular weight (LMW) HA and HA oligosaccharides that may trigger inflammation in VF. Thus, the purpose of the current study was to understand the molecular steps regulating HA degradation and HA catabolic enzyme hyaluronidase and how HA degradation releasing LMW HA orchestrates vocal fold inflammation under stress. **Method:** The immortalized hVFF cell line was exposed to cigarette smoke extract (CSE). WST-1 assay determined cell survivability and the half maximal inhibitory concentration (IC_{50}) was calculated. qPCR and western blot assessed gene and protein expression respectively. CD44/HYAL2 interaction was studied by co-immunoprecipitation. **Results:** CSE suppressed the growth of hVFF in a dose-dependent manner and the upregulation of ECM marker (collagen) and inflammation marker (TGFβ1) confirmed the effectiveness of hVFF cells in CSE to mimic injured vocal fold. Significant upregulation of *HAS3* synthesizing LMW HA and *HYAL2* catabolizing HA ($p \leq 0.05$), suggests accumulation of LMW HA in the injured hVFF. Upregulation of HA receptor CD44 suggested that CD44 might play an important role in regulating HA catabolism. HYAL2 binds to CD44 ectodomain and CD44/HYAL2 interaction regulates HA degradation inside the cell. The enzymes (MT1-MMP and ADAM10) regulating

cleavage of CD44 ectodomain were upregulated in hVFF in CSE. Regulation of CD44 cleavage, CD44/HYAL2 binding, and inhibition of lysosomal degradation of HA was thought to regulate HA degradation in hVFF under stress. **Conclusion:** The study identified the principal steps regulating hyaluronidase activity and HA degradation in the VF inflammation. This is important to make a strategy to increase the stability of the HA-based biomaterials and hydrogel for VF and control inflammation.

B570/P2243

Conductive Electrospun Polymer Platforms Improve Stem Cell-Derived Cardiomyocyte Function and Maturation

G. Gonzalez[1], A. C. Nelson[1], A. J. Whitehead[1], E. LaMontagne[1], R. Vatsyayan[2], S. Dayeh[2], A. J. Engler[1]; [1]Bioengineering, University of California, San Diego, San Diego, CA, [2]Electrical and Computer Engineering, University of California, San Diego, San Diego, CA.

Despite efforts to mature human pluripotent stem cell-derived cardiomyocytes (PSC-CMs) for disease modeling and high throughput screening, cells remain immature and may not reflect adult biology. Recent advancements utilize electro-mechanical and paracrine stimulation to functionally mature cardiomyocytes but continue to lack mature electrical conduction. Conductive matrices may facilitate electrical maturation between gaps in sparse PSC-CM clusters or between PSC-CMs that model conduction defects; hence, we electrospun and crosslinked poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) blended with 8% w/v poly(vinyl alcohol) (PVA) scaffolds. Matrix fiber structure remained stable over 4 weeks in buffer, stiffness remains near cardiac stiffness *in vivo,* and electrical conductivity scaled with PEDOT:PSS concentration. Electrically excitable PSC-CMs were cultured sub-confluently or as a monolayer. Cells adhered to all scaffolds, with increased desmoplakin (DSP) localization on conductive scaffolds, indicating an improvement in the mechanical stability of our PSC-CMs. Sarcomere organization also scaled with increasing PEDOT:PSS concentration, even in the absence of a uniform monolayer, suggesting that improved organization of the contractile machinery in these cells was due to the electrical condition of the matrix. Calcium handling indicated higher calcium flux with a shorter time to peak, further suggesting improved electrical maturity, even when sub-confluent. Taken together, these data suggest that PEDOT:PSS/PVA scaffolds are stable, of a stiffness relevant to cardiomyocytes, and supportive of electrical coupling even in the absence of a monolayer, which may improve cardiac disease modeling and drug development.

B571/P2244

RGD peptide and glutamic acid crosslinked chitosan hydrogel promotes salivary gland cell acinar formation

K. Hozumi[1], C. Takahashi[2], M. Nomizu[3]; [1]Shigakkan University, Obu, JAPAN, [2]Kitasato Junior College of Health and Hygienic Science, Minamiuonuma, JAPAN, [3]Tokyo University of Pharmacy and Life Sciences, Hachioji, JAPAN.

Extracellular matrix (ECM) provides suitable biochemical and structural support to the tissue mimicked cell culture platforms, such as Matrigel. Designing the cell culture platforms mimicking the environment of the cells in vivo is useful strategy to develop the cell culture materials, and many materials have been used to mimic the structural and biological functions of basement membrane and ECM. Chitosan-based biomaterials have been used to perform as cell culture platforms. Here, we crosslinked chitosan, a polysaccharide containing many free amine groups, using dicarboxylic acids and conjugated ECM

derived active peptides to the chitosan hydrogel. Ten dicarboxylic acids were examined to obtain the chitosan solution and found that seven liner saturated dicarboxylic acids could solve the chitosan to the water. Adipic acid effectively generated transparent 3D hydrogels through the chitosan crosslinking. Dicarboxylic amino acids of glutamic acid (Glu) and aspartic acid (Asp) did not promote chitosan hydrogel by crosslinking, but mixed adipic acid/Glu generated hydrogels and its stiffness was regulated by the mixing ratio of adipic acid and Glu. These suggest that the stiffness of chitosan hydrogels is related and can control by the amount of Glu. Next, we conjugated ECM peptides to the chitosan hydrogel and examined its biological activities. We used ECM derived five ECM receptor specific binding peptides, such as fibronectin derived RGD (integrin alpahvbeta5 binding peptide), laminin alpha1 derived AG10 (integrin alpah6beta1 binding peptide), and laminin alpha1 derived AG73 (syndecan binding peptide). The peptide-chitosan/dicarboxylic acid hydrogel effectively promoted human salivary gland cell attachment. The biological activities of peptide-chitosan/dicarboxylic acid hydrogels were different depending on the ECM derived active peptide. After cell attachment, we cultured salivary gland cells for seven days on the peptide-chitosan/dicarboxylic acid hydrogel, RGD-chitosan/dicarboxylic acid hydrogel promoted differentiation and formation of acinar like structure as salivary gland cells cultured on Matrigel. The peptide-chitosan/dicarboxylic acid hydrogel could use to analyze the cellular functions mediated by specific cell surface receptors and have the potential to use as cell culture matrices of salivary gland cells.

B572/P2245

The shape of a microfabricated scaffold can control cell protrusion and migration direction

H. Sunami; Faculty of Medicine, University of the Ryukyus, Okinawa, JAPAN.

The present study demonstrates unidirectional cell migration using a novel three-dimensional microfabricated scaffold, as revealed by the uneven sorting of cells into an area of 1 mm x 1 mm. To induce unidirectional cell migration, it is important to determine the optimal arrangement of three-dimensional edges, and thus, the anisotropic periodic structures of micropatterns were adjusted appropriately. The cells put forth protrusions directionally along the sharp edges of these micropatterns, and migrated in the protruding direction. There are three advantages to this novel system. First, the range of applications is wide, because this system effectively induces unidirectional migration as long as three-dimensional shapes of the scaffolds are maintained. Second, this system can contribute to the field of cell biology as a novel taxis assay. Third, this system is highly applicable to the development of medical devices. In the present report, we describe unique three-dimensional microfabricated scaffolds that provoked unidirectional migration of NIH3T3 cells. The three-dimensional scaffolds could provoke cells to accumulate in a single target location, or could provoke a dissipated cell distribution. Because the shapes are very simple, they could be applied to the surfaces of various medical devices. Their utilization as a cell separation technology is also anticipated.

B573/P2246

Biomimetic extracellular matrix nanofibers electrospun with calreticulin promote synergistic activity for tissue regeneration

S. Mishra[1], M. E. Stack[2], M. P. C. Ratnamani[2], H. Wang[2], H. Wang[2,2,2], L. I. Gold[1]; [1]NYU Langone Health, New York, NY, [2]Stevens Institute of Technology, Hoboken, NJ

Electrospun nanofibers (NFs) are engineered to simulate extracellular matrix (ECM) scaffolds that provide biophysical and biochemical cues to elicit cellular responses favoring wound repair. Polycaprolactone-collagen I (PCL-Col1) NFs were electrospun with calreticulin (CRT; CRT-NFs), a protein that possesses numerous vulnerary effects including functioning as a chemoattractant for keratinocytes, fibroblasts and macrophages, the most important cells for wound healing. CRT potently enhances the rate and quality of wound healing in porcine and diabetic murine models; mice treated with CRT demonstrated tissue regeneration of excisional wounds marked by epidermal appendage neogenesis and lack of scarring. Poor healing chronic wounds, such as diabetic foot ulcers, is an unmet need due to lack of successful treatments. A major problem associated with chronic wounds is the paucity of granulation tissue from lack of cellular recruitment/migration, cell proliferation and ECM induction required for resurfacing and reconstructing the wound defect. We proposed that CRT sequestered into NFs would offer tunable delivery of CRT and protect the protein from proteolytic digestion by the abundant enzymes in wounds. Results show that following electrospinning 100 ng fluoresceinated CRT (Fl-CRT) into PCL-Col1 NFs, the NFs are architecturally unaltered with release kinetics of 50% in 24h, 77% in 96h with sustained release. Remarkably, CRT-NFs retained the ability to induce migration and proliferation of human keratinocytes and fibroblasts seeded onto the CRT-NFs equal to, or with statistically higher potency than CRT added exogenously to NFs. In addition, the dynamic expression of ECM proteins and integrins for migration was observed by fibroblasts seeded onto CRT-NFs: TGF-β1, TGF-β3, fibronectin, elastin, integrin β1, and integrin α5. Furthermore, NFs protected sequestered CRT from proteolytic digestion by common wound enzymes, Cathepsin G, elastase, lysozyme, and subtilisin [from bacteria] as shown by release of Fl-CRT electrospun into NFs compared Fl-CRT alone. Notable synergistic effects of CRT-NFs compared to NFs or exogenous CRT alone were CRT-NFs effect on basal exposure to fibroblasts and keratinocytes, which adapted a clear elongated migratory phenotype with increased expression of vinculin, stress fibers, and abundant migrasomes released by the cells. CRT within NFs is released over time, protected from proteolysis, and together CRT-NFs have synergistic activities providing a potentially improved topical therapeutic for tissue regeneration to compensate the lack of granulation associated with difficult to heal chronic wounds. In summary, we show that CRT can be electrospun into PCL-Col1 NFs without loss of wound healing related biological activities.

B574/P2247

cRGD Surface Activity - How It Can Be Quantified, Compromised, and Controlled for Live-Cell Experiments

M. Robitaille[1], J. Christodoulides[1], P. Calhoun[2], J. Byers[1], M. Raphael[1]; [1]US Naval Research Laboratory, Washington, DC, [2]Nanocrine, Inc., Frederick, MD

RGD peptides play a crucial role in diverse areas of biological research, yet their applications in terms of surface functionalization are typically based on a set of assumptions rather than directly measured. To address this, we outline a methodology that can quantitatively measure the amount of RGD available to interface with cells (surface activity) and show that surface activity 1) can be significantly reduced by in-

vitro media constituents irrespective of RGD surface concentration, 2) directly influences cell behavior with regards to adhesion and migration, and 3) can be preserved/regenerated. First, surface activity is quantified by using identical surface functionalization protocols on a Surface Plasmonic Resonance (SPR) chip to serve as a concurrent control. Recombinant $\alpha_V\beta_3$ integrins serve as cell-free sensors to quantify cyclic RGD (cRGD) surface activity in a reliable and reproducible manner via SPR. Next, we show that commonly used agents in-vitro such as Bovine Serum Albumin (BSA), PLL-g-PEG, and Fetal Bovine Serum (FBS) are effective blocking molecules cRGD surface activity, highlighted by the addition of 1% FBS reducing cRGD surface activity 33-fold. We show that SPR surface activity measurements reflect the behavior of MDA-MB-231 cells atop similarly functionalized surfaces/environments in-vitro, in which changes in surface activity alter their migration and adhesion. Finally, we show that cRGD activity can be preserved in the presence of blocking molecules by utilizing recombinant $\alpha_V\beta_3$ integrins as a protective cap in-vitro.

B575/P2248

Suspended Nanonets for Quantitative Cell Biology

N. E. Grega Larson, B. Behkam, A. Nain; Virginia Tech, Blacksburg, VA

Cell migration is defined as the directed movement of a cell or group of cells in response to chemical and/or mechanical signals. The extracellular matrix (ECM) acts as the “track” on which cells extend protrusions that sense these signals, and is comprised of individual and bundles of protein fibers that vary in diameter (nanoscale-to-microscale), orientation (random-to-aligned), and mechanical stiffness (soft-to-stiff). Thus, the ECM presents various curvatures and other topographical cues that in some cases lead to the initiation of cell migration. While the process of cell migration is essential for physiological and pathological processes in development, wound healing, the clearance of infections, and cancer metastasis, our understanding of how cell migration is controlled by the physical properties of the ECM is still lacking. Here, we demonstrate the ability to quantitatively measure protrusive, migratory, and force response behaviors of cells attached to various patterns of ECM-mimicking suspended fibrous nanonets, which are fabricated using our non-electrospinning Spinneret-based Tunable Engineered Parameters (STEP) platform. By visualizing cells cultured on suspended nanonets, we discovered that the tips of cell protrusions *wrap around* ECM fibers, termed coiling, and the amount of coiling and the size of protrusions increase as fiber diameter increases. Furthermore, differences in directed cell migration can be measured as a function of ECM architecture by depositing fiber networks in various organizations (aligned vs. crosshatched). For example, we observe differences in cell behavior on suspended nanonets versus flat 2D culture in contact inhibition of locomotion and chemotaxis, suggesting that differences in the physical attachments a cell makes with its surroundings are sufficient to control its behavior. Finally, we have pioneered Nanonet Force Microscopy (NFM) to measure cell forces originating at focal adhesion sites that direct along actin stress fibers. NFM data show that force increase with fiber diameter due to an increase in both focal adhesion cluster length and stress fiber number. Overall, our low-cost, well-defined and repeatable, and high throughput platform quantitatively evaluates cell behavior at single-cell resolution, and better recapitulates the environment that cells experience *in vivo* as compared to flat 2D culture methods.

B576/P2249

Developing Decellularized Plant Scaffolds as a 3D in vitro Cell Culture Platform

C. A. Sword, K. E. Kubow; James Madison University, Harrisonburg, VA

Current 3D in vitro cell culture models are typically simple gels of biological and/or synthetic components or precisely structured synthetic scaffolds. While both approaches have benefits, the former are often too structurally simplistic while the latter require equipment and expertise not typically found within biological laboratories. One simpler and more easily accessible option is to create cellulose-based scaffolds with complex 3D structures by decellularizing plant tissues. Our aim was to develop such a scaffold from the tissue of the stem of a common broccoli plant. We selected broccoli stem because of its open mesh structure, which could provide a mimetic for connective tissue. The scaffolds were produced by decellularizing thin slices of broccoli stem with concentrated sodium dodecyl sulfate, followed by washing and sterilization in a series of water, ethanol, and PBS solutions. The successful removal of plant cells was assessed by staining with a live/dead stain (calcein AM and propidium iodide). Only a small number of plant cell nuclei remained along the edges of the scaffolds, likely due to the thicker exterior tissues of the stem. These areas were easily trimmed. The scaffolds consisted of a meshwork of pockets with diameters of 24.4 +/- 5.9 µm (mean +/- standard deviation) as assessed using calcofluor stain and confocal microscopy. Rheological measurements revealed that the scaffolds had a bulk shear modulus on the order of kilopascals.We cultured MEFs (mouse embryonic fibroblasts) on the cellulose-derived scaffolds for up to one week to demonstrate the scaffolds' ability to support cell growth. The cells colonized the pockets left behind by the broccoli cells and remained viable. Although most cells were localized to the outer edges of the scaffold, we found that minor mechanical disruption of the scaffold structure enabled greater cell attachment and invasion. Future experiments will include optimizing cell seeding and studying extracellular matrix deposition.

Regulation of Aging

B578/P2250

Determining Parkinson's Disease causing variants in PRKN using a pooled FACS-based screen

A. J. Gilsrud, J. A. Thayer, D. P. Narendra; NINDS, NIH, Bethesda, MD.

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's Disease. The characteristic motor symptoms in PD are due to degeneration of dopamine neurons within a region of the midbrain called the *substantia nigra pars compacta*. Approximately 500,000 Americans are diagnosed with PD. 5-10% of Parkinson's disease cases are caused by genetic mutations, with loss of function mutations in *PRKN* (coding for the protein Parkin) being the most common cause of autosomal recessive Parkinson's disease. The pathogenicity of many *PRKN* missense variants, however, remains unknown. Indeed, we recently found that approximately 1% of individuals carry a rare *PRKN* missense variant of unknown significance, complicating genetic counseling (Zhu et al., in press). To resolve this uncertainty, we generated a pooled cDNA library of all rare Parkin missense variants in publicly available databases as well as alanine substitutions of critical Parkin residues (599 variants in total). The Parkin variants in the library were additionally tagged with YFP. Therefore, the effect of each mutation on Parkin stability can be determined. Finally, we developed a novel FACS based screening approach to resolve the functional status of each variant, using a single-cell reporter of Parkin function. As an initial assessment of feasibility, we single cell sorted the high and low Parkin activity populations and grew up

the resulting single cell clones. As expected, all (3/3) of the high single-cell clones had Parkin function, whereas 60% (3/5) of the low function single-cell clones did not. From four of these clones, we identified two novel loss of function Parkin variants and two benign Parkin variants. We are now developing a next-generation sequencing approach to test the library as a pool to differentiate benign mutations from loss of function mutations. The structural relationship of these variants will be assessed by mapping them onto the solved structures of Parkin in its auto-inhibited and active states. We anticipate the results of this study will help resolve the functional consequence of *PRKN* variants in the population and additionally reveal novel structural requirements for Parkin activity.

B579/P2251

Lewy-body associated proteins suppress α-synuclein toxicity under respiratory growth

B. Gillespie, R. P. Almazan, A. P. Almazan, P. Shirahatti, R. Santhanakrishnan, I. Haider, Z. Paxhia-poppaw, E. Manishimwe, S. Ju, Q. Zhong; Wright State University, Dayton, OH.

A pathological hallmark of Parkinson's disease is the formation of Lewy bodies, primarily composed of abnormal protein aggregates of α-synuclein. The accumulation of α-synuclein in neurons causes dosage-dependent cytotoxicity. This toxic phenotype has been recapitulated in a simple eukaryote, *Saccharomyces cerevisiae.* Genetics screens using yeast models have led to the discovery of several hundred modifier genes and critical cellular processes driving α-synuclein toxicity. Most of these yeast models rely on the use of the *GAL1* promoter to induce gene expression. The *GAL1* promoter is activated by a fermentable carbon source, galactose. Yeast cells do not require full mitochondrial function, such as oxidative phosphorylation, to grow on galactose. Accumulating evidence supports a role of mitochondrial dysfunction in neurodegenerative disease. The dispensable mitochondria in yeast models grown on galactose represents a missing cellular context for studying the toxicity of α-synuclein. Here, we developed a new yeast model to express α-synuclein when cells are forced to respire on a non-fermentable carbon source. We found that α-synuclein is more prone to form aggregated cytoplasmic structures and to induce mitochondrial damage and cell death under respiratory growth. We reasoned that previous genetic screens in yeast have not uncovered the full spectrum of genetic modifiers of α-synuclein toxicity. By performing a genome-wide overexpression screen, we identified a set of Lewy-body associated proteins and their corresponding yeast homologs exhibiting conserved protective effects on cells against α-synuclein toxicity. These Lewy-body associated proteins are not uncovered in previous genetic screens. Expression of these proteins also reduces the accumulation of cytoplasmic structures and mitochondrial defects induced by α-synuclein. Our data support that mitochondrial function is a critical cellular context for understanding α-synuclein pathology.

B580/P2252

Relevance of Rhomboid-like Protease-4 mediated Amyloid Precursor Protein Processing in Alzheimer's Disease.

Y. Penalva, S. Recinto, L. Munter; McGill University, Montréal, QC, CANADA.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive impairments in cognitive functions and affects 32 million people worldwide. Autosomal dominant inherited mutations causatively link the amyloid precursor protein (APP) to AD and one of its proteolytic cleavage products via the canonical amyloidogenic pathway, Aβ peptides, is a hallmark of AD. While APP's relevance in the pathogenesis of the disease is clear, its physiological functions remain poorly characterized. Unveiling

APP's functions and defining the triggers leading to Aβ production are crucial to determining the cellular conditions underlying AD. We have discovered a novel processing pathway of APP mediated by the endoplasmic reticulum resident rhomboid-like protease-4 (RHBDL4). We have shown in Human Embryonic Kidney 293T (HEK293T) cells that RHBDL4 cleavage results in decreased total APP and Aβ levels. In this study, we aim to determine the physiological relevance of this pathway in AD. We crossed an AD mouse model expressing a mutated form of APP only in neurons (hAPP J20) to a RHBDL4 global knockout (KO) mouse. hAPP J20 mice show cognitive defects at 4 months of age and increased hippocampal Aβ production starting 6 to 36 weeks of age. We assessed cognition using the Y maze and the Novel Object Recognition (NOR) test and found a rescue of cognition in female hAPP J20 mice in the absence of RHBDL4 at 5 months of age. Interestingly, when we quantified total APP and Aβ levels from brain samples using western blot and Enzyme Linked Immunosorbent Assay (ELISA) respectively, we found a significant increase for both when knocking out RHBDL4 in the hAPP J20 females. These results confirmed the absence of correlation between Aβ and cognition, but most importantly RHBDL4's relevance for APP physiology *in vivo*. We aim to further confirm the effect of RHBDL4 on APP total levels and processing as well as on cognition using RHBDL4 KO and knockdown models in mouse embryonic fibroblasts (MEFs) and HEK293T cells. Specifically, we have shown in RHBDL4 KO MEFs that β-catenin protein expression is increased, possibly hinting at a greater activation of the Wnt/β-catenin pathway whose decreased signaling has been published to underlie cognitive defects in the hAPP J20 model. Herein, we propose to use RHBDL4 as a new tool to further our knowledge on APP's functions and AD pathology.

B581/P2253

Reduced expression of the cell intrinsic clock protein Bmal1 in myeloid cells accelerates cognitive decline and alters microglial function in aging mice

C. Iweka, E. Seigneur, A. Latif-Hernandez, S. Herrera Paredes, M. Cabrera, E. Blacher, Q. Wang, F. Longo, L. DeLecea, K. Andreasson; Stanford University, Stanford, CA.

Neuron-microglia interactions are critical to maintaining neural circuitry and function. Microglia are myeloid-lineage cells that possess an intrinsic time-keeping system that regulates its function to anticipate changes in its external environment. This time-keeping system, the circadian clock, diminishes with aging, and disruption of the circadian rhythmicity impairs neuroinflammatory responses in microglia. Several studies have demonstrated that tissue-specific deletion of Bmal1, a transcription factor and regulator of circadian rhythms, can accelerate aging, for example the development of sarcopenia in muscle, bone calcification in bone and cataracts in the eye. Here, we tested the hypothesis that Bmal1-deficient microglia disrupts essential neuron-microglia interactions that ultimately impair neuronal circuits and function in aged mice. We show increased microglial activation with decreased expression of lysosomal proteins, CD68 and LAMP1. Bmal1-deficient microglia also exhibited alterations in expression of complement proteins, C1q and C3. Altogether, this resulted in decreased microglia-mediated synaptic engulfment and consequently increased dendritic spine density in the hippocampus that caused deficits in LTP and cognitive impairment. Furthermore, the sleep-wake cycle, critical for synaptic remodeling was significantly impaired. These results highlight the significance of maintaining the cell-intrinsic circadian clock system in myeloid cells, and its contribution to neuron-microglia interactions.

B582/P2254

Exploiting age-dependent biomechanical features of single cells for disease detection and prevention.

S. Hinz[1], M. Miyano[1], A. Manousopoulou[1], R. W. Sayaman[1,2], K. Y. Aguilera[3], M. E. Todhunter[1], J. C. Lopez[1], L. D. Wang[1], L. L. Sohn[4], M. A. LaBarge[1]; [1]City of Hope, Duarte, CA, [2]University of California, San Francisco, CA, [3]David Geffen School of Medicine at UCLA, Los Angeles, CA, [4]University of California at Berkeley, Berkeley, CA.

Age is the major risk factor in most carcinomas, yet little is known about the specific reasons aging increases cancer susceptibility. In mammary gland, luminal epithelial cells are thought to be the breast cancer cell of origin. Dysregulation of keratin intermediate filament proteins is a hallmark age-dependent change in luminal cells, which implicates mechanical states that are unique to cancer susceptible cells. We implemented mechano-node-pore sensing (mechano-NPS), which is a powerful tool in multi-parametric single-cell analysis that simultaneously measures cell diameter, resistance to compressive deformation, transverse deformation under constant strain, and recovery time after deformation. We identified mechanical phenotypes that distinguish human mammary epithelial cells by lineage, age, and cancer progression stage. A machine learning model accurately predicted the chronological age of average risk epithelial cells based exclusively on mechanical properties of single cells. Application of the model to cells from women who are germline carriers of high-risk cancer-causing mutations showed that they are mechanically old irrespective of their chronological age, suggesting that mechanical states could be a window into detection and prevention of cancer susceptible states. Mass spectrometry and cell-based functional assays were used to dissect the molecular underpinnings of age-dependent changes in the mammary epithelium. Cytoskeleton related proteins keratin 14 (KRT14) and pseudopodium enriched atypical kinase 1 (PEAK1) were key drivers of the age-dependent mechanical signatures. Pharmacological and gene silencing approaches that targeted KRT14 and PEAK1 were able to modulate the mechanical age of HMEC and, in the case of PEAK1 modulation, ablate luminal epithelial cells in an age and lineage dependent manner. Our work has assessed the intersection between mechanical phenotypes and novel age-dependent proteins that can be exploited to assess breast cancer susceptibility and reveal vulnerabilities that can be exploited for prevention.

B583/P2255

Missorting of Plasma miRNAs in Aging and Alzheimer's Disease

M. Čarna[1], J. S. Novotný[1], N. Dragišič[1], H. Slavik[2,3], K. Sheardova[1,4], Y. E. Geda[5], M. Vyhnalek[1,6], J. Laczo[1,6], J. Hort[1,6], Z. Mao[7], R. A. Rissman[8], M. Hajduch[9], E. B. Dammer[10], G. B. Stokin[1,11,12]; [1]International Clinical Research Centre, St. Anne's University Hospital, Brno, Czech Republic, St. Anne's University Hospital Brno, Brno, CZECH REPUBLIC, [2]Institute for Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, CZECH REPUBLIC, [3]Department of Neurology, University Hospital Olomouc, Olomouc, Czech Republic, Olomouc, CZECH REPUBLIC, [4]1st Department of Neurology, Masaryk University, Brno, Czech Republic, Brno, CZECH REPUBLIC, [5]Department of Neurobiology, Barrow Neurological Institute, Phoenix, AZ, USA, Phoenix, AZ, [6]Memory Clinic, Department of Neurology, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic, Prague, CZECH REPUBLIC, [7]Department of Pharmacology and Chemical Biology, Emory University School of Medicine, Atlanta, GA, USA, Atlanta, GA, [8]Department of Neurosciences, University of California San Diego, La Jolla, CA, USA, San Diego, CA, [9]nstitute for Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, CZECH

REPUBLIC, [10]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, USA, Atlanta, GA, [11]Division of Neurology, University Medical Centre, Ljubljana, Slovenia, Ljubljana, SLOVENIA, [12]Department of Neurosciences, Mayo Clinic, Rochester, MN, USA, Rochester, MN.

Aging is the greatest risk factor for Alzheimer's disease (AD)[1], a pervasive cognitive disorder with unsettled etiology. The precise role of aging in AD, however, remains poorly understood. Accumulating evidence documents the dysregulation of circulating microRNAs (miRNA) separately in aging[2,3] and AD[4]. Considering miRNAs play a role in aging and longevity[5,6], we comprehensively test which aging-associated miRNA changes are observed in AD, and change in AD beyond aging in the circulating miRNA network. Here we show that plasma miRNAs in aging are downregulated and preferentially targeted to extracellular vesicle (EV) content, while in AD, miRNAs are further downregulated and of exclusive EV origin. We further show that miRNAs in AD display altered proportions of motifs relevant to their loading into EVs[7,8] and secretion propensity[9]. Considering endosomes play a role in the genesis of EVs[10,11] and are compromised early in AD[12], these findings implicate endosomal pathology underlying the AD plasma miRNA profile and its potential as a mechanistic AD biomarker. Striking similarities between plasma miRNA profiles in aging and AD further suggest that the AD miRNA network profile reflects a pathological exacerbation of the aging process whereby physiological suppression of AD pathology by plasma miRNAs becomes insufficient.

B584/P2256

Effect of fine particles driven pollution combined to UV exposure on keratinocytes 2D culture and reconstructed 3D skin models

E. Leblanc-Noblesse, L. Sobilo, J. Sage, K. Pays, C. Nizard; LVMH Recherche, Saint Jean de Braye, FRANCE.

INTRODUCTION: Skin is the largest and outermost organ of the human body, which makes it an important barrier against external aggressions. Due to its stratified epithelium formed by layers of keratinocytes and the lipophilic film on its surface, skin is impermeable to most molecules and microorganisms. However, to date, there are some pieces of evidence that describe the adverse effects of pollutants on keratinocyte physiology and metabolism. These pollutants and particularly fine particles associated with UV Irradiation are known to disrupt skin homeostasis. Indeed, in addition to oxidative stress and the ensuing inflammation, we have brought to light numerous structural modifications such as alteration of the skin barrier and metabolic activity as well as a degradation of the extra-cellular matrix.

MATERIALS AND METHODS: Different categories depending on the size of the fine particles were tested in this study: PM2.5, PM10 and Urban Dust. These particles associated or not with UVB Stress were either applied to a cell culture of normal human keratinocytes (NHK) or on a reconstructed skin model and different end-point analyses were carried out: A gene expression profiling (TLDA technology) where genes were specifically selected for their biological functions relevant to pollution, ATP quantification, inflammatory cytokines assay using the multiplex technology and imaging techniques (immunostaining and confocal microscopy) to assess the protein expression of cutaneous markers.

RESULTS: The results of the various experiments showed that pollution driven by fine particles coupled to UV irradiation is able to induce numerous skin deleterious phenomena such as a strong induction of pro-inflammatory cytokines, a decrease in cellular energy, a high oxidative stress and the degradation of extracellular matrix components. These results allow us to bring forward that chronic exposure to pollutants, associated with solar irradiation, enhances premature skin aging.

B585/P2257

Cell size and growth rate affect the proteome and modulate cell senescence

E. Zatulovskiy[1], M. Lanz[1,2], S. Zhang[1], J. Elias[2], J. Skotheim[1]; [1]Department of Biology, Stanford University, Stanford, CA, [2]Chan Zuckerberg Biohub, Stanford, CA.

Cell size is tightly controlled in healthy tissues, but it is unclear how deviations in cell size affect cell physiology. To address this, we measured how cell proteome changes with cell size. We found that size-dependent protein concentration changes are widespread and predicted by subcellular localization, size-dependent mRNA concentrations, and protein turnover. As proliferating cells grow larger, concentration changes typically associated with cellular senescence are increasingly pronounced, suggesting that large size may be a cause rather than just a consequence of cell senescence. Consistent with this hypothesis, larger cells are prone to replicative, DNA damage-, and CDK4/6i-induced senescence. Moreover, we found that size-dependent changes to the proteome, including those associated with senescence, are not observed when an increase in cell size is accompanied by an increase in ploidy. In addition to the proteomic changes, large cells also have lower specific growth rates. That both the cell's proteome and growth rate change with cell size suggests they may be interdependent. To test the role of size-dependent growth rate decrease in proteome remodeling, we used quantitative mass spectrometry to measure how the cell proteome changes in response to the mTOR inhibitor rapamycin, which decreases cellular growth rate and has only minimal effect on cell size. We found that large cell size and mTOR inhibition, both of which lower the growth rate of a cell, remodel the proteome in similar ways. This suggests that many of the effects of cell size are mediated by the size-dependent slowdown of the cellular growth rate. Importantly, size-dependent changes to the cell's growth rate and proteome composition are still apparent in cells continually exposed to a saturating dose of rapamycin, indicating that cell size can affect the proteome independently of mTORC1 signaling. Taken together, our results clarify the dependencies between cell size, growth, mTOR activity, and the proteome remodeling that ultimately controls many aspects of cell physiology.

B586/P2258

Metabolic gating of misfolded protein import into mitochondria

Y. Wang[1], L. Ruan[1], J. Zhu[2], A. Chang[3], X. Zhang[1], A. Tomaszewski[1], R. Li[1,2,3]; [1]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, [2]Mechanobiology Institute, National University of Singapore, Singapore, SINGAPORE, [3]Department of Chemical and Biomolecular Engineering, Johns Hopkins University Whiting School of Engineering, Baltimore, MD.

Metabolic imbalance and loss of proteostasis are common hallmarks of ageing. At the nexus of them are mitochondria, the key organelles that control cellular metabolism and influence proteostasis. The 'mitochondria as guardian in cytosol' (MAGIC) pathway represents an example in which mitochondria import and degrade misfolded proteins (MPs) concentrated on the cytosolic surface of mitochondria. This study investigates how mitochondria maintain a delicate balance of metabolic and proteostasis functions. By performing a genome-wide screening in yeast, we identified the yeast Snf1 protein kinase and its mammalian ortholog AMP-activated protein kinase (AMPK) as the master regulator of the permissibility for MPs to enter mitochondria. Activation of Snf1 upregulates the expression of nuclear encoded mitochondrial genes and inhibits the import of MPs in a manner dependent on downstream transcription factors. This inhibition is orchestrated through multiple mechanisms, including substrate

competition, translocon selectivity, and elevated expression of two outer membrane proteins. By limiting the mitochondrial entry of MPs under Snf1 activation and glucose restriction, cellular fitness and mitochondrial capacity are protected under stress associated with MPs. We also show that Snf1/AMPK prevents the mitochondrial accumulation of disease-related misfolding-prone proteins such as FUS, suggesting a novel link between metabolic states and proteinopathies.

B587/P2259

Regulation of age-associated insulin resistance by MT1-MMP-mediated cleavage of insulin receptor

X. Guo, Z. Bian, H. Wong; Hong Kong Baptist University, Hong Kong, Hong Kong

Insulin sensitivity progressively declines with age. Currently, the mechanism underlying age-associated insulin resistance remains unknown. Here, we identify membrane-bound matrix metalloproteinase 14 (MT1-MMP/MMP14) as a central regulator of insulin sensitivity during ageing. Ageing promotes MMP14 activation in insulin-sensitive tissues, which cleaves Insulin Receptor to suppress insulin signaling. MT1-MMP inhibition restores Insulin Receptor expression, improving insulin sensitivity in aged mice. The cleavage of Insulin Receptor by MT1-MMP also contributes to obesity-induced insulin resistance and inhibition of MT1-MMP activities normalizes metabolic dysfunctions in diabetic mouse models. Conversely, overexpression of MT1-MMP in the liver reduces the level of Insulin Receptor, impairing hepatic insulin sensitivity in young mice. The soluble Insulin Receptor and circulating MT1-MMP are positively correlated in plasma from aged human subjects and non-human primates. Our findings provide mechanistic insights into regulation of insulin sensitivity during physiological ageing and highlight MT1-MMP as a promising target for therapeutic avenue against diabetes.

B588/P2260

Differential transcriptional and splicing profile in POLR3A-mutated Wiedemann-Rautenstrauch syndrome fibroblasts

G. Arboleda, D. Santos-Gil, P. Gaete, C. Arboleda-Bustos, H. Arboleda; Universidad Nacional De Colombia, Bogota, Colombia

Introduction: Wiedemann-Rautenstrauch syndrome (WRS) is a rare disease characterized by premature neonatal aging, caused by mutations in the RNA polymerase 3 subunit A (POLR3A) gene. POLR3A has important roles in regulating the transcription of small RNAs, including tRNAs, 5S rRNA and snRNA 7SK. However, the mechanisms of cellular senescence and accelerated aging in WRS are not fully understood. **Aim:** To determined differentially expressed genes and intron retention events, as well as key metabolic pathways underlying WRS from RNA-Seq data by using bioinformatic methods. **Methodology:** Primary fibroblasts from forearm skin biopsies of two WRS patients (WRS1 and WRS2) with distinct monoallelic heterozygous POLR3A mutations and control cells matched for age and gender were cultured. Total RNA was extracted and Paired-ended RNA-seq libraries were constructed using the TruSeq Strand mRNA LT Sample Prep Kit and sequenced using the Illumina NovaSeq 6000. Differential gene expression analysis was performed using DESeq2 to identify differentially expressed genes (DEGs) between WRS datasets and controls. Intron retention detection was analyzed using IRFinder. Enrichment analysis was performed by Gene Ontology and the Kyoto encyclopedia of genes and genomes pathways. **Results:** 592 DEGs for WRS1 and 984 DEGs for WRS2 were obtained compared to the control group. Additionally, 204 genes were found in common between the two WRS datasets, of which 136 were upregulated and 68 downregulated. The functional enrichment analysis showed that the PI3K-Akt signaling pathways,

organization of the extracellular matrix, retinol metabolism and regulation of the inflammatory response were found to be commonly enriched in the patients. In addition, 24 differentially expressed introns were detected between WRS patients and control. The percentage of genes with intron retention was 8,14% in WRS1, 6,28% in WRS2 and 7,8% in control. Transcripts with intron retention in WRS patients were involved in RNA-binding, cell cycle regulation and transcription regulation. **Conclusions:** This study identified key genes and signal pathways which might help us improve our understanding of the mechanisms involving POLR3A in the physiopathology of WRS.
Acknowledgements: Funded by DIB-Universidad Nacional de Colombia and MINCIENCIAS (#844-2019)

B589/P2261

Analysis of the role of the POLR3A gene in cellular senescence: Wiedemann-Rautenstrauch syndrome as a model

G. Arboleda, K. Velásquez-Méndez, H. Arboleda; Universidad Nacional De Colombia, Bogotá, Colombia

Analysis of the role of the POLR3A gene in cellular senescence: Wiedemann-Rautenstrauch syndrome as a modelK.L. Velásquez-Méndez[1], H. Arboleda[1,2], G. Arboleda[1,3,*1]Grupos de Neurociencias y Muerte Celular, Instituto de Genética y Facultad de Medicina; [2]Departamento de Pediatría; [3]Departamento de Patología, Universidad Nacional de Colombia, Bogotá, Colombia.
*gharboledab@unal.edu.co**Introduction:** Wiedemann-Rautenstrauch syndrome (WRS), also called neonatal progeria, is an extremely rare disease characterized by premature aging at birth, pseudo-hydrocephalus, triangular face with prominent forehead and veins, intrauterine and postnatal growth retardation, as well as absence of subcutaneous fat, osteopenia, presence of neonatal front teeth and scoliosis. In the scientific literature, at least 35 cases have been described, of which 60% are Colombian cases. Previous studies have associated WRS with biallelic mutations in the *POLR3A* gene as responsible for the senescent phenotype. **Aim:** Here we report the results of gene expression level analysis of the RPC1 protein encoded by the *POLR3A* gene for two WRS patients whose mutations were previously reported in the literature, as well as the effect of these mutations on the expression of the senescence-associated protein p53. **Methodology:** Through the expression levels of the RNA polymerase III reporter genes (5SrRNA, tRNA-Leu-CAA and 7SK RNA), the effect of WRS mutations in the transcription of the RNA polymerase III complex was analyzed, as well as the effect on the expression of 18S and 28S ribosomal subunits to assess ribosomal biogenesis. Measurements of the mitochondrial membrane potential were also performed. Finally, using the fibrillarin nucleolar protein, the size of the nuclei and nucleoli and their relationship with premature aging were also analyzed. **Results:** It was found that the *POLR3A* gene mutations c.3772_3773delCT (p. Leu1258Gly*) and c.3G>T (p. Met1?) cause destabilization of the RNA polymerase III complex, causing deregulation of the transcription of its target genes which affects vital processes for the cell, such as ribosomal biogenesis and mitochondrial function. Furthermore, fibroblasts from WRS patients show nucleolar disruption, deregulation of ribosomal biogenesis affecting global protein synthesis as well as mitochondrial dysfunction. In addition, mutations in the *POLR3A* gene induce activation of p53 in the nucleus, as well as activation of pH2AX in response to DNA damage, which is associated with normal human aging. **Conclusions:** These results open new alternatives to study cell senesce and human aging.**Acknowledgements:** Funded by DIB-Universidad Nacional de Colombia and MINCIENCIAS (#844-2019).

B590/P2262

Lovastatin enhances follicular development in mice ovaries

Y. Kim[1], Y. Cho[2], J. Jang[3], Y. Koo[3], S. Park[1], J. Lee[1,3]; [1]CHA Fertility Center Seoul Station, Seoul, Korea, Republic of, [2]Wonju Severance Christian Hospital, Wonju-si, Korea, Republic of, [3]CHA University, Pocheon, Korea, Republic of

Ovarian aging is not reversible through the pathophysiological mechanism underlying the poor follicular development. Steroidogenesis in the oogenesis and follicular development for ovulation is highly complicated processing and several factors are involved depending on the follicle stage. The starting point of steroidogenesis is an influx of cholesterol from extracellular fluid using LDLR signaling. The up-regulation of low-density lipoprotein receptor (LDLR) correlates with clinical pregnancy during in vitro fertilization for humans. Therefore, we investigated that Lovastatin is a LDLR enhancer for hyper-ovulated stimulation for promoted ovulation and steroidogenic activity of the ovary. We injected the lovastatin to hyper-stimulated mouse ovaries and analyzed its follicular development. We confirmed the follicular development and the number of oocytes through the histological analysis and counting of ovulated oocytes. And the mRNA and protein isolated from the ovary were compared and analyzed gene expression through real time-PCR and western blot regarding follicular development and steroidogenesis genes. Body weight increased less in the lovastatin treated group than control. LDLR was expressed in ovaries and was 40% higher in lovastatin-treated ovaries than control ovaries. The number of oocytes increased 20% for lovastatin-treated ovaries than control. And maturation ratios of lovastatin-treated GV oocytes significantly increased compared to the control group. The hyper-ovulated mouse ovaries treated with lovastatin increased the expression of LDLR and StAR (Steroidogenic acute regulatory protein) which are components of the steroidogenesis pathway. In conclusion, we found that the role of lovastatin promotes follicular development by increasing the LDLR genes in the ovary of mice. Previous data shows that activation of LDLR and StAR was associated with follicular development in infertile women. Therefore, further investigation is needed to test the effect of lovastatin on human ovary. Our data suggests that lovastatin has stimulated LDLR expression to enhance ratio of follicular development in the ovaries.

B591/P2263

Characterization of intra and sub-cellular deposits in mono and polygenic retinal degenerations using iPSC-derived retinal pigment epithelium

R. Sharma, M. Farnoodian, D. Bose, D. Ortolan, K. Miyagishima, K. Bharti; NEI/NIH, Bethesda, MD

Retinal pigment epithelium (RPE) is a monolayer of hexagonal cells located at the back of the eye; it provides nourishment and support to photoreceptors (PR) and choroidal capillaries (CC), phagocytosis of photoreceptor outer segments (OS) and polarized cytokine secretion for maintaining outer retina homeostasis and normal vision. Dysfunctional RPE, which is likely caused by aging and environmental factors, results in the degeneration of other retinal layers and causes vision loss. The hallmark phenotypic features of degenerating RPE includes intra and sub-cellular deposits. These are the common phenotypes across different retinal degenerative disorders. The underlying factors and pathways involved the biogenesis of lipid and protein-rich intra and sub-cellular deposits are still not entirely well understood. We have established induced pluripotent stem cell (iPSC) derived RPE model for monogenic (Stargardt, L-ORD) and polygenic (AMD) retinal degenerations to study lipids and protein deposits. In addition, we exploited LC-MS and MALDI-TOF imaging to understand the composition of these deposits

in different retinal degenerative diseases. iPSC-RPE are seeded on a porous plastic membrane and cultured for five weeks. Mature RPE cells from Stargardt and L-ORD patients were fed with OS for seven days and tested for the accumulation of sub-RPE deposits. The AMD model was developed by using an activated alternate complement stressor. SubRPE deposits can be labeled for APOE and lipid deposits by Nile red and BODIPY dyes. In the case of LC-MS, cells were collected and analyzed for lipidomics, while MALDI-TOF imaging was carried out on a porous decellularized membrane. Our data revealed that RPE cells from Stargardt and L-ORD patients recapitulated the intra and sub-cellular lipid deposits. With LC-MS on Stargardt patient-derived iPSC-RPE, we discovered higher amounts of sub-species of ceramide. While MALDI-TOF revealed differences in subRPE deposit composition between different diseases. In conclusion, we created RPE models to study the RPE-deposit biogenesis and their composition in different retinal degenerative diseases.

B592/P2264
The functional landscape of an Alzheimer's disease risk factor
Z. March, P. Narayan; NIH/NIDDK, Bethesda, MD

APOE alleles are the strongest genetic modifiers of late-onset AD risk. *APOE* exists in three common alleles (*E2, E3,* and *E4*) that encode three apolipoprotein E (APOE) variants, which differ from one another at only two amino acid sites (112 and 158). Whereas the most common allele, *APOE3*, is neutral with respect to AD risk, *APOE4* is the most highly-validated risk factor for sporadic late-onset AD. In contrast, *APOE2* is considered protective due to its decreased association with AD. Moreover, rare variants have recently been uncovered that appear extraordinarily protective even against familial AD. Here, I present our progress to define the functional landscape of APOE. We have identified multiple rare coding variants as determinants of APOE conformation and have begun to delineate how specific variants affect neurodegenerative disease-associated phenotypes in human iPSC-derived neural subtypes. These studies begin to comprehensively address how genetic variation within *APOE* affects AD risk and resilience.

Cell Death

B593/P2265
Associate Professor
Y. Wan[1], J. M. Salinas[2], X. Li[3], T. Li[3], Y. Zhang[3], E. Slevin[2], **F. Meng**[2]; [1]Dept. of Pathophysiology, Southwest Medical University, Luzhou, CHINA, [2]Indiana University School of Medicine, Indianapolis, IN, [3]Southwest Medical University, Luzhou, CHINA.

Transcribed ultraconserved regions (T-UCRs) are long non-coding RNAs (lncRNAs) that are transcribed from ultraconserved regions (UCRs) in the human genome. Accumulation of senescent hepatocytes may contribute to loss of functional hepatic mass and lead to alcoholic liver decompensation and fibrosis. The current study aimed to characterize the functional role of T-UCR regulated cellular senescence during alcoholic-associated liver diseases (ALDs). Senescence related T-UCR and gene expression was assessed using microArray, the cellular senescence PCR Array/Assays in ethanol- and LPS-treated normal human hepatocytes (N- Heps), as well as in liver specimens from a mouse model of chronic and binge ethanol feeding (the NIAAA model) relative to control liver tissue. Cellular senescence was measured by SA-β-gal Activity assay. We identified that chronic and binge ethanol feeding significantly increased the

expression of uc.189, total liver histopathology score and hepatocellular senescence by PCR array and SA-β-gal assay, along with the reduced expression of let-7a microRNA. Overexpressing of let-7a has significantly reduced uc.189 expression and blocked enhanced cellular senescence in ethanol and LPS treated N-Heps. Silencing of uc.189 has also decreased ethanol and LPS-induced senescence and increased viability in N-Hep cells. TLR4 knockout mice and lin28 knockout mice with reduced uc.189 /enhanced let-7a expression displayed less sensitivity to alcoholic injury, along with enhanced SA-β-gal activity and reduced expressions of α-SMA, Col1A1 and TIMP-1. Silencing uc.189 by TLR4/lin28 depletion also significantly reduced hepatic p53, PAI-1 and EGR1 expressions in chronic and binge ethanol feeding mice, suggests a critical role of specific T-UCR regulated cellular senescence during ALD. Our findings provide new insight into the function of long non-coding RNA regulated cellular senescence and testing novel therapeutic approaches for human alcoholic liver diseases. Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} <![endif]-->

B594/P2266

Mitochondrial localized novel protein Cux1 (p75) regulates mitophagy & ER stress induced cell death

A. Paripati, A. Pal, N. Sepuri; University of Hyderabad, Hyderabad, INDIA.

Mitochondria, apart from its most prominent function in ATP generation, are also involved in key cellular events like calcium signaling, lipid and amino acid metabolism, ROS signaling, regulation of cell death. Given the importance of mitochondria in cellular homeostasis, it is required to maintain mitochondria in healthy state. Mitochondria communicates with other organelles for its functions and quality control. Mitochondria and ER are known to form closely associated networks that are structurally and functionally essential to maintain cellular homeostasis and the fate of cell under various pathological conditions depends on this network. In this study we report that one of the isoforms of homeodomain transcription factor Cux1(p75) localizes to outer membrane of mitochondria. Functional studies in knock-down and overexpression of Cux1 show that it regulates the mitophagy by stabilizing the PINK1 upon mitochondrial depolarization. Additionally, it is also involved in ER-Mitochondrial interaction and ER-stress mediated cell death by specifically interacting with Mfn2. This study uncovers the possible new mechanism of ER-Mitochondrial interaction and mitophagy.

B595/P2267

Cell survival following direct executioner caspase activation

M. Nano[1], J. A. Mondo[1], V. Balasanyan[2], G. Sun[3], D. J. Montell[1]; [1]UCSB, Santa Barbara, CA, [2]UCLA, Los Angeles, CA, [3]Shandong University, Jinan, CHINA.

Executioner caspase activation has long been considered a point-of-no-return in apoptotic cells. However, some studies report that cells can recover from caspase activation after treatment with drugs or radiation. An open question is whether cells can recover from direct caspase activation without drug-induced pro-survival stress responses. To address this question, we engineered a HeLa cell line to

express an inducible caspase-3 instead of the endogenous enzyme together with a quantitative caspase activity reporter. We measured the level and rate of caspase activity in individual cells over time and demonstrate that cells can survive direct effector caspase activation. Furthermore, we show that the dynamics and amount of caspase activity is insufficient to predict cell death vs. survival. These results indicate that pre-existing differences in cellular state modify the threshold of caspase activity required to irreversibly commit cells to die. Such differences may underlie incomplete tumor cell killing in response to apoptosis-inducing cancer treatments such as radiation and chemotherapy.

B596/P2268

Silencing BAX Inhibitor 1 (BI-1) sensitizes senescent pulmonary fibroblasts to ER stress mediated apoptosis

D. W. Moore[1], J. G. Fernandez-Davila[1], S. Bui[1], S. D. Nathan[2], L. R. Rodriguez[1], G. M. Grant[1]; [1]Department of Biology, George Mason Univeristy, Manassas, VA, [2]Advanced Lung Disease and Transplant Program, Inova Fairfax Hospital, Falls Curch, VA.

Idiopathic pulmonary fibrosis is characterized by an abundance of highly secretory apoptotic resistant fibroblasts. We have previously demonstrated that IPF fibroblasts (IPF-F) are also resistant to oxidative stress and ER stress. The IRE1α; arm of the unfolded protein response (UPR) is critical to the fate of highly secretory cells under ER stress. UPR activation promotes IRE1α; dimerization, leading to the degradation of mRNA transcripts and splicing of the XBP-1 transcription factor. Multiple IRE1α; modulating proteins including BAX Inhibitor 1 (BI-1) are upregulated in IPF-F. As an inhibitor of IRE1α; BI-1 is a potential mediator for apoptosis resistance in IPF-F under ER stress. We hypothesis that inhibition of this pathway may alter apoptosis resistance.Senescence in primary fibroblasts was measured by β-Galactosidase staining and telomere length analysis via QPCR. Immunohistochemistry was performed to measure expression of BI-1 in donor lung tissue. IPF-F and normal human lung fibroblasts (NHLF) were transfected with siRNAs targeting BI-1, IRE1α;, and BAX. IRE1α; signaling was assayed via QPCR of: *XBP*, *HO1*, *SEC61A*, *ERO1A*, and *ERDJ4*. Cell proliferation markers *p21, Bcl-xL,* and *Cyclin D* were measured by qPCR and western blot. Cell viability was measured via Cell Titer Glo viability assay.IPF-F as compared to NHLF showed increased senescence, decreased expression of proliferation associated genes, increased expression of cell cycle arrest genes. Increased BI-1 expression was observed in primary IPF lung tissue and fibroblasts. Silencing BI-1 under conditions of ER stress reduced the percentage of senescent cells. BI-1 deficient IPF-F and NHLF demonstrated an increase in percent XBP-1 splicing. Measurement of RNA expression in BI-1 silenced fibroblasts showed an upregulation in *HO1* and *ERDJ4* and no change in *SEC61A* and *ERO1A*. Silencing of BAX and IRE1α; under conditions of ER stress induced by tunicamycin challenge resulted in decreased cell viability of both IPF-F and NHLF.The silencing of BI-1 under conditions of ER stress promoted the preferential clearance of senescent IPF-F. A potential mechanism for this may be the increased IRE1α; signaling activity under silencing conditions. In comparison, NHLF showed only a mild increase in IRE1α; activity under the same conditions. These data reinforce that BI-1 acts as a survival mechanism against ER stress in IPF-F and the differential response to BI-1 silencing suggests that this could serve as a potential target for future therapeutics.

B597/P2269

Identification of genes that alter cancer cell sensitivity to DNA damage/OXPHOS inhibition using CRISPR screening

X. Kong, A. Bayrakci, C. Umemura, K. Yokomori; UC Irvine, Irvine, CA.

A major characteristic of cancer cells is their increasedreliance on glycolysis to maintain cellular homeostasis (Warburg effect).Recent evidence indicates, however, that the oxidative phosphorylation (OXPHOS)pathway remains critical for cancer cell survival. Our recent study indicatesthat cancer cells increase their metabolic reliance on OXPHOS in order tosurvive in the presence of DNA damage when poly(ADP-ribose) polymerase 1 (PARP1)is activated. To understand the molecular pathways underlying the metabolic switchthat promotes cell survival in response to DNA damage, we performed CRISPR libraryscreening in cells with DNA damage (MMS) with and without OXPHOS inhibitor rotenone(R). We found several PARP1 sgRNAs as the top hits for enrichment for MMS+R treatedcells, which is consistent with our previous study, confirming that OXPHOSsuppresses parthanatos (PARP-dependent cell death). We also found sgRNAsagainst MIR378G in the drop-out screen in MMS-treated cells whose mutation isknown to increase DNA damage sensitivity. These results provide strong supportfor the authenticity of the screening. Interestingly, we found that multiplesgRNAs specific for hexokinase 2 (HK2) and Phosphofructokinase, platelet (PFKP),two critical enzymes in the glycolysis pathway, were among the top hits on theenrichment list for MMS treatment (but not for MMS+R), suggesting thatsuppression of the glycolysis pathway promotes damaged cell survival. PARP1 wasshown to inhibit HK in response to stress/damage, which was simply thought to causedeleterious energy deprivation and cell death. Our results strongly suggestthat glycolysis inhibition actually has pro-survival effect, possibly byenforcing the metabolic switch to OXPHOS. For MMS+R, we found enrichment ofmultiple sgRNAs against subunits of two distinct ubiquitin (Ub) E3 ligasecomplexes that both mediate histone ubiquitination. We also found threesubunits of an Ub-dependent histone methyltransferase (HMTase) complex. Theresults were further confirmed using individual sgRNAs against candidate genesin HeLa cells expressing Cas9. Ourfindings raise the possibility that coordinated functions of these histonemodification enzyme complexes may be a major driver of parthanatos, which isnormally suppressed by OXPHOS. Ourresults reveal a link between metabolic and epigenetic regulations in responsesto DNA damage critical for cell survival.

B598/P2270

Analysis of a role for Mitochondria Associated ER Membranes in Compartmentalized Cell Elimination

A. Elkhalil, R. Sharmin, K. Juanez, Y. Mohamed, G. Clark, P. Ghose; University of Texas At Arlington - Arlington, TX, Arlington, TX.

Programmed cell death is a vital cell fate for normal development and homeostasis. Morphologically complex cells are known to have elaborate processes, such as axons and dendrites in neurons. While complex cells are commonplace, how their programmed elimination takes place is not well understood. Also elusive is how their elimination under pathological conditions or following injury occurs. We introduce a 'tripartite' killing program called Compartmentalized Cell Elimination (CCE) that eliminates two cells of the nematode *C. elegans* during its embryonic development-the morphologically complex tail-spike cell (TSC) and the sex-specific CEM neurons. CCE is characterized by three cell compartments dying in three different ways. Strikingly, the single process/dendrite of these cells demonstrates two completely different elimination modalities in its two segments, each reminiscent of different types of

developmental pruning. The proximal segment fragments in a manner similar to injury-induced Wallerian degeneration of axons, while the distal segment withdraws/retracts into itself. We find that soma-directed transport and confinement of mitochondria are necessary for CCE initiation. We have also found that genes promoting endoplasmic reticulum (ER) network stability promote process degeneration during CCE.Mitochondria-Associated ER Membranes (MAMs) are regions of the ER tethered to mitochondria that mediate communication between ER and mitochondria. MAMs are known to be involved in calcium homeostasis, lipid synthesis and cell death signaling. Recent studies also show the connection of MAMs to neurodegenerative diseases. We seek to look at the roles of MAMs in CCE. Our preliminary results show exaggerated mitochondria when ER network stability is compromised. Using tri-color imaging of the ER, mitochondria, and TSC, we plan to further look at the nature of mitochondria in these ER stability mutants and look at the involvement of the mitochondrial fusion machinery in mitochondrial elaboration. Conversely, we also plan to study the nature of the ER in mitochondria trafficking and dynamics mutants. Together we aim to understand novel roles of MAMs in CCE and by extension developmental pruning and neurodegeneration.

B599/P2271

Mitochondria-targeted anti-oxidant mitoquinone suppresses benign prostatic hyperplasia via regulation of AR-NLRP3 pathway

B. Jin[1], H. Kim[2], T. Gil[1], H. An[1]; [1]Kyung Hee University, Seoul, KOREA, REPUBLIC OF, [2]Sangji University, Wonjusi, KOREA, REPUBLIC OF.

Mitoquinone (MitoQ), a mitochondria-targeted anti-oxidant agent, has been estimated in several diseases. Previous studies have demonstrated its anti-inflammatory and anti-proliferative potential. Nevertheless, the effect of MitoQ on the development and progression of BPH remains unclear. The present study aimed to investigate the therapeutic effects of MitoQ on BPH models and molecular mechanism(s) underlying its effects on BPH. Anti-proliferative and anti-oxidant functions of MitoQ were evaluated in dihydrotestosterone (DHT)-stimulated prostatic cells. The binding between MitoQ and NLRP3 was estimated using docking assay. The effects of MitoQ were estimated in testosterone-induced BPH animal model. The levels of AR and NLRP3 were analyzed using immunoblotting, qRT-PCR and IF assay. MitoQ inhibited AR/NLRP3 signaling and mitochondrial ROS induced by DHT in prostatic epithelial cells. Molecular modeling revealed the interaction between NLRP3 NACHT and MitoQ. Prostatic cell proliferation induced by testosterone was suppressed by oral administration of MitoQ in BPH rats model. In addition, MitoQ exerted pro-apoptotic activity and anti-oxidant effects via inhibition of AR and NLRP3 signaling pathway. Our results present anti-proliferative, anti-oxidant and pro-apoptotic effects of MitoQ in androgen-induced BPH models, suggesting MitoQ as a direct inhibitor of NLPR3 and therapeutic agent for treatment of BPH. ▣ This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Grant No. NRF-2022R1A4A3019157 and NRF-2021R1A6A3A01086659).

B600/P2272

Pituitary tumor-transforming gene 1 (PTTG1) modulates the growth of oral squamous cell carcinoma by regulating double-strand DNA break

S. Park[1], J. Jung[2], S. Lee[1], J. Choi[1]; [1]Oral Pathology, Gangneung-Wonju National Universityniversity, Gangneung-si, KOREA, REPUBLIC OF, [2]Gangneung-Wonju National Universityniversity, Gangneung-si, KOREA, REPUBLIC OF.

Human PTTG1 is known as onco-protein and involved with sister chromatid separation in normal cells. PTTG1 was subsequently revealed to be highly expressed in diverse type of malignant tumors, which regulates the growth and metastatic properties of tumor. Oral squamous cell carcinoma (OSCC) is a one of the malignant tumor including oropharynx, larynx and oral cavity. Although improved detection and treatment methods of OSCC have been discovered in recent years, however, 5-year survival of patients with OSCC steadily be closed to 50%. However, the diagnostic biomarkers to detection of OSCC and molecular mechanisms underlying the regulation of PTTG1 in OSCC largely unknown. In the present study, we first investigated the role of PTTG1 in growth of OSCC. Then we explored the regulation of double-strand DNA break (DSB) depend on PTTG1 expression in OSCC growth. To investigate the function of PTTG1 in OSCC growth, we analyzed the cell viability and cellular senescence by EdU assay and SA-β-gal assay, respectively. To explain the DNA damage-induced senescence of PTTG1, we analyzed the chromosomal damage in OSCC cell lines *in vitro*. Finally, we confirmed the effect of PTTG1 on tumor growth and gene expression related to cell viability and DNA damaged-induced senescence in mice xenograft model. The expression of PTTG1 in OSCC tissues was remarkably higher than adjacent normal tissues. In addition, PTTG1 knockdown suppressed the cell viability and promoted the DNA damage-induced senescence in OSCC cell lines by accumulating chromosomal damage. In vivo mice model, tumor growth was significantly decreased in PTTG1 knockdown group compared with Mock control group via DSB. Taken together, our results suggest that PTTG1 expression modulates OSCC growth via DSB. Those finding may provide new strategies to develop more reliable diagnostic markers for patients with OSCC.

B601/P2273

The DNA Damage Response regulates epithelial cell dynamics in the infected gut

P. Nagy, B. Hixson, N. Buchon; Department of Entomology, Cornell University, Ithaca, NY.

Intestinal microbes are central to host health but can also constitute an important etiological factor for the initiation and progression of diseases. However, the mechanisms by which pathogenic microbes influence intestinal tissue health remain largely unknown. Our overarching goal is to understand how pathogenic microbes inflict stress on the intestinal epithelium in *Drosophila melanogaster* and to characterize the impact of the molecular dialogue on cell dynamics. This is particularly important in the gut tissue where intestinal barrier, immune and digestive functions need to be coordinated with cell turnover. We and others have demonstrated that microbes stimulate epithelial turnover and initiate a feedback loop in which the elimination of enterocytes (ECs) activates intestinal stem cell proliferation in order to replace stressed/dying cells, balancing cell loss with tissue renewal. However, the molecular mechanisms that lead to EC loss in response to infection or innate immune activation are still not understood. We have found that infection induces an immediate host response in a subpopulation of midgut ECs which accumulate the DNA damage histone mark, γH2av (the phosphorylated form of H2av). Importantly, we have shown that pathogens, but not the commensal microbiota, activate the DNA damage repair (DDR) pathway which modulates epithelial cell elimination, cytokine production and stem

cell proliferation. Functional/bacterial genetics and microscopy imaging techniques allowed us to determine the microbial characteristics and epithelial cell state that influence DDR pathway activation. Gut transcriptome analyses and cell lineage-tracing methods previously developed in our lab enable us to identify how DDR pathway activation influences both cell elimination and pro-survival pathways. This work expands our understanding of how extrinsic factors and intrinsic cellular processes predispose cells to stress inflicted by infection-induced damage. Our study demonstrates that DDR pathway activation is a novel signaling route modulating cell loss and cytokine production, which is critical for health and homeostasis in a high-turnover tissue such as the intestine.

B602/P2274

Ferroptosis in myocardial infarction

M. Mohr, S. Li, A. Trouten, R. Stairley, G. Tao; Medical University of South Carolina, Charleston, SC

Every 40 seconds, a person in the United States experiences a myocardial infarction (MI), or heart attack. Following MI, mature mammalian cardiomyocytes (CMs) are unable to repopulate the injured myocardium due to a low turnover rate. After MI, fibrosis occurs to prevent rupture leaving the heart with decreased contractility and function, potentially leading to heart failure. However, the neonatal heart retains a regenerative capacity during the first week of life. We attempted to determine the main source of CM loss after MI to gain insights into potential mechanisms to preserve pre-existing CMs after injury. Regenerative postnatal day 1 (P1) and non-regenerative postnatal day 7 (P7) mice were subjected to permanent left anterior descending artery occlusion surgery (LAD-O) and hearts were collected at multiple timepoints to profile scar progression and cell death. We examined three types of cell death using confocal microscopy including previously reported apoptosis and necroptosis, as well as ferroptosis. We identified ferroptosis as the main contributor to CM death in both P1 and P7 mouse hearts with non-regenerative hearts experiencing more ferroptosis. Ferroptosis is an iron-dependent form of cell death that occurs after the peroxidation of lipid radicals leading to increased reactive oxygen species (ROS) and thus cellular damage and death. Interestingly, developmental gene paired-like homeodomain 2 (*Pitx2*) is required for regeneration after cardiac injury and activates antioxidant genes including glutathione peroxidase 4 (*Gpx4*), a key regulator of ferroptosis. We hypothesized that *Pitx2* negatively regulates ferroptosis through upregulation of *Gpx4* and other ferroptotic genes. Using confocal microscopy, we analyzed markers of ferroptosis in *Pitx2* conditional knockout hearts (cKO) and *Pitx2* overexpression hearts (OE). OE hearts showed decreased lipid peroxidation compared to controls and cKO hearts. Additionally, we performed RNA sequencing on cardiomyocytes derived from human induced pluripotent stem cells (iCMs) with siRNA of *Pitx2* and found decreased expression of genes involved in ferroptosis resistance. In conclusion, we found that ferroptosis is the main form of CM death after MI in both regenerative and non-regenerative hearts. As well, *Pitx2* can regulate ferroptosis and may be important in regeneration after MI.

Physical Approaches to Cell Biology 2

B604/P2275

Condensates of Disordered Proteins Have Small-World Network Structures and Interfaces Defined by Expanded Conformations

M. Farag[1], S. R. Cohen[1], W. M. Borcherds[2], A. Bremer[2], T. Mittag[2], R. V. Pappu[1]; [1]Biomedical Engineering, Washington University in St. Louis, Saint Louis, MO, [2]Structural Biology, St Jude Children's Research Hospital, Memphis, TN.

Biomolecular condensates are thought to form via phase separation coupled to percolation (PSCP) driven by multivalent protein and RNA molecules. PSCP combines phase separation, a density transition, and percolation, a networking transition. What kind of mesoscale and molecular scale structures arise from PSCP based processes and do the interfaces formed via PSCP of flexible, intrinsically disordered proteins have special features? Here, we answer these questions by modeling PSCP using a coarse-grained *stickers*-and-*spacers* model implemented in LaSSI, a lattice-based simulation engine. Specifically, we model the low complexity domain of hnRNPA1 and designed variants thereof. The LaSSI model at single bead per residue resolution was parameterized using experimental data for chain conformations in dilute solutions. We show that the model generates phase diagrams that agree with experimental measurements. We find that individual chains are more expanded inside condensates when compared to the coexisting dilute phases. This arises from the preference for intermolecular physical crosslinks within condensates vs. the preference for intramolecular physical crosslinks in dilute phases. The network structures of molecules within condensates have small world topologies, implying a hub-and-spoke-like organization and significant spatial inhomogeneities that explain why condensates are viscoelastic network fluids. Our results also show that the interfaces of condensates have distinct molecular and mesoscale features. The interfacial width scales with the polymer length at low temperatures and increases exponentially with increasing temperatures, regardless of polymer length. Strikingly, we find that chains at the condensate interface have uncharacteristically high radii of gyration - larger than those of chains in the dense or dilute phases. Further, chains at the interface are oriented perpendicular to the surface of the condensate. Our results regarding the structures of interfaces have a direct bearing on recent discoveries demonstrating the importance of interfaces in mediating condensate mechanics and surface interactions, including polymerization and fibrillation reactions.

B605/P2276

RNA Structure Controls RNA Multimerization, Phase Separation with Protein, and Time-Dependent Condensate Viscoelasticity

I. Seim[1], A. Graham[2], A. Mathijssen[3], A. Gladfelter[1]; [1]University of North Carolina At Chapel Hill, Chapel Hill, NC, [2]University of California, San Francisco, San Francisco, CA, [3]University of Pennsylvania, Philadelphia, PA.

Biomolecular condensates can form by phase separation and likely serve myriad functions across cell biology. Many studies have characterized protein condensates, sometimes including model RNAs that are usually nonspecific, short, and/or homopolymeric. However, the majority of biomolecular condensates in cells likely comprise mixtures of proteins and complex RNAs including mRNAs. Thus, characterization of the role of RNA sequence and structure with respect to protein phase behavior is paramount to understanding biologically relevant condensates. We leveraged our understanding of the

Whi3 protein and its condensation with the G1 cyclin-encoding *CLN3* mRNA to characterize the contributions of mRNA structure to phase separation. Whi3-*CLN3* condensates influence nuclear division in syncytial cells and Whi3 preferentially phase separates with this target RNA. We first created a directed evolution algorithm that designs shuffled *CLN3* RNA sequences which either maximize or minimize predicted free energies of folding while preserving mass, known Whi3 binding sites, and overall nucleotide composition. RNAs with minimized free energies of folding contain many stable duplexes, while RNAs with maximized energies have unstable structures with long single-stranded regions. Using mass photometry, we show that RNAs with stable structures are mostly monomeric, while RNAs with unstable structures multimerize more favorably. When mixed with protein, all RNAs undergo phase separation, but condensate compositions are altered. Unstable RNAs that multimerize more favorably form condensates with high RNA concentrations and low protein concentrations, while RNAs with stable structures and a low tendency to multimerize form condensates with low RNA concentrations and high protein concentrations. Finally, we used passive microbead rheology to characterize the time-dependent viscoelasticity of condensates formed by the different RNAs. Unstable RNAs form condensates that undergo significant aging with long viscoelastic relaxation times indicating solid-like properties at many timescales. However, stable RNAs form more liquid-like condensates that undergo less aging and have shorter viscoelastic relaxation times. Thus, RNA structure can have multi-scale consequences for biomolecular condensates, with impacts on RNA multimerization and condensate composition and material properties.

B606/P2277

Effects of delamination treatment time and fluid shear force on the maintenance of pluripotency in mouse ES cells during the passage process

K. ABE[1], K. Ueno[2], S. Miyata[3]; [1]Center for Environmental Engineering, Medical Environment Engineering Group, Shimizu Corporation, Institute of technology, Tokyo, JAPAN, [2]Graduate School of Science and Technology, Keio University, Tokyo, JAPAN, [3]Faculty of Science and Technology, Keio University, Yokohama, JAPAN.

Since iPS cells, ES cells, have the ability to differentiate into various cells and are available for various treatments and studies, they have received great attention as cell products. How efficient and large-scale production of cells, a commodity, reduces treatment costs and leads to popularization, but many of the cultures are performed by humans. Automated culture equipments have also been developed, but in the maintenance culture of pluripotent stem cells, in order to proliferate cells efficiently while preserving their differentiation potential, cultured experts often use empirically constructed operations as a reference standard, and few cases have been comprehensively investigated. It is crucial for the development of the culture equipment to engineer the manual cell culture manipulation until now, and to clarify the impact of various conditions in the operation on the cell. Therefore, this study focused on the cell detachment process of the passage work in the culture of pluripotent stem cells, and clarified the impacts of cell detachment treatment time and enzyme treatment time on the differentiation potential of cultured pluripotent stem cells.

B607/P2278

Biophysical Characterization of Cytotoxic T cell Heterogeneity in Cancer Progression

Y. Zhang[1], J. Yu[1], T. Dinter[1,2], R. Wu[1,3], S. Duquette[1,3], T. Miettinen[1], A. Miller[1,4], S. Spranger[1,2,5], S. R. Manalis[1,3,6]; [1]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, [3]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, [4]Department of Health Sciences and Technology, Harvard University - Massachusetts Institute of Technology, Cambridge, MA, [5]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, [6]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA.

Cytotoxic T cells are key modulators of immune response and exhibit significant heterogeneity in terms of phenotype and function. Although previous studies have shown that T cell biophysical properties vary during cell activation and become altered by inflammatory conditions, the extent to which differential T cell function is associated with distinct biophysical properties is unknown. To address this, we are using a microfluidic device called suspended microchannel resonator (SMR) that measures the buoyant mass (BM) of individual cells with a precision near 0.1%. BM is defined as the product between a cell's volume and its density difference relative to the surrounding solution. Upon measuring BM of resting T cells from PBMCs of healthy donors, we consistently observed a bimodal distribution consisting of heavy (10-20 pg) and light (<10 pg) cells. We verified through scRNA-Seq and flow-based assays that both populations consisted of viable cells. We observed similar bimodal distribution from CD3+CD8+ T cells isolated from spleen of healthy mice. Interestingly, both the volume and density distributions of these T cells showed only one population, indicating that neither of these parameters alone was responsible for the bimodality of T cell BM. Thus, although these two T cell populations cannot be distinguished by conventional methods such as light scattering and density gradient centrifugation, high precision BM measurements uniquely enabled by the SMR can clearly distinguish these two populations. To investigate how the light and heavy populations relate to T cell state, we inoculated C57BL/6 mice with KP-SIY cells and separated exhausted tumor-reactive T cells (PD1+/Tim3+) from tumor followed by BM measurements. We found that exhausted T cells contained mixed populations of light and heavy cells while both effector and central memory T cells consisted primarily of heavy cells. To further compare the functional differences between heavy and light populations, we developed a non-invasive cell sorting platform for SMR based on BM gating and sorted exhausted T cells into light and heavy bins followed by bulk RNA sequencing. We found that genes associated with chemotaxis (Ccl3, Ccl4), activation (Ifng) and terminal differentiation (Rps8) are downregulated in light cells, while Nap1l3, which is predominantly expressed in hematopoietic stem cells, is upregulated. These results suggest that heavy cells may represent a population that tends to be long-lived and retain proliferative capacity, while the light cells could be T cells within a dormant state that can be further differentiated. Ultimately we envision that single-cell biophysical measurements with high precision may provide informative biomarkers for distinguishing between functional and dysfunctional T cells.

B608/P2279

Fibrotic wound healing as a function of fluid viscosity

Y. Chen[1], M. Pittman[2], D. Yan[2], H. Qureshi[2]; [1]Johns Hopkins University, Baltimore, MD, [2]Mechanical Engineering, Johns Hopkins University, Baltimore, MD.

Cells are surrounded by and move through fluids that span orders of magnitudes of viscosity *in vivo*, including mucus, saliva, blood, and synovial fluid, among others. We observed that some adherent cells migrate at higher speeds in highly viscous fluids, with the consistency similar to honey. We found that an actively ruffling lamellipodium when immersed in low- viscosity fluids is required for this counter-intuitive response. Upon viscosity increase, viscous drag immediately redistributes the membrane originally stored in the ruffles, resulting in larger spread area, faster focal adhesions formation, greater traction forces, and faster cell migration. Fibroblasts, neutrophils and macrophages exhibit ruffling lamellipodia thus visco-sensitive; whereas minimal ruffles are observed in inter-connected epithelial cells within an epithelium. Consequently, at high viscosity fibroblasts, neutrophils and macrophages move faster than at low viscosity, while inter-connected epithelial cells do not increase the migration speed. In the context of wound healing, faster moving fibroblasts, neutrophils and macrophages disproportionately occupy the wound site before epithelial cells arrive, depositing excessive ECM, exaggerating inflammatory responses and disrupting reepithelization. Furthermore, YAP translocates to the nucleus as viscosity increases. YAP promotes expression of the genes associated with proliferation, inflammation and tissue infiltration in both macrophages and neutrophils; YAP also upregulates genes in fibroblasts promoting myofibroblast phenotypes, leading to increase in contractility, ECM deposition, and pro-inflammatory cytokine secretion. In summary, increased viscosity in body fluids (mucus, exudate) are likely to promote fibrosis during wound healing. Reducing viscosity might suppress the fibrotic process which is an important part of disease progression in chronic lung diseases and cancer.

B609/P2280

Intracellular diffusion scales with cell size

C. Tan, F. Chang; University of California, San Francisco, San Francisco, CA.

Cells exist in a wide variety of sizes yet cells within acertain cell type exhibit a narrow range of size - does this suggest an optimalcell size? What happens when cells are the “wrong” size? How do cellularproperties change in different cells sizes? For instance, previous studiessuggest that in abnormally large or quiescent cells, the cytoplasm appears moredilute than in normal-sized cells. Here, we use genetically-encoded fluorescent40-nm nanoparticles (GEMs) (Delarue et al. 2018) to measure intracellulardiffusion in various-sized cells in the fission yeast *Schizosaccharomycespombe*. Using genetic mutants to alter cell size (e.g. *cdc25* and *wee1*mutants), we found that the GEMs diffusion coefficient increased in larger cellsand decreased in smaller cells. These size-dependent effects were present bothin the cytoplasm and nucleoplasm. We are currently testing the effects of theDNA-to-cytoplasm ratio on diffusion. Future studies will focus on probing therelationships between these size-dependent changes in diffusion with cellularcomposition and functions.

B610/P2281

A multiscale whole-cell theory for mechano-sensitive migration on viscoelastic substrates

N. Kaplan, W. Shu; Virginia Tech, Blacksburg, VA.

Increasing experimental evidence validates that both the elastic stiffness and viscosity of the extracellular matrix regulate mesenchymal cell behavior, such as the rational switch between durotaxis (cell migration to stiffer regions), anti-durotaxis (migration to softer regions), and adurotaxis (stiffness-insensitive migration). To reveal the mechanisms underlying the crossover between these motility regimes, we have developed a multiscale chemo-mechanical whole-cell theory for mesenchymal migration. Our framework couples the sub-cellular focal adhesion dynamics at the cell-substrate interface with the cellular cytoskeletal mechanics and the chemical signaling pathways involving Rho GTPase proteins. Upon polarization by the Rho GTPase gradients, our simulated cell migrates by concerted peripheral protrusions and contractions, a hallmark of the mesenchymal mode. The resulting cell dynamics quantitatively reproduces the experimental migration speed as a function of the uniform substrate stiffness and explains the influence of viscosity on the migration efficiency. In the presence of stiffness gradients and absence of chemical polarization, our simulated cell can exhibit durotaxis, anti-durotaxis, and adurotaxis respectively with increasing substrate stiffness or viscosity. The cell moves toward an optimally stiff region from softer regions during durotaxis and from stiffer regions during anti-durotaxis. We show that cell polarization through steep Rho GTPase gradients can reverse the migration direction dictated by the mechanical cues. Overall, our theory demonstrates that opposing durotactic behaviors emerge via the interplay between intracellular signaling and cell-medium mechanical interactions in agreement with experiments, thereby elucidating complex mechano-sensing at the single-cell level.

B611/P2282

Configurational entropy as a driver of structural heterogeneity in tissues

V. Srivastava[1], J. L. Hu[1], S. F. Shalabi[2], J. C. Garbe[1], M. R. Stampfer[3], M. A. LaBarge[2], Z. J. Gartner[1]; [1]Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA, [2]Beckman Research Institute, City of Hope, Duarte, CA, [3]Life Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA.

Tissues are comprised of multiple cell types, embedded in a complex and heterogeneous microenvironment. A hallmark of tissues is the ability of their cells to maintain complex structures in vivo that are central to their function. Tissue structure emerges and is maintained by programs of self-organization that derive from cell mechanics and cell-cell signaling. Most existing models predict that these driving forces promote a single correct tissue structure. However, the structure of many tissues, while possessing a well-defined average structure, remain spatially heterogeneous where the local arrangement of cells can deviate significantly from the population average. This observation is difficult to reconcile with models of tissue self-organization based on mechanics or cell-cell signaling alone. Using the human mammary gland as a model, we quantified the structural heterogeneity in the relative position of the primary cell lineages (luminal and myoepithelial cells) with respect to the basement membrane. We observed significant local structural heterogeneity even after correcting for differences in tissue composition. We investigated the fundamental sources of structural heterogeneity using reconstituted human mammary organoids after carefully controlling for their composition, geometry and microenvironment. These organoids self-organized to largely exclude luminal cells from the tissue

edge, but maintained a highly heterogeneous ensemble structure centered around a reproducible mean. We show that the observed structural distribution follows Boltzmann statistics, corresponding to a maximum entropy distribution given the energetic constraints from lineage-specific interfacial mechanics. We experimentally validated the relative entropy of different tissue configurations using spheroids containing a single cell type, in the absence of a mechanical potential. We used this statistical mechanical framework to systematically engineer the structure of organoid ensembles by either varying their mechanical energy (cell-ECM and cell-cell adhesions) or entropy (composition and geometry). Together, these experiments reveal that the configurational entropy of cell arrangements imposes a theoretical maximum limit to structural order at the tissue level.

B612/P2283

Multi-gene Co-Transfection is Dependent on Transfection Method

U. Baliga, D. Dean; University of Rochester, Rochester, NY.

Many research approaches require the delivery of multiple different genes to a single cell. These approaches include iPSC generation, viral reverse genetics, and molecular pathway analyses. However, as increasing numbers of distinct genes are delivered, the efficiency of every distinct gene being delivered to a given cell is reduced. This creates an expensive and frustrating bottleneck in multigene studies and may complicate analysis when some cells only receive part of the total desired genetic cargo. Various non-viral transfection methods are used to deliver multiple genetic cargoes, commonly lipofection and electroporation. We analyzed co-transfection of both methods through their ability to deliver plasmids expressing different fluorescent proteins. We further tested increasing the amount of plasmid used in the transfection and observed how co-transfection and cytotoxicity were affected by increasing the amount of transfected plasmids. Co-transfection was analyzed using spectral flow cytometry and fluorescence microscopy, and cytotoxicity was analyzed using an LDH assay. We found that increasing numbers of plasmids resulted in fewer co-transfected cells, regardless of method, in both HEK293T and A549 cells. Lipofection resulted in greater co-transfection of HEK293Ts, however electroporation resulted in greater co-transfection of A549 cells. Using lipofection in either HEK293T or A549, increasing the number or quantity of plasmids delivered resulted in minor increases of cytotoxicity, paralleled by a similar increase in co-transfection. Electroporation did not show this increased cytotoxicity with increased plasmid number or quantity, despite a higher baseline cytotoxicity. Increased amount of plasmid in electroporation resulted in greater improvement of co-transfection. These data indicate that (1) the choice of transfection agent affects co-transfection between different cell lines, and (2) increasing plasmid quantity in electroporation improves co-transfection more than lipofection does, without an increase in cytotoxicity. In the future, these co-transfection data will be tested functionally for treatment of acute respiratory distress syndrome, co-transfection an MRCKa plasmid to improve lung barrier function, and a plasmid expressing a STING shRNA to reduce inflammation.

B613/P2284

How Cells Program Mineral Shape in Sea Cucumbers - Part 2

C. Brannon[1], P. Vyas[2], L. Formery[3], C. Lowe[1], M. Prakash[1]; [1]Biology, Stanford University, Stanford, CA, [2]Bioengineering, Stanford University, Stanford, CA, [3]Biology, Stanford, Stanford, CA.

Biomineralization is a widespread phenomenon in the tree of life yet remains poorly understood. While the chemical processes underlying biomineralization have been studied in a range of organisms, the fundamentals of biomineral morphological programming remain unknown. We utilize holothurians as an exciting new model system for studying the biology of biomineral morphological programming. Holothurian biominerals exhibit striking morphological diversity both within and across species. Further, this morphology differs substantially from its abiotic counterpart. This leads us to wonder, how are holothurian skeletogenic cells controlling biomineral construction? We hypothesize that proteins secreted by associated skeletogenic cells into the biomineral growth compartment may play a role in shaping biomineral morphology. We explore this hypothesis through Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS) on protein extracted from biomineral. We identify known biomineralization proteins embedded in holothurian biomineral and explore the spatial distribution of embedded protein using Nanoscale secondary ion mass spectrometry (NanoSIMS). In parallel to establishing the spatial patterning of protein deposition in the biomineral itself, we are developing approaches for single-cell RNA-sequencing on holothurian skeletogenic cells, and searching for transcriptomic diversity which may correspond to biomineral morphological diversity. By applying modern techniques in cell and molecular biology to the holothurian system, we aim to both answer this biological question and reveal novel mechanisms for programming shape into mineral structures.

B614/P2285

What's the move? Novel counter-rotational flow in Xenopus embryonic epithelia creates heterogeneous strain fields and long range alignment of fibronectin.

G. Masak, L. Davidson; Integrative Systems Biology, Bioengineering, Developmental Biology, University of Pittsburgh, Pittsburgh, PA.

We report a novel form of counter-rotational, or 'Polonaise' flow in the late-neurula early-tailbud stage in *Xenopus laevis* embryos. Rotational or counter-rotational flows occur when collective migration or directed rearrangement is confined by physical barriers, either in embryonic or engineered contexts. Such flows are typified by vortices or swirls in cell trajectories. First described near the anterior end of the primitive streak in avians, counter-rotational movements have also been described in hair follicle morphogenesis in mammals. These flow patterns are thought to play an important role in patterning cell identities and setting up subsequent stages of morphogenesis and organogenesis. Live stereoscope timelapse sequences of the posterior end of embryos revealed unique tissue movements around the blastopore beginning in late neurula stages, similar to those described during chick gastrulation. We expressed a fluorescent nuclear reporter (H2B-mScarlet) to facilitate cell tracking through high-resolution confocal microscopy, enabling us to more accurately define patterns of individual cell movements. Image analysis of stereoscope and confocal timelapse sequences with our custom 'StrainMapper' macros reveal similar patterns of displacement, deformation, and strain. This analysis reports highly heterogeneous patterns of anisotropic strain, compression, and dilation in the dorsal and ventral tissues surrounding the blastopore. These counter-rotational flows occur in the cell and mechanical context of the blastopore during late neural to early tailbud stages. Dorsal axial tissues

continue to undergo convergent extension as fusion of neural folds is completed. By contrast, ventral tissues are more static and only initiate convergent extension during mid tailbud stages. To explore the origins of the heterogeneous strains, we carried out immunostaining of fibronectin, collagen and fibrillin extracellular matrix together with cytoskeletal factors actomyosin and cytokeratin within posterior tissues. Fibronectin fibril orientation between surface and deep cell layers suggests highly coordinated development of long range tension within the ventral tissues while apical surface cytoskeleton and cell shapes in surface cells suggest compression. We do not observe structures that might 'confine' epithelial in the plane but instead propose that counter-rotational flow is the result of more rigid confinement between layers as ventral tissues accommodate dorsal extensional movements. The polonaise movements at the posterior of the Xenopus embryo provide a third model system to investigate principles that leads to formation of the movement and to explore its role in establishing posterior neural and mesodermal cell fates in posterior stem cells.

B615/P2286

Contractile Actomyosin Cables and Pulling Forces from Adjacent Tissue Cooperate to Drive *Drosophila* Germ-band Extension

T. Zhu, H. Zhu, B. O'Shaughnessy; Chemical Engineering, Columbia University, New York, NY.

Germ-band extension in *Drosophila* is a major event during embryogenesis, when convergent-extension dramatically reshapes the germ-band. Fluid-like cell motions narrow the germ-band in one direction (dorsal-ventral axis, DV) and elongate it in the other (anterior-posterior axis, AP).
What forces drive germ-band extension? This is controversial. (1) One driving force is from planar polarized myosin at cell-cell junctions parallel to the DV axis. Blocking of myosin activation with optogenetic RhoGAP (optoGAP) activation reduced germ-band elongation ~1/3 and suppressed cell intercalation (Herrera-Perez et al., 2021). (2) Another contribution is the pulling force from the invaginating posterior midgut, adjacent to the posterior end of the germ-band. Blocking invagination with the *torso* mutation reduced germ-band elongation ~1/3 (Collinet et al., 2015). Further, the degree of DV elongation of germ-band cells is reduced with the *torso-like* mutation that blocks pulling forces (Lye et al., 2015), but is little affected by optoGAP-mediated blocking of myosin activation (Herrera-Perez et al., 2021).
Thus, experiment implicates both myosin cables and pulling forces from adjacent tissue, but their respective contributions and the underlying mechanisms are poorly understood. Thus, we developed a biophysical model of *Drosophila* germ-band extension, with junctional actomyosin contractility, elastic restoring forces, internal viscous forces, extracellular drag, forces from adjacent tissues and cell intercalation either by T1 transitions, when DV cell edges shrink to zero and merge two vertices, or by many-cell rosette formation (Herrera-Perez et al., 2022).
Our results quantitatively reveal that planar polarized myosin and posterior midgut pulling are major driving forces for convergent extension in the DV and AP directions, respectively. Actomyosin cables shrink junctions and intercalate cells, but cannot elongate tissue at wild-type rates, consistent with the phenotype of *torso* mutants (Collinet et al., 2015). Normal tissue elongation required both myosin cables and posterior midgut pulling, and in simulations with both, the profile of cell lengths reproduced the experimental monotonic increase in the AP direction (Lye et al., 2015). These results suggest convergent extension is cooperatively driven by myosin contractility and active pulling from adjacent tissue.

B616/P2287

Nanomechanical surface characterization of T lymphocyte viscoelasticity during formation of the immune synapse

K. Do, C. H. Parvini, A. X. Cartagena-Rivera; National Institutes of Health, Bethesda, MD

The cell-cell interface that forms between a T cell and an antigen-presenting cell (APC) is widely known as the immunological synapse (IS), where engagement of the T-cell receptor with its specific peptide antigen drives T cell activation. A growing body of work has shown that the mechanical properties of T cells during activation significantly influence essential cellular functions including proliferation, migration, APC detection, and killing. The formation and maintenance of the IS itself have been shown to be facilitated by force generation through actomyosin and microtubule cytoskeletal dynamics. However, the mechanism by which this force generation affects a T cell's mechanical properties is not well understood. Here, we demonstrate the use of a recently published discrete integral transform technique on force-distance datasets collected from T cells during IS formation using high-resolution atomic force microscopy. This approach enables quantitative, multi-timescale observation of the local viscoelastic response via common mechanical parameters, namely the storage modulus, loss modulus, and loss angle at the nanometer length scale. Our viscoelastic measurements reveal that T cells at the nanoscale level exhibit structurally heterogeneous nanoscale viscoelastic properties in response to forming an IS induced by CD3/CD28 stimulation, as indicated by stiff and viscous edge and central regions, with a simultaneously softer and more fluid peripheral transition region. These observations correlate well with actomyosin cytoskeletal changes within those regions and with the understanding that the peripheral transition region is known to cluster LFA-1 ligands. This study reveals the complex dorsal viscoelastic behavior of T cells during IS formation, which could be leveraged to better understand T cell activation.

B617/P2288

The study of preimplantation embryo development with 3D-bioprinted gelatin methacrylate as oviduct environment mimics systems

J. JANG[1], D. Kim[2], M.-H. Kang[3], M. Cho[3], Y. Kim[3], Y. Koo[1], S. Yeo[4], S. Park[4], J. Lee[1,3]; [1]CHA University, Pocheon, Korea, Republic of, [2]Seoul National University, Seoul, Korea, Republic of, [3]CHA Fertility Center, Seoul, Korea, Republic of, [4]Korea Institute of Machinery and Materials (KIMM), Daejeon, Korea, Republic of

In vivo condition is not a plastic dish but a soft tissue. Therefore, optimal soft tissue like oviduct physical condition develops for the optimal culture of mouse oocyte and embryo. Mechanical properties are fundamental properties of the cells and tissues of living organisms. The mechanical properties of a single cell as a biocomponent are determined by the interdependent combination of cellular components' mechanical properties. In this study, we investigate that the development of the oviduct's environment mimics the optimal culture dish for optimal reproductive cell culture. We developed an oviduct tube mimic culture system using gelatin methacrylate (GelMa) solution by 3D-bioprinting a set-up mechanical property from 2kPa, 4kPa and 10kPa. Quantitative estimate of the mechanical properties of hydrogel depends on the state, method of measurement, and the theoretical model that has been used. Then we used the B6D2F1 female for embryo collection through superovulation. Then we culture in a time lapse system incubator with 5% CO2, 37℃ condition in the SAGE in vitro blastocyst media with different hydrogel culture dish. We acquired images every 5 minutes for each culture dish. And we evaluated the

embryo development during each examination and recorded it through images. We checked up development ratios and cleavage time for the point of embryo during time lapse culture. Then we classified the quality of the embryo depending on the cleavage speed and blastocyst ratios. 10kPa culture dish shows fast development and high ratios of blastocyst formation compared to the control dish. Also hatched out ratios of blastocyst significantly increases on the 10kPa culture dish compared to the control dish. Then the blastocyst on the hydrogel culture dish reveals significantly higher Oct4, Sox, Nanog as fetal formation related gene expression ratios then control group. Hydrogel culture system induced inner cell mass in the embryo compared to the control group. In conclusion, Hydrogel promoted the speed of blastocyst development and hatched out ratios. It also significantly increased inner cell mass size and ratios compared to the control group. Therefore, optimal extracellular mechanical stimulation enhanced blastocyst developmental ratios. And optimal cell hydrogel base mechanical culture system could apply to improved clinical outcome of in vitro fertilization patient to high pregnancy and healthy live birth.

B618/P2289

Ionic and Cytoskeletal Control of Cell Protein Density

G. J. Fu[1,1], Q. Ni[1,1], Z. Ge[1,1], S. X. Sun[1,1,2]; [1]Johns Hopkins University, Baltimore, MD, [2]Johns Hopkins School of Medicine, Baltimore, MD

Cellular processes depend on the concentration of intracellular proteins in the cytoplasm. In adherent mammalian cells, protein concentration or protein density (protein mass / cell volume) is tightly regulated during the cell cycle and is critical for proper cell metabolism and physiological activities. Cells actively monitor and maintain cell volume via ion channels and cytoskeleton. Changing extracellular ionic content will disrupt the transmembrane osmotic gradient and change cell volume. On the other hand, ions can influence protein synthesis and protein function both physically and epigenetically. However, a quantitative study of ionic and cytoskeletal control of cell protein density is still lacking. In this work, we combined quantitative phase microscopy and the Fluorescence Exclusion method to investigate how different ions and cytoskeletal components regulate cell protein density. We find that depletion of extracellular sodium and chloride can alter cytoplasmic protein density, however, the alterations are variable, depending on the timescale. At the timescale of hours, depleting either sodium or chloride (without changing osmolarity) leads to protein density increase by decreasing cell volume. At the time scale of a cell cycle, chloride depletion increases cell volume while leaving protein mass intact, resulting in a cell protein density decrease. Sodium depletion also reduces protein density, but both cell volume and protein mass are increased. Extracellular calcium, potassium, and the actin cytoskeleton also impact cell volume and protein mass but do not alter the protein density. Our work reveals the central role of ions in controlling cell protein density, providing fundamental understandings of cell size and cell cycle regulation.

B619/P2290

A mechanical cusp catastrophe imposes a universal constraint on the shapes of tip growing cells

M. E. Ohairwe, E. R. Rojas; New York University, New York, NY

Understanding the mechanistic and evolutionary bases for cellular morphology are central problems in biology. Evolution has converged on tip growth many times, yielding filamentous cells, yet tip-growing cells display a range of apical morphologies. To understand this range, we measured the spatial

gradients of the principal tensions and expansion rates in the cell walls of tip-growing cells from three kingdoms and found that, in each case, cell growth and apical morphology are consequences of mechanical stretching of the cell wall by turgor pressure. As a result, we could use continuum-mechanics theory to describe the entire space of possible apical morphologies that could be generated using this mechanical mechanism. Surprisingly, we discovered that the experimentally measured morphologies of a wide range of tip-growing species populate only a small region of this theoretical "morphospace.'' Through further analysis, we discovered that natural morphologies are constrained by a "cusp bifurcation'' in the morphospace. Specifically, the bistable region of the morphospace associated with the bifurcation imposes a boundary that separates fast-growing naturally occurring morphologies from unobserved morphologies that are predicted to grow more slowly. We tested this prediction by pharmacologically perturbing the tip growth of species with apical morphologies close to the boundary, which indeed resulted in both slow growth and the non-natural apical morphologies associated with the other side of the boundary. Collectively, our results reveal how the interplay between natural selection for fast cell-growth and the convergent evolution of turgor-driven growth has resulted in a universal developmental constraint on the shapes of a broad class of cells.

B620/P2291

Cancer-associated mucins provide sustained physical protection against Natural Killer cell attack

J. H. Paek, S. Park, M. J. Paszek[1,1]; Cornell University, Ithaca, NY

Cancer is often associated with aberrant expression of cell surface mucins, resulting in a thick cellular glycocalyx that coats the cancer cell membrane and governs its interactions with immune cells. Several biochemical studies have uncovered an immunosuppressive role of the O-glycans that decorate cancer-associated mucins. However, densely packed mucin biopolymers on the cell surface may also physically protect cells from immune recognition and attack. Here, we investigate how the physical properties of a mucin-rich glycocalyx impact effector-target interactions. Using CRISPR-Cas9 gene editing to manipulate the physical structure of the glycocalyx, we show that the cancer-specific glycocalyx can act as a nanometer-scale shield that provides resistance to Natural Killer (NK) cell-mediated cytotoxicity. We also develop a 3D co-culture platform coupled with live-cell imaging to investigate the dynamics and mechanisms of immune cell engagement and killing of target cells. We show that NK cells are highly motile and exhibit classical amoeboid migration through complex collagen matrices as they search for their target cells. We find that target cells with a thin mucin barrier can be killed within minutes of contact with an NK cell. Meanwhile, cells with high mucin surface density have a remarkable ability to resist NK cell attack for several hours of continuous engagement. Lastly, we show how equipping NK cells with mucin-digesting enzymes endows them with the ability to overcome this barrier and enhances their cytolytic activity against target cells that exhibit a mucin-rich glycocalyx. These results suggest that NK cells can be engineered to utilize mucinases to boost their activity against target cells, leading to a novel strategy for cancer immunotherapy.

Chemical Cell Biology

B621/P2292

Nucleus uptake of water soluble C_{60} derivatives: a molecular platform for targeting cargo to diseased cells

R. Biswas, S. Yang, Z. Shi, J. Zhang; Chemistry and Chemical Biology, Rutgers University, New Brunswick, NJ.

Entry of cargo into the nucleus is challenging because of the size and molecular weight cutoff of the nuclear membrane, yet the nucleus is a significant target for therapeutics as alterations to the native genetic material can lead to pathological conditions such as cancer among others. Here we investigated the ability of water-soluble C_{60} β-cyclodextrin derivatives carrying doxorubicin to enter the nucleus of HeLa cells. By selectively labelling the nuclei and lysosomes of HeLa cells and using fluorescence microscopy, we established that one of the molecules with a hydroxylated C_{60} cage was able to efficiently target the drug into the nucleus whereas the one with an intact hydrophobic cage remained trapped in the lysosome. Additionally, by using transmission electron microscopy, we showed that the C_{60} derivatives were able to penetrate the nucleus. We tested through cell viability assays that the C_{60} derivatives themselves did not reduce cell viability, however the doxorubicin loaded versions significantly reduced cell viability under optimal doxorubicin loading. The above observations led us to investigate the effect of these molecules on stress granules as they are implicated in neurodegenerative diseases such as amyotrophic lateral sclerosis and Alzheimer's, which is currently under investigation. As discussed above, the ability of C_{60} derivatives to cross different layers of the cell could be attributed to the unique structure of C_{60} which belongs to a broad family of carbon materials called fullerenes. The hydrophobic carbon cage helps C_{60} or its derivatives to effectively partition into the lipid bilayer of the cell membrane. Currently, we are developing a bi-functional C_{60} derivative with orthogonal functional groups which will have the potential to be functionalized with fluorescent tags and other bioactive molecules such as peptides, sugars or nucleic acids. It is worthwhile to point out that the C_{60} cage has functional purposes beyond its use as a structural scaffold. Depending on the functional groups and the nature of conjugation of the fullerene cage, C_{60} derivatives are known to quench reactive oxygen species (ROS) which we are currently investigating. This is important from a therapeutic standpoint to treat ROS induced inflammation which is prevalent in many diseases such as intervertebral disc disease. To conclude, the primary focus of this presentation will be on the cellular uptake behavior of C_{60} derivatives into HeLa cells and their specific targeting to the nucleus and its implications. In addition to this, current efforts to develop bi-functional C_{60} derivatives which can be potentially used for imaging and delivering essential bioactive molecules such as chemotherapeutics or anti-inflammatory compounds to diseased cells will be discussed.

B622/P2293

Bioorthogonal ligation-activated fluorogenic FRET dyads

E. Albitz, D. Kern, A. Kormos, M. Bojtár, G. Török, A. Biró, Á. Szatmári, K. Németh, P. Kele; Research Centre for Natural Sciences, Budapest, HUNGARY.

We explored a concept that enables the transformation of the excellent tetrazine-ligation dependent fluorogenicity of blue-excitable cores to biologically more preferable red emission. The synthesized energy transfer (FRET) dyads were studied under different conditions. Intracellular protein labelling

studies of live cells with subsequent confocal fluorescence microscopy imaging served evidence for the applicability of these fluorogenic dyads. Results have shown that the relay of bioorthogonally activatable blue fluorogenicity indeed results in improved yellow/red fluorogenicities. Furthermore, the large apparent Stokes-shifts of the dyads in combination with a spectrally matching fluorogenic coumarin enabled multicolor imaging of distinct intracellular structures using a single excitation wavelength with distinct emission ranges. Moreover, due to the improved photostability of the dyads their use in STED imaging experiments was also demonstrated.
The modularity of the design allows a generalizable approach that can be applied to various fluorogenic scaffolds in combination with suitable acceptors. Thus, we believe that such energy transfer-based relay of bioorthogonal reaction aided fluorogenicity may address the wavelength limitation of tetrazine modulated fluorogenic probes.

B623/P2294

Analyzing Site-specific and Direct Interactions of AAA Proteins with Cofactors and Substrates in Living Cells

L. E. Vostal[1,2], W. Zhang[3], B. T. Chait[3], T. M. Kapoor[1]; [1]Laboratory of Chemistry and Cell Biology, The Rockefeller University, New York, NY, [2]Tri-Institutional PhD Program in Chemical Biology, The Rockefeller University, New York, NY, [3]Laboratory of Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, New York, NY.

Members of the ATPases associated with diverse cellular activities (AAA) protein family are macromolecular machines that require interaction with cofactors or adaptors to remodel target substrates across a variety of cellular processes, including cytoskeleton regulation, membrane biogenesis, and proteostasis. In particular, valosin-containing protein (VCP/p97) is a hexameric AAA protein that interacts with ~30 cofactors that recruit poly-ubiquitinated and non-ubiquitinated substrates. These substrates are likely unfolded by threading them through the central pore of the VCP hexamer for subsequent recycling or degradation. Previous studies have used proteomic and structural approaches to characterize VCP-cofactor and VCP-substrate interactions *in vitro*. However, the binding modes of known VCP interactors to the AAA protein hexamer have not been analyzed in living cells. Here, we adapt iCLASPI, an approach relying on amber suppression, photo-crosslinking, and quantitative proteomics, to probe site-specific and direct VCP protein-protein interactions in living cells. We have focused on examining VCP-cofactor interactions, which structural studies map to a hydrophobic groove in the VCP N-terminal domain or above the entrance into the VCP central pore. We find that a known cofactor, Npl4, crosslinks directly to VCP L278 positioned at the entrance to the central pore, but not within the central pore near residues E314 and D592 in living cells. Additional cofactors, p47 and p37, crosslink to the same region of VCP as Npl4, consistent with a model in which mutually exclusive regulation of the AAA protein can be achieved in cells. Our modified iCLASPI approach will be used to map the native binding sites of VCP interactors and to examine the temporal dynamics of these interactions during cell division. In ongoing work we are applying our approach to VPS4, a AAA protein involved in membrane remodeling. Together, our findings introduce a robust method for probing site-specific and direct AAA protein-protein interactions in living cells, where these interactions are essential for several cellular processes.

B624/P2295

Should I Stay of Should I Go?: Investigating Repellent Chemoanemotactic Responses in Crickets

R. O. Alzyoud, **V. D. C. Shields**; Biological Sciences, Towson University, Towson, MD.

Insects, like most organisms, use their olfactory (smell) system to detect and analyze environmental cues. Their antennae bear olfactory sensory organs (sensilla) containing neurons that encode and process olfactory stimuli. Such stimuli can trigger behaviors, such as orientation toward food, mating partners, and avoidance of predators. House crickets make excellent model systems in neuroscience as they, like other insects, have moderately complex nervous systems and bear a rich behavioral repertoire. House crickets are generally considered to be pests. Due to their omnivorous feeding preference, they can potentially contaminate food sources with their fecal matter. Additionally, their audible chirping noises are very annoying. The aim of this study was to determine the behavior elicited by several essential oils known to elicit repellency or movement away from the odorant source (i.e., negative anemotaxis) in some insects. More specifically, we tested the essential oils of peppermint, citronellal, eucalyptus, lavender, lemon eucalyptus, wintergreen, and orange. We hypothesized that some of these essential oils would act as repellents. We found that peppermint oil and lemon eucalyptus oil were similarly strongly repellent. Eucalyptus oil and citronellal oil were only moderately repellent individually, but when combined (lemon eucalyptus oil), were strongly repellent. Lavender oil was strongly repellent but not as potent as lemon eucalyptus and peppermint. Wintergreen oil and orange oil appeared to be similarly moderately repellent. Some of the repellent compounds may be useful for applied researchers to develop olfactory repellent traps to deter crickets from entering homes and contaminating food sources. This research was funded by NIH grant 5-R25-GMO5826416 and NSF MRI grant 1626326 to V.D.C.S.

B625/P2296

Bioorthogonal diagnostic fluorophores for imaging oxidative stress-induced carbonylation in live cells

J. Argueta[1], A. Franklyn[1], T. Shirley[1], B. Jones[2], E. Krawczyk[2], S. Ranjit[2], M. Levi[2], **O. Dilek**[1]; [1]University of the District of Columbia, Washington, DC, [2]Georgetown University, Washington, DC.

Oxidative stress is reported to associated with the development of cellular damage, cancer, aging and several age-related disorders. Oxidative stress often induces carbonylation of biomolecules in cellular systems. Detection of carbonyl-containing biomolecules in live cells with novel bioorthogonal fluorophores provides a fast, selective, and stable approach for imaging. To introduce fluorophores into the cellular systems, these fluorophores should have distinct chemical and photophysical characteristics to achieve successful imaging: high stability, less toxicity, fast kinetics, good spectral properties (*e.g.,* large Stokes shifts, reasonable quantum yields, drastic changes on absorption and emission spectra). In this work, we developed small molecule-based fluorophores that have a reactive hydrazine functional group, which rapidly formed a fluorescent turn-on hydrazone product upon reacting with the carbonyl groups in biological system—is called bioorthogonal fluorescent labeling so that we were able to visualize carbonylation process in various cancer cell lines. Our spectroscopic and confocal microscopy results showed that newly synthesized fluorophores can be labeled successfully in human dermal fibroblasts along with renal cell carcinoma (RRC) cell lines and prostate cancer (PC) cells. In addition, synthesized fluorophores can selectively detect carbonylation level that differs in response to exogenous and endogenous stress in healthy and cancer cells. Use of click chemistry method to monitor carbonylation with a bioorthogonal fluorophore has proven itself to be superior in satisfying many

criteria (e.g., biocompatibility, selectivity, yield, stability, and so forth); our results will therefore provide a powerful probe technology that can label carbonyl moieties in live cells. We anticipate that development of new library of fluorophores will not only advance the discovery of future drug therapeutics targeting carbonylation process, but also enable the development of better imaging agent technologies that have potential to diagnose early stages of selective diseases.

B626/P2297

Saccharomyces cerevisiae* Proteins Exhibit Benzil Reductase Activity *in vitro

B. Garcia, **K. Riley**; Chemistry, Rollins College, Winter Park, FL.

IRC24 and NRE1 encode putative oxidoreductases separated by 284 bp on chromosome IX of *Saccharomyces cerevisiae*. Little is known about either enzyme, though an unpublished structure for NRE1 was deposited into the Protein Data Bank as part of a high throughput study. The IRC24 and NRE1 proteins are relatively small (263 and 254 amino acids, respectively), cytoplasmic, and are 52% identical/70% similar in sequence. Bioinformatic predictions identified likely NAD(P)-binding Rossmann folds in both proteins, implicating NAD or NADP as a likely cofactor. Comparisons of the NRE1 protein structure to homologous protein structures KRED1-Pglu from the yeast *Ogataea glucozyma* and carbonyl reductase variant 4 from the bacterium *Serratia marcescens*, further confirmed a conserved the Rossmann fold and illuminated a conserved active site motif thought to bind to diketone compounds such as benzil. His-tagged IRC24 and NRE1 purified from *E. coli* were compared in terms of their substrate/cofactor preference and catalytic parameters. Both enzymes catalyze redox reactions in vitro with measurable kinetics and substrate/cofactor preferences. These *in vitro* studies will hopefully inform our understanding of the role of these probable paralogs *in vivo.*

B627/P2298

Effects of Protein Binding Site Valence in RNA on Biological Condensates

S. J. Cole[1], C. Roden[2], A. Gladfelter[2]; [1]Biochemistry and Biophysics, University of North Carolina Chapel Hill, Chapel Hill, NC, [2]University of North Carolina Chapel Hill, Chapel Hill, NC.

Spatial and temporal control of biochemistry is central to cell function. One way the cell spatially controls reactions is through formation of biomolecular condensates, the mismanagement of which is tied to several disease states. Most condensates are a mixture of RNAs and RNA binding proteins (RBP), yet it is still mysterious how RNAs impact the form and function of condensates. One key question is how RNA properties, such as sequence and structure effect condensate properties. Key insights into the role of RNA sequence and structure in condensates have come from study of the Whi3 RNA-binding protein within the multinucleate filamentous fungus Ashbya gossypii. Whi3 forms three distinct condensates with the RNAs encoding WHI3 itself, BNI1, and CLN3 RNA. Despite all three condensates containing Whi3 protein, these condensates are essential for different functions and found in distinct locations in the cell. Previous work showed that reconstituted Whi3 droplets had different material properties depending on which RNA was present and we predict is important for the distinct local functions. BNI1 and CLN3 condensates display different relative concentrations of Whi3 protein in the condensate and this could be one basis for the differences in material properties. However, this is a surprising difference to observe since each of these RNAs have the same number of Whi3 binding sites. This led us to hypothesize that there are functional differences amongst the binding sites that vary somehow in their ability to interact with Whi3 protein to drive phase separation. This project examines

multiple mechanisms underlying this difference 1) spacing of the binding motifs on the RNA, 2) the local structure of the sites in the RNA or 3) other differences in the sequence between these RNAs (potentially unidentified Whi3 binding sites) that may contribute to their ability to recruit Whi3. We first hypothesized that there are specific sequence or structural elements surrounding Whi3 binding motifs that effect the binding affinity of Whi3 for the site. We tested this with CLN3 transcripts with individual binding sites removed in in vitro phase separation assays and find evidence that not all Whi3 binding sites are created equal indicating heterogeneity and potentially cooperativity in Whi3 interactions with specific sites on target RNAs. We are now testing the sites individually for affinity differences. This system is revealing how valence in RBP/Protein interactions can be fine-tuned based on subtle affinity differences that arise from RNA sequence context. This work will shed light on how the structure and sequence-encoded features surrounding protein binding sites on RNA control assembly of biomolecular condensates.

B628/P2299

Controlling the Activity of Polo-like Kinase 1 with Light

V. von Glasenapp[1,2], D. Chang[3,4,2], N. Winssinger[3,2], M. Gotta[1,2]; [1]Department of Physiology and Metabolism, University of Geneva, Geneva, SWITZERLAND, [2]NCCR Chemical Biology, University of Geneva, Geneva, SWITZERLAND, [3]Department of Organic Chemistry, University of Geneva, Geneva, SWITZERLAND, [4]Current address: Institute of Chemical Science and Engineering, EPFL, Lausanne, SWITZERLAND.

Polo-like kinase 1 (Plk1) is a conserved mitotic kinase necessary for several processes during cell division, such as mitotic entry, bipolar spindle assembly and cytokinesis. The activity of the kinase is regulated during the course of mitosis to be active at specific time points. The small molecule inhibitor BI2536 has been widely used to study the functions of Plk1. We have developed a Plk1 inhibitor based on BI2536 that can be controlled with light to have precise temporal control. We have attached a coumarin based photoremovable protecting group to the inhibitor at a position important for interaction with the kinase. In HeLa and hTERT-RPE cells the caged inhibitor is stable and does not interfere with the cell cycle even at high concentrations. It can be rapidly uncaged and activated under the confocal microscope as well as wide field microscopes equipped with a laser or a lamp of a wavelength of 488 nm. Once uncaged, the inhibitor efficiently interferes with Plk1 activity. We have successfully used the caged inhibitor in high-content microscopy and achieved precise temporal control of Plk1 inhibition. We are now functionalizing the inhibitor to introduce spatial control. This tool will allow us to dissect the roles of Plk1 at different locations and in individual cells of developing organisms and organoids.

B629/P2300

Assessment of side effects of chemotherapy drugs on a patient derived small intestine organoid model

J. S. Boyd, V. Chu; Cellular Assays R&D, MilliporeSigma, Temecula, CA.

Patient-derived organoids are increasing in interest for their utility as relevant pharmacological models for drug screening. In contrast to 2D cellular models, 3D organoid models more closely represent the structure and types of differentiated cells, more accurately recapitulating in vivo physiology and response to therapeutic agents. Drug-induced gastrointestinal toxicity is a common adverse effect that impairs efficacy and reduces treatment options for patients, thus making relevant assays using in vitro

cellular models highly desirable. We tested a panel of chemotherapeutics known to induce varying degrees of diarrhea incidence using a commercially-available human small intestine duodenum model via organoid viability assay. Drug toxicity values for the human patient-derived duodenum small intestine organoids closely mirrored published values for human ileum small intestine organoids. Toxicity data were correlated with effects on membrane integrity assessed via epithelial transmembrane epithelial resistance (TEER). This study reinforces the relevance of patient-derived organoid models for drug toxicity and in vitro risk assessment of drug side effects.

B630/P2301

Chemoproteomic-guided development of SLC15A4 inhibitors with anti-inflammatory activity

T.-Y. Chiu, C. G. Parker; The Scripps Research Institute, San Diego, CA

SLC15A4 is an endolysosome-resident transporter that is intimately linked with autoinflammation and autoimmunity. Specifically, SLC15A4 is critical for Toll-like receptor (TLR) 7, 8, and 9 as well as the nucleotide-binding oligomerization domain-containing protein (NOD) 2 signaling in several immune cell subsets. Notably, SLC15A4 is essential for the development of systemic lupus erythematosus in murine models and is associated with autoimmune conditions in humans. Despite its therapeutic potential, to our knowledge no pharmacological tools have been developed that target SLC15A4. Here, we use an integrated chemical proteomics approach to develop a suite of chemical tools, including first-in-class functional inhibitors, for SLC15A4. We demonstrate SLC15A4 inhibitors suppress endosomal TLR and NOD functions in a variety of human and mouse immune cells and provide early evidence of their ability to suppress inflammation *in vivo* and in clinical settings. Our findings establish SLC15A4 as a druggable target for the treatment of autoimmune/autoinflammatory conditions.

B631/P2302

Development of MDM2-targeting PROTAC System for Hard Tissue Regeneration

S. Jeong, S. Kang, J. Kim; Seoul National University, Seoul, Korea, Republic of

MDM2 is a negative p53 regulator, and various inhibitors targeting MDM2 has been developed as anticancer drugs. Our previous study confirmed that p53 activation through inhibition of MDM2 induces osteogenic differentiation in multipotent mesenchymal stromal cells (MSCs). MDM2 inhibitor remarkably induced biomineralization in MSCs, but p53 activation was insufficient due to an MDM2-p53 autoregulatory feedback loop. To overcome this limitation of MDM2 inhibitors, we applied proteolysis targeting chimera (PROTAC), a technology that degrades a protein of interest (POI) through an intracellular ubiquitin-proteasome system. Hence, we propose a strategy to induce hard tissue regeneration by MDM2-targeting PROTAC technology. We selected POI ligand among various MDM2 inhibitors by Alizarin Red S (ARS) based screening process, and the selected ligands were applied to CRBN or VHL ligands. The MDM2-PROTAC synthesis platform was designed to include each ligand combination, and 16 compounds of MDM2-PROTAC were synthesized. We discovered multiple candidates that effectively degraded MDM2 protein by immunoblot assay. By performing the degradation test following concentration of selected compounds, the maximal degradation concentration (DC_{max}) and the half maximal degradation concentration (DC_{50}) were identified. In addition, timelapse live cell imaging was conducted to visualize the degradation dynamics and efficiency of MDM2-PROTAC in EGFP-MDM2-expressing cells. For functional validation in biological systems, we demonstrated that MDM2-PROTAC induced a robust effect on biomineralization compared to the

MDM2 inhibitor. MDM2-PROTAC significantly increased mRNA levels of osteogenic differentiation marker genes. Also, the effect of MDM2-PROTAC was validated in animal models of hard tissue defect. In this study, we demonstrated the potent protein degradation effects of newly developed MDM2-PROTAC and an intracellular MDM2 regulation strategy using PROTAC technology customized to induce biomineralization. These results are expected to lead to development of a new therapeutic modalitiy for hard tissue regeneration, expanding the application of the PROTAC system.

B632/P2303

Pipeline development for a fast quantitative kinetic understanding of nucleotide addition catalyzed by RNA/DNA polymerases

Z. I. Carter, L. Saleh, A. F. Gardner; New England Biolabs, Ipswich, MA

The 99 kDa single subunit enzyme, T7 RNA Polymerase, is a well-studied enzyme with manuscripts dating back to the late 1950's. Despite this fact, many mechanistic questions still exist. A few include: Does T7 RNAP incorporate the four conical nucleotides with an identical kinetic mechanism? Are particular nucleotides misincorporated more readily? How does the sequence context impact the rates of misincorporation? Etc. Many traditional Polymerase assays still in use today use sequencing gels to resolve elongated DNA/RNA transcripts. Sequencing gels are both slow and low throughput. Here we have adapted a traditional T7 RNA polymerase assay for fluorescent detection. We report the development of a pipeline to measure single nucleotide incorporation kinetics, misincorporation kinetics, modified nucleotide incorporation kinetics, and multi-nucleotide incorporation kinetics. Time courses monitoring nucleotide addition were generated using transient-state kinetic techniques in combination with a high-throughput capillary electrophoresis system. This experimental methodology allowed 20+ time courses to be collected and analyzed in hours compared to days or perhaps weeks using standard denaturing sequencing gels. Rate-constants were measured describing the incorporation of the four canonical nucleotides into a nascent RNA strand. The hypermodified N1-methyl-psudouridine, which is used in the production of the COVID-19 vaccine, was also investigated. Additionally, we generated estimates for the rate-constants describing misincorporation in a variety of sequence contexts. Model-independent and model-dependent analyses were performed for each nucleotide addition revealing mechanistic insight for this critical enzyme. We expect this novel pipeline and the corresponding analysis strategies to be broadly applicable to many RNA/DNA polymerase researchers.

B633/P2304

1,4-Dioxane prevents LPS-induced macrophage inflammatory response in a NF-κB-independent manner

Z. Cabail; SUNY-Old Westbury, Old Westbury, NY

1,4 dioxane is an emerging environmental hazard that has been reported to elicit carcinogenic effects to humans. 1,4 dioxane is a Synthetic Organic Compound (SOC) found in several daily-life products, including shampoos, body washes, baby products, laundry detergents, hand and dish soaps. A wide range of evidence demonstrate that environmental toxic products are associated with the onset, development, and progression of cancer. Macrophages are cells of the innate immune system responsible for mounting an inflammatory response. Macrophages that infiltrate the tumor stroma and become tumor-associated macrophages (TAMs) polarized towards a pro-inflammatory state. It has been

reported that environmental toxicants alter the balance macrophage polarizations from inflammatory M1 toward anti-inflammatory M2 and this may be contributed to tumor growth. Recent evaluation of public water suppliers across Long Island, NY, where our college (SUNY-Old Westbury) is located, reported the highest levels in the nation of 1,4-dioxane contamination in groundwater and drinking water. This prompted our lab to investigate the molecular mechanisms of macrophage polarization in response to 1,4 dioxane. Therefore, the goal of this study is to achieve a mechanistic understanding of how 1,4 dioxane affects the inflammatory response. We used a murine macrophage cell line, RAW264.7, and a murine microglia -a specialized population of macrophages found in the central nervous system- cell line, BV-2. These cells were exposed to different concentrations of 1,4 dioxane in a time-course manner to mimic the plasma concentration of 1,4 dioxane after exposure. Immunoblotting showed that LPS-induced macrophage/microglia activation after pre-treating cells with different concentrations of 1,4 dioxane, and at different time points, resulted in the downregulation of the functional marker of M1 pro-inflammatory phenotype inducible nitric oxide synthase (iNOS). This was accompanied by decrease levels of liver Arginase, a signature protein of M2 anti-inflammatory activation. This polarization was not mediated by the key regulator of the M1 program NF-κB. Further investigation of the cellular signaling mediating the inhibition of LPS-induced inflammatory response by 1,4 dioxane is warrant.

Cell Fate Determination 1

B635/P2305

The conserved RNA binding protein Orb2 upregulates rare codon biased transcripts during differentiation of *Drosophila* neurons.

R. K. Stewart, S. Allen, D. Fox; Duke University, Durham, NC.

Many processes drive differentiation, including changes in protein expression from RNA transcripts. One process that is understudied for its effect on protein expression during differentiation is codon bias. Codon bias occurs when certain codons are rarely used compared to their synonymous counterparts and is present throughout all forms of life. To begin to understand how codon bias impacts development and differentiation, we completed an animal wide reporter-based screen in the model organism *Drosophila.* This screen revealed cell type and tissue-specific responses to codon bias. Specifically, we found the testis and brain are unique in their ability to express protein derived from rare-codon enriched reporters. In these tissues, we find robust protein expression from reporters enriched in rarely used codons, whereas the other tissues in the fly do not express such reporters. Upon further investigation in the brain, we found differential regulation of protein expression from rare codon enriched transcripts within a single stem cell lineage. Neural stem cells are unable to express reporters or endogenous genes encoded by rare-codon enriched genes, while differentiated neurons do so robustly. Differentiation is tied to the ability to express transcripts enriched in rare codons, as experimentally blocking neuronal differentiation drastically reduces rare-codon enriched reporter protein. To uncover molecular regulators that enable neurons to specifically express rare codons, we conducted a reverse genetic screen of 54 candidate regulators. This screen identified the conserved cytoplasmic polyadenylation element binding (CPEB) protein Orb2 and its translational coregulator CG4612 as regulators of rare codon specific protein expression in neurons. Using both RNA FISH and RNA-seq we then identified endogenously expressed rare-codon enriched mRNAs regulated by Orb2. These rare-codon enriched mRNAs are linked to Orb2's function in long term memory in specific neuronal cell types. Using our *Drosophila* model, we have uncovered an Orb2-dependent mechanism that critically regulates rare

codon-dependent expression in neurons. Our findings reveal dynamic central dogma regulation within a defined stem cell lineage that drives cellular differentiation and function.

B636/P2306

The Epithelial to Mesenchymal Transition (EMT) in early differentiation: integrating and interrogating cell organization, cell behavior, and cell identity

A. Borensztejn[1], .. Allen Institute for Cell Science[1], J. A. Theriot[2], C. Hookway[1], S. M. Rafelski[1]; [1]Allen Institute for Cell Science, Seattle, WA, [2]Department of Biology and Howard Hughes Medical Institute, University of Washington, Seattle, WA.

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSC) establish and maintain robust dynamic localization of cellular structures and how cells transition between states during differentiation and disease. The epithelial to mesenchymal transition (EMT) is a state change that occurs in both normal and pathological contexts, including development and cancer metastasis respectively. The EMT has been described as a behavioral change from largely non-motile to migratory, a change in organization from apical-basal to front-back polarity, and a change in identity by protein/transcript expression profile. However, the dependencies and relationships between all three of these aspects of EMT are not well understood and require a large-scale, multi-modal data integration approach. We are working to develop and standardize a framework to combine live-cell imaging of dynamic cell behavior with cell identity characterization via multiplexed immunolabeling or RNA-FISH. We have found subpopulations of migratory cells for which the expression of particular markers is associated with being more or less stationary. A standardized framework will allow us to assess how widespread results like these are, and to probe relationships between additional markers and behaviors. In parallel, we have obtained a single cell RNA-seq (scRNA-seq) dataset with high temporal resolution over the duration of EMT, which has allowed pseudotime trajectory analysis. We find more variation in the pseudotime assignment of cells collected at (real-time) timepoints associated with dramatic behavioral changes. We are additionally using this data to identify novel probes to feed back into the live-cell/fixed and labeled cell framework described above. This integrative approach allows us to formally compare EMT in different contexts, including examples in perturbed cells or when EMT is induced by different methods. We believe that this multi-modal, multi-scale approach might serve as a template for studying EMT in many contexts and aid in understanding cell states and transitions more generally.

B637/P2307

Toxicant-responsive miRNAs skews human embryonic stem cell osteoblastogenesis

N. R. L. Sparks[1], D. Williams[2], N. zur Nieden[2,3]; [1]Environmental and Occupational Health, University of California Irvine, Irvine, CA, [2]Molecular, Cell & Systems Biology, University of California Riverside, Riverside, CA, [3]Stem Cell Center, University of California Riverside, Riverside, CA.

Birth defects that affect skeletal tissues are a major public health concern causing a life-long impact on the individual and their families. Insults from environmental chemicals can disrupt the dynamic genetic regulatory processes that can affect tissues of the neural crest and/or mesoderm which would manifest skeletal disorders of the skull or short/long bones, respectively. The number of chemicals that need evaluation of risk to the developing skeleton cannot be met with traditional testing methodologies. Here, we aimed to identify microRNAs (miRNAs) as biomarkers associated with impaired skeletal

development caused by toxicant exposure in an *in vitro* human embryonic stem cell (hESC) osteoblast differentiation model. Osteogenically differentiating hESCs were exposed to test chemicals known for their toxic effects on *in vivo* skeletal development. All tested chemicals inhibited hESC osteoblast differentiation. In addition, altered skeletal fate commitment determined by *RUNX2* upregulation and downregulation of *PAX7*, *SNAI2*, *TBX6*, and *CCN1*. Global miRNA profiling provided evidence of toxicant-responsive miRNA signatures. Chemicals cyclopamine, cyclophosphamide, methotrexate, methoxyacetic acid, ogremorphin, triadimenol, valproic acid, and 5-flurouracil identified 202, 425, 404, 313, 518, 394, 354, and 259 differentially expressed miRNAs, respectively, after exposure. Global profiling revealed 65 differentially expressed miRNAs shared between all chemicals. MiRNAs involved with ossification were deregulated by each chemical. Gene ontology (GO) indicated that the overlapping miRNAs are involved in skeletal system development, neural crest developmental/differentiation, and mesoderm development, and associated with key signaling pathways such as canonical WNT, BMP, and TGF-beta. To phenocopy the toxicants effects, selected miRNAs were knockdown or overexpressed during hESC-osteoblastogenesis. Candidate miRNA inhibitors and mimics representing down and up regulated miRNAs, respectively, attenuated hESC osteogenic differentiation, mimicking the toxicant's inhibitory effects. Further, hESC-osteoblast differentiation was rescued with miRNA mimics and inhibitors to restore miRNA expression levels during toxicant exposure. These results suggests that disruption of these necessary processes interferes with osteogenic development and provides new insights into using miRNA expression profiles as a toxicological molecular endpoint for osteotoxicity. These dynamic toxicant-induced changes in miRNA signatures reflect crucial roles of miRNAs during skeletal development and can serve as a biomarker for skeletal defects.

B638/P2308

MicroRNA-124 plays a dual role to modulate neurogenesis and mesodermal development

K. Konrad[1], S. Suarez[2], J. Song[1]; [1]University of Delaware, Newark, DE, [2]Regeneron, Terryville, NY.

MicroRNA-124 (miR-124) plays a conserved role in regulating neurogenesis; however, its additional function in various developmental processes is less defined. We use the purple sea urchin as a model to reveal the function of miR-124. The sea urchin has one of the most well-documented gene regulatory networks (GRN), making it an excellent model to integrate the regulatory role of miR-124 into its GRN. We observed that inhibition of miR-124 resulted in decreased larval gut contractions and swimming velocity, indicating potential neuronal defects. Inhibition of miR-124 resulted in an increased number of cells expressing transcription factors associated with progenitor neurons and a concurrent decrease of mature and functional neurons. Results revealed that in the early blastula/gastrula stages, miR-124 regulates undefined factors during neuronal specification and differentiation. In the late gastrula/larval stages, miR-124 regulates *Notch* and *NeuroD1* during the transition between neuronal differentiation and maturation. In addition, we observed that miR-124 has a novel role in regulating the differentiation of mesodermally-derived immune cells, by targeting both Notch and Nodal signaling pathways. The sea urchin immune cells consist of blastocoelar cells (BCs) and pigment cells (PCs), which are derived from the same progenitor cells. Inhibition of miR-124 resulted in an increase of BC-specific gene expression and a concurrent decrease in differentiated PCs. Removing miR-124's suppression of *Nodal* leads to increased BCs and decreased PCs, while removing miR-124's suppression of *Notch* leads to increased BCs and PCs. In summary, we systematically examined the conserved function of miR-124 in neuronal development and identified miR-124's novel role in mesodermal differentiation.

B639/P2309

Optogenetic manipulation of brain cell development in the early embryo

K. Fenelon, S. Abbasi, A. Chandrasekhar, P. H. Borad, T. Koromila; Biology, University of Texas Arlington, Arlington, TX.

Embryonic cells experience distinct signaling dynamics, levels, and combinations that control differentiation into the diverse cell fates needed in healthy adults. To determine how signaling is decoded during development, experimental control over signaling inputs is required. Pioneer-like factors activate a multitude of genes simultaneously to produce antecedent cells for burgeoning common lineages of distinct germ layers or tissue primordia. Neural progenitor specification and differentiation occur early in development with detectable neuroblasts arising as early as stage 8/9 early of *Drosophila* embryogenesis. The roles of transcription factors (TFs) that activate the zygotic genome such as Zelda (Zld)/POU5F1, Odd-paired (Opa)/ZIC, and Ocelliless (Oc)/OTX1/2 are largely conserved across the animal kingdom. Opa drives the transcriptional landscape to undergo a dramatic shift to prepare the syncytial nuclei for cellular sovereignty rounding out the blastula stage and transitioning the embryo into gastrulation. After this, subpopulations of cells destined for exponentially expanding fates begin to arise driven by more local TF, like Oc. Our hypothesis is that Opa and Oc are driving brain cell specification in the early embryo. Using super resolution microscopy, we find that opa and oc expression domains overlap, with both factors being simultaneously transcribed within neuron cells in the overlapping region. Further, analysis of scRNA-seq data reveals enrichment of several known neural developmental genes in cells containing both opa and oc transcripts. Interrogation of these genes against Opa, Oc and Zld ChIP-seq datasets and expression databases suggests that Oc and/or Opa regulate their expression. Also, we are developed a blue light-activated optogenetic tool to orthogonally, reversibly activate TFs in Drosophila embryos. We are further able to optogenetically control protein function through using photo-inducible nuclear export tools, providing further temporal control. In addition, we are using live RNA transcript imaging to visualize gene expression of multiple neuroblast specific genes at a single cell level. Our data demonstrate novel combinatorial, pioneering functions of Opa and Oc in the establishment of the neural cell lineage in the early *Drosophila* embryo. Additionally, Oc binds to a subgroup of both DV and AP enhancers independently and activates them in a third wave of zygotic transcription. The study of these dynamics in vivo in a multicellular animal like *Drosophila* presents an opportunity to understand the role of transcription dynamics for tissue differentiation and patterning. This work is vital to understanding the cell fate determinations which beget the coordinated cellular diversity of mature animalia. Funding: STARs UTA

B640/P2310

The fly larval nerve cord on a budget: preparing for the future while taking care of the present needs

T. T. NGUYEN, R. Vicidomini, S. Choudhury, T. Han, T. Brody, M. Serpe; NICHD/NIH, Rockville, MD.

The insects ventral nerve cord (VNC), the equivalent of mammal the spinal cord, coordinates and integrates neural signaling from brain and periphery nervous system to produce a variety of locomotor outputs. Holometabolous insects, which undergo complete metamorphosis, need very different VNC functions, one for controlling crawling during the larval stages and another one for enabling the adult complex locomotor activities. How do insects develop and manage two different VNC? In flies, an atlas of the adult VNC has been assembled. However, the molecular characterization of the cells in third instar VNC remains to be determined.Here we use scRNA-seq to profile the transcriptome of 31,040

single cells from *Drosophila* third instar VNCs. Surprisingly, we found that almost 60% of these cells (17,920) are immature and undergoing a developmental program that will generate the adult VNC. These populations of cells include newborn secondary interneurons (INs) and motor neurons (MNs), glial precursors. The remaining cells are mature primary INs, MNs, and glia that fulfil various functions during the larval stages of development. Among the immature cells, we identified a transcription progression from neuroblasts to newborn secondary neurons. We found that all 21 hemilineages (LINs) reported in adult VNCs have correspondent clusters of secondary neurons in the larval VNC. Moreover, each larval secondary LIN has already acquired neurotransmitter identity and has its own repertoire of cell fate determinants. Almost 40% of the larval VNC cells are mature neurons and glia that control larval behavior and function. We identified over 40 subtypes of differentiated primary INs, each expressing unique combinations of transcription factors and neurotransmitters. Interestingly, primary INs are very different from secondary neurons. Finally, we identified and compared immature secondary and mature primary MNs. Adult newborn secondary MNs have low level of glycolysis related genes, low level of genes encoding synaptic proteins, and express no BMP target genes. In contrast, larval MNs are metabolically active (high level of glycolytic genes) are connected and functional (high level of synaptic genes) and are shaped by retrograde BMP signaling (express BMP target genes). Our studies uncover a surprising partition of the larval VNC which prepares the cells for the adult VNC while ensuring that larval needs are met. This larval VNC atlas provide a valuable resource for future studies of neurodevelopment and behavior.

B641/P2311

Mechanosensation via NompC Regulates Sensory Progenitor Differentiation in Maintaining Proprioception of Adult *Drosophila*

R. Zhu, S. Younger, K. Li, P. Jin, L. Y. Jan, Y. N. Jan; Department of Physiology, UCSF/HHMI, San Francisco, CA.

The “sixth sense” proprioception is mediated by mechanosensory neurons in muscles, tendons, and ligaments. Mechanosensitive channels (MSCs) are essential components for these neurons. MSC gene variant could result in proprioception defects in human. While consequences of chronically losing the mammalian MSCs are hard to study, we turned to *Drosophila melanogaster* MSC NompC, a Transient Receptor Potential family protein. The survival flies with *nompC* knocked out (KO) by CRISPR significantly decreased compared to w1118 control after eclosion. Further, the KO adult flies exhibited wing held-up defect and uncoordinated movement after eclosion. Re-expression of the full-length NompC protein by using binary Gal4 drivers revealed that NompC is required in class I/bd dendritic arborization (da) neurons, but not in other da neurons, motor neurons or muscles to regulated wing held-up phenotype. Localization of nompC by nompC-Gal4 overexpression of fluorescent marker and endogenous knocked-in *gfp* in *nompC* , together with rescue experiments pin pointed that NompC in type I small sensillum campaniformium of dorsal radius Sc12 at wing hinge is important for normal wing posture. Re-expression of NompC mutants that lost their mechanosensitivity in Sc12 cells failed to rescue the held-up phenotype observed in the KO, suggesting mechanosensation via NompC is important for maintaining the wing posture. Lineage analysis of Sc12 cells by LacZ and Gal4 systems suggested a defect in sensory progenitor cell number at early pupal formation. Consequently, the neuron number and glial number are reduced in the Sc12 niche of NompC-depleted adults. Mutants of NompA, a putative NompC binding partner in the extracellular matrix, displayed a similar help-up/held-out proprioceptive wing posture defect, and we are currently testing whether NompA is also involved in sensory progenitor’s

development. Our studies suggest that external force sensed by NompC in sensory progenitors is important for their differentiation, which in turn is responsible for maintaining normal wing posture.

B642/P2312

Profiling of differently reproductive-aged ovaries from marmoset monkey

S. Ku[1], Y. Kim[2]; [1]Dept of OBGYN, Seoul Natl University Hospital, Seoul, KOREA, REPUBLIC OF, [2]Biomedical Research Institute, Seoul Natl University Hospital, Seoul, KOREA, REPUBLIC OF.

Ovarian reserve decreased naturally with age or by chemotherapy. However, the exact mechanism is not known and is difficult to elucidate due to the ethical limits of the human sample. Marmoset monkey (*Callithrix jacchus)* is a rising non-human primate (NHP) model of biomedical research and an optimal model for reproductive medicine due to their close reproductive physiology and short gestation period. In this study, we profiled the mRNA expressions of 84 genes, related to female infertility, in differentially aged marmoset monkey ovaries. Ovariectomy of each aged marmoset monkey was performed and the ovaries were cut into small pieces (1x1 cm^2). Then, the pieces were enzymatically dissociated with Col. IV (1 mg/ml) and filtered through 40 μm strainers. Collected cells were replated and processed for further RNA extraction and qRT-PCR. The results were analyzed using databases. The expression profile of a total of 84 genes related to the female infertility of each aged ovary was analyzed. Among the female Infertility related genes, TGFβ, IL1 α, IL5, VEGFα, IL1β, Bcl2, ITGAV, TNF, MMP9, AKT1, MMP2, EGF, TIMP1, and ITGB, are involved in fibrosis and increased as reproductively aged. This study analyzed the female infertility-related genes in pre-pubertal, reproductive, and menopausal aged marmoset ovaries. The results indicated that the decreased ovarian reserve is strongly related to the onset of fibrosis (2020R1A2C1010293 and 2022R1A2B5B01002541).

B643/P2313

Characterization of Calcium Binding and Coiled Coil Domain 1 (Calcoco1) in Skeletal Muscle

A. Kajdic, D. Waddell; Biology, University of North Florida, Jacksonville, FL.

Skeletal muscle atrophy results in loss of muscle mass and strength and is caused by a range of physiological conditions including aging, cancer, corticosteroid use, and denervation. To further our understanding of the molecular genetic events associated with muscle atrophy, a previous study isolated skeletal muscle from mice following 3 days and 14 days of denervation and the gene expression profile was analyzed by microarray and compared to control muscle to identify novel, atrophy-induced genes. The microarray revealed that Calcium Binding and Coiled Coil Domain 1 (Calcoco1) is expressed in skeletal muscle and induced in response to denervation. The cDNA of Calcoco1 was successfully amplified and cloned from cultured C_2C_{12} cells, demonstrating that this gene is expressed in muscle cells. Quantitative PCR was subsequently conducted using RNA isolated from proliferating and differentiating muscle cells to determine the expression profile of Calcoco1 at the transcriptional level. We observed moderate activation of Calcoco1 through myoblast proliferation and early differentiation, followed by a robust increase in expression at later stages of myotube differentiation. These results were mirrored at the protein level where it was observed that Calcoco1 is expressed in early proliferation and increases as differentiation progresses. To elucidate the function of Calcoco1 in muscle, we transfected cultured muscle cells with a Calcoco1 expression plasmid and then harvested the cells at timepoints ranging from proliferation through late differentiation. The cell lysates were probed by Western blot for markers of muscle cell differentiation, including Myosin Heavy Chain (MyHC) and myogenin, which both show

significant repression in response to Calcoco1 overexpression. The Calcoco1 protein is predicted to contain both a calcium binding domain and a coiled-coiled domain, suggesting that it may play a role in protein-protein interactions or as a putative transcription factor. To begin to investigate these possibilities, we sought to determine the sub-cellular localization of Calcoco1 in muscle cells by fusing Calcoco1 cDNA to Green Fluorescent Protein (GFP) and expressing it in muscle cells. Visualization by confocal microscopy revealed clear nuclear-exclusion of Calcoco1 protein in unchallenged myoblast cells, suggesting that it may not participate directly in gene regulation. The discovery that Calcoco1 is expressed in skeletal muscle and is induced in response to neurogenic atrophy, in combination with its apparent role in muscle cell differentiation, helps further our understanding of the molecular and cellular events of muscle atrophy and may eventually contribute to the identification of new therapeutic targets for the treatment of muscle wasting.

B644/P2314

***Prdm1* Drives a Sensory Hair Cell Fate Switch in Zebrafish**

J. E. Sandler, N. T. T. Tran, S. Chen, T. Piotrowski; Stowers Institute for Medical Research, Kansas City, MO.

One of the causes of permanent hearing loss in mammals is the lack of regeneration in the cochlea following damage to mechanosensory hair cells. Regenerating hair cells is a central strategy for restoring hearing, but triggering their proliferative regeneration and maturation in mammals remains elusive. The zebrafish *Danio rerio* has hair cells both in an array along the trunk, called the lateral line, and in the inner ear. While sharing genetic, functional, and structural similarity with mammalian inner ear hair cells, zebrafish hair cells differ in that they readily and rapidly regenerate following death to restore full function. Therefore, it is our objective to understand the genetic networks driving zebrafish hair cell regeneration to identify gene targets for triggering regeneration in mammals. The transcription factor *prdm1a* is expressed in hair cells of the zebrafish lateral line, but not in hair cells of zebrafish or mammalian inner ears. Previously, mammalian *Prdm1* has been shown to control a fate switch in various cell types, including B lymphocytes and photoreceptors in the retina. The combination of both its hair cell-specific expression and its involvement in cell fate decisions led us to investigate *prdm1a* for its role in hair cell development and regeneration. We mutated *prdm1a* in zebrafish and found a drastically reduced number of hair cells and cell proliferation in the lateral line during development and regeneration. Single cell RNA-seq on *prdm1a* mutants and siblings revealed a cell type fate switch between lateral line and inner ear hair cells, with a multitude of inner ear hair cell-specific genes ectopically expressed in mutant lateral line hair cells. Performing ATAC-seq and ChIP-seq on zebrafish lateral line cells, we found Prdm1 binding sites highly enriched in the promoters and enhancers of ectopically expressed inner ear hair cell genes. Using these enhancers to drive GFP expression in zebrafish confirmed their ectopic expression in *prdm1a* mutant hair cells, but not in siblings. These findings show that Prdm1a plays a crucial role in repressing an inner ear hair cell fate in lateral line organs and highlight Prdm1 as a potential driver of hair cell type specification and regeneration in other vertebrates. Combined, our data show that *Prdm1* is an essential gene to consider in future regeneration attempts in the mammalian cochlea.

B645/P2315

Planarian glia in the context of whole brain regeneration

B. Chandra[1], M. Voas[2], E. Davies[2], **R. Roberts-Galbraith**[1]; [1]Cellular Biology, University of Georgia, Athens, GA, [2]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD.

Non-neuronal cells collectively called glia play diverse roles in neurodevelopment and in regulation of neural function across diverse animal species. Roles for glia in regeneration are more complex, with glia playing inhibitory and/or permissive roles in regeneration depending on the species and the injury context. The discovery of astrocyte-like glial cells in planarians—freshwater flatworms with extraordinary regenerative abilities—presents the opportunity to understand glial regeneration and function in the context of successful, whole-brain regeneration. In our laboratory, we are interested in using planarians to uncover mechanisms underlying glial regeneration, to discover functions of glia in a new animal species, and to explore the interplay between glia and neurons in the context of brain regeneration. Here we show that transcription factor *ets-1*regulates glial cell regeneration and cell state in planarians. With a gene in hand to perturb glia, we also explored the effects of glial reduction on neurons and showed that loss of *ets-1* results in locomotion defects, changes gene expression in neurons, and impacts markers of neural connectivity. Finally, we show that planarian neurons regenerate and develop before glia. We are currently testing whether planarian glia require neurons for their specification and proper localization within the nervous system. Our results establish planarians as a model for studying evolution of glia/neuron interactions and interplay between these critical cell types during brain regeneration.

B646/P2316

An Essential Role of Polyhomeotic Homolog 1 (Phc1) in Early Neural Development

A. L. C. Ong[1], M. Shirai[2], N. Sasai[1]; [1]Laboratory of Developmental Biomedical Science, Nara Institute of Science and Technology, Ikoma, Nara, JAPAN, [2]Omics Research Center, National Cerebral and Cardiovascular Center, Osaka, JAPAN.

Polyhomeotic homolog 1 (PHC1), is a component of the polycomb repressive complex 1 (PRC1) that has a role in transcription repression during developmental stages. Phc proteins mainly regulate the PRC1 assembly and polymerization that help in chromatin modification and lead to gene silencing. Previous studies have shown that deficiency of the *Phc1* gene in mice caused eye abnormalities during early embryonic stages. However, little is known about the detailed mechanisms of Phc1. With that, we set to investigate Phc1 and its role in early neural development.For this purpose, we utilized CRISPR/Cas9 system and ES cells-derived differentiation system to generate *Phc1*-knockout (*Phc1*-KO) mouse embryonic stem cells, then differentiate into early-stage neural tissues. *Phc1*-KO cells were unable to differentiate into neural progenitor cells, whereby the neural markers, Sox1 and Pax6, failed to be expressed. The expression of pluripotent genes (Nanog, Klf4 and Zfp42) were upregulated in the absence of *Phc1*. We hypothesized that the high expression of pluripotency markers prevented the *Phc1*-KO cells from early neural differentiation. We then performed CRISPR-mediated targeted knock-in to insert the complete coding sequence of *Phc1* into the *Phc1*-KO genome. The early neural differentiation phenotypes were partially recovered with the exogenous *Phc1* gene. Besides Phc1, there are several protein subunits in the PRC1, including Ring1A/B, Pcgf2/4, Cbx2/4/6/7/8 and Phc1/2/3. Several knockout cells of these subunits were generated and we found that absence of these subunits did not prevent the cells from differentiation into early neural progenitor cells. This suggested that they

could be compensated by their respective paralogs. As Phc1 and PRC1 play critical roles in histone modification and chromatin remodeling, we are now evaluating the genome wide chromatin accessibility, modification and target gene binding with ATAC-sequencing and chromatin immunoprecipitation quantitative real-time PCR.

B647/P2317

Intracellular pH dynamics regulates intestinal stem cell lineage specification

Y. Liu[1], E. Reyes[1], D. Castillo-Azofeifa[1,2], O. D. Klein[1], T. Nystul[1], D. L. Barber[1]; [1]University of California, San Francisco, San Francisco, CA, [2]Genentech, Inc., South San Francisco, CA

Intracellular pH (pHi) dynamics is increasingly recognized to regulate myriad cell behaviors, including proliferation, migration, differentiation, and transformation. Here we report a new finding that pHi dynamics also regulates adult stem cell lineage specification. In mouse small intestinal organoids, we identify a pHi gradient along the crypt axis, lower at the crypt base and higher toward the villus, and find that dissipating this gradient by inhibiting Na^+-H^+ exchanger 1 (NHE1) activity genetically or pharmacologically abolishes crypt budding. Using single-cell RNA sequencing and lineage tracing we demonstrate that pHi dynamics acts downstream of ATOH1, with increased pHi promoting differentiation toward the secretory lineage, while reduced pHi biases differentiation into the absorptive lineage. Consistent with these results, disrupting the pHi gradient blocks new Paneth cell differentiation. Paneth cells provide an essential WNT signal to ISCs in organoids, and we find that the loss of crypt budding with inhibiting NHE1 activity is rescued with exogenous WNTs. Our findings indicate that pHi dynamics is tightly regulated in the ISC lineage and that an increase in pHi is required for the specification of secretory lineage, including Paneth cell differentiation that contributes to crypt maintenance. These observations reveal a previously unreported role for pHi dynamics in cell fate decisions within an adult stem cell lineage.

B648/P2318

Intracellular pH Dynamics Regulates Wnt Signaling for Stem Cell Differentiation

H. Virk[1,2], K. Kisor[1], T. Nystul[1], D. L. Barber[1]; [1]University of California, San Francisco, San Francisco, CA, [2]San Francisco State University, San Francisco, CA

Intracellular pH (pHi) dynamics is now recognized as a signal regulating stem cell differentiation and cell fate decisions. Increased pHi is required for the differentiation of mouse embryonic stem cells (mESCs) and adult intestinal stem cells as well Drosophila follicle stem cells (FSCs), although the mechanisms mediating these effects remain unknown. Decreased Wnt pathway activity is necessary for the differentiation of multiple types of stem cells, and we previously reported that increasing pHi attenuates Wnt signaling in epithelial cells by decreasing stability of the effector β-catenin. We reported that a higher pHi increases β-catenin binding to the E3-ligase β-TrCP by deprotonating β-catenin-His35 at the binding site. We now report two advances in our understanding of pHi dynamics, Wnt signaling, and stem cell differentiation. First is that β-catenin binding to BCL9, which is a means for translocating β-catenin to the nucleus for transcriptional responses, is attenuated at higher pH. The crystal structure of BCL9 in complex with β-catenin indicates a salt bridge between BCL9-His358 and β-catenin-Asp162 at the binding interface, and we confirmed with *in vitro* binding using recombinant proteins that a neutral histidine at pH $\geq$ 7.5 decreases binding compared with pH < 7.5. Second, using *in vivo* differentiation of Drosophila FSCs, which requires increased pHi and decreased Wnt pathway activity, we found that a

mutant armadillo-His42R (Drosophila β-catenin-His42R) that is insensitive to pH-regulated stability results in severe morphological defects in the FSC lineage through the expansion of epithelial cells and improper budding of germline cysts. These findings support our previous reports on increased pHi being necessary for stem cell differentiation, and they add mechanistic insight by suggesting the effect is due to a higher pHi in differentiating cells decreasing Wnt pathway activity.

Stem Cells and Pluripotency

B649/P2319

PSME4 degrades acetylated YAP1 in the nucleus of mesenchymal stem cells to induce cardiac commitment

G. Kang[1], **G. Eom**[2]; [1]Chonnam National University Hospital, Gwangju, KOREA, REPUBLIC OF, [2]Pharmacology, Chonnam National University Medical School, Hwasun, KOREA, REPUBLIC OF.

Intensive research has focused on minimizing the infarct area and stimulating endogenous regeneration after myocardial infarction. Our group elucidated that apicidin, a histone deacetylase (HDAC) inhibitor, robustly stimulates cardiac commitment of mesenchymal stem cells (MSCs) through acute loss of YAP1. Here we further studied the mechanism of this role of YAP1 in MSCs. We found that acute loss of YAP1 after apicidin treatment resulted in the mixed effects of transcriptional arrest and proteosomal degradation. Subcellular fractionation revealed that YAP1 was primarily localized in the cytoplasm. YAP1 was acutely relocalized into the nucleus and underwent proteosomal degradation. Interestingly, phosphor-S127 YAP1 was shuttled into the nucleus, suggesting that a mechanism other than phosphorylation governed subcellular localization of YAP1. Apicidin successfully induced acetylation and subsequent dissociation of YAP1 from 14-3-3, an essential molecule for cytoplasmic restriction. HDAC6 regulated both acetylation and subcellular localization of YAP1. An acetylation-dead mutant of YAP1 retarded nuclear redistribution upon apicidin treatment. We failed to acquire convincing evidence for polyubiquitination-dependent degradation of YAP1, suggesting that a polyubiquitination-independent regulator determined YAP1 fate. Nuclear PSME4, a subunit of the 26S proteasome, recognized and degraded acetyl YAP1 in the nucleus. MSCs from PSME4-null mice were injected into infarcted heart and aberrant sudden death was observed. Injection of immortalized human MSCs after knocking down PSME4 failed to improve either cardiac function or the fibrotic scar area. Our data suggest that acute ablation of YAP1 in the nucleus by the acetylation-dependent proteasome subunit PSME4 is mandatory for cardiac commitment of MSCs.

B650/P2320

Golgi organization is a determinant of stem cell signaling in the small intestine

S. Scharaw[1], A. Sola Cavajal[1], A. Webb[1], I. Belevich[2], N. Pentinmikko[2], S. Das[1], E. Jokitalo[2], R. Coffey[3], P. Katajisto[1]; [1]Karolinska Institute, Solna, Sweden, [2]University of Helsinki, Helsinki, Finland, [3]Vanderbilt University, Nashville, TN

Cell-to-cell signaling between stem and niche cells regulates stem cell function and tissue regeneration. Lgr5+ small intestinal stem cells (ISCs) reside at the intestinal crypt bottom and communicate with their neighboring niche Paneth cells by growth factor signaling. Whereas the key signals have been extensively characterized, the secretory transport of ISC signal proteins is largely unknown. Here, we set up a microscopy-based secretion assay in crypt cells of intestinal organoids and quantified the secretion

efficiency of Epidermal growth factor receptor (Egfr), a key receptor for stem-to-Paneth cell signaling. This showed that ISCs at the crypt center secreted Egfr with a higher efficiency to the cell surface than ISCs at the crypt border. Serial block-face scanning electron microscopy on intestinal crypts revealed ISCs to consist of lateral Golgi complex mini-stacks, and two mini-stacks in center ISCs accounted for their higher secretion efficiency compared to border ISCs with one mini-stack. Mechanistically, Golgi complex mini-stack morphology of ISCs requires A-kinase anchor protein 9 (AKAP9), and AKAP9 inhibition altered Egfr secretion and *in vitro* regeneration capacity of ISCs. Moreover, in aged mice Golgi mini-stack differences between ISCs at the center and border were lost, which caused signal secretion efficiencies of center ISCs similar to those of border ISCs. Our results reveal a Golgi complex morphology arrangement that facilitates a secretion efficiency gradient of ISCs from the center towards the crypt border, which is required for tissue regeneration and is lost in the old epithelium.

B651/P2321

PLEKHA4/*kramer*regulates intestinal homeostasis via wnt signaling in *drosophila*

H. Sun[1], A. Shami Shah[2], A. Bonfini[3], N. S. Buchon[3], J. M. Baskin[1]; [1]Weill Institute for Cell & Molecular Biology, Cornell University, Ithaca, NY, [2]Weill Institute for Cell & Molecular Biology and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, [3]Departments of Entomology, Cornell University, Ithaca, NY.

Wnt/β-catenin signaling is required in animals for the regulation of intestinal stem cells (ISCs) during homeostasis and tissue regeneration. We have previously demonstrated that the phosphoinositide-binding protein PLEKHA4 sequesters the Cullin-3 (CUL3) E3 ubiquitin ligase substrate adaptor Kelch-like protein 12 (KLHL12) in an inactive state at the plasma membrane and thus enhances Wnt/β-catenin signaling by preventing polyubiquitination of Dishevelled (Dvl), a key activator of Wnt/β-catenin signaling, by CUL3-KLHL12 in mammalian cells. However, the potential effects of PLEKHA4 on Wnt/β-catenin signaling has not been examined in vivo. Here, we investigated whether the *Drosophila* ortholog of PLEKHA4, *kramer*, regulates Wnt/β-catenin in vivo using a model for intestinal stem cell (ISC) regeneration following non-lethal infection. We infected wildtype, *kramer* knockout, or tissue-specific *kramer* knockdown *Drosophila melanogaster* with *E. carotovora carotovora 15* (Ecc15), a gram-negative bacterium, and we assessed ISC proliferation in the midgut, the fly equivalent of the small intestine. Knockout and knockdown of *kramer* decreased proliferation of ISCs and certain other cell populations in the intestinal epithelium; moreover, *kramer* knockout also downregulated the expression of Frizzled 3, a Wnt/β-catenin target gene, specifically in the posterior midgut. Our results indicate that *kramer* regulates ISC proliferation via Wnt/β-catenin signaling. Elucidation of Wnt/β-catenin activation in ISCs, as well as additional roles for non-canonical Wnt signaling pathways impacted by *kramer*, will provide insights for identifying novel therapeutic targets for the treatment of diseases caused by the dysregulation of Wnt signaling pathways.

B652/P2322

The role of Wnt pathway in activation of Hair Follicle Stem Cells during cyclic hair regeneration

P. Mazurek, K. Tchórz, K. Łukaszyk, K. Kobielak; Laboratory of Stem Cells, Tissue Development and Regeneration, Centre of New Technologies University of Warsaw, Warsaw, POLAND.

In the sense of a mini-organ, the hair follicle is an excellent model for studying the molecular mechanisms that govern the transition between quiescence and cyclic activation of the hair follicle stem

cells (hfSCs) during hair growth. Our previous finding revealed that a competitive balance between two well-known molecular pathways, wingless/integrated pathway (Wnt) and bone morphogenetic protein pathway (BMP) is crucial for hfSCs homeostasis *in vivo*. We found that hfSCs with suppressed BMP signaling show up-regulation of several WNT activators, including the Wnt7b ligand. This ligand was identified as a potential target of BMP signaling in hfSCs *in vivo*. Although we revealed that loss of Wnt7b function during the hair cycle results in a delay of hfSCs activation, there has been no evidence regarding how Wnt7b works at the molecular level on hfSCs regulation during the hair regeneration cycle. To answer this question, we created a genetic mouse model to simultaneously label and specifically inactivate the Wnt7b pathways within the hfSCs. Using this approach, we were able to isolate living Wnt7b knockout hfSCs marked by eYFP using fluorescence-activated cell sorting (FACS). Total RNAs from isolated cells were used to perform RNA-seq analyses, which allowed us to analyse the expression of hfSCs genes after inactivation of Wnt7b and thus characterize target genes relevant for Wnt7b signaling. We identified 2240 gene expression changes after the inactivation of the Wnt7b ligand. Bioinformatic analysis showed that more than 90% of the axial genes of the hfSCs internal oscillator were altered. Interestingly, more than 83% of the genes are altered in the same way as in the BMP pathway inactivation, resulting in premature activation of stem cells. Surprisingly, the molecular mechanism we discovered shows that the deletion of the Wnt7b ligand for the WNT canonical pathway in hfSCs is compensated for by activating the expression of two other ligands for the WNT canonical pathway, which correlates with the inhibition of two other ligands and Fzds receptors for the non-canonical WNT pathway. In summary, our discovery fits into the internal oscillator model we previously discovered, which explains that this naturally occurring mechanism of compensation and coupling of genes responsible for regulating hair regeneration adapts to changing molecular conditions. Moreover, to investigate the mechanism of action of other WNT pathway modulators during hfSCs activation, we are also using the CRISPR/Cas9 system. This approach allows us to inactivate several genes of interest within the hfSCs in vitro at the same time and then further investigate their biological functions, again, returning to the *in vivo* model using the chamber graft assay.

B653/P2323

Deletion of the von-Hippel Lindau (Vhl) gene in bone cells leads to aberrant hematopoiesis in mice

B. Chicana Romero[1,2], H. Taglinao[2], J. O. Manilay[2]; [1]Fred Hutch Cancer Reserach Center, Seattle, WA, [2]University of California Merced, Merced, CA.

Stabilizers of hypoxia-inducible factors (HIFs) are proposed treatments to alleviate bone loss. One strategy to stabilize HIF is via inhibition of the von-Hippel Lindau protein (VHL). VHL regulates hypoxia-inducible factor degradation and is involved in responses to low oxygen environments. Osteoblasts (OBs) and osteocytes (OCYs) are bone cells known to support hematopoiesis in the bone marrow. However, the specific interaction of bone cells and local bone marrow microenvironments that contribute to the regulation of hematopoiesis are not fully understood. We utilized *Dmp1*-Cre;*Vhl* conditional knockout mice (cKO), in which *Vhl* is deleted in OBs and OCYs. When a longitudinal analyses of cKO mice were performed at 3, 6, 10 and 24 weeks of age, a time dependent increase of high bone mass and reduction of the bone marrow cavity was gradually observed. In transplantation experiments, reciprocal bone marrow chimeras showed significant reduction of % B cells in the WT→cKO, while B cell development was normal in the cKO→WT chimeras, suggesting a cell-extrinsic effect of *Vhl* on B cells development within the cKO bone marrow microenvironment. Observed drastic defects in B cell development were associated with reduced levels of CXCL12 and IL7, increased apoptosis and

quiescence of early B cell progenitors, and indications of hypoxia in local B cell niches. We also hypothesized that distinct subsets of myeloid cells were differentially affected in the cKO bone marrow. Using flow cytometry, we discovered increased frequencies (%) of common myeloid progenitors (CMP) and granulocyte-monocyte progenitors (GMP) but no difference in % frequency of neutrophils, dendritic cells and monocytes. In contrast, we observed similar % of myeloerythroid progenitors (MEP) in cKO and controls, but enhanced erythropoiesis in the cKO, including a significant increase in CD71- TER119+ orthochromatic erythroblasts in the bone marrow. These discoveries provided a potential mechanism by which VHL/HIF signaling in osteocytes serves as a regulatory niche for distinct hematopoietic lymphoid and myeloid lineages in the bone marrow. Additionally, these studies are important for understanding how HIF stabilization in skeletal cells can affect hematopoiesis.

B654/P2324

Dynamic patterns of ERK activity encode skeletal tissue size during zebrafish fin regeneration

A. Rich[1], Z. Lu[1], A. De Simone[2], K. D. Poss[1], S. Di Talia[1]; [1]Cell Biology, Duke University, Durham, NC, [2]Department of Genetics & Evolution, University of Geneva, Geneva, SWITZERLAND.

Mammals possess a limited capacity to regenerate appendages following traumatic injury and amputation, including regenerating digit tips and healing bone fractures. In contrast, zebrafish can regenerate entire appendages following amputation. As in development, regenerated appendages are scaled appropriately to body size. Extensive genetic and pharmacological experiments have established that canonical signaling pathways, such as Fgf and Wnt, are reactivated following injury to promote the cell proliferation, migration, and differentiation that drives appendage regeneration. However, how these pathways encode size and shape is unclear. Here, we study the molecular underpinnings of size control during the regeneration of caudal fin osteoblasts, bone matrix secreting cells. Osteoblast proliferation must be tightly controlled in time and space to regenerate bony rays of appropriate length. Extracellular signal-regulated kinase (ERK) is an established regulator of cell proliferation and is known to regulate osteoblast differentiation. ERK is also a canonical downstream target of Fgf signaling, which is required for caudal fin regeneration. We developed a live-imaging platform to quantitatively investigate whether ERK activity controls osteoblast proliferation and encodes tissue size during caudal fin bone regeneration. We find dynamic, long-range gradients of ERK activity that persist throughout regeneration, and we show that the probability of proliferation within a region of regenerating osteoblast tissue quantitatively depends on ERK activity. Furthermore, both average ERK activity and osteoblast proliferation decrease as regeneration progresses. Importantly, these relationships are conserved between longer lateral and shorter medial rays. Using these experimental observations, we modeled ray growth rate as a function of fraction of regeneration completed and found that our prediction of this relationship agrees with previously published measurements of ray growth during fin regeneration. Altogether these results suggest a universal law, molecularly controlled by ERK activity, describes skeletal tissue growth during fin regeneration.

B655/P2325

In Vitro Culture of Skin Samples Excised from an Accidentally Deceased One Month Old Goat Kid up to 14 Days of Postmortem Interval Upon Storage at 4°C

M. Singh, T. Stephens, X. Ma, A. Abosede; Animal Science, Fort Valley State University, Fort Valley, GA.

The objective of this study was to assess the postmortem time limits within which live and proliferative cells can be recovered from newborn goat kid skin tissues stored in refrigerator (4°C) after their death. Small (3-4 mm^2) explants (n = 20) from ear skin stored at 4°C postmortem were cultured in 35 mm dish after 0, 7, 14, 21 and 28 days of storage in DMEM media supplemented with 10% FBS, 50 units/mL of penicillin, 50 µg/mL of streptomycin and 2.5 µg/mL of fungizone. Presence or absence of the fibroblast cell outgrowth around the explants was scored. We observed outgrowth of cells around explants up to 14 days of postmortem interval in all the explants (100%) that adhered to dish surface. Cells started outgrowing from the explants at day 3, 5, and 8 in 0-dpm, 7-dpm and 14-dpm time intervals, respectively. The level of confluence, as recorded on day 10-12 of culture initiation, decreased with increasing postmortem time interval. The outgrowing cultures were trypsinized to recover the cells upon reaching around 90% confluence, in each time point, and the number of recovered cells counted using trypan blue dye exclusion method. The average number of cells recovered from each explant was calculated to be 26.11 x 10*4, 7.10 x 10*4 and 2.40 x 10*4 cells/mL for 0-dpm, 7-dpm and 14-dpm cultures, respectively. When equal number of these passage 0 primary cells sub-cultured into a new dish for each postmortem time interval, we observed comparable growth profile and morphology to that of fresh tissue derived cell populations. Taken together, these results suggest that live, proliferative, and usable cells can be recovered from newborn goat kid skin tissues up to 14 days of postmortem interval, if the skin tissues are stored at 4°C. Reprogramming of these cells to clone the dead goat genetics remain to be seen in future studies.

B656/P2326

Mechanisms of vascular maturation and maintenance captured by longitudinal imaging of live mouse skin

C. Kam[1], I. D. Singh[1], C. Matte-Martone[1], D. G. Gonzalez[1], P. Sola[2], G. Solanas[2], J. Bonjoch[2], E. D. Marsh[1], K. K. Hirschi[3], V. Greco[1]; [1]Genetics, Yale University, New Haven, CT, [2]IRB Barcelona, Barcelona, SPAIN, [3]Cell Biology, University of Virginia, Charlottesville, VA.

A functional network of blood vessels is essential for organ growth and homeostasis. Yet, how the vasculature matures and maintains adult homeostasis remains elusive in live mice. To shed light on these fundamental biological processes, we performed longitudinal imaging in the skin of live mice using 2-photon microscopy, which allows for non-invasive tracking of cell populations over long periods of time. We systematically evaluated the same capillaries and endothelial cells (ECs) in neonatal mice over periods of days to weeks, tracking their behaviors as the skin plexus expanded with organismal growth and reached adulthood. Counterintuitively, we found that capillary plexus expansion is driven by network-wide vessel regression and transient angiogenesis during neonatal stages. These processes involve migratory ECs that relocate from regressed segments to neighboring vessels. Furthermore, we observed that all ECs dynamically rearrange their positions within maturing capillaries, independent of their remodeling status. These rearrangements regulate EC density by ensuring an even cellular distribution during plexus expansion. As the skin approaches adulthood, plexus regression rates decrease, and ECs become positionally stable. However, upon discrete local injury via targeted laser

ablation, neighboring ECs do not proliferate but collectively elongate or migrate towards damaged sites to repair vessels. This mechanism is in direct contrast to the proliferative repair observed during large scale ablation of blood vessels. Remarkably, we found that injured adult ECs, rather than dying, survive through the activation of a plasmalemmal self-repair response whereby ECs are able to excise and discard damaged portions of their cell membranes prior to regenerating. In contrast, neonatal ECs are disposed to be eliminated via apoptosis in response to injury, causing the regression of the vessel segment in which they reside. Lastly, neonatal vessel regression and adult vascular maintenance are orchestrated by temporally restricted VEGFR2 dependent signaling. Acute blockade of VEGFR2 during neonatal stages accelerates the rate of vessel regression and network maturation. Surprisingly, when revisited in adulthood, these animals display widespread vessel tortuosity, an indicator of unhealthy vasculature. Our work sheds light on fundamental tissue, cellular, and molecular mechanisms that underlie both vascular maturation and adult homeostasis in vivo.

B657/P2327

Activated platelet derived growth factor signaling pathway enhances ovarian function in injured rat ovary *via* pericyte recruitment

H. Park[1,2], D. Lee[1,2], K. Na[1], W. Jeong[1], J. Seok[3], J. Lim[4], G. Kim[1,2]; [1]CHA University, Seongnam-si, KOREA, REPUBLIC OF, [2]PLABiologics.Co.Ltd, Seongnam-si, KOREA, REPUBLIC OF, [3]University of Chicago, Chicago, MD, [4]Hyejeon College, Hongsung-gun, KOREA, REPUBLIC OF.

Vascular abnormalities in the ovary cause ovarian dysfunction due to a microenvironment of barren ovarian tissues. Platelet-derived growth factor signaling is critical pathway in maturation stage of follicular development and ovarian function. It is major factor in vascular stabilization and maturation by pericyte recruitment. In previous reports, we demonstrated placenta-derived mesenchymal stem cells (PD-MSCs) overexpressing phosphatase regenerating liver-1 (PD-MSCs^{PRL-1}) restores ovarian function via antioxidants effect. However, there is still unclear the therapeutic mechanism between ovarian function and vascular function (e.g., vascular structure, vascular permeability, and so on). Therefore, we examined whether PD-MSCs^{PRL-1}, which is correlated with angiogenesis in reproductive systems, could maximize the angiogenic effects in an injured ovarian rat model and regulates the PDGF signaling by enhanced vascular function via pericyte recruitment. One week after Sprague-Dawley (SD) rats with ovariectomy, PD-MSCs and PD-MSCs^{PRL-1} were transplanted into the injured rat model by tail vein. After transplantation, the expressions of factors for angiogenic and follicular development were analyzed. Vascular structures in ovarian tissues (e.g., thickness and lumen area) showed changes in the PD-MSCs and PD-MSCs^{PRL-1} transplantation (Tx) groups compared to the non-transplantation (NTx) group. Especially, PD-MSCs^{PRL-1} induce to increase the expression of PDGF compared to the NTx (*p<0.05). The pericyte recruitment (NG2) was significantly enhanced in PD-MSCs^{PRL-1} compared to the NTx and PD-MSCs *via* activated PDGF (*p<0.05). The expression of genes related to vascular permeability (e.g., erg-3) and follicular development (e.g., Nobox, BMP15 and EGFR) in ovarian tissues was significantly improved in the PD-MSCs^{PRL-1} compared to the NTx and PD-MSCs (*p<0.05). Also, PD-MSCs^{PRL-1} enhanced the vascular formation and decreased permeability of human umbilical vein endothelial cells (HUVECs) *via* activated the PDGF signaling. The expression of PDGF was significantly decreased in si-PRL-1 or PDGFR inhibitor treatment compared to control. However, their expression was significantly increased in PD-MSCs and PD-MSCs^{PRL-1} cocultivation group compared to si-PRL-1 or PDGFR inhibitor treatment (*p<0.05). Our results show that increased PRL-1 restored ovarian function by enhanced vascular function *via* activated pericyte and PDGF signaling pathway. These findings offer new insight into the

effects of functionally enhanced stem cell therapy for reproductive systems and should provide new avenues to develop more efficient therapies in degenerative medicine.

B658/P2328

Interpreting geometric rules of early kidney formation for synthetic morphogenesis

J. Viola, J. Liu, L. Prahl, A. Huang, T. Chan, G. Hayward-Lara, C. Porter, **A. Hughes**; University of Pennsylvania, Philadelphia, PA

The kidney develops through coordinated growth of ureteric epithelial tubules (the future urinary collecting ducts), stroma, and nephron progenitors in the cap mesenchyme that surrounds each ureteric tip as they branch. Dynamic interactions between these tissues set nephron numbers for life, impacting the probability of adult disease. How then are the rates of nephron formation and ureteric tubule duplication balanced? Here we study the geometric and mechanical consequences of tubule tip packing at the embryonic kidney surface for tip organization and nephron formation. We find that over developmental time, kidney curvature reduces and 'tip domains' pack more closely, which together create a semi-crystalline tip geometry at the kidney surface. We apply a geometric parameter of tip domains called the shape index to predict a rigidity transition to more solid-like tissue properties at later developmental stages and confirm by micromechanical measurements. At the level of individual tips we use the shape index to define a tip 'life-cycle' between branching events, and find that nephrogenesis rate varies over this life-cycle. Applying force inference techniques adapted from a cell vertex model and validating with laser ablation shows that tip domains experience a cyclical mechanical transient over each life-cycle. We then hypothesized that tip duplication periodically creates a mechanical microenvironment permissive to nephrogenesis. Indeed, mimicking a mechanical transient in human iPSC-derived nephron progenitor organoids increased Wnt-driven commitment to early nephron cell aggregates. The data suggest that temporal waves of mechanical stress within nephron progenitor populations could constitute a clock that synchronizes nephron formation and ureteric tubule duplication. Ongoing work to understand the spatial and temporal regulation of nephron induction will clarify variation in nephron endowment between kidneys and advance engineered replacement kidney tissues for regenerative medicine.

B659/P2329

Coupled oscillators in growth and patterning- cell cycle and the segmentation clock

U. Kadiyala, Q. Yang; University of Michigan, Ann Arbor, MI.

Coordinated regulation of cell proliferation and differentiation is fundamental to the growth and patterning of multicellular structures. During vertebrate development, the process of somitogenesis captures this delicate balance of cell proliferation and differentiation in an intricate manner. Here, several molecular oscillators and spatial gradients temporally pattern the formation of somites. The details of how the patterning and growth of somites are regulated is less understood. Here we study the interactions between the cell cycle and the segmentation clock to understand how cell proliferation and patterning are coupled during somitogenesis. Using a 3D zebrafish embryonic tissue model and confocal microscopy, we demonstrate that the phase dynamics of the cell cycle and segmentation clock are spatially coupled. The two oscillators appear to be in phase at the anterior end and out of phase at the posterior end of the elongating tailbud. We find that the number of posteriorly positioned progenitor cells and the rate at which they differentiate and move into the presomitic mesoderm (PSM) is tightly

regulated. A ratio of 1:3, progenitors to PSM cells, appears to be maintained throughout development. Here, we propose a general framework where the spatially coupled phase dynamics of the cell cycle and the segmentation clock could maintain the ratio and distribution of cell types and their proliferation rate during segmentation.

B660/P2330

Activated HGF signal pathway by PRL1 regulate glucose metabolism *via* ATF5/PDX1 interaction in STZ injured liver rat model

W. Jeong[1], S. Kim[1], J. Kim[2], J. Jun[3], S. Bae[4], G. Kim[1]; [1]CHA University, Seongnam-si, KOREA, REPUBLIC OF, [2]University of California, San Diego, CA, [3]Duke University, Durham, NC, [4]Catholic University, Seoul, KOREA, REPUBLIC OF.

The precise balance of glucose metabolism is one of the important factors involved in various cellular events such as proliferation, metabolism, differentiation and cell death. Glucose metabolism that is not homeostatically controlled causes various diseases including diabetes as well as liver disease. In particular, abnormal glucose metabolism not only causes oxidative stress in mitochondria, but also causes abnormal ATP production, which affects the normal survival of cells. Recently, it was reported that hepatocyte growth factor (HGF) protects the survival of islet beta cells and regulates glucose transport and metabolism. In previous reports, it was confirmed that the efficacies for anti-fibrotic effect and liver regeneration were increased when placenta-derived mesenchymal stem cells overexpressed with phosphatase of regenerating liver-1 (PRL-1) known as a marker for liver regeneration (PD-MSCs^{PRL-1}) were transplanted into an animal model of hepatic cirrhosis. However, their mechanism between PRL-1 and glucose metabolism was still unclear. The objectives of the present study are to analyze the expression of HGF and demonstrate glucose metabolic pathway in diabetes model after PD-MSCs^{PRL-1} transplantation. If the HGF signaling activated by PD-MSCs^{PRL-1} regulates glucose metabolism, we demonstrated how it improve hepatic functions regulating the interaction between ATF5/PDX1 in the liver of diabetes model after PD-MSCs^{PRL-1} transplantation (Tx). One weeks after intraperitoneal injection (I.P) by streptozotocin (STZ, 60mg/Kg), PD-MSCs and PD-MSCs^{PRL-1} were transplanted into the diabetes rat model by tail vein.. The expression of HGF was significantly increased in PD-MSCs^{PRL-1} compared to PD-MSCs (*p<0.05).The expression of gene related to islet beta cells (e.g., ATF5, PDX1) was significantly increased in PD-MSCs and PD-MSCs^{PRL-1} Tx groups compared to non-transplantation group (NTx) (*p<0.05). The levels of mitochondrial stress of PD-MSCs^{PRL-1} Tx group was significantly decreased compared to PD-MSCs (*p<0.05). Also, the expression of genes related to regeneration of hepatocytes (e.g., albumin, cyclin D1, HNF1a, HNF4a) were increased in PD-MSCs and PD-MSCs^{PRL-1} Tx groups compared to those of NTx group. Taken together, PD-MSCs^{PRL-1} improved hepatic function through activated HGF signaling via ATF5/PDX1 interaction in liver of STZ-injured rat model. Therefore, these results provide useful data for the development of functional enhanced MSC-based stem cell therapy for hepatic diseases in diabetes. **Funding:** This research was supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Health & Welfare). (RS-2022-00070304)

B661/P2331

Cytonemes coordinate asymmetric signaling and organization in the*Drosophila*muscle progenitor niche.

A. Patel[1], Y. Wu[2], X. Han[2], Y. Su[2], T. Maugel[1], S. Roy[1]; [1]University of Maryland, College Park, MD, [2]Laboratory of High-Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD.

Tissue development and homeostasis rely on the ability of stem cells to efficiently balance their fates between self-renewal and differentiation. These stem cell fate decisions are made in the context of the niche and are governed by asymmetric signaling and organization in the niche. Niche cells present self-renewal growth factors selectively only to stem cells, but not to their neighboring non-stem cell daughters, located just one cell diameter away. Understanding how this signaling asymmetry arises is critical to understanding how stem cells determine their identity and prime differentiation in an organized pattern to generate/regenerate tissues. We investigated the basis of asymmetric signaling and cell organization using the Drosophila wing disc that creates an adult muscle progenitor (AMP) niche. We discovered that AMPs extend polarized actin-based filopodia, named cytonemes, to contact the wing-disc epithelial junctions and physically adhere themselves to the niche. AMP cytonemes localize FGF-receptor (FGFR) to selectively adhere to the FGF-producing niche and receive FGFs in a contact-dependent manner. Activation of FGF signaling in AMPs, in turn, reinforces the niche-specific cytoneme polarity/adhesion to maintain niche occupancy. This interdependence between the cause and effect of cytoneme-mediated polarized signaling enables AMPs to sense different FGF-producing niches and self-organize diverse niche/signal-specific patterns. The intrinsic self-regulation of cytoneme polarity by signaling activation is spatiotemporally coordinated by extrinsic patterns of localized expression and restricted presentation of different FGFs in distinct wing-disc niches. Loss of cytoneme adhesion to an FGF-producing niche promotes AMPs to lose niche occupancy and FGF signaling and enables AMPs to occupy a disc-distal position to acquire morphological hallmarks of differentiation. These findings provide new insights into how contact-mediated polarized FGF signaling via cytonemes can play critical roles in generating and maintaining asymmetric signaling and organizations. This work is funded by NIH R35GM124878 to S.Roy.

B662/P2332

Mechanisms and Function of Sibling Cell Size Asymmetry in *Drosophila* Neural Stem Cells

M. K. Delgado, C. Cabernard; University of Washington, Seattle, WA

Asymmetric cell division (ACD) is a widely conserved process which creates diverse cell populations. ACD can be molecular, physical or a combination of the two. Molecular asymmetry generates daughter cells inheriting distinct sets of RNA and proteins while physical asymmetry is manifested in sibling cells differing in their size. Although much is known regarding molecular ACD, we know very little about the mechanisms and function of sibling cell size asymmetry. A useful system to study sibling cell size asymmetry is *Drosophila* neural stem cells (called neuroblasts; NBs). During mitosis, *Drosophila* NBs exhibit a spatiotemporally regulated flow of Non-muscle Myosin II (Myosin, hereafter), producing a large self-renewing NB and a small differentiating Ganglion Mother Cell (GMC). Our lab has identified a poorly characterized serine-threonine kinase, Protein Kinase N (Pkn), that induces the flow of Myosin towards the basal NB cortex in early anaphase. However, the mechanisms by which Pkn initiates this apical to basal Myosin flow and the function of sibling cell size asymmetry are not known.

We found Pkn's kinase domain is required for proper spatiotemporal dynamics of Myosin and without this domain abnormal cell shape changes during ACD occur. To gain a better understanding of the molecular mechanisms underlying sibling cell size asymmetry, we conducted pull-downs on eGFP tagged Pkn *Drosophila* embryos followed by mass spectrometry. Using this approach over 50 potential binding partners were identified. Currently, we are genetically characterizing the top candidates Lethal Giant Larvae and Shaggy to determine their role in Myosin flow and sibling cell size asymmetry.
To understand the function of sibling cell size asymmetry, we altered sibling cell size via a nanobody approach. In *Drosophila* larval brains, we found that permanently trapping Myosin to the apical cortex alters sibling cell size resulting in cases of inverted as well as symmetric ACD. We also determined alterations to sibling cell size affects cell cycle timing. Lastly, our preliminary immunohistochemistry data suggests that changes in sibling cell size asymmetry alter the number and nuclear size of cells postulated to have an NB fate. Future research will be conducted to determine if changes to sibling cell size result in abnormal neurogenesis. Taken together, these findings begin to elucidate the mechanisms and function of sibling cell size asymmetry which may be conserved in other organisms that undergo physical ACD.

B663/P2333

Smurf ubiquitin ligases regulate development processes in embryonic stem cells

J.-H. Shin[1], X. Xu[1], Y. Tang[1], Y. Du[2], C. Liu[2], Y. E. Zhang[1]; [1]National Cancer Institute, NIH, Bethesda, MD, [2]National Heart, Lung, and Blood Institute, NIH, Bethesda, MD

Smad ubiquitination regulatory factors (Smurfs), are HECT domain containing E3 ubiquitin ligases. There are two Smurf genes, Smurf1 and 2 in mammals, and which are originally found to be negative regulators of TGFβ/BMP signaling. Subsequently, Smurfs have been shown to play key roles in many biological processes. Mouse lacking Smurf1 and Smurf2 are embryonic lethal, and more than one third of them displayed gastrulation defects. To understand the role of Smurfs in early embryonic development, we established ESC lines using E3.5 blastocytes with *Smurf2*$^{fl/fl}$ (herein referred to as "*control*" or "*WT*") and *Smurf1*$^{-/-}$*;Smurf2*$^{fl/fl}$ (referred as "*SF1KO*") alleles. Then, the floxed Smurf2 alleles in above ESC lines were removed after treating them with TAT-CRE recombinase to establish ESC lines with *Smurf2*$^{\Delta/\Delta}$ (referred as "*SF2KO*") and *Smurf1*$^{-/-}$*; Smurf2*$^{\Delta/\Delta}$ (referred as "*DKO*") alleles. We found that *Smurf*-deletion had no effect on ESC morphology, alkaline phosphatase staining, or pluripotent marker expression as assessed by real-time quantitative PCR (RT-qPCR) or immunofluorescence. Moreover, control and *Smurf*-deficient ESCs supported teratoma formation to a similar degree in vivo, confirming that Smurf1 and Smurf2 are not required for maintaining pluripotent potential of ESCs. However, when cultured these ESCs during embryoid body (EB) formation, we observed that the size of DKO EBs were considerably smaller than that of control, SF1KO or SF2KO EBs. H&E staining revealed that the DKO EBs were less differentiated than that of control EBs. Further RNAseq and RT-qPCR assays revealed that depletion of both Smurf1 and Smurf2 causes the developmental delay and arrests cells at the gastrulation stage. These observations were also confirmed using monolayer neuronal differentiation assay. Besides higher level of Smad-dependent TGFβ signaling in DKO EBs or differentiated cells, several other developmental signaling pathways were also affected by Smurf DKO. These results indicating that Smurfs regulate developmental process in embryonic stem cells by affecting multiple signaling pathways, including TGFβ signaling.

B664/P2334

Injury induces wild-type cell proliferation to suppress oncogenic Ras cells

S. Gallini, V. Greco; Yale University, New Haven, CT

Healthy skin is a tapestry of wild-type and mutant clones. Although injury can cooperate with Ras mutations to promote tumorigenesis, the consequences in genetically mosaic skin are unknown. Here, we show that wild-type cells suppress oncogenic Ras-induced aberrant growth after injury. Although $Hras^{G12V/+}$ and $Kras^{G12D/+}$ cells outcompete wild-type cells in uninjured, mosaic tissue, their expansion is prevented after injury due to a selective increase in wild-type cell proliferation. Mechanistically, we show that, unlike $Hras^{G12V/+}$ cells, wild-type cells respond to autocrine and paracrine secretion of EGFR ligands and their differential activation of EGFR pathway explains the competitive switch during-injury repair. Inhibition of EGFR by both drug and genetic approaches specifically abolishes the proliferative advantage of wild-type cells after injury leading to the expansion of $Hras^{G12V/+}$ cells. Increased wild-type cell proliferation through a global loss of the cell cycle inhibitor p21 counteracts the expansion of $Hras^{G12V/+}$ cells even without injury. Thus, injury plays an unanticipated role in switching the competitive balance between oncogenic and wild-type cells in genetically mosaic skin.

B665/P2335

Temporal Transcriptome Analysis of Epithelial to Mesenchymal Transition (EMT) in Human iPSCs reveals Greater Variation in Lineage Progression at Time Points Associated with Key EMT Behaviors

N. C. Gomez, . Allen Institute for Cell Science, T. Knijnenburg; The Allen Institute for Cell Science, Seattle, WA

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how cells transition between states during differentiation and disease. EMT is a hallmark cellular state change from non-motile to motile in both normal and pathological conditions. Apart from the switch in cell behavior, it's also characterized by changes in protein expression and cellular organization. To understand the interplay between these aspects, we use large-scale imaging and multimodal quantitative assays on gene-edited hiPSCs from the Allen Cell Collection (allencell.org). Our study is based on the finding that increasing WNT signaling, via a small molecule (GSK-3β inhibitor) directs hiPSCs into a mesodermal fate, marked by consistent colony-wide contraction and cellular migration by 24 and 30 hours, respectively. To correlate transcription with cellular behavior, we collected GSK3-inhibited hiPSCs every three hours over 48 hours and performed scRNA-seq. First, we confirmed that cells cluster by time point using dimensionality reduction of our high-resolution temporal dataset. Next, we reconstructed a continuum of hiPSC differentiation during EMT via pseudotime analysis. We found that time points associated with major cell behavior changes, such as cellular migration at 24 hours, demonstrated greater variation in lineage progression than earlier time points. Using differential expression analysis, we found that groups of genes with distinct expression patterns cluster throughout EMT and are enriched in specific biological processes such as junctional proteins during migration. To relate transcriptional changes with cellular behavior, we are implementing a sparse regression model approach. This will identify one or more core gene signatures that could then be used by multiplexed RNA-FISH to classify and identify cells undergoing behavioral changes such as migration. Applying our transcriptional signature to other models of EMT will allow us to assay the similarities and differences between them. Integrating our large-scale imaging data with transcriptomics will ultimately allow us to

associate gene expression with distinct cellular shape and behavior changes, and in turn reveal the fundamental mechanisms that occur during cell state transitions.

Patterning

B667/P2336

Smart glues: coordinating robust pattern formation and morphogenesis with an adhesion code

M. Ryan, C. Chiu, **T. Tsai**; Developmental Biology, Washington University in St. Louis, St. Louis, MO.

An outstanding question in embryo development is how pattern formation and tissue morphogenesis are coordinated to generate tissues with reproducible pattern and shape. The zebrafish spinal cord is a remarkable example. In just 4 hours, the tissue undergoes dramatic shape changes from a plate to a tube, altering its height and width by 5-fold. At the same time, cells are instructed by opposing spatial gradients of morphogen signals (Shh/BMP/Wnt) to choose among 13 distinct cell fates and form stereotypic stripe patterns. Despite the dramatic changes in tissue shape and pattern, the developmental outcome is highly reproducible. We found a cell-type-specific adhesion code is instrumental to coordinate patterning with morphogenesis during zebrafish spinal cord development. The adhesion specificity for each cell type promotes cohesion among cells of the same type to improve patterning precision. Extending from our published work (Tsai et al, 2020), we have combined single cell transcriptomics with multiplex *in situ* hybridization to identify several additional cadherin-family adhesion molecules with cell-type-specific expression patterns. Our expanded adhesion code significantly increased the orthogonal adhesion modes available for adhesion specificity of all thirteen cell types in the spinal cord. In addition to the roles in patterning, we found coordination among these cadherin genes are critical to ensure robust spinal cord morphogenesis, as combinatorial loss of these cadherin genes result in defective tissue convergence and greater fluctuations in the final tissue shape. We also identified genetic conditions where the whole neural tube tissue failed to maintain cohesion and split into two neural tubes, a previously undocumented phenotype. Our ongoing studies will illuminate deeper cellular mechanisms of how different cadherin genes collaborate to simultaneously ensure robust pattern formation and morphogenesis during zebrafish spinal cord development.

B668/P2337

Sonic Hedgehog Stimulated Endocytic Recycling Drives Cytoneme Loading for Mammalian Tissue Patterning

E. T. Hall[1], E. R. Cleverdon[1], M. E. Dillard[1], Y. Zhang[1], R. Wakefield[2], C. G. Robinson[2], S. M. Pruett-Miller[1], S. S. Ansari[1], Y. Wang[1], D. Stewart[1], A. Johnson[2], M. Tanes[2], Y. Ryu[2], J. Steinberg[2], S. K. Ogden[1]; [1]Cell and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, [2]St. Jude Children's Research Hospital, Memphis, TN.

During development, morphogens like Sonic Hedgehog (SHH) instruct cell fate by inducing distinct transcriptional programs through graded concentrations and durations of signal exposure. Directly visualizing morphogen gradient establishment *in situ* has been impossible, so the molecular mechanisms by which target cells achieve appropriate signal thresholds for tissue patterning remain ambiguous. To tackle this longstanding problem, we developed a mouse model for compromised SHH morphogen dispersion. We mutated an essential Furin cleavage site in the SHH release protein Dispatched (DISP), which attenuates its ability to release ligand from producing cells. DISP mutation led to severe

developmental phenotypes including hydrocephalus, cerebellar dysfunction, and failure to thrive. Cell culture analysis revealed altered membrane localization of DISP mutants. Live imaging and biochemical assays of cultured cells revealed that DISP mutants were unable to undergo effective endocytic recycling with SHH, which attenuated SHH vesicular loading for entry into signaling filopodia called cytonemes. We optimized methods to preserve cytonemes in embryonic tissue for advanced microscopy and discovered that cytonemes containing endogenously expressed SHH connect signal-producing and -responding cell populations in developing neural tubes. SHH is depleted from cytonemes of DISP mutant mice, which corrupts the signaling gradient and compromises progenitor fate specification. Thus, cytonemes are essential conduits for SHH dispersion and may provide similar functionality for other morphogens contributing to mammalian tissue development.

B669/P2338

Analysis of the individual and collective behaviors of signaling filopodia during Notch-mediated patterning

S. Kundu, T. Smith, **G. L. Hunter**; Department of Biology, Clarkson University, Potsdam, NY.

Signaling filopodia, or cytonemes, are long, dynamic cellular protrusions that facilitate cell-cell communication. During the formation of the bristle spot pattern in the Drosophila thorax, actin-rich signaling filopodia extend from the basal surface of all epithelial cells in order to support long-range, Notch-mediated, lateral inhibition. The mechanisms that regulate signaling filopodia formation, as well as how signaling filopodia interact with each other in order to propagate signaling, are not well understood. The objective of this work is to analyze signaling filopodia in individual cells and their interactions across a patterning tissue, using a combination of Drosophila genetics and quantitative microscopy. We have found that an unconventional MyTH4-FERM myosin, Myosin XV, plays a role in regulating the formation of signaling filopodia in individual cells. Myosin XV localizes along the length of these structures, in a manner that is dependent on specific MyTH4-FERM domains. We find that signaling filopodia make complex interactions with each other during patterning. We observe transient collective filopodia structures that move across small regions of the tissue, and use a particle image velocimetry based strategy to analyze their movements. We find that these structures move without alignment to any specific body axis throughout late patterning stages. We are currently working on an ex vivo strategy to block the collective movement of signaling filopodia in order to test if they are required for late stage patterning. Altogether, these results support the role of signaling filopodia in long-range lateral inhibition, and indicate that the range of Notch signaling may not only result from the activity of individual cells, but from their collective behavior

B670/P2339

The expression pattern of *calca* in the zebrafish

T. Liang[1], J. Zhang[2], J. Y. Jang[1], **D. W. Jang**[1], S. Y. Choi[1]; [1]Biomedical Sciences, Chonnam National University Medical School, Hwasun 58128, KOREA, REPUBLIC OF, [2]Neurology, Tangdu Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710038, CHINA.

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide, which has two major forms (α and β). These peptides act on an unusual receptor family, consisting of calcitonin receptor-like receptor (CLR) linked to an essential receptor activity modifying protein (RAMP) that is necessary for full functionality. CGRP is a highly potent vasodilator and possesses protective mechanisms that are

important for cardiovascular system and wound healing. CGRP is primarily released from sensory nerves and thus is implicated in pain pathways. The proven ability of CGRP antagonists to alleviate migraine has been of most interest in terms of drug development. To determine the expression pattern of *calca (calcitonin related peptide α)* in zebrafish, we performed whole-mount in situ hybridization on wild-type zebrafish embryos and found that *calca* is predominantly expressed in the nervous system. Then we cloned a 5-kb *calca* promoter region and generated a *calca* reporter line, *Tg(calca:gfp)*. GFP expression was observed in the central nervous system, lips, nose, barbel, gill and skin. In particular, the expression pattern of skin GFP in *Tg(calca:gfp)* was reminiscent of that of Merkel cells, which are oval receptor cells found in the skin of vertebrates that have synaptic contacts with somatosensory afferents. Currently, we are generating a Ca^{2+} reporter line, *Tg(calca: GCaMP6s-P2A-mCherry)*, *Tg(calca:GFP-NTR)* line based on Nitroreductase/Metronidazole (NTR/MTZ) cell ablation system which will cause the death of the $calca^{+}$ cell when exposed to the prodrug MTZ, and $calca^{-/-}$ line using CRIPSR-Cas9. These lines would help us identify the nature of $calca^{+}$ cells in zebrafish.

B671/P2340

How the gut got its spots: mesenchymal dewetting drives tissue patterning and interfacial folding to build intestinal villi

T. R. Huycke[1], H. Miyazaki[1], T. J. Häkkinen[1], C. S. McGinnis[1], A. Kalantari[1], E. Barruet[1], D. Boffelli[2], O. D. Klein[2], Z. J. Gartner[1]; [1]Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA, [2]Orofacial Sciences, University of California, San Francisco, San Francisco, CA.

Intestinal villi arise during development as a consequence of signals and forces that sculpt an initially flat epithelium into a periodic pattern of folds. The detailed mechanisms driving villus formation are thought to differ between mammals and birds, and have been the subject of considerable debate. Here we identify a biomechanical mechanism essential for villus patterning and folding in both species, but which operates in unique ways and at distinct stages of intestinal morphogenesis in each organism. In mice, we find that the aggregation of subepithelial mesenchymal cells into condensates is sufficient to initiate villus formation by generating the forces that produce curvature of overlying tissue interfaces and specify villus location. Cellular condensation initiates upon fluidization of the subepithelial mesenchyme, a process that is dependent on metalloproteinase activity and characterized by a loss of anisotropy in cell shape and matrix alignment, the acquisition of enhanced actomyosin-dependent motility and neighbor exchange, and the expression of a unique cell-matrix adhesion program. In chick, the same program acts at the late stages of intestinal morphogenesis to sculpt zig-zag patterned ridges into villi. To gain further insight into this unique biomechanical program, we parameterize both cell-based and continuum models of mesenchymal condensation and interface bending using quantitative live imaging data from mouse tissue explants. These models predict multiple and unanticipated aspects of condensate formation, patterning, and dynamics that we validate experimentally. Together, our data suggest that villus initiation is analogous to the behavior of thin liquid films, which de-wet from surrounding interfaces when decreasing their surface-to-volume ratio minimizes the total surface energy of the system. Similar tissue-scale dewetting might be common across developmental systems as a mechanism to organize morphogenesis at tissue interfaces.

B672/P2341

Biomechanical regulation of adhesion-mediated cell patterning

R. M. Garner, S. G. Megason; Systems Biology, Harvard Medical School, Boston, MA.

The self-organization of cells into patterns is essential for proper development. One widespread mechanism that is critical for patterning dozens of embryonic tissues across diverse organisms is adhesion-mediated sorting. In this scheme, distinct adhesion molecules are differentially expressed on the surface of different cell types, driving cells to group together based on compatible adhesion molecule expression (see our recent review on this topic: Tsai, Garner, & Megason, ARCDB, 2022). However, while there is ample evidence that cells *can* sort by adhesion compatibility, almost nothing is known about how cells move through the tissue, find partners with similar adhesion protein expression, and eventually stop moving to lock in the sorted pattern. In particular, we fundamentally lack an integrative understanding of how cell mechanical and migratory properties control the ability of cells to sort. Here, I present my progress towards developing a combined experimental and theoretical approach to address this gap, using developing zebrafish embryos as a model system. First, I will discuss the "in toto" imaging and image analysis pipeline I have established to (1) visualize the dynamics of cells and their interfaces at high resolution, (2) perform automated 3D segmentation and tracking of *all* of the cells within a developing tissue, and (3) quantify cellular rearrangements in the tissue over time. Second, I will show how I utilized this approach to measure how perturbations to adhesions and actin biomechanics affect the ability of cells to move within the tissue and exchange neighbors. Finally, I will present my efforts to develop a physically-realistic computational model of cellular patterning in 3D multicellular systems, which integrates both adhesion-mediated sorting and actin biomechanics.

B673/P2342

Development drives changes in tight junction morphology and myosin II patterning

R. E. Stephenson, A. L. Miller; Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI.

Tight junctions closely adhere epithelial cells to one another, creating a barrier that regulates the passage of ions, water, and macromolecules across the paracellular space. When viewed by fluorescence microscopy, tight junctions typically appear as a relatively straight, narrow band that encircles the apical most planes of each epithelial cell. Change in tight junction appearance (e.g., increased waviness) is frequently observed following alteration to the actin cytoskeleton or protein composition of the tight junction. However, the molecular regulation and functional consequence of changes to tight junction morphology are poorly understood. Previously, we used gastrulating *Xenopus laevis* embryos to study how cell shape change impacts tight junction integrity, and we identified that local breaks in tight junctions are repaired by Rho flares. Rho flares are local accumulations of active RhoA, F-actin, and myosin II that repair tight junction breaks via junction contraction. When analyzed individually, the size and shape of Rho flares varies dramatically. Here we present population-level analysis of Rho flares suggesting that tight junction morphology and myosin II patterning are correlated with changes in Rho flare morphology and frequency, as well as the developmental stage of the embryo. Using a semi-automated image analysis pipeline, we generated a categorization framework of *Xenopus* epithelial tissue dynamics based on analysis of Rho flare characteristics and corresponding changes in myosin II patterning, tight junction morphology, and cell geometry. We speculate that these changes in epithelial tissue properties and junction organization occur in order to meet the challenge of

maintaining epithelial integrity as the embryo transitions from a hollow ball of cells to an elongated form with distinct germ layers. Understanding the relationship between tight junction morphology and local repair events will further our understanding of the functional consequence of changes in tight junction morphology.

Host-pathogen/Host-commensal Interactions 1

B675/P2343

An ancestral mycobacterial effector promotes dissemination of infection

M. I. Sweeney[1], J. W. Saelens[1], G. Viswanathan[1], A. M. Xet-Mull[1], J. Coers[1], J. E. Stout[2,3], D. M. Tobin[1]; [1]Department of Molecular Genetics and Microbiology, Duke University, Durham, NC, [2]Department of Medicine, Duke University, Durham, NC, [3]Department of Infectious Diseases and International Health, Duke University, Durham, NC.

Mycobacterium tuberculosis (*Mtb*), the human-adapted pathogen that causes tuberculosis disease (TB), has evolved to subvert the human immune system and become one of the most successful pathogens in human history. We analyzed a clinical TB outbreak in North Carolina and identified an ancestral secreted *Mtb* effector that induces a dramatic phenotypic change in infected host cells. Clinically, *Mtb* strains that express the full-length effector, EsxM, cause a high rate of disseminated disease. The full-length effector is expressed in ancestral strains of *Mtb* but is truncated by an early stop codon in modern strains. We find that EsxM mediates changes in infected macrophages via the modulation of the host actin cytoskeleton. We first show that expression of full-length EsxM but not the modern truncated version in zebrafish macrophages, a key host cell niche for infecting mycobacteria, induces increased motility and migration. Utilizing a macrophage-specific F-actin reporter line, we identified changes in the macrophage cytoskeleton during migration and infection. Additionally, *M. marinum* zebrafish infection experiments demonstrated that EsxM-expressing *M. marinum* possesses an increased capacity for dissemination from established sites of infection and a tropism for bone infection, consistent with human clinical data from the outbreak. In human cells, we find that infection with EsxM-expressing *Mtb* leads to dramatic filopodial projections, F-actin and Arp2/3 rearrangement, and increased cell migration when compared to infection with *Mtb* strains lacking the effector. Currently, we are following up on the potential host interacting partner of EsxM, a member of the WASH complex. This Arp2/3 nucleation promoting factor has been studied primarily for its role in endocytic trafficking, but less is known about its potential role in macrophage migration, or as a target of intracellular bacteria. We hypothesize that EsxM may be mediating its effects via an interaction with the WASH complex, subverting its normal function in cells. Overall, these studies uncover an evolutionary relationship between a secreted effector and the transition of *Mtb* from a generalist pathogen causing disseminated infection to a specialized inhabitant of the human lung. Furthermore, we identify a potential interaction between mycobacteria and the host cytoskeleton that leads to dysregulation of cell motility and ultimately disseminated infection.

B676/P2344

ISG15 modification of the Arp2/3 complex restricts pathogen spread

Y. Zhang[1], B. Ripley[1], W. Ouyang[2], R. Coloma[3], M. Sturtz[1], E. Upton[1], E. Luhmann[1], N. Schwery[1], S. Anthony[4,5], A. Goeken[4], T. Moninger[6], J. Harty[4], A. Klingelhutz[1], E. Lundberg[2], D. Meyerholz[4], C. Stipp[7], B. Manicassamy[1], S. Guerra[3], L. Radoshevich[1]; [1]Department of Microbiology and Immunology, University of Iowa, Iowa City, IA, [2]Science for Life Laboratory, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, Stockholm, SWEDEN, [3]Department of Preventive Medicine, Public Health and Microbiology, Universidad Autónoma, Madrid, SPAIN, [4]Department of Pathology, University of Iowa, Iowa City, IA, [5]Integrated Research Facility at Fort Detrick, Frederick, MD, [6]Central Microscopy Research Facility, University of Iowa, Iowa City, IA, [7]Department of Biology, University of Iowa, Iowa City, IA.

During infection the ubiquitin-like protein, ISG15, can act as a cytokine or can covalently modify host and pathogenic proteins. While progress has been made in identifying sites of modification on target proteins, the molecular consequences of ISGylation on individual protein substrates are still unknown. Here by using a model of enhanced ISGylation, we identify that ISG15 modifies the ARP2/3 complex following both bacterial and viral infection. ISGylation significantly slows *Listeria*-actin comet tail speed leading to morphologically shorter and denser comet tails. For Vaccinia virus, this results in reduced spread, whereas while *Listeria* is initially restricted, over time the bacteria divide while remaining tightly attached to actin filaments resulting in multi-headed comet tails that move as a group. These structures contribute to spread in both human and murine cells and in vivo in mice with unchecked ISGylation, which leads to increased mortality. ISG15 modification of the Arp2/3 complex also affects cortical actin density, cell motility, and adhesion. Furthermore, ISG15-deficient neonates have aberrant epidermal epithelia, which correlates with observed defects in wound healing in a subset of human patients who lack ISG15. Our discovery identifies a conserved molecular mechanism of ISG15 modification of the Arp2/3 complex which directly restricts pathogen spread.

B677/P2345

The Role of Bacterial Filamentation in the Protection Against Human Cathelicidin Antimicrobial Peptide LL-37

N. Porco, M. Tomlinson, R. J. Botelho, J. B. McPhee; Toronto Metropolitan University, Toronto, ON, CANADA.

Urinary tract infections are one of the most common type of bacterial infection. Uropathogenic E. coli (UPEC) are the primary bacterial species that cause persistent urinary tract infections. Infection begins via colonization of bladder epithelial cells by UPEC forming intracellular bacterial communities. The bacteria inside these intracellular bacterial communities go through morphological changes including filamentation, which prevents them from dividing but allows them to keep growing. Filamentation can be triggered in response to environmental stressors like reactive oxygen species which are produced during the immune response. The immune response against urinary tract infections is complex but, includes recruitment of macrophages and neutrophils and the release of antimicrobial peptides such as LL-37. Since filamentation is a stress response and LL-37 is secreted during infection, we hypothesized that bacterial filamentation may protect bacteria against LL-37. Our data support this hypothesis since filamentation of UPEC strains and non-pathogenic bacteria driven by different mechanisms all promoted bacterial survival in the presence of LL-37. Next, we analysed LL-37 binding patterns in filamentous and

non-filamentous cells to understand why filamentous cells are better protected. We found that LL-37 appears to target regions of significant negative curvature in non-filamentous bacteria, while LL-37 bound more randomly in filamentous bacteria. We postulate that filamentation reduces membrane curvature, which may be required for LL-37 to insert into bacteria membrane to promote killing. Lastly, we also observed that filamentous bacteria are ingested more slowly by macrophages than coccal forms, further aiding in bacterial survival. This work aims to provide insight into how filamentation acts as a crucial virulence strategy for specific microbes like UPEC.

B678/P2346

BMP signaling in regulation of *C. elegans* lipid homeostasis and immune response

K. Yamamoto, C. Savage-Dunn; Queens College & The Graduate Center, CUNY, New York, NY.

Research on innate immunity has focused on aspects that decrease pathogen load, including physical barriers and the upregulation of antimicrobial peptides (AMPs). Less is known, however, about the role of host metabolism in supporting survival independently of anti-bacterial responses. Bone Morphogenetic Proteins (BMPs) are secreted peptide growth factors in the TGF-β family, well known for their roles in development and differentiation, but emerging as homeostasis modulators. The genetically tractable model system *C. elegans* has revealed signaling mechanisms that regulate innate immunity. DBL-1 is the BMP2/4 ortholog in *C. elegans*. Impairment of DBL-1, or components of its signaling pathway, result in increased infection susceptibility and decreased survival after exposure to bacterial pathogens *Serratia marcescens* and *Photorhabdus luminescens*. The BMP pathway also regulates lipid metabolism in *C. elegans*, where changes in DBL-1 signaling result in decreased quantities of lipid stores, and fewer, smaller lipid droplets. Altered lipid metabolism is one potential mechanism for immune tolerance, an alternative response that does not depend on reducing pathogen load. Here we begin to explore the relationship between host metabolism and pathogen tolerance. We hypothesized that DBL-1/BMP regulation of lipid metabolism in *C. elegans* complements its role in the transcriptional regulation of AMP genes and contributes to survival on pathogenic bacteria. We used Oil Red O to conduct lipid staining of animals after exposure to bacterial pathogens *S. marcescens* and *P. luminescens*. In wild type animals, we observed a small, but significant, decrease in lipids. However, in *sma-3* null mutants, exposure to either bacterial pathogen resulted in decreased lipid levels by more than 50%. We conclude that pathogen exposure elicits a lipid mobilization response in the host and that this response is BMP-dependent. To complement our investigation into the effect of lipid metabolism on immune tolerance, we have been more robustly characterizing BMP's regulation of lipid homeostasis. To this end, we conducted a genetic screen to identify novel genes that function downstream of DBL-1 by identifying suppressors of the low-fat phenotype of *dbl-1* using BODIPY lipid stain. We analyzed 8224 genomes and isolated 71 mutants with high-fat phenotypes. Our goal is to define the molecular pathways that act downstream of DBL-1 to regulate lipid metabolism and immune response.

B679/P2347

***Cryptosporidium parvum* highjacks a host lncRNA NR_0033483 to suppress intestinal epithelial cell-autonomous antiparasitic defense**

M. Graham[1], M. Li[2], A. Gong[1], S. Deng[2], K. Jin[1], X. Chen[1]; [1]Microbial Pathogens and Immunity, Rush University, Chicago, IL, [2]Medical Microbiology and Immunology, Creighton University, Omaha, NE.

Cryptosporidium is a zoonotic apicomplexan parasite that infects the gastrointestinal epithelium and other mucosal surfaces in humans. It is an important opportunistic pathogen in AIDs patients and the second most common cause of diarrhea in young children in developing countries. The intestinal epithelial cells provide the first line of defense against *Cryptosporidium* infection and play a central role in activating and regulating the host immune response. Increasing evidence suggests that long noncoding RNAs (lncRNA) participate in host-pathogen interactions and play a regulatory role in the pathogenesis of diseases but the underlying molecular mechanisms are not fully understood. The lab previously identified a panel of host lncRNAs that are upregulated in murine intestinal epithelial cells following *Cryptosporidium* infection, including NR_0033483. We demonstrate here that NR_0033483 is upregulated in response to *Cryptosporidium* infection but is not upregulated in response to the immune signaling pathways tested. Additionally, inhibition of NR_0033483 resulted in a decreased infection burden of the parasite while overexpression of NR_0033483 showed an increase in infection burden, suggesting NR_0033483 is acting in a pro-parasitic manner. Various sequencing methods revealed that NR_0033483 suppressed transcription of host defense genes involved in controlling *Cryptosporidium* infection including *Aebp1*, a potent modulator of inflammation and NF-κβ signaling. Interestingly, *Cryptosporidium* carries the *Cryptosporidium* virus 1 (CSpV1), a dsRNA virus coding two dsRNA fragments, CSpV1-dsRdRp and CSpV1-dsCA. Both CSpV1-dsRdRp and CSpV1-dsCA can be delivered into infected cells. We found that cells with *in vitro* transcribed CSpV1-dsCA or CSpV1-dsRdRp displayed increased levels of NR_0033483, suggesting CSpV1 is involved in the upregulation of NR_0033483 during *Cryptosporidium* infection. Our study highlights a new strategy by *Cryptosporidium* to hijack a host lncRNA to suppress epithelial cell-autonomous antiparasitic defense and allow for a robust infection.

B680/P2348

***Mycobacterium tuberculosis* utilizes serine/threonine kinase PknF to evade NLRP3 inflammasome dependent interleukin-1beta secretion.**

S. Rastogi, V. Briken; Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, College Park, MD.

Mycobacterium tuberculosis (Mtb) has evolved strategies to evade host innate immunity and persist inside host cells. Dendritic cells (DCs) act as a bridge between innate and adaptive immunity and thus are the key regulators of immune response against infectious diseases included tuberculosis. Both macrophages and DCs encode the cytosolic sensor proteins, NOD like receptor (NLRs), which recognizes pathogen/danger associated molecular patterns and lead to assembly of the inflammasome signaling complex that activates caspase-1 and results in release of mature IL-1β and host cell death via pyroptosis. Here we show that Mtb inhibits NLRP3 inflammasome activation and subsequent production of IL-1β in an ESX-1 independent manner. We identified the serine/threonine kinase PknF as one protein of Mtb involved in the NLRP3 inflammasome inhibition, since the pknF deletion mutant of Mtb compared to Mtb-infected cells induces increased production of IL-1β and increased pyroptosis in BMDMs and BMDCs. The increased production of IL-1β was found to be dependent on the NLRP3, the

adaptor protein, ASC, and the proteases caspase-1 and -11, as revealed by studies performed in BMDMs and BMDCs derived from the corresponding knockout mice. In addition to NLRP3, we show that Mtb pknF mutant also mediates RIPK3/Caspase8 dependent IL-1β production in BMDCs. Consistently, infection with the Mtb pknF mutant results in increased activation of caspase-1 and caspase-8 in BMDCs when compared to Mtb. The IL-6 production by Mtb pknF mutant remained unchanged compared to Mtb-infected cells, suggesting that the mutant did not affect the priming step of inflammasome activation. In contrast, the activation step was affected since potassium efflux and calcium influx played a significant role in Mtb pknF mutant induced inflammasome activation in BMDCs. However, in Mtb pknF mutant infected BMDMs, potassium efflux, chloride efflux and the generation of reactive oxygen species played a significant role in inflammasome activation but there was no role for calcium fluxes. In conclusion, we reveal a novel role for Mtb PknF in innate immune evasion through inhibition of the NLRP3 inflammasome.

B681/P2349

Spatio-temporal analysis of LC3-associated responses in macrophages during*Mycobacterium tuberculosis* infection

J. Augenstreich, A. T. Phan, C. N. S. Allan, V. Briken; Cellular Biology and Molecular Genetics, University of Maryland College Park, College Park, MD.

Mycobacterium tuberculosis (Mtb) is the causative agent of the pulmonary disease tuberculosis which is still one of the deadliest infections worldwide. Macrophages are its main niche of replication inside the host, so it is crucial to decipher how Mtb survive and evade the bactericidal responses deployed by these cells. Among them, the autophagy could be triggered and eventually control Mtb survival inside macrophages. Specifically, xenophagy can target bacteria after phagosome rupture or escape of the bacterium. However, others showed that Mtb can inhibit autophagic signaling. These results suggest a highly dynamic interplay between the autophagic responses and Mtb that is still poorly understood. We used live-cells imaging approaches to monitor the dynamics of autophagic responses during Mtb infection. The results showed that a large part of Mtb-containing vacuoles (MCVs) exhibited at least one temporary LC3 recruitment during the acquisition. Indeed, about half of the MCVs ended losing LC3 signal, either by disappearance of the LC3 signal or Mtb egress out of the autophagosome, thus showing an active escape from autophagic responses. In term of dynamics, MCVs either showed an early LC3 recruitment (≤ 20min post phagocytosis) or later, from a time ≥ 1h post phagocytosis, up to 10h post phagocytosis. Single cells analysis of the dynamics of LC3 recruitment in regard of the bacterial burden revealed that timing was not a major factor governing LC3 recruitment. However, the amount of MCVs phagocytosed or already present inside the cells when a new MCV is internalized would rather be the trigger. Finally, the maturation level of MCV positive for LC3 was assessed by measuring the acidification at 16h post-infection. Results showed that LC3 positive MCVs did not acidify and that acidified MCVs didn't show LC3 recruitment. In conclusion, we observed that after internalization Mtb largely triggers an autophagic response that the bacteria can successfully avoid or escape, and if not, inhibit its maturation. So, our results did not show that Mtb was actively being killed by autophagic responses in our cell system.

B682/P2350

Inhibition of IFN-I signaling by *Salmonella enterica* serovar Typhimurium

M. Shuster, D. Banks, Z. Lyu, J. Ling, V. Briken; University of Maryland, College Park, MD.

Interferons (IFNs) play an important role in the host response to pathogens. Interferon signaling results in the expression of hundreds of genes that upregulate, modulate, and inhibit host immunity. While IFN-II (IFNγ) is well established as beneficial to host immunity in bacterial infections, IFN-I (IFNβ) has a more ambivalent role. In the context of *Salmonella enterica* serovar Typhimurium (STm) infection, IFNβ has a dual effect, being both beneficial and detrimental to host immunity. In the current study, we investigated the interaction of STm with respect to IFNβ-mediated signaling.We show that STm inhibits IFNβ signaling in murine macrophages independent of IFNβ dosage. This inhibition is specific to IFNβ signaling as IFNγ signaling is unaffected by STm infection. Additionally, sustained IFNβ signaling restricts STm growth. This restriction is dependent on reactive nitrogen intermediates (RNIs) as *Nos2* deficient, STm-infected bone marrow derived macrophages have significantly higher bacterial burdens with respect to wild type macrophages. Production of RNIs is dependent on IFNβ signaling as antibody-based blockage of signaling results in reduced RNIs. Inhibition of IFNβ signaling by STm is independent of Salmonella pathogenicity island (SPI)-1, SPI-2, and SPI-6 as mutants for these secretion systems show no loss of inhibition with respect to wild type STm.

B683/P2351

Examination of Endogenous CD147 during Enteropathogenic*Escherichia coli*and*Salmonella enterica*Serovar Typhimurium Cell Culture Infections

A. Dhanda, C. Yu, **J. Guttman**; Simon Fraser University, Burnaby, BC, CANADA.

Many metazoan cell biological processes, such as cell migration and endocytosis, are driven by a highly conserved actin cytoskeletal network. However, any insidious access to the cytoskeleton represents an open avenue for disease. Enteropathogenic *Escherichia coli* (EPEC) and *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) are two highly infectious gastrointestinal human pathogens that exploit the actin cytoskeleton as part of their disease processes. To initiate infection, EPEC attaches onto the surface of an effaced host cell and generates a characteristic actin-rich membranous protrusion (termed pedestal) to remain on and move atop the host epithelia. *S.* Typhimurium on the other hand triggers large actin-based membrane ruffles to gain access to the host cytoplasm. Interestingly, several non-actin-associated proteins, such as the prolyl *cis-trans* isomerase cyclophilin A (CypA), make up the repertoire of these complex bacterially-induced actin-rich structures. In an effort to expand on this catalog, we set out to examine the distribution of CD147, a host plasma membrane protein and cognate receptor of CypA, during EPEC and *S.* Typhimurium infections. We show that endogenous CD147 clusters at the basolateral membrane regions of EPEC pedestals, but, unlike its cognate ligand, remains absent from the actin-rich core of the structures. Using EPEC genetic mutants, we show that CD147 clustering depends on the pedestal-forming process. Conversely, siRNA-mediated depletion of CD147 reveals that pedestal formation itself does not require CD147 expression in host cells. Lastly, we show that CD147 is also highly enriched throughout *S.* Typhimurium membrane ruffles. In summary, our findings establish CD147 as another host factor that is enriched at pathogen-induced actin-based structures.

B684/P2352

Salmonella actively regulates TFEB in macrophages in a growth-phase and time-dependent mode

S. Inpanathan, E. Ospina Escobar, Y. Cho, N. Porco, J. B. McPhee, R. J. Botelho; Chemistry & Biology, Toronto Metropolitan University, Toronto, ON, CANADA.

Lysosomes are degradative organelles responsible for degrading and recycling endocytic, phagocytic, and autophagic cargo. The transcription factor EB (TFEB) is a scaling transcription factor that can adapt the expression and activity of genes associated with endosomal, lysosomal, autophagic, and immune-protective functions in response to stresses like starvation and infection. In the immune context, TFEB plays important roles in microbe killing, immune cell migration, antigen presentation, and pro-inflammatory transcriptional programs. In fact, we and others previously showed that phagocytosis of bacteria activates TFEB and boosts degradative capacity of macrophages to enhance bacterial killing and that certain microbes like *Salmonella* and *S. aureus* suppress TFEB. However, *Salmonella* has also been suggested to actively stimulate TFEB, which implies a benefit to *Salmonella*. These results appear contradictory at first sight. Thus, we sought to elucidate the complex interplay between *Salmonella* infection and TFEB activity by examining if Salmonella had different effects on TFEB under different contexts such as bacterial growth phase and time of infection. To test this, we compared *Salmonella* grown to late-log versus stationary phases. Late-log Salmonella are invasive and promote their own uptake, while stationary Salmonella are thought to be engulfed by macrophages primarily. In fact, our observations suggest that the effect of Salmonella depends on infection conditions. While late-log grown *Salmonella* caused immediate activation of TFEB in macrophages, phagocytosis of stationary-grown *Salmonella* did not stimulate TFEB after engulfment within the first hour of infection. This was time dependent though and eventually TFEB entered the nucleus of macrophages. Importantly, this early repression of TFEB during phagocytosis of stationary-grown *Salmonella* is driven by the bacteria since i) dead *Salmonella* could not repress TFEB in the first hour of engulfment and ii) we discovered several mutants of *Salmonella* that could not repress TFEB, including mutants of the Type III Secretion Systems required for infection, PhoPQ system, required for bacterial stress adaptation, and other factors that allow *Salmonella* to usurp macrophages. Overall, these observations suggest that *Salmonella* may be able to finetune TFEB activation for its benefit in a manner dependent on the infection conditions. In agreement with this, forced activation of TFEB with phagocytosis, which bypasses the ability of the Salmonella to control TFEB, caused macrophages to kill Salmonella more effectively. This suggests that drugs that can promote TFEB and bypass Salmonella's ability to modulate this protein may have therapeutic value to help resolve infections.

B685/P2353

A siRNA screen to uncover the mechanisms underlying plasma membrane repair of mammalian cells damaged by bacterial pore-forming toxins

M. Prislusky, J. Lam, M. Ng, S. Seveau; Ohio State University, Columbus, OH.

Plasma membrane disruption by pore-forming toxins is a most common and ancient strategy used by bacterial pathogens to infect their host and evade the host immune responses. *L. monocytogenes* is a facultative intracellular pathogen that infects a large variety of host cells including epithelial cells and macrophages. *L. monocytogenes* major virulence factor is a 57 kDa pore-forming toxin called listeriolysin O (LLO) that binds cholesterol-rich membranes to assemble a large transmembrane pore complex. LLO targets the host cell plasma membrane and the phagosome membrane to allow for bacterial

proliferation in the host cell cytosol. Despite LLO attack, the infected cells repair their plasma membrane to maintain homeostasis. To uncover the plasma membrane repair machineries of cells exposed to LLO, we developed a fluorescence-based siRNA screen. The selected library targets 287 host genes controlling endocytosis, exocytosis, intracellular trafficking, autophagy and cytoskeletal assemblies. HeLa cells stably expressing Histone-2B-GFP were reverse transfected with siRNAs (three 11-mer siRNAs per target were pooled in four wells) in 96-well plates for 72 hours and were exposed to 0.5 nM LLO and 1 µM TO-PRO-3 (a small dye that only labeled damaged cells) in Ca^{2+}-containing (repair permissive conditions) and Ca^{2+}-free (repair restrictive conditions). The fluorescence intensities of TO-PRO-3 and GFP were then recorded for 30 min at 37ºC in a multimode plate reader. TO-PRO-3 fluorescence intensity was used to assess plasma membrane integrity and GFP fluorescence to enumerate cells via image cytometry. The dual GFP/TOPRO-3 fluorescence recording allowed to account for potential differences in the proliferation of siRNA-transfected HeLa cells. Although some siRNAs affect final cell density, none of the siRNA treatment conditions exhibited cell detachment after exposure to LLO in the Ca2+-containing and in the Ca2+-free media. The average cell count ratio, defined as the cell count post-kinetic relative to the cell count pre-kinetic, across all siRNA treatment conditions was 1.0061 ± 0.0065 and 1.0059 ± 0.0058 for Ca2+-containing and Ca2+-free conditions, respectively. The Z-factor and SSMD (strictly standardized mean difference) confirmed that this assay has the robustness necessary to identify potential “hits”. In addition, the mixed effects model was used as a statistical tool to evaluate “hits” within a single assay. In conclusion, this screen identified 70/287 targets, that when knocked down, altered membrane resealing efficiency. We identified genes encoding proteins controlling lysosomal biogenesis, membrane fusion, intracellular trafficking, exocytosis, and autophagy.

B686/P2354

The bacterial pathogen *Shigella flexneri* co-opts the human exocyst complex to promote cell-to-cell spread

K. Ireton, T. U. B. Herath, A. Gianfelice, N. Lee; Microbiology and Immunology, University of Otago, Dunedin, NEW ZEALAND.

Shigella flexneri is a gram-negative bacterial pathogen that causes dysentery. Critical for disease is the ability of *Shigella* to use an actin-based motility (ABM) process to spread between cells of the colonic epithelium. ABM transports bacteria to the periphery of host cells, allowing the formation of plasma membrane protrusions that mediate spread to adjacent cells. How these protrusions form is not fully understood. The objective of this study was to test the hypothesis that that efficient protrusion formation and cell-to-cell spread of *Shigella* involves bacterial stimulation of the host process of polarized exocytosis through exploitation of the human exocyst complex. Using an exocytic probe, we found that exocytosis is locally upregulated in bacterial protrusions in a manner that depends on the exocyst. In addition, the exocyst component Exo70 and the exocyst regulator RalA were recruited to *Shigella* protrusions, suggesting that bacteria manipulate exocyst function. Importantly, RNA interference (RNAi)-mediated depletion of exocyst proteins or RalA reduced the frequency of *Shigella* protrusion formation and also the lengths of protrusions, demonstrating that the exocyst controls both the initiation and elongation of protrusions. RNAi-induced knockdown of exocyst components or RalA also impaired cell-to-cell spread of bacteria. The ability of *Shigella* to infect human cells requires a bacterial type III secretion system (T3SS), which injects ~ 25 microbial effector proteins into the host cytosol. Using mutants defective in genes encoding structural components of the T3SS, we found that this secretion apparatus is needed for bacterial-induced recruitment of Exo70 and stimulation of

exocytosis in protrusions. In addition, we have screened a collection of *Shigella* effector mutant strains, and identified at least two effector proteins (IcsB and IpgD) that contribute to stimulation of host exocytosis. Collectively, our results reveal that *Shigella* uses its T3SS to co-opt the exocyst, thereby enhancing dissemination in host cell monolayers. Future work should focus on elucidating molecular mechanisms by which particular *Shigella* effector proteins manipulate exocyst function.

B687/P2355

Tracking Extracellular Vesicles (EVs) and their sRNA Cargoes in plant

D. Adekanye[1], T. Chaya[1], P. Snee[2], M. Batish[1], J. Caplan[1]; [1]University of Delaware, Newark, DE, [2]University of Illinois at Chicago, Chicago, IL

Eukaryotic animal cells secrete three types of extracellular vesicles (EVs), each with a definite mechanism of biogenesis- apoptotic bodies, microvesicles, and exosomes. Exosomes are employed by the cell to mediate intercellular communication by shuttling mRNAs and various species of small noncoding RNAs between cells. The shuttled materials remain functional after delivery and can elicit their functions on the recipient cell. The function of EVs in plants has only recently been studied, with a major focus on plant-pathogen interactions. Even though there is evidence of the lipids, protein and RNAs transport between the plant and the fungal cell, it is still unclear as to how these materials cross the plasma membrane and cell wall of both species. Plant EVs have been implicated to be the shuttle in this interkingdom communication. Using quantum dots (QD) based molecular beacon (MB) targeted at sRNAs in EVs, we aim to determine if EVs mediate the cross kingdom communication between plants and fungi using *Sorghum bicolor* and the *Colletotrichum sublineola* as the plant pathosystem interaction, by tracking the movement of sorghum EVs into the fungal cells. Our MB uses click chemistry to attach an oligonucleotide with complementary sequence to a sRNA to a QD. A short oligonucleotide containing a black hole quencher uses Förster resonance energy transfer (FRET) to quench the QD. When the sRNA binds, the oligo with the black hole quencher is released, resulting in an increase in fluorescence. Our QD-based MB detected target sRNA in-vitro and we are now using cell penetrating peptides to load QDs into the EVs isolated from sorghum to track sorghum EV uptake into *C. sublineola*. We have developed a functional molecular beacon that can detect target sequence in-vitro, the MB has been characterized using transmission electron microscope(TEM), Quantum dots electrophoretic mobility shift assay(QEMSA), High performance liquid chromatography(HPLC). We have imaging evidence that suggests efficient loading of EVs with quantum using cell penetrating peptides. We have evidence of sorghum EVs taken up by isolated *C.sublineola* spores.

B688/P2356

Unexpected Roles of Toll-like Receptor Adaptors TRIF and Mal/ MyD88 in Microglia Response to Neurotropic Murine BetaCoronaviruses

E. Davis, S. Navas-Martin; Drexel University, Philadelphia, PA

Background: The COVID-19 pandemic highlights the impact of betacoronaviruses (β-CoVs) in human health. Endemic CoVs, as well as the highly pathogenic β-CoVs (SARS-, MERS-, and SARS-CoV-2) cause acute, mild to severe, and fatal respiratory tract illnesses. SARS-CoV-2, however, is associated with frequent neurologic symptoms despite unclear neuroinvasive properties. Murine β-CoVs MHV-JHM and -A59 strains are models of acute encephalitis and chronic demyelination. Microglia, the resident innate immune cell of the central nervous system (CNS), function in immunosurveillance and initiating immune

responses upon infection. Their role in β-CoV neuropathogenesis, however, is poorly understood. While microglia are required for protection against lethal murine CoV encephalitis, they do not restrict SARS-CoV-2 infection in the K18-human ACE2 transgenic mouse model. Importantly, microglia contribute to neuroinflammation in CoV-infected mice. The innate immune sensors that determine how microglia influence host protection and disease progression in response to β-CoV infections remain ill-defined. Here, we assess the role of Toll-like Receptor (TLR) adaptor proteins TIR-domain-containing adapter-inducing interferon-β (TRIF) and Myeloid differentiation primary response 88 (MYD88) in antiviral and inflammatory response following infection with MHV-A59 and -JHM in microglia.
Methods: Murine-derived immunocompetent and TLR-adaptor deficient microglia were challenged with MHV strains A59 and JHM. Constituents of viral replication were examined via immunoblot, plaque assay, and immunofluorescence. Additionally, proteomic secretory profiles were assessed to determine pro-inflammatory cytokine responses.
Results: Surprisingly, CoV replication was drastically reduced in TLR - adaptor deficient microglia. This reduction was most prominent in cells lacking MyD88, with viral titers undetectable upon MHV-JHM challenge. This inhibition correlated with significantly reduced double-stranded RNA intermediates and viral nucleocapsid protein expression. Loss of either adaptor increased the expression of cytosolic dsRNA sensor, MDA-5. Moreover, TRIF was shown to negatively regulate several pro-inflammatory molecules, such as IL-6 and MIP-1β during β-CoV infection. These results highlight several novel, unexpected roles of TLR adaptors as negative regulators of microglia antiviral responses during β-CoV-infection.

B689/P2357
Identification of novel phosphoinositide binding *Legionella pneumophila* effector proteins
A. Bolt, M. Neunuebel; University of Delaware, Newark, DE

Phosphoinositide (PIP) lipids serve as molecular beacons directing localization and function as key regulators coordinating many eukaryotic cellular processes. Intracellular bacterial pathogens target PIPs to promote their survival within the host cell. The human pathogen, *Legionella pneumophila,* infects and proliferates within human lung macrophages. The bacterium employs an arsenal of over 300 effector proteins which aid the bacterium in resisting degradation by establishing a replication permissive compartment. Previous studies revealed a growing number of *L. pneumophila* effectors target host PIPs to subvert host vesicular trafficking. However, the full repertoire of PIP-binding *L. pneumophila* effectors is unknown. Here, we performed a screen of 241 effectors with PIP-coated agarose beads. We employed an *E. coli* expression library of His-tagged effectors and optimized effector expression levels using MagicMedia™. Additionally, we tested various additives during cell lysis to improve their stability. Our screen identified previously known PIP-binding effectors and an additional 94 candidates. We then analyzed a subset of effectors in transiently transfected mammalian cells with PIP biosensors and thus far have found that over 10 candidates colocalize with PIP biosensors. These results indicate *L. pneumophila* harbors more PIP-binding effectors, providing an opportunity to better understand how this bacterium manipulates host membrane compartments to establish a replicative niche.

B690/P2358

Characterizing*L. pneumophila*effector Lem8 and its interaction with host cytoskeletal regulators

S. Hackenburg, M. Neunuebel; University of Delaware, Newark, DE

Cytoskeletal rearrangement plays a pivotal role in cell proliferation, migration, and phagocytosis. Given its critical function in coordinating eukaryotic cellular processes, the cytoskeleton and its regulators are often key targets of bacterial pathogens seeking to establish a replicative niche. A recent study found that *Legionella pneumophila* effector Lem8 (Lpg1290), a predicted cysteine protease, interacts with 14-3-3 isoform zeta, a master regulator in cytoskeletal arrangement and cell proliferation. Here, we confirmed this interaction, and we reveal that Lem8 also interacts with host Rho GTPases. Furthermore, we show HaloTag-Lem8 binds lipids *in vitro* and colocalizes with autophagosomes. Remarkably, fluorescence microscopy of RAW264.7 cell infected with *L. pneumophila* expressing HaloTag-Lem8 efficiently detected translocated effector Lem8 early during bacterial uptake. These findings suggest Lem8 acts during the early stages of vacuole formation and biogenesis.

B691/P2359

Delineating the Molecular Basis of Acidic pH and Antibiotic Tolerance in Uropathogenic *Escherichia coli*

F. Guice[1], M. Wiebe[2], T. Bermudez[2], M. Hadjifrangiskou[2]; [1]Biology, Prairie View A&M University, Prairie View, TX, [2]Vanderbilt University, Nashville, TN.

One of the most common bacterial infections in humans is urinary tract infections (UTIs) which affect over 150 million people worldwide every year. Over 75% of UTIs are caused by uropathogenic E. coli (UPEC). UPEC's ability to withstand various environmental stresses is required for its successful pathogenesis. Our lab is interested in two stresses specifically: antibiotic and pH stress. Due to increasing rates of drug resistance toward first-line antibiotics, use of the last-line antibiotic fosfomycin is on the rise. To investigate antibiotic resistance pathways towards fosfomycin, our lab has isolated nonsusceptible subpopulations of UPEC clinical isolates. I have evaluated the relative increase of resistance these subpopulations display and monitored relevant growth defects previously associated with fosfomycin resistance. This will allow for the prioritization of sequencing efforts for possibly uncharacterized routes to fosfomycin resistance. With my efforts I have identified 6 out of 18 NICs that demonstrate atypical growth in nutrient-deficient media, suggesting they contain mutations in genes previously unassociated with fosfomycin resistance. To investigate how UPEC combats pH stress, our lab has generated various strains of UPEC with mutations in acid resistance mechanisms. I have begun to characterize the growth of these mutant strains under normal laboratory conditions and plan to evaluate their growth in conditions that replicate the bladder environment. My work will generate a better understanding of how UPEC adapts to stress encountered in the host caused by last-line antibiotics or acidic conditions.

B692/P2360

The intracellular bacterial pathogen, *Brucella* targets host deubiquitin protease through the effector protein, TcpB for facilitating infection of macrophages.

K. Joshi, V. Muzumdar, G. Radhakrishnan; National Institute of Animal Biotechnology, Hyderabad, India

Brucella species are Gram negative intracellular bacterial pathogens that cause the world-wide zoonotic disease, brucellosis. *Brucella* can infect a wide range of mammals, including humans and domestic and wild animals. *Brucella* manipulates various cellular processes to successfully invade and multiply in the professional and non-professional phagocytic cells. However, the host targets and the mechanism behind their modulation remain obscure. Here, we report that *Brucella* downregulates the expression of a host ubiquitin peptidase at its early stage of infection that results in an enhanced uptake of *Brucella* by macrophages through the membrane receptor. Upon silencing and chemical inhibition of ubiquitin peptidase, the membrane localization of the receptor was enriched, which augmented invasion of *Brucella* into macrophages. Further, we found that only live *Brucell*a was capable of suppressing the expression of ubiquitin peptidase suggesting the role of secreted effector proteins of *Brucella* in modulating the gene expression. Subsequent studies revealed that the *Brucella* effector protein, TIR-domain containing protein from *Brucella* (TcpB) plays a major role in downregulating the expression of ubiquitin peptidase by targeting its transcriptional regulators. The TcpB knock-out *Brucella* was unable to modulate the expression of ubiquitin peptidase and this defect was restored when the TcpB expression plasmid was introduced into TcpB knock-out *Brucella*. Besides regulating the invasion of *Brucella*, the host ubiquitin peptidase enhanced the expression of late endosomal markers, suggesting its role in promoting lysosomal killing. Our *in vitro* and *in vivo* studies showed an enhanced multiplication of *Brucella* in the presence of an inhibitor of the ubiquitin peptidase. The present research uncovered a mechanism by which *Brucella* imparts suppression of host ubiquitin peptidase for its enhanced invasion and intracellular survival. Further, our experimental data demonstrate a novel role of ubiquitin peptidase in the host defense against the microbial intrusion. The present study provides insights into the microbial subversion of host defenses and this information may ultimately help to develop novel therapeutic intervention for brucellosis.

B693/P2361

Crippled Coronavirus: 5’-PolyU Targeted Oligo Inhibits Development of Infectious Virions

D. Davis, H. Ullah; Howard University, Washington, DC

We are currently facing a global pandemic caused by a strain of virus from the Coronaviridae family, coronavirus disease-2019 (COVID-19). The additional variants have prolonged the effects of the pandemic by enhancing their ability to evade their host immune systems and decreasing the effectiveness of current pharmaceutical treatments. Identification of viral targets that are indispensable for the virus can be targeted to inhibit mutation-based new escape variant development. The 5’-polyU tract of the antigenome offers such a target. Host cells do not harbor 5’-polyU tracts on any of their transcripts, making the tract an attractive, virus-specific target. Inhibiting the 5’-polyU can limit the use of the tract as template to generate 3’ polyA tails of +RNAs of coronaviruses. To counter host evading property, we reported that by targeting the unique 5’ polyU tag on the mouse coronavirus (MHV) anigenome (negative RNA strand) with a complementary oligonucleotide, the virus replication could be prevented (patent pending). As the tag is indispensable for virus replication, it is likely that the virus would not be able to develop escape variants by mutating the tag. This proof-of-concept result shows a

unique mode of action against mouse coronavirus without affecting host cells and can be used for the development of novel classes of drugs that inhibit coronavirus infection in host cells, specifically by the COVID-19-causing virus SARS-CoV-2.

B694/P2362

Effects of Decompression Using Drain on Bone Healing of S. aureus Infected Osteomyelitis Mandibular in Rat

S. Kim, B. Sodnom-Ish, K. Mustakim, M. Eo, Y. Cho; Seoul National University, Seoul, Korea, Republic of

Osteomyelitis (OM) of the jaw is usually caused by odontogenic infection or by a variety of other reasons which result in jaw bone destruction. Microorganisms such as Staphylococcus aureus (S. aureus) are known to be significant pathogens at the pathogenesis of OM. Decompression using a drain is a reliable technique, which releases the intraluminal pressure causing reduction of cyst and allows gradual bone regeneration from the periphery. The aim of this study was to analyze the effectiveness of decompression in an OM rat mandible model. A 4 mm diameter defect was made on the mandibles of Sprague-Dawley rats (8 weeks old) and 20 µl of $1x10^7$ CFU/ml S. aureus was inoculated. Two weeks later, the animals were randomly divided into non-treatment control (C1), curettage only control (C2), curettage and decompression using drain (E1), and curettage and decompression using drain with normal saline irrigation (E2) groups. After four weeks, the animals were assessed with micro-computed tomography (micro-CT), histology stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT), and immunohistochemistry (IHC) stained with inflammation-related IL-6 and TNF-α, angiogenesis-related VEGF-A and TGF-β1, and osteogenesis-related OPN and ALP antibodies, followed by statistical analysis. S. aureus infected osteomyelitis model was successfully created in a rat mandible. Most parameters showed significantly lower bone healing in the C1 and C2 groups in micro-CT analysis. Especially in the E2 group, bone mineral density with bone volume was significantly enhanced compared to that seen in the C1 or C2 groups, and bone volume/volume of interest parameter was significantly higher ($p < 0.05$) in the E2 group. In the histological analysis, the E1 and E2 groups showed the most prominent bone healing with a significantly high number of osteocytes found in the defect area ($p < 0.05$). In IHC staining, the E2 group had the weakest expression of IL-6 compared to that of the C1 group. The TNF-α antibody was stained strongly in the E1 group compared to that of other groups. The expression of VEGF-A was the highest in the C1 group compared to the E2 group. The TGF-β1, ALP, and OPN expressions were markedly high in the E1 group, while the C1 group had no TGF-β1, ALP, and OPN expression compared to that of the other groups. The micro-CT, H&E, and MT stained histological analysis, and IHC analyses showed that decompression drains exhibited superior bone healing results compared with that of conventional surgical treatment alone in an OM rat mandible model. Based on these results, it is recommended that clinicians use decompression with drainage and irrigation to treat jaw OM.

Fungi

B695/P2363

Genome analysis of deadly poisonous mushroom *Amanita virosa* using nanopore sequencing technology

K. Soga, S. Yoshiba, J. Narushima, N. Shibata, K. Kondo; National Institute of Health Sciences, Kawasaki, Kanagawa, JAPAN.

The fungus *Amanita virosa (A. virosa),* commonly known as destroying angel and originated in Europe, is one of the deadly poisonous mushrooms in the genus *Amanita*. The mushroom is thought to have genes producing amatoxins and phallotoxins. However, so far the detailed genomic information has not been revealed, and other toxins remain unclear. The reasons are due to (1) the diversity and the difficulty of culturing wild mushrooms in a laboratory; (2) few reference sequences and information concerning related species. In this study, the high-quality genome of *A. virosa* was assembled using nanopore-based long-read sequencing technology and genes associated with poisonings were predicted. *A. virosa* fruit bodies were collected from the fields in Hokkaido. A cap of fruit body was ground in liquid nitrogen. High molecular weight genomic DNA was extracted by the modified CTAB method and purified using a short read eliminator Kit (Circulomics). The DNA library was prepared using Ligation Sequencing Kit. DNA sequencing was performed using the PromethION, nanopore sequencer (Oxford Nanopore Technologies). Obtained raw sequence data (FAST5 format) were base-called using Guppy basecaller v5. The reads including more than 200×deep sequencing data were *de novo* assembled using NECAT v0.0.1, a long read assembler. The assembly was polished using Racon v1.4.20, Medaka v1.5.0, and hypo v1.0.3. The completeness of the assembled genome was evaluated using BUSCO v5.3.1 (fungi_odb10). Homology-based and RNA-seq-assisted gene prediction and annotation were performed using RepeatModeler v2.0.3, RepeatMasker v4.1.2, BRAKER2 v2.16, GeMoMa v1.8, and EnTAP v0.10.8. The resulting assembly of *A.virosa* yielded 69.3 Mb comprising 61 contigs. The N50 and N90 lengths were 2.6 and 0.6Mb, respectively. A total of 97.8% of the 1698 BUSCO genes were detected in the genome, indicating the completeness of the assembly. More than half of the total sequence was occupied by retroelements. *A. virosa* contained more than s 10,000 genes, many of which were DNA replication and repair-related genes. Our data could provide the first report of the draft genome of *A. virosa* and serve as a reference for poisonous mushrooms. We plan to use this genome to search for novel toxic genes.

B696/P2364

Exploring the unconventional cell biology of multi budding yeast, *Aureobasidium pullulans*

C. Petrucco[1], O. Gorman[2], D. J. Lew[2]; [1]Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, [2]Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.

Cells of the same type often display characteristic sizes, shapes, and organelle compositions. Budding yeast (*Saccharomyces cerevisiae*) have sophisticated organelle segregation strategies to ensure that new buds receive appropriate complements of organelles like nuclei, mitochondria, vacuoles and peroxisomes. They also have checkpoints and compensatory pathways so that errors in organelle segregation can be corrected. Unlike *Saccharomyces cerevisiae*, *Candida albicans*, *Cryptococcus neoformans*, *Ustilago maydis*, and other well-studied yeasts, *Aureobasidium pullulans* mother cells can be multinucleate and produce multiple buds within a single division cycle. Initial observations suggest that buds made from a single mother in the same cell cycle have comparable growth rates and final

volumes, but it is not yet clear whether they have uniform or variable complements of different organelles. We are interested in asking whether all sister-buds are endowed with comparable complements of each organelle (which would require precise and potentially novel inheritance mechanisms), or whether sloppy inheritance pathways produce a heterogeneous population (which may reveal how cells deal with unusual organelle compositions). To investigate this interesting cell biology, we are developing a molecular genetics toolbox, including efficient transformation and fluorescent probes for intracellular structures of interest in order to make *A. pullulans* a tractable system for basic cell biology research. We found that most buds inherit exactly one nucleus regardless of the number of nuclei in the mother or the number of sibling buds. However, occasional buds received two nuclei, so that comparably-sized sibling cells could differ two-fold in DNA content. These findings suggest that *A. pullulans* mother cells have a mechanism to ensure that each bud gets at least one nucleus, and that daughter cells can thrive despite large differences in the DNA-to-cytoplasm ratio.

B697/P2365

Characterization of an ORFan and Putative Nitrilase from *Saccharomyces cerevisiae*

E. Koyama, A. Nguyen, S. S. Tadesse, S. Walsh; Life Sciences, Soka University of America, Aliso Viejo, CA.

While *Saccharomyces cerevisiae* is a well-studied organism with its genome fully sequenced, there are still over 100 uncharacterized open reading frames, ORFans, with no significant sequence homology to other genes. As ORFan roles cannot be determined solely through sequence homology, their functions remain a mystery until both in vitro and in vivo biochemical and cell biology experiments are performed. In collaboration with the Yeast ORFan Gene Project, we used bioinformatics modules to initially characterize one such ORFan, *YIL165C*. Together with the gene immediately preceding it, *YIL164C* (*NIT1*), these two genes have significant homology to the nitrilase superfamily of proteins, but have low sequence homology to the other two *S. cerevisiae* nitrilases *NIT2* and *NIT3*. We experimentally verified that lab strains of *S. cerevisiae* have a bonafide stop codon breaking up the two proteins in clones from both genomic DNA and mRNA, but the brewing yeast strain WLP001 (White Labs) and other related yeast strains have a SNP (A>G), causing these two proteins to be expressed as a single, continuous polypeptide chain. The YIL164C protein alone contains both the conserved catalytic triad and a region that shares homology with prokaryotic nitrilases that specifically target aromatic, rather than aliphatic, nitrile substrates. To understand the function of both the full-length YIL164C/YIL165C and the individual YIL164C protein, we expressed the proteins in bacteria and began enzymatic assays to understand their substrate preference. We hope to identify distinctions in activity, or the lack thereof, between the longer and the truncated proteins. In addition, we generated yeast which either overexpress the full-length YIL164C/YIL165C or the truncated YIL164C protein or knockout both genes at once. Neither the overexpression or knockout strains have an obvious phenotype distinct from wild-type laboratory strains, particularly in the realm of mitophagy, where knockout of *YIL165C* alone was previously reported to have a strain-specific mitophagy defect. Taken altogether, understanding these enzymes will provide clues to the evolutionary relationships among genes in this superfamily.

B698/P2366

Crosstalk between titanization in the*Cryptotococcus neoformans/gattii*complex and macrophages

Y. Loperena-Alvarez[1], R. J. Colón-Reyes[2], L. Kozobowski[2]; [1]Biology, Pontifical Catholic University of Puerto Rico, Mayaguez, PR, [2]Eukaryotic Pathogens Innovation Center, Clemson University, Clemson, SC.

Cryptococcosis is a fun`gal infection of the lungs and brain that affects both immunocompromised and immune-competent individuals, caused by *Cryptococcus neoformans and Cryptococcus gattii complex* (*C.neo/gat*). The capsule is primarily responsible for the phagocytosis evasion. *C. neo/gat* has the ability to adapt and produce an enlarge capsule called titan cells, a mechanism that limits the attack of macrophages. In the host, it has been observed that TC were engaged, but not engulfed by phagocytic cells such as macrophages, showing a new strategy of the yeast to evade the immune system. Proliferation of cryptococcosis have been characterize by the high levels of M2 macrophages in its chronic stage.The purpose of this research is to better understand the mechanisms that drive the pathogen/host interaction between *C.neo/gat* complex with macrophages. J774 monocytes were allowed to grow for 24 and 48 hrs on DMEM without FBS, following by collection of the media. The media was used to determine which cytokines are present and to treat *C. neoformans* H99 and *C. gattii* 265 for 24hr to determine if the secreted product of macrophages induce titanization. After treatment, it was observed titanization in both strains, being in a higher quantity and size the ones of *C. gattii*. Expression of key genes related to titanization such as cAMP and GPR-5 was significantly affected in the strains, without affecting RIM101. In another set of experiments, J744 cells were treated with regular morphotypes, mixed morphotypes and the titan morphotype in order study the effect of *C.neo/gat* in the phenotype switching. After 24h of treatment, it was observed a population of M2b with both mixed and titan C. neo/gat morphotype, while the regular morphotypes showed M1 phenotype of macrophages. Our data suggest that macrophages secreted products induce titanization in *C. neo/gat* using two different signaling cascades. Additionally, our data indicates that the titan cells have the capacity of switch the phenotype of macrophages to and antiinflammatory one, explaining the phagocytosis evasion of the yeast.

Acknowledgement: PR-INBRE IDeA grant number P20 GM103475-15 under NIH, Eukaryotic Pathogen Innovation Center at Clemson University under COBRE-P20GM109094, Pontifical Catholic University of Puerto Rico-Mayagüez Campus,

B699/P2367

The fungal pathogen *Cryptococcus* actively manipulates maturation of its phagosome in an immune signal-dependent way.

P. Stuckey[1], E. Santiago-Burgos[2], F. Santiago-Tirado[1]; [1]University of Notre Dame, Notre Dame, IN, [2]Imanis Life Sciences, LLC, Rochester, MN.

Cryptococcus neoformans is a common fungal pathogen responsible for nearly 200,000 deaths yearly. This ubiquitously present yeast affects a diverse group of immunocompromised individuals, which left untreated is invariably fatal. Once *C. neoformans* particles are inhaled and make their way into the lungs they encounter alveolar macrophages. Interactions between these host cells and the fungi will influence whether the infection is cleared or disseminates to the central nervous system. Since *C. neoformans* can survive and replicate inside host macrophages, they represent a safe haven, hidden from the immune response of the body, as well as a vehicle to move throughout the organism, even allowing movement across the blood-brain barrier. The mechanisms *C. neoformans* use to mediate this intracellular

parasitism are not fully understood. Even less is known about how immune signals impact this replicative niche. Therefore, we aim to gain a better understanding of the macrophage-cryptococcal interactions and decipher the properties of the cryptococcal-containing phagosome (CCP), which we believe may be key to determining infection outcome.
Using high-resolution microscopy, we have identified a population of CCPs that display both early endosomal lipid (PI3P) and lysosomal protein (LAMP1) characteristics, a combination that is not normally observed. Using a pH-sensitive dye, we have identified three populations of CCPs with different behaviors: one that gains acidification after phagocytosis but subsequently loses it; some that acidify and remain acidic; and some that never acidify. This is in contrast to phagosomes containing the non-pathogenic yeast *S. cerevisiae*, which rapidly acidify and stay acidic. This suggests that *C. neoformans* can alter its phagosome, explaining the confounding literature regarding the intracellular niche of *C. neoformans* and provide a potential mechanism for intracellular survival that may be driving cryptococcal pathogenesis.
We have demonstrated a delay in acquisition of lysosomal markers to CCPs in comparison to *S. cerevisiae* and latex bead controls. However, when macrophages are stimulated with interferon-γ, acquisition of lysosomal markers occurs more rapidly. Host Rab20 appears to be critical for this promotion of phago-lysosomal fusion and may be key in influencing the infection outcome. Rab20 has been shown to be interferon-γ inducible and promote phagosome fusion and maturation, thereby linking host cell immune status and phagosomal properties during cryptococcal infection.
Our results suggest that *Cryptococcus* actively manipulates the phagosomal environment to avoid killing by targeting Rab GTPases, phosphoinositides, or both, and modulating the pH of its phagosome in a way that is dependent on the host's immune status.

B700/P2368

The *wtf* question: How do extremely diverged Wtf proteins do the same job?

A. Nidamangala Srinivasa[1,2], S. Campbell[1], M. De Carvalho[1], S. Zanders[1,2]; [1]Stowers Institute for Medical Research, Kansas City, MO, [2]Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Meiotic drivers are genetic parasites that manipulate gametogenesis to be transmitted into more than 50% of the viable gametes produced by a heterozygote. The *wtf* gene family in the *Schizosaccharomycete* fission yeasts includes multiple meiotic drive genes. Each *wtf* driver encodes an aggregating poison protein that targets all the developing gametes in addition to an antidote protein that only rescues *wtf*+ gametes. In one characterized *wtf* driver, *wtf4*, the antidote acts by co-assembling with the poison and promoting its trafficking to the vacuole. Strikingly, Wtf proteins that share less than 17% amino acid identity with those of Wtf4 still function as poisons and antidotes. We are exploring how these Wtf proteins can support the same molecular functions, despite extreme divergence. We propose that low functional constraint on the *wtf* gene family evolution could contribute to their evolvability and promote their long-term success. Additionally, the results of this work could broaden the knowledge on the breadth of sequences that facilitate protein self-assembly and aggregation.

B701/P2369

Quantifying and expanding the halotolerance of the black yeast *Knufia petricola*

G. Murphy[1], G. Hamilton[2], A. Gladfelter[2]; [1]University of Richmond, Richmond, VA, [2]University of North Carolina, Chapel Hill, NC

Knufia petricola is a black yeast and an emerging model organism for the study of eukaryotic extremotolerance. It is found in diverse hostile environments, from bare rock faces to the ocean. *Knufia* exhibit the typical characteristics of extremotolerant black fungi, such as slow yeast-like growth, highly melanized cell walls, the absence of specialized reproductive structures, and the production of orange to red carotenoids. Concurrent with studies exploring other facets of this fungus's polyextremotolerance, this study specifically sought to define the limits of *Knufia*'s halotolerance. We also set out to compare the halotolerance of a terrestrial isolate obtained from marble in Greece (A95) and a marine isolate obtained from a plankton tow off the coast of Woods Hole (WH). We hypothesized that *Knufia* could survive from 5-10% NaCl and that the marine isolate would have a higher tolerance due to the isolate already being subjected to NaCl in the ocean. To test this hypothesis, we created liquid and solid cultures with varying salt conditions and grew A95 and WH strains to compare their growth and morphology. Survival on solid media was quantified using a drop dilution assay. Maximum growth was observed at 5-8% NaCl with limited growth at 9-10% NaCl. For reference the ocean has around 3.5% NaCl, and *Saccharomyces cerevisiae* cannot survive even 3% NaCl. We also observed that the marine isolate, WH, did in fact have a higher survival rate in higher NaCl concentrations when compared to the terrestrial isolate. With the use of directed evolution, I was able to progress the salt limits that *Knufia* could survive in, generating a slightly more halotolerant strain. I also involved another osmotic stress, glycerol, in our experiment. Our preliminary data suggest that osmotic stress from glycerol, an organic solute, and NaCl, an inorganic salt, functioned additively and prevented the *Knufia* from surviving at all. We will continue our directed evolution experiments in order to push and understand the limits of halotolerance in *Knufia*, and CRISPR knockouts will allow us to test the importance of specific genes to *Knufia*'s remarkable extremotolerance. Understanding how a eukaryotic organism evolved to survive in environments as hostile as bare rock faces will help us understand the limits of life on Earth.

B702/P2370

Structural cell biology of the invasion organelle from microsporidian parasites

M. Usmani[1], N. Coudray[1], H. Ramchandani[1], M. Kopylov[2], D. Bobe[2], D. Ekiert[1], G. Bhabha[1]; [1]NYU School of Medicine, New York, NY, [2]New York Structural Biology Center, New York, NY

Intracellular pathogens have developed diverse ways to enter and infect host cells. Microsporidia are single-celled eukaryotes that form a sister group to fungi, and are obligate intracellular parasites that have evolved a unique, harpoon-like organelle called the polar tube (PT) to invade host cells. Outside the host, microsporidia exist as dormant spores, in which the long PT is tightly coiled. Upon firing, the PT undergoes a tremendous conformational change to a long, linear tube extruding out of the spore and is thought to pierce the plasma membrane of a target cell. The PT then serves as a tunnel which delivers infectious cargo into the host cell to initiate infection. This entire process occurs on a millisecond timescale and the extruded PT is 20 times the length of the spore it fires from. The architecture of the PT and the large structural rearrangement that occurs upon firing is poorly understood. To gain an understanding of the PT firing mechanism we are using cryo-electron tomography (cryo-ET) to study the 3D architecture of the PT in its pre-firing and post-firing states. By annotating tomograms, we have

gathered insights into how the PT interacts with other organelles in the spore that may play a role in its firing mechanism. We are also using subtomogram averaging (STA) on the PT to gain higher resolution insight into its structure. Using cryo-ET we have been able to resolve new features of the PT in both its coiled and linear states, shedding new structural insights into this unique invasion organelle.

B703/P2371

Development of a Genetic Transformation System for the Frog-Killing Chytrid Fungus *Batrachochytrium dendrobatidis*

E. Kalinka, A. J. M. Swafford, E. M. Medina, L. K. Fritz-Laylin; University of Massachusetts Amherst, Amherst, MA

Chytrids are early-diverging fungi that display both animal- and yeast-like cellular features (e.g., actin cytoskeletal components), providing a unique opportunity to study early fungal evolution. Two chytrid species, *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans*, are decimating amphibian populations around the world. Efforts to mitigate the damage and spread of *Bd* infections have been limited by the lack of knowledge about its basic biology and molecular genetic tools with which to test core hypotheses about amphibian-chytrid symbiosis. To date, the non-pathogenic species *Spizellomyces punctatus* is the only genetically tractable chytrid (Medina, E.M., 2020). Here, we adapted a previously reported electroporation protocol (Swafford, A.J.M., 2020) for the delivery of exogenous DNA into *Bd* zoospores and quantified transgene expression by performing luciferase assays on transfected cells. We generated DNA constructs that facilitate transient expression of an antibiotic resistance marker and various fluorescent proteins and observed transgene expression for up to four life cycles (12 days). By expressing a LifeAct-tdTomato fusion and using live-cell imaging, we visualized the distribution and dynamics of polymerized actin in *Bd* at each stage of its life cycle. We are now developing a suite of genetic tools to facilitate subcellular localization analysis of endogenous proteins, stable transgenesis, and CRISPR-mediated genome editing in *Bd*. These new tools will pave the way for addressing fundamental questions of chytrid cell and evolutionary biology by molecular genetic approaches, and aid in the establishment of ecological mitigation strategies.

B704/P2372

Bioassay System for Evaluating Branched-chain Higher Alcohols Titerby Using Yeast Double Deletion Strains with Isobutanol Hypersensitivity

G. Sato, K. Kuroda; Graduate School of Agriculture, Kyoto University, Kyoto, Japan

[Objective]: Branched-chain higher alcohols (BCHAs) production by yeast is a promising process for providing advanced biofuels toward sustainable society. However, the further development of yeasts producing BCHAs requires costly and time-consuming titer-evaluation processes using chromatography instruments. The transcription factor-based biosensor was recently proposed as a rapid BCHAs titer-evaluation process. Nevertheless, it was limited to intracellular detection of intermediate of BCHAs biosynthesis [1], and direct detection of BCHAs is still unavailable in yeasts. Therefore, we propose a novel bioassay system for the direct evaluation of BCHAs titer in the yeast fermentation broth.
[Methods and Results]: The biomolecules that can detect BCHAs are unknown in yeasts. Thus, we conceived a novel bioassay system based on the inhibition of yeast growth by BCHAs. We previously identified deletion strains of *Saccharomyces cerevisiae* that are highly sensitive to isobutanol (IbOH), one of the BCHAs, while maintaining ethanol tolerance [2]. Based on our knowledge, we constructed

various double deletion strains as candidates for BCHAs sensors. By evaluating growth inhibition of candidate sensors by IbOH in the yeast fermentation broth, a strain with sensitivity to under 0.2% IbOH was identified, and its sensitivity was maintained in the fermentation broth. As a bioassay system, the sensor was added to filter-sterilized fermentation broths produced by each strain of the yeast IbOH producer library and cultured to measure growth inhibition of the sensor. The growth inhibition of the sensor showed a significant correlation to IbOH titer of producers. Furthermore, to examine the direct detection of BCHAs, partial least square regression (PLSR) model of the sensor growth was constructed from high-performance liquid chromatography and refractive index detector chromatogram peak areas of fermentation broths. As a result, IbOH concentration was the most important factor in the PLSR model of sensor growth among all detected metabolites in the fermentation broths, suggesting direct detection of BCHAs. **[Conclusion]:** Measuring growth of the double deletion strain with IbOH hypersensitivity was proposed as a novel bioassay system for evaluating the IbOH titer of yeast producers. [1] Zhang, Y., *et.al*. (2022). *Nat Commun*., **13**, 270 [2] K. Kuroda, *et.al*. (2019) *Cell Sys*., **9**, 534-547

B705/P2373

Investigating the Effectiveness of Antimicrobial Reagents on Environmental Mold Samples

V. Adams, K. Hodges, A. Shukwit, C. Davis, C. Jones Carter, M. Van Stry; Lane College, Jackson, TN

There are millions of mold species found on Earth that grow in the damp places of homes, college dorms, and classrooms. Indoor mold such as *Alternaria alternata*, *Aspergillus fumigatus*, and *Cladisporium cladosporioïdes* have a greater effect on human health. After spores have been released into the environment and inhaled, illnesses such as respiratory diseases, asthma, and bronchitis can occur. The greater amount of mold infestation the more extensive the damages and associated cost-related restoration. The goal of this study is to isolate mold found on the college campus and to test the antimicrobial properties of cost effective treatments. The analysis and experimentation of indoor mold species benefits the school and other historical black colleges or universities (HBCU's) who are struggling with this problem. Seventeen (17) mold samples were isolated from three (3) locations on campus, and three (3) of six (6) samples were used based on phenotypic interest. Antimicrobial reagents such as bleach, hydrogen peroxide, mold armor, vinegar, and tea tree oil were tested using two (2) different methods: treating one group immediately with each reagent and the other group after three (3) days of growth. The results showed the most effective reagent at preventing mold growth was Mold Armor and the least effective was tea tree oil. The potential hydrogen (pH) of each reagent was also tested to determine a possible cause and effect relationship. Future directions include the three (3) remaining samples to determine any of the extent relationship amongst samples.

Immune System

B707/P2374

NOX2-derived Reactive Oxygen Species shape the inflammatory response through GSDMD post-translational regulation

D. Stanga, F. Touzot; Immunology and Cancer, CHU Sainte-Justine Research Center, Montreal, QC, CANADA.

Redox signaling is known to be the principal driver of inflammation. This is mainly achieved through transcriptional modulation and post-translational modifications of the multiprotein complexes of the inflammasomes, which are involved in mediating cellular inflammation in response to various damaging agents. Redox signaling is induced by reactive oxygen species (ROS) that are produced either by the mitochondrial respiratory chain also called mitochondrial ROS or by the NADPH oxidases called cytosolic ROS. While excessive ROS production is a well-recognized trigger of an excessive inflammatory response, little is known about the consequences of low redox signaling on inflammation. The need for fine-tuning of ROS production is well exemplified by the NOX2 deficiency model, an inborn error of immunity that abolishes the function of the NADPH oxidase complex 2. Interestingly, the defective cytosolic ROS production observed in NOX2 deficiency leads to an enhanced inflammatory response. This biological picture is intriguingly similar to the one observed in pathological conditions with excessive ROS production.In our study, we investigate the biological consequences of defective cytosolic ROS production on the inflammatory response in humans using primary cells from NOX2-deficient patients and a CRISPR-engineered NOX2-deficient CYBBko THP-1 cell line. We provide evidence that low cytosolic ROS production increases the transcriptional priming of NLRP3 and IL-1β. This priming is IL-1β dependent through auto/paracrine stimulation of the NF-KB pathway downstream of the IL-1β receptor. We also show that, in NOX2-deficient human macrophages, the NLRP3 inflammasome activation is triggered by increased K+ efflux and cytosolic release of mitochondrial DNA, which are enhanced by the presence of cleaved Gasdermin D (GSDMD) at the plasma and mitochondrial membranes. This observation refines the role of GSDMD as an enhancer of the inflammatory response rather than only a terminator through pyroptosis. Finally, our deep study of the NOX2 deficiency model unveils the crucial role of GSDMD Serine 252 residue, whose phosphorylation is critical in enhancing GSDMD cleavage and membrane pore formation.Thus, our study brings two key messages: (1) revealing the role of NOX2-derived ROS in tempering the inflammasome cascade at the transcriptional and post-translational levels, thus rebalancing our view on the function of the redox signaling in regulating the inflammatory response (2) evidencing GSDMD S252 phosphorylation as an important event in regulating GSDMD pyroptotic activity and offering a new drug target for inflammasome -driven pathologies.

B708/P2375

Rac-mediated hyperphagocytosis in mammalian immunodeficiency

M. Rodriguez; MCDB, University of California, Santa Barbara, Santa Barbara, CA.

Rac2 is amongst the numerous signaling molecules essential for engulfment of fluid, apoptotic bodies, and pathogens. Loss of function mutations in the hematopoietic-expressed Rac2 gene causes immunodeficiencies due to impaired migration and survival of neutrophils, macrophages, and lymphocytes. Human patients with germline mutations that hyperactivate the Rac2 protein exhibit unexplained clinical features ranging from lymphopenia to severe combined immunodeficiency.

Specifically, patients who carry a $Rac2^{E62K}$ amino acid substitution exhibit repeated respiratory infections and B and T cell lymphopenia. There are no defects in the development of B and T cells, thus the cause of the lymphopenia in patients remains a mystery. Recently, our lab discovered that hyperactive Rac is sufficient to drive one cell to engulf another living cell in a process known as cell cannibalism. First discovered in *Drosophila*, we found this effect is conserved in mammals. The objective of this study is to decipher how a dominant activating mutation in Rac causes mammalian immunodeficiency. Normally, phagocytes engulf and kill activated B and T cells to resolve inflammatory responses and to turn over cells at the end of their life cycle. So, we hypothesized that $Rac2^{E62K/+}$-expressing macrophages might prematurely engulf and kill lymphocytes, just as border cells expressing active Rac prematurely engulf and kill nurse cells in the *Drosophila* ovary model. Using the $Rac2^{E62K}$ mouse model that phenocopies the human genetic disease, we found that $Rac2^{E62K}$ macrophages engulf and kill significantly more T cells than wildtype macrophages. We discovered that $Rac2^{E62K}$ macrophages are not only hyperphagocytic, but T cells are more vulnerable to engulfment compared to B cells. In addition, despite the loss of circulating B cells we found a striking increase in B cell numbers in the spleens of $Rac2^{E62K}$ mice compared to wildtype littermates. These data suggests that the dominant activating Rac2 mutation functions cell-autonomously in these immune cells to cause the lymphopenia. Together, these results demonstrate that hyperactive Rac enhances engulfment of target living cells when those cells express a specific ligand and when the Rac-expressing phagocyte expresses the receptor. This work builds on a discovery made in *Drosophila* to provide a novel cellular and molecular explanation for an otherwise mysterious rare immunodeficiency in patients. We propose that though harmful as a germline mutation, we can harness this Rac-mediated behavior in macrophages to improve CAR macrophage (CAR-M) cancer immunotherapies.

B709/P2376

Defining the activation of CD16 by phosphorylation of CD3ζ in human natural killer cells

H. Fatima, M. J. Shannon, E. M. Mace; Columbia University Irving Medical Center, New York, NY.

The Fc receptor CD16 (FcγRIIIa) is expressed on $CD56^{dim}$ peripheral blood natural killer (NK) cells and a subset of human blood monocytes. CD16 mediates antibody-dependent cellular cytotoxicity (ADCC) upon binding the Fc region of antibodies coating abnormal cells. CD16 ligation at the immunological synapse initiates the exocytosis of perforin and granzyme resulting in the lysis of target cells. This response is important for the clearance of tumor cells and pathogens and in the context of monoclonal antibody (mAb) treatment. Rituximab is a mAb that binds CD20 on B-cells, which become targets for NK cell directed ADCC. CD16-mediated clearance of tumor cells by NK cells mediates the clinical response to Rituximab treatment in lymphoma patients. CD3ζ activation and kinase-based signal amplification is coupled to CD16-Rituximab binding, but little is known about the spatiotemporal organization of these molecules required for their function.

Here, we use detection of CD3ζ by confocal microscopy as a proximal marker for CD16 activation and demonstrate that Rituximab-induced NK cell activation leads to increased levels of phosphorylated CD3ζ compared to immobilized ICAM-1, the ligand for LFA-1 expressed on NK cells. This was concluded by quantifying the number of foci of phosphorylated CD3ζ at the plane of the activated glass surface. The existence of large CD3ζ foci around smaller instances of CD16 visible by confocal microscopy has encouraged us to employ single molecule imaging to better understand how CD16 spatial localization and activation changes in response to Fc binding. Preliminary results demonstrate that we can visualize CD16 at single molecule resolution. We used direct stochastic optical reconstruction microscopy

(dSTORM) and the Python-based localization software 'Picasso' to acquire nanoscale point maps of molecular positions. Our results provide new insight into the first steps of CD16 activation during the process of ADCC and have exciting implications for better understanding how monoclonal antibody therapies exert function.

B710/P2377

Honey Mediated Gold Nanoparticles Attenuate Macrophage Secretion of IL-6 Following LPS Exposure

J. Duncan, S. Basu, P. Vivekanand; Susquehanna University, Selinsgrove, PA.

Interleukin-6 (IL-6) is a pleiotropic cytokine that coordinates host immune responses to infection. Though essential to the acute phase response, prolonged IL-6-mediated recruitment of mononuclear cells has been implicated in chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, and Chron's disease. Accordingly, identifying novel therapeutics that diminish circulating IL-6 levels should benefit individuals suffering from chronic inflammation. Under normal circumstances, in immunocompetent hosts, bacterial lipopolysaccharide (LPS) recognition by toll-like receptor 4 (TLR4) prompts macrophage secretion of IL-6. Interestingly, both citrate-stabilized and 'green' synthesized gold nanoparticles (AuNPs) have been shown to modulate the cytokine responses of LPS-induced macrophages. Here, we demonstrate that AuNPs, synthesized with commercial and locally sourced honey, downregulate LPS-induced macrophage secretion of IL-6 *in vitro*. After 1 hr. of LPS exposure, macrophages derived from a THP-1 human monocytic cell line were treated with 25 and 50 µg/ml concentrations of commercial, locally sourced dark, and light honey mediated AuNPs. Compared to LPS-only controls, a concentration dependent inhibition of IL-6 levels was observed for all three types of honey AuNPs. Further investigation into the anti-inflammatory properties of honey AuNPs may yield novel therapeutics for the treatment of chronic inflammation.

B711/P2378

Ataxin-1 is epigenetically regulated in B cells upon multiple sclerosis and exerts an immunomodulatory function against autoimmune demyelination

Q. Ma[1], J. R. Oksenberg[1], **A. Didonna**[2]; [1]Neurology, University of California San Francisco, San Francisco, CA, [2]Anatomy and Cell Biology, East Carolina University - Greenville, NC, Greenville, NC.

More than 200 genetic loci have been found convincingly associated with an increased risk of developing multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS) characterized by lymphocyte infiltration, demyelination and axonal injury. Among them, the strongest signal in the risk locus mapping at chromosome 6p22.3 lies within the *ATXN1* gene, which encodes the polyglutamine protein ataxin-1. This chromatin-binding protein works as a transcriptional co-repressor in a large multimeric complex, and it was previously found implicated in the etiology of the neurodegenerative disorders spinocerebellar ataxia type 1 (SCA1) and Alzheimer's disease (AD). On the contrary, very little is known about the function and the regulation of ataxin-1 in the context of CNS autoimmunity. To fill this gap, we analyzed via bisulfate DNA sequencing (BS-seq) four peripheral immune cytotypes (CD4+ and CD8+ T cells, CD19+ B cells, and CD14+ monocytes) isolated from a cohort of untreated MS patients at symptoms onset. Here, we show that *ATXN1* is hypomethylated upon MS at four different sites, exclusively in the B cell population. Importantly, these sites overlap with other regulatory epigenetic marks and their hypomethylation leads to an increase in *ATXN1* expression as confirmed by luciferase assays and RNA-seq transcriptomic profiling. In the MS animal model

experimental autoimmune encephalomyelitis (EAE) ataxin-1 is upregulated in the immune system at the peak of disease and its ablation results in an aggravated EAE course. Consistent with the human data, immunophenotyping and cell activation studies on ataxin-1 null mouse splenocytes narrowed down the main target of ataxin-1 activity to the B cell compartment. In detail, we found that ataxin-1 controls specific genetic programs underlying B cell proliferation, activation, antigen presentation, and antibody secretion. In this light, conditional ablation of the ataxin-1 interactor capicua (CIC) in B cells was able to recapitulate the exacerbated EAE phenotype of ataxin-1 null mice. Altogether, our results pinpoint a previously undocumented key role of ataxin-1 in B cell biology as part of a novel homeostatic mechanism to control the aberrant immune response.

B712/P2379

Immunomodulatory effects of resolvin D2 in a 2-hit infection model

P. Kadiyam Sundarasivarao, J. Walker, A. Rodriguez, B. Spur, K. Yin; Rowan University, Stratford, NJ

Dysregulated hyperinflammatory host immune response to underlying bacterial infections can lead to sepsis. In sepsis, bacteria often trigger abnormal hyperinflammatory responses which can cause multiple organ failure and if sustained can lead to an immunosuppressive phase where the host is susceptible to secondary infections caused by opportunistic bacteria like *Pseudomonas aeruginosa* (*P. aeruginosa*). In our study, we used a 2-hit model of cecal ligation and puncture (CLP) followed by *P. aeruginosa* secondary lung infection to investigate cellular and molecular mechanism in the beneficial action of resolvin D2 (RvD2). Resolvins of the D-series are a group of Specialized Pro-resolving Mediators (SPMs) synthesized endogenously from docosahexaenoic acid (DHA) by different immune cells including neutrophils and macrophages during infection and inflammation. In CLP sepsis, we observed that production of RvD2 in spleens had returned to basal levels by 48 hours after CLP surgery. Administering RvD2 (100 ng/mouse, i.v.) in CLP mice at this time point where endogenous RvD2 synthesis is low enabled CLP mice to clear blood bacteria more efficiently at 24 hours after administration compared to saline controls. There was no significant difference in plasma cytokine production. To further understand cellular and molecular mechanisms, we used flow cytometry to identify different immune cells in spleen and found that RvD2 treatment increased splenic neutrophils ($Ly6G^+$) and myeloid-derived suppressor cells (MDSCs: $CD11b^+Ly6G^+Ly6C^+$) compared to vehicle treated mice. RvD2 treatment increased oxidative burst in splenic neutrophils but not in splenic MDSCs compared to vehicle treated mice. These results provide evidence that RvD2 can expand splenic neutrophil and MDSC numbers to effect greater blood bacterial clearance even when given 48 hours after primary infection. When mice were challenged with *P. aeruginosa* (intranasally; 24 h after RvD2 treatment) we found RvD2 increased bacterial clearance in lungs, increases alveolar macrophage numbers and reduced IL-23. These results suggest that late RvD2 administration boosted host defense to reduce infection and inflammation. This study provides insight into immunomodulatory effects of RvD2 in a 2-hit infection model of sepsis.

B713/P2380

ACE and ACE-2 as potential biomarkers in multiple sclerosis

M. S. Al-Keilani[1], H. M. Abdelrazeq[2], S. Jaradat[2]; [1]Clinical pharmacy, Jordan University of Science and Technology, Irbid, JORDAN, [2]Genetics and biotechnology, Jordan University of Science and Technology, Irbid, JORDAN.

Background: Multiple sclerosis (MS), one of the common diseases in the Eastern Mediterranean region, is a chronic autoimmune neurodegenerative disease characterized by demyelination in the centralnervous system (CNS). It is thought to result from a complex interaction between multiplepredisposing genes; the majority of them are related to the immune function in addition to severalenvironmental factors. However, the exact etiology remains a mystery yet.Due to the genetic differences between individuals, MS patients show great heterogeneity in termsof clinical expression, histopathological and radiological changes, and response to therapy. Thisemphasizes the urgent need for the identification of novel biomarkers to allow the earlierdiagnosis, better prediction of disease severity and progression, as well as to allow personalizationof therapy in complement with MRI and clinical markers of MS.Renin-Angiotensin System (RAS) has a pivotal role in autoimmune inflammation in the CNS.Angiotensin-converting enzyme (ACE) and its homolog ACE-2 are considered two of the major enzymes in the RAS system. The results of the previous studies showed that there are differences between MS patients and healthy individuals in terms of gene and protein expression levels of these enzymes.In the current study, we aimed to measure protein levels of ACE and ACE2 in the serum obtained from MS patients and healthy controls, and to find significant associations with demographics and clinical characteristics of patients.**Methods**: This was a case-control study that was carried out on 74 MS patients and 73 age and sex matched healthy controls. Serum levels of the target markers was measured using human enzyme-linked immunosorbent assay (ELISA) technique.**Results**: Median serum ACE and ACE-2 levels were significantly higher in MS patients (206.66 pg/ml and 2026.21 pg/ml, respectively) compared to healthy controls (142.04 pg/ml and 938.30 pg/ml, respectively) (p-value<0.001). Cut-off values of 170 pg/ml for ACE and 1395 pg/ml for ACE2 were found to discriminate MS patients from healthy controls (sensitivity = 68.9% and 89.2%, and specificity = 61.6% and 83.6%, respectively). Significant correlations were revealed between serum ACE level and EDSS level (p-value=0.028), and MS disease duration (p-value=0.001).**Conclusion**: Serum ACE and ACE-2 can be potential diagnostic biomarkers for MS, and serum ACE level may predict disease severity in MS patients.

B714/P2381

Is Green Tea All It Is Brewed Up To Be? Our Results Suggest It May Not Be.

A. Mattern, C. Azar, J. B. Slee; Biology, DeSales University, Center Valley, PA.

Inflammation underlies multiple disorders such as cardiovascular disease, diabetes mellitus, and cancer. Naturally occurring compounds have historically been investigated for potential medicinal benefit. Published literature indicates that Epigallocatechin gallate (EGCG), derived from green tea leaves, has antioxidant, anti-inflammatory, anti-cancer, and anti-microbial properties. Through multiple experiments, we determined that EGCG is strongly proinflammatory at 10 µM and cytotoxic at 50 µM, concentrations utilized in multiple published reports, in both vascular endothelial cells and monocyte-derived macrophages. Furthermore, EGCG suppressed wound healing in vascular endothelial cells at both 10 µM and 50 µM. 1 µM EGCG treatment appeared to have no effect on vascular endothelial cells

or monocyte-derived macrophages when treated for less than 3 days. 1 µM, 10 µM, and 50 µM EGCG were assessed for the ability to promote the biocompatibility of polymers using a THP-1 cell adhesion assay and a Chandler Loop *ex vivo* model of blood perfusion using whole equine blood. No significant decrease in the number of adhered cells were seen in either assay, suggesting that EGCG does not promote the biocompatibility of commonly used polymers in medical devices. Taken together, our results indicate that EGCG is proinflammatory and not suitable as a therapy to promote biocompatibility.

B715/P2382

Investigation of inflammation-mediated secondary organ damage as a consequence of mast cell tumor degranulation.

R. M. Edwards, S. L. McNally, J. Fellmeth; Biology, Millersville University, Millersville, PA.

Benign mast cell tumors (MCTs) are rare in humans, but relatively common in dogs. Under typical circumstances, mast cells release proinflammatory components as part of an antigen-mediated immune response, which is normal and crucial to survival. During tumorigenesis, however, there is the potential to cause secondary damage through the excess release of pro-inflammatory components, a process referred to as mass degranulation. As of current, correlations have been found suggesting that there is a functional link between the existence of the benign MCT and inflammatory diseases, like chronic renal failure. We propose that during episodes of mass degranulation, these tumors secrete specific cytokines and instigate a cascade of intercellular signals, which result in immune-mediated organ dysfunction. One such cytokine, tumor necrosis factor (TNF-α), has been found to mediate this type of damage through Th1 T-cells. We expect to reiterate similar findings within mast cells and aim to expand current knowledge of mast cell involvement in secondary organ damage, with an emphasis on renal damage. To test this hypothesis, we collected canine MCT samples with paired healthy tissue from local veterinary offices. qRT-PCR was performed to probe for specific cytokines, proteases, and interleukins, all having been found to participate in inflammatory cascades. By comparison to healthy control tissue, we expect to see a notable increase in expression of these components across specimens. To identify specific cellular populations containing these factors, it is our hope to further probe our samples via immunohistochemistry. In its essence, this research has the potential to provide the veterinary medical community with further insight into secondary immune-mediated organ damage.

B716/P2383

Adaptive immune receptor repertoire profiling for biomarker discovery

A. Chenchik[1], M. Makhanov[1], T. Liu[1], D. Hu[1], **K. Ghias**[1], P. Diehl[2], L. Kobzik[1]; [1]Cellecta, Inc., MOUNTAIN VIEW, CA, [2]Cellecta, Inc., Mountain View, CA.

Adaptive Immune Receptor (AIR) Repertoire profiling and characterization of antigen-activated immune cells is essential for the discovery of novel prognostic and predictive biomarkers and for studying immune response mechanisms in cancer, auto-immune and other diseases.To facilitate these studies, we developed a novel technology for combined, unbiased profiling of all human TCR and BCR variable regions; and phenotypic characterization of immune cells in bulk, sorted fraction, and single immune cells. This method involves multiplex RT-PCR amplification and sequencing of CDR3 regions of TCR and BCR genes along with immunophenotyping with a set 500 highly expressed T- and B-cell subtyping and activation marker genes. Bioinformatic analysis of NGS data allows comprehensive AIR repertoire profiling, identification of antigen-activated TCR and BCR clonotypes, and detailed phenotypic

characterization of T and B cells induced by adaptive immune responses. Preliminary phenotypic AIR profiling studies in metastatic tumor samples and Humira®-treated rheumatoid arthritis blood samples indicate that AIR immunophenotyping technology has unparalleled throughput and sensitivity for the discovery of immunity biomarkers.

B717/P2384

Identifying Novel Intracellular Pathogen Response (IPR) triggers by intestinal wounding in C. elegans

A. Alvarado, S. Snipes, J. N. Sowa; Biology, West Chester University of Pennsylv, West Chester, PA.

The development and maintenance of immune responses to various stressors are crucial to organismal survival. Due to their simplicity, *Caenorhabditis elegans* serve as useful model organisms to study host-pathogen interactions since they rely on innate immunity, eliminating the complexity associated with adaptive immune responses. The Intracellular Pathogen Response (IPR) is an immune response that serves as a defense mechanism in *C. elegans* against stress and pathogens. Localized to the intestinal epithelial tissue of the organism, the IPR is triggered in response to invasion from viral pathogens in an attempt to eliminate the threat. Currently, viral infection, proteasome stress, and heat stress are known triggers of the IPR. Preliminary evidence from our lab has shown that intestinal wounding via feeding *C. elegans* sulfate-based detergents or ethanol triggers IPR gene upregulation. As a result, we hypothesize that intestinal wounding may be a separate branch of the IPR activation pathway. However, what induces and characterizes this pathway is currently not known. To determine those factors, we pursued an ethanol-based treatment that showed promise for high-throughput IPR activation. To quantify the level of activation, we use a nanoluciferase dual reporter assay to measure expression of nanoluciferase, which is expressed from the *pals-5p* IPR promoter. In addition, our transgenic strains contain an internal control of firefly luciferase expressed from the *sur-5* promoter. Through our analysis, we hope to identify a novel intestinal wounding method that causes IPR induction and can be applied in a high-throughput manner to facilitate genome-wide screening.

B718/P2385

Improving Bacteriophage Compatibility with the Mammalian Immune System for Therapeutic Applications

C. Carmody, S. Nugen; Cornell University, Ithaca, NY.

The World Health Organization has deemed antimicrobial resistance a global crisis. Bacteriophages (phages) are viruses that infect bacteria hosts and kill them upon completion of their lytic replication cycle. Phages are highly abundant, do not show viral tropism for mammalian cells, and are continuously evolving alongside their bacteria hosts, offering a sustainable long-term option for pathogenic bacteria mitigation. A limitation to phage therapy is the strong mammalian immune response elicited by phages resulting in their rapid clearance from the body by immune cells. Immune system clearance relies on recognition and adherence to structural features of substances, influenced by their size, shape, hydrophobicity, and surface charge. For phages, the capsid is a key structural feature in determining bloodstream half-life with certain capsid proteins allowing for increased interactions with immune cells and proteins. To overcome this issue my hypothesis for this study is, phage capsid modifications to reduce interactions with mammalian immune cells and proteins will decrease strong immune response and rapid clearance of phage. This will be assessed via two objectives. (1) Creating a phage with a synthetic polymer, polyethylene glycol, shielding the capsid. (2) Creating a phage capable of selectively

binding a native mammalian protein, albumin, to camouflage the capsid. My approach is to utilize CRISPR/Cas9 for phage genome editing to translationally fuse affinity peptide genes to capsid genes, allowing for site-specific binding of PEG or albumin. Immune response to modified phages will be evaluated in mouse model. The overall goal of this study is to develop broadly applicable, site-specific phage capsid modifications that increase phages' therapeutic potential. Results to date include successful design and screening of CIRSPR/Cas9 gRNA that cleave the targeted gene, sanger sequencing confirmed donor DNA plasmids for creating modified phages, whole genome sequencing confirmed modified phage genome, approval of the mouse study experimental plan, and optimization of PEG length for lowest modified phage infectivity reduction (30kDa). Based on previous studies in the literature utilizing PEG and albumin to modify virus capsids of similar size and structure, we predict our modified phages to result in increased bloodstream half-life and decreased immune response compared to wild type.

B719/P2386

Deciphering the underlying dynamics of cells and gene expression during injury-induced skin inflammation using unannotated single-cell data

R. Chatterjee, A. Rahnavard, B. Shook; The George Washington University, Washington, DC.

In response to damage, tissues must undergo a complex series of cellular responses that promote inflammation and subsequent repair to restore tissue function. In particular, efficient skin wound healing requires the communication of numerous tissue resident cells with infiltrating immune cells. While recent studies have identified cellular communication that supports later reparative processes, little is known about mechanisms that promote and resolve early inflammatory processes. Given that manipulating early inflammatory events can impact progression through the healing process, there is a great need to understand the cellular communication networks and their relationships dictating the recovery process. The recent development of single-cell RNA-sequencing has provided the power to extract transcriptomic, spatial, and lineage information for each individual cell and affords a unique opportunity to study the interactions occurring during skin wound healing. In this study, we elucidate the precise cell populations and their early changes in gene expression caused by injury to the skin using non-injured tissue as a comparator. We developed a pipeline "cellSight", which utilizes standard tools for targeted clustering based on marker genes, and integration and analysis of cells from both uninjured and injured skin samples for differentially expressed genes across both conditions. The pipeline also utilizes differential effect size calculation using Tweedie models, infers cell to cell communication occurring during early inflammation, and identifies key genes marking biological changes due to injury.

B720/P2387

The role of *N*-acetyl aspartate in inflammation

M. Nassef, K. Hiller; TU - Braunschweig, Braunschweig, GERMANY.

N-acetylaspartate (NAA) is an amino acid derivative synthesized from aspartic acid and acetyl-coA by the enzyme *N*-acetyltransferase 8-like protein (NAT8L). NAA is highly abundant in the brain and it is a major transport and storage form of acetyl-coA. Moreover, it plays a role outside the CNS in brown adipose tissue and in cancer cells. Similar to the CNS, NAA was found to be a storage and transport form of acetate. Moreover, NAA might be providing an alternative pathway of lipogenesis in adipocytes. Apart from adipose tissue and cancer cells, NAA might be also playing a role in macrophages. To investigate

the function and the role of NAA in macrophages, RAW 264.7 cells were virally transduced with lentivirus containing the *NAT8L* overexpression plasmid. After successfully transducing the cells and the selection procedure, it was possible to detect the increased NAA production on metabolic level through gas chromatography - mass spectrometry (GCMS) analysis. Moreover, the mRNA levels of *NAT8L* showed to be highly upregulated. To have a proper control for the *NAT8L* overexpression cell line, further RAW 264.7 cells were virally transduced with lentivirus containing the β-galactosidase gene (*LacZ*) plasmid. After further investigation of the RAW 264.7 *NAT8L* overexpression cell line, the high levels of NAA showed to affect metabolism of the cells in various ways. Furthermore, experiments with labeled [U-13C]-glucose and [U-13C]-glutamine showed high relative abundance of labelled carbons in NAA. This proves that the cells use glucose and glutamine as carbon sources to provide for the production of acetyl-coA and aspartic acid needed for the synthesis of NAA. To sum up, NAA seems to be involved with immunometabolism and mounting the inflammatory response. Further research is needed to have a better understanding of the function of NAA in immunity.

B721/P2388

Phenotypic Evaluation of TREX1 Null Mice as a Model for Lupus-like Autoimmune Disease

J. Ling[1], Z. Chen[1], S. Xu[1], **Z. Li**[2], H. Tong[3], A. Zong[3], C. Ju[2], J. Zhao[3], X. Gao[3]; [1]GemPharmatech Co. Ltd., Nanjing, CHINA, [2]GemPharmatech, Wilmington, DE, [3]GemPharmatech, Nanjing, CHINA.

TREX1 (DNase III) is the major 3'-5' DNA exonuclease of mammalian cells. It has been proposed to have a major role in cell death and genomic DNA degradation. It has been suggested that dysfunction of Trex1 may activate immune response by self DNA as suggested by the TREX1 null mice which develop an inflammatory myocarditis similar to autoimmune cardiomyopathy and produce type 1 IFN. Moreover, TREX1 D18N mutation causes a monogenic, cutaneous form of lupus called familial chilblain lupus, and the TREX1 D18N enzyme exhibits dysfunctioned dsDNA-degrading activity, providing a link between dsDNA degradation and nucleic acid-mediated autoimmune disease. Here we generated the $Trex1^{-/-}$ mice and performed comprehensive phenotyping to evaluation the loss of function of TREX1 and its potential application in SLE study.

$Trex^{-/-}$ null mice were generated by knocking out the coding sequence in exon 2. There was a dramatically increased morbidity of both female and male Trex1 null mice post weaning. Serum ALT, AST and LDH levels were dramatically increased in both male and female $Trex^{-/-}$ mice compared with wild type and $TREX^{+/-}$ mice at 6 weeks of age, indicating injuries of liver and heart. Pathohistological analysis showed inflammatory cell infiltration in liver, heart, spleen, kidney, lung and skin. Interestingly, the level of ALT, AST and LDH gradually deceased from 8 weeks to 10 weeks of age. Since Trex1 is the major DNA exonuclease, we hypothesized that dsDNA was the antigen that caused the organ injury. We found that serum total anti-dsDNA antibody levels were significantly increased in $TREX^{-/-}$ male and female mice compared with wildtype and heterozygous mice, which was kept high from 7 to 13 weeks of age. Protein to creatinine ratio measured in the urine showed no difference between $Trex^{-/-}$ and wildtype mice.

In conclusion, the $Trex^{-/-}$ null mice we generated in this work showed lupus-like inflammatory autoimmune response with increased anti-dsDNA levels and inflammation in multiple organs, providing a useful tool to study TREX1-mediated autoimmunity and to evaluate potential treatments for lupus.

B722/P2389

Anti-inflammatory effect of CSS extract in RAW264.7 cells

J. SHIM; Semyung Univ., Jecheon, KOREA, REPUBLIC OF.

This research was designed to evaluate the possible anti-inflammatory effects of CSS extract on the skin using RAW 264.7 cells. The assessments of these effects were based on cell viability assay, mRNA expression levels of interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), IL-6, tumor necrosis factor alpha (TNFα), and levels of nitric oxide (NO)/prostaglandin E2 (PGE2) production. Quantitative real-time polymerase chain reaction showed that treatment with CSS extract decreased the mRNA levels of iNOS, COX2, IL-1α, IL-1β, IL-6, and TNFα. Furthermore, from the production levels of PGE2/NO, it can be inferred that CSS extract exhibited anti-inflammatory properties. These results suggest that CSS extract contains anti-inflammatory compound(s). Consequently, the extract may be a potent cosmeceutical material for anti-inflammatory effects against atopic dermatitis. Taken together, further studies on the anti-inflammatory mechanisms of CSS extract would not only facilitate the development of cosmeceutical but also engender pharmacological treatments.

B723/P2390

Macrophages phase separate and cooperate to phagocytose cohesive cellular targets

L. J. Dooling, J. C. Andrechak, B. H. Hayes, S. Kadu, D. E. Discher; University of Pennsylvania, Philadelphia, PA.

Macrophages are abundant in many solid tumors and are potential effector cells for monoclonal antibody-based immunotherapies because they could phagocytose IgG-opsonized cancer cells. However, it remains unknown whether phagocytic macrophages can overcome the cohesive forces among phagocytic targets in solid tumors. We show that maximum phagocytosis of B16 melanoma cells cultured as cohesive ‘tumoroids’ requires IgG opsonization and knockout of the macrophage checkpoint ligand CD47. Under these conditions, phagocytic macrophages cluster or ‘phase separate’ and eliminate B16 cells with kinetics that indicate cooperativity with respect to macrophage number (i.e. Hill exponent $|n| > 1$). Experiments using chelating agents to disrupt phagocytic macrophage clusters specifically implicate Mg^{2+}, which forms part of the ligand binding site of macrophage integrins. Cytokine treatments and myosin-II inhibition further indicate roles for integrins and phagocytic surface receptors as well as the actomyosin cytoskeleton in macrophage clustering that can impact tumoroid phagocytosis. Although tumor-associated macrophages correlate with negative prognoses in many cancers, our findings are the first to reveal the advantage of high macrophage numbers, macrophage clusters, and resulting cooperative effects when combined with opsonizing antibodies and CD47 blockade.

B724/P2391

Phagocytosis-dependent activation of macrophage Nrf2 curbs intracellular ROS levels to strengthen the inflammatory response.

G. Clemente, H. Weavers; University of Bristol, Bristol, UNITED KINGDOM.

The production of reactive oxygen species (ROS) during the ‘respiratory burst’ is an effective weapon exploited by leukocytes to neutralize dangerous pathogens and degrade cellular debris. Nevertheless, because of their exceptionally reactive nature, ROS could induce indiscriminate bystander damage,

causing a progressive deterioration of cellular functions. Therefore, to sustain full functionality under these hostile conditions, immune cells must orchestrate a complex and robust self-protection network. This program allows leukocytes to mitigate the undesirable side-effects of the constant oxidative challenge while allowing low levels of ROS for crucial cellular signalling. Here, we investigate the molecular nature of these self-protective strategies, exploring *in vivo* their precise mechanism of activation and physiological relevance. We show that, in *Drosophila* macrophages, a conserved Calcium-PI3K-NOX axis drives the release of ROS downstream of corpse uptake and consequent activation of the redox-sensitive transcription factor Nrf2. By performing RNAi experiments, we find that the Nrf2-mediated antioxidant response is vital to promote *Drosophila* macrophage tolerance to oxidative stress during normal homeostatic behaviour, preserving efficient basal motility and allowing a robust and timely detection of epithelial wounds. Moreover, we find that, by reducing the oxidative burden, macrophage Nrf2 crucially restrains the accumulation of dangerous oxidative damage and delays the onset of immune senescence. Finally, we propose that, by preserving a healthy immune system, leukocyte Nrf2 might exert important paracrine effects by delaying premature systemic aging and improving organismal lifespan. We suggest that future therapeutic interventions aimed at boosting immune self-protection may be beneficial to delay immune aging and alleviate correlated morbidities.

B725/P2392

HIV-1 p55 Gag Immunization mediated via Imiquimod by adjuvant effects on dendritic cells

A. De Freitas-Suarez[1], J. Roldan-Kalil[2], S. Vendrell-Gonzalez[1], F. Reyes-Ortiz[1], G. Jimenez-Jordan[1], J. Alves[1], M. Inyushin[1]; [1]University of Central Del Caribe, Bayamon, PR, [2]University of Puerto Rico Medical Sciences Campus, San Juan, PR

The ravaging HIV pandemic has been claiming lives since the 1980's. This incurable disease has long been a threat due to danger it poses to the immunological system. A key to halting this threat may lie in developing an effective HIV vaccine. A difficulty for developing an effective HIV vaccine results in the ability of the virus to suppress the specific immunity, rendering it ineffective if the response is slow. The most notable expected benefit of an effective HIV vaccine resides in the ability to prevent acute infection by rapidly diminishing the viral load. This project takes on a different scope for developing such vaccine by targeting dendritic cells (DC). The purpose of our work is to pre-activate in-vivo dendritic cells before HIV vaccinations using certain adjuvants that interact with DC's in order to achieve rapid enhancement of immune response. These DC's play a crucial role in maintaining T cell (TC) immunity. DC's are antigen presenting cells that can alter TC's by activating them (or attenuating during hyperimmunization). For this reason DC's have been gathering interest for additional vaccination intentions. DC's contain G-protein coupled Toll-like receptors (TLR7) which have specific agonist, Imiquimod. Thus, Imiquimod is an imidazoquinoline amine with properties capable of modifying immune response. To carry out the procedure in-vivo models used were 4-6 week old *BALB/c* mice. DNA plasmids were constructed resulting in our p55-Gag-HIV plasmid. Imiquimod was employed as an adjuvant in ranging concentrations of 25, 50 and 100nM to properly observe the effect of DC stimulation on the effectiveness of an HIV-1 p55 Gag DNA vaccine in-vivo. Subsequently a cell culture was performed in order to quantify the p55 protein production after the immunization via Western blot analysis. To understand the immune response of TC's an ELIspot assay and ELISA were performed to measure the frequency and production of IFN-γ -secreting cells, IFN-γ and IL-4. Our results show that the adjuvant effects of Imiquimod rely on concentration. We observed that low concentrations of Imiquimod were found to effectively stimulate Gag production by up to 25% as well as the magnitude of

T-cells response, whereas higher concentrations reduced vaccination effects up to 55%.
Acknowledgements: Supported by Universidad Central Del Caribe (UCC) and the office of the Associate Dean for Research and Graduate Studies, The Alliance-NIMHD-NIH, Expanding Undergraduate Students Education, Opportunities and Options in Clinical and Translational Research Supported by the US Department of Education: Title V Grant Award #P031S160068 and MAC-FRED Program 2018.

B726/P2393

Assessment of Anti-SARS-CoV-2 Spike RBD Antibody Responses Using a Multiplexed Serological Assay and Advanced Flow Cytometry

M. Carter[1], J. Lovchik[1], A. Munitz[2], M. Gerlic[2]; [1]Sartorius BioAnalytical Instruments, Inc., Albuquerque, NM, [2]Tel Aviv University, Tel Aviv, Israel

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, continues to be a global public health threat. SARS-CoV-2 initiates entry into target cells through the interaction of the receptor binding domain (RBD) of its envelope Spike protein with the angiotensin-converting enzyme 2 (ACE2) on human cells. Antibodies directed against the RBD of the SARS-CoV-2 Spike protein demonstrate potent neutralizing activity by blocking virus interaction and entry into host cells. Several isotypes (IgM, IgG and IgA) of these neutralizing antibodies have been identified. In this study, we examined the anti-Spike RBD antibody isotype profiles induced in COVID-19 patients and individuals vaccinated with COVID-19 mRNA vaccines. In order to quickly assess the levels of all three major isotypes of anti-Spike RBD antibodies, we utilized the iQue® SARS-CoV-2 kit which provides a rapid, robust, and sensitive flow cytometry assay for the simultaneous measurement of IgG, IgM and IgA anti-Spike RBD antibodies in a single sample of human blood (serum or plasma) in either a 96 or 384-well plate format. The results revealed the heterogeneity of anti-Spike RBD antibody responses induced in both COVID-19 patients and vaccine recipients. In COVID-19 patients, the levels of all three isotypes of anti-Spike RBD antibodies generally increased as the disease progressed, with some individuals producing very high levels of IgA anti-Spike RBD antibodies. IgM and IgA anti-Spike RBD antibody levels then started to decline after 56 days-post symptom onset (DPS), while IgG levels remained relatively stable out to 84 DPS. Variable levels of IgG, IgM and IgA anti-Spike RBD antibodies were also observed in response to vaccination with the Moderna (Spikevax) or Pfizer-BioNTech (Comirnaty) mRNA COVID-19 vaccines. Notably, accurate detection of seroconversion in both COVID-19 patients and vaccine recipients was found to depend on analysis of multiple isotypes since not all isotypes were present in every individual. Overall, this study shows the importance of measuring the quantity and kinetics of all three major isotypes of anti-Spike RBD antibodies to accurately assess the antibody response against SARS-CoV-2 in COVID-19 patients, as well as to evaluate the induction and duration of the anti-Spike RBD antibody response in vaccinated individuals.

B727/P2394

High-carbohydrate diet impacts hepatic IL1beta expression in rainbow trout

A. Vasil'eva[1,2], S. Matrosova[2], E. Borvinskjaya[3], **I. Sukhovskaya**[1,2]; [1]Institute of Biology of Karelia Research Centre of RAS, Petrozavodsk, Russian Federation, [2]Petrozavodsk State University, Petrozavodsk, Russian Federation, [3]Irkutsk State University, Irkutsk, Russian Federation

Cytokines arethe polypeptide mediators involved in regulation of immune defense responses through binding specific cellular receptor complexesin vertebrates. Interleukin-1β (IL1β), a key pro-inflammatory

cytokine, is produced by many cell types including hepatic Kupffer cells. To assess the effect of the feed composition on the artificial rearingtrout, the juvenile rainbow trout (average weight 70-76 g) were fed either a balanced diet (control group) or a diet enriched with carbohydrates (experimental group) during54 days; both rationswere ofthe same energy value. The IL1βtranscripts werequantifiedby RT-PCR technique withelongation factor and β-actin as reference genes. Being acarnivorespecies, trout prefers a high-protein diet, and experimental high-carbohydrate feedingwas expected to induce inflammation at the morphological and molecular levels. We observed morpho-pathological changes in 65% of high-carbohydrate fed fish, including surfaceerosions, liverdiscoloration and petechia, and posterior intestineinflammation. Despite well-documentedIL1βvalue asindicator of inflammation inhumans, our study showed no significant differences in the hepatic IL1β expressionbetween the trout with or without ichthyopathological signs of inflammation. We can assume both low inflammatory response to non-infectious alimentary agentand low sensitivity of IL1βas inflammatory markerin fish.Consideringtissue-specific cell signaling pathwaysof cytokineexpression,other tissues may be more responsive to carbohydrate-induced enteritis. The interdisciplinary research in the partnership with Irkutsk State Universitywas supported bythe Russian Science Foundationgrant No. 20-66-47012.

B728/P2395

Skin fibroblast subsets contribute to injury-induced inflammation through spatiotemporal changes in pro- and anti-inflammatory gene expression.

V. Balick, B. Shook; The George Washington University, Washington, DC

Skin wound healing requires an initial phase of myeloid cell recruitment and inflammation, which must subsequently resolve for the tissue to transition to a reparative state. Pro-inflammatory signals are known to arise from epithelial and immune cells; however, the contribution of mesenchymal cells to injury-induced inflammation remains under-studied in acute wound healing. Since fibroblasts contribute to acute inflammation in other tissues and chronic inflammatory skin diseases, we hypothesized that dermal fibroblasts support acute inflammation after skin injury. To identify fibroblast-derived factors which could mediate this effect, we characterized fibroblast gene expression with RT-qPCR and RNA sequencing in a murine skin wound model at multiple time points throughout acute inflammation. Dermal fibroblasts acquired a pro-inflammatory gene expression profile at time points representing early and peak inflammation, upregulating cytokines and chemokines known to contribute to an inflammatory environment and immune cell recruitment. These factors influence the quantity of the immune cells in the tissue, as fibroblast-specific deletion of the chemokine *Ccl2* significantly reduced the number of monocytes and macrophages present in wounds at the peak of inflammation. As inflammation resolved, the fibroblast gene expression profile changed to reflect the tissue's shift to an anti-inflammatory, pro-remodeling environment. At this late inflammation time point, fibroblasts expressed secreted factors associated with extracellular matrix formation and the dampening of immune responses. To complement our gene expression analysis, we performed RNA fluorescence *in situ* hybridization to spatially map fibroblast inflammatory gene expression at the wound periphery throughout tissue inflammation. We identified distinct patterns of spatial bias for numerous cytokines, chemokines, and tissue remodeling enzymes. Since fibroblast subsets can be defined based on their location in the skin, these biases suggest that distinct fibroblast populations contribute to the inflammatory environment, cell recruitment, and tissue organization after injury. Overall, these results convey that dynamic changes in gene expression allow multiple populations of skin-resident fibroblasts to actively participate in the induction and resolution of inflammation during wound healing.

B729/P2396

CRISPR-Cas9 Whole-Genome Screens Identify Genes Critical for Neutral Lipid Metabolism in Bone-Marrow Derived Macrophages and Highlights mTOR Regulating Genes as Mediators of Lipid Droplet Degradation

K. Wanniarachchi; South Dakota State University, Brookings, SD

Atherosclerosis-related heart diseases are the leading causes of death in the United States and worldwide. Atherosclerosis is primarily caused by the over-accumulation of modified low-density lipoprotein (LDL) cholesterol within artery walls. Macrophages serve as critical contributors to atherosclerotic plaque formation. Macrophages internalize modified LDL-cholesterol within the artery walls and store neutral lipids in lipid droplet organelles. While macrophages efficiently degrade lipid droplets and efflux free cholesterol in healthy circumstances, these processes become pathological in the disease condition. However, the molecular mechanisms of neutral lipid uptake, storage, lipid droplet degradation and cholesterol efflux are not clearly understood in macrophages. Here, we report CRISPR/Cas9 whole-genome screens results to identify critical genes for neutral lipid metabolism in macrophages using a combination of fluorescence-activated cell sorting (FACS) and next-generation sequencing methods. We identified hits and ranked them using model-based analysis of genome-wide CRISPR/Cas9 knockout (MAGeCK). Using gene set enrichment analysis (GSEA), we identified mTOR signaling and autophagy as key pathways in neutral lipid metabolism. We validated screen hits and successfully recapitulated screen phenotype using a targeted CRISPR/Cas9 gene disruption of screen hits. Identified hits might regulate cellular processes such as neutral lipid or cholesterol accumulation, lipid droplet degradation, or cholesterol efflux. Our screens identified mTOR-regulating genes including *Rraga, Rragc, Flcn*, and *Lamtor4* as positive regulators of lipid droplet degradation. To investigate the role of these genes, we disrupted them with CRISPR/Cas9 and studied proteins of the mTOR and autophagy pathway. Currently, there is limited understanding about the role of mTOR regulation of lipid droplet degradation in macrophages. In this study, we interrogate how genes regulating mTOR activity mediate lipid droplet degradation in macrophages. We observed that macrophages have high mTOR activity in the absence of *Rraga, Rragc, Flcn,* and *Lamtor4*, thus leading to defective autophagy and persistent lipid droplets. Specifically, we observed increased p62 accumulation in these cells, indicating reduced autophagy flux. Interestingly, mTOR inhibition with rapamycin restored normal p62 levels and lipid droplet degradation in macrophages. This study demonstrates that *Rraga, Rragc, Flcn,* and *Lamtor4* regulate lipid droplet degradation via an autophagic process that is inhibited by mTor activity. Overall, this work can help expand our current mechanistic understanding of cholesterol metabolism and lipid droplet dynamics in macrophages.

Blood Cells and Vessels

B730/P2397

The Link Between Altered Secondary Hemoglobin Structure and Augmented Hypoxia-Induced ATP Release from Erythrocytes in ApoE/LDLR Double-Deficient Mice

F. C. Alcicek[1], J. Dybas[1], A. Blat[1,2], K. Bulat[1,3], T. Mohaissen[1,4], D. Y. Dogan[5], I. Hornung[5], W. Rutkowska[1,2], A. U. Steinbicker[5], K. M. Marzec[1,6]; [1]Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Krakow, POLAND, [2]Faculty of Chemistry, Jagiellonian University, Krakow, POLAND, [3]Łukasiewicz Research Network - Krakow Institute of Technology, Krakow, POLAND, [4]Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, POLAND, [5]Department of Anesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt, Goethe University, Frankfurt am Main, GERMANY, [6]Łukasiewicz Research Network, Krakow Institute of Technology, Krakow, POLAND.

Red blood cells (RBCs) play a role in the regulation of vascular tone via release of adenosine triphosphate (ATP) into the vasculature. ATP release occurs in response to various stimuli including lower oxygen tension and causes vasodilation and increased blood flow. Interestingly, ApoE/LDLR double deficient (ApoE/LDLR$^{-/-}$) mice, a murine model of atherosclerosis, display higher exercise capacity compared to age-matched controls. However, it is not known whether increased exercise capacity in ApoE/LDLR$^{-/-}$ mice is linked to altered ATP release from RBCs. Therefore, we characterized the ATP release feature of RBCs by exposing them to 3V cocktail (contains 10 µM isoproterenol, 30 µM forskolin, and 100 µM papaverine) and hypoxia in female and male 8- and 24-week-old ApoE/LDLR$^{-/-}$ mice compared to their age-matched controls (N=4-13). Also, alterations in secondary structure of hemoglobin (Hb) and 2,3-diphosphoglycerate (2,3-DPG) levels in RBCs were measured. In addition, we analyzed the expression of pannexin 1 (PANX1) protein on RBC membranes as a conduit for ATP release. The results indicate notable changes in the secondary structure of Hb and higher 2,3-DPG levels in RBCs from ApoE/LDLR$^{-/-}$ mice compared to their age-matched controls. This may suggest that alterations in Hb structure in ApoE/LDLR$^{-/-}$ mice are irreversible. 3V-induced ATP release from RBCs occurred at comparable levels for all groups, which indicates the activity of adenylyl cyclase and the components of upstream signal-transduction pathway are intact. Moreover, hypoxia-induced ATP release from RBCs was higher in ApoE/LDLR$^{-/-}$ mice compared to their age-matched controls, a potential contributing factor and a finding in line with the higher exercise capacity. Taken together, augmented hypoxia-induced ATP release from RBCs in ApoE/LDLR$^{-/-}$ mice indicates a possible altered component in the ATP release pathway. Previously, the role of confirmational change of Hb in hypoxia-induced ATP release from RBCs had been reported. We speculate that the altered Hb structure, or another component related, could lead to the exhibited results in ApoE/LDLR$^{-/-}$ mice. Additionally, the expression of PANX1 protein confirmed its function as the conduit for ATP release. Further studies to characterize the altered components of the ATP release mechanism in ApoE/LDLR$^{-/-}$ could lead to pharmacological modification of pathologically impaired microcirculation. **Acknowledgments:** This research was funded by the Polish National Science Centre, No. UMO- 2020/38/E/ST4/00197.

B731/P2398

M6A Epitranscriptome Mediated RNA Stress Gradule Assembly Governs Blood Development and Regeneration

R. Gunage; Boston children's hospital, Boston, MA

Hematopoietic stem cell (HSC) form diverse cell types and requires transcription and translation regulation. Using CD34+ human hematopoietic stem and progenitor cells combined with N6-methyladenosine (m6A) seq we identified a novel, stress granule regulation during erythropoiesis. Transcriptome-wide single-nucleotide resolution m6A analysis identified ~4800 transcripts to be m6A modified and showed demethylation (>2-14 fold) during erythropoiesis. Genome-wide CHIP-seq of CD34+ cell erythropoiesis with anti-Gata1 showed an enhancer enrichment at the Alkbh5 RNA demethylase gene. CD34+ cell Alk5 CRISPR Cas9 KO led to erythropoiesis failure validating Alk5 role in erythropoiesis. CD34+ Alk5 KO cells showed a failure of RNA m6A demethylation and we discovered stress granule (SGs) transcripts m6A levels to be upregulated (>2-4 fold, p=0.01). SGs are membrane-less organelles composed of various RNA-binding proteins. SGs form a hub in the cytoplasm, govern RNA metabolism and global proteome by translational regulation. We show ATXN2, G3BP1/2, Tia-1, and PABPC1 SGs transcripts are m6a modified at 3'UTR. K562 cell Alk5 KO erythropoiesis model led to massive m6A upregulation on ATXN2 3'UTR (>14 fold, p=0.001) and other SGs transcripts. Mass spectrometry of CD 34+ Alk5 KO cell proteome showed ATXN2 downregulation (>2 fold, -log P value2), confirming 3' UTR m6A mediated regulation. Alk5 KO led to the aggregation of SGs protein in the cytoplasm due to altered SG protein stoichiometry(>4-10 fold, p=0.001). Our Zebrafish Alk5 KO has defective blood development characterized by anemia, and increased myeloid and lymphoid lineages indicating early lineage bias. CD34+ ATXN2 KO cells with colony formation assay showed defects in erythroid and myeloid lineages. Super-resolution microscopy of CD34+ ATXN2 KO cells phenocopied the presence of disease-associated ALS (amyotrophic lateral sclerosis) stress granules with TDP-43, G3BP1/2, PABPC1, and Tia-1 enriched for m6A mRNAs. Next, the K562 Alk5 KO erythropoiesis model and ATXN2 lentiviral overexpression effectively resolved stress granules and rescued erythropoiesis. In summary, ATXN2 mRNA m6A regulates SG biology to affect HSC function, impacting blood development. SG- mediated regulation of HSC fate may highlight new therapeutic strategies for blood disorders.

B732/P2399

How does the red blood cell lose its nucleus: intrinsic and extrinsic factors regulating erythroid enucleation

L. M. Newton[1], C. B. Wölwer[1], K. Lim[1], E. D. Hawkins[2], P. O. Humbert[1]; [1]La Trobe University, Melbourne, Australia, [2]The Walter and Eliza Hall Institute, Melbourne, Australia

Red blood cells (RBCs) are produced rapidly in the bone-marrow of mammals, and undergo a series of cell divisions during development, culminating in the asymmetric separation of the future erythrocyte and its nucleus, in a process termed enucleation. Enucleation is the rate limiting step in the production of erythrocytes *in vitro* and our work aims to better understand enucleation to improve the production of RBCs for transfusion medicine. Our lab identified cyclin-dependent kinase-9 (CDK9) as a novel cell-intrinsic regulator of the enucleation process. To understand how CDK9 drives enucleation, I have utilised primary mouse models and human cell line models of erythroid differentiation to characterise the expression and localisation of CDK9 during erythroid differentiation and enucleation and have identified CDK9-associated proteins using mass spectrometry. Surprisingly, our data suggests a novel

role for CDK9 in enucleation independent of its usual role in RNA polymerase II-mediated transcription. In this talk, I will share how CDK9 localises to the future erythrocyte during enucleation and acts upstream of other enucleation regulators, including actin polymerisation and calcium signalling. To examine cell-extrinsic factors in enucleation, we have conducted studies to identify the enucleation "niche" for erythroblasts within the bone marrow. Enucleation is thought to occur in structures termed erythroblastic islands which consist of a central macrophage surrounded by developing erythroblasts. Importantly, it is unclear if or how these erythroblastic islands modulate enucleation, and how they respond to various disease states like anaemia. Here, we have used confocal microscopy in optically clear mouse bone marrow with erythroid and macrophage lineage transgenic fluorescent reporter mice to quantitate sites of enucleation and their proximity to vascular sinuses and bone marrow resident macrophages within the enucleation "niche" *in vivo*. This analysis has been complemented with live imaging of erythroblast enucleation *in vitro* by scanning-disc microscopy and *in vivo* using intravital imaging of mouse bone marrow. These studies provide novel insights into the dynamic nature and heterogeneity of erythroblastic islands and the intrinsic and extrinsic factors regulating erythroid enucleation.

Tuesday, December 6, 2022

New Technologies for Cell Biology

B1/P2400

Highly Multiplexed Tracking of Signaling Dynamics in Organoids Using Biosensor Barcoding

J. Yang, C. Chen, C. Huang, S. Wang, L. Wood, T. Larman, C. Huang; Pathology, Johns Hopkins Unive, Baltimore, MD.

To understand cellular processes at the molecular level, it is often necessary to monitor the temporal dynamics of multiple signaling, metabolic, and other molecular activities as well as their responses to perturbations. Genetically encoded fluorescent biosensors are powerful tools to track these activities in real time in live cells. However, the availability of spectral space limits the number of biosensors that can be simultaneously imaged. We recently overcame this problem by developing the "biosensor barcoding" method. In this method, a pair of barcoding proteins composed of spectrally separable fluorescent proteins targeted to distinct subcellular locations are used to label cells expressing different biosensors. Our method achieves simultaneous tracking of dozens of signaling activities in live cells, but it has only been tested in immortalized 2D cell lines, which do not preserve the 3D cell-cell interactions that govern various cell behaviors. In this regard, organoids derived from primary tissues or tumors recapitulate the 3D cell connections and are better models to address a range of questions such as development, tumor heterogeneity, drug screening, and tumor/microenvironment interactions. The biological and morphological features of organoids pose unique challenges when applying the methods and analysis pipeline developed in 2D cell lines. To extend biosensor barcoding to 3D organoids, the objectives are: 1) to establish a biosensor barcoding expression system in organoids; 2) to train deep learning models for efficient and accurate identification of barcoded organoids; and 3) to simultaneously image barcoded organoids expressing different biosensors. Using a set of lentiviral vectors, we successfully expressed barcoding proteins in two types of organoids with different morphologies: those derived from normal mouse intestinal epithelium and those derived from human pancreatic ductal adenocarcinoma. Images

of barcoded organoids revealed distinct subcellular localization of barcoding proteins that are used for optimizing deep learning models. Moreover, co-expression of barcoding proteins and biosensors in organoids showed robust spectral separation. In conclusion, our results demonstrate the feasibility of biosensor barcoding in organoids and pave the way for highly multiplexed imaging of the complex dynamics of molecular networks in cells interacting in a 3D environment.

B2/P2401

High-dimensional morphology analysis enables identification and label-free enrichment of heterogeneous tumor cell populations

A. Jovic, K. Saini, T. Pham, C. Corona, J. Mei, M. Phelan, S. Boutet, K. B. Jacobs, J. Kim, C. Johnson, V. Lu, N. Li, M. Salek, M. Masaeli; Deepcell, Inc, Menlo Park, CA.

Studying cancer at single cell resolution is crucial in elucidating tumor heterogeneity and guiding targeted therapy options. However, current isolation of tumor cells from tissue typically relies on biomarkers, such as EpCAM, which miss EpCAM-negative but interesting populations. The Deepcell platform enables high-dimensional morphology analysis of unlabeled single cells using artificial intelligence (AI), advanced imaging, and microfluidics allowing for higher resolution of population heterogeneity beyond protein expression markers. We report application of the Deepcell platform by training a convolutional neural network classifier to identify and enrich for carcinoma cells from non-small cell lung cancer (NSCLC) dissociated tumor cell (DTC) samples based on morphology. The enriched NSCLC cells are label-free, unperturbed, and viable, making them amenable to diverse molecular and functional analyses. The cell images are used to generate high-dimensional morphological profiles that can be visualized by UMAP to uncover heterogeneity within cancer cell populations. Additionally, these morphological signatures can also be used to sort out subpopulations for further molecular and discovery work.

We verified enrichment of malignant cells by performing genomic analysis on Deepcell-sorted samples. scRNA-Seq analysis showed cell populations with high levels of EpCAM expression in sorted, versus pre-sorted cells. CNV analysis demonstrated increased amplitude of deletion and amplification peaks relative to pre-sorted DTC samples. Further, mutational analysis showed increased allele frequency of mutations, including P53 and KRAS, in sorted versus pre-sorted samples.

Multiple clusters of morphologically distinct tumor cell populations were detected by UMAP analysis. We further trained the AI classifier to detect and enrich for each of these subpopulations, and subsequent CNV, mutation, bulk RNA-Seq, and scRNA-Seq analyses revealed molecular differences between the morphologically distinct subgroups. Together, our data suggests AI-detected morphological differences between cell populations may establish a biologically significant link between morphology and molecular signatures. Thus, we demonstrate the use of the Deepcell platform to perform high-dimensional morphological profiling of NSCLC tumor cells from DTCs, revealing a new dimension for identifying heterogeneous tumor cell populations.

B3/P2402

T cell expansion and viability from filtered media

A. Basu[1], J. Smith[1], J. Broe[2], A. Chatterjee[2]; [1]MilliporeSigma, Burlington, MA, [2]MilliporeSigma, Bedford, MA.

Contamination-free medium containing cytokines, serum, and related supplements keep T cells viable and ready for therapies and implantation. Filter-sterilization is the preferred method using sterile, vacuum-based filter cups to remove particulates and contaminants (ex. microorganisms, bacteria, etc.) greater than the rated pore size. Preserving key growth factors and supplements post-filtration is paramount for T cells to function. Cytokines like Interleukin-2 (IL-2), when added to growth medium, help in expansion, viability, and related functional activities like abating inflammatory responses and equally priming naïve T cells for regulating immune responses. The objective of this study was to evaluate filtered prepared media for T cell culturing. We formulated T cell medium (Hematopoietic cell medium, Human Serum, and IL-2) and filtered the media once (1x), five times (5x), and up to ten times (10x) using sterile, low protein binding filter cups containing 0.22 µm polyethersulfone (PES) or polyvinylidene fluoride (PVDF) membranes. We looked at the impact of filtration of key media components for T cell expansion and viability by utilizing tools like flow cytometry (multiplex flow assays and growth curve examination), IL-2 ELISA, metabolite and glucose culture parameter detection, and hand-held automated cell counters. The results show that the different T cell phenotype markers, $CD3^+$ (≥ 99%) and $CD4^+/CD8^+$ (≈65%/≈35%), are consistently expressed regardless of filtration type and frequency after initial experimentation and at day 5. IL-2 concentration in control and all filtered media (up to 6 days) remained comparable (≈ 2 ng/ml). T cell viable cell density, concentration, and diameter (average: 12.7 ± 0.4 µm) also showed no significant deviation from each filtered media set and different culture periods. Our data highlights the ability to expand T cells in PES- and PVDF-filtered media without a noticeable effect on T cell viability. Vacuum-based filtered media can present a worry-free expansion and constant viability for T cells, aiding in the production of T cell therapeutics.

B4/P2403

A framework for high-throughput and comparative cell biology

P. Avasthi, D. Matus, D. Mets, **R. York**; Arcadia Science, Berkeley, CA.

Cells display an astonishing amount of phenotypic variation. Mapping the extent of this diversity - and the evolutionary processes sculpting it - could accelerate the discovery of novel cellular mechanisms, organisms, and, potentially, general biological principles. A central need in this effort are tools for the unbiased measurement and comparison of cellular traits (which are often high-dimensional, time varying, and complex). Here we present our attempts toward generating some of these tools, focusing first on an especially nuanced functional trait: cellular motility. Using both publicly available and novel data sets, we show that the movement of diverse cell types can be represented with a discrete set of canonical cellular shapes. These shapes can be used to generate a low-dimensional universal 'motility space' that captures the full range of cellular dynamics available. Variation across many aspects of movement can be mapped using this space, identifying broad differences within and among cell types. Furthermore, complex cellular structure-function relationships can be uncovered and we develop a metric for comparing these relationships across evolution. Finally, we discuss this framework in the context of a larger effort to perform high-throughput phenotypic and genomic measurements among diverse microbial eukaryotes. This work intends to provide open and broadly-available tools for

comparing cellular phenotypes across evolutionary scales, potentially opening new pathways for dissecting the origins and generation of cellular diversity.

B5/P2404

SmartHeart - a novel 3D *in vitro* assay for improved assessment of cardiac drug efficacy and toxicity

M. Seguret[1], P. Davidson[2], S. Robben[2], C. Jouve[1], C. Cerveau[2], J. Hulot[3], M. Le Berre[2], **R. S. R. Ribeiro**[2]; [1]INSERM, PARCC, Université de Paris, Paris, FRANCE, [2]4Dcell, Montreuil, FRANCE, [3]INSERM, PARCC, Université de Paris and CIC1418 and DMU CARTE, Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Européen Georges-Pompidou, Paris, FRANCE.

According to WHO, every year there is an average of 18 million people dying out of cardiac diseases. A substantial part of these diseases is due to cardiomyopathies that affect many young people. Despite all the advances made in medicine in the last decades, there is still a long way to go before having robust disease models and efficient development of new therapies. Nowadays, cardiac tissue engineering is becoming one of the most common ways to mimic the main events encountered in these types of pathologies. To answer to these questions, we have developed a novel 3D cardiac assay, which enables both the formation and maturation of cardiac organoids based on cardiomyocytes derived from hiPSC as well as the acquisition of the most relevant redouts, all in single platform. To form the cardiac organoids, cell culture plates (96WP) are coated with a 3D structured PEG hydrogel that has an array of conical-shaped microwells containing a central pillar. When cells are seeded on top of these microstructures, they are guided towards the ring-shaped cavity, self-assembling into a circular organoid, which surrounds a hydrogel central pillar. Cell contractility force, beating rate and beating amplitude are easily measured through the contraction of the central pillar, whose stiffness is known and can be adjusted to the cell model. Using this model contraction of the tissue, i.e. restored cell beating, was observed in less than 24 hours after cell seeding. In addition to this, the effect of calcium channel blockers (Verapamil) was investigated, showing an inotropic effect in agreement with the literature. The effect of calcium concentration increase in the medium was also studied, demonstrating an increased contraction force until a plateau is reached around 1 mM. Due to the design of the microwells and the optical transparency of the hydrogel, the assay is completely compatible with image-based acquisition techniques. Therefore, besides the readouts previously stated, ionic exchanges such as calcium transients have been observed using a calcium sensitive dye, revealing an expected increase of intensity during CMs contraction. Similarly, spatial organization of cells and intracellular morphology was observed using immunofluorescence, showing for instance, the elongation of the CMs in the tissue. To summarize, the SmartHeart assay enables the fast assembly and maturation of functional cardiac organoids and the acquisition of many relevant read-outs, all of this in a single platform. In the future it can be used to test new compounds or assess toxicity of drugs targeting other types of pathologies, as it can be simply scaled to fit HTS and HCS requirements.

B6/P2405

Toward long-term lineage tracing and event recording in mammalian cells

T. B. Loveless, C. C. Carlson, C. A. Dentzel Helmy, V. J. Hu, C. C. Liu; Department of Biomedical Engineering, University of California-Irvine, Irvine, CA.

During the development of multicellular organisms, and in aberrant developmental processes such as cancer, cells undergo a series of stresses, gene expression changes, and other transient events, some of

which determine their ultimate fates. The historical events preceding most cell fates are unknown. Even less is known about which historical events *cause* a cell fate and which merely accompany it. DNA recorders are synthetic biology tools that enable cells to make a durable DNA record of ephemeral events in their history, in principle enabling biologists to correlate any type of cellular event to long term changes in the cell's phenotype. Moreover, by recording the relative timings of the events and the fate changes, DNA recorders should make it possible to form better hypotheses about which events cause which fate changes. Our DNA recorder, peCHYRON, uses prime editor to make insertion mutations at a genomic locus in temporal order. The mutations are directed by a collection of prime editor guide RNAs (pegRNAs); each pegRNA can add to the locus, and the exact mutation added indicates exactly which pegRNA directed it. Therefore, peCHYRON could in principle be used to record, in parallel, many different types of events. Here, we will report peCHYRON recording over weeks to months.

B7/P2406

Learning a meaningful representation of cell fate using variational autoencoders

A. R. Lowe[1], C. Soelistyo[2], G. Vallardi[2], G. Charras[2]; [1]Alan Turing Institute/UCL, London, UNITED KINGDOM, [2]UCL, London, UNITED KINGDOM.

Cell fate has an important role in many cellular quality control mechanisms. However, our understanding of how cell-scale topological and physical parameters determine cell fate remains elusive, largely due to the complexity of manually analyzing a time varying system of many cells. Here, we explore the idea of using machine learning to decipher the biophysical determinants of cell fate directly from a large corpus of time-lapse microscopy data. Our approach uses unsupervised deep learning to extract interpretable features of the image data, coupled to a temporal model to predict the fate of each cell in a mixed epithelium. Remarkably, once trained, our model is able to accurately predict the fate of each cell, given only a sequence of images prior to a fate being observed. Further, we show that the model is interpretable; learning to represent cell type, local tissue topology and nuclear morphology amongst many other parameters, directly from the image data. Remarkably, in the case of mechanical cell competition, our model learns to represent local cellular density, and use this to make accurate predictions of cell fate. Finally, we use genetic perturbations and drugs that block signaling pathways involved in the mechanism to test the learned representation. Since the model generates hypotheses for the fate of each cell based on the observations, we can use it to identify systematic deviations arising from perturbations of the underlying mechanism. Our results suggest that machine learning can be a powerful new tool to decipher the timescale and determinants of cell fate, enabling a new platform for automated hypothesis generation and directed experimental studies.

B8/P2407

Classification and enrichment of carcinoma cells in real-time based on high-dimensional morphology enables increased sensitivity of molecular and cytology analysis

A. Mavropoulos[1], J. Cruz[1], J. Nieto[1], K. Saini[1], W. Austin[1], M. Phelan[1], J. Mei[1], J. Kim[1], W. Yu[2], C. Johnson[1], V. Lu[1], S. Boutet[1], T. D. Lee[2], N. Li[1], J. Rao[2], M. Salek[1], M. Masaeli[1]; [1]Deepcell, Inc, Menlo Park, CA, [2]University of California, Los Angeles, Los Angeles, CA.

Cytopathology studies and diagnoses disease at the cellular level, but it is currently restricted by low-throughput, subjective, laborious methods and low sensitivity. For example, the low abundance of malignant cells in a high non-malignant cell background limits the use of effusion samples for liquid

biopsy purposes. The Deepcell platform combines artificial intelligence (AI) and advanced imaging to classify and sort unlabelled cells in real-time, and generate quantitative morphology data. Here, we applied the Deepcell platform to the sensitivity challenges of effusion samples and demonstrated the ability to image, classify, and enrich carcinoma cells from patient-derived ascites and pleural effusions based on high-dimensional morphology and increase sensitivity of cytology and molecular analysis.
The Deepcell model was trained using >3 million cell images from malignant effusion samples, and in silico validation showed high model classification performance to identify malignant cells (AUC=0.96). The model detected carcinoma cells in 100% of cytologist-defined malignant effusion specimens (n=22), and percent frequency was concordant to ground truth. High-dimensional, quantitative morphology data from the cell images were used for UMAP analysis of the embeddings, which showed carcinoma and benign cells clustered distinctly. This suggests the AI captured morphological differences in carcinoma and benign cells, and more interestingly, that these morpholomic signatures correspond to cell biology. Furthermore, Papanicolaou staining showed higher purities in sorted versus pre-sorted samples, verifying carcinoma cell enrichment by the platform. CNV analysis and targeted sequencing of sorted samples showed increased amplitude of deletion/amplification peaks and increased TP53 mutation frequency, respectively. scRNA-seq analysis showed EpCAM+ tumor cell enrichment, and interestingly, EpCAM-Claudin4+CD24+ tumor cell subsets.
Thus, results of the Deepcell platform are consistent with cytology and biomarker-based results, with added benefits of greater sensitivity and enrichment of live carcinoma cells present in effusion samples. Additionally, high-dimensional morphology analysis provides a new dimension that can be integrated into single cell multi-omic modalities to elucidate tumor cell heterogeneity within effusions to ultimately improve precision medicine.

B9/P2408

Combined Immunofluorescence - In Resin Fluorescence Morphology (IF-FIRM) Microscopy provides histologic and cellular context to highly resolved fluorescence markers

C. Chalouni, A. Kumar Katakam, M. Sagolla, M. Reichelt; GENENTECH, South San Francisco, CA.

Immunofluorescence labeling and imaging of cell and organelle markers in cells and tissues is a key method of cell biology and biomedical research. Single, multiplex or superplexed immunofluorescence microscopy is used in biomedical research involving tissues in addition to more classic approaches that combine immunohistochemistry with chromogenic stains and morphological stains like H&E. One advantage of using IF isthe ability to perform higher order multiplexing of targets of interest.A major limitation of performing immunofluorescence imaging in complex tissues is the lack of histological cellular and subcellular morphological context. Similar to landmarks in a geographical map, morphological context provides valuable information about the localization of a fluorescence marker in a particular tissue region, cell type or relative to histopathological changes.To address these shortcomings, we have established IF-FIRM (Fluorescence in Resin Morphology) that combines immunofluorescence labeling of resin-embedded tissue sections with the staining of the sections with the organic dye cresyl violet (chemical formula: $C_{19}H_{18}ClN_3O$) to visualize tissue morphology. To establish and evaluate our IF-FIRM method we used resin-embedded pancreas or kidney tissue, and labeled it with antibodies against insulin and pancreatic alpha-amylase, or the kidney marker podocalyxin, respectively. In addition, the labeled sections were stained with the commercially available FIRM stain (cresyl violet) purchased from Electron microscopy sciences (EMS). For imaging we used an epi-fluorescence microscope (Zeiss Axioimager) or a fully spectral confocal microscope (Leica SP8 with white

light laser andspectral detectors) to enable better spectral separation of the FIRM fluorescent signal from the fluorophores used in the IF staining.With this approach we could readily identify by morphology particular tissue regions, such as the Islet of Langerhans in the pancreas, or glomeruli in the kidney sections. In addition, blood vessels, endothelium, epithelium, erythrocytes and subcellular structures and organelles such as nuclei, nucleolus, heterochromatin, basement membranes are unequivocally identifiable by the morphological FIRM stain.Combination of morphology signal (image) with the specific immunofluorescence stains (insulin, amylase, podocalyxin) localized these markers correctly within the histologic context of the Islet of Langerhans or kidney glomeruli.In conclusion, IF-FIRM provides valuable histological and cellular context to highly resolved immunofluorescence markers for fluorescence microscopy applications in tissue research.

B10/P2409

CRISPR-Cas9 genome editing in iPSCs for functional genetic screening

N. Isachenko[1], D. Hu[1], A. Chenchik[2], **P. Diehl**[1], D. Tedesco[1]; [1]Cellecta, Inc., Mountain View, CA, [2]Cellecta, Inc., MOUNTAIN VIEW, CA.

Induced pluripotent stem cells (iPSCs) are widely used for disease modeling, drug discovery, and cell therapy development. However, iPSCs are difficult to engineer with an efficient CRISPR/Cas9 system for functional genetic screens. We used WTC11 human iPS cells to generate functionally validated lentiviral transduced Cas9 lines, characterized by high gene-editing activity and sustained potential to differentiate. Flow cytometry was used to check for the expression of pluripotency markers Oct3/4, TRA1-60, and SSEA-4 in the iPS-Cas9 cells, and their actual pluripotency was then confirmed by testing the ability to differentiate into the three germ layers: ectoderm, mesoderm, and endoderm. Furthermore, genome-wide transcriptome analysis was performed to confirm the expression of germ layer-specific markers in the differentiated cells. Sustained Cas9 activity was confirmed in the differentiated cells. This study provides proof-of-principle that patient-derived iPSCs can be used to enable CRISPR/Cas9 functional genetic screening technology in reconstituted patient-specific tissues/ disease models.

B11/P2410

The Cyto R1: A Multifunctional Biophysical Sample Preparation Tool for Sorting, Filtering, and Enrichment

R. Barua[1], K. E. Degen[1], D. Thomas[1], D. Keck[1], A. Straka[2], S. Manns[3], K. Kinskie[2], C. Coogan[4], A. Nelson[2], K. David[2], R. V. Davalos[2], A. R. Hyler[1]; [1]CytoRecovery, Inc., Blacksburg, VA, [2]Biomedical Engineering and Mechanics, Virginia Tech, Blacksburg, VA, [3]Bioengineering, George Mason University, Fairfax, VA, [4]Biochemistry, Virginia Tech, Blacksburg, VA.

The Cyto R1 is an integrated benchtop platform providing multifunctional solutions for sample preparation. The core of the platform is a microfluidic device, Cyto Chip, that utilizes a unique array of over 25,000 insulating microcylindrical posts positioned between two parallel electrodes in order to generate a nonuniform electric field, a technique called dielectrophoresis (DEP). The unique architectural design and positioning of the micro-posts and electrodes facilitates the creation of electrical traps that can be used to capture and hold a targeted cell subpopulation while sustaining a sterile and untouched sample with high viability. This dielectrophoretic Cyto Chip exploits the interaction of the targeted cell with a nonuniform electric field to exploit biophysical properties of cells

such as cell size, membrane morphology, and nuclear size. The strength and direction of the DEP force on a targeted cell is dependent on the frequency of the applied electric field as well as the dielectric properties of the cell and suspending media. Preliminary experiments with the Cyto R1 Platform have demonstrated the use of our technology for cell sorting, filtration, and enrichment. The Cyto R1 successfully facilitated the separation of peripheral blood mononuclear cells (PBMCs) from a co-culture with ovarian cancer stem cells (CSCs), both from mice. From a 56.2% CSCs and 43.8% PMBCs co-culture, we were able to deplete 50% of the PMBCs while retaining the original CSC population. Similarly, the Cyto R1 chip has demonstrated its potential for filtering biological debris and satellite glial cells in order to purify a sample of murine trigeminal ganglia (TG) neurons. Lastly preliminary evidence has indicated the use of the Cyto R1 to enrich cell concentrations from 10^3 to 10^5. Ongoing work is the continued development of the Cyto R1's enrichment capabilities and quantifying the throughput and effectiveness of enrichment.

B12/P2411

Bioelectric regulation of tissue hydrostatic pressure in tissue spheres and organoids

G. Shim[1], I. Breinyn[1], A. Calvo[1], D. J. Cohen[2]; [1]Princeton University, Princeton, NJ, [2]Princeton Bioengineering Institute; Mechanical Engineering, Princeton University, Princeton, NJ.

Direct-Current (DC) electric fields exist throughout the body and are involved in a range of cellular responses, from directed cell migration (electrotaxis) to transcriptional regulation. New approaches to use electrical stimulation to program and control biological systems will therefore be very useful. Previously we focused on electrical control of collective migration in epithelial monolayers, but here we demonstrate how those same electrical cues reprogram 3D growth in lumenized tissues such as hollow kidney spheroids and intestinal organoids. In particular we found that electrical stimulation induced a rapid, powerful, and reversible swelling in these tissues by regulating water transport. Here, we discuss how external electrical stimulation can control the inflation of lumenized structures and cellular mechanisms mediating the response.We first studied hollow kidney spheroids (MDCK) within a hydrogel (Geltrex) and integrated into our SCHEEPDOG electrobioreactor. This allowed complete timelapse imaging (phase contrast, epi-fluor, and confocal) during electrical stimulation. Lumenized MDCK spheroids inflated significantly with 4 hrs of electrical stimulation with the volume almost doubling due to water transport. The rate and magnitude of inflation increased as the magnitude of the electrical field increased, with overly strong field gradients inducing 'popping'. We hypothesized that the dense actomyosin network at the apical surface of the structures provided a physical limit to the inflation response, and showed that inhibiting actomyosin contractility prevented rupture and increased the maximum swelling volume. We used ion channel inhibitors to determine that the majority of the response can be attributed to the CFTR ion channel (apical chloride channel) and NKCC channel (basal sodium/potassium channel) ultimately driving water flux into the tissue lumen and generating pressure. We have also developed a computational model relating applied direct-current electric fields, electrochemistry, and tissue hydrostatic pressure. This model can accurately describe the bulk swelling response, and also predicts an asymmetric distribution of ions around the spheroid. This is consistent with 3D imaging data demonstrating that cells on the cathode-side of the spheroid swell slightly while cells on the anode-side dramatically thin and stretch. Finally, we are now exploring how general electrically mediated swelling is, and we have recently replicated key swelling data using mouse intestinal organoids. That ionic electric currents can regulate or program tissue hydrostatic pressure

could help inform our understanding of how environmental factors regulate multicellular development, as well as possibly providing a new tool for tissue engineering.

B13/P2412

Polymerized Laminin-521 Promotes Large-Scale Human iPSC Expansion at Low Protein Concentrations

C. Hochman-Mendez; Regenerative Medicine Research, Texas Heart Institute, Houston, TX.

Laminins (LN) play a central role in the self-assembly and maintenance of basement membranes and are involved in critical interactions with cells and other extracellular matrix proteins. Among defined and xeno-free ECM culture matrices, LNs, namely LN-511 and LN-521, have emerged as promising coating systems for large-scale expansion of induced pluripotent stem cells (iPSCs). LN's biological activity stems from its self-polymerization into a cell-associated network. Polylaminin (polyLN) is an acidification-induced LN polymer that can recapitulate the native-like polymeric array in a cell-free system. Here, we show for the first time that polyLN-521 displays a hexagonal-like structure and that polyLN-521 permits large-scale human iPSC expansion at basal and low concentrations. Human iPSCs expanded with polyLN-521 maintained the pluripotent state, and the karyotype stability and telomere length were not impaired. These results suggest that polyLN-521 is a stable and cost-effective coating for large-scale iPSC expansion and illustrate the ability of polyLN-521 to reproduce a native-like polymeric structure.

B14/P2413

A New Service Network for Cryo-Electron Tomography

A. Hoenger, K. Luger, M. H. B. Stowell; Mol. Cell. and Dev. Biology, University of Colorado-Boulder, Boulder, CO.

The CU Boulder Center for Electron Tomography or CCET is part of an NIH common fund initiative to broaden access to high resolution cryo-electron microscopy and tomography. More information about the initiative can be found at the URL shown https://www.cryoemcenters.org/. CCET is part of a network four centers including the Stanford SLAC Specimen Preparation Center (SCSC) the National Center for In-Situ Tomographic Ultramicroscopy (NCITU) and the Midwest Center for Cryo-Electron Tomography (MCCET) which serves as the network hub.CCET has a dedicated staff for assisting users in sample preparation for cryo-ET. Staff members are trained in the use of modern cryo-ET methods including high-pressure freezing, plunge freezing, FIB-SEM milling, correlative light and electron microscopy (CLEM), tomographic data collection and modelling. We welcome relevant projects from a nationwide audience for consulting and help with data collection. We host visitors for training and education.

B15/P2414

Probing mechanotransduction pathways through visualization and control of vinculin conformation

M. Choi*, J. M. Szulczewski*, S. Hu, K. M. Hahn; Pharmacology, University of North Carolina At Chapel Hill, Chapel Hill, NC.

*These authors contributed equally.

Vinculin is important in multiple contexts where cells must measure and respond to mechanical force. It acts via a conformational latch mechanism where stretching produces an open conformation that enables binding to downstream effectors. We are specifically studying how cells use vinculin-containing structures called podosomes to probe the rigidity of potential targets during phagocytosis, and how

cancer cells navigate between tumors and the vasculature by sensing the rigidity of aligned collagen. Podosomes oscillate like pistons against phagocytic targets, with frequency dependent on target stiffness. Metastatic cells use vinculin-containing focal adhesions to monitor and respond to the rigidity of collagen fibers. In each case it is difficult to probe hypotheses coupling mechanical changes in vinculin to downstream signaling because interactions are transient and localized. We are therefore developing vinculin analogs for use in living cells to 1) report vinculin conformational state, and 2) control vinculin activity with light. To visualize the exposure of hidden binding sites in vinculin, a short peptide chain was inserted where it is exposed only in the open, "stretched" conformation. When exposed, the tag can be accessed by a small fluorescent protein, so simple colocalization of vinculin and this protein indicates the open conformation. To control vinculin's transmission of force, we sought to allosterically modulate vinculin-actin interaction. Previous studies have shown that protein activity can be controlled by inserting the LOV2 domain, which undergoes conformational changes when irradiated. We have thus made photoinhibitory and photoactivated vinculin analogs. We will show initial application of these analogs and fluorescent biosensors to study cell motility on micropatterned substrates that mimic the tumor microenvironment.

B16/P2415

Spatial and temporal localization of nucleic acid targets in FFPE tissue samples with the novel loopRNA™ ISH technology

D. M. Holzapfel, PhD, J. Coleman, PhD; Enzo Biochem, Farmingdale, NY

Spatial and temporal localization of DNA or RNA targets in formalin-fixed, paraffin-embedded (FFPE) cells and tissue samples can be achieved with *in situ* hybridization (ISH) technique, while preserving the morphology of the cell or tissue. ISH is used for the identification and localization of viral infections, analysis of transcriptionally active nucleic acid and its distribution in cells and tissues and identify sites of gene expression. While immunohistochemistry (IHC) shows protein localization in cells and tissue samples, ISH can help identify the cell of origin. In order to achieve successful results, we used AMPIVIEW™ ISH probes, which combines the precision of targeted, sequence-specific RNA probes with a superior sensitivity of loopRNA™ technology, making them compatible with nanopolymer detection systems used in IHC. This powerful tool can be designed to detect the nucleic acid expression patterns and spatial localization of individual targets or DNA-RNA, RNA-RNA interactions. In addition, RNA-protein interactions in tissues can be studied when combined ISH and IHC. This study explains how these probes are used not only for the detection of viruses such as SARS-CoV-2 and human papillomavirus (HPV), but also the expression of endogenous biomarkers such as HER2/neu in FFPE tissue samples.

B17/P2416

An Expandable FLP-ON::TIR1 System for Precise Spatiotemporal Protein Degradation in *C. elegans*

Y. Xiao[1], C. Yee[2], M. A. Q. Martinez[1], C. Zhao[1], W. Zhang[1], D. Q. Matus[3], C. Hammell[4]; [1]Stony Brook University, Stony Brook, NY, [2]Stanford University/HHMI, Stanford, CA, [3]Arcadia Science, Berkeley, CA, [4]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Organismal development relies on the proper spatial and temporal control of gene expression. The auxin-inducible degradation system has been widely adopted in *C. elegans* for its ability to control the spatiotemporal patterns of target protein expression. While the AID system has provided a key step forward in controlling endogenous protein activity, it still needs further optimization, especially for cell-

type-specific protein degradation. Previous studies reported insufficient depletion of multiple AID*-tagged proteins using tissue or cell type specific promoters which fails to recapitulate the genetic null phenotype, leaving only hypomorphic defects to be detected. Given the modular nature of the AID system we hypothesize that one of the potential limiting factors in these experiments is insufficient expression of TIR1 when driven via spatially restricted promoters with dynamic activity. Here, we aim to boost TIR1 level using a FLP/FRT based recombination binary system. In this study, we engineered a hybrid transgenic system that programs cell type specific FLP activity to animate a dormant, high-powered promoter to drive optimized $_{At}$TIR1(F79G) expression in a cell type specific fashion. This composite system enables high activity TIR1-dependent degradation to be achieved in specific cell types without a dependency on cell-type specific promoter strength. We benchmark the utility of this system by generating a number of tissue specific FLP-ON::TIR1 drivers to reveal genetically separable cell-type specific phenotypes for a variety of proteins expressed in multiple tissues during *C. elegans* post-embryonic reproductive development. We also demonstrate that the FLP-ON::TIR1 system is compatible with other AID degron sequences, like mIAA7, which is optimized for faster degradation kinetics. Finally, we generate an expandable toolkit utilizing the basic FLP-ON::TIR1 system that can be adapted to drive optimized $_{At}$TIR1(F79G) expression in any tissue or cell type of interest for targeted protein degradation. We concluded that our FLP-ON::TIR1 system could further facilitate the expansion of the genetic toolbox for *C. elegans*, enabling more sophisticated temporal studies of cell and developmental processes to be addressed.

B18/P2417

Automated Tissue Culture Workflow for Plating, Maintenance, and Differentiation of hiPSCs on Glass Bottom Plates for 3D Live Cell Microscopy, and their Cryovial Banking for Freezing and Distribution

B. Gregor, A. AICS, N. Gaudreault; Allen Institute for Cell Science, Seattle, WA

Title: Automated Tissue Culture Workflow for Plating, Maintenance, and Differentiation of hiPSCs on Glass Bottom Plates for 3D Live Cell Microscopy, and their Cryovial Banking for Freezing and Distribution

Authors:Benjamin Gregor, Allen Institute for Cell Science, Nathalie Gaudreault

Abstract:

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. We do this by visualizing hiPSC lines expressing fluorescently tagged proteins that represent a specific cellular structure or organelle using 3D live cell microscopy. These gene-edited hiPSCs and all our other openly available scientific resources can be found at allencell.org.. To produce a large number of standardized cell images for analysis, we have developed a fully automated workflow on a Hamilton Star. This robotic liquid-handling platform can perform hiPSC culture and cryovial banking seamlessly. We have also automated the seeding, maintenance, passage, and differentiation of edited hiPSCs at timed intervals, on 6-well plastic plates for propagation, and 96-well glass bottom plates conducive to high-resolution 3D microscopy. Here, we describe this automated workflow that has been instrumental in the successful scale-up and optimization of our work, along with details such as aspiration and dispense speeds, movements of plates, and seeding strategies. Upon validating our methods through a side-by-side comparison of quality control results obtained from manual and automated modes, we found that automation considerably improved our pipeline by providing more uniformity, reproducibility, and overall cell quality while eliminating operator-to-operator variability and the need for weekend

maintenance of cell lines. We have also developed an automated image-based colony segmentation and feature extraction pipeline to streamline the process of predicting cell count and selecting wells with consistent morphology for high-resolution 3D microscopy. Overall, the automation of our cell culture platform with robotic equipment and the use of image processing algorithms not only increased consistency and reproducibility, but also ensured standardization and scalability. We found that our method considerably decreased error and variability introduced by manual repetitive handling of cultures and reduced the need for stringent training standards.

B19/P2418

Simplified, open-source analysis of DNA-binding proteins

M. Dilsaver; Lumicks B.V., Waltham, MA

The structure, regulation, and maintenance of DNA is critical to cellular function. Errors in these underlying mechanisms play a major role in diseases such as cancer. As a result, insights into the molecular details are needed to further our understanding, improve biological models, and develop novel therapeutics. Single-molecule techniques have emerged as useful tools for uncovering the activity and function of DNA-binding proteins in real time. Furthermore, the data from single-molecule techniques allows for an accurate quantification of many molecular properties, such as binding location, kinetics, etc. For many researchers, however, developing an analysis pipeline to quantify single-molecule data is challenging due to the required time, resources, and expertise. To address these obstacles, we developed an automated, open-source image processing and data quantification toolbox. We show that our toolbox allows for easy, rapid, and intuitive processing of single-molecule data. Using systems like the gene editing CRISPR/Cas9 system, we demonstrate that our toolbox 1) reports DNA binding specificity at the base-pair level, 2) measures binding constants (kon/koff) from the direct visualization of individual complexes interacting with DNA, and 3) allows for the accurate quantification of enzymatic processivity and protein diffusion. Overall, we show that our simplified, open-source data analysis platform allows for automated processing of single-molecule data and rapid characterization of virtually any DNA-binding protein or polymerase. This toolbox is accessible to all researchers and will enhance efforts to understand a wide range of normal and pathological DNA processes.

B20/P2419

Survey of Cell Line Compatibility with Cells-to-C_T™ for Direct RT-qPCR Analysis of Gene Expression

E. Quandt, J. Bhandari, L. Chapman; Thermo Fisher Scientific, Austin, TX

Cells-to-C_T™ is a direct lysis method that accelerates and simplifies gene expression analysis by real-time reverse transcription quantitation PCR (RT-qPCR) by eliminating the need for traditional RNA purification, instead a cell lysate is used directly as a template for RT-qPCR. This method has been shown to be compatible with many, but not all, commonly used cell types. For this reason, it is recommended that each new cell type be evaluated for compatibility before proceeding with experimentation. In order to expand the utility of Cells-to-C_T as well as to determine the underlying reasons why some cells lines are incompatible, we evaluated the performance of Cells-to-C_T on a panel of previously untested cell lines. We find that the majority of cell lines tested are compatible with Cells-to-C_T, but that a subset, primarily enriched with immune cell types, were incompatible with the method. The expanded list of cell lines that are compatible with Cells-to-C_T will enable researchers to better determine if Cells-to-C_T is suitable for their cell line of interest.

B21/P2420

FluidFM technology: a technology to revolutionize precision CRISPR editing, cell-based *in vitro* models, single cell adhesion and Live-seq transcriptomics.

T. Gerecsei, A. Kerjouan, A. Migenda Herranz, S. Mailand, M. Milla, K. Keevend, T. Pekeč, D. Ossola, T. A. Beyer; Cytosurge AG, Zürich, Switzerland

Objectives: Single-cell technologies have greatly impacted our understanding of population heterogeneity and cellular mechanisms. Our FluidFM technology addresses many hurdles in single cell research linked to CRISPR genome engineering, adhesion behavior and repetitive sampling of single cells for transcriptomics. Our objective was to use FluidFM to develop novel approaches for these applications.
Methods: The FluidFM technology is integrating atomic force microscopy with microfluidic probes, creating the world smallest syringe. Here, we present our FluidFM OMNIUM system enabling precise injection, extraction, and relocation of single cells. The technology can be used for diverse applications including precision CRISPR mediated gene engineering, semi-automated single cell adhesion measurements, and the recently published Live-seq approach1. The latter allows serial RNA extraction from single cells without impairing their viability enabling tracking of temporal trajectories.
Results: Direct intranuclear injection of CRISPR RNPs into a single cell circumvents harsh transfection methods and permits gene editing in hard-to-transfect and sensitive cells. Fine-tuning of the injection parameters enables to minimize the amount of CRISPR RNPs and deliver optimized amounts of HDR-templates. This enhances homologous recombination and minimize off-target editing effects. To illustrate the power of FluidFM mediated genome engineering we show deletion of SOX17 in hiPSCs. Further, we will present our effort to semi-automated single-cell force spectroscopy though reversable attachment of cells to the FluidFM cantilever. We show how we can measure adhesion forces up to 3 µN- in HeLa and CHO cells, with an unprecedented throughout of 50 cells/2h. Lastly, we will introduce our newest feature for serial RNA extractions (biopsies) from a single cell to support Live-seq workflows.
Conclusion: The FluidFM OMNIUM platform will improve the quality and ease in which single cell level projects in biomedical research are conducted. Intranuclear injection, enables precision gene editing, even in hard-to-transfect and sensitive cells. Semi-automated adhesion workflows enable measurements of cell-cell and cell-matrix interactions with high accuracy and unprecedented throughput. The single cell biopsy workflow allows the serial and non-destructive extraction of cellular material, conserving the physiological context.

B22/P2421

Illuminating dynamic structural states in hiPSCs through endogenous gene tagging

B. Roberts, R. N. Gunawardane; Allen Institute for Cell Science, Seattle, WA

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. As a first step, we have created a collection of endogenously tagged hiPSC lines to illuminate cell organization. The tagged proteins include notable membrane-bound and membrane-less cellular organelles, signaling complexes, phase transition markers, transcription factors, and structural markers specific to differentiated cells. To date, the Allen Cell Collection consists of 54 single- or dual-edited lines that have undergone extensive

quality control testing to ensure genomic, cell biological, and stem cell integrity. Here, we highlight our gene-editing and quality control workflow for mono- and biallelic editing of expressed or silent genes that are expressed specifically during differentiation and function as reporters of cellular state. Furthermore, we underscore our efforts to increase efficiency of the current workflow by utilizing Adeno-Associated Virus (AAV) to deliver donor DNAs and multiplexing transfection strategies for gene tagging at multiple loci. Our most recently released lines are CTCF-mEGFP and PCNA-mEGFP, for dynamic visualization of chromatin domains and replication foci, G3BP1-mEGFP to label stress granules, mono- and bi-allelic E-cadherin-mEGFP to visualize adherens junctions and DCP1a-mEGFP for P-bodies. Currently, we are working on getting gene-edited cell populations for N-and VE-cadherin ready for single cell sorting. Our overarching goal is to make these tools openly available to the cell biology community to help accelerate biomedical research. In addition to our cell lines, the donor plasmids, segmented 3D images of about 200,000 cells from our lines, image analysis, visualization tools, integrated cell models and biological findings are all openly available to the research community (www.allencell.org).

B23/P2422

A critical comparison of bulk and single-cell manipulation technologies for genome editing

S. Patange, S. Maragh; National Institute of Standards and Technology, Gaithersburg, MD

The NIST Genome Editing Program within the U.S. Department of Commerce leads international efforts with academia, industry, and government stakeholders to identify and address existing gaps in the standards and measurement needs of the genome editing field. One major project area is to evaluate the performance of technologies that deliver genome editing reagents to human cells. Here, we present our evaluation using HEK293 cells to compare a traditional bulk delivery technology (Nucleofection) with an emerging single-cell manipulation technology (FluidFM). We use several metrics including fluorescence quantification and genomic sequencing to assess the efficiency of reagent delivery and genome editing at the on-target locus These results provide guidance for research applications where it is advantageous to have controlled dose delivery of a reagent or high-resolution data on the distribution of genomic and phenotypic outcomes of a small population of cells. The outcome of this study is a critical step towards addressing future needs and technological advancements that would allow precision manipulation to be coupled with precision measurement for applications in human genome editing.

B24/P2423

Diazaborine-based bioconjugation: A uniquely versatile bioorthogonal reaction for biochemistry and cell biology

S. Ghosh, S. C. Mallojjala, J. Hirschi, **S. L. Bane**; Binghamton University, Binghamton, NY

With an abundance of functional groups and reactive species present in biological systems, precise site-specific chemical bond formation is an ongoing challenge. Biorthogonal chemical reactions meet this challenge by incorporating exogenous reaction functional groups into a target biomolecule of interest. Great success has been achieved with the popular “click” reactions, particularly the strain-promoted azide-alkyne conjugation (SPAAC) and the tetrazine-based inverse electron demand Diels-Alder reaction (IEDDA). A common feature of these reactions is that the conjugation products contain large, hydrophobic moieties, which is undesirable for in vivo applications such as drug delivery. A recently uncovered bioorthogonal reaction has potential for broad applications in biochemistry and cell biology.

The diazaborine (DAB) reaction occurs between a hydrazine derivative and an ortho-carbonyl substituted phenylboronic acid. The boronic acid moiety accelerates the usually sluggish hydrazone-forming reaction by several orders of magnitude and yields various forms of a boron-nitrogen heterocycle as the product. Many of these products are zwitterionic; thus, unwanted hydrophobicity is not conveyed into the product. Importantly, the reaction is orthogonal to the SPAAC and IEDDA processes, allowing for its simultaneous deployment for multiple site-specific conjugation applications. The accessibility of hydrazine derivatives from commercial sources coupled with the availability of chemical and enzymatic methods for incorporating these functional groups into biomolecules makes this bioconjugation reaction quite accessible to biological researchers. Our group is investigating the mechanism of formation of this highly unusual heterocycle as well as the stability and reversibility of the various forms of the DAB, with a goal of deploying the optimal reactive pairs for the desired application. We will present results of chemical and computational studies that define the mechanism of this process and biochemical studies that inform the suitability of this chemistry for cellular applications.

B25/P2424

New Generation of Fluorescent probes for cell-based measurements of Caspase Activation and Mitochondrial Superoxide

B. S. Mandavilli, D. Beacham, Y. Hu, A. Chen; Thermo Fisher Scientific, Eugene, OR

Neural cell health and stress readouts are critical indicators of altered or impaired function in normal and diseased states of excitable cells, and work has been underway to develop improved small molecule sensor dyes compatible with traditional imaging and High Content Analysis (HCA) interrogation of apoptotic and mitochondrial stress pathways.
The CellEvent™ Caspase Green dye effectively reports caspase activation, but suffers complications in assay configuration when attempting to multiplex with the Green Fluorescent Protein (GFP), calcein, or other 488 laser line tools in fluorescence microscopy. Here, we describe the testing and functional characterization of a new candidate molecule for measuring apoptosis in living cells. Our sensor is comprised of a fluorogenic reporter dye that is liberated from a DEVD peptide substrate by caspase activation, but operates in the Texas Red, 590nm excitation band, with an emission peak near 610 nm, permitting easy multiplex with GFP or calcein stained neurons in both traditional and HCA microscopy configurations. Similarly, mitochondrial superoxide accompanying cell stress is probed in microscopy with the MitoSOX™ Red Mitochondrial Superoxide Indicator dye, which localizes to mitochondria and reports superoxide generation, ignoring other Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). This dye has an unusually long Stokes' shift, requiring specialized microscopy and HCA filters that excite at 405nm, and capture emission at 610nm for specific superoxide detection. This unconventional spectroscopic profile prevents the dye's use on many imaging platforms and promotes phototoxicity. To this end, our team has produced a dye with the same level of specificity for superoxide that will operate in one of the traditional fluorescence microscopy channels. Our candidate dye, here named MitoSOX™ Green Mitochondrial Superoxide Indicator also localizes to mitochondria of live cells and selectively reports superoxide generation, while ignoring other ROS and RNS species in ex vivo testing. With an Excitation/Emission profile in the GFP/FITC microscopy channel, a series of comparative studies in immortalized and neural cells are shown, highlighting photostability, specificity and signal amplitude from the dye.

B26/P2425

New Approaches Towards Targeted Protein Degradation

J. Yang, A. Ting, C. Bertozzi; Stanford University, Stanford, CA

Our lab has pioneered extra cellular approaches towards degrading protein via an approach called Lysosomal Targeting Chimeras (LYTACs). These molecules have worked very well in the lab, but have a long and complex chemical synthesis. In this new work we have made a new version that also traffics and degrades proteins in the lysosome, but can be readily made in e. Coli in one step. We demonstrate the efficacy of this biologic as an extra culler protein degrader.

B27/P2426

Development and application of the ratiometric calcium indicator specific to an inter-organelle communication

Y. Woo, E. Cho, Y. Suh, B. Suh, S. Kim, T. Nhung, T. Nghi, S. Lee, D. Mun, S. Park; Pohang University of Science and Technology, Pohang, Korea, Republic of

Mitochondria-associated ER membrane (MAM) is the structure where these two major calcium-regulating organelles form the close contact site, so it provides physical site for efficient Ca^{2+} crosstalk between them. Due to the technical limitation, it was hard to directly and specifically measure Ca^{2+} concentrations inside the MAM with a poor/limited understanding about MAM Ca^{2+} dynamics. Here, we developed an MAM-specific BRET-based Ca^{2+} indicator, MAM-Calflux, to visualize Ca^{2+} dynamics at this inter-organellar compartment. Application of the bimolecular fluorescence complementation (BiFC) concept in the fluorescent domain successfully spotlighted Ca^{2+}-responsible BRET signals in MAM. Taking advantage of the ratiometric Ca^{2+} indicator, MAM-Calflux could estimate steady-state Ca^{2+} level inside MAM. Also, it achieved the dual functionality as the MAM Ca^{2+} sensor and the MAM structure marker and could visualize the uneven intracellular distribution of MAM Ca^{2+}. Eventually, it unveiled abnormal MAM calcium cross-talk in neurons of neurodegenerative disease model mice. Thereby, we propose a new sensor ratiometrically and specifically indicating the Ca^{2+} concentration in an inter-organelle communication.

B28/P2427

Exploring the perspectives of using the D-lldR protein from *Pseudomonas fluorescens* as a biosensor for D-lactic acid *in vitro*

E. Indosova, E. Zolotovskaya, A. Saranchina, P. Drozdova; Institute of Biology At Irkutsk State University, Irkutsk, Russian Federation

Background. Lactic acid (LA) is a common substance widely found in nature and used in industry. It has a chiral carbon atom and thus occurs as two enantiomers. While L(+)-LA acid is common in all living cells, D(—)-LA is a specific metabolite of some bacteria. In human or farmed animals, blood accumulation of D-LA is a marker of bacterial infection. However, a simple, inexpensive and effective chiral-specific method of D-lactic acid detection and quantification is still lacking. Recently, Singh *et al.* (2019; doi: 10.1016/j.synbio.2019.08.004) have discovered and characterized D-lldR, a D-LA-responsive transcription factor from *Pseudomonas fluorescens*. The genetic construct encoding this factor under a constitutive promoter and GFP under the D-lldR-driven promoter proved to be a working biosensor in *P. denitrificans*. However, its dynamic range was 35-100 mM (Singh *et al.*, 2019), which is much higher

than even the 3 mM concentration threshold for D-lactic acidosis, a serious neurological condition (Petersen, 2005; doi: 10.1177/0115426505020006634). In this work, we aimed at determining whether D-lldR can be used to construct a more sensitive system *in vitro*. Methods and Results. To do so, we recloned the sequence encoding D-lldR from the pUCPK'-D-lldR-P_{lldP}-gfp vector (Singh *et al*., 2019) to the pET20b(+) vector, obtaining the expression construct for production of D-lldR with a C-terminal His6 tag. This construct was used to transform *Escherichia coli* strains BL21(DE3)pLysS, C41(DE3) or C43(DE3). In C41(DE3) or C43(DE3) strains, the protein was successfully produced. These cells were used to extract protein and then purify D-lldR with Ni-NTA agarose resin (Thermo). Both crude extract and purified protein were used to check for D-lactic acid-dependent DNA-binding activity with electrophoretic mobility shift assay in a native 5% TBE polyacrilamide gel (Hsieh *et al.*, 2016; doi: 10.3791/54863) with the ROX-labeled oligonucleotide corresponding to the predicted binding site (Singh *et al.*, 2019). However, so far we have not observed the expected shift of the DNA band. This may mean that other cofactors are needed for DNA binding in the *Pseudomonas* cells. This research was funded by the Russian Science Foundation; grant number 20-64-47011 for interdisciplinary project performed in association with the Petrozavodsk State University.

B29/P2428

3D print your own lab - 3D printers in service of biological laboratories

Z. Baster; National Heart, Lung, and Blood Institute, NIH, Bethesda, MD

Within the past 10 years 3D printing gain in popularity, due to expiration of several key patents. The main application for 3D printing is in different branches of Engineering and manufacturing prostheses and customized surgical tools. Interestingly, specialized 3D printers can be used in almost any field of life, from printing buildings to printing biocompatible scaffolds designed for seeding cells. Currently more and more research groups use 3D printers to design unique parts of instruments to use in their projects. One of the main advantages of current 3D printers is their availability, both in the case of number of 3D printers in the community, as well as in the cost of the printer, which is less than $1000 for high quality entry level printer, and the printing material.

Many labs struggle with acquiring small lab equipment, like shakers or sample holders, due to their high cost. The solution to that problem, that I would like introduce, is using 3D printers to make such equipment. With basic knowledge in 3D printing and electronics, this approach can reduce the cost of small laboratory devices 10-fold and more.

Nonetheless, the main problem behind 3D printing is a need to provide a 3D model to the printer. Such a design usually requires good background in 3D computer-aided design software. There are many services allowing to share design files between people, yet they are usually low specialized and not popular in biological science community.

The goal of this work is to reach out to our community, both to makers and people in need. Firstly, to popularize the use of 3D printers in making every-day small lab equipment, allowing to reduce the cost of running the lab. Secondly, to assess the need for a such solution in our community. Finally, to connect scientist interested in exchanging knowledge and 3D print designs.

B30/P2429

Development of a new All-In-One inducible lentiviral shRNA/gRNA Vector

L. Wu, H. Su, J. Fellows, M. Fu, J. Heller, B. Park, D. Yin; Origene, Rockville, MD

Tet-On is a powerful inducible system and a classical tool to regulate gene expression in mammalian cells. It has also been applied to regulate Pol III-driven transcription, such as shRNA or gRNA driven by a U6 or H1 promoter. However, all of the [JF1] current versions of Tet-On shRNA vectors are based on H1-2O2 or U6-2O2 promoters, which are only compatible with first-generation tetracycline repressor TetR.[JF2] In addition, these promoters have the problem of driving downstream transcription without the binding of the Tet regulatory protein. Here, we developed a new system that is built upon tetracycline activator protein, Tet-On 3G, combined with a new structure of Tet responsive promoter H1-4O4, which tightly regulates [JF3] the downstream transcription of gRNA or shRNA. The responsiveness of our system to Doxycycline regulation is dramatically improved compared with the current versions. The new Tet-On system is further optimized into a compact structure to be compatible with the lentivirus package (All-In-One Lenti-Tet-On system), which still keeps the leaky expression at an undetectable level. Combined with all these features, the new generation of the Tet-On system offers broad applications in gene knocking down and genomic editing.

B31/P2430

Improved design of kinase translocation reporters allows simultaneous measurement of cAMP-dependent kinase (PKA) and extracellular signal-regulated protein kinases (ERK) activities

S.-J. Tsai[1], Y. Gong[2], J. Xu[2], F. Zahra[1], M. J. Caterina[1,1,1,1], S. J. Gould[1]; [1]Johns Hopkins University School of Medicine, Baltimore, MD, [2]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD

Signal transduction through protein kinases mediates a vast array of physiological processes and disease pathologies. Live-cell biosensors for protein kinase activities have led to significant advances in our understanding of human health and disease. Of these, kinase translocation reporters (KTRs) are among the most useful, as they allow for facile monitoring of kinase activity based on movement of a fluorescent protein (FP) between the nucleus and the cytoplasm. A working KTR relies on phosphorylatable sites within a vicinal pair of strong bipartite nuclear localization signal (bNLS) and weak nuclear export signal (NES). When the sites are phosphorylated, the added negative charges inhibit bNLS activity and stimulate NES activity, reversing the net strength and triggering movement of the KTR from nucleus to cytoplasm. While many of the original KTRs are useful, some are less so, suggesting room for improvements. Here we describe principles to redesign KTRs that yielded enhanced performance characteristics. First we increased KTR size, thereby minimizing their back-diffusion through the nuclear pore complex. Another improvement was to restructure the bNLS to render tuning to various levels of kinase activity. Third, we found that marking the nucleus/cytoplasm boundary with endoplasmic reticulum-localized FPs greatly eliminated image analysis and biological artifacts caused by other commonly used marker proteins. Importantly, integration of these design principles allowed us to develop a PKA-sensitive KTR (PKA-KTR) with greatly enhanced sensitivity and response amplitude to forskolin and other PKA stimuli, that was functional in multiple cell lines and primary cells. We applied the same principles to optimize an existing KTR for ERK (ERK-KTR) and derived a set of new sensors with tunable sensitivity for different cultured and primary cell types. We also showed that our improved PKA-KTR and ERK-KTR can be used to simultaneously record both signaling pathways. Importantly, these sensors are designed such that they can be multiplexed with other indicators to achieve even higher-

dimensional, real-time monitoring of diverse signaling pathways. In summary, we demonstrated a set of generalizable principles to optimize and improve KTR sensors for higher sensitivity, enhanced response amplitude, and expanded versatility in cellular contexts and applications.

Regulation of Actin Dynamics 2

B33/P2431

Dissecting the role of cortical actomyosin dynamics during Wnt-dependent asymmetric cell division

M. Taher[1,2], V. Juciute[1,2], D. Qi[1,2], E. Wang[1,2], K. Sugioka[1,2]; [1]Department of Zoology, University of British Columbia, Vancouver, BC, CANADA, [2]Life Sciences Institute, University of British Columbia, Vancouver, BC, CANADA.

Asymmetric cell division plays a crucial role in controlling cell type diversification during metazoan development by producing two daughter cells with different fates, shapes, and sizes. In *Caenorhabditis elegans* embryo, asymmetric cell division of endomesodermal precursor EMS requires a Wnt ligand expressed from the posteriorly located P_2 cell. Although previous studies report that Wnt signalling pathway components localize asymmetrically in a dividing cell and specify mitotic spindle orientation, microtubule organization asymmetry, and cell fate, the precise mechanism underlying the Wnt-dependent asymmetric cell division remains elusive. Here, we investigated the role of cortical actomyosin in the EMS asymmetric cell division. We quantitatively analyzed the cortical actomyosin dynamics by visualizing non-muscle myosin II/NMY-2::GFP and lifeact::mKate2 (F-actin marker) using a spinning disk confocal microscope. We observed the polarized cortical flow from the posterior cell cortex toward the cleavage furrow during cytokinesis, as we reported previously (Sugioka and Bowerman 2018). On the other hand, the anterior cell cortex did not exhibit the pronounced furrow-directed cortical flow. Moreover, we found that the dividing EMS cell exhibits asymmetric actin orientation during cytokinesis: actin filaments were randomly oriented throughout the cortex at anaphase onset, then over time, actin filaments oriented as mesh-like at the anterior, mostly vertical at the cleavage furrow, and they tend to be horizontal at posterior. Interestingly, these asymmetries of cortical flow and actin orientation are lost in *wnt/mom*-2 and *frizzled/mom*-5 mutants, suggesting the Wnt-dependent regulation of cortical actomyosin dynamics. We are currently investigating a potential actomyosin regulator downstream of the Wnt pathway. We are also investigating how the loss of asymmetric cortical flow affects asymmetric cell division by introducing actin and myosin inhibitors. Our study should reveal new mechanisms underlying the Wnt-dependent regulation of asymmetric cell division.

B34/P2432

Exploring SLK effects on adhesion cytoskeletons

Y. Lin[1], H. Lin[2], Y. Jo[1], C. Fan[1], F. Tsai[1,3]; [1]Department of Pharmacology, National Taiwan University College of Medicine, Taipei, TAIWAN, [2]Department of Immunology, National Taiwan University College of Medicine, Taipei, TAIWAN, [3]Department of Internal Medicine, National Taiwan University Hospital, Taipei, TAIWAN.

SLK(Ste20-like kinase) is a serine/threonine protein kinase involved in many biological processes including cell migration, apoptosis and cell proliferation. It was identified as one of the candidates from our recent screen of synthetic dysmobility, as collective cell migration could be synergistically increased

by simultaneous suppression of myosin activator ROCK and actin modulator SLK. Although previous research suggested that SLK might directly suppress RhoA-ROCK activities in cardiomyocytes, our results did not reveal direct signaling interactions between SLK and ROCK during cell migration. Interestingly, recent reports revealed that SLK activated ERM to regulate apical-basal polarity in epithelial cell sheets. Our preliminary data also showed that knockdown of SLK improved cell alignment in head and neck cancer SAS cells, and cell sheets with SLK knockdown persisted to grow in single layer rather than multiple layers. Therefore, we hypothesized that by activating ERM, SLK regulates actin cytoskeletons to remodel cell-cell and cell-matrix adhesion, both of which are critical for cell sheet integrity and collective cell migration. We are now elucidating whether and how SLK regulates adhesion cytoskeletons, with the ultimate goal of understanding its mode of synergistic collaboration with ROCK during collective cell migration.

B35/P2433

Anillin Regulates Rho Activity during Cell-Cell Junction Remodeling

Z. Craig, A. Walkon, R. Stephenson, T. Arnold, A. L. Miller; University of Michigan, Ann Arbor, MI.

Epithelial tissues require apical cell-cell junctions to adhere cells to one another and generate specialized barriers that are essential for homeostasis, while disrupted barrier function is linked to disease pathogenesis. As cells change shape and tissues experience mechanical forces, cell-cell junctions must be continually remodeled while maintaining a functional barrier. However, the mechanisms underlying junction maintenance, repair, and remodeling are not well understood. Using *Xenopus* embryonic epithelial cells as a model epithelium, our lab has shown that tight junction repair following local leaks in the barrier following cell shape change involves Rho flares, short-lived local accumulations of active RhoA, which trigger actomyosin contractility to restore barrier function. Anillin is a scaffolding protein that is known to regulate RhoA during cytokinesis, where it forms a tension-dependent complex with p190RhoGAP (p190) to terminate Rho contractility after cytokinetic furrow ingression is complete (Manukyan et al. 2014, J. Cell Sci.). We and others have shown that Anillin is also present at cell-cell junctions, where it regulates junction integrity, organizes junctional actomyosin, and perpetuates Rho activity by increasing Rho's membrane retention time and thus its availability to downstream effector proteins (Arnold et al. 2019, eLife; Budnar et al. 2019, Dev. Cell; Reyes et al. 2014, Curr. Biol.). Here, we demonstrate that Anillin and p190 both localize to Rho flares. We hypothesize that Anillin coordinates contractility at Rho flares by first promoting membrane retention of active Rho and then recruiting p190 to terminate contractility. Recent data shows that Anillin knockdown enhances the intensity of Rho flares, which suggests that Anillin is involved in negative regulation of Rho. Interestingly, Anillin knockdown also reduces tight junction reinforcement following tight junction breaks. Ongoing work is focused on investigating the mechanism of p190's recruitment to Rho flares, and whether Anillin binding is required for p190 localization at Rho flares. Additionally, we are investigating the function of p190 at Rho flares by knocking down p190 or disrupting p190's GAP activity and measuring effects on local Rho activation and tight junction remodeling. Together, this work will reveal how Anillin and p190 coordinate contractility during RhoA-dependent tight junction repair in polarized epithelia.

B36/P2434

Shaping the presynaptic compartment and cytoskeleton via Tropomyosin

B. Ermanoska, A. A. Rodal; Brandeis University, Waltham, Massachusetts, MA.

Meter-long axons of human peripheral nerves endure the mechanical strain of a lifetime of muscle contraction. A submembranous cytoskeleton, composed of periodic repeats of spectrin and actin rings and non-muscle myosin II (NMMII), is suggested to provide a mechanical scaffold for axons. The organization and composition of the presynaptic actin cytoskeleton remain largely unknown due to the small size of the presynaptic compartment and the numerous membrane specializations and actin structures it contains. To address this question, we examined the presynaptic actin cytoskeleton at the *Drosophila* larval neuromuscular junction (NMJ) - a model synapse with presynaptic boutons having multiple active zones that innervate the mechanically active postsynaptic muscle. We identified different actin subpopulations, including an actin core traversing the NMJ, cable-like structures, and persistent patches, in addition to the previously characterized endocytic actin patches. We assessed the organization of the presynaptic actin upon neuronal down-regulation of non-muscle Tropomyosin (Tm1), a key actin regulator which we found to localize along the NMJ, partially overlapping with the presynaptic actin core. The coil-coiled tropomyosin dimer binds along actin filaments and acts as an inhibitor of Arp2/3 nucleation. In line with this function, neuronal decrease of Tm1 levels induced up-regulation of presynaptic actin assemblies, which correlated with increased levels of the Arp2/3 nucleation complex. Tropomyosin is also crucial for the recruitment of NMMII to actin filaments, and upon neuronal Tm1 down-regulation we found a correlating decrease of NMMII not only in the presynaptic compartment but also in the postsynaptic muscle proximal to boutons. This finding suggests that neuronal actomyosin rearrangements could propagate transsynaptically, and possibly tune synaptic function. In addition, we mimicked the presynaptic actin disorganization observed upon neuronal down-regulation of Tm1 by performing axotomy, which indicates that both physical and genetic defects could lead to similar disruptions of the neuronal mechanical continuum. Finally, we demonstrate that Tm1 regulates synaptic morphology as its neuronal down-regulation induces ectopic growth of synaptic protrusions. Thus, our study provides unique insight of the role of Tropomyosin in shaping the presynaptic compartment and regulating of F-actin and contractile apparatus organization, addressed in their *in vivo* context in the animal.

B37/P2435

Positive Feedback is Essential for Rho Amplification in Cortical Excitability

D. Chomchai, W. Bement; Cellular and Molecular Biology, University of Wisconsin - Madison, Madison, WI.

Recent studies have revealed that the cell cortex - the plasma membrane and the cytoskeleton-rich layer of cytoplasm just beneath it - behaves as an excitable medium. Specifically, during cytokinesis propagating waves of Rho GTPase activity and F-actin assembly and disassembly are concentrated in the equatorial cortex, where they direct the formation of the contractile ring. Rho wave propagation is proposed to be the result of an excitable circuit: interdependent positive and negative feedback loops. Positive feedback is suggested to depend on the Rho Guanine nucleotide exchange factor (GEF) Ect2, and negative feedback depends on the Rho GTPase activating protein (GAP) RGA-3/4. Similar dynamics have been observed with other GTPases, such as Cdc42 during cell locomotion and cell polarization. Collectively, these dynamics are termed cortical excitability.

Modeling of cortical excitability predicts that positive feedback is essential for Rho wave generation, but this has yet to be directly tested *in vivo*. We have previously shown that cortical excitability can be induced in immature Xenopus laevis (frog) oocytes by co-expressing mutant Ect2 and RGA-3/4. Using this system as an *in vivo* model for cortical excitability, I found that two Rho GEFs capable of positive feedback, GEF-H1 and Net1, can support cortical excitability in the place of Ect2 whereas two others that are not capable of positive feedback, Abr and ArhGEF10, did not support cortical excitability. Additionally, I also found that Ect2 modified to lack the capacity to participate in positive feedback did not support cortical excitability.
Another model prediction that has yet to be tested *in vivo* is that Rho wave generation is all-or-none. To test this, I held RGA-3/4 levels constant while titrating Ect2. I found that cells with low expression of Ect2 generated Rho waves with characteristics (amplitude, period, width) identical to those with high expression of Ect2, suggesting that Rho wave generation is indeed all-or-none. I also found that there is a threshold of ~50ng/uL Ect2 expression necessary to produce Rho waves. Just beyond the threshold, the likelihood of a cell to exhibit cortical excitability increases sharply, as predicted by the model. Together, these data provide direct *in vivo* evidence that Rho amplification by positive feedback is a feature of cortical excitability.

B38/P2436

Spatiotemporally Precise Optogenetics Reveal Ras/mTorC2/Akt Pathway Tune Polarity and Organize Protrusions in Human Neutrophil Migration

D. S. Pal, P. N. Devreotes; Department of Cell Biology and Center for Cell Dynamics, School of Medicine, Johns Hopkins University, Baltimore, MD.

In the last several decades, biochemical and genetic investigations have revealed that Ras GTPases promote cell growth, energy metabolism, and innate immunity functions of neutrophils. However, their role in cell motility is still unexplored. Although Ras mediates multiple signaling pathways which control neutrophil chemotaxis, there is no conclusive evidence to show Ras activity on the plasma membrane directly regulates immune cell migration. To prove that activation of Ras could generate cellular protrusions, we employed a spatiotemporally precise, cryptochrome-based optogenetic system in migratory HL-60 human neutrophils. We recruited GTP-bound or constitutively active Ras to the cell membrane. K- or H-Ras recruitment during global illumination experiments promoted actin polymerization at the ‘front’ or leading edge resulting in sustained protrusions and increased random motility in neutrophils. These findings were corroborated by transient recruitment of GTP-bound Ras to ‘back’ or inactive regions of the cell membrane which led to polarity breaking and localized protrusion generation. Furthermore, activating endogenous Ras by recruiting RasGRP4, a physiologically relevant RasGEF in neutrophils, was very effective in generating protrusions. Pharmacological inhibition studies suggested that these cytoskeletal effects of Ras activation are predominantly mediated through mTorC2 pathway. These findings led us to investigate a crucial downstream Ras-mTORC2 effector, Akt1, whose role in cell migration is still unidentified. Global or localized activation of Akt1 on the membrane showed that it is a positive regulator of F-actin polymerization, protrusive activity, and migration. Interestingly, we also discovered that Akt1 activation occurs via a yet uncharacterized PI3K-independent mechanism in neutrophils. Altogether, this study is the first of its kind in human neutrophils where we dynamically perturbed activities of established players of classical growth control pathways, on a sub-cellular level, and visualized their direct involvement on actin assembly, cell shape, and migration modes.

B39/P2437

Cell metabolism regulates contractility

L. D. Troughton, J. R. Beach, P. W. Oakes; Loyola University Chicago, Chicago, IL

Cell migration is a dynamic process that requires extensive protein turnover, extracellular-matrix interaction and remodeling, and a dynamic actomyosin cytoskeleton. The actomyosin motor is vital for the force generation required to initiate and maintain motile behaviour. Interestingly, different cell types utilize this machinery in different ways to promote either mesenchymal or amoeboid-like migration. Regardless, these processes rely heavily on ATP hydrolysis, and availability of ATP is thus likely to tune both the mode of migration and the underlying mechanical interactions. Moreover, migration mode plasticity often accompanies pathological states such as cancer, where a dysregulated metabolism is often a defining feature. Surprisingly, how metabolic activities affect the cellular contractile machinery and the rest of the cytoskeleton remains unclear. Here we investigate how small-scale changes to cellular metabolism (and ultimately ATP:ADP ratios) alter the contractile behaviour of fibroblasts and immune cells. Using traction force microscopy to measure the contractile energy the cells exert on their substrate while simultaneously imaging intracellular ATP, we find that contractile energy tracks with rapidly fluctuating ATP levels. We observe a near instantaneous decrease in contractile energy when intracellular ATP is reduced by perturbing glycolysis with 2-deoxy-D-glucose or by reducing culture supplement concentrations (glucose, sodium pyruvate, and L-glutamine). Moreover, we observe reduced actin retrograde flow rates, cell spreading and membrane protrusion dynamics during the reduced intracellular ATP phase, which can be rescued by increasing supplement concentrations. Surprisingly, increasing the availability of the metabolites has no effect. This suggests that cellular contractile energy is tightly coupled to the availability of ATP. Furthermore, we demonstrate that the actin dynamics that drive cell membrane/ blebbing activity consume more ATP than the myosin motors. This suggests amoeboid-like migration has a higher bioenergetic demand than mesenchymal migration. Taken together, these observations demonstrate that cell migration is intricately regulated via the metabolic demand of the cytoskeletal components.

B40/P2438

Contractility patterns in actomyosin networks with turnover are controlled by system geometry

M. Savinov[1], A. Krishna[2], N. Ierushalmi[2], A. Mogilner[1], K. Keren[2]; [1]Courant Institute of Mathematical Sciences, New York University, New York, NY, [2]Department of Physics, Technion--Israel Institute of Technology, Haifa, ISRAEL.

Actomyosin network contraction plays an essential role in many cellular processes including motility, division, and intracellular transport. How cells regulate their actomyosin cytoskeleton to generate distinct architectures and contractile dynamics from the same underlying molecular components is still not well understood. Here we tackle this challenging question using a combined experimental and modeling approach.

Our experimental efforts employed a reconstituted actomyosin system, in which cell extracts are encapsulated in water-in-oil droplets. Without any upstream regulation, this system spontaneously self-organizes and generates stable contractile patterns, with flow persisting for hours. Interestingly, we found the contractile behavior was strongly dependent on droplet size. In small droplets, these systems exhibit a dynamic steady-state characterized by radially symmetric inward flow. As the droplet size increases, the networks transition to a state of contractile waves or spirals.

In order to determine the mechanism underlying this size-dependent transition, we developed a mathematical model of the actomyosin network as a compressible, viscous fluid with active stresses representing the local network contraction due to myosin. Whether the network flows due to contraction or experiences viscous drag due to filament linkage is density-dependent; thus, the model considers the coexistence of different local mechanical states with distinct rheological properties. Analysis and numerical simulation demonstrate that our model successfully reproduces key experimental results, most notably that the transition between continuous and periodic contraction occurs at a characteristic length scale that is inversely dependent on the network contraction rate. This model shows how large-scale contractile behaviors emerge from the interplay of network connectivity, essential for long-range force transmission, with motor activity and turnover, which continually rearrange the network.
In conclusion, we demonstrate how varied contraction patterns can arise from the same microscopic constituents, without invoking specific biochemical regulation, merely by a change in the system's geometry. This work has broad implications for how contractile actin networks might behave in different parts of the cell, different stages of the cell cycle, or in cells of different size.

B41/P2439

Non-degradative ubiquitination of VASP regulates actin dynamics

L. E. McCormick[1], L. Herring[2], N. G. Brown[2], S. L. Gupton[1]; [1]Cell Biology and Physiology, University of North Carolina At Chapel Hill, Chapel Hill, NC, [2]Pharmacology, University of North Carolina At Chapel Hill, Chapel Hill, NC.

Filopodia are dynamic, actin-rich structures that extend outward from the cell to explore and respond to cues in the local environment. The actin polymerase VASP is a component of the filopodial tip complex, where it regulates actin polymerization and filopodial dynamics. Previously, we showed that VASP transiently co-localizes with the brain-enriched E3 ubiquitin ligase TRIM9 at the tips of neuronal filopodia. TRIM9 was required for the reversible, non-degradative ubiquitination of VASP and this modification was associated with decreased filopodia number and stability. Furthermore, the axon guidance cue netrin promoted deubiquitination of VASP. We hypothesize VASP ubiquitination is a mechanism to negatively regulate actin dynamics by blocking VASP and actin interactions. Through mass spectrometry, we identified numerous lysine residues that are ubiquitinated in VASP. We selected several of these residues for follow-up investigation. Using chemical ubiquitination, we created purified, ubiquitinated VASP to evaluate its activity through in vitro assays. We observe no changes in the tetramerization of ubiquitinated VASP through mass photometry. In preliminary assays, we do not see any changes in the bundling or binding of ubiquitinated VASP to actin filaments through cosedimentation assays. Currently, we are testing the ability of ubiquitinated VASP to accelerate the elongation of actin filaments through actin pyrene polymerization assays. Future work will visualize actin polymerization in the presence of ubiquitinated VASP through TIRF microscopy assays.

B42/P2440

Mechanochemical simulation of stress fiber

H. Ni[1], Q. Ni[2], G. A. Papoian[3], Y. Jiang[4], A. Trache[5]; [1]Institute for Physical Science and Technology, University of Maryland, College Park, College Park, MD, [2]Institute for NanoBioTechnology, John Hopkins University, Baltimore, MD, [3]Department of Chemistry and Biochemistry, University of Maryland, College Park, College Park, MD, [4]Department of Mathematics and Statistics, Georgia State University, Atlanta, GA, [5]Department of Medical Physiology, Texas A&M University Health Science Center, Bryan, TX.

Stress fibers are contractile actomyosin bundles that sense and transduce mechanical force by connecting to extracellular matrix through focal adhesions. Under external mechanical stimuli, stress fibers actively remodel and display properties of viscoelastic materials. For example, experiments show that mechanical stretching immediately increases the contractile force generated by stress fibers, which slowly drops afterwards. The molecular origin for such behavior is not well understood. In this work, we simulated mechanochemical dynamics and force generation of stress fibers using molecular simulation platform MEDYAN. We model the stress fiber as two connecting bipolar bundles attaching to focal adhesions. In the presence of myosin motors and alpha-actinin crosslinkers, the simulated stress fiber is able to generate contractile force that resembles its biomechanical functions. When the stress fiber is extended by tensile pulling, we found instant increase of the contractile force which slowly decreases until reaching a new steady state, in close agreement with experiments. We further show that the characterized timescale of contractile force relaxation is not determined by the external stimuli, instead, is determined by the intrinsic property of the stress fiber: the degree of crosslinking as well as the dynamics of the actomyosin network. Our results provide a molecular explanation on how actomyosin networks respond to mechanical stimuli and have strong implications in understanding the mechanobiology of cells.

B43/P2441

CAP1 and cofilin1: an intimate duet that governs neuronal actin dynamics

M. Rust[1], A. Heinze[1], F. Schneider[1], C. Schuldt[1], S. Khudayberdiev[1], T. Duong[1], R. Stringhi[2], E. Marcello[2]; [1]Institute of Physiological Chemistry, Philipps-University of Marburg, Marburg, GERMANY, [2]University of Milan, Milan, ITALY.

Cyclase-associated proteins (CAP) are evolutionary conserved actin-binding proteins (ABP). *In vitro* studies of the past few years unraveled important functions for CAP in actin treadmilling, as they demonstrated that it accelerates both dissociation of actin subunits from filamentous actin (F-actin) and nucleotide exchange on actin monomers. Mammals express to CAP family members, namely CAP1 and CAP2. Analyses of systemic knockout (KO) mice by us and others unraveled important functions for CAP2 in heart physiology and myofibril differentiation during skeletal muscle development. Instead, due to the lack of appropriate mouse models, the physiological functions of CAP1 in mammals largely remained unknown. We have generated a conditional KO mouse model for CAP1 to study its function in the brain. Brain-specific CAP1-KO mice displayed a defect in neuron connectivity, while other important aspects of brain development were not affected. Impaired neuron connectivity was caused by delayed neuron differentiation, which we demonstrated for hippocampal neurons isolated from CAP1-KO mice. Mechanistically, we found that CAP1 controlled actin turnover and F-actin dynamics during neuron differentiation and that it cooperated with the key actin regulator cofilin1. Moreover, rescue experiments in double KO neurons lacking CAP1 and cofilin1 revealed mutual functional dependence of

both ABP in neuronal actin dynamics and neuron differentiation. Further, we found that CAP1 was relevant for actin regulation not only during neuron differentiation, but also in dendritic spines, the postsynaptic compartment of most excitatory synapses in the vertebrate brain. Consequently, CAP1 inactivation altered dendritic spine density and morphology as well as synaptic function in hippocampal neurons. We showed that CAP1 cooperated with cofilin1 in actin regulation in dendritic spines and that both ABP were functionally dependent on each other at synapses. Together, we identified CAP1 as a crucial regulator for neuron differentiation and synaptic function, and we provide strong evidence for the conclusion that CAP1 and cofilin1 form an intimate duet that governs neuronal actin dynamics.

B44/P2442

Simultaneous Inhibition of the Arp2/3 Complex & Formins Enhances *de novo* Actin Filament Nucleation in *Arabidopsis thaliana*

L. Xu[1], L. Cao[1], J. Li[1], C. J. Staiger[1,2,3]; [1]Department of Biological Sciences, Purdue University, West Lafayette, IN, [2]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, [3]EMBRIO Institute, Purdue University, West Lafayette, IN.

Precise control over when, where, and how actin filaments are created in eukaryotic cells leads to the construction of unique cytoskeletal arrays with specific functions within a common cytoplasm. Actin filament nucleators are key players in this activity, which include the conserved Actin-Related Protein 2/3 (Arp2/3) complex that creates dendritic networks of branched filaments as well as a large family of formins that typically generate long, unbranched filaments and bundles. In some eukaryotic cells, these nucleators compete for a common pool of actin monomers and loss of one favors the activity of the other. However, it remains unclear whether this is a common mechanism across kingdoms. In plant epidermal cells, the cortical actin array plays a key role in cell morphology establisment, response to pathogens, and supporting symbiotic microbe interactions; however, the exact role of filament nucleation and the function of different nucleators in coordinating actin filament dynamics in the plant cortical actin is still poorly understood. Here, we combined the ability to image single filament dynamics in living plant epidermal cells with genetic and/or small molecule inhibitor approaches to stably or acutely disrupt nucleator activity. We found that Arp2/3 mutants, *arp2-1* and *arpc2*, or acute CK-666 treatment resulted in a marked reduction of actin filament abundance as well as significantly decreased side-branched nucleation frequency. We also confirmed that both plant Arp2/3 complex and formins were capable of generating side-branched filaments *in vivo*, but Arp2/3-nucleated filaments grew slower and were shorter than formin-nucleated ones. Surprisingly, simultaneous inhibition of both nucleators increased the overall actin filament abundance and enhanced the frequency of *de novo* nucleation events. Collectively, these observations suggest that plant cells have a unique actin filament nucleation mechanism to maintain the dynamic behavior of the homeostatic network of actin filaments in the cortical cytoskeleton.

B45/P2443

Augmented Actin Cable Fragmentation and Slow Turnover Rate of Abp1-GFP with Core-Shelled Quantum dots.

N. Le, J. Routh, C. Kirk, K. Kim; Biology, Missouri State University, Springfield, MO.

Quantum dots (QDs) are nano-semiconductor crystals that emit a broad range of tunable fluorescence. Due to its unique characteristics, QDs have shown vast potential for various industrial and biomedical

applications. However, the lack of knowledge in QDs and its toxicity mechanism has been a great concern for large scale adoption. The goal of our research is to investigate the toxicity of QDs and uncover the mechanism of its toxicity using budding yeast, Saccharomyces cerevisiae, as a model organism. We first tracked quantum dots intracellular trafficking and found that post-internalized QDs are distributed at the early endosome and the late Golgi/ trans Golgi network. Our RNA sequence data revealed a downregulation of AP2, a gene that is important for the initiation of clathrin-coated pit. Consistently, QDs exposure resulted in a longer lifespan of Abp1-GFP patches at the membrane and a slower Abp1-GFP turnover rate after internalization as well as altered polarization of GFP-Snc1. Furthermore, markedly increased levels of fragmented actin cables were observed upon QD treatment for 6 hours. We then tested whether QDs alter Cof1 activity. Using a temperature sensitive mutant strain cof1-22, we found that at permissive temperature, QDs exposure rescued the depolarization of actin patches caused by cof1-22 mutation. We, therefore, came to the conclusion that QDs may directly or indirectly regulate Cof1-mediated actin severing activity. The possibility of QD interaction with Cof1 and QD-mediated elevation of Cof1 expression via protein shotgun identification and iTRAQ-based proteome analysis, respectively, is currently being investigated.

B46/P2444

Regulation of Actin Organization by Clathrin Light Chains in Mouse Embryonic Stem Cells

M. Tiwari[1], M. Dingankar[2], A. Solanki[1], D. Subramanyam[1]; [1]National Centre for Cell Science, Pune, INDIA, [2]Indian Institute of Science Education and Research, Pune, Pune, INDIA.

Clathrin mediated endocytosis (CME) is a major studied endocytic pathway for the internalization of macromolecules, proteins and lipids from the plasma membrane. It is implicated in various cellular and biological processes including cellular signaling, motility and polarity. Recent studies demonstrate the importance of CME in the maintenance of mouse embryonic stem cell (mESC) pluripotency. ESCs are derived from the early pre-implantation embryo and are a valuable system to study early developmental cell fate decisions. The major component of CME is the clathrin triskelion, which is composed of three molecules of the clathrin heavy chain (CHC), each of which is associated with the smaller clathrin light chain (CLC). In mammals, there are two clathrin light chains, CLCa and CLCb whose individual physiological roles are poorly understand. CLCs have been shown to help recruit and organize actin to sites of endocytosis in differentiated cells, especially under conditions of high membrane tension. However, their role in mESCs remains unknown. Using the CRISPR-Cas9 genome editing system, we have generated CLC knock out mESC lines. We show that in the absence of only CLCa, or both light chains, actin is disorganized, forming patches in mESCs. Quantitative analysis of actin structures in Clta^{KO} mESCs show lower actin mesh density, increased actin patches, and lower actin branch points compared to WT mESCs. We also observe an enrichment of actin binding proteins Arp3 and cortactin in the patches, suggesting altered actin organization in the absence of clathrin light chains in mESCs. Our results demonstrate a unique role for CLCa in the organization of the actin cytoskeleton in the context of mESCs, and are critical for providing insight into early developmental processes.

B47/P2445

F-actin processive polymerization at the minus end

E. Kudryashova[1], A. Ankita[2], H. Ulrichs[2], S. Shekhar[2], D. S. Kudryashov[1]; [1]Chemistry and Biochemistry, The Ohio State University, Columbus, OH, [2]Emory University, Atlanta, GA.

Polarity is a key property of filamentous actin required for its barrier functions, cell migration, and cargo transport. It underlies the ability of the actin cytoskeleton to do productive work either via directional polymerization or the activity of myosin motors. At the functional level, the polarity-dependent filament dynamics are enhanced by actin-binding proteins, whose combined effects favor elongation from the barbed (plus) ends and shortening at the pointed (minus) ends. Pointed-end elongation is disfavored by the logic of cellular organization, the abundance of profilin, and the structural properties of the minus end. Accordingly, all the proteins currently known to promote F-actin polymerization do so at the filament barbed ends. Bacterial and viral pathogens that hijack the actin machinery for self-propelling in the cell cytoplasm also promote actin polymerization at the barbed ends. In striking exception, we found that VopF and VopL bacterial toxins produced by pathogenic *Vibrio* species promote unconventional minus-end F-actin elongation and, therefore, are the first *bona fide* pointed-end processive polymerases. Upon nucleation, the toxins remain associated with the filament pointed end, promoting its elongation even in the presence of profilin, as revealed by single-molecule live-cell and *in vitro* reconstituted TIRFM analyses. The VopF/L-induced nucleation and filament elongation at both ends disrupt cell polarity, undermining the defensive functions of the actin cytoskeleton. Beyond the role in the pathogenesis, the paradigm-shifting discovery of the pointed-end actin processive elongation expands the current view on the functional possibilities achievable with the actin cytoskeleton design.

B48/P2446

Unraveling the effects of Mical-induced oxidation on actin isoforms

E. E. Grintsevich, J. L. Martin; California State University, Long Beach, Long Beach, CA.

The Mical family of enzymes are unusual actin regulators that prime filaments (F-actin) for disassembly via site-specific oxidation of M44/M47. Filamentous actin acts as a substrate of Mical enzymes as well as an activator of their NADPH oxidase activity which leads to hydrogen peroxide generation. Mical enzymes are required for cytokinesis, muscle and heart development, dendritic pruning, and axonal guidance among other processes. Thus, it is critical to understand how this family of actin regulators functions in different cells and tissues. Humans express multiple actin isoforms in cell-specific manner but Mical's impact on their intrinsic properties was never systematically investigated. Our data reveal the differences in intrinsic stability of Mical-oxidized actin isoforms. Furthermore, our results establish a link between actin stability and its activation of NADPH oxidase activity of Mical. Our data suggest that differential properties of actin isoforms translate into the distinct patterns of hydrogen peroxide generation in Mical/NADPH-containing systems. Altogether our results suggest that Mical activities (posttranslational modification of actin and its NADPH-oxidase activity) may have different functional outputs in different cells and tissues depending on the isoform-related identities of local actin networks.

B49/P2447

Cytotoxic T Lymphocyte Activation Signals Modulate Cytoskeletal Dynamics and Mechanical Forces

A. Pathni, A. Özçelikkale, I. Rey-Suarez, S. Davis, N. Rogers, L. Li, Z. Xiao, A. Upadhyaya; University of Maryland, College Park, MD.

CD8+ cytotoxic T lymphocytes (CTLs) play a vital role in the adaptive immune response by killing tumor cells and cells infected with pathogens. During infection, the first signal for naïve CD8+ T cell activation is provided by antigen presenting cells which present pathogenic peptides to the T cell receptor (TCR). Complete activation requires a second signal, provided by the binding of co-stimulatory proteins to the CD28 receptor on the CTL membrane. Inflammatory cytokines, such as interleukin-12 (IL-12), have recently been characterized as a third signal for CTL activation, enhancing proliferation and effector function. Activated CTLs secrete lytic granules containing perforin and granzymes at the CTL-target synapse or the immune synapse (IS), triggering target cell death. The CTL cytoskeleton is instrumental in aiding the killing of target cells: within minutes of contact, the microtubule cytoskeleton reorganizes to allow centrosome translocation to the IS. Lytic granules are transported along microtubules to the IS where granule secretion is regulated by actin depletion and recovery. The cytoskeleton further promotes target killing by mediating mechanical force exertion at the IS. In this study, we hypothesize that the third signal for CTL activation, IL-12, modulates cytoskeletal dynamics and force exertion at the IS, thus potentiating effector function. We use total internal reflection fluorescence (TIRF) microscopy to study actomyosin flows and microtubule tip speeds at the IS of murine CTLs activated with TCR engagement and co-stimulation alone (two signals, 2SI), or additionally with IL-12 (three signals, 3SI). We find that 3SI CTLs have enhanced actin depletion, altered actomyosin dynamics and microtubule growth rates as compared to 2SI CTLs. Rapid TIRF imaging of lytic granules in activated CTLs revealed that granules in 3SI CTLs have altered accumulation patterns and lower velocities than in 2SI CTLs. We further characterize and compare centrosome polarization in 2SI and 3SI CTLs using confocal microscopy. Finally, we use traction force microscopy to show that 3SI CTLs exert greater forces than 2SI CTLs. Our results demonstrate that activation of CTLs in the presence of IL-12 results in differential modulation of the cytoskeleton, thus indicating a potential mechanism via which the third signal can augment the T lymphocyte biophysical response.

B50/P2448

PIKfyve regulates lamellipodia dynamics via Rac1 GTPase activation and Kindlin-2 recruitment

G. Luo[1], **H. Karaburk**[1], R. Benyair[1], G. Kreider-Letterman[2], R. Garcia-Mata[2], L. Weisman[1]; [1]University of Michigan, Ann Arbor, MI, [2]The University of Toledo, Toledo, OH.

Cell migration is an essential phenomenon in diverse biological processes including embryonicdevelopment, immune responses, and tissue repair. This directed movement of cells is controlledby chemical cues and signaling pathways. The force generated by actin polymerization within thecells drives cell migration by projecting membrane protrusions at the leading edge of cells. Amongthose protrusions, lamellipodia are the major driver of cell migration and are controlled by actindynamics. There are multiple signaling pathways that drive actin polymerization in lamelloipodiaduring cell migration. One key signaling pathway is based on phosphorylated inositol lipids whichact by interacting with various effector proteins, which in turn organize protein complexes atspecific membrane subdomains. To date, Class I phosphoinositide 3-kinases (PI3Ks), have beenshown to regulate actin polymerization to control cell migration. Importantly, new preliminarydata from our lab

suggests that another lipid signaling pathway may also regulate cell migrationvia actin polymerization at the plasma membrane. Our data show that PIKfyve, a PI3P 5-kinase,which has key roles at the lysosome, additionally localizes to the cell leading edge. Moreover, weobserve that an acute 5-10 minute chemical inhibition of PIKfyve reduces actin levels at cellprotrusions; and impairs lamellipodia dynamics in living cells. Using live-cell imaging, we alsodetect that acute inhibition of PIKfyve results in the concomitant impairment of actin dynamics aswell as loss of Rac1 GTPase activation in lamellipodia. Furthermore, we observe that acuteinhibition of PIKfyve impairs the localization of Kindlin-2, which regulates actin dynamics via atleast two mechanisms. We also observe that Arp2/3 complex recruitment to the plasma membranerequires PIKfyve activity. Together, these findings suggest that PIKfyve regulates lamellipodiadynamics via direct regulation of actin dynamics, and that this occurs in part by activation of Rac1and the recruitment of Kindlin2 to plasma membrane.

Actin Dynamics in Development

B51/P2449

Evolutionary diversification of Arp2 separated somatic versus germline roles

K. A. Stromberg[1], T. Spain[1], S. A. Tomlin[2,3], C. M. Schroeder[1]; [1]Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX, [2]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, [3]Howard Hughes Medical Institute, Seattle, WA.

The actin cytoskeleton often forms branched networks that are critical in many fundamental cellular processes, including cell motility and division. The Arp2/3 complex, a 7-membered protein complex including actin-related proteins (Arps) 2 and 3, is responsible for generating branched actin. Arp2 is evolutionarily ancient among eukaryotes and under stringent sequence conservation, yet we surprisingly discovered two clade-specific gene duplications of *Arp2* in *Drosophila*: *Arp2D* in the *obscura* clade and *Arp2D2* in the *montium* clade. Our targeted sequencing and phylogenetic analyses show these duplicates evolved independently about 14 million years ago and were retained throughout speciation within their respective clades. The two duplicates exhibit distinct sequence diversification from canonical Arp2 with Arp2D being the most divergent. Because Arp2 is normally conserved, we investigated the gene duplications to determine how evolution can diversify Arp2 structure and function. We first replaced canonical *Arp2* in *D. melanogaster* with *Arp2D* or *Arp2D2* to test whether both duplicates can polymerize branched actin networks similar to their parental gene. Surprisingly, we found both rescue the *Arp2* knockout lethality phenotype despite their sequence divergence. However, *Arp2D*-expressing males were subfertile and had lower sperm production. Cytological analyses revealed defects in the early stages of sperm development. We identified two structural regions in Arp2D that differ from canonical Arp2 and found one novel domain is critical for rescuing lethality, whereas a divergent loop contributes to the testis defects observed in *Arp2D*-expressing males. Based on our findings, we hypothesize that evolutionary diversification of Arp2 led to separation of function between somatic and germline cells, and specific structural elements in Arp2 tune actin branching for these different contexts.

B52/P2450

Dual control of formin-nucleated actin assembly by the chromatin and ER in mouse oocytes

H. Wang[1], **J. HU**[1], K. Yi[2], Z. Ma[3], X. Song[1], Y. Lee[1], P. Kalab[4], A. Bershadsky[1], Y. Miao[3], R. Li[1]; [1]Mechanobiology Institute, National University of Singapore, Singapre, SINGAPORE, [2]Stowers Institute for Medical Research, Kansas, MO, [3]School of Biological Sciences, Nanyang Technological University, Singapre, SINGAPORE, [4]Johns Hopkins University, Baltimore, MD.

The first asymmetric meiotic cell divisions in mouse oocytes are driven by formin 2 (FMN2)-nucleated actin polymerization around the spindle. In this study, we investigated how FMN2 is recruited to the spindle peripheral ER and how its activity is regulated in mouse meiosis I (MI) oocytes. FMN2 contains a nuclear localization sequence (NLS) within an N-terminal spindle localization domain (SLD) previously shown to be required for FMN localization to the spindle periphery. Combining mass spectrometry from oocyte extract and pulldown assay using recombinant proteins, we confirmed FMN2 NLS is bound to the importin α1/β complex. Furthermore, disruption of FMN2-importin interaction in oocytes revealed that RanGTP is required for FMN2 accumulation proximal to the chromatin and the MI spindle. Revisiting FMN2 SLD interactome, along with in vitro pulldown, in vivo Co-IP, and Fluoppi experiments, we confirmed the physical interaction between FMN2 SLD and an ER membrane protein VAPA. Thus, after being recruited and unloaded at the spindle periphery, the importin-free FMN2 is then anchored to the surface of ER around the spindle through SLD-VAPA interaction. Taking advantage of actin polymerization assays (pyrene and TIRF), we also show that FMN2 is autoinhibited through an intramolecular interaction between the N-terminal SLD with the C-terminal formin homology 2 (FH2) domain, which nucleates actin filaments. Surprisingly, we found VAPA binding to SLD relieves the autoinhibition of FMN2, leading to increased actin polymerization. This dual control of formin-mediated actin assembly allows actin polymerization to initiate the movement of the meiotic spindle toward the cortex, an essential step in the maturation of the mammalian female gamete.

B53/P2451

The prophase oocyte nucleus is a homeostatic G-actin buffer

K. Scheffler[1], **F. Giannini**[1,2], T. Lemonnier[1], B. Mogessie[1]; [1]MCDB, Yale University, New Haven, CT, [2]School of Biochemistry, University of Bristol, Bristol, UNITED KINGDOM.

Formation of healthy mammalian eggs from oocytes requires specialised F-actin structures. F-actin disruption produces aneuploid eggs, which are a leading cause of human embryo deaths, genetic disorders, and infertility. We found that oocytes contain prominent nuclear F-actin structures that are correlated with meiotic developmental capacity. We demonstrate that nuclear F-actin is a conserved feature of healthy mammalian oocytes and declines significantly with female reproductive ageing. Actin monomers used for nuclear F-actin assembly are sourced from an excess pool in the oocyte cytoplasm. Increasing monomeric G-actin transfer from the cytoplasm to the nucleus or directly enriching the nucleus with monomers led to assembly of stable nuclear F-actin bundles that significantly restrict chromatin mobility. By contrast, reducing G-actin monomer transfer by blocking nuclear import triggered assembly of a dense cytoplasmic F-actin network that is incompatible with healthy oocyte development. Overall, our data suggest that the large oocyte nucleus helps to maintain cytoplasmic F-actin organisation and that defects in this function are linked with reproductive age-related female infertility.

B54/P2452

The role of nuclear F-actin in oocyte genomic integrity

S. Ghadaouia, F. Giannini, K. Scheffler, B. Mogessie; Yale University, New Haven, CT.

Efficient DNA repair is critical for animal development. Mammalian oocytes are remarkably susceptible to DNA damage and accumulate DNA lesions with advancing maternal age, a phenomenon whose molecular basis remain unknown. The actin cytoskeleton has recently emerged as a protector of oocytes against chromosomal abnormalities. We recently discovered that mammalian oocyte nuclei contain prominent actin filaments that are significantly reduced in reproductively older females. Our new preliminary data show that oocyte genome damage causes excessive assembly and bundling of nuclear F-actin cables. Based on these observations, we hypothesize that nuclear F-actin structures promote DNA repair and that their disruption underlies reproductive aging-related accumulation of DNA damage in oocytes. We will test this exciting hypothesis using a combination of advanced microscopy, localized nuclear F-actin degradation, and DNA damage induction assays. We are combining pharmacological approaches of generating moderate levels of DNA damage with high-resolution live and immunofluorescence microscopy assays to analyze DNA damage repair. These integrated approaches could reveal new actin-based mechanisms of genomic integrity and provide new insights into the causes of reproductive age-related female infertility.

B55/P2453

Investigating mechanisms regulating actin in the early *Drosophila* embryo

A. R. Yeh, A. C. Martin; Biology, MIT, Cambridge, MA.

Actin networks undergo dynamic rearrangements that promote cell and tissue morphogenesis during development. The range of mechanisms that control actin network organization during development is still not fully understood. The *Drosophila* Synaptotagmin-like protein, Bitesize (Btsz), has been shown to organize actin at epithelial cell apical junctions in a manner that depends on its interaction with the actin-binding protein, Moesin. Here, we discovered that Btsz also functions at earlier, syncytial stages of development. In the syncytial embryo, actin organization changes in a cell cycle-regulated manner. In interphase, actin forms structures called caps, which are located above syncytial nuclei. These actin caps grow and collide such that pseudocleavage furrows form between mitotic nuclei and prevent spindles from colliding during the synchronous mitotic divisions. Btsz-RNAi disrupts these pseudocleavage furrows, leading to spindle collisions and other nuclear division defects. Previous studies have focused on how Btsz regulates actin through Moesin. However, there are multiple Btsz isoforms, only some of which contain the Moesin Binding Domain (MBD). Mulitple isoforms are transcriptionally upregulated at the maternal-zygotic transition. We used existing *btsz* alleles and CRISPR/Cas9 to engineer new *btsz* alleles to test the requirement of different Btsz isoforms during syncytial divisions. Our data suggest that non-MBD isoforms are important for proper syncytial development and preventing spindle collisions. We found that the C-terminal half of the Btsz1 isoform can bind F-actin with micromolar affinity which suggests that Btsz can regulate actin directly. This work suggests a novel mechanism for a Synaptotagmin-like protein regulating actin organization during animal development.

B56/P2454

Localization Mechanism of The Arf-GEF Steppke During Initial Junction Formation in the Drosophila Embryo

L. Xu, T. Harris; Department of Cell and Systems Biology, University of Toronto-47731885, Toronto, ON, CANADA.

Cytohesins are a class of Arf guanine nucleotide exchange factors (Arf GEFs) that can regulate actin networks and membrane trafficking in several contexts. Steppke (Step) is the sole Drosophila cytohesin, and it has been shown to negatively regulate myosin in several Drosophila developmental processes. Step and its two candidate adaptor proteins, Stepping Stone (Sstn) and Ajuba (Jub), have been shown to be enriched at myosin-rich adherens junctions (AJs), and depend on the actomyosin network for their localization. However, it is unknown when Step is first recruited to AJs or whether Step has the same degree of association with Sstn and Jub. Thus, analyzing embryos from cellularization, where myosin is minimally associated with AJs, to full extension of the germband, where myosin is highly enriched at AJs, we investigated the localization of Step, Sstn and Jub using spinning disk confocal microscopy of fluorescently tagged proteins expressed by endogenous promoters. During cellularization, all three proteins are evenly distributed along the plasma membrane. They first become enriched at tricellular junctions as junctions gain more actomyosin network during Drosophila embryogenesis stage six. All three proteins become gradually more enriched at tricellular junctions as the embryo develops. Other myosin-rich regions that Step, Sstn and Jub are enriched at include apical regions of invaginating tissues such as the ventral furrow and posterior midgut, and the ventral midline. In the amnioserosa, where myosin is gradually lost as the embryo develops, Step, Sstn, and Jub follows a similar pattern, losing junctional signal as time goes on. In addition to having similar localization patterns, we show that Sstn is required specifically for Step enrichment to tricellular junctions during gastrulation, but with Sstn RNAi depletion, Step is still seen evenly distributed along bicellular junctions. Furthermore, we show that Sstn depletion leads to distorted cell-cell interactions across the ectoderm. We are currently investigating the role of Jub in Step localization at these stages. Thus far, our results show that Step and its adaptor proteins are first enriched at AJs as they associate with actomyosin networks, exhibit very similar localization patterns, and that Sstn is required for Step's enrichment to these junctions.

B57/P2455

Adipose triglyceride lipase promotes prostaglandin-dependent actin remodeling by regulating substrate release from lipid droplets

M. Giedt[1], J. Thomalla[2], M. Johnson[2], Z. Lai[3], **T. Tootle**[1], M. Welte[2]; [1]Anatomy and Cell Biology, University of Iowa-Carver College Med, Iowa City, IA, [2]Biology, University of Rochester, Rochester, NY, [3]Harvard T.H. Chan Advanced Multi-omics Platform, Harvard T.H. Chan School of Public Health, Boston, MA.

A key factor controlling oocyte quality and fertility is lipids. Even though lipid droplets (LDs) are crucial regulators of lipid metabolism, their roles in fertility are poorly understood. During *Drosophila* oogenesis, LD accumulation in nurse cells coincides with dynamic actin remodeling necessary for late-stage follicle morphogenesis and fertility. Loss of the LD-associated Adipose Triglyceride Lipase (ATGL) disrupts both actin bundle formation and cortical actin integrity, an unusual phenotype also seen when Pxt, the enzyme responsible for prostaglandin (PG) synthesis, is missing. Dominant genetic interactions and PG treatment of follicles *in vitro* reveal that ATGL and Pxt act in the same pathway to regulate actin remodeling, with ATGL upstream of Pxt. Further, lipidomic analysis detects arachidonic acid (AA)

containing triglycerides in ovaries. Because AA is the substrate for Pxt, we propose that ATGL releases AA from LDs to drive PG synthesis necessary for follicle development. We also find that exogenous AA is toxic to follicles *in vitro*, and LDs modulate this toxicity. This leads to the model that LDs both sequester AA to limit toxicity, and release AA via ATGL to drive PG production. We speculate that the same pathways are conserved across organisms to regulate oocyte development and promote fertility.

B58/P2456

E-cadherin dependent molecular and biophysical mechanism of epithelial phagocytic clearance in the early vertebrate embryo

H. M. Häkkinen[1], E. Hoijman[2], M. Batet Palau[1,3], L. Bianchi[1,2], S. Wieser[4], V. Ruprecht[1]; [1]Cell and Developmental Biology, Centre for Genomic Regulation (CRG), Barcelona, SPAIN, [2]Universitat de Barcelona (UB), Barcelona, SPAIN, [3]Universitat Pompeu Fabra, Barcelona, SPAIN, [4]The Institute of Photonic Sciences (ICFO), Barcelona, SPAIN.

Cellular errors can lead to spontaneous cell death in the earliest stages of development. We identified that the embryonic surface epithelium mediates efficient phagocytic clearance of apoptotic cells in the early blastula-gastrula embryo and that this mechanism is conserved between zebrafish and mouse (Hoijman *et al.* Nature 2021). We found that epithelial cells form two types of actin-based protrusions during target interactions: 1) phagocytic cups responsible for apoptotic target uptake and 2) “epithelial arms” that are able to exert mechanical pushing forces on targets. Combining experiments and mathematical modeling we showed that both types of protrusions are required for phagocytic clearance by non-motile epithelial cells. We here address the role of cell-cell adhesion in phagocytic clearance by the embryonic epithelium. Using high-resolution quantitative live imaging and genetic tools we identified that E-cadherin, a central regulator of epithelial tissue connectivity, also controls phagocytic tissue clearance. Our results suggest that E-cadherin is required for both phagocytic cup and epithelial arm formation. We further show that E-cadherin is not required for a direct *trans*-binding at the target-epithelial cell interface, but is rather acting upstream of actin polymerization upon target interaction. Pharmacological interference with actin polymerization regulators reveals that Arp2/3 is essential for both phagocytic cup and epithelial arm formation, while Formins are dispensable from epithelial arms. Our data suggest that a lack of Formin prevents the final closure of the phagocytic cup, while a lack of Arp2/3 inhibits its entire formation. These findings reveal distinct functions of actin regulators in controlling epithelial protrusions plasticity and establish a new function of E-cadherin mediated tissue connectivity that controls efficient phagocytic tissue clearance.

B59/P2457

The F-actin disassembly factors, Capulet and Flare, regulate distinct pools of actomyosin dynamics to promote epithelial remodeling during *Drosophila* gastrulation

M. A. Collins, A. C. Martin; Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

During development, epithelial tissues undergo extensive remodeling events that transform an embryo from a simple sheet of cells to a complex three-dimensional organism. Such morphogenetic changes are driven by the intrinsic mechanical properties of individual cells within the tissue as well as the forces these cells actively generate, both of which are regulated by the contractile actomyosin cytoskeleton. While the spatial and temporal coordination of actomyosin assembly and force generation has been

extensively studied in morphogenesis, regulation of actomyosin disassembly, and its impact on cellular mechanics, is less well understood. Using *Drosophila* gastrulation as a model, we found that two different mediators of actin disassembly, Capulet and Flare, have strikingly distinct localization patterns during mesoderm invagination. Capulet and Flare are both implicated in modulating Cofilin's activity to sever and disassemble actin filaments (F-actin), suggesting they have pivotal roles in controlling where F-actin is disassembled. Mesoderm invagination involves the inward folding and internalization of a subset of cells along the ventral surface of the embryo. Before invagination onset, Capulet protein is confined to dense patches on the medioapical surface of cells throughout the epithelium. Furthermore, Capulet patch size scales with apical surface area; Capulet accumulations shrink in constricting cells (mesoderm) while those in non-constricting cells (ectoderm) do not change in size. In contrast to Capulet, Flare is only recruited to the cell cortex as the mesodermal cells (*Twist*-expressing) begin to fold inward. Once the mesoderm is internalized, Flare becomes enriched along the closure seam and junctional in the neighboring ectoderm tissue (*Twist*-negative). Pharmacological inhibition of Rho-kinase results in diffuse Flare, suggesting that Flare recruitment to junctions within the ectoderm may depend on intercellular forces. Finally, depletion of Capulet or Flare disrupted closure of the folding mesoderm, highlighting a key role for actin disassembly during this process. Together, these data suggest that Capulet and Flare spatially regulate the dynamics of distinct apical and junctional F-actin pools to promote mesoderm invagination.

B60/P2458

AID-ing our understanding of the *Drosophila* actin mesh

H. M. Bailey, M. E. Quinlan; Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA.

The process of egg development, oogenesis, is highly conserved and crucial for producing offspring. *Drosophila melanogaster* have long served as a model system to understand aspects of egg development including stem cell and germ cell development, meiosis, cell migration, intercellular signaling and mRNA localization. An essential component of oogenesis in *Drosophila* is the presence of a cytoplasmic actin meshwork that spans the oocyte and persists during mid-oogenesis. This complex actin network is built by the collaboration of actin nucleators, Spire and Cappuccino (Spir and Capu). The composition, organization, stabilization, and removal of the mesh remain unknown. This is, in part, due to the requirement for actin binding proteins in early oogenesis and our inability to visualize removal of the actin mesh because egg chambers expire *ex vivo* just prior to this transition. To overcome these obstacles, I am developing methods to directly observe this meshwork and study the underlying regulatory mechanisms of the mid to late oogenesis transition. I am utilizing the Auxin Inducible Degradation (AID) system to test the requirement of candidates in mesh regulation. AID is composed of three components; a protein tagged with the AID motif (candidate), Tir1 (a transgenic auxin receptor), and the plant hormone auxin. When cells have all three components, the candidate is ubiquitinated on the AID motif and sent to the proteasome for degradation. Using this system in mid-oogenesis will allow for temporal control and removal of the candidate proteins and determining their role in mesh stabilization, maintenance, organization, and removal. Thus, far I have endogenously tagged Spir, Capu, and Myosin V (didum) with the AID degron. I have been working to optimize conditions for removal of the candidates on a biologically meaningful timescale. I am taking multiple approaches to visualize the effects of candidate removal on the mesh in real time. These include using flow chambers for drug treatment and using an adapted Windowmount (O'Brien lab) method to visualize oogenesis. Altogether,

my work will facilitate a detailed characterization of the actin mesh that can translate to studies in other organisms and provide a powerful tool to the *Drosophila* research community.

B61/P2459

The cortical actomyosin cytoskeleton is spatiotemporally dynamic in the C. elegans oogenic germline syncytium over short and developmental time scales

J. Linehan, M. Werner, D. Lee, S. Marron, A. Maddox; University of North Carolina, Chapel Hill, NC.

The production of a female gamete with sufficient cytoplasmic volume to support embryogenesis involves the spatial-temporal coordination of intercellular signaling, cell architecture, differentiation, and cell cycle progression. Oocytes are produced by syncytia, tissue-like cells consisting of many partially compartmentalized nuclei connected to a common cytoplasm by stable actomyosin rings. In *C. elegans*, oogenesis is carried out in a U-shaped germline. Mitotic germline stem cell compartments divide at one end of the syncytium, and compartments are gradually displaced along the length of the syncytium as they proceed through stages of prophase of meiosis I, over the course of 40+ hours. Cytoplasm flows into the syncytial central core from nurse cell-like compartments and apoptotic compartments, and into rapidly growing presumptive oocytes proceeding around the bend. It remains unknown how cortical contractility and the size of compartments and their actomyosin rings are differentially regulated in enlarging, cellularizing and apoptotic germ cells. Here we report that distribution of actomyosin is highly variable throughout the germline and that levels of actomyosin on intercellular bridge rings are not sufficient to explain ring size dynamics during oogenesis, suggesting that this process is regulated by a balance of mechanical forces. While the actomyosin rings rimming cytoplasmic bridges are relatively stable, actomyosin rings close in several contexts: when stem cells divide mitotically, when compartments undergo apoptosis, and when presumptive oocytes cellularize. To analyze actomyosin ring dynamics in the germline, we developed a method of mounting *C. elegans* that sustains reproductive health for multiple hours. Using this technique, we collected multi-hour time-lapse fluorescence image series of ring closure during stem cell cytokinesis, compartment apoptosis, and cellularization. Closure speed does not scale with initial ring size for these three different kinds of rings, as we and others have shown occurs in other *C. elegans* cell types. This indicated that germline rings ingress via distinct and novel mechanisms. The density dynamics of the scaffolding protein anillin (ANI-1) and of non-muscle myosin II (NMY-2) throughout ingression are also distinct among germline ring types. Using a theoretical framework, we found that fitting ring protein density dynamics depended on a unique combination of parameter values (cortical contributions, positive and negative feedback loops, and ring size) for each ring type. Together, our findings support the conclusion that the mechanisms of ring intrinsic contractility are distinct among the three contexts of ring closure in the shared cytoplasm of the *C. elegans* oogenic germline syncytium.

B62/P2460

RhoA regulator-mediated control of cortical actomyosin patterning in the *Drosophila* egg chamber and embryo

J. A. Jackson[1], M. Denk-Lobnig[2], A. C. Martin[1]; [1]Biology, Massachusetts Institute of Technology, Cambridge, MA, [2]Department of Biopyhsics, University of Michigan – Ann Arbor, Ann Arbor, MI.

Spatiotemporal regulation and polarization of force generation within cells by cortical actomyosin is critical to development. As a result, actomyosin contractility is subject to multiple layers of regulation

and feedback, notably through the RhoA GTPase/Rho kinase pathway. We discovered traveling waves of actomyosin in nurse cells in late-stage *Drosophila* egg chambers, during which these fifteen support cells rapidly transfer their contents to the growing oocyte. These cell-scale waves lead to heavy shape deformation and initiate after each cell has shrunk to 25% its original volume. We show that actomyosin waves are regulated by the GTPase-activating protein (GAP) RhoGAP15B and the guanine nucleotide exchange factor (GEF) Pbl/Ect2. Both RhoGAP15B and Ect2 localize to the nucleus in the earlier stages of oogenesis and become cytoplasmic prior to wave onset, suggesting that release of GAP and GEF is responsible for wave onset. Furthermore, we found that we could induce similar actomyosin waves in the embryo during gastrulation, in which actomyosin behavior is normally pulsatile, by simultaneously overexpressing a GEF (RhoGEF2) and GAP (RhoGAP71E/C-GAP). Overall, these results suggest that transitions between cortical actomyosin patterns are controlled through dynamic activation and inactivation of RhoA and its effectors rather than by overall RhoA activity levels. We are continuing to investigate how systematic perturbation of GAP and GEF levels affects spatiotemporal actomyosin behavior in different developmental contexts.

B63/P2461

Growth of a *Drosophila* embryo actin cap begins with apparent coupling of Arp2/3 network induction and exocytosis at plasma membrane infolds organized by centrosomes

R. Tam, N. Vergara Ruiz, T. Harris; Cell and Systems Biology, University of Toronto-47731885, Toronto, ON, CANADA.

Centrosomes spatially organize the actin cortex and membrane secretion in various cellular processes, but the coupling of these effects is incompletely understood. In the syncytial *Drosophila* embryo, nucleus-associated centrosomes induce cortical actin caps which grow into dome-like compartments to house each mitotic apparatus. The actin cap grows to meet an actomyosin border which exerts tension on the actin cap. Compartment formation requires Arp2/3 actin networks and membrane exocytosis, and early caps have a folded topography. Thus, we hypothesized that plasma membrane infolding is coupled with the earliest stages of Arp2/3 network induction and membrane secretion organized by the centrosome. Live imaging the nascent cap revealed plasma membrane folds and tubules in proximity to centrosomal microtubules. As the cap grew, the folds and tubules shifted to its periphery and apparently joined the sides of the forming dome while the dome surface flattened. Centrosome disruption by centrosomin RNAi reduced the initial plasma membrane folds and tubules, suggesting they are pulled inward by centrosomal microtubules. This local infolding seems to overcome a general cortical tension, since weakening the actomyosin cortex by myosin depletion resulted in ectopic folding between centrosomes. The tubules extended from the base of folds and colocalized with Rab8-positive membranes, suggesting sites of exocytosis. Moreover, an Arp2/3 induction pathway (the Rac-GEF Sponge, Rac-GTP, and SCAR), Arp3, and F-actin were enriched at the centrosome-proximal folds and tubules relative to non-centrosomal-associated natural and ectopically-induced folds. Depletion of Arp3 resulted in abnormal aggregation of the centrosome-associated plasma membrane folds, indicating that local induction Arp2/3 networks normally separates the folds and tubules. We propose that centrosome microtubules pull the plasma membrane inward to couple exocytosis and Arp2/3-induction pathways, and that local Arp2/3-based actin polymerization may push the membrane structures apart to optimize exocytosis and promote subsequent expansion of the cap.

B64/P2462

Plasma membrane organization and dynamics in relation to the actomyosin network of the early syncytial Drosophila embryo cortex

R. K. Naidoo, T. J. C. Harris; University of Toronto, Toronto, ON, CANADA.

The cell cortex is a thin layer of actin cytoskeleton that associates with the inner leaflet of the plasma membrane (PM). The primary functions of the cell cortex are to provide structure and force generation, giving the cell highly polymorphic and dynamic remodelling properties essential for processes such as cell division, migration, and cell-cell adhesion. Studying how materials organize at the cortex and how they interact as a composite during dynamic events provides holistic understanding of cortical remodelling processes. The syncytial preblastoderm stage of Drosophila embryogenesis provides a system to study the structure of the PM and actomyosin across a large and relatively uniform cortex, and additionally how the structure behaves during actomyosin assembly/disassembly cycles. Here, using a combination of spinning disk confocal microscopy, 3D reconstruction soft- ware, and genetic perturbation we have characterized structural interaction between the PM, actomyosin network, and endoplasmic reticulum (ER) membrane during cyclic preblastodermal contraction. We dual-imaged the PM and myosin and observed that the PM structure is organized into three distinct layers which fold above and below the actomyosin cortex. The continuity of this membrane was verified using 3D reconstruction analyses. The folded structure, identity of the three structural layers, and positioning of the myosin network within the middle PM layer, were supported with additional probes. We then asked if the two materials were interacting with each other during myosin contractile activity and observed that myosin recruitment to the cortex correlated with condensation of PM undulation in XY and Z. Next we asked if the intertwined recruitment of myosin with the PM played a role in PM structure by disrupting heavy chain myosin using shRNA. Myosin perturbation resulted in abnormal structure of the PM where the size of undulation and the thickness and depth of the membrane significantly increased. Together, these results show that the myosin network affects the folded structure of the PM, indicating that they are an integrated material. We then investigated the bottom layer of the PM structure below the actomyosin cortex by dual-imaging the PM with the ER membrane. We found that the bottom layer of the PM overlaps with ER membrane tubules and together they follow contractile dynamics of the cortex. We propose a model of the cell cortex in which the PM undulates above and below the actomyosin cortex and its bottom layer folds meet with ER membranes. We hypothesize that the intertwined relationship between the PM and myosin network, and contact between the PM and ER regulate myosin network translocation during graded cortical contractility.

B65/P2463

Interrogating Differential Contributions of Adhesion Proteins BOC and CDON to Sonic Hedgehog Cytoneme Formation

C. A. Daly, E. Hall, D. Stewart, S. Ogden; Cell and Molecular Biology, St Jude Children's Research Hospital, Memphis, TN.

Proper physiological development relies on cell-to-cell communication via secreted morphogens. These signaling molecules facilitate long distance cellular communication in temporal- and tissue-specific manners to instruct cell fate and pattern tissues. Sonic Hedgehog (SHH) morphogens play integral roles in a variety of developmental processes including neural tube patterning and limb development. SHH-producing cells extend thin, actin-based projections termed “cytonemes” that facilitate direct transport

of ligand to signal-receiving cells. To permit mechanistic studies on cytonemes in mammalian systems, our lab developed protocols that allow preservation of cytonemes for confocal analysis in cultured cells and murine embryos. Using these techniques, we found that expression of SHH promotes cytoneme initiation in cultured murine fibroblasts. We determined that while Dispatched (DISP) deployment receptor is largely dispensable for cytoneme formation, it is critical for efficient loading of SHH into cytonemes both *in vitro* and *in vivo*. Notably, the structurally related SHH coreceptors CDON (Cell adhesion-associated Down-regulated by Oncogenes) and BOC (Brother of CDON) mediate SHH-induced cytoneme formation and stability. Further study of these three receptors led to identification of an interaction between DISP and BOC that occurs independent of SHH ligand status. Conversely, DISP and CDON interact in the absence of SHH and disengage upon SHH binding to DISP. These results, along with the different expression domains of BOC and CDON in the developing embryo, point towards distinct roles of the coreceptors in signal relay from the SHH-producing tissue. Since loss of both BOC and CDON ablates SHH-induced cytonemes, one of these functions appears to be activation of cytoneme-initiating signaling occurring downstream of SHH-coreceptor engagement. However, the specific interactions and intracellular signaling pathways that result in cytoneme initiation have yet to be identified. My focus is to clarify the SHH-activated signals occurring in response to its association with CDON, BOC, and/or DISP that drive cytoneme-mediated transport of SHH during embryonic development.

B66/P2464

Investigating tropomyosin isoform localization and function during sea star development

V. Montenegro, C. B. Shuster; Department of Biology, New Mexico State University, Las Cruces, NM.

Spatial segregation of viscoelastic and contractile actin networks helps drive critical cell shape changes such as polarized cell migration and cytokinesis. Tropomyosin (Tpm) represents an actin-binding protein whose differential effects on filament branching, actin depolymerization and myosin contractility may help define these different cytoskeletal networks. In vertebrates, four genes are alternatively spliced to generate 28 different isoforms, which display different effects on actin organization, diverse expression patterns and differing levels functional redundancy. However, the isoform-specific roles of Tpm splice forms in regulating cellular mechanics during cell division and early development remain unclear. In the sea star Patiria miniata, a single Tpm gene gives rise to three long and two short isoforms, and in oocytes, mRNA for one long (X3) and both short isoforms (X4 and X5) were detected. To explore the spatial dynamics of these proteins, fluorescent fusions for Tpm X3 and X4 were expressed in Patiria oocytes and embryos. Both isoforms localized to the cortex prior to meiosis I and were recruited to actin flares upon hormone addition. Upon germinal vesicle breakdown, both isoforms were largely cleared from the cortex, tracking with previously described decreases in cortical actin. Surprisingly, neither isoform was enriched in the polar body contractile ring, where it has been long assumed to be essential, but both isoforms were enriched in the polar body. Throughout early embryogenesis, X3 and X4 became increasingly restricted to the apical cortex and adherent junctions, but could also be detected on the mitotic spindle and at the nuclear envelop just prior to NEB. During mitosis, X3 and X4 remained largely cortical, and neither isoform could be detected in the contractile ring. To determine the specific functions of the three expressed Tpm isoforms during meiosis and early development, morpholinos against X3-X5 were designed. Oocyte maturation, fertilization and early cleavage were normal in morphants, but depletion of either long or short Tpm isoforms failed to form a midgut, which normally expands dramatically during late gastrulation. While these results support a role for Tpm in ensuring the integrity of the apical actin cytoskeleton in epithelia, current efforts are focused on achieving

phenotypes earlier in development to better understand the role of these proteins in determining the cellular mechanics of meiosis and early embryonic cleavages.

B67/P2465

Transgelin as a Regulator of Myofibroblast Differentiation in Epithelial to Mesenchymal Transition

G. Emin; University of Delaware, Newark, DE.

Cellular differentiation into highly contractile myofibroblasts can be a major underlying cause of morbidity and mortality in a large number of fibrotic diseases. Filamentous (F-)actin reorganization into stress fibers and the incorporation of alpha smooth muscle actin (αSMA) into these stress fibers play a critical role in myofibroblast differentiation. An understanding on the regulation of F-actin reorganization could lead to the development of therapeutic interventions against fibrosis. Transgelin (TAGLN), is an actin associated molecule that is upregulated early during fibrotic EMT. It is speculated that TAGLN is required to cross-link F-actin for stress fibers formation, but this remains unclear. In addition, it is not certain if TAGLN regulates the incorporation of αSMA. This led us to test the hypotheses that Tagln is required for the differentiation of epithelial cells to myofibroblasts by promoting (1) stress fiber formation and (2) the incorporation of αsma into the stress fibers.
We developed an ex vivo whole organ model of fibrosis by placing isolated mouse ocular lenses in culture. In this model we saw F-actin reorganization into stress fibers within 1 hour. This also resulted in an increase in αsma and Tagln mRNA levels, demonstrating co-regulation. Next we used an in vitro model of fibrosis to determine the association of Tagln with stress fibers and asma. Exposure of cultured immortalized mouse lens epithelial cells (imLECs) to TGFβ2 resulted in co-upregulation of Tagln and αsma mRNA and protein levels. Using fluorescence confocal microscopy, we find that of the cells that organized F-actin into stress fibers, 40% do not express TAGLN. Intriguingly we found that all the cells that incorporated αSMA express TAGLN. Exposure of imLECs to TGFβ2 increases the proportion of cells, from 17% to 26%, that have αSMA and TAGLN in stress fibers.
Our data thus far suggests that stress fiber formation is not necessarily dependent on TAGLN, but that αSMA incorporation may be dependent on TAGLN. Ongoing TAGLN knockdown/knockout studies will determine if TAGLN binding to F-actin is required for αSMA incorporation. Understanding the role of TAGLN in myofibroblast differentiation and the generation of contractile forces will provide insight into whether targeting TAGLN could be a means of preventing the transition of cells into myofibroblasts during fibrosis.

B68/P2466

Comprehensive analysis of actin binding proteins and actin nucleation factors in erythropoiesis reveals Tensin1 as a novel actin regulator in erythroid terminal differentiation

A. Ghosh[1,2], S. Barndt[3], M. Coffin[4], J. Mason[4], V. Fowler[4], S. Lo[5]; [1]Biological Sciences, University of Delaware, Newark, DE, [2]Delaware Biotechnology Insititute, Newark, DE, [3]Medical and Molecular Sciences, University of Delaware, Newark, DE, [4]University of Delaware, Newark, DE, [5]School of Medicine, University of California, Davis, CA.

Mammalian red blood cells (RBCs) are generated in a terminal erythroid differentiation pathway culminating in cell polarization and expulsion of the nucleus (enucleation). Actin filament polymerization is critical for enucleation, but the molecular mechanisms for either are not well understood. We utilized publicly available RNA-seq and proteomics datasets to analyze actin-binding proteins and actin

nucleation factors differentially expressed during erythroid terminal differentiation. We discovered that the focal adhesion protein, Tensin1 (TNS1), shows a dramatic increase in expression during terminal erythroid differentiation at both transcriptional and translational levels. This contrasts with other actin-binding proteins that either decreased or showed a minor increase in expression during erythroid differentiation when compared to TNS1. Tensins are a family of proteins typically found at specialized cell-matrix focal adhesions. TNS1 is the largest of the four proteins in this group with a predicted molecular weight of ~185 kDa, containing N-terminal and internal actin-binding domains, and C-terminal SH2 and PTB domains. We found that human CD34+ cells differentiated to erythroid cells express a novel truncated form of TNS1 (e-TNS1) (predicted MW ~125 kDa) missing the N-terminal actin-binding domain, likely due to an internal TNS1 mRNA translation start site. We performed CRISPR/Cas9 gene editing of the e- TNS1 gene in CD34+ cells prior to differentiation and examined the effects of loss of e-TNS1 in terminally differentiated erythroid cells via a combination of cellular and biochemical analyses. In cells with TNS1, confocal microscopy revealed that e-TNS1 is localized in the cytoplasm during terminal erythroid differentiation, with no apparent membrane association or focal adhesion formation. Similar to our previous studies, we observed distinct foci of F-actin in differentiated cells with a polarized nucleus, and in enucleating cells. By contrast, cells lacking e-TNS1 post-Cas9-mediated gene editing were found to have greatly reduced F-actin foci. Interestingly, we also observed that the Glycophorin-positive erythroblasts lacking TNS1 were significantly larger than cells with TNS1, suggesting a role for TNS1-regulated F-actin polymerization in cell size reduction late in terminal differentiation. In conclusion, our study shows that e-TNS1 is a novel regulator of erythropoiesis with a critical role in terminal erythroid differentiation of human CD34+ hematopoietic stem cells via regulating F-actin polymerization as well as cellular size reduction. We speculate that e-TNS1 signaling pathways enable the F-actin reorganization necessary for enucleation and formation of mature RBCs.

B69/P2467

Myo1c regulates neurite formation in mouse cortical neuron through regulating F-actin aggregation and actin-membrane interplay

X. Liu, S. Bennison, K. Toyooka; Drexel University School of Medicine, Philadelphia, PA.

Myosins are actin-binding proteins that are most notable for functioning as actin motor proteins. MYO1C belongs to the class 1 myosin, and it is encoded in the Miller-Dieker syndrome critical region on human chromosome 17p13.3. Neurodevelopmental defects including impaired neurite formation are common in MDS patients, however, MYO1C's roles in neurons and neurodevelopment are unclear. We found mouse Myo1c is expressed in the cerebral cortex from embryonic to neonatal stages. To study Myo1c functions, we knocked down Myo1c in layer 2/3 cortical pyramidal neurons using *in utero* electroporation and discovered defects in neuronal morphogenesis in Myo1c-depleted neurons, including shorter apical dendrites at P3 and decreased neurite number at P12. *Ex vivo* live imaging reveals that Myo1c KD neurons in layer2/3 grow neurites faster than the control, suggesting a disruption in the polarity due to lack of Myo1c. In *in vitro* mouse cortical neurons, besides a reduced number of neurites, Myo1c KD also causes a shorter axon. These together suggest that Myo1c plays essential roles in neurite outgrowth. In neuromorphogenesis research, an ongoing question is how actin and cell membrane cooperate to extend protrusions and neurites. Immunocytochemical analysis shows Myo1c is highly expressed at the filopodial and lamellipodial region in stages 1 and 2 of neurite outgrowth colocalizing with F-actin aggregation, which is an essential step in neurite outgrowth. This indicates the association of Myo1c with F-actin organization during the early stages of neurite formation. Myo1c KD in

cortical neurons in DIV0 shows a reduction of F-actin aggregates in the soma area, suggesting a role of Myo1c in promoting F-actin aggregation. On the other hand, Myo1c can link to the lipid rafts in the cell membrane through interaction with phosphoinositide PIP2. Lipid raft isolation followed by western blot shows that Myo1c level in lipid raft is high during neurite initiation. The treatment of neurons with MBCD, a lipid raft disruptor, shows similar phenotypes as seen in Myo1c KD neurons. These include the disrupted Myo1c localization as well as F-actin localization.To identify the molecular signaling pathway, we performed the co-immunoprecipitation analysis and we identified Myo1c bound to ADNP, a strong autism risk protein as well as a responsible gene for ADNP syndrome. This further indicates the importance of Myo1c in neurodevelopment and neurodevelopmental disorders. Together, these shed light on Myo1c's novel role in the regulation of actin and potentially serve as a promoter in the membrane-actin coupling during neurite initiation.

B70/P2468

The Roles and Regulation of Capping Protein in Cortical F-actin Network Organization in the *C. elegans* Zygote

S. E. Yde[1], R. S. Kadzik[1], E. Munro[1,2,3], D. R. Kovar[1,2,3,4]; [1]Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, [2]Committee on Development, Regeneration, and Stem Cell Biology, University of Chicago, Chicago, IL, [3]Institute for Biophysical Dynamics, University of Chicago, Chicago, IL, [4]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL.

The actin cytoskeleton is highly dynamic and continually remodeled during organism development in order to accomplish many fundamental tasks, including polarity establishment, cell division, morphogenesis, and endocytosis. To perform these functions, architecturally distinct filamentous (F-) actin networks must coexist within a cell. Thus, how cells assemble, maintain, and disassemble functionally diverse F-actin networks from a common pool of actin and actin-binding proteins (ABPs) in a shared cytoplasm is an important question. A key determinant of a specific network's architecture and dynamics is the length of actin filaments within the network. Capping protein (CP) acts as an important regulator of actin filament length, by binding the fast-growing barbed ends of actin filaments to prevent further assembly or disassembly.We are combining *in vitro* reconstitution with *in vivo* analysis to investigate the mechanistic role(s) that *C. elegans* CP (*Ce*CP) plays in actin cytoskeleton self-organization in the one-cell *C. elegans* embryo (zygote). In polarized zygotes, two distinct F-actin structures assemble at the anterior cortex, both of which contain *Ce*CP. Filopodia assemble during early mitosis, and mini-comets assemble during late mitosis. In zygotes depleted of *Ce*CP via RNAi, the size and number of filopodia is significantly increased, the time period of filopodia appearance is extended, and there is a corresponding decrease in the assembly of mini-comets. This suggests that in addition to maintaining the architecture, abundance, and timing of the assembly of individual networks, *Ce*CP also plays an important role in controlling the balance of actin incorporation into competing structures.In addition to *Ce*CP's distinct roles in different F-actin networks, we have determined drastic differences in the lifetimes of *Ce*CP-actin barbed end-binding events *in vitro* (t_{half}=~650 s) and *in vivo* (~8 s). These data suggest that there are additional factors that regulate the association of *Ce*CP with actin filaments *in vivo*. We are currently investigating the role of multiple putative CP regulators in the *C. elegans* zygote. We hypothesize that these regulators are differentially active in different networks in order to assemble filaments of varying lengths. Interestingly, we have observed that CARMIL, a canonical CP regulator, localizes preferentially to the shafts of the filopodia. We are currently investigating the differential

regulation of CP within different F-actin networks using *in vivo* imaging of endogenously tagged proteins, RNAi knockdowns, and *in vitro* reconstitution with purified proteins.

Actin-Membrane Interactions

B71/P2469

Phase separation properties determine the*in vivo*function of adaptor proteins in myoblast fusion.

R. Zhang, **D. Tong**, P. Pandey, Z. Ruan, D. Lee, E. Chen; Univeristy of Texas Southwestern Medical Center, Dallas, TX.

Cell surface transmembrane receptors often form nanometer- to micrometer-scale clusters to initiate signal transduction in response to environmental cues. At the sites of Drosophila myoblast fusion known as the fusogenic synapse, Ig domain-containing cell adhesion molecules (CAMs) accumulate in clusters and recruit over a dozen additional proteins to facilitate cell membrane fusion. These proteins are enriched in a “focus” to promote actin polymerization and invasive membrane protrusions. The SH2 and SH3 domain-containing adaptor proteins, Crk, Drk, and Dock, are known to link CAMs and the actin cytoskeleton. However, which adaptor protein replays the cell fusion signal from the CAM, Sticks and stones (Sns), to the actin cytoskeleton remains a longstanding question. Using an *in vitro* phase separation assay, we found that the cytodomain of Sns phase separated by itself and that Crk, but not Drk or Dock, partitioned into the Sns condensates. Correspondingly, Crk was recruited to the liquid droplets formed by Sns cytodomain in cultured S2R+ cells. Moreover, *crk*, but not *drk* and *dock*, mutant embryos exhibited a severe myoblast fusion defect, despite the accumulation of Sns and F-actin at the fusogenic synapse. EM analysis showed that in *crk* mutant, the invasive protrusions were abnormally wider, indicating disorganized actin filaments within these protrusions. We further show that the actin-binding WASP-interacting protein Solitary (Sltr) was no longer recruited to the fusogenic synapse in crk mutant embryos. Taken together, our study demonstrates that the distinct phase separation properties of the small adaptor proteins determine their *in vivo* function in myoblast fusion, and suggests that Sltr is recruited to the Sns-Crk condensate at the fusogenic synapse to increase the mechanical strength of the actin cytoskeleton, allowing the formation of narrow invasive protrusions to promote cell membrane fusion.

B72/P2470

Self-organized Rho and F-actin patterning in an artificial cortex

J. Landino[1], M. Leda[2], A. Michaud[3], Z. T. Swider[3], M. Prom[3], C. M. Field[4], W. M. Bement[3], A. G. Vecchiarelli[1], A. B. Goryachev[2], A. L. Miller[1]; [1]University of Michigan, Ann Arbor, MI, [2]University of Ediburgh, Edinburgh, UNITED KINGDOM, [3]University of Wisconsin, Madison, WI, [4]Harvard Medical School, Boston, MA.

The cell cortex, comprised of the plasma membrane and an underlying meshwork of filamentous actin (F-actin), is remodeled during a variety of essential biological processes including cell adhesion, cell migration, and cell division. Previous work has shown that prior to large-scale remodeling, the cell cortex is dynamically patterned with subcellular waves of F-actin assembly and disassembly, a phenomenon termed “cortical excitability”. In developing embryos, cortical excitability is generated through coupled positive and negative feedback, with rapid activation of F-actin assembly driven by the small GTPase Rho, followed in space and time by inhibition of Rho activity Investigating the mechanisms that support

and regulate cortical patterning is currently limited by a lack of technical approaches that can bridge our understanding of biochemical feedback signaling and cortical pattern formation, including the molecular regulation of signaling molecules, membrane dynamics, and cytoskeletal remodeling. A breakthrough in this gap in knowledge has been the development of an “artificial cortex”, made from supported lipid bilayers (SLBs) and *Xenopus* egg extract, which successfully reconstitutes active Rho and F-actin dynamics in a cell-free system. This reconstituted system spontaneously develops two distinct types of self-organized cortical dynamics: singular excitable Rho and F-actin waves, and non-traveling oscillatory Rho and F-actin patches. Like *in vivo* cortical excitability, patterning in the artificial cortex depends on Rho activity and F-actin polymerization. We also find that SLB fluidity directly influences the propensity for pattern formation in the artificial cortex, which suggests dynamic membranes support cortical patterning. These findings reveal that the cell cortex is a self-organizing structure and present a novel approach for investigating mechanisms of Rho-GTPase-mediated cortical dynamics.

B73/P2471

Actin cortex drives membrane tension propagation in cells

H. De Belly[1], S. Yan[2], H. Borja da Rocha[3], S. Ichbiah[3], J. Town[1], H. Turlier[3], C. Bustamante[2], O. Weiner[1]; [1]UCSF, San Francisco, CA, [2]UC Berkeley, Berkeley, CA, [3]College de France, Paris, France

For proper regulation of physiology, cells need a way to bridge local biochemical signalling events to cell-wide responses. Membrane tension has been proposed serve this integrative role for cell migration, cell cycle, and cell fate. How far membrane tension propagates in cells is not well understood, with conflicting data in the field, some indicating that membrane tension rapidly propagates across the entire cell and other studies indicating that membrane tension is locally constrained. We hypothesized that some of these discrepancies could be due to methodological differences, such as exogenous force application that may not accurately mimic endogenous forces. To help resolve this controversy, we are using surgical optogenetic tools to trigger focal endogenous mechanical perturbations (such as cell protrusion) in combination with precise multipoint force measurements using a dual optical tweezer setup. Our work reveals that actin driven mechanical perturbations, like those occurring during cell protrusion, lead to rapid membrane tension propagation across the cell with little to no attenuation. In stark contrast, exogenous mechanical perturbations including pulling on the membrane only (such as tether pulling) are locally constrained and do not propagate across the cell. We develop a simple mathematical mechanical model of the cell that captures these key observations and suggests that the cortex and membrane act as an integrated system for propagating mechanical forces across the cell. Our work reveals that the plasma membrane is indeed a long-range integrator of mechanical information across the cell, but only when engaged in a context where forces are applied to both the membrane and cortex, as in a cellular protrusion.

B74/P2472

Wiskott-Aldrich Syndrome protein promotes antigen-specific selection of germinal center B-cells by modulating antigen presentation and mitochondrial function

Y. Li[1], R. Dai[1], J. van Haaren[2], A. Bhanja[2], X. Zhao[1], **W. Song**[3]; [1]Chongqing Key Laboratory of Child Infection and Immunity, Children's Hospital of Chongqing Medical University, Chongqing, CHINA, [2]Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, [3]Chongqing Key Laboratory of Child Infection and Immunity, University of Maryland, College Park, MD.

Wiskott-Aldrich Syndrome protein (WASp)-deficiency causes severe immune disorders, characterized by poor antibody responses to infections and elevated autoantibodies. However, the cellular mechanisms by which WASp regulates antigen affinity-dependent selection of B-cells in germinal centers remain elusive. Using WASp knockout (WKO) and bone marrow chimeric mice, we demonstrated germinal center B-cell (GCB)-intrinsic roles for WASp in selecting high-affinity antigen-specific GCBs. Antigen-specific IgG in the serum, their relative affinity, and the percentages of antigen-specific GCBs were all drastically reduced in WKO and WKO chimeric mice, compared to WT and WT chimeric mice. While the B-cell receptor internalized antigen at a similar rate in WKO and WT GCBs, the ability of WKO GCBs to present antigen and interact with cognate T-cells was defective. Surprisingly, WKO GCBs displayed a higher survival and a lower apoptosis rate than WT GCBs in the absence of antigenic stimulation and T-cells. This increased survival rate was associated with reduced ROS and mitochondrial superoxide production in WKO GCBs compared to WT GCBs. Mitochondria in WKO GCBs exhibited lower levels of dysfunction indicators, higher membrane potential, larger volumes, and longer shapes than those in WT GCBs. WKO significantly reduced the cellular level of F-actin and the recruitment of Drp1, a dynamin-related protein required for mitochondrial fission, to mitochondria. Our data demonstrate essential and B-cell-intrinsic roles for WASp in interacting with T-cells to activate antigen-specific GCB and in promoting the apoptosis of non-specific GCBs through mitochondrial stress.

B75/P2473

Pivotal roles of branched actin in promoting B-cell receptor signaling attenuation

A. Bhanja[1], M. Lazzaro[1], I. Rey-Suarez[2], A. Upadhyaya[2], **W. Song**[1]; [1]Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, [2]Physics, University of Maryland, College Park, MD.

B-cell receptor (BCR) signaling in response to antigenic stimulation is a critical initiation and regulatory step for B-cell activation and differentiation. The actin cytoskeleton plays essential roles in modulating BCR signaling. When encountering cell-surface antigens, actin-driven B-cell spreading on the antigen-presenting surface amplifies signaling by promoting surface BCR clustering, while B-cell contraction following the spreading promotes signal attenuation by driving the merger of BCR clusters. This signal attenuation curtails the activation of autoreactive B-cells. However, the mechanism by which the actin cytoskeleton switches BCR signaling from the amplification to attenuation mode was unknown. Here, we show that branched actin polymerized by Arp2/3 is required for B-cell contraction in addition to its role in B-cell spreading. Contracting B-cells exhibit centripetally moving inner actin foci in the B-cell membrane region contacting antigen-presenting surfaces. These inner actin foci are derived from the lamellipodial actin network. N-WASP, but not WASP, activated branched actin polymerization forms these actin foci by sustaining their lifetime and centripetal movement. N-WASP-dependent inner actin foci are necessary for recruiting non-muscle myosin II to create an actomyosin ring-like structure at the periphery of the membrane contact region, driving contraction. B-cell contraction particularly increases

the BCR molecular density of individual clusters, compared to B-cell spreading and post-contraction phases. Increased molecular density in BCR clusters leads to decreases in the phosphorylation of the BCR, the stimulatory kinase Syk, and the inhibitory phosphatase SHIP1. These results suggest that the actin cytoskeleton reorganizes from the lamellipodial branched actin networks to centripetally moving actin foci, enabling actomyosin ring-like structure formation, through N-WASP-activated Arp2/3. Actomyosin-mediated B-cell contraction attenuates BCR signaling by increasing receptor molecular density in individual BCR clusters.

B76/P2474

Epithelial polarity requires WAVE-dependent transport of E-Cadherin/HMR-1

M. Soto[1], L. Cordova Burgos[1], D. Rao[1], J. Egwuonwu[1], S. Borinskaya[2]; [1]Rutgers - Robert Wood Johnson Medical Sch, Piscataway, NJ, [2]Saint Elizabeth University, Morristown, NJ.

Cadherin dynamics drive morphogenesis, while defects in Cadherin polarity contribute to diseases, including cancers. However, the forces polarizing Cadherin membrane distribution are not well understood. We previously showed that WAVE-dependent branched actin polarizes Cadherin distribution in the embryonic and adult *C. elegans* intestine, and suggested one mechanism is protein transport. While previous studies suggested that WAVE is enriched at various endocytic compartments, the role of WAVE in protein traffic is understudied. Here we test the model that WAVE regulates Cadherin by polarizing its transport. In support of this model we show that: 1) endogenously tagged WAVE accumulates in vivo at several endocytic compartments, including recycling endosomes and Golgi; 2) likewise, Cadherin protein accumulates at recycling endosomes and Golgi; 3) loss of WAVE components reduces Cadherin accumulation at apically directed RAB-11-positive recycling endosomes and increases accumulation at Golgi. In addition, live imaging illustrates that dynamics and velocity of recycling endosomes enriched for RAB-11::GFP and RFP::RME-1 are reduced in animals depleted of WAVE components, and their movements are misdirected, suggesting WAVE powers and directs their movements. This *in vivo* study demonstrates the importance of WAVE in promoting polarized transport in epithelia, and supports that WAVE promotes cell-cell adhesion and epithelial polarity by promoting Cadherin transport.

B77/P2475

Mechanisms underlying the slow axonal transport of spectrin and its assembly into the membrane associated peroidic skeleton

O. Glomb[1], G. Swaim[2], S. Yogev[3]; [1]Neuroscience, Yale University, New Haven, CT, [2]Cell Biology, Yale University, New Haven, CT, [3]Neuroscience, Cell Biology, Yale University, New Haven, CT.

How the size of the cytoskeleton is scaled to the size of the cell remains poorly understood, particularly in neurons. In the axon, the delivery of cytoskeletal proteins from the cell body is the main mechanism determining overall cytoskeletal mass. This delivery is believed to consist of fast movements interspersed by long pauses, consistent with the known rates of molecular motors. We developed a probe to visualize the axonal delivery of endogenous spectrin and its assembly into the membrane periodic cytoskeleton (MPS) during axon stretch-growth, when axon size scales with the growing organism. We find that, contrary to current models of axonal delivery of cytoskeletal proteins, spectrin can move processively at rates ~100-fold slower than that of molecular motors. We identified two conserved coiled-coil proteins that link spectrin to kinesin-1 and are required to generate processive

slow movement and to form the MPS. Spectrin was delivered to hotspots along the axon, where it locally incorporated into the MPS lattice to drive its expansion. Interestingly, the rate of spectrin integration into the lattice and hotspot expansion was set by the rate of animal growth and hence axon stretch growth. Thus, slow processive transport and local expansion scale the cytoskeleton to the size of the axon.

B78/P2476

INF2 Regulates Organelle Motility via ER-associated Actin

C. R. Schiavon[1], Y. Wang[1], A. Manole[1], T. Sung[1], Y. Dayn[1], P. Latour[2], A. Echaniz-Laguna[3], M. E. Shy[4], B. J. Zuchero[5], G. S. Shadel[1], U. Manor[1]; [1]Salk Institute for Biological Studies, La Jolla, CA, [2]Hospices Civils de Lyon, Bron, FRANCE, [3]Paris-Sud University, Le Kremlin Bicêtre, FRANCE, [4]University of Iowa, Iowa City, IA, [5]Stanford University, Stanford, CA.

Charcot-Marie-Tooth (CMT) disease is the most common inherited neurological disorder, affecting ~1 in 2500 people. It is a peripheral, length-dependent neuropathy defined by progressive degeneration of the nerves in the distal parts of the body. Mutations in a wide variety of proteins cause CMT. Many of these proteins play a direct or indirect role in organelle mobility. Given this fact and the fact that cells affected in CMT (peripheral neurons) are the longest cells in the body, it is hypothesized that CMT is a result of impaired organelle mobility. INF2 is one of the proteins mutated in CMT. It is an ER-anchored formin protein which promotes actin assembly and modulates mitochondrial morphology and motility through this actin polymerizing activity. All CMT-causing INF2 mutations result in a constitutively active form of INF2. We predicted that this aberrant activity causes excess accumulation of ER-associated actin around mitochondria, restricting their mobility. We have demonstrated that mitochondrial mobility is reduced by exogenous expression of two CMT-causing INF2 mutations. We also studied the mobility of other organelles and found that expression of these mutants also reduces the mobility of endosomes and lysosomes, broadening the potential organelles that may play a role in the pathomechanism of CMT. We further validated these results in primary human fibroblasts from two CMT patients harboring endogenous INF2 mutations as well as neurons directly converted from these fibroblasts. Neurons derived from CMT patient fibroblasts also display neuronal swellings, consistent with a neurodegenerative phenotype. The reduction in organelle mobility is reversible by pharmacologically depolymerizing actin, supporting the model of excessive actin polymerization limiting organelle movement. To avoid the toxic side-effects of wholesale actin depolymerization, we developed a novel tool to specifically disrupt ER-associated actin. We found that this tool results in rescue of organelle mobility without causing obvious toxicity and reverses the neuronal swellings observed in derived CMT neurons. These findings demonstrate a potential mechanism by which INF2 mutations lead to excessive ER-associated actin, impaired organellar mobility, and ultimately, peripheral neuron degeneration.

Motors: Kinesins in Neurons

B80/P2477

Selective axonal transport through branch junctions is directed by growth cone signaling and mediated by KIF1/kinesin-3 motors

S. Tymanskyj, L. Ma; Department of Neuroscience, Thomas Jefferson University, Philadelphia, PA.

Development of a functioning nervous system requires the establishment of individual neurons communicating with multiple targets, achieved by a single axon generating multiple branches. These branches differ in lengths and targets, and as such have different metabolic and protein needs. Delivery of proteins from the cell body to the correct targets rely on the orchestration of microtubule-based transport by the motor protein kinesin family, but if and how protein or membrane cargos are regulated along the axon is unknown. Here we demonstrate that cargo delivery is in fact regulated along the axon, specifically through branch junctions where they can target specific branches. We show that anterograde transport of LAMP-1 and synaptic vesicles through axonal branch junctions is highly selective, influenced by branch length and more strongly by growth cone motility. We further show that not all cargos are responsive to the same cues with secretory vesicles such as BDNF being largely unresponsive. We developed an optogenetic tool based on mimicking the guidance receptor activity of positive (TrkA) and negative (PlexinA4) cues and focally activated one specific growth cone. We were able to demonstrate that signaling from the growth cone can rapidly direct transport through branch junctions located a distance away from the site of activation. We further demonstrate that such transport selectivity is differentially regulated and mediated by the KIF1/kinesin-3 family motors. We propose that this transport regulation through branch junctions could broadly impact neuronal development, function, and regeneration.

B81/P2478

Differential regulation of kinesin and dynein motility by microtubule-associated septin complexes

Y. Suber, K. Nakos, E. Spiliotis; Drexel University, Philadelphia, PA.

Septins are a large family of GTP-binding proteins that oligomerize into higher order complexes, which associate with microtubules (MTs). Microtubule motors kinesin and dynein mediate plus-end and minus-end directed intracellular transport respectively, and their activity and directionality are regulated by several different factors including microtubule-associated proteins (MAPs). In previous work (Karasmanis et al, Dev Cell 46:204, 2018), we found that the MT-associated septin 9 (SEPT9) impedes the motility of kinesin-1/KIF5C and enhances kinesin-3/KIF1A, and through this differential regulation promotes neuronal axon-dendrite membrane polarity. Here, we explored the hypothesis that different septin complexes exert different effects on kinesin and dynein motors. Using in vitro single molecule motility assays, we compared the effects of the MT-associated SEPT2/6/7, SEPT5/7/11 and SEPT2/6/7/9 on the motility of kinesin-1/KIF5C, kinesin-3/KIF1A and the dynein-dynactin-BicD2 (DDB) motor complex. MT-associated SEPT2/SEPT6/SEPT7 and SEPT5/SEPT7/SEPT11 impeded the motility of kinesin-1/KIF5C in a similar manner to SEPT9. Strikingly, we found that the motility of kinesin-1/KIF1A is differentially modulated by SEPT9, SEPT2/6/7 and SEPT5/7/11. MT-associated SEPT2/6/7 decreased the binding, velocity and run-lengths of kinesin-3/KIF1A, which are all enhanced by SEPT9. In contrast, SEPT5/7/11 did not affect the binding and run-lengths of kinesin-3/KIF1A, but had a mild impact on velocity and pausing. DDB velocity and run lengths were significantly reduced by MT-associated SEPT2/6/7/9,

SEPT2/6/7 and SEPT5/7/11. Although SEPT2/6/7/9, SEPT2/6/7 and SEPT5/7/11 inhibited the MT landing rates of DDB, we found that only SEPT2/6/7/9 and SEPT5/7/11 enhanced the number of immotile DDB particles, which was indicative of a decrease in DDB detachment. SEPT5/7/11 was similarly unique in impeding the detachment of immotile kinesin-1/KIF5 and kinesin-3/KIF1A motors. Taken together, these data demonstrate that MT-associated septins fine tune motor motility by modulating individual parameters such as motor detachment in a septin paralog and complex-specific manner.

B82/P2479

Kinesin-1, -2 and -3 motors use family-specific mechanochemical strategies to effectively compete with dynein during bidirectional transport

W. O. Hancock[1], A. M. Gicking[1], T. Ma[1], Q. Feng[2], R. Jiang[3], S. Badieyan[4], M. A. Cianfrocco[4]; [1]Biomedical Engineering, Penn State University, University Park, PA, [2]Cell Biology, Yale University, New Haven, CT, [3]Molecular & Cell Biology, UC Berkeley, Berkeley, CA, [4]Biological Chemistry, University of Michigan, Ann Arbor, MI.

Bidirectional cargo transport in neurons requires competing activity of motors from the kinesin-1, -2 and -3 superfamilies against cytoplasmic dynein-1. Previous studies demonstrated that when kinesin-1 attached to dynein-dynactin-BicD2 (DDB) complex, the tethered motors move slowly with a slight plus-end bias, suggesting kinesin-1 overpowers DDB but DDB generates a substantial hindering load. Compared to kinesin-1, motors from the kinesin-2 and -3 families display a higher sensitivity to load in single-molecule assays and are thus predicted to be overpowered by dynein complexes in cargo transport. To test this prediction, we used a DNA scaffold to pair DDB with members of the kinesin-1, -2 and -3 families to recreate bidirectional transport in vitro, and tracked the motor pairs using two-channel TIRF microscopy. Unexpectedly, we find that when both kinesin and dynein are engaged and stepping on the microtubule, kinesin-1, -2, and -3 motors are able to effectively withstand hindering loads generated by DDB. Stochastic stepping simulations reveal that kinesin-2 and -3 motors compensate for their faster detachment rates under load with faster reattachment kinetics. The similar performance between the three kinesin transport families highlights how motor kinetics play critical roles in balancing forces between kinesin and dynein, and emphasizes the importance of motor regulation by cargo adaptors, regulatory proteins, and the microtubule track for tuning the speed and directionality of cargo transport in cells.

B83/P2480

The CFEOM-causing β-tubulin-R380C mutation activates kinesin motor activity

P. Sinha Roy[1], A. Luchniak[1], A. Kumar[1], I. Schneider[1], V. Gelfand[2], R. Jernigan[1], **M. Gupta, Jr**[1]; [1]Iowa State University, Ames, IA, [2]Northwestern University, Chicago, IL.

Kinesin-mediated transport on microtubules is critical for axon development and health. Mutations in the kinesin Kif21a, or the microtubule subunit β-tubulin, inhibit axon growth and/or maintenance producing the eye-movement disorder congenital fibrosis of the extraocular muscles (CFEOM). While most CFEOM-causing β-tubulin mutations examined inhibit kinesin-microtubule interactions, Kif21a mutations hyperactivate the motor. These contrasting observations led to opposed models of inhibited or hyperactive Kif21a in CFEOM. We show that, contrary to other CFEOM-causing β-tubulin mutations, R380C enhances kinesin activity. β-tubulin-R380C expression increases kinesin-mediated peroxisome transport in S2 cells. The binding frequency, percent motile engagements, run length and plus-end dwell

time of Kif21a are higher on β-tubulin-R380C than wildtype microtubules reconstituted in vitro. This conserved effect persists across tubulins from multiple species. Using molecular dynamics, we visualize how β-tubulin-R380C allosterically alters structural elements within kinesin that underlie processive motility. These findings resolve the disparate models and confirm both inhibited or hyperactive kinesins are involved in CFEOM. They also demonstrate the microtubule's role in regulating kinesin and highlight the importance of balanced transport for cellular and organismal health.

B84/P2481

Entering the primary cilia of sensory neurons in *C. elegans*: A single-molecule perspective

A. Mitra, E. Loseva, E. J. G. Peterman; Department of Physics and Astronomy, Vrije Universiteit Amsterdam, Amsterdam, NETHERLANDS.

Intraflagellar transport (IFT) along a microtubule-based axoneme orchestrates the growth and maintenance of primary cilia, elongated signalling hubs protruding out of eukaryotic cells. Kinesin-2 motors drive anterograde IFT trains, assembled at the cilia base, into the cilium, importing cargo proteins hitchhiking on the trains. In this study, we employ a single-molecule fluorescence microscopy approach involving localized illumination, to visualize and quantify the entry of individual anterograde kinesin-2 motors, kinesin-II and OSM-3, and cargo proteins associated with anterograde IFT trains, IFT dynein and tubulin, into cilia located at the tip of dendrites of chemosensory neurons in *C. elegans*. Single-molecule tracking reveals docking of the different proteins to IFT trains, as well as pausing and the entry into the cilium. At the ciliary base, where the transition zone acts as a diffusion barrier for larger proteins, IFT dynein docks on to assembling IFT trains, which appear stuck for a significant duration. Trains are picked up at the base by Kinesin-II, transporting them through the transition zone, into the cilium. The other kinesin motor, OSM-3, hops, just like the cargo protein, tubulin, onto these moving IFT trains throughout the transition zone. Pooling the localization information of many single molecules allows mapping of the 3D ultrastructure of the axoneme with a precision of several tens of nanometers, in wild-type *C. elegans* as well as mutant strains lacking kinesin-2 motor and/or transition zone function. These maps provide new insights in ciliary structure at the base, train-docking dynamics and the interplay of the kinesin-2 motors, kinesin-II and OSM-3. In summary, using single-molecule live-cell imaging in *C. elegans*, we illuminate structural and dynamic features at the base of primary cilia.

B85/P2482

An ALS-associated KIF5A mutant forms oligomers and aggregates and induces neuronal toxicity

K. Chiba, J. Nakano, S. Niwa; Tohoku University, Sendai, Japan

KIF5A is a kinesin superfamily motor protein that transports various cargos in neurons. Mutations in Kif5a cause familial amyotrophic lateral sclerosis (ALS). It has been suggested that ALS is caused by loss of function of KIF5A. However, the precise mechanisms regarding how mutations in KIF5A cause ALS remain unclear. Here, we show that an ALS-associated mutant of KIF5A, KIF5A(Δexon27), is predisposed to form oligomers and aggregates in vivo and in vitro. Moreover, KIF5A(Δexon27)-expressing Caenorhabditis elegans neurons showed morphological defects. These data collectively suggest that ALS-associated mutations of KIF5A are toxic gain-offunction mutations rather than simple loss-of-function mutations.

Motors: Kinesins in Regulation of Microtubule Dynamics

B86/P2483

Understanding the role of the beta tubulin tail as a mechanism to control kinesin-8 activity

K. Alemany, J. Moore; University of Colorado AMC, Aurora, CO.

The microtubule surface is a molecularly complex landscape, decorated by the heterogeneous carboxy-terminal tails (CTTs) of tubulin proteins, which serve as potential sites of regulation. The 'tubulin code' hypothesis proposes that differences in CTTs, such as changes in amino acid sequences that are encoded by different genes called tubulin isotypes and post-translational modifications, present a molecular code for specifying interactions with proteins that regulate microtubules. We previously conducted a genetic interaction screen to identify genes that require CTTs for function. This screen identified Kip3, a kinesin-8 family motor, as potentially requiring the β-tubulin CTT for function. Kinesin-8 members are unique motors that combine two activities to regulate aspects of dynamic instability: processive motility towards the plus end of the microtubule and depolymerizing activity. Here we seek to define the mechanism through which the β-CTT promotes Kip3 function. We address these questions using budding yeast to assess Kip3 function and define the features of β-CTT and Kip3 that are important for this interaction. From our results we draw three major conclusions. First, the β-CTT is not necessary for Kip3 binding and motility along the microtubule. Second, in mutant cells that lack β-CTT there is increased accumulation of Kip3 at the plus end and across the microtubule. Third, the β-CTT is necessary to promote Kip3 mediated catastrophe. Furthermore, we find that high levels of Kip3 induce tubulin accumulations that are unable to form microtubules, and this effect requires β-CTT. Our findings suggest that the β-CTT is important for Kip3 depolymerase activity at the microtubule plus end by supporting a Kip3-tubulin binding state that inhibits the normal tubulin-tubulin interactions that occur in microtubules. To further define this binding state, we mapped and analyzed potential interaction sites on both the β-CTT and Kip3. Our results support a model in which the negatively charged residues of the β-CTT may interact with positively charged residues found in Kip3's Loop12 that are well-conserved across kinesin-8 family members. We further propose that β-CTTs are tunable platforms for regulating kinesin-8 motor's ability to depolymerize microtubules.

B87/P2484

Liquid-liquid phase separation generates KIF1C clusters that drive microtubule sliding, knotting, and breakage in cells

Q. Geng, R. Prasad, K. Verhey; The University of Michigan, Ann Arbor, MI.

Kinesin proteins are exquisite nanomachines that convert chemical energy into mechanical work. Transport kinesins can carry cargoes along microtubule (MT) tracks and can slide anti-parallel microtubules. Here, we find that overexpression of the kinesin-3 family member KIF1C in cells leads to heavily curved, knotted, and fragmented MTs in regions where KIF1C accumulates and forms puncta. Interestingly, the KIF1C puncta display properties of liquid-liquid phase separation (LLPS). A highly disordered proline-rich domain (PRD) in the KIF1C tail is necessary for driving LLPS and for KIF1C's ability to induce MT destruction. Furthermore, a split kinesin assay demonstrates that the PRD is sufficient for LLPS in cells and for MT destruction when combined with a kinesin motor head. Together, these experiments indicate a strong correlation between KIF1C LLPS and MT destruction. Live imaging in cells shows that groups of KIF1C motors in the puncta slide anti-parallel microtubules into dense knots that

break. To further understand how LLPS of KIF1C promotes MT destruction, we substituted the KIF1C tail with constructs that undergo LLPS, clustering, or anchoring to organelle surfaces. All substitutions recapitulate KIF1C-induced MT destruction, supporting our working model that clustered KIF1C motors lead to active sliding and extensive crosslinking of the MT network, eventually causing MT breakage. Overall, our work reveals that the unique LLPS property of the KIF1C PRD clusters multiple motors and affects MT network integrity. These results suggest that clustering of many kinesin motors, including on an organelle, can have deleterious effects on the MT network in cells.

B88/P2485

Importin a/β Promote Kif18B Microtubule Association to Spatially Control Microtubule Destabilization Activity

S. C. Ems-McClung[1], S. Shrestha[1], M. A. Hazelbaker[1], S. L. Shaw[2], **C. E. Walczak**[1]; [1]Indiana University School of Medicine-Bloomington, Bloomingrton, IN, [2]Indiana University, Bloomington, IN

Tight regulation of microtubule (MT) dynamics and organization is necessary for proper spindle assembly and chromosome segregation. The microtubule destabilizing Kinesin-8, Kif18B, controls astral MT dynamics and spindle positioning. Kif18B interacts with importin α/β as well as with the plus-tip tracking protein EB1, but how these associations modulate Kif18B *in vivo* is not known. We mapped the key binding sites on Kif18B, made residue-specific mutations, and assessed their impact on Kif18B function. Blocking EB1 interaction disrupted Kif18B MT plus-end accumulation and inhibited its ability to control astral microtubule length in cells. Blocking importin α/β interaction reduced Kif18B plus-end accumulation on astral MTs but did not inhibit its ability to control astral MT length. *In vitro*, importin α/β increased Kif18B binding to the MT lattice by increasing the on-rate and decreasing the off-rate, which stimulated MT destabilization activity. In contrast, EB1 promoted Kif18B-mediated MT destabilization without increasing lattice binding *in vitro*, which suggests that EB1 and importin α/β have distinct roles in the regulation of Kif18B-mediated MT destabilization. We propose that importin α/β-spatially modulate Kif18B association with MTs to facilitate its MT destabilization activity. Our results suggest that Ran-regulation is important not only to control molecular motor function near chromatin but also provides a spatial control mechanism to modulate MT binding of NLS-containing spindle assembly factors.

Motors: Myosins in Contraction

B89/P2486

motile properties of nonmuscle myosin 2c

M. Rahman, J. R. Sellers; NIH, Bethesda, MD.

Nonmuscle myosin 2 (NM2) paralogs are members of actomyosin cytoskeletal complex where they hydrolyze ATP to convert chemical energy into mechanical functions in cells. NM2 are expressed in all mammalian cells where they contribute to many subcellular functions including cell migration, adhesion and cytokinesis. Mutations in NM2 proteins causes cancer, hearing loss, bleeding disorders etc. NM2 has three paralogs, namely NM2A, B and C. Both NM2A and NM2B are well investigated regards to their roles in disease and development with extensive characterization of their biochemical, kinetic and structural properties. NM2C, on the other hand, is the most recently identified isoform and less is known about its roles, biochemical and kinetic properties compared to its other two counterparts.

Recent studies showed the involvement of NM2C in disease and development. For example, NM2C is associated with sensorineural hearing impairment and peripheral neuropathy. Other studies show roles of NM2C in cell dynamics, e.g. formation of sarcomere like architecture in the tricellular apical junction and control of microvilli growth. Such roles of NM2C in cells are critically dependent on NM2C generated motility. However, the motile properties of NM2C are not studied in detail like NM2A or NM2B. Thus, study of the motile properties of NM2C will bring information that will be required to understand its subcellular function in greater detail. Here, we study the motile properties of NM2C e.g. speed at which it translocates actin, processive movement of NM2C filaments on actin and on actin-fascin bundles. We find from the in vitro motility assays that NM2C translocates actin at 46.1±0.4 (mean ± SEM) nm/s which is slowest among all three NM2 paralogues. We also show that GFP attachment at the NM2C regulatory light chain site reduces (~ 15%) actin gliding speed in vitro which is consistent with earlier data of NM2A. From single filament assays, we also show that NM2C filaments move processively on both actin (27.8±0.45 nm/s) and actin-fascin bundles (22.1±0.86 nm/s) in the presence of methyl cellulose. We find that NM2C filaments makes large stacks in vitro which is consistent with recent observation in cells.

B90/P2487

In vitro assembly and tension formation of a synthetic sarcomere

P. Bleicher[1], N. Billington[1], R. G. Hart[2], I. Chandrasekar[2], J. R. Sellers[1]; [1]National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, [2]Enabling Technologies Group, Sanford Research, Sioux Falls, SD.

Myosin 2 is the motor that powers muscle contraction and various cytoskeletal functions such as adhesion, cell motility and cytokinesis. Muscle fibers are arranged into repetitive sarcomeres where bipolar myosin filaments interact with actin filaments to bring about shortening. These systems are amenable for mechanical studies at the level of whole muscle, individual muscle fibers and even as isolated myofibrils. In contrast myosin 2 in nonmuscle cells is localized in many places including just behind the leading edge, in stress fibers and during cytokinesis in the contractile ring. Recently several super resolution light microscopic studies of various cells have shown evidence for a sarcomere-like arrangement of actin and myosin in certain parts of cells. However, even here these structures are not easily amenable to mechanical studies to probe the properties of myosin. To address this in vitro we are using micropatterning to create sarcomeric arrangements of actin and nonmuscle myosin 2 paralogs. These model sarcomeres can self-organize into tense, anti-parallel fibers with the motile nonmuscle myosin 2 filaments in their center as observed by total internal reflection fluorescence microscopy (TIRFM). A fluorescence resonance energy transfer (FRET)-based force sensor is used to quantify the tension formation of the paralogs, revealing the single-molecular force and ratio of active and relaxed myosin heads. Alpha-actinin reduces the contraction velocity without affecting the force during tension formation. Taken together, this minimal system provides a mechanistic foundation for intracellular sarcomere contractility where the effect of accessory proteins can easily be probed.

B91/P2488

Smooth muscle myosin 2 filaments dynamically assemble and activate during induced contractility

M. Bennett[1], S. Sala[1], S. Demeulenaere[2], H. Patel[1], P. Oakes[1], J. Beach[1]; [1]Loyola University, Chicago, IL, [2]Loyola University, Wilmington, IL.

Vascular smooth muscle cells (SMCs) line blood vessels throughout the body, where they dynamically alter vessel diameter to regulate blood pressure, provide structural integrity, and absorb shock on a

beat-to-beat timescale. Interactions between actin and the molecular motor smooth muscle myosin 2 (SM2) drive contractility within the cell and allow smooth muscle to perform these vital functions. As smooth muscle function fails, profound vascular disease can ensue with tragic results- even death. Integral to SM2's ability to function is the formation of SM2 filaments and the interaction with these filaments with actin. Although smooth muscle contraction is integral to health, there are many unknowns about SM2 filament assembly and dynamics and the role these dynamics play in SMC contraction. Our goals were first, to determine equilibrium kinetics between SM2 monomer and filaments, and second, to determine if this equilibrium is shifted during SMC activation to drive contraction. Fluorescence recovery after photobleaching (FRAP) of SM2 in A7R5 (rat aortic smooth muscle cells) shows SM2 in filaments are highly dynamic, with recovery halftimes on the order of 10s of seconds with SMC activation stabilizing filaments. This suggests SM2 filament dynamics are more similar to non-muscle myosin 2 (NM2) than striated myosin 2. Consistently, structured illumination microscopy of SM2 and NM2 suggests that these closely-related myosin 2s can co-assemble into filaments. To further understand SM2 during cell contractility, we analyzed assembly levels. The prevailing model in SMC contraction suggests that activation of existing filaments leads to cell wide contractile forces, as opposed to increased filament assembly. However, our data and recent literature demonstrate a significant steady-state SM2 monomer pool (~25% of total SM2). By using an imaging-based assembly assay, the percent of filamentous and monomeric SM2 can be measured. Our results indicate the monomer pool decreases while filamentous myosin increases upon SMC activation. This indicates the filamentous population is tunable, with additional monomers being assembled into filaments upon activation of SMCs by carbachol. Traction force microscopy in A7R5 cells transfected with a calcium sensor confirms that the increase in SM2 filament formation coincides with both intracellular Ca response to stimulus and increase in contractile force production. This supports a model of SMC contraction in which both existing filaments are activated and new filaments are formed to produce cell level contractile forces.

B92/P2489

Cellular Contractile Structures are Organized by Assembly and Coalescence of Myosin Contractile Units

W.-h. Chou[1,1], M. Molaei[1,1], H. Wu[2], P. Oakes[2], J. Beach[2], M. Gardel[1,1,1,1]; [1]University of Chicago, Chicago, IL, [2]Loyola University Chicago, Chicago, IL

Various cellular processes are powered by mechanical forces, such as cell migration, cell division, and mechanosensing. At the molecular scale, mechanical forces are generated by myosin filaments sliding adjacent actin filaments past each other. Inside cells, the actomyosin cytoskeleton further builds higher-order contractile structures, such as stress fibers, where arrays of myosin contractile units contract crosslinked F-actin bundles to produce contractile forces. While the molecular regulation of myosin is well-understood, much less is known about myosin contractile units. Recent studies have identified that myosin contractile units arise from myosin stacks that nucleate and amplify at the leading edge of the cell. However, the organization and regulation of myosin contractile units across the cell remain poorly understood. In this work, we quantified myosin contractile units across the cell using quantitative microscopy. We label non-muscle myosin IIA in the cell using the CRISPR knock-in approach, which allows us to quantify the number of myosin filaments associated with myosin contractile units at the endogenous level. We find that myosin form contractile units of various sizes, ranging from 5 to 50 myosin filaments per contractile unit. Large contractile units tend to be on large F-actin bundles, such as

on mature transverse arcs or ventral stress fibers. However, F-actin does not play a role in organizing the contractile units. Instead, we show that the sizes of myosin contractile units are regulated by their assembly and coalescence, similar to size control mechanisms of cellular organelles. Combining live cell imaging, pharmacological perturbations, and quantitative analysis, we find that myosin filament assembly is required to assemble myosin contractile units. In addition, myosin motor activity can drive the coalescence between contractile units, which is not required for the assembly of contractile units but is required for the maturation of contractile units. With a simple agent-based simulation, we demonstrate that the assembly and coalescence of myosin contractile units underlie their organization in cells. Together our data provide new insight into myosin regulation in cells, revealing an additional regulatory layer of cellular contractility.

B93/P2490

Robust mechanism of tension production compensates for increasing the dosage of myosin Myp2 in the cytokinetic contractile ring

J. Martinez-Baird, K. Bellingham-Johnstun, C. Laplante; North Carolina State University, Raleigh, NC

Cytokinesis partitions a mother cell into two daughter cells by the constriction of a contractile ring of actin and myosin. In this contractile ring, myosin motor proteins can generate tension by binding to and pulling on actin filaments. Yet, how these myosins drive the production of tension during cytokinesis remains unknown. In fission yeast, two type II myosins cooperate to drive cytokinesis: Myo2 and Myp2. Myp2 contributes to ~50% of the total tension of the constricting contractile ring. Based on this role, we hypothesized that the amount of tension generated by the contractile ring scales up with increasing amounts of Myp2. We tested this hypothesis by measuring the impact of doubling the genetic dosage of *myp2* (2x*myp2*) on cytokinesis. We integrated a second copy of *mEGFP-myp2* into the *leu1* locus of cells expressing *mEGFP-myp2* at their endogenous locus. Fluorescence intensity comparisons showed that adding a second copy of *myp2* increased the amount of fluorescence signal from mEGFP-Myp2p to ~1.27X. Unsurprisingly, this incremental increase in Myp2p had no measurable impact on the constriction rate of rings in 2x*myp2* cells. Furthermore, the comparable constriction rates suggests that the total amount of tension generated by 2x*myp2* rings is comparable to wild types. However, increasing Myp2 by ~25% causes its arrangement around the ring to shift from an uneven crescent shape in wild types to a relatively uniform circle in 2x*myp2*. This organizational re-arrangement suggests an underlying change in the mechanical proprieties of contractile rings in 2x*myp2* cells. We measured the impact of increasing Myp2 on the mechanical properties of the contractile ring using laser ablation. The displacement of the severed tips of ablated constricting contractile rings follows an exponential profile as expected of a viscoelastic material under tension. Our data showed 2x*myp2* rings had a dramatic increase of ~90% in viscous drag, consistent with Myp2 acting as a crosslinker of actin filaments. These results highlight both the strong relationship between Myp2 and viscous drag, such that a 25% increase of Myp2p in the ring resulted in a dramatic increase in viscous drag, and the robust nature of the compensatory mechanisms in the contractile ring that can overcome significant increases in viscous drag to conserve constriction rate.

B94/P2491

Control of actomyosin contractility by spectrin maintains the distribution of axonal microtubules and F-actin

G. L. Swaim, O. Glomb, S. Yogev; Yale University, New Haven, CT

The axonal cytoskeleton is subject to exterior forces acting on the axon itself as well as to interior forces generated by molecular motors. How cytoskeletal organization is maintained despite these forces is poorly understood. From a candidate approach screen for mutants that disrupt cytoskeletal organization, we identified that mutations in *unc-76*/FEZ1 lead to a drastic misdistribution of axonal microtubules (MTs), where the distal axon contains areas with no MT polymers interspersed among accumulations of excessive MT polymers. We determined that this misdistribution is not due aberrant MT dynamics, but rather to uncontrolled movement of whole polymers, which in control animals are static. Using additional screening, single cell knock down approaches, live microscopy, and genome engineering we determined that UNC-76/FEZ1, along with the short coiled coil protein UNC-69/SCOC, act as a kinesin-1 adaptor for spectrin transport. The spectrin cytoskeleton is severely disrupted in either *unc-76* or *unc-69* mutants, and microtubules are misdistributed not only in *unc-76* mutants, but also upon cell-specific knock down of *unc-69* and spectrin. To investigate the mechanisms that lead to microtubule disorganization in the absence of the spectrin cytoskeleton we conducted *in vivo* live imaging of polymer transport in combination with genetic and pharmacological perturbations. Surprisingly, we found only a minor contribution of MT-based motors to MT polymer movement, but an important role for actomyosin contractility: acute inhibition of myosin II with blebbistatin arrested MT movement and long-term treatment rescued the steady-state misdistribution of MTs. Consistent with a role for the spectrin cytoskeleton sequestering actomyosin, we found that upon disruption of the spectrin cytoskeleton ectopic F-actin accumulates around microtubule ends . Taken together, our data suggest a major role for the axonal spectrin cytoskeleton in organizing the distribution of F-actin and MTs, partially by regulating myosin II activity. Myosin II is known to be a part of the spectrin cytoskeleton, where it enables the axon to withstand external forces. We propose that restricting actomyosin contractility to the sub-membranal spectrin lattice allows the axon to counteract external forces without applying uncontrolled forces on its other cytoskeletal elements.

B95/P2492

Reconstitution of the calcium-triggered contractile apparatus of the ciliated protozoa *Spirostomum*

J. Honts[1], T. Bartolome[1], F. Melzl[1], C. O'Shea[1], S. Bhamla[2]; [1]Drake University, Des Moines, IA, [2]Georgia Institute of Technology, Atlanta, GA

Protists, notably ciliated protozoa, exhibit a novel form of contraction that is rapidly triggered by calcium ions but does not require ATP hydrolysis. Ultrafast forms of calcium-triggered contraction have been described for the *Vorticella* stalk and the *Spirostomum* cell body. In heterotrich ciliates *Stentor* and *Spirostomum*, contraction is mediated by branching, spindle-shaped structures called myonemes. The goal of these studies is to reconstitute this novel contractile system in vitro. Through mass spectrometric analysis of *Spirostomum ambiguum* cytoskeletal preparations, a family of EF-hand proteins closely related to the calcium-binding protein centrin have been identified in several *Spirostomum* species. Bioinformatics analysis of published and derived proteome data suggests the existence of a distinct subgroup of centrin-like calcium-binding contractile proteins in a variety of ciliated protozoa. In addition, several candidates for a centrin-binding Sfi1-like scaffold protein have been identified in the

Spirostomum proteome. Synthetic genes encoding *Spirostomum* centrin and Sfi1-like centrin-binding proteins have been engineered for expression in *E. coli.* Expression of the Sfi1-like repeat protein in bacteria yielded little protein, while co-expression of *Spirostomum* centrin and the Sfi1-like repeat protein yielded both proteins in milligram quantities. These proteins have been purified by IMAC and gel filtration chromatography. Gel filtration chromatographic separations revealed a single peak containing the two proteins, with a molecular mass consistent with the formation of a 1:1 complex. Electrophoretic mobility shift experiments showed both centrin and the centrin-Sfi1 complex undergo a conformational change upon the addition of calcium. Microscopic observations revealed that concentrated mixtures of these two proteins assembled to form unusual branching networks when calcium was added. Current efforts are aimed at engineering a self-assembling form of centrin-Sfi1 filament bundles that can be induced to contract in response to calcium ions. Our ultimate goal is to engineer *Spirostomum* contractile proteins to fully reconstitute in vitro the ultrafast form of contraction seen in living *Spirostomum* cells. These studies should yield insight into the structure, mechanism and regulation of these novel contractile systems in living cells.

B96/P2493

Defining the functions of myosin 2 isoforms in epithelia using intestinal organoids

M. Yousafzai, K. Remmert, L. Boley, C. Alexander, A. Pedrosa, J. Hammer; National Heart, Lung, and Blood Institute, Bethesda, MD

Mouse intestinal organoids provide a physiological context for the investigation of fundamental aspects of epithelial cell biology (e.g. cell:cell adhesion, maintenance of barrier function, cell division, and extrusion of apoptotic/aneuploid cells) because they retain a folded, crypt-villus topology in the form of a three-dimensional, regenerative, stem cell-driven tissue model. Moreover, they are amenable to structure-function analyses using knockout (KO) and knockin (KI) mice, shRNA-mediated protein knockdown (KD), the expression of fluorescent reporters via lenti and/or Piggybac-mediated recombination, and imaging using several super-resolution modalities. Here we imaged the localization and dynamics of endogenous myosin 2A (M2A) and myosin 2C (M2C) using organoids made from GFP-M2A and GFP-M2C KI mice. M2A localizes along the lateral surface of organoids, consistent with its known roles in supporting cadherin-based cell: cell adhesion at adherent junctions (AJs) and ZO-1-based cell: cell adhesion at tight junctions (TJs). M2A also localizes in stress fibers at the basal surface, consistent with a role in supporting integrin-based cell: ECM adhesion as well. Interestingly, M2A is seen not only in the cleavage furrow of dividing enterocytes, but also in evenly-spaced strings of cortical mini-filaments that span the long axis of the dividing cell. shRNA-mediated knockdown of M2A in GFP-M2A KI organoids leads to the appearance of a multilobed phenotype that scales with the degree of KD (the dimmer the GFP-M2A signal, the more multilobed the organoid appears). Current efforts are directed at understanding the physical basis of this robust phenomenon. Finally, endogenous M2C localizes exclusively at the apex of organoid, where it appears as a belt of mini-filaments that resides directly across from ZO-1 in TJs and is brightest at tricellular junctions. Current efforts are directed at creating confluent 2D organoids from WT and M2C KO mice to assess the role of M2C in maintaining barrier function using a variety of permeability assays.

B97/P2494

Mmsdh as a bridge between metabolic activity and cortical dynamics

M. C. Jacob, D. N. Robinson; Johns Hopkins School of Medicine, Baltimore, MD

The cytoskeleton is an energy intensive system responsible for many cellular processes such as cell motility, morphology, and division. In the case of Dictyostelium discoideum, the cortical cytoskeleton is a highly dynamic network that actively responds to sites of mechanical stress via accumulation and disassembly of prefabricated contractility kits (CKs) at the cortex. These CKs are comprised of cytoskeletal proteins known as non-muscle myosin II (NMII), cortexillin I (cortI), and IQGAP1 and/or IQGAP2. Depending on which IQGAP is bound, these CK proteins can be mechanoresponsive or non-mechanoresponsive. Interestingly, the components of the CKs also interact with proteins outside the traditional cytoskeletal system. In our lab, past genetic selections and proteomic studies done in Dictyostelium discoideum have revealed metabolically related proteins that interact with cytoskeletal proteins like NMII and cortI. An exemplar protein that came about from these studies was the mitochondria-localized ATP/ADP antiporter, ancA. AncA was identified as a genetic suppressor of a cytokinesis-defective phenotype caused by a myosin II phosphomimetic scenario. Interestingly, ANT, the human analogue of ancA, was shown to be a genetic protector against cigarette smoke in the lung tissue of COPD patients when overexpressed - with cigarette smoke exposure known to be a disrupter actin and myosin II organization. In the same vein, methyl-malonate semialdehyde dehydrogenase (mmsdh) was found to be a genetic interactor of NMII and a biochemical interactor of cortI and IQGAP1. Mmsdh is an enzyme downstream of the valine catabolism pathway that is localized in the cytoplasm and mitochondria and that catalyzes the production of propionyl-CoA. Though much is known about mmsdh's function in metabolism, not much is known about its interaction with the cytoskeleton. Here, I am characterizing mmsdh's impact on the cytoskeleton and how these cytoskeletal interactions may contribute to mmsdh's metabolic role. To this end, we are generating an mmsdh knockout in Dictyostelium discoideum and am employing several assays to probe alterations in metabolic activity and/or cytoskeletal dynamics. Collectively, we aim to define how mmsdh contributes to cortical cytoskeleton function and how this activity may contribute to mmsdh's metabolism role.

Motors: Myosins in Cell Migration

B98/P2495

Microtubule-binding Myosin-X (Myo10) is required for efficient dendritic cell migration in complex environments

A. Gasilina, P. Rezvan Sangsari, N. Y. Morgan, J. A. Hammer; National Institutes of Health, Bethesda, MD

Activation of naïve T cells by antigen-presenting dendritic cells is a critical determinant of efficient adaptive immune responses. Following pathogenic antigen capture, dendritic cells use a CCL19/CCL21 chemokine gradient to guide their migration from the primary sampling site to the closest lymph node. During migration dendritic cells must negotiate passage through basement membranes, extracellular matrices, cell junctions, and branched vessel networks, all while maintaining speed, persistence and directionality. Efficient navigation through these complex microenvironments involves coordinated movements of the cell's nucleus, actin-rich extensions, and microtubule cytoskeleton, which together regulate space exploration, path finding, and pore size selection. In this study we are using imaging to define the contribution that the MyTH4/FERM domain-containing molecular motor myosin-X (Myo10)

makes to dendritic cell migration. Using murine bone marrow-derived and splenic dendritic cells, we show that Myo10 is dispensable for dendritic cell differentiation, maturation, and CCL19/CCL21 chemokine sensing, but is essential for efficient directed cell migration in confined and complex environments. Specifically, Myo10-depleted dendritic cells exhibit increased velocity but impaired directionality and persistence during directed migration in both confined 2D environments and complex branched networks. In complex networks especially, Myo10-depleted dendritic cells are ineffective at using their nucleus as the pore size and path selector, and they cannot resolve competing leading edges. These deficiencies result in cell entanglement and fragmentation. Current efforts are directed at determining whether Myo10 requires its microtubule-binding MyTH4 domain to control dendritic cell navigation through complex environments.

B99/P2496

Mechanoresponsive Adaptability in Pancreatic Cancer Cells and Tissue

E. Parajon, D. N. Robinson; Johns Hopkins School of Medicine, Baltimore, MD

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer characterized by early systemic dissemination and poor patient prognosis. Our lab identified a PDAC program of upregulation of key highly mechanoresponsive cytoskeletal proteins that accumulate in response to mechanical stress. Cancer cells are highly adaptive and must continuously adjust to their ever-changing environment, including nutrient and oxygen availability, stiffness of the local matrix, and the neighboring cells. The mechanoresponsive proteins compose an adaptability program that allows cells to respond to alterations in many of these parameters. In humans, this mechanoresponsive machinery encompasses nonmuscle myosin II proteins (NMIIA, NMIIB, and NMIIC), alpha-actinins (ACTN4, but not ACTN1) and filamin (FLNB with FLNA to a much smaller extent). However, it is still unclear how the mechanoresponsive cytoskeletal machinery drives PDAC dissemination and metastasis. NMIIA, NMIIC, ACTN4, and FLNB, are expressed at low levels in normal pancreatic ductal epithelia but become highly expressed in PDAC. We are utilizing a three-dimensional, reconstituted tissue model with engineered cells where we have altered the adaptability program using the CRISPR-Cas9 system. We are also investigating how these engineered cells influence neighboring cell behaviors and tissue mechanics. This study has revealed that knocking out NMIIC and ACTN4 leads to significant changes in cortical tension, cell roundness, and cell motility of metastasis derived pancreatic cancer cells. This effort will decipher how alterations in these proteins impact cell growth, shape, motility, and invasiveness and will guide future strategies for manipulating these proteins towards a therapeutic end.

B100/P2497

Identifying mechanisms of non-muscle myosin II filament assembly and amplification

M. Quintanilla[1], H. Wu[1], K. Sochacki[2], M. Akamatsu[3], J. Rotty[4], F. Korobova[5], J. Bear[6], J. Taraska[2], P. Oakes[1], J. Beach[1]; [1]Loyola University Chicago, Maywood, IL, [2]National Heart, Lung, and Blood Institute, Bethesda, MD, [3]University of Washington, Seattle, WA, [4]Uniformed Services University of the Health Sciences, Bethesda, MD, [5]Northwestern, Evanston, IL, [6]University of North Carolina, Chapel Hill, Chapel Hill, NC.

The majority of contractile forces in non-muscle cells are generated by non-muscle myosin 2-driven contraction of actin networks. Previous high-resolution imaging in the lamella of migrating fibroblasts revealed myosin filaments appear and amplify by an actin-dependent partitioning process, feeding into

larger actomyosin networks. We believe this filament amplification drives requisite physiological levels of contraction in cells and tissues. Our goals were to delineate mechanisms by which new filaments are established in the lamella, and then define, with molecular precision, how filament partitioning enables the formation of contractile networks. We fail to observe calcium- or Rho-mediated activation in the immediate vicinity of a new myosin filament, suggesting monomer activation occurs with low spatial precision. However, consistent with published work, we observe filament appearance following leading edge retraction. Appearance stalls when actin dynamics are pharmacologically halted, but rescued by myosin monomer release from posterior stress fibers via ROCK inhibition. This suggests that filament formation is a low-precision, stochastic event dependent on regional increases in available monomer. Consistently, when local concentrations of myosin monomer were artificially enriched via photo-recruitment to the plasma membrane, we observed de novo filament formation. Interestingly, once the photo-recruitable myosin initiated filament assembly, endogenous myosin also incorporated into expanding filament clusters, suggesting myosin filaments are capable of self-propagation once an initial filament is established. We then used molecular counting to determine that new lamellar filaments are ~100x more likely to form at pre-existing myosin filaments than to form a nascent filament cluster, despite ample actin available in the lamella. Finally, we demonstrate that prior to partitioning into clusters, bipolar myosin structures are already small sub-resolution filament stacks in register. Taken together, we propose a model whereby increased actin network density during retraction events acts as a kinetic trap for myosin monomers, resulting in nascent filament assembly events. Once established, myosin filaments act as a diffusion trap for assembly-competent monomers and possibly other cytoplasmic myosin filaments, resulting in sub-resolution stacks and clusters. These established filaments and filament clusters then mature into a contractile network to enable cell migration. We anticipate similar mechanisms exist in more complex contractile networks throughout cell and tissue biology.

B101/P2498

Myosin Waves and a Mechanical Asymmetry Guide the Oscillatory Migration of *Drosophila* Cardiac Progenitors

N. Balaghi[1,2], G. Erdemci-Tandogan[1,2], R. Fernandez-Gonzalez[1,2,3,4]; [1]Institute of Biomedical Engineering, University of Toronto, Toronto, ON, CANADA, [2]Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, Toronto, ON, CANADA, [3]Department of Cell and Systems Biology, University of Toronto, Toronto, ON, CANADA, [4]Developmental and Stem Cell Biology Program, Hospital for Sick Kids, Toronto, ON, CANADA.

Heart development begins with the formation of a tube, as cardiac progenitors migrate collectively from opposite sides of the embryo and meet medially. Defective cardiac progenitor migration results in congenital heart defects. However, the mechanisms of cardiac progenitor movement and coordination thereof remain largely unknown. In *Drosophila*, the embryonic heart is a linear structure composed of 52 pairs of contralateral cardiac precursors (cardioblasts, CB) that migrate dorsally and medially to form the heart tube. We developed machine learning tools to quantify the dynamics of CB migration from confocal microscopy movies. We found that CB velocities oscillated between positive (forward) and negative (backward) values. The forward steps were greater in both amplitude and duration, resulting in net forward movement of the cells. Using quantitative time-lapse microscopy, we found that the molecular motor non-muscle myosin II displayed an alternating pattern of localization between the leading and trailing ends of migrating CBs, forming oscillatory waves that traversed the cells. Notably,

the cyclic myosin flows were anti-correlated with oscillations in the position of the CB nucleus. Myosin inhibition delayed CB migration and the timely formation of the heart tube. Live imaging of embryos co-expressing fluorescent myosin and CB-CB contact markers revealed that the alternating pattern of myosin polarity in CBs was associated with alternating contraction and relaxation of the leading and trailing edges of the cell, respectively. Mathematical modeling predicted that the forward migration of CBs using alternating contraction and relaxation of both leading and trailing edges requires the presence of a boundary at the trailing edge of the cell that restricts backward movement. Consistent with this, we found a supracellular actin cable at the trailing edge of the CBs. When we used targeted laser ablation to release the tension sustained by the actin cable, we found that the amplitude of the CB backward steps increased significantly, thus reducing the speed of migration. Together, our results indicate that alternative and periodic cell shape changes generated by oscillatory myosin waves coupled with a supracellular actin cable that resists backward movements, result in a rectified form of cell migration that guides cardiac morphogenesis.

Microtubule Networks

B103/P2499

A gamma-tubulin complex-dependent pathway suppresses unscheduled ciliogenesis by promoting cilia disassembly

S. Shankar[1], Z. Hsu[2], A. Ezquerra[3], C. Li[2], T. Huang[1], E. Coyaud[4], R. Viais[3], C. Grauffel[1], B. Raught[4], C. Lim[1], J. Lüders[3], S. Tsai[2], **K. Hsia**[1]; [1]Institute of Molecular Biology, Academia Sinica, Taipei, TAIWAN, [2]National Taiwan University, Taipei, TAIWAN, [3]The Barcelona Institute of Science and Technology, Barcelona, SPAIN, [4]University of Toronto, Toronto, ON, CANADA.

The γ-tubulin ring complex (γ-TuRC) serves as a master template for microtubule nucleation, and its activity is canonically associated with the generation of microtubules to build cellular structures such as primary cilia. Here, we report a γ-TuRC-dependent pathway that promotes cilia disassembly, thereby preventing unscheduled cilia formation and ensuring cell cycle progression. Using proteomics-based mass spectrometry and immunofluorescence analyses, we demonstrate that Kif2A—a kinesin motor that bears microtubule-depolymerizing activity—is recruited to the cilium basal body in a γ-TuRC-dependent manner. Our structural and biochemical data uncover how γ-TuRC specifically recruits Kif2A via the GCP2 subunit and its binding partner Mzt2. Hence, γαμμα-TuRC functional heterogeneity at the basal body facilitates both microtubule nucleation and, through recruitment of KIF2A, control of ciliogenesis.

B104/P2500

Microtubule Severing Proteins Play Distinct Roles in Drosophila Neurons

Y. Shen, M. M. Rolls; Pennsylvania State University, State College, PA.

Microtubule severing proteins have similar protein architecture and include a microtubule-interacting domain and an AAA (ATPases Associated with diverse cellular Activities) ATPase domain that catalyzes removal of tubulin heterodimers from a microtubule using ATP as an energy source. There are four severing proteins in Drosophila neurons: Katanin, spastin, Kat60L1 and Fidgetin. The specific role of each severing protein in neurons remains unclear. In our previous study we found Fidgetin promotes degeneration of dendrites after injury, but does not seem to function in uninjured neurons. Kat60L1 has

been shown to contribute to microtubule dynamics in Drosophila dendrites, however, whether the remaining two function in dendrites is not clear. Here, we show that Katanin and spastin have distinct roles in controlling microtubule dynamics in Drosophila sensory neuron dendrites. In *Katanin* mutant neurons, very few microtubule plus and minus ends labeled with EB1-GFP are present. We considered that the strong reduction in EB1-GFP-labeled microtubule ends could be due to a deficit in microtubule growth in *Katanin* mutants. To test this hypothesis, we severed dendrites and assayed EB1-GFP in the cut off dendrite. As in control neurons, dendrite severing increased the number of microtubule ends recognized by EB1 suggesting that when ends are present in Katanin mutants they can be detected. When we overexpressed Katanin, we saw strong increases in both plus end and minus end number, suggesting Katanin functions to maintain number of microtubule ends, and thus presumably microtubule length, in neurons. While overexpression of spastin also increased the number of microtubule ends, *spastin* mutant neurons had similar numbers of growing plus ends to control neurons, however, the number of minus ends labeled with EB1-GFP was strongly reduced. We therefore hypothesized that minus ends remain capped by the g-Tubulin ring complex (g-TuRC) at dendrite branch points. To test this hypothesis, we examined the layout of microtubules at dendrite branch points using fluorescently labeled tubulin. In control neurons, microtubules pass through the branch point in a V shape along the outside and leave an empty space in the middle of the branch point. In *spastin* mutant neurons, the space in middle of the branch point was frequently occupied by microtubules, consistent with continued attachment of the minus end to the nucleation site. Together, we show that Katanin and spastin play different roles in neurons, with spastin mediating the release of new microtubules from nucleation sites and Katanin maintaining overall microtubule dynamics

B105/P2501

Dissecting the Function of the Microtubule-Associated Protein, Doublecortin

R. L. Agulto, K. Ori-McKenney; Department of Molecular and Cellular Biology, University of California, Davis, Davis, CA.

Cellular function and health rely on the precise arrangement of underlying cytoskeletal networks. Microtubule-associated proteins (MAPs) regulate microtubule dynamics, bundling, and motor transport for specific cellular processes. Doublecortin (DCX) is a MAP implicated in the neurodevelopmental diseases: subcortical laminar heterotopia (SCLH), also called “double cortex syndrome”, in females and lissencephaly in males[1]. With both SCLH and lissencephaly, affected individuals exhibit intellectual disability and epilepsy of varying severity. Though it has been shown that defects in neuronal migration cause SCLH and lissencephaly, the actual function of DCX within neurons, and how disease mutations alter this function are open areas of investigation. Recent studies have shown that the microtubule lattice can exist in an expanded or compacted state, depending on a variety of factors[2]. Certain proteins can alter the lattice as well. Kinesin-1 prefers an expanded lattice and can actively expand the lattice[3]. Other MAPs such as tau and MAP2 prefer a compacted lattice and can actively compact the lattice[4]. Microtubule lattices stabilized in the presence of taxol have been shown to be in an expanded state[5,6]. Utilizing SiR-tubulin, a fluorescent taxol derivative that binds the expanded lattice, we find that DCX binds cooperatively by actively compacting the lattice, displacing SiR-tubulin from the compacted lattice[4]. We have made several DCX constructs to dissect which domains of DCX are responsible for this cooperative binding and lattice compaction and find that the conserved linker region that connects the tandem microtubule-binding domains of DCX is important for this ability. Our experiments with DCX and different kinesin motors show that DCX can indirectly inhibit certain kinesins that prefer an expanded

lattice by compacting the lattice, providing one explanation for why DCX inhibits kinesin-1 processivity even though they have different binding sites[7]. Our observations have led us to hypothesize that compaction of the microtubule lattice by DCX can affect the local microtubule environment. Patient mutations disrupt the cooperative binding behavior of DCX[8]. Consequently, understanding why DCX displays cooperative behavior, the domains responsible for that cooperativity, and its differences on compacted and expanded microtubule lattices will provide significant insight into how DCX behaves on the microtubule to regulate diverse microtubule processes and how its function becomes altered in disease.

B106/P2502

The TOG5 domain of XMAP215 facilitates interaction with F-actin and contributes to microtubule advancement in neurons

T. O'Brien[1], G. Cammarata[2], A. Thawani[3], **L. Lowery**[1]; [1]Boston University, Boston, MA, [2]Boston College, Newton, MA, [3]Princeton University, Princeton, NJ.

Microtubule and F-actin cytoskeletal cross talk and reorganization are important aspects of cell migration and axonal guidance, but the mechanisms by which associated proteins facilitate this are still being revealed. While XMAP215 (CKAP5 / ch-TOG) has been best characterized for its microtubule polymerase function, our previous studies have highlighted a novel role for XMAP215 in facilitating interactions between microtubules and F-actin in the embryonic neuronal growth cone, the dynamic structure at the tip of the growing axon. Here, we utilized biochemical assays in combination with high resolution light microscopy, including super resolution imaging, to determine how XMAP215 links microtubules to F-actin in the growth cone. We first established the TOG5 domain as a necessary region that is responsible for direct interactions with F-actin. In addition, we determined that XMAP215 microtubule-lattice-binding activity as well as TOG5-mediated F-actin binding domains are essential for influencing microtubule guidance into the growth cone peripheral domain. Finally, we discovered that these dual cytoskeletal roles of XMAP215 are independent from, and do not require, its classical microtubule polymerase activity. Thus, our work highlights the novel and important role that XMAP215 plays in directing microtubule extension into targeted regions of the growth cone through F-actin interaction, facilitating protrusion and steering of the axon during outgrowth.

B107/P2503

Microtubule remodeling transforms the cell morphology of activated microglia

M. Adrian[1], M. Weber[1], M. Tsai[1], L. Phu[2], T. Cheung[2], W. J. Meilandt[1], C. Rose[2], C. C. Hoogenraad[1]; [1]Neuroscience, Genentech, South San Francisco, CA, [2]Microchemistry, Proteomics & Lipidomics, Genentech, South San Francisco, CA.

Microglial activation is a pathological hallmark in many neurodegenerative diseases. During activation, microglia undergo complex morphological changes, including loss of their characteristic ramified cell morphology, which is routinely used to detect and quantify inflammation in the brain. However, the molecular mechanisms enabling this morphological change and the relation between microglia cell morphology and its pathophysiological function are not understood. Here, proteomic profiling of activated microglia cells identified parallel microtubule remodeling pathways that drive the morphological change and subsequently control cytokine release. We found that activated microglia cell increase their microtubule dynamics to form a stable and centrosomally anchored microtubule array to

facilitate efficient cytokine trafficking. Moreover, we identified cyclin-dependent kinase 1 (Cdk-1) as a critical upstream regulator of microtubule remodeling, cytokine release, and morphological change in vitro and in vivo. These results demonstrate a critical role for microtubule dynamics and reorganization in microglial activation and modulating cytokine-mediated inflammatory responses.

B108/P2504

Anoxia-induced Rearrangements of Cellular Networks

J. ZHU, K. YUEN; The University of Hong Kong, Hong Kong, CHINA.

Oxygen plays a crucial role in cellular energy production because it acts as the terminal electron acceptor in mitochondrial oxidative phosphorylation. Acquiring and utilizing oxygen is essential for protozoans and metazoans on earth. In many organisms, including budding yeast, worms, and zebrafish, cells can enter a reversible suspended animation state under anoxia (0-100 ppm O_2), an extreme form of hypoxia, but the specific molecular mechanisms involved are unclear, and the responses appear to be different from hypoxia. Our current research is centred on elucidating cell molecular mechanisms and pathways in the adaptation of cellular organizations in anoxia-induced suspended animation, in which many organelles rearrange to facilitate the arrest. We have performed a genome-wide deletion gene mutant screening in budding yeast and identified 34 AIS (anoxia-induced suspended animation-required) candidate genes that can be grouped into different functional categories, such as cell cycle regulation, nuclear regulation, and chromosome organization. We have observed polarization of spindle pole body, docking of microtubules and nucleolus, changes in nuclear envelope shape and chromosome condensation by time-lapse live-cell imaging. Specifically, we identified a conserved cell division cycle gene *CDC50* that may be involved in cell polarization when entering anoxia-induced arrest and in polarized proliferation during recovery. We will further investigate how such spindle pole polarization affects kinetochore-microtubule attachment and chromosome segregation accuracy after anoxia. In conclusion, anoxia-induced rearrangements of the cellular network are important for cell survival from anoxia and such understanding will provide insights into therapeutic approaches that may prevent the reactivation of proliferation and metastasis of dormant cancer cells due to oxygen availability.

B109/P2505

C53 Interacting with UFM1-Protein Ligase 1 Regulates Microtubule Nucleation in Response to ER Stress

V. Sulimenko[1], A. Klebanovych[1], S. Vinopal[1], E. Dráberová[1], V. Sládková[1], T. Sulimenko[1], V. Vosecká[1], L. Macůrek[1], A. Legido[2], P. Dráber[1]; [1]Institute of Molecular Genetics, Prague, CZECH REPUBLIC, [2]Drexel University College of Medicine, Philadelphia, PA.

ER distribution depends on microtubules, and ER homeostasis disturbance activates the unfolded protein response resulting in ER remodeling. CDK5RAP3 (C53) implicated in various signaling pathways interacts with UFM1-protein ligase 1 (UFL1), which mediates the ufmylation of proteins in response to ER stress. Here we find that UFL1 and C53 associate with γ-tubulin ring complex proteins. Knockout of *UFL1* or *C53* in human osteosarcoma cells induces ER stress and boosts centrosomal microtubule nucleation accompanied by γ-tubulin accumulation, microtubule formation, and ER expansion. C53, which is stabilized by UFL1, associates with the centrosome and rescues microtubule nucleation in cells lacking UFL1. Pharmacological induction of ER stress by tunicamycin also leads to increased microtubule nucleation and ER expansion. Furthermore, tunicamycin suppresses the association of C53 with the

centrosome. These findings point to a novel mechanism for the relief of ER stress by stimulation of centrosomal microtubule nucleation.
This work was supported by grant 21-30281S from the Czech Science Foundation, and grants LTAUSA17052 and LX22NPO5102 from the Ministry of Education, Youth and Sports of the Czech Republic

B110/P2506

Marine sponge extract produces unique microtubule-related structural properties in myofibroblasts

M. B. Vaughan[1], S. Ko[1], A. L. Waters[2], D. Hashemi[1]; [1]Biology, University of Central Oklahoma, Edmond, OK, [2]Chemistry, University of Central Oklahoma, Edmond, OK.

Myofibroblasts are present in fibroses and contractures; these diseases are difficult to treat, and new therapies are needed. Marine sponges are known for producing bioactive compounds with therapeutic promise. We tested numerous marine sponge crude extracts for their effects on fibroblast proliferation, migration, and differentiation into myofibroblasts. One extract from a New Guinea sponge inhibited migration, but also truncated the bipolar cells' long axis, leaving cells somewhat cuboidal; this effect was similar in all 6 cell strains tested. We conducted staining to determine whether the cytoskeleton was affected. Microfilaments arranged in stress fibers were grossly unaffected by the treatment, while microtubules were severely disorganized, leaving a characteristic pattern of 4-8 foci of intensely stained microtubule tangles that developed over a period of 24-48 hours. Tubulin expression was largely unaffected. TGF-beta treatment increased alpha-smooth muscle expression by western blot and immunostaining, although the extract slightly reduced the overall effect. Proliferation was decreased, and certain cell strains were apoptotic. Fractionation will be conducted to isolate purified compounds to determine if these effects are due to a single compound or mixtures of compounds.

B111/P2507

Conserved mechanisms of branched cytoskeletal network growth in the Rhizarian amoeba, *Filoreta ramosa*

S. L. Guest, S. C. Dawson; University of California, Davis, Davis, CA.

Multicellularity involving colonial cell organization and differentiation generates emergent morphological complexity though the development of specialized cytoskeletal structures and cell types. Multicellularity has evolved at least five times across eukaryotic supergroups, including members of the SAR (Stramenopiles/Alveolates/Rhizaria). Our Rhizarian isolate *Filoreta ramosa* exhibits "aggregative multicellularity" in which amoebae fuse to form a reticulate network of branching and anastomosing pseudopodia. The resultant syncytium grows to span multiple centimeters in diameter and shares a contiguous cytoplasm, cell membrane, and cytoskeleton components. *Filoreta* uses branching mechanisms reminiscent of neuronal growth cone extension and dendritic arborization, while self-recognition enables continuous fusion events for rapid repair and dynamic morphology.
How and when have the cytoskeletal mechanisms enabling polarized growth cones and arborized morphology evolved in Eukarya? We are using our recently completed genome combined with super-resolution imaging and morphometric analyses to quantify cytoskeletal elements of complex network growth and development. We use antibodies to ɣ-TURC components and EB1 to visualize non-centrosomal MT nucleation and polarity throughout the network. We have also compared morphological perturbations in the network following treatment with various drugs affecting actin and

MT dynamics. As in neuronal growth cones, the branched network is organized with longitudinal MT arrays and actin-rich pseudopodia that initiate branch formation. Cytoskeletal drugs alter syncytial network development, underscoring the critical role of actin and MT dynamics, and actin-MT interactions in generating the branched morphology in Rhizaria and in Metazoa. We predict that the mechanisms governing the intricate cytoskeletal networks in *F. ramosa* are emergent properties of simple branch and anastomosis patterns involving widely conserved cytoskeletal proteins. Thus, the extensions and branching mechanisms typified by neuronal growth cones are conserved in Rhizaria and thus many pre-date the divergence of major eukaryotic lineages.

B112/P2508

Septin association with microtubules is regulated by phosphorylation of the microtubule-binding domain of septin9

P. Bhakt[1], I. A. Kesisova[2], E. T. Spiliotis[1]; [1]DREXEL UNIVERSITY, PHILADELPHIA, PA, [2]Massachusetts Institute of Technology, Cambridge, MA.

Septins are a family of GTP-binding proteins, which assemble into higher order oligomers and filamentous polymers, and function in cell morphogenesis, mitosis, cell migration, and host defense against pathogens. Septins are associated with the actin and microtubule (MT) cytoskeleton, and cellular membranes, but the mechanisms and signaling pathways that regulate these interactions are poorly understood. Previous work has identified putative phosphorylation sites on the N-terminal MT-binding domain of septin 9 (SEPT9), which mediates the interaction of septins with MTs. Here, we have focused on the phosphorylation of the SEPT9 residues S82 and S85 by the glycogen synthase kinase-3 beta (GSK3b), and the phosphorylation of residues S80 and S89 by the citron kinase (CK) and protein kinase A/C (PKA/PKC), respectively. S82 and S85 are also putative PKA/PKC sites, and S89 can function as a priming phosphosite for the phosphorylation of S85 by GSK3b. Notably, all serine residues are positioned between MT-binding motifs (K/R-R/x-x-D/E) of SEPT9. To investigate whether phosphorylation of these residues impact septin-MT association, we performed in vitro phosphorylation of purified recombinant Sept9, and generated a Sept9 construct with phosphomimetic mutations in S80/82/85, which were converted to aspartate (D; Sept9-S80/82/85D). We found that phosphorylation of Sept9 by PKA reduced MT-binding in vitro, and Sept9-S80/82/85D exhibited diminished interaction with MTs in vitro and in MDCK cells. Additionally, expression of the constitutively active kinases GSK3b-S9A and PKAcs-GGSD5X altered the localization of Sept9 to MTs and endomembranes. Notably, expression of Sept9-S80/82/85D in Sept7-depleted cells resulted in significant loss of MT mass, indicating that Sept9 association with MTs is critical for MT polymerization and stability. Ongoing work aims at characterizing the phosphorylation effects on SEPT9 dimerization and heteromeric assembly, its physiological significance for MT organization and functions in polarized traffic and cell polarity.

B113/P2509

AUG8 function in the *Arabidopsis* interphase cortical microtubule array

N. Chana, T. Cioffi, S. L. Shaw; Indiana University Bloomington, Bloomington, IN

The genetic mechanisms controlling cellular morphogenesis are largely unknown. These mechanisms are of particular importance in plants, which adapt to changing environmental stimuli through irreversible cell growth. Plant cell shape is defined by a semi-rigid cellulosic cell wall. Cellulose deposition is guided by a plasma-membrane associated network of microtubules nucleated by γ-tubulin ring complexes. This

cortical microtubule array organizes into specific patterns in response to developmental and environmental cues that pattern the cellulose deposition, influencing the material properties of the cell wall. Thus, studying the mechanisms regulating cortical array organization are integral to elucidating how cellular morphogenesis occurs.
In animal cells, branching microtubule-dependent microtubule nucleation occurs almost exclusively during mitosis. The hetero-octameric Augmin complex facilitates this nucleation type by connecting γ-tubulin ring complexes to pre-existing microtubules. The Augmin complex and its mitotic function are conserved in vascular plants, which create bipolar mitotic spindles in the absence of centrosomes. Bioinformatic analyses indicate that *Arabidopsis* contains four paralogous genes closely related to human HAUS8, *X. laevis* haus8.L, and *D. melanogaster* Dgt4. *Arabidopsis* EDE1 is most homologous to HAUS8 and is only expressed in mitosis. The three remaining *Arabidopsis* orthologs, SCO3, QWRF4, and QWRF8(AUG8), are expressed during interphase in growing tissues. While all HAUS8 orthologs maintain strong C-terminal conservation, QWRF4 and AUG8 contain an elaborated N-terminal domain going back to liverworts. We hypothesize that QWRF4 and AUG8 function in the organization of the interphase cortical microtubule array and may have specific roles related to the parallel and antiparallel microtubule bundles arising in different cortical array patterns. To test our hypothesis, we have identified mutants for both genes and characterized defects in the axial growth of the seedling hypocotyl. Mutants for both genes are viable and display hypocotyl hyper-elongation when grown in the dark. To characterize the molecular function of these genes, we have generated fluorescent protein fusions and observed puncta localizing along microtubules.

B114/P2510
Elucidating the molecular mechanism of tubulin isotype function
L. Savoy, M. Gupta; GDCB, Iowa State University, AMES, IA.

Microtubules (MTs) are dynamically unstable cytoskeletal filaments, polymerized from tubulin, an obligate heterodimer of α- and β- subunits. They play an essential role in preserving genome stability and cell viability by mediating a multitude of diverse and conserved cellular functions, like cell division, cell migration, force generation and intracellular cargo transport. Naturally, a variety of regulators underlie these precise MT functions, namely, microtubule associated proteins (MAPs), post translational modifications, and relatively understudied, multiple tubulin isotypes. Why multiple tubulin isotypes exist, remains a fundamental and elusive question in cytoskeleton biology. Unfortunately, studying this question in most metazoan models, possessing multiple isotypes, risk introducing masking effects and hence biased observation. However, Saccharomyces cerevisae, harboring only 2 α- and 1 β-tubulin tubulin isotypes, offers a simple, robust, genetically tractable and superior model system. Our recent study demonstrated that each of the two *S. cerevisiae* α-tubulin isotypes (*TUB1* and *TUB3*) distinctly optimizes the overall efficiency of the two mutually redundant, Dynein- and Kar9-dependent mitotic spindle positioning pathways. Additionally, *TUB1* and *TUB3* drive differential recruitment of MAPs involved in these pathways on cytoplasmic MTs. But the underlying molecular mechanism(s) of differential MAP recruitment by distinct α-tubulin isotypes is presently unknown. In this study, by applying yeast CRISPR-Cas9 toolbox, live cell imaging and cell-based assays we aimed to elucidate the specific structure-function effects between tubulin isotypes and their preferred MAPs (Tub1 vs Dynein- and Tub3 vs Kar9-pathway proteins) by interchanging different functional domains of the two α-tubulin isotypes for the first time to elucidate what portion of each produce their specific functions.

Microtubule Dynamics: Modeling and Reconstitution

B115/P2511

Building order from disorder: mechanistic and regulatory roles for phase condensation in the assembly of microtubule architectures by Tubulin polymerization-promoting protein

C.-C. CHANG, S. M. Coyle; The University of Wisconsin-madison, Madison, WI

Microtubule filaments (MTs) provide the foundation for many higher-order cytoskeletal structures within the cell, ranging from the mitotic spindle to ciliary axonemes to the axons and dendrites in neuron cells. These structures require the coordinated action of specific microtubule-associated proteins (MAPs) to organize the microtubules in a precise manner across cellular length-scales. In oligodendrocytes, tubulin polymerization promoting protein (TPPP) is a microtubule-associated protein (MAP) and has been proposed to nucleate and stabilize microtubules at Golgi-outpost to promote the elongation, branching, and polarization of myelin sheaths. However, the molecular mechanism of microtubule bundling by TPPP and its regulation has not been defined. Here, we demonstrate that TPPP assembles higher-order microtubule architectures through a multivalent bind-and-condense mechanism. We find that a structured “core” domain of TPPP can bind MTs in vitro, but that bundle-formation requires the presence of flanking intrinsically disordered regions (IDRs) that drive TPPP droplet formation. The combination of MT-binding and condensation activity was sufficient for assembling MT-bundles, as TPPP-core bunding activity could be functionally rescued by fusion with condensate-forming IDRs from unrelated proteins. Electron-microscopy revealed that these MT-bundles are held together by a combination of direct binding to individual MTs and condensate-bridges between adjacent MTs. To test the relevance of this mechanism in living cells, we reconstituted TPPP-bundle formation synthetically in NIH 3T3 cells. We found that optogenetic clustering of full-length TPPP, but not TPPP-core, led to rapid droplet-formation and MT-association. Moreover, we could induce persistent and extensive MT-bundling in the cell by coupling TPPP to other droplet-forming IDRs. Strikingly, the extent of this bundling scaled with the strength of the IDR, suggesting that regulation of TPPP condensation can provide a mechanism for organizing higher-order microtubule architectures in space and time. Our study provides mechanistic evidence that IDRs enable TPPP to form high valency phase-separated liquid droplets that can enhance its ability to bind, coat, and bundle microtubules. This work provides a new perspective on how LLPS cooperates with classic microtubule-directed activities to allow microtubule architectures to be coordinated across cellular length scales.

B116/P2512

Dynamic Instability from Non-equilibrium Structural Transitions on the Energy Landscape of Microtubule

S. F. Stewman, K. Tsui, **A. Ma**; University of Illinois Chicago, Chicago, IL.

Microtubules are the backbone of the cytoskeleton and vital to numerous cellular processes. All the functions of microtubules are driven by dynamic instability, but its mechanism has remained unresolved for over 30 years because of conceptual difficulties inherent in the prevalent GTP-cap framework. We present a physically rigorous structural mechano-chemical model: dynamic instability is driven by non-equilibrium transitions between the bent (B), straight (S), and curved (C) structures of tubulin monomers and longitudinal interfaces in the two-dimensional lattice of microtubule. All the different phenomena (growth, shortening, catastrophe, rescue, and pausing) are controlled by the kinetic pathways for B-to-S

and S-to-C transitions and their corresponding energy landscapes. Different kinetics of dynamic instability at plus and minus ends are due to different B-to-S and S-to-C pathways necessitated by the polarity of microtubule lattice. This model enables us to reproduce all the observed phenomena of dynamic instability of purified tubulins in kinetic simulations and provide the detailed physical mechanisms behind them.

B117/P2513

Investigating the biochemical mechanisms of microtubule depolymerization with simulations and experiments

L. A. McCormick, E. Bonventre, A. Brudnicka, L. M. Rice; UT Southwestern Medical Center, Dallas, TX.

Microtubules are essential polymers of αβ-tubulin that form key components of the eukaryotic cytoskeleton. Despite decades of study, we lack a complete understanding of how microtubules depolymerize, or "shrink". Are shrinking rates driven purely by the one-at-a-time dissociation of αβ-tubulin subunits from the microtubule end, or might other mechanisms play a role? In previous work, we identified a β-tubulin mutation that slows the shrinking rates of yeast and human microtubules without directly altering tubulin polymerization interfaces. The buried, allosteric β:T238A mutation is a unique tool to study how microtubules depolymerize. Here I use Interference Reflection Microscopy (IRM) and recombinant human tubulin to determine how "doses" of the slow-shrinking mutant affect the dynamics of mixed mutant-wild type (WT) microtubules. Microtubules were polymerized from mixtures of 5%, 10% or 20% T238A (95%, 90% or 80% WT, respectively) at 37 °C. To establish a baseline for the dynamics of WT and mutant, I also measured the polymerization dynamics of pure WT and pure mutant microtubules. Relative to WT, substantial changes in growth and shrinking rates were observed for mixed mutant-WT microtubules, and a decrease in catastrophe frequency was observed for both the plus and minus ends. The mutant-correlated decrease in catastrophe frequency was not accompanied by an increase in rescue frequency. In previous work, we developed kinetic Monte Carlo simulations that create a "biochemical movie" of microtubule dynamics with one reaction - association, dissociation, GTPase - per frame. To test mechanistic interpretations of my data, I have extended our plus end code to model the minus end, and have added the capability to model mixed mutant-WT microtubules. Current work is focused on using my experimental data to constrain kinetic simulations of microtubule dynamics. By combining simulations with experiments, I hope to produce new, quantitative insight into the biochemical mechanism of shrinking.

B118/P2514

Self-organization of microtubules through hydrodynamic interactions drives cell-spanning rotational flows

D. B. Stein[1], A. B. Sami[2], G. Kabacaoğlu[3], M. J. Shelley[1], J. C. Gatlin[2]; [1]Center for Computational Biology, Flatiron Institute, New York City, NY, [2]Molecular Biology, University of Wyoming, Laramie, WY, [3]Bilkent University, Ankara, TURKEY.

The piconewton forces generated by molecular motors carrying cargo along microtubules, or by microtubules polymerizing against the cell cortex or artificial boundaries, are sufficient to deform long microtubules. When microtubules are sparse in the cytoplasm, their deformations are disordered, characterized by high-frequency buckling and inducing only localized cytoplasmic flows. When the microtubules are instead arranged in a dense forest, the nature of the microtubule deformations and

induced cytoplasmic flows can change dramatically, giving rise to long-range order and coherent flows. Using a combination of experiments, large-scale simulations of microtubules interacting hydrodynamically through a viscous fluid, and a coarse-grained theory for dense beds of filaments, we elucidate the mechanisms that underlie the self-organization of microtubule ensembles and their subsequent generation of cell-spanning rotation in two examples: cytoplasmic streaming in the Drosophila Melanogaster oocyte, and spontaneous rotation of artificially confined asters in Xenopus Laevis extract.

B119/P2515

A structural mechano-chemical model of microtubule nucleation

K. K. Tsui, S. F. Stewman, A. Ma; Biomedical Engineering, University of Illinois At Chicago, Chicago, IL.

Microtubules (MTs) are the backbone of cytoskeleton and crucial for many essential processes, such as mitosis, morphogenesis and migration. A critical step in the assembly and regulation of cellular MT network is nucleation. There are two types of templates for MT nucleation in cells: existing MT and γ-tubulin ring complex. Both types of nucleations have been reconstituted in vitro, but their mechanisms remain poorly understood. Here, we developed a physically rigorous structure-based mechano-chemical model that provides an unified mechanism for both types of nucleations. For nucleation from seed MTs, the key observations are: 1) the critical tubulin concentration for nucleation is much higher than that for growth, 2) the concentration-dependent time lags. Our model shows that the bottleneck for nucleation is frequent catastrophes (i.e. the switching from MT growth to shortening) that keep removing nascent MTs from the seed, which is reduced as tubulin concentration increases. The observed time lag is due to repeated assembly and removal of nascent MTs, which delay nucleation. For nucleation from γ-tubulin ring, the most intriguing question is the effects of ring closure, a complex-wide conformational change required for the ring complex to match the tube-like geometry of MT. Our model shows that ring closure helps tubulins to first form a sheet, which is then closed into tube with the help of the ring closure process. Because catastrophe cannot develop while sheet is open, MTs more likely grow longer, increasing the chance of nucleation. Thus, ring closure allows more regulated and efficient MT nucleation.

B120/P2516

Mechanism of Microtubule Assembly by CKAP2

T. McAlear, M. Wozny, M. Strauss, **S. Bechstedt**; McGill University, Montreal, QC, CANADA.

Our lab has recently described the mitotic spindle protein CKAP2 (Cytoskeleton-associated protein 2) as a potent microtubule assembly factor.We now investigate, how CKAP2 can increase the nucleation and assembly rate of microtubules.As working hypotheses, we consider that CKAP2 could either activate tubulin, the microtubule end, affect GTP hydrolysis, or concentrate and capture tubulin to achieve the observed effects. Here, we discuss experiments including structure-function studies for the interaction of CKAP2 with microtubules and tubulin in vitro to confirm or exclude working hypotheses.Strikingly, we find that CKAP2 can grow microtubules in the absence of GTP. We present cryo-EM data showing structures assembled in the absence and presence of tubulin.

B121/P2517

The g-tubulin ring complex (g-TuRC) regulates microtubule protofilament number

A. Aher, L. Urnavicius, A. C. Xue, T. M. Kapoor; Laboratory of Chemistry and Cell Biology, The Rockefeller University, New York, NY.

Microtubules are cytoskeletal polymers comprised of α-β tubulin heterodimers. Tubulin dimers associate in a head-to-tail fashion to form protofilaments, several of which interact laterally to assemble the cylindrical microtubule filament. Microtubules nucleated spontaneously *in vitro* can have 9-16 protofilaments, and 14 protofilament microtubules are the most abundant. In contrast, microtubules in many human cell types have been found to have 13 protofilaments. It has been proposed that the γ-tubulin ring complex (γ-TuRC), a ~2.3 MD cone-shaped assembly comprised of γ-tubulins and associated proteins, can template the nucleation of microtubules with 13 protofilaments in cells. However, it is unclear if the γ-TuRC can specify microtubule protofilament number as structural studies have revealed a mismatch between the helical arrangement of γ-tubulins in γ-TuRC and that of tubulin dimers in a 13 protofilament microtubule. To examine the regulation of microtubule architecture by γ-TuRC, we established assays with purified proteins and employed cryo-electron microscopy (cryo-EM). We first optimized conditions for microtubule nucleation by native human γ-TuRC in TIRF microscopy-based assays. We then established conditions to directly nucleate microtubules on EM grids in the presence of γ-TuRC alone or with additional accessory proteins. We used reference-free 2D classification to segregate individual microtubule segments and measure respective widths. In addition, the segments were subjected to 3D classification with alignment to synthetic references of microtubules with differing protofilament numbers. Together, these analyses indicate that γ-TuRC nucleates microtubules with predominantly 13 protofilaments. Our findings suggest that the association of tubulin subunits with the γ-tubulin subunits might induce changes in the γ-TuRC's conformation and regulate the architecture of the microtubule lattice. The methods we have developed, along with recombinant forms of γ-TuRC we have generated, will help further dissect the regulation of microtubule architecture and nucleation.

B122/P2518

CLASPs dictate the tubulin off-rate to regulate microtubule dynamics

E. J. Lawrence, M. Zanic; Vanderbilt University, Nashville, TN.

CLASPs constitute a family of conserved TOG-domain proteins that regulate microtubules in many fundamental cellular processes. CLASPs stabilize dynamic microtubules by suppressing catastrophe and promoting rescue, the switch-like transitions between growth and shrinkage. However, the molecular mechanisms underlying CLASPs' activity are not fully understood. Here, we report that human CLASP1 depolymerizes stable microtubules in the absence of tubulin, an activity previously reported for another TOG-domain protein XMAP215. Surprisingly, we find that, unlike XMAP215, CLASP activity is nucleotide-dependent, depolymerizing stable microtubules in the presence of GTP and GDP, but not in the presence of GMPCPP nor in the absence of nucleotide. We demonstrate that other human CLASP family members (CLASP2α and CLASP2γ) also exhibit nucleotide-dependent depolymerase activity on stable microtubules, as does a TOG2-domain construct, the minimal unit required for regulating microtubule dynamics. By investigating CLASP's effects on microtubules grown with GTP, GMPCPP, or a mixture of the two nucleotides, we find that CLASP1 dictates the rate of microtubule depolymerization regardless of microtubule substrate. Therefore, we conclude that CLASP1 regulates the tubulin-off rate at microtubule ends. The unanticipated finding that CLASPs dictate the tubulin-off rate in a nucleotide-

dependent manner underlies CLASPs activity on dynamic microtubules and provides critical mechanistic insights into an important family of microtubule regulatory proteins.

B123/P2519

CLASP2 facilitates dynamic actin filament organization along the microtubule lattice

N. Rodgers, E. Lawrence, N. Efimova, G. Gonzalez-Vasquez, T. Hickman, I. Kaverina, M. Zanic; Vanderbilt University, Nashville, TN.

Coordination between the microtubule and actin networks is essential for cell motility, neuronal growth cone guidance, and wound healing. Members of the CLASP (Cytoplasmic Linker-Associated Protein) family of proteins have been implicated in the cytoskeletal crosstalk between microtubules and actin networks, however, the molecular mechanisms underlying CLASPs role in cytoskeletal coordination are unclear. Here, we investigate CLASP2α's crosslinking function with microtubules and F-actin. Our results demonstrate that CLASP2α crosslinks F-actin to the microtubule lattice in vitro. We find that the crosslinking ability is retained by L-TOG2-S, a minimal construct containing the TOG2 domain and serine-arginine rich region of CLASP2α. Furthermore, CLASP2α promotes the accumulation of multiple actin filaments along the microtubule, supporting up to 11 F-actin landing events on a single microtubule lattice region. CLASP2α also facilitates dynamic organization of polymerizing actin filaments templated by the microtubule network, with F-actin forming bridges between individual microtubules. Finally, we find that depletion of CLASPs in vascular smooth muscle cells results in disorganized actin fibers, suggesting that CLASP and microtubules contribute to higher-order actin structures. Taken together, our results indicate that CLASP2α can directly crosslink F-actin to microtubules, and that this microtubule-CLASP-actin interaction may influence overall cytoskeletal organization in cells.

B124/P2520

A chemical genetics approach to examine cytoskeleton remodeling functions of AAA proteins

N. H. Jones, T. Cupido, M. J. Grasso, R. Pisa, F. Anson, T. M. Kapoor; Laboratory of Chemistry and Cell Biology, The Rockefeller University, New York, NY.

AAA (ATPases associated with diverse cellular activities) proteins such as katanin and spastin can sever microtubules and contribute to cytoskeletal organization and function. Cell-permeable, selective, and potent chemical probes can be useful tools to study the functions of these proteins in dynamic cellular processes including cell division, cell migration, and the assembly/disassembly of the primary cilium. However, structural conservation across the AAA protein family makes designing selective chemical inhibitors challenging. Here, we used a structure-based approach to design ASPIRe-1 (allele-specific, proximity-induced reactivity-based inhibitor-1), a compound that selectively inhibits katanin with an engineered mutation (D210C). Structural studies confirm our model for compound binding at the AAA ATPase site and the proximity-induced reactivity-based inhibition. ASPIRe-1 treatment (1.25 μM, 4 hours) of cells expressing katanin D210C (in addition to the native protein) increases the length of CAMSAP2 'stretches' at microtubule minus ends by ~1.5-fold, consistent with katanin knockdown studies. Importantly, ASPIRe-1 treatment does not result in cell toxicity, nor does it cause elongation of CAMSAP2 stretches in cells expressing WT katanin, indicating inhibitor specificity. Our findings also suggest that the ATPase activity of katanin contributes to its function in CAMSAP2 regulation. Together, our findings introduce a selective tool to decipher the role of katanin in cytoskeletal dynamics in cell division, cell migration, and ciliary biology. Since ASPIRe-1 binds the conserved ATPase site, our

approach can also be used to study the functions of other AAA proteins, such as VSP4, that regulate membrane dynamics.

B125/P2521

Regulating Microtubule Severing by Katanin Physically and Chemically

T. M. Finegan, K. A. Lindsay, N. Abdelhamid, K. M. Primeau, J. L. Ross; Department of Physics, Syracuse University, Syracuse, NY.

We are investigating the physical and chemical regulation of the microtubule severing enzyme, katanin. Katanin is a AAA+ enzyme that hexamerizes to build a functional unit that severs tubulin filaments via the removal of tubulin monomers. Unregulated activity of katanin in cells can destroy the entire microtubule network, thus precise spatial and temporal regulation is essential. We present novel data illustrating that the carboxy-terminal tail sequence of tubulin acts as a code to control katanin severing activity. We are also using quantitative fluorescence microscopy to test the hypothesis that oligomerization of katanin at microtubules is the rate-limiting step for tubulin severing. We are utilizing tools that precisely target katanin localization and expression in cells to locally disrupt the microtubule cytoskeleton and investigate the biochemistry of multitude microtubule-regulated processes. We present novel data quantifying katanin concentration and the microtubule filament density as a function of time to elucidate the dynamics of biochemical control of microtubules by katanin in cells for the first time.

B126/P2522

Combinatorial readout of the tubulin code by katanin

E. Szczesna[1], E. A. Zehr[1], S. W. Cummings[1], A. Szyk[1], K. K. Mahalingan[1], Y. Li[2], A. Roll-Mecak[1,3]; [1]Cell Biology and Biophysics Unit, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, [2]Proteomic Core Facility, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, [3]Biochemistry and Biophysics Center, National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

Cells functionalize microtubules with spatiotemporally complex patterns of posttranslational modifications. How effectors interpret this tubulin modification code is largely unknown. Here, we show that katanin, a microtubule severing AAA ATPase mutated in microcephaly and critical for cell division, axonal elongation and cilia biogenesis, responds precisely, differentially and combinatorially to three chemically distinct modifications: glycylation, glutamylation, and tyrosination, but is insensitive to tubulin acetylation. Glutamylation and glycylation act as antagonistic rheostats with glycylation being protective of microtubules. Katanin exhibits graded and divergent responses to glutamylation on the α- and β-tubulin tails, and these act combinatorially. The structure of the katanin hexamer central pore constrains the polyglutamate chain patterns on β-tails that can productively be recognized. In contrast, elements distal to the katanin AAA core sense α-tubulin tyrosination, and detyrosination downregulates severing. The multivalent microtubule recognition that enables katanin to read multiple tubulin modification inputs explains *in vivo* observations and illustrates how effectors can integrate tubulin code signals to produce diverse functional outcomes. A.R.M is supported by the intramural programs of the National Institute of Neurological Disorders and Stroke (NINDS) and the National Heart, Heart and Blood Institute (NHLBI).

B127/P2523

Ultrasensitivity of microtubule severing rate on the concentration of free tubulin

C. E. Shiff[1], J. Kondev[2], **L. Mohapatra**[3]; [1]Stanford University, Stanford, CA, [2]Brandeis University, Waltham, MA, [3]Rochester Institute of Technology, Rochester, NY

Cytoskeletal structures aid in cell polarization, motility, and intracellular transport. Their functions are predicated on the rapid turnover of cytoskeleton proteins, which is achieved by the coordinated effort of multiple regulatory proteins whose dynamics are not well understood. In-vitro experiments have shown that free tubulin can repair nanoscale damages of microtubules created by severing proteins. Based on this observation, we propose a model for microtubule severing as a competition between the processes of damage spreading and tubulin-induced repair. Using theory and simulations, we demonstrate that this model is in quantitative agreement with in vitro experiments. We predict the existence of a critical tubulin concentration above which severing becomes rare but fast, and hypersensitive to the concentration of free tubulin. Further we show that this hypersensitivity leads to a dramatic increase in the dynamic range of steady-state microtubule lengths, when lengths are controlled by severing. Our work demonstrates how synergy between tubulin and severing proteins can lead to novel dynamical properties of microtubules.

B128/P2524

Making a microtubule: structural analysis of microtubule nucleation by the gamma-tubulin ring complex (γTuRC)

B. Romer[1], B. Mahon[1], M. Rale[1], N. Coudray[2], G. Bhabha[2], S. Petry[1]; [1]Princeton University, Princeton, NJ, [2]New York University, New York, NY

Microtubules (MTs) are dynamic cylindrical polymers made of α/β-tubulin dimers that function in many diverse and essential cellular processes such as cell division, intracellular transport, and organelle positioning. Because of their importance, it is imperative that microtubules are nucleated at the correct time and place. A protein known to be essential for MT nucleation is γ-tubulin, which together with other proteins in the cell, forms a ring-like template called the γ-tubulin ring complex (γTuRC). Despite its importance, little is known about how the γTuRC nucleates MTs. To address this open question, our work is aimed at determining the structure of the γTuRC in its activated, nucleation-prone state. Using single-particle cryo-EM of γTuRC bound to a known activating factor, we will uncover if structural changes occur in the complex to facilitate MT nucleation. Altogether, these studies will further our understanding of how the universal MT nucleator functions to regulate the production of MTs.

B129/P2525

Acentrosomal spindles assemble from branching microtubule nucleation near chromosomes in *Xenopus laevis* egg extract

B. Gouveia, S. U. Setru, M. R. King, A. Hamlin, H. A. Stone, J. W. Shaevitz, S. Petry; Princeton University, Princeton, NJ

Microtubules are generated at centrosomes, chromosomes, and within spindles during cell division. Whereas microtubule nucleation at the centrosome is well characterized, much remains unknown about where, when, and how microtubules are nucleated at chromosomes. To address these questions, we reconstituted microtubule nucleation from purified chromosomes in meiotic *Xenopus* egg extract and

found that chromosomes alone can form spindles. We visualized microtubule nucleation near chromosomes using total internal reflection fluorescence microscopy to find that this occurs through branching microtubule nucleation. The initial branches nucleate near and towards chromosomes, helping explain how kinetochores might be efficiently captured. By inhibiting molecular motors, we find that the organization of the resultant polar branched networks is consistent with a theoretical model where the effectors for branching nucleation are released by chromosomes, forming a concentration gradient that spatially biases branching nucleation. In the presence of motors, these branched networks are ultimately organized into spindles.

B130/P2526

The role of microtubule severing protein UNC-45A in microtubule curvature

A. Hoshino, V. Clemente, M. Shetty, M. Bazzaro; Masonic Cancer Center and Department of Obstetrics, Gynecology and Women's Health, University of Minnesota, Minneapolis, MN.

Microtubules (MTs) are the least flexible of the cytoskeletal filaments. In cells MTs frequently curve and break (*1*). We have recently identified UNC-45A as novel ATP-independent MT severing protein (***2-5***). Here we wanted to study the contribution of UNC-45A to MT curvature. We used total internal reflection fluorescence (TIRF) and live cell imaging to examine the localization and effect of UNC-45A on MT curvature. We found that UNC-45A preferentially binds the curved regions of the MTs. Furthermore, UNC-45A increased MTs curvature independent of actomyosin. This is consistent with the fact that MT severing by UNC-45A is preceded by MT "kinks" (***3***). In conclusion, UNC-45A preferentially binds to curved MTs and causes exacerbation of this curvature leading to severing. Curved MTs are known to have lattice defects (*6*). Our result suggests that UNC-45A may bind to MT defects. The implication is that UNC-45A may also selectively affect MTs in paclitaxel and oxidative exposed cells (*7, 8*). Thus, UNC-45A may be particularly relevant in human diseases.

References:1.D. J. Odde, L. Ma, A. H. Briggs, A. DeMarco, M. W. Kirschner, Microtubule bending and breaking in living fibroblast cells. *J Cell Sci* **112 (Pt 19)**, 3283-3288 (1999).2.J. Habicht *et al.*, UNC-45A is preferentially expressed in epithelial cells and binds to and co-localizes with interphase MTs. *Cancer Biol Ther* **20**, 1304-1313 (2019).3.J. Habicht *et al.*, UNC-45A breaks the microtubule lattice independently of its effects on non-muscle myosin II. *J Cell Sci* **134**, (2021).4.A. Mooneyham *et al.*, UNC-45A Is a Novel Microtubule-Associated Protein and Regulator of Paclitaxel Sensitivity in Ovarian Cancer Cells. *Mol Cancer Res* **17**, 370-383 (2019).5.V. Clemente *et al.*, UNC-45A Is Highly Expressed in the Proliferative Cells of the Mouse Genital Tract and in the Microtubule-Rich Areas of the Mouse Nervous System. *Cells* **10**, (2021).6.S. Chakraborty, J. Mahamid, W. Baumeister, Cryoelectron Tomography Reveals Nanoscale Organization of the Cytoskeleton and Its Relation to Microtubule Curvature Inside Cells. *Structure* (2020).7.R. R. Goldblum *et al.*, Oxidative stress pathogenically remodels the cardiac myocyte cytoskeleton via structural alterations to the microtubule lattice. *Dev Cell*, 2252-2266 e2256 (2021).8.A. Rai *et al.*, Taxanes convert regions of perturbed microtubule growth into rescue sites. *Nat Mater***,** (2020).

B131/P2527

Integrated model of the vertebrate augmin complex

S. M. Travis[1], B. P. Mahon[1], W. Huang[2], M. Ma[3], M. J. Rale[1], J. S. Kraus[1], D. J. Taylor[2], R. Zhang[3], S. Petry[1]; [1]Department of Molecular Biology, Princeton University, Princeton, NJ, [2]Department of Pharmacology, Case Western Reserve University, Cleveland, OH, [3]Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis, School of Medicine, St. Louis, MO.

Accurate segregation of chromosomes is required to maintain genome integrity during cell division. This feat is accomplished by the microtubule-based spindle. To build a spindle rapidly and with high fidelity, cells take advantage of branching microtubule nucleation, which exponentially amplifies microtubules during cell division. Branching microtubule nucleation relies on the hetero-octameric augmin complex, but understanding how augmin promotes branching has been hindered by a lack of structural information about the complex. Here, we report an integrated model of vertebrate augmin, combining cryo-electron microscopy, advanced protein structural prediction, and the visualization of fused bulky tags via negative stain electron microscopy. This strategy allowed us to identify the location and orientation of each subunit within the structure. Evolutionary analysis of augmin's structure reveals that it is highly conserved across diverse eukaryotes, and that augmin contains a previously-unidentified microtubule binding site. Moreover, we identify homology with the kinetochore-localized NDC80 complex. This new model of the augmin complex provides insight towards the mechanism and evolution of branching microtubule nucleation.

B132/P2528

The Augmin Complex is Regulated by RanGTP via its Microtubule Binding Domain

J. Kraus[1], S. Travis[1], M. King[2], S. Petry[1]; [1]Princeton University, Princeton, NJ, [2]Washington University in St. Louis, St. Louis, MO

The metaphase spindle is composed from microtubules (MT) that must be nucleated at exactly the right place and time for mitosis to occur efficiently without errors. The chromosome-localized RanGTP controls MT nucleation by sequestering spindle assembly factors (SAFs) via its effectors, importin-α and importin-β. Branching MT nucleation is a primary source of spindle MTs, and is dependent on the proteins augmin and TPX2, which recruit the universal nucleator, the γ-tubulin ring complex (γ-TuRC), to the sides of pre-existing MTs. While TPX2 is a Ran-regulated SAF, it is not required in all species, which leaves the regulation of branching MT nucleation an open question. Here, we use *in-vitro* pulldowns and TIRF microscopy assays to show that augmin is a bona-fide SAF. Augmin directly interacts with both importin-α and importin-β through two unique nuclear localization sequences (NLS) on augmin. Moreover, the localization of augmin to MTs in both *Xenopus* egg extract and *in-vitro* is dependent on the presence of RanGTP. Our work shows that augmin function is directly regulated by Ran, independently of TPX2, pointing to a conserved role for Ran in controlling branching MT nucleation during spindle assembly and beyond.

B133/P2529

Using the tubulin-like phage protein PhuZ to study the fundamental properties of biological energy utilizing polymers

Y. A. P. Huang, H. V. Goodson; University of Notre Dame, Notre Dame, IN

Microtubules (MTs) are energy utilizing polymers of α&β tubulins that form part of the cytoskeleton. A character of MTs is that individual MTs in the same cell constantly change randomly between phases of growth and shortening, a behavior known as Dynamic Instability (DI). Understanding the mechanism of DI and its regulation is essential to understand the role of MTs in cell organization, but DI is still poorly understood. Our lab is working to develop mathematical models (i.e., equations) that quantitatively connect the biochemical characteristics of subunits to the observed dynamic instability behaviors. Traditionally, the critical concentration (CC) is defined to be the lowest concentration of free subunits required to obtain polymer filaments. However, the traditional theory is based on equilibrium polymers and fails to incorporate energy utilization or the DI displayed by dynamically unstable polymers like MTs. Our previous studies based on computational MTs model indicated that MTs have (at least) two experimentally relevant CCs: $CC_{Elongation,}$ above which growth phases of individual filaments can occur transiently. $CC_{NetAssembly}$, above which the population's polymer mass will increase persistently. Here, we use a tubulin-like bacteriophage protein called PhuZ to establish a simplified experimental system to test the predictions of our computational models. Because PhuZ has sufficiently similar characteristics to MTs, studying $CC_{Elongation}$ and $CC_{NetAssembly}$ of wild-type and mutant PhuZs will experimentally contribute to establish a predictive understanding of the relationship between the characteristics of the subunits, the behaviors of the filaments and the attributes of the dynamically unstable biological polymer systems.

Sensory and Signaling Functions of Cilia

B135/P2530

Kinesin-2 motors differentially impact biogenesis of distinct extracellular vesicle subpopulations shed from *C. elegans* sensory cilia

M. Clupper, R. Gill, M. Elsayyid, D. Touroutine, J. Caplan, **J. Tanis**; Department of Biological Sciences, University of Delaware, Newark, DE.

Secreted extracellular vesicles (EVs) play a critical role in communication between cells during physiological processes and under pathological conditions. A single cell can release multiple distinct EV subpopulations, each with different cargo enrichment and functionalities. How an individual cell packages and sheds distinct populations of EVs is unclear. In *C. elegans*, EVs bud from male sensory neuron cilia and then are either up-taken by surrounding glia or discharged into the environment where they play a role in animal-to-animal communication. We developed a model to interrogate EV cargo enrichment mechanisms by expressing different fluorescently-labeled EV cargoes in *C. elegans* at single copy and imaging with total internal reflection fluorescence (TIRF) microscopy. We discovered the ion channel CLHM-1 as a cargo in EVs released from the ciliary base into the environment. tdTomato-tagged CLHM-1 and GFP-tagged PKD-2 TRPP channel, a well described EV cargo, colocalize in the ciliary base and middle segment of the cilium proper. However, we found that these proteins are enriched in distinct EV subpopulations, with PKD-2 alone located in the cilium distal tip and EVs shed from this site. Shedding of CLHM-1 containing EVs increased in response to the presence of hermaphrodite mating partners, while release of tip-derived PKD-2 EVs decreased under these conditions, suggesting that

these subpopulations are differentially shed in response to physiological stimulus. We discovered that the heterotrimeric KLP-11-containing and homomeric OSM-3 kinesin-2 motors play distinct roles in CLHM-1 EV biogenesis, colocalization of ciliary proteins, and EV cargo enrichment. Complete elimination of all kinesin-2 motors decreased shedding of EVs containing PKD-2, but did not inhibit CLHM-1 cilia entry or inclusion in EVs, indicating that intraflagellar transport differentially impacts biogenesis of discrete EV subpopulations. This work shows that an individual cilium generates heterogeneous EVs with different signaling potentials and that spatial determinants regulate both EV cargo enrichment and biogenesis.

B136/P2531

Characterization of ciliary associated renal extracellular vesicles

A. Frei[1], **H. May-Simera**[1], A. Volz[1], J. Weinmann-Menke[1], M. Ueffing[2], K. Boldt[2], E. Albers[1]; [1]Johannes Gutenberg University, Mainz, GERMANY, [2]University of Tübingen, Tübingen, GERMANY.

Primary cilia are required for a variety of cellular processes such as signal transduction and cell communication during development and tissue homeostasis. Defects in ciliary function lead to a broad range of diseases, with renal dysfunction as a common feature representing a significant cause of morbidity and mortality in patients. Recent studies showed that primary cilia are able to transmit signals via the release of extracellular vesicles (EVs). Although the putative role of EVs in processes during organ development and homeostasis is not well know, it is emerging that EVs can influence key signalling pathways involved in kidney development and pathogenesis. Changes in EV-mediated signalling due to ciliary dysfunction could thus impact tissue homeostasis leading to disease pathogenicity such as renal cyst formation. In our study we focused on the release of small EVs from a variety of ciliated kidney cell lines to better understand ciliary EV biogenesis and bioactivity in renal tissues. We observed differences not only in the release but also in the composition of EVs depending on cell type as well as ciliation. In addition, we shed light on the origin of small EVs by inhibition of various release pathways. To further investigate EV based signalling mechanisms in renal pathogenesis associated with ciliopathies, we compared EVs from control and ciliary mutant cells derived from both mouse tissue and patient urine. Ciliary dysfunction greatly affected renal EV release and composition, which was differently influenced by modulation of release pathways. These results provide us with insights into EV-related ciliary signaling mechanisms which might underly ciliopathy disease pathogenesis.

B137/P2532

A serotonergic axon-cilium synapse drives nuclear signaling to alter chromatin accessibility

S. SHEU[1], S. Upadhyayula[2], V. Dupuy[3], S. Pang[1], F. Deng[4], J. Wan[4], D. Walpita[1], H. Pasolli[1], J. Houser[5], S. Sanchez-Martinez[1], S. E. Brauchi[1], S. Banala[6], M. Freeman[1], C. Xu[1], T. Kirchhausen[5], H. F. Hess[1], L. Lavis[6], Y. Li[4], S. Chaumont-Dubel[3], D. E. Clapham[6]; [1]Janelia Research Campus/Howard Hughes Medical Institute, Ashburn, VA, [2]UC Berkeley, Berkeley, CA, [3]Université de Montpellier, Montpellier, FRANCE, [4]Peking University, Beijing, CHINA, [5]Boston Children's Hospital, Boston, MA, [6]Janelia Research Campus of Howard Hughes Medical Institute, Ashburn, VA.

Chemical synapses between axons and dendrites mediate neuronal intercellular communication. Here we describe a synapse between axons and primary cilia; the axo-ciliary synapse. Using enhanced focused ion beam-scanning electron microscopy on samples with optimally preserved ultrastructure, we discovered synapses between brainstem serotonergic axons and the primary cilia of hippocampal CA1

pyramidal neurons. Functionally, these cilia are enriched in a ciliary-restricted serotonin receptor, the 5-hydroxytryptamine receptor 6 (5-HTR6). Using a cilia-targeted serotonin sensor, we show that opto- and chemogenetic stimulation of serotonergic axons releases serotonin onto cilia. Ciliary 5-HTR6 stimulation activates a non-canonical $G_{\alpha q/11}$-RhoA pathway, which modulates nuclear actin and increases histone acetylation and chromatin accessibility. Ablation of this pathway reduces chromatin accessibility in CA1 pyramidal neurons. As a signaling apparatus with proximity to the nucleus, axo-ciliary synapses short circuit neurotransmission to alter the postsynaptic neuron's epigenetic state.

B138/P2533

Differentiated dynamic response in*C. elegans*chemosensory cilia

C. Bruggeman, **E. J. G. Peterman**, G. H. Haasnoot; Physics and Astronomy, Vrije Universiteit, Amsterdam, NETHERLANDS.

Cilia are membrane-enveloped organelles that protrude from the surface of most eurokaryotic cells and play crucial roles in sensing the external environment. For maintenance and function, cilia are dependent on intraflagellar transport (IFT). Here we investigate the response of the *Caenorhabditis elegans* phasmid chemosensory cilia to chemical stimuli to better understand the process of chemosensation. To do so we use a combination of microfluidics, to apply stimuli with a high degree of temporal control, and fluorescence microscopy, to visualize structure and dynamics of the cilia, as well as the neuronal response. We found that chemical stimulation resulted in unexpected changes in IFT and ciliary structure. Notably, stimulation with hyperosmotic solutions or chemical repellents resulted in different responses, not only in IFT, ciliary structure and cargo distribution, but also in neuronal activity. We found that in response to hyperosmotic stimuli, IFT components accumulate at the ciliary tip due to inhibition of retrograde transport. In response to chemical repellents, such as SDS or Cu^{2+}, the ciliary axoneme shrinks and IFT components are redistributed towards the ciliary base. In response to SDS we observe shedding of extracellular vesicles containing IFT components and transmembrane proteins. Repetitive stimulation with chemical repellents results in lower neuronal activity every subsequent exposure, pointing towards a mechanism of desensitization. This suggests that IFT plays a role in regulating the chemosensory response. Currently we are trying to unravel how IFT and neuronal activity are precisely linked on the molecular level. Taken together, our findings show that cilia are able to sense and respond to different external cues in distinct ways, highlighting the flexible nature of cilia as sensing hubs.

B139/P2534

Processing and Secretion of a Ciliary Peptidergic Signal

R. Luxmi[1], R. E. Mains[2], B. A. Eipper[3], S. M. King[1]; [1]Molecular Biology and Biophysics, UCONN Health, Farmington, CT, [2]Neuroscience, UCONN Health, Farmington, CT, [3]Molecular Biology and Biophysics, Neuroscience, UCONN Health, Farmington, CT.

Cilia are membrane bound microtubule-based sensory and secretory organelles that transmit and receive information from the extracellular environment. Cilia-derived vesicles (ectosomes), formed by outward budding of the ciliary membrane, contain bioactive proteins that mediate signals between individual cells and organisms. Ectosomal content is distinct from that of the cilium from which they derive and is regulated by both trafficking proteins through the transition zone and subsequently sorting them into nascent ectosomes. Peptidergic intercellular communication is evolutionary conserved, and

controls a broad array of physiological and behavioral responses. Many secreted peptides require C-terminal α-amidation in order to be bioactive. The *Chlamydomonas* genome encodes numerous genes with the potential properties of prepropeptides, and these cells secrete the peptide amidating enzyme and bioactive amidated products in ciliary ectosomes. An amidated synthetic peptide (GATI-*amide*) generated from a 91-kDa proGATI precursor acts as a chemotactic modulator, attracting *minus* gametes while repelling *plus* gametes. Full-length proGATI is heavily glycosylated and contains three stable folded domains interconnected by long, proline-rich linkers. Biochemical analysis revealed that proGATI is trafficked to the ciliary membrane, where *plus* gametes cleave it, producing a C-terminal glycosylated amidated product that is released in ciliary ectosomes during mating. Analysis of a potential prohormone convertase and the proGATI-derived products in cilia and ectosomes, links endoproteolytic cleavage to ectosome entry. Analysis of this pathway affords insight into the evolution of peptidergic signaling through regulated secretion, and will facilitate study of protein secretion through cilia in metazoans. To locate the cell surface receptor for GATI-*amide*, we designed a biotinylated GATI-*amide* synthetic peptide containing a benzoyl-phenylalanine residue that can be photo-activated by exposure to UV light. Preliminary studies revealed that this probe labels a ~70-kDa protein in isolated gametic cilia from *Chlamydomonas*. Formation of the biotinylated product is UV-dependent and competed out by addition of unlabeled GATI-*amide* peptide demonstrating probe specificity and indicating that a GATI-*amide* binding protein is localized to cilia.

B140/P2535

The tectonic complex forms a membrane diffusional barrier in mouse rod outer segments

H. M. Truong, K. O. Cruz-Colón, J. Y. Martínez-Márquez, J. R. Willer, A. M. Travis, J. N. Pearring; Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI.

Primary cilia are thin, microtubule-based organelles that relay extracellular signals to the cell through ciliary membrane receptors. The distinct protein composition of the cilium is maintained by a membrane gate formed by the transition zone, located at the base of the cilium. In primary cilium, the transition zone generally retains receptors in this unique organelle; however, in the specialized light-sensing photoreceptor cilium (aka the outer segment) the transition zone acts to prevent non-resident proteins from accumulating in the outer segment compartment. The transition zone is delineated by the presence of electron-dense connections between the axoneme and ciliary membrane, called Y-links, as well as extracellular membrane densities, known as the ciliary necklace. While previous work has shown that membrane proteins utilize ciliary transport carriers to cross the transition zone, the molecular components forming the ciliary gate and its mechanisms regulating membrane protein composition remain ambiguous. The tectonic complex, Tctn1/2/3, is a component of the transition zone that has been shown to concentrate ciliary membrane proteins in primary cilia. We hypothesize that the tectonic complex helps form a diffusion barrier at the transition zone to maintain the unique membrane-associated protein composition of the photoreceptor outer segment. To study Tctn1 in rod photoreceptors, we generated a rod-specific Tctn1 knock-out mouse (*iCre;Tctn1*$^{f/f}$) and show that the entire tectonic complex - Tctn1/2/3 - is lost from the transition zone of rod outer segments. In the absence of the tectonic complex, outer segment morphology appears normal, however, membrane proteins typically excluded from the outer segment begin to accumulate within the outer segment. Mislocalization of these proteins is detrimental to rod health, resulting in photoreceptor degeneration by 6 months. Interestingly, we find that ciliary transport carriers are not affected by loss of tectonics but instead, the rate of membrane protein diffusion within the transition zone is increased. We conclude

that the tectonic complex helps form the membrane diffusional barrier at the transition zone to prevent non-resident membrane proteins from mislocalizing to the outer segment. Ultimately, these results establish how the transition zone functions as a membrane ciliary gate both within photoreceptors and primary cilia.

B141/P2536

Mutations in the microcephaly gene,*RRP7A*, converge on defective TGFB/BMP signaling and associated trafficking events at the primary cilium

J. L. Fialová[1], J. Stensbøl[1], S. Antony[2], E. Dyke[3], L. B. Pedersen[1], N. N. Kasri[3], K. Boldt[2], L. A. Larsen[4], S. T. Christensen[1]; [1]Department of Biology, University of Copenhagen, Copenhagen, DENMARK, [2]Molecular Biology of Retinal Degenerations, Institute for Ophthalmic Research, University of Tübingen, Tübingen, GERMANY, [3]Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, Nijmegen, NETHERLANDS, [4]Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, DENMARK.

Background and objective: We previously identified a homozygous missense mutation (W155C) in Ribosomal RNA (rRNA) Processing 7 Homolog A, RRP7A, segregating with primary microcephaly (MCPH; MIM #251200) in a consanguineous family with 10 affected individuals. RRP7A is highly expressed at the ventricular zone in the developing human neocortex in apical radial glial cells (aRGCs) to control rRNA processing, neurogenesis and cell cycle entry, which in part is associated with defects in the timely resorption of primary cilia [Farooq et al. (2020) Nat. Comm. 11, 5816]. Here we addressed the possible function of RRP7A at the primary cilium to regulate cellular signaling events that converge on neurogenesis and ciliary resorption to control brain development. **Methods and results:** Live imaging of RPE-1 cells stably expressing GFP-tagged RRP7A validated localization of RRP7A to the primary cilium, and immunofluorescence microscopy (IFM) showed prominent RRP7A localization to primary cilia laminating the neural rosette of cerebral organoids, marking the developmental signature of aRGCs, which expands the pool of neural progenitors and produces post-mitotic neurons during neocortex development. Proteomics of the RRP7A interactome in HEK293T and RPE-1 cells revealed novel interactors that converge on ciliary trafficking events and canonical TGFB/BMP signaling; interactions all affected by the patient mutation. IFM and western blotting analyses showed that TGFB/BMP-mediated R-SMAD signaling, which regulates aRGC self-renewal and differentiation, and which we show to induce ciliary resorption in human dermal fibroblasts and RPE-1 cells, is reduced at the primary cilium in HDFs carrying the patient mutation in RRP7A and in RPE-1 cells stably expressing the mutant version of RRP7A. In support of a ciliary link to regulation of canonical TGFB/BMP signaling during neocortex development, IFM analysis of cerebral organoids showed that R-SMAD activation at the neural rosette is confined to primary cilia of the aRGCs. In contrast, non-canonical TGFB/BMP pathways as well as PDGFRA signaling at the cilium are not perturbed in patient HDFs and RPE-1 cells expressing mutant RRP7A. These signaling phenotypes were recapitulated in RPE-1 cells subjected to siRNA-mediated depletion of RRP7A. **Conclusions:** We propose that RRP7A regulates key functions of primary cilia, including canonical TGFB/BMP signaling, to control the expansion of neural progenitors and ultimately the number of cortical neurons, providing novel insight into regulation of developmental signaling, brain development and MCPH. **Funding:** This project has received funding from the European Union's Horizon 2020 research & innovation program Marie Sklodowska-Curie Innovative Training Networks (ITN) grant No. 861329.

B142/P2537

MRAP2: A universal adapter for ciliary GPCR localization

M. Konjikusic, J. Reiter; University of California, San Francisco, San Francisco, CA.

Growing evidence indicates that GPCRs, the largest class of receptor proteins, function differently in different subcellular locations, including the plasma membrane, endosome and Golgi. Recently, my lab and others have found that a select number of GPCRs localize to the membrane of the primary cilium. The primary cilium is a solitary, immotile, antenna-like projection that can transduce multiple types of intercellular cues. While we have a growing understanding of which GPCRs localize to cilia, how these GPCRs localized to cilia remains unknown. Recently, in collaboration with the Vaisse lab, we have shown that MC4R, integral for energy homeostasis and the single most common mutated gene in monogenic obesity, relies on a mysterious single-pass transmembrane receptor, MRAP2, for localization to the cilium. *MRAP2* has also been implicated in human obesity and female infertility. More recently, we have found that MRAP2 can traffic multiple other GPCRs to the cilium, outside of MC4R. Additionally, we report that MRAP2 expression is much wider than MC4R, implicating broader functions than that of MC4R localization in energy homeostasis. Future experiments will focus on identifying phenotypes associated with loss of *Mrap2* function in mouse other than obesity, and specifically identifying which GPCRs *Mrap2* can traffic to the cilium endogenously. We will also work to identify how exactly MRAP2 is trafficking multiple GPCRs to the cilium through structure function approaches *in vitro*.

B143/P2538

A novel role for the conserved G protein regulator RIC-8/Synembryn in *C. elegans* neuronal cilia biology

C. Campagna, I. Nechipurenko; Worcester Polytechnic Institute, Worcester, MA

G protein coupled receptors (GPCRs) modulate key physiological processes via heterotrimeric G (αβγ) proteins. Many GPCRs and their downstream effectors localize to specialized cellular compartments called primary cilia, which mediate transduction of all major signaling pathways in mammals. Defects in cilia structure or function result in a spectrum of genetic disorders called ciliopathies that are commonly associated with neurological deficits. Likewise, genetic perturbation of select cilia-localized GPCRs and G protein alpha subunits (Gα) in experimental models modulates cilia morphology and impairs neuronal functions. What remains incomplete is our knowledge of the mechanisms by which G proteins shape cilia morphology, and the extent to which disruption of ciliary Gα signaling contributes to neurodevelopmental phenotypes.

We discovered that RIC-8, a non-canonical activator of Gα, localizes to cilia and regulates cilia membrane morphology in *C. elegans* sensory neurons – a previously unreported function for this highly conserved protein (ortholog of human *RIC8A* and *RIC8B*). RIC-8 has been demonstrated to function as a guanine-nucleotide exchange factor (GEF) to activate monomeric Gα independently of GPCRs and as a chaperone to fold nascent Gα. We show that the RIC-8 C-terminus containing GEF and chaperone activities is required for ciliogenesis and identify $G\alpha_{i/o}$ ODR-3 as a client Gα of RIC-8 in this context. Specifically, *odr-3* mutants recapitulate the *ric-8*-dependent cilia phenotypes, and RIC-8 physically associates with and regulates levels of ODR-3 in sensory neurons. We will present our ongoing efforts to molecularly define the novel *ric-8*-dependent ciliogenic pathway in the *C. elegans* model.

Chromosome Organization

B145/P2539

Condensation Defects of Pericentromeric Major Satellite DNA Underlie Subfertility in Hybrid Female Mice

W. El Yakoubi, T. Akera; Cell and Developmental Biology Center, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD.

One of the goal in the evolutionary biology is to understand the genetic control of hybrid sterility that lead to postzygotic reproductive isolation between closely related species. Following Haldane's rule, hybrid sterility is limited to heterogametic sex which is the male in mammals and flies. According to this law much more attention has been paid to the nature of reproductive isolation in male than in female. By using the F1 hybrid between *Mus musculus domesticus* (Hereafter *musculus*) and *Mus spretus* (hereafter *spretus*) we provide the first cell biological mechanism of female subfertility leading to reproductive isolation between these two species. It consists on defective chromosomal condensation due to less condensin II enrichment on the chromosomes in hybrid oocytes. Especially, the *musculus* centromeres, containing higher copy number of the major satellites repeats than *spretus* centromeres, are much more sensitive to low dosage of condensin II and are prone to stretching. Therefore, the stretching of the *musculus* centromeres causes lagging of only *musculus* chromosomes at anaphase of meiosis I and high rate of aneuploid eggs. Interestingly, the centromere stretching and aneuploidy rate phenotypes are both rescued by the overexpression of the condensin II specific subunit NCAPG2 which is found down regulated in the hybrid oocytes. Together, we propose that differences in both condensin II regulation and major satellite copy number between closely related species can cause reproductive isolation.

B146/P2540

Investigating the Mechanisms Underlying Meiotic Chromosome-specific Differences

K. Billmyre[1], Z. Yu[1], E. Kesler[1], R. Hawley[1,2]; [1]Stowers Institute for Medical Research, Kansas City, MO, [2]University of Kansas Medical Center, Kansas City, KS.

Inheritance of a complete set of chromosomes is critical for fertility and production of viable offspring. However, chromosomes are vastly different in size and structure, and thus cells must be capable of accurately segregating an array of diverse chromosomes. For example, the largest human chromosome can be as much as 5 times larger than the smallest and in *Drosophila* 46 times larger. Currently, the mechanisms underlying meiotic recombination and segregation of specific chromosomes are not understood. One key component of the meiotic machinery is a large multi-protein structure called the synaptonemal complex (SC). During meiosis, the SC holds homologous chromosomes together and is necessary for proper recombination and chromosome segregation. In yeast, in-frame deletions within the transverse filament (TF) protein, a key SC component, decrease the width of the SC and cause chromosome missegregation. In *Drosophila melanogaster*, we find that small in-frame deletions in the TF protein of the SC result in fragmentation of the SC and chromosome-specific defects in meiotic recombination and pairing. To determine if the width of the SC is altered in our mutants with chromosome-specific meiotic defects, we imaged meiotic chromosome spreads with super-resolution STED microscopy. In these TF deletion mutants, the width of the SC is reduced significantly more than is predicted based on the size of the deletions. Further work is necessary to determine if changes in the

width and fragmentation of the SC play a role in the chromosome-specific recombination defects. Therefore, we are currently examining pairing, double-strand break placement, and recombination on individual chromosomes in TF deletions mutants using the Lac*O*/Lac*I* system combined with super-resolution imaging and chromosome tracing. Additionally, we are creating point mutants to identify residues within the small deletions that are responsible for SC phenotypes seen in these mutants. Our work has generated a fly model that exhibits chromosome-specific meiotic behaviors and defects, which could lead to a greater understanding of the basics of meiotic biology and human infertility.

B147/P2541

Novel mechanism for spontaneous chromosome 12 aneuploidy in hiPSCs

M. Narozna, C. O. DuBose, J. R. Daum, G. J. Gorbsky; Cell Cycle and Cancer Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK.

Human induced pluripotent stem cells (hiPSCs) offer tremendous potential in replacing damaged tissues and organs. They are generated from the patient's cells and reprogrammed under the influence of pluripotency-inducing factors. After differentiation into various cell types, they may be reintroduced for therapy with limited chances of immune rejection. However, to be used for therapy cells must be propagated in culture both before and after reprogramming. Previous work has shown that hiPSCs can accumulate genetic aberrations, potentially limiting their usefulness in therapy and in research. An often detected abnormality is trisomy (gain of an additional copy of the entire chromosome) of chromosome 12. Normally, chromosome 12 trisomic cells become dominant in the culture, but how the trisomy arises and how it becomes dominant remain unknown. Although trisomic cells proliferated slightly faster than their diploid precursors, mathematical modeling showed that the difference in growth rates was insufficient to account for the rapid dominance of the trisomic cells. To understand potential mechanisms, we performed dual color fluorescent in-situ hybridization (FISH) and the whole chromosome 12 paint in hundreds of mitotic and interphase cells from various passages of hiPSCs passages, including the critical transition passages where the trisomic cells gained dominance. Surprisingly, analyses of cells from the transition passages (during which trisomy arises) in anaphase showed many cells with three signals from chromosome 12 oriented to one pole and two oriented to the other pole. We also detected single, potentially unpaired chr12 chromatids apart from aligned chromosomes in metaphase cells. Chromosome 10, mapped as a control, never showed these abnormalities. These data suggest that transition passages show a high proportion of cells entering mitosis with two normal chromosome 12's and one half chromosome 12 (single chromatid). We propose that this unusual chromosome content may come about through incorporation of a missegregating chromosome 12 in micronuclei that then fail to replicate. In support, we found by FISH that chromosome 12 was more often detected in micronuclei than would be expected by random incorporation of chromosomes. Our findings implicate a completely novel mechanism of aneuploidy that may be of profound importance for understanding genome instability in stem cell biology and that has strong implications for the use of hiPSCs in therapy and research.

Funding: Support was provided by the Oklahoma Center for Adult Stem Cell Research and by the National Institute of General Medical Sciences, grant no. R35GM126980.

B148/P2542

Mitotic R-loops direct RBMX and Aurora B kinase to maintain centromeric cohesion

T. Stukenberg; Biochemistry and Molecular Genetics, University of Virginia, School of Medicine, Charlottesville, VA.

Recent work has shown that R-loops exist at mitotic centromeres, but the dynamics of R-loops and their functions during mitosis are not well understood. To address this, we mapped R-loops during synchronous mitotic progression. During prophase R-loops associated with transcription are reduced, while R-loops associated with repetitive sequences, particularly α-satellite regions increase. These dynamics were dependent on Aurora B kinase activity. We also found an enrichment of R-loops at CTCF/Cohesin sites in interphase cells and these are also dynamically controlled during mitosis by Aurora B. Over 80 percent of CTCF sites that also bind cohesin have R-loops and these R-loop sites are often cell type specific suggesting that R-loops control the generation of TADs during differentiation. Mitotic R-loops are required to maintain sister chromatid cohesion demonstrating a functional link between cohesin and R-loops. This requires a pathway where R-loops localize RBMX to centromeres, which in turn binds Aurora B to localize Sgo1 and maintain cohesion. We conclude that R-loops are dynamically rearranged by Aurora B during mitosis and R-loops play important roles in controlling cohesin and higher order chromatin structure throughout the cell cycle.

B149/P2543

Oligopaint FISH reveals transcription-associated meiotic pairing initiation in Lepidopteran insects

L. F. Rosin, E. P. Lei; NIH/NIDDK, Bethesda, MD.

Precise homolog pairing is essential for accurate meiotic chromosome segregation in species from plants to humans. Failure to accurately segregate chromosomes in meiosis can lead to nondisjunction and infertility or aneuploidy in progeny. Yet, the mechanisms regulating homolog recognition and pairing are unknown. In many species, it is thought that DNA double-strand breaks (DSBs) facilitate the search for homology. However, some species including insects like fruit flies (Diptera) and moths (Lepidoptera) are able to accurately pair their homologs before DSB formation or without the need to ever form crossovers. Here, we employ the highly specific Oligopaint fluorescence *in situ* hybridization (FISH) technology to interrogate homolog recognition and the homolog pairing process in the silkworm moth *Bombyx mori* and the pantry moth *Plodia interpunctella*. Our studies demonstrate that meiotic pairing in male moths occurs asynchronously through numerous partially paired intermediate structures. Similar to findings from the holocentric nematode *C. elegans*, we find that meiotic pairing in holocentric moths is initiated at chromosome ends. However, unlike *C. elegans*, chromosome ends in moths are not necessarily gene-poor, and gene-rich chromosome ends pair earlier than gene-poor ends. Finally, our preliminary studies employing RNA FISH suggest that genes near early-pairing chromosome ends are indeed transcribed during early meiotic prophase. Together, our data support a model in which transcription, the transcription machinery, or transcripts themselves facilitate homolog recognition and meiotic pairing initiation in Lepidopteran insects. Whether or not these mechanisms are more broadly conserved remains an area for future study.

B150/P2544

Chromosome Atlas: A trip around chromosome formation during mitosis

F. Cisneros Soberanis[1], E. Simpson[2], A. Beckett[3], N. Pucekova[1], I. Prior[3], D. Booth[2], W. Earnshaw[1]; [1]Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, UNITED KINGDOM, [2]Biodiscovery Institute, University of Nottingham, Nottingham, UNITED KINGDOM, [3]Biomedical Electron Microscopy Facility, University of Liverpool, Liverpool, UNITED KINGDOM.

INTRODUCTION. During mitosis, cells compact their DNA to segregate it equally to their daughter cells. Chromosome compaction starts during prophase and continues along prometaphase. During metaphase, the chromosomes are compacted and localise at the metaphase plate pushed/pulled by the microtubules. In anaphase, the microtubules pull the chromosomes to the spindle poles and the chromosomes start to decompact. This work explores the changes in chromosome structure along the different stages of mitosis using volume electron microscopy and 3D reconstruction. **METHODS.** To enrich the mitotic population, we genetically modified RPE1 hTERT cell line to introduce a CDK1 mutant sensitive to an ATP analogue. We pre-synchronised them in G1 using a CDK4/6 inhibitor. Then, we released and blocked them in G2 using an ATP analogue. Finally, we washed out the ATP analogue to let them go to mitosis. We processed the cells for electron microscopy using *en block* staining and we imaged them using SBF-SEM. From the 3D reconstruction analysis, chromosomes 1-5 and 19-22 were identified by size and centromere position. We measured morphological characteristics such as volume, surface and length. Also, we evaluated the interaction with other subcellular structures. **RESULTS.** In prophase cells, the chromosomes were forming attached to the nuclear envelope, their morphology was supple and the chromosome surface was rough. After the nuclear envelope break-down, in prometaphase, the chromosomes detached from the nuclear envelope. The chromosome surface looked smoother. The chromosome lengths were variable between cells and centromeres showed a severe stretching caused by the microtubule interactions. During metaphase, the chromosomes looked stiffer. The centromere stretching decreased and the volume and length kept homogeneous between the cells. In anaphase, when the sister chromatids are separated, the chromosomes start to decompact. Some chromosomes showed an S shape caused by two kinetochores in the same chromosome. However, the segregation was correct as the microtubules were pulling in the same direction, this event hasn't been shown by light microscopy due to the limited resolution. **CONCLUSION.** We concluded that the nuclear envelope could be important for the early stages of chromosome compaction. The centromere stretching could be decreasing in subsequent mitotic stages due to the increase in chromosome stiffness. The increase in chromosome stiffness could be associated with the increase of chromosome scaffold proteins interacting with the chromatin and rearrangement in the structure.

B151/P2545

Mechanical regulation of chromosome loss in lung cancer spheroids

A. Anlas, B. Hayes, D. E. Discher; University of Pennsylvania, Philadelphia, PA.

During cancer progression, changes in the tumor microenvironment could lead to increased physical confinement and drive chromosome segregation errors in cancer cells. While most solid tumors exhibit chromosomal instability, the mechanisms that lead to chromosome missegregation and loss are unknown. Here, we used the CRISPR/Cas9 system to engineer H23 lung adenocarcinoma cells that carry a monoallelic chromosome reporter for the constitutively active Lamin-B1 gene to track chromosome loss. H23 cells with the LaminB1-GFP reporter were cultured in 1% methylcellulose or agarose to

generate spheroids. We analyzed mitotic events, cellular contractility and chromosome loss at different regions within spheroids. Our data suggest that chromosome loss is increased in cancer cells at the physically confined core of lung cancer spheroids. Future work will determine the mechanotransduction pathways that regulate chromosome missegregation in lung adenocarcinoma spheroids growing under confinement.

B152/P2546

The Skp1-Cullin-F-box ubiquitin ligase complex is dynamically regulated throughout mouse spermatogenesis

I. D. Wolff[1], J. Moore[2], S. Gray[2], P. E. Cohen[1]; [1]Biomedical Sciences, Cornell University, Ithaca, NY, [2]School of Life Sciences, University of Nottingham, Nottingham, UNITED KINGDOM.

Sexually reproducing animals generate gametes through the process of meiosis, a reductional cell division that yields a haploid oocyte or sperm. Meiosis I proceeds through two distinct stages: prophase I, when homologous chromosomes are physically tethered through the formation of crossovers, and M-phase, when homologs and sister chromatids are separated in the first and second meiotic divisions, respectively. To prevent errors in chromosome segregation, meiotic progression is strictly regulated to ensure that major events are completed before subsequent downstream events initiate, however, a distinct molecular cue that senses completed crossover formation then triggers cell cycle progression has not yet been found. Recently, the SCF (Skp1-Cullin-F-box) E3 ubiquitin ligase complex has been implicated in meiotic cell cycle progression: a core SCF component, SKP1, is essential for progression from prophase I to M-phase, and SCF may be regulated by the crossover protein CNTD1 to promote downstream degradation of cell cycle inhibitors. However, it remains unclear how the SCF complex is biochemically regulated throughout the meiotic program. In this work, we investigate the molecular composition of the SCF complex during spermatogenesis in mouse. We identified a testis-specific post-translational modification on SKP1 that is lost in two null mutants, *Cntd1* and *Mlh1*, both of which reduce crossover frequency to ~5-10% of wild type levels. Gravitational cell sorting of spermatocytes revealed that this modification appears during the zygotene and pachytene substages of prophase I, correlating to the period of crossover maturation. In addition, we identified a crossover independent phosphorylation event on SKP1 that is enriched in M-phase spermatocytes and may be important for SCF complex assembly, particularly the interaction between SKP1 and F-box proteins. Altogether, these data indicate that the SCF ubiquitin ligase complex is biochemically regulated within different meiotic contexts in a dynamic fashion to achieve differing signaling outcomes. Our work also implicates post-translational modifications on SCF subunits as a critical mechanism for direct molecular crosstalk between crossover formation and meiotic cell cycle progression.

B153/P2547

Centromere Innovations within a Mouse Species

N. Pandey[1], C. Gambogi[1], J. Dawicki-McKenna[1], U. Arora[2,3], G. Logsdon[4], J. Ma[5], P. Lamelza[5], M. Lampson[5], B. Dumont[2,3], B. Black[1]; [1]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [2]The Jackson Laboratory, 600 Main Street, Bar Harbor, ME, [3]Tufts University, Boston, MA, [4]University of Washington School of Medicine, Seattle, WA, [5]School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA

Mammalian centromeres direct faithful genetic inheritance during cell division. Despite this critical function, the highly repetitive DNA sequence at centromeres is rapidly evolving. The prevailing model for this rapid evolution invokes competition during female meiosis wherein selfish centromeres are preferentially inherited. Intraspecific and interspecific hybrid mice have recently provided molecular clues into meiotic competition (Iwata-Otsubo, *et al.*, 2017, ***Curr. Biol.***, 27:2365-73; Akera *et al*., 2019, ***Cell***, 178:1132-44), yet the strains/species investigated to date have relatively homogeneous centromeres between chromosomes. Transient evolutionary centromere intermediates are predicted yet largely unexplored. At the molecular level, the only known sequence-specific centromere DNA binding protein, CENP-B, can modulate centromere competition (Kumon, *et al*., 2021, ***Cell***, 184:4904-18) and is thus a prime candidate to be impacted by rapid centromere DNA sequence changes. Here we focus on a mouse species, *M. pahari,* which diverged from *M. musculus* ~5 M years ago. We find two different types of *pahari* centromeres: one present on most of the autosomes and largely devoid of the recognition element for CENP-B (the CENP-B box) and one found on a single autosome that has ~100-fold greater number of CENP-B boxes. Both types of centromeres have related sequence that we define as "π-sat". We report the full, contiguous (6-16 Mbp) sequences for both types of centromeres. The centromere with high levels of CENP-B has larger kinetochores, potentially strengthening microtubule attachments to the spindle. We find this is counterbalanced by relatively high levels of pericentromeric heterochromatin (i.e. H3K9me3 enrichment) and accumulation of the microtubule destabilizing kinesin, MCAK. Indeed, this balance leads to similar fidelity in mitotic chromosome segregation between *M. pahari* chromosomes despite a large divergence in molecular composition. Thus, this mouse species naturally provides an example of how highly divergent centromeres transiently co-exist prior to homogenizing during mammalian chromosome evolution.

B154/P2548

Persistently unaligned polar chromosomes link increased microtubule stability to biased aneuploidy landscape in tumor cells

K. Vukušić, S. Kodba, P. Risteski, I. M. Tolić; Ruder Boskovic Institute, Zagreb, CROATIA.

Congression is a movement of chromosomes toward the equatorial plane of the mitotic spindle during which kinetochores acquire end-on attachments to microtubules, a key step for error-free mitosis. It was recently reported that interphase nuclear chromosome organization dictates probability of chromosome mis-segregation during mitosis by biasing mis-segregations toward chromosomes positioned at nuclear periphery and behind spindle poles in non-transformed cells with weakened mitotic checkpoint. However, the mechanisms that induce a biased aneuploidy landscape in tumor cells, in which the mitotic checkpoint is rarely defective, are unclear. Here, by establishing a database of multiple live human cell lines imaged through mitosis, we show that congression frequently failed specifically in tumor cells, resulting in chronically unaligned polar chromosomes, which were a major

contributor to strong mitotic delays. Furthermore, in a minority of tumor cells, unaligned chromosomes remained at the pole through mitosis, resulting in aneuploidy. By tracking chronically unaligned chromosomes in U-2 OS cells, we show that they originated preferentially from the nuclear periphery and were situated behind spindle poles during early mitosis, contrary to pairs that rapidly aligned, indicating that delayed passage across polar region impedes speed of chromosome congression. Furthermore, delayed alignment of chromosomes severely abolished their stability within the metaphase plate after the congression attempt, contrary to pairs that aligned rapidly during prometaphase, including the subset of peripheral polar chromosomes. Even after successful alignment, persistent laggards repeatedly followed polar chromosomes, whereas chromatin bridges were not related to this phenomenon, indicating a connection between delayed alignment and the higher incidence of merotelic attachments. Intriguingly, by combining live cell imaging with STED microscopy, we report that treatment of non-transformed cells with nanomolar doses of nocodazole increased the stability of the kinetochore microtubules and produced chronically unaligned polar chromosomes characterized by complex types of end-on attachments, thus mimicking the phenotype of tumor cells. We conclude that increased microtubule stability, a known hallmark of tumorigenesis, preferentially affects the congression of peripheral polar chromosomes, giving rise to chronically unaligned polar chromosomes that enter a vicious cycle of hampered alignment that results in strong mitotic delays and biased aneuploidy landscape in tumor cells. Therefore, both in the case of reduced and increased mitotic duration, the peripheral polar chromosomes are biased toward mis-segregation and micronuclear entrapment.

B155/P2549

Fragmented chromosomes from micronuclei are repaired by non-homologous end joining to generate complex genome rearrangements

Q. Hu[1], J. E. Valle-Inclan[2], A. Guyer[1], E. G. Maurais[1], Y. Lin[1], I. Cortés-Ciriano[2], P. Ly[1]; [1]Department of Pathology, Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX, [2]European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, UNITED KINGDOM.

Chromosome segregation errors during mitosis can generate abnormal nuclear structures called micronuclei. The accumulation of DNA double-strand breaks (DSBs) within micronuclei triggers the catastrophic shattering of the mis-segregated chromosome through a process termed chromothripsis. Error-prone DSB repair of chromosome fragments can subsequently generate a spectrum of genomic alterations, including both simple and complex rearrangements found in cancers and developmental disorders. How distinct DSB repair pathways recognize and process these lesions remain poorly understood. Here we used CRISPR/Cas9 to systematically inactivate genes spanning distinct DSB processing or repair pathways and interrogated the structural rearrangement landscape of mis-segregated chromosomes. Deletion of core non-homologous end joining (NHEJ) components - including DNA ligase 4, DNA-PKcs, and XLF, as well as the NHEJ-promoting factor 53BP1 - substantially reduced the formation of complex rearrangements, suggesting that NHEJ is essential for re-ligating fragmented chromosomes. By contrast, inactivation of alternative end-joining or recombination-based DSB repair did not alter the frequency and types of rearrangements induced by micronucleation. Whole-genome sequencing revealed that rearranged chromosomes from cells lacking NHEJ were comprised of relatively simple rearrangements without the characteristic patterns of cancer-associated chromothripsis. In the absence of NHEJ, chromosome fragments were rarely engaged by other DSB repair pathways, resulting

in the accumulation of persistent DNA damage in the nucleus. Thus, we provide evidence supporting NHEJ as the exclusive DSB repair pathway generating complex rearrangements following chromothripsis from mitotic errors.

B156/P2550

Reorganisationof Centromere Chromatin During the G2-Mitosis Transition Requires Condensin

K. Samejima[1], C. A. Brackley[2], N. Gilbert[3], D. Marenduzzo[4], W. C. Earnshaw[1]; [1]Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, United Kingdom, [2]School of Physics and Anatomy, University of Edinburgh, Edinburgh, United Kingdom, [3]IGMM, University of Edinburgh, Edinburgh, United Kingdom, [4]School of Phyics and Astronomy, University of Edinburgh, Edinburgh, United Kingdom

Abstract: Structural analysis of the DNA and chromatin organisation of vertebrate centromeres is complicated by the presence of complex highly-repetitive satellite DNA arrays. However, several species, including equids and chickens, have one or several chromosomes with evolutionarily new centromeres assembled on non-repetitive DNA. Chickens have such centromeres on chromosomes Z, 5 and 27. The DT40 chicken B lymphocyte cell line is a particularly favourable system in which to study the three-dimensional organisation of centromere DNA as it is female (Z/W), so has a single copy of the Z chromosome. The Z chromosome is the 5th largest (83 Mb) and is metacentric, so its centromere conformation is not influenced by telomere proximity. As an additional benefit, synchronisation of DT40 CDK1^{as} cells in late G_2 and mitosis is straightforward and reproducible.We have previously published a detailed study of mitotic entry in DT40 CDK1^{as} cells using Hi-C. Unfortunately, the usual Hi-C resolution (40-100 kb) is not sufficient to resolve structural detail within chicken core centromeres (CENP-A domain ~30 kb) and the sequence cost to obtain 1 kb resolution would be prohibitive. Therefore, we have applied Capture-C analysis to construct a high-resolution interaction map of the Z centromere in G_2 and mitosis in wild type and cells depleted of condensin and cohesin, taking advantage of our CDK1as/AID tagged cell lines. Our Capture analysis of WT cells indicated that core centromere chromatin exhibits strong inter core interactions during G_2. In contrast, a striking bi-partite organisation appears in mitosis. Furthermore, our analysis demonstrated that condensin is essential for the structural transition of centromeric chromatin from G_2 to mitosis.

B157/P2551

Actin limits egg aneuploidies associated with female reproductive ageing

S. Dunkley[1], B. Mogessie[2]; [1]School of Biochemistry, University of Bristol, Bristol, UNITED KINGDOM, [2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT.

Ageing is accompanied by aneuploidy in mammalian eggs, which underlies common pregnancy failures in reproductively older females. This is consistent with gradual, ageing-related depletion of centromeric cohesion proteins leading to premature separation of sister chromatids. However, such progressive cohesion loss does not satisfactorily explain the sharp rise in egg aneuploidy near the end of female reproductive life. Here we show that F-actin helps to keep most sister chromatids together after centromeric cohesion has deteriorated in ageing mammalian eggs. By combining targeted protein degradation with advanced microscopy of chromosomal dynamics in eggs of young and aged females, we demonstrate that actin mitigates premature sister chromatid separation (PSSC) arising from centromeric cohesion loss by limiting microtubule-dependent chromatid disengagement. We propose that impairment of this function underlies ageing-related exponential rise in egg aneuploidy.

B158/P2552

Defining CLS-2 and SPD-1 Function in Central Spindle Assembly during *C. elegans* Sperm Meiosis

S. Gomez, 1st, M. M. Garcia, C. Huynh, B. R. Lobos, D. Chu; Cellular and Molecular Biology, San Francisco State University, San Francisco, CA.

Male infertility is a serious concern for many Americans. To elucidate how infertility arises, researchers are using the Caenorhabditis elegans nematode to study chromosome segregation. In C. elegans, different forms of cell division use different mechanisms to ensure proper chromosome segregation. In the case of mitosis and oocyte meiosis, a central spindle structure composed of microtubules assembles in the midzone between segregating chromosomes. The central spindle produces pushing and pulling forces in both to coordinate chromosome segregation. In the case of C. elegans sperm meiosis, few microtubules are present in the midzone except those tied to the lagging unpaired X chromosome, a unique feature of male sperm meiosis. The central spindle structure is initiated by the kinetochore protein, CLS-2, at the midzone region of the segregating chromosomes. CLS-2 then recruits SPD-1, a microtubule bundler protein, to elongate the spindle. It is unknown whether the presence of the lagging unpaired X chromosome implies that there is a distinctive mechanism of chromosome segregation in sperm meiosis. We hypothesize that CLS-2 and SPD-1 do not play a significant role in central spindle assembly in sperm meiosis. Instead, sperm meiosis relies on the pulling forces of the kinetochore-connected microtubules rather than the push/pull forces generated by the central spindle. We will deplete CLS-2 and SPD-1 in young adult C. elegans males using the novel auxin-inducible degradation system and apply immunostaining techniques to observe our proteins of interest. Our preliminary data shows that CLS-2 and SPD-1 fail to localize to the midzone during sperm meiosis except to the lagging unpaired X chromosome. In males depleted of CLS-2 and SPD-1, any influence of a present central spindle will be removed. By removing the push/pull influence of the central spindle and using confocal live-imaging to observe the resulting phenotype, we expect to see DNA segregate at a similar rate to wild-type which will confirm that sperm meiosis does not rely on a central spindle to coordinate chromosome segregation. Understanding SPD-1 and CLS-2 function can elucidate the molecular mechanisms required for forming healthy sperm.

B159/P2553

From Surfactant to glue: how Ki-67 regulates chromosome surface properties

A. Hernandez-Armendariz[1,2], S. Cuylen-Haering[1]; [1]EMBL, Heidelberg, Germany, [2]Heidelberg University, Heidelberg, Germany

Compartmentalization into functional units is a key principle of cellular life. In addition to membrane-bound organelles, eukaryotic cells utilize membrane-less biomolecular condensates to locally concentrate proteins and nucleic acids. While we are beginning to understand how membrane-less condensates assemble and disassemble, we know very little about the biological processes that take place at the surface of such condensates.

The surface of the largest membrane-less cellular assembly, the mitotic chromosome, is covered by the intrinsically disordered protein Ki-67. Our previous studies have revealed that Ki-67 has dual functionality. In early mitosis, Ki-67 functions as a surfactant to prevent chromosomes from collapsing into a single chromatin mass, whereas it actively promotes chromosome clustering during exit from mitosis. How Ki-67 switches between these two opposing processes - chromosome dispersal and

chromosome clustering - has remained unknown.
Here, we demonstrate that Ki-67's biophysical properties radically change during anaphase onset, when all chromosomes merge into a single cluster. Ki-67's amphiphilic character is lost as its molecular brush structure collapses and the soluble pool of the protein forms condensates. Our study uncovers a cell-cycle-regulated mechanism that controls individualization and coalescence of chromosomes during mitosis.

B160/P2554

Naturally occurring variants in human chromosome segregation genes reveal novel Chromosomal Instability aiding Variant (CIVa) in kinetochore-microtubule bridges

A. Islam, C. Manjarrez, X. Song, T. Gore, **V. Draviam**; Queen Mary University of London, London, United Kingdom

Chromosomal Instability (CIN) is a hallmark of several developmental abnormalities, intellectual disabilities and aggressive cancers. Yet, in a vast majority of CIN syndromes, the underlying molecular drivers remain unknown. To address this knowledge gap, we are collating a comprehensive map of Chromosomal Instability aiding Variants (CIVa) taking advantage of human genome sequencing efforts. We first assessed the prevalence of Loss-of-Function (LoF) variants in 135 chromosome segregation genes from over 150,000 humans across multiple ethnicities [https://draviam-lab.github.io/CIVa/]. The assessment of CIVa predictions revealed a surprisingly high incidence of heterozygous and homozygous LoF variants in three chromosome-microtubule attachment bridging proteins: Ndc80, Astrin and SKA3. Next, we systematically probed the impact of CIVas using a quantitative framework, including single-cell microscopy and population-level isoform expression studies. Analysing subcellular dynamics following controlled protein variant expression revealed the naturally occurring Astrin p.Q1012* as a harmful variant. The Astrin p.Q1012* variant fails to localise normally at kinetochores; induces chromosome misalignment; prolongs the process of cell division, and promotes chromosome missegregation and DNA damage. Other frameshift variants observed in Ndc80, Astrin and SKA3 genes are likely to generate shorter isoforms which do not compromise chromosome segregation in monoallelic forms. Thus, we present a scalable quantitative framework to stratify CIVa predictions - an essential step towards precision healthcare for CIN syndromes.

B161/P2555

Chromosome Atlas: A trip around chromosome formation during mitosis

F. Cisneros Soberanis[1], E. Simpson[2], A. Beckett[3], N. Pucekova[1], I. Prior[3], D. Booth[2], W. Earnshaw[1]; [1]Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, UNITED KINGDOM, [2]Biodiscovery Institute, University of Nottingham, Nottingham, UNITED KINGDOM, [3]Biomedical Electron Microscopy Facility, University of Liverpool, Liverpool, UNITED KINGDOM.

INTRODUCTION. During mitosis, cells compact their DNA to segregate it equally to their daughter cells. Chromosome compaction starts during prophase and continues along prometaphase. During metaphase, the chromosomes are compacted and localise at the metaphase plate pushed/pulled by the microtubules. In anaphase, the microtubules pull the chromosomes to the spindle poles and the chromosomes start to decompact. This work explores the changes in chromosome structure along the different stages of mitosis using volume electron microscopy and 3D reconstruction. **METHODS.** To enrich the mitotic population, we genetically modified RPE1 hTERT cell line to introduce a CDK1 mutant

sensitive to an ATP analogue. We pre-synchronised them in G1 using a CDK4/6 inhibitor. Then, we released and blocked them in G2 using an ATP analogue. Finally, we washed out the ATP analogue to let them go to mitosis. We processed the cells for electron microscopy using *en block* staining and we imaged them using SBF-SEM. From the 3D reconstruction analysis, chromosomes 1-5 and 19-22 were identified by size and centromere position. We measured morphological characteristics such as volume, surface and length. Also, we evaluated the interaction with other subcellular structures. **RESULTS.** In prophase cells, the chromosomes were forming attached to the nuclear envelope, their morphology was supple and the chromosome surface was rough. After the nuclear envelope break-down, in prometaphase, the chromosomes detached from the nuclear envelope. The chromosome surface looked smoother. The chromosome lengths were variable between cells and centromeres showed a severe stretching caused by the microtubule interactions. During metaphase, the chromosomes looked stiffer. The centromere stretching decreased and the volume and length kept homogeneous between the cells. In anaphase, when the sister chromatids are separated, the chromosomes start to decompact. Some chromosomes showed an S shape caused by two kinetochores in the same chromosome. However, the segregation was correct as the microtubules were pulling in the same direction, this event hasn't been shown by light microscopy due to the limited resolution. **CONCLUSION.** We concluded that the nuclear envelope could be important for the early stages of chromosome compaction. The centromere stretching could be decreasing in subsequent mitotic stages due to the increase in chromosome stiffness. The increase in chromosome stiffness could be associated with the increase of chromosome scaffold proteins interacting with the chromatin and rearrangement in the structure.

B162/P2556

Argonautes from the AGO family are key players in sex chromosome inactivation during spermatogenesis

M. Carro; Cornell University - Ithaca, NY, Ithaca, NY

Production of healthy sperm requires highly regulated gene expression for each stage of spermatogenesis. Argonaute proteins are RNA binding proteins that associate with small non-coding RNAs (smRNAs) and coordinate downstream post-transcriptional gene-silencing events in the cytoplasm. The four members of the AGO clade of Argonaute proteins are expressed in the germline during spermatogenesis. Previous work from our lab supports for a role of Argonautes in Meiotic Silencing of Unpaired Chromatin (MSUC) and Meiotic Sex Chromosome Inactivation (MSCI). AGO3 and 4 localize in the nuclei of pachytene spermatocytes, at sites of asynapsis and in the transcriptionally silenced XY subdomain named the sex body. Loss of *Ago4* results in aberrant sex body morphology and upregulation of Ago3, suggesting that these proteins might have overlapping functions. Triple Ago314 knockout and heterozygous males have a subtle fertility phenotype with decreased testis size, reduced sperm counts, and increased germ cell apoptosis. Study of prophase I progression revealed that knockout spermatocytes show mislocalization of sex body markers, including γH2AX and TOPBP1. To investigate how the lack of AGOs could alter gene regulation during spermatogenesis, we performed RNA seq and smRNA seq in spermatocytes from different genotypes. Loss of AGO3,1 and 4 causes differential expression of Prophase I associated genes and upregulation of sex-linked genes, along with ingression of RNA pol II to the sex body, supporting a role of AGOs in MSCI. SmRNA seq revealed differential expression of micro RNAs from a cluster located in the Fragile X region that could act as candidate AGO binding partners. In addition, we performed a co-immunoprecipitation of AGO3, followed by Mass spectrometry and smRNA seq. Interestingly, we found several proteins involved in chromatin

remodeling as potential interactors, suggesting that AGO3 function resides at the nuclear level, and thus may involve transcriptional control rather than the more traditional role of Argonautes in post-transcriptional mechanisms in the cytoplasm.

B163/P2557

Identification of molecular targets of PICH remodeling activity for faithful chromosome segregation

B. Lama; University of Kansas, Lawrence, KS

Mitosis is a complex process with many regulatory mechanisms involved for faithful chromosome segregation. Different proteins and protein modifications help ensure correct mitosis, one such important protein modification is SUMOylation, a post-translational modification. Loss of mitotic SUMOylation shows aberrant chromosome structure, chromosome separation defects, and delayed mitosis in the eukaryotic cell. Either inhibiting SUMOylation or stabilizing SUMOylation showed a comparable defect in chromosome segregation, hence this transient /dynamism of SUMOylation of mitotic proteins is critical for faithful chromosome segregation. Polo-like kinase 1 interacting checkpoint helicase (PICH) is a DNA translocase belonging to an SNF2 nucleosome remodeling family protein that does not have robust histone remodeling activity. Loss of PICH function increase chromosome segregation defects thus its remodeling activity is required for preventing genome instability. We identified PICH has a SUMO-interacting motif (SIM), which is required for preventing chromosome bridge formation. The conditional PICH depletion and replacement to mutant showed that PICH's translocase activity and SIM are required for preventing chromosome bridge formation and for controlling the abundance/localization of chromosomal SUMOylated proteins. These observations provide the hypothesis that PICH is the remodeling factor of mitotic chromosomal SUMOylated proteins that are required for faithful chromosome segregation. Here, we aim to identify the chromosomal target protein of PICH's remodeling function, which could be SUMOylated or non-SUMOylated. Using the His6-tag on endogenous SUMO-2 protein in different PICH-replaced cell lines, we isolated chromosomal SUMOylated proteins under loss of PICH function. Identification of purified protein using label-free quantitative mass spectrometry revealed several candidate proteins specifically increased in loss of PICH function. These are the potential SUMOylated target of PICH remodeling activity. Conditional PICH-depletion using the AID system results in mis regulation of chromosomal structural protein and defects in progression in mitosis, and identified proteins are known to involve in these phenotypes. With these observations, we propose potential molecular targets and functions of PICH's remodeling activity in mitosis for faithful chromosome segregation.

B164/P2558

The Chromosomal Passenger Complex Stabilizes the Inner Kinetochore in Mitosis

M. Bonner, **J. Haase**, A. E. Kelly; NIH/National Cancer Institute, Bethesda, MD

The kinetochore is assembled at centromeres on specialized nucleosomes that contain the histone H3 variant, CENP-A. The inner kinetochore, made up of sub-complexes referred to as the constitutive centromere-associated network (CCAN), directly links the centromeric chromatin to the microtubule-binding proteins at the outer kinetochore, which in higher eukaryotes are assembled upon entry into mitosis. Recent cell biological and structural work has demonstrated that the CCAN undergoes a reorganization upon entry into mitosis, but the mechanistic underpinnings of this process are not completely understood. We have previously shown that the chromosomal passenger complex (CPC) is

required to maintain proper CCAN levels at centromeres in mitosis, independently of its kinase subunit Aurora B, and that this process is required to detect errors in kinetochore-microtubule attachment. However, it remains unclear how the CPC regulates CCAN levels at centromeres. Here, using Xenopus egg extracts, we found that CCAN proteins such as CENP-C and the CENP-N/L complex display dynamic exchange at centromeres during interphase but rapidly become immobilized upon entry into mitosis. Strikingly, CPC depletion or mutations that affect its interactions with chromatin result in persistent CCAN turnover throughout mitosis, which leads to a substantial decrease in CCAN concentration at centromeres. Inhibition of the phosphatase PP1, but not of PP2A, rescues the effects of CPC depletion on CCAN stability, implicating another kinase in this process. These results suggest that the CPC promotes the stabilization of the CCAN at centromeres in early prophase to promote the proper attachment of microtubules to kinetochores.

B165/P2559

A quantitative survey of current and prospective measures of chromosomal instability in model systems identifies strengths and weaknesses

A. Lynch, S. Bradford, R. Lera, A. Zhou, B. Weaver[,1], M. Burkard[,1]; University of Wisconsin - Madison, Madison, WI

Chromosomal instability (CIN) is the persistent reshuffling of cancer karyotypes via chromosome mis-segregation over time. In cancer, CIN can exist at different levels, and these levels determine whether it restrains or promotes tumor progression. Quantitative measures of CIN may predict metastases and response to the chemotherapeutic paclitaxel. Despite the potential clinical significance mis-segregation rates remain challenging to assess in human cancer despite an array of proposed measurement methods. To evaluate measures of CIN, we compared quantitative methods with defined CIN models. We implemented multiple inducible cell culture models of CIN by distinct mechanisms and comprehensively applied multiple CIN measures including fixed and timelapse fluorescence microscopy, chromosome spreads, 6-centromere FISH, bulk transcriptomics, and single cell DNA sequencing (scDNAseq). To induce multipolar spindles and polar chromosomes, we overexpressed PLK4-WT or sequentially inhibited CENP-E and MPS1 respectively in MCF10A-PLK4-WT-tetOn cells. To induce bridging chromosomes, we expressed a dominant negative TRF2 mutant in CAL51-TRF2-DN-tetOn cells. While fixed and timelapse imaging correlated well in most cases ($R = 0.76$; $p < 0.001$), fixed imaging underestimated the presence of mis-segregated polar chromosomes in anaphase. Of the commonly used cytogenetics methods we tested, chromosome spreads and 6-centromere FISH correlated well ($R = 0.77$; $p < 0.01$) and exhibited baseline non-modality rates of ~50% and ~5% respectively. Yet neither method was sensitive to structural CIN or mis-segregation of only a few chromosomes. We used scDNAseq to measure CIN and propose a measure of CIN, Mis-segregations per Diploid Division (MDD), which accounts for differences in proliferation rates and ploidy levels between tumors and correlates very well with imaging methods ($R > 0.75$; $p < 0.05$). Accordingly, we propose CIN in tumors be measured using MDD as a primary metric. This analysis of scDNAseq data also validated an increase in CIN caused by HSET inhibition in the setting of widespread multipolar spindle formation (PLK4 overexpression), as reported by previous imaging studies. Importantly, we found no increase in CIN70/HET70 scores in any of our inducible models, indicating these widely cited transcriptional CIN measures are not indicative of ongoing CIN. This systematic analysis of common CIN measures' respective sensitivity will guide the measure of CIN in the clinical setting and highlights the primacy of single-cell methods.

B166/P2560

A non-centromeric locus experiences biased segregation during female meiosis in mice

F. Clark, T. Akera; NHLBI, NIH, Bethesda, MD

Proper chromosome segregation is a key component of meiosis. Multiple cellular mechanisms exist to ensure the equal and random segregation of homologous chromosomes, producing complete haploid genomes and genetic diversity among gametes. However, some genetic elements exhibit selfish mechanisms, altering chromosome segregation for their own benefit, often at the cost of organismal fitness and fertility. Mouse chromosome 2 harbors such a genetic element, a repeat with a 127 kb monomer, called R2d2. Known to experience strong transmission ratio distortion (TRD) in heterozygous females, R2d2 is thought to preferentially segregate to the egg in female meiosis. This TRD is also correlated with decreased fertility. We found that in heterozygous mice, the chromosome with the R2d2 repeat lags during anaphase of meiosis. In female meiosis, cytokinesis is asymmetric, incorporating most of the cytoplasm into the egg. Therefore, this lagging behavior could allow R2d2 to avoid segregation to the polar body by remaining near the spindle midzone long enough to be incorporated into the egg. The question remains, what mechanism regulates this chromosome-specific lagging behavior. To better examine this, cytogenetic studies of mouse oocytes provide a tractable tool to test the hypothesis that R2d2 alters its own segregation by acting as a neo-centromere. Surprisingly, a FISH-based technology, OligoPaint, revealed no co-localization between R2d2 and canonical centromere proteins, implying that R2d2 may have evolved another way of facilitating chromosome-spindle interactions to bias segregation.

B167/P2561

Defining the Independence and Localization ofMaintenance Genome Stability Protein 1

B. R. Jordan, **J. S. Glavy**; University of Texas At Tyler Fisch College of Pharmacy, Tyler, TX

Genome Stability is fundamental for the preservation and proper transmission of genetic material through generations. Maintenance of Genome Stability Protein 1 (MGSP1) is a promising modulator for safeguarding DNA. MGSP1 promotes replication fork maintenance and antiviral signaling. Our investigations establish MGSP1 to be independent of its previous classification as a progeria-associated protein and more in line with its yeast Maintenance of Genome Stability (MGS). Key to MGSP1's function is its localization in the nucleus, specifically at nuclear pore complexes (NPCs). In our study, we map its nuclear localization signal (NLS) domain that directs the protein nuclear positioning except during stages of mitosis. While MGSP1 connection to p53 and ataxia-telangiectasia mutated (ATM) pathway is known, we further show that MGSP1 maintains associates with these proteins in aneuploidy tripolar mitotic cells. Its cellular location and intersecting partners may play an important role in tempering DNA stresses to preserve cell division.

Spindle Assembly 2

B169/P2562

Mechanics of the anaphase spindle

P. Real Calderon[1], J. Lemière[1], R. Daga[2], F. Chang[1]; [1]University of California, San Francisco, San Francisco, CA, [2]CABD, Seville, SPAIN.

In the fission yeast *Schizosaccharomyces pombe*, the mitotic spindle can be regarded as a simple bundle of overlapping microtubules that elongates during anaphase to push the nuclei and chromosomes apart. We have discovered conditions under which the majority of cells fail during anaphase. In these cells, the spindle continues to elongate but the poles cannot move past a certain point, so that the spindle gradually adopts a bent shape rather than its normal straight shape. Ultimately, these spindles fail abruptly, by either breaking near the spindle mid-zone or near the spindle pole body. We hypothesize that these spindles exert pushing forces to elongate and bend their microtubules into this shape. Using simple beam theory, preliminary estimates suggest that the spindle exerts 100's picoNewton forces to bend itself, and that similar forces are needed to break the spindle. The cross-linker protein Ase1 (PRC1 orthologue) contributes to spindle stability, while kinesin motor like proteins (Cut7, Pkl1, Klp9) are individually dispensable for formation of the bent spindles. We are currently analyzing spindle curvature to discern the effects of Ase1 and the spindle midzone region on the rigidity of the spindle. Initial results show that the bent spindles preferentially break at the edge of the Ase1/MT overlap, identifying this as a fragile site. Our studies develop a tractable system to probe the mechanics and robustness of a simple spindle.

B170/P2563

Self organization of the metaphase human mitotic spindle

W. Conway, 2022, G. Fabig, R. Kiewsiz, T. Mueller-Reichert, D. J. Needleman; Harvard University, Cambridge, MA.

During eukaryotic cell division, the mitotic spindle is responsible for segregating chromosomes to the newly formed daughter cells. The spindle is a bipolar, self-assembled structure primarily composed of microtubules, molecular motors, and other microtubule associated proteins. The manner by which the spindle's constituents give rise to its structure and behaviors remains poorly understood. We employed a combination of serial section electron tomography, live cell polarized light microscopy, and biophysical modeling to develop an active liquid crystal theory that describes how forces shape metaphase human mitotic spindles. In the spindle interior, we found that local interactions between neighboring microtubules align the microtubules along well-defined trajectories of growth and motion. By examining how the aspect ratio of the spindle boundary scales with spindle volume, we found that the ellipsoid spindle boundary is the result of a balance of surface tension, due to microtubule crosslinking, and extensile active stress, from molecular motor activity. Taken together, this provides a framework for understanding the self-organization of human mitotic spindles, and the contributions of different molecular constituents and different classes of microtubules. We are in the process of extending this work to understand the monopolar spindles that result when the molecular motor, kinesn-5, is inhibited.

B171/P2564

Protein Regulator of Cytokinesis 1 assists Kinesin-12 in a Kinesin-5 independent pathway of mitotic spindle assembly

B. M. Salazar[1,2,3], R. Ohi[2]; [1]Doctoral Program in Cancer Biology, University of Michigan, Ann Arbor, MI, [2]Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, [3]Rogel Cancer Center, University of Michigan, Ann Arbor, MI.

The mitotic spindle is a bipolar microtubule-based machine that segregates chromosomes during cell division. Mistakes in spindle assembly result in cell death and/or the generation of aneuploid daughter cells. Spindle bipolarity stems from the activity of Eg5, a tetrameric motor that slides anti-parallel microtubules apart. Inhibition of Eg5 leads to mitotic arrest and apoptosis, making Eg5 a promising target for anti-mitotic cancer therapies. However, cells can develop resistance to Eg5 inhibitors (Kinesin-5 inhibitors (K5Is)) by deploying an alternate Kinesin-12 motor, Kif15, to drive spindle assembly. Like Eg5, Kif15 slides anti-parallel microtubules apart, suggesting that Kif15 uses this mechanism to promote spindle assembly. We model K5I resistance by selecting for cells that grow in the presence of K5Is, referred to as kinesin-5 inhibitor resistant cells (KIRCs). Interestingly, we observe that Kif15 becomes enriched at the spindle equator in KIRCs, a site expected to be enriched in anti-parallel microtubule overlaps. We hypothesized that this zone of anti-parallel microtubule overlaps is expanded in KIRCs, providing an optimal architecture for Kif15 to work as a spindle assembly motor. We further speculated that this KIRC-specific microtubule architecture may be facilitated by specific protein factors and to investigate this possibility, we focused on Protein Regulator of Cytokinesis 1 (PRC1), a microtubule-associated protein well known to bundle anti-parallel microtubules. PRC1 is best known for its cytokinetic functions, but growing evidence indicates that PRC1 is important during pre-anaphase mitosis. Here we show that KIRCs require PRC1 for spindle assembly, as 1) depletion of PRC1 by RNAi reduces the efficiency of spindle assembly in KIRCs, and 2) overexpression of PRC1 greatly improves spindle assembly in KIRCs. These results suggest a model in which PRC1-mediated bundling of spindle microtubules creates a more favorable architectural environment for Kif15-driven mitotic spindle assembly in the context of Eg5 inhibition.

B172/P2565

Investigating centriole-based assembly of spindle microtubules in the absence of pericentriolar material matrix in human cells

J. Xu[1], F. Metinger[1,2,3,4], A. Desai[1,2,3], K. Oegema[1,2,3]; [1]Department of Cell and Developmental Biology, School of Biological Sciences, University of California, San Diego, La Jolla, CA, [2]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, [3]Ludwig Institute for Cancer Research, La Jolla, CA, [4]Cell proliferation and gene editing unit, Okinawa Institute of Science and Technology, Onna, JAPAN.

Centrioles are small cylindrical structures composed of a nine-fold symmetric array of stabilized microtubules. Centrioles recruit a much larger pericentriolar material (PCM) matrix assembled from the coiled-coil protein CDK5RAP2 to form centrosomes. During mitosis, the main function of centrosomes is to catalyze the assembly of microtubules for spindle formation. It was long assumed that microtubule generation was performed by the PCM matrix. In particular, the main component of the PCM matrix CDK5RAP2 contains docking sites (CM1 motifs) that recruit microtubule-nucleating γ-tubulin complexes. In recent work, we have shown that in addition to the PCM matrix, there is a second pathway anchored

to centrioles that is sufficient to generate microtubules for spindle assembly. Spindles can form using the PCM matrix-based pathway when centrioles are removed and using the centriole-anchored pathway when the PCM matrix is removed. However, spindle assembly fails when both centrioles and the PCM matrix are removed (Watanabe, Meitinger et al. J Cell Biol., 219(12):e202006010, 2020). Although potent for the generation of spindle microtubules, the centriole-based pathway remains poorly understood. To dissect this pathway, we are investigating foci comprised of centriole-proximal components that form when the centrosomal ubiquitin ligase TRIM37 is deleted and centrioles are removed by chemical inhibition. These foci lack PCM matrix, but function as robust MTOCs that accelerate spindle assembly in the absence of centrosomes; notably, while these foci contain centriole-proximal components such as PLK4 and SAS-6 and have been shown to require PLK4 for their assembly, they do not contain the PCM matrix component CDK5RAP2 (Meitinger et al., Nature 585(7825):440-446, 2020). We are thus using these foci to understand microtubule generation by centriole-proximal components. Specifically, we are performing systematic inducible gene deletions under conditions where these foci form (TRIM37Δ RPE1 cells treated with the PLK4 inhibitor centrinone). This effort should help elucidate the centriole-based pathway that supports spindle formation when the PCM matrix is absent and yield a deeper understanding of the microtubule generation pathways that contribute to spindle assembly in human cells.

B173/P2566

Pushing the envelope: force balance in fission yeast closed mitosis

M. A. Begley[1], P. Zareiesfandabadi[2], A. Sharma[1], M. B. Rapp[1], M. W. Elting[1,3]; [1]Department of Physics, North Carolina State University, Raleigh, NC, [2]Department of Biology, Duke University, Durham, NC, [3]Quantitative and Computational Developmental Biology Cluster, North Carolina State University, Raleigh, NC.

In the fission yeast *S. pombe*, the mitotic spindle consists of a single bundle of parallel and antiparallel microtubules, crosslinked by motors and non-motor MAPs, that segregates chromosomes inside a closed nuclear envelope. Forces provided by both motors and microtubule dynamics work to elongate the spindle and segregate the cell's chromosomes during mitosis. Meanwhile, the nucleus morphs from a spheroid, through a peanut-shaped intermediate, and finally into a barbell shape before separating into two daughter nuclei. While it is assumed that spindle elongation drives nuclear shape changes, the mechanics underlying these transitions are very poorly understood. To examine the mechanics underlying closed cell division, we molecularly and mechanically perturb mitotic *S. pombe* cells. We sever specific mitotic structures, including the spindle and the nuclear envelope, by laser ablation, and we quantify the subsequent response to these acute perturbations using live cell confocal microscopy. We observe that motor-based force on the spindle that is recruited in response to laser ablation is able to dynamically re-shape the nuclear envelope. It has previously been shown that increasing envelope tension can cause the spindle to bow or curve, and we find that relieving envelope tension by laser ablation allows the spindle to straighten. The dynamics of this process report on the material properties of the spindle, nuclear envelope, and the nucleoplasm. We also perturb the long term mechanics of the spindle (by genetic deletion of crosslinkers and motors) and of the nuclear envelope (by altering membrane synthesis), and combine these changes with laser ablation. Finally, we perform a similar set of experiments in *S. japonicus*, the phylogenetic cousin of *S. pombe*, which goes through semi-open mitosis, in which nuclear envelope closure is not required. We find differences in spindle mechanics

between the two species, suggesting possible feedback between spindle and nuclear envelope structure and mechanics.

B174/P2567

RNA Localization to the Mitotic Spindle

C. Remsburg, K. Konrad, J. Song; University of Delaware, Newark, DE.

Mitosis is a fundamental and highly regulated process that acts to faithfully segregate chromosomes into two identical daughter cells. The timing of mitosis is essential, as prolonged mitosis can result in a myriad of disorders, including chromosome nondisjunction. Transcript localization of genes involved in mitosis at the mitotic spindle may be one way to ensure that mitosis occurs in a timely manner. We identified the transcripts of several genes involved in mitosis, including *AuroraB*, *Polo kinase*, and *Cyclin B* to be localized at the mitotic midzone in dividing cells of sea urchin and mammalian cell lines. Using the sea urchin as a model, we found that disruption of actin dynamics with cytochalasin D does not affect the localization, but disruption of microtubule polymerization with colchicine results in lack of localization of these RNA transcripts. These data suggest that localization of these transcripts is microtubule-dependent. Treating embryos with kinesore, which prevents kinesin-1 from binding to its cargo, results in a more diffused localization of these RNA transcripts. Additionally, treating embryos with ciliobrevin D, a dynein inhibitor, results in a change in localization of these RNA transcripts. These preliminary results indicate that kinesin-1 and dynein are likely motors responsible for localization of the RNA. We also identified that the cytoplasmic polyadenylation element (CPE) within the 3'UTR of *Aurora B*, a recognition sequence for the CPEB protein, is essential for localization of the transcript to the mitotic spindle. Blocking this sequence results in arrested development during early cleavage stages, indicating that transcript localization is important for early development. Understanding how RNA localization occurs to the mitotic spindle is critical for the fundamental understanding of cell division, as this has consequences in causing birth defects and predisposition to cancer.

B175/P2568

Evolutionary and functional divergence in the uncoupled microtubule networks of Naegleria

A. S. Kennard[1], K. B. Velle[1], C. Ballatore[2], L. K. Fritz-Laylin[1]; [1]University of Massachusetts, Amherst, Amherst, MA, [2]University of California San Diego, La Jolla, CA

The mitotic spindle is an essential component of cell division machinery throughout eukarya, but the organization and dynamics of this microtubule-based structure vary significantly across species. We aim to understand the organization and function of the mitotic spindle in the unicellular eukaryote *Naegleria gruberi*, which last shared an ancestor with animals and fungi over a billion years ago. *Naegleria* grows and divides as an amoeba, but under stress can rapidly differentiate into a transient flagellate state. Intriguingly, unlike nearly all eukaryotes studied to date, interphase *Naegleria* amoebae lack any microtubules whatsoever; instead, *Naegleria* transcribes and translates complete microtubule cytoskeletons de novo for mitosis and flagellar differentiation. Using RNA-Seq of cells undergoing synchronized cell division or flagellar differentiation, we find that the microtubule-associated genes upregulated during each of these processes are largely distinct. This genetic uncoupling of mitotic and flagellar gene repertoires in *Naegleria* provides a unique opportunity to study how tubulins and microtubule associated proteins diverge when constrained by only one of these two ubiquitous functional requirements. In line with this, we have shown that the *Naegleria* tubulins expressed during

mitosis are significantly divergent relative to model tubulins as well as *Naegleria* flagellar tubulins, with variation at key residues that suggest distinct microtubule properties. We are currently extending this investigation to *Naegleria*'s 42 kinesins. Consistent with the extreme divergence of *Naegleria* tubulins relative to model tubulins, we find that many conventional microtubule inhibiting drugs do not affect *Naegleria* growth, implying that these inhibitors may not bind to the divergent mitotic tubulins. We have identified microtubule stabilizing agents called phenylpyrimidines that block *Naegleria* cell division, arrest the spindle in metaphase, and lead to the formation of multipolar spindles, suggesting microtubule dynamics contribute to in maintenance of *Naegleria* spindle bipolarity. The conserved and divergent properties of *Naegleria* spindles make *Naegleria* a unique system for uncovering core principles underlying spindle assembly and function in diverse evolutionary contexts.

B176/P2569

A mechanical signal regulates Dynein loading on the nuclear envelope

V. Nunes[1], D. Castro[1], M. Dantas[1], M. Piel[2], P. Aguiar[1], H. Maiato[1], J. Ferreira[1]; [1]i3S - UP, Porto, PORTUGAL, [2]Institut Curie, Paris, FRANCE.

Increasing evidence shows that the nucleus is an active player during mitotic entry. Accordingly, chromosomes and other nucleus-associated components, which directly contribute to mitotic spindle assembly, also regulate nuclear mechanics. Importantly, in preparation for mitosis, cells undergo an extensive cytoplasmic and nuclear reorganization. These nuclear modifications are expected to elicit changes in the mechanical properties of the nucleus.
To address how these changes directly impact early spindle assembly, we followed mitotic entry in cells with compromised nuclear organization and structure, measured nuclear envelope (NE) fluctuations and extracted high-resolution quantitative data for both centrosome and nucleus-related metrics.
Our results allow us to propose a model where mitotic chromosome condensation increases intranuclear stiffness. This increased stiffness is transmitted by telomeres to SUN proteins at NE. In turn, this SUN protein-mediated strain on the nucleus is transmitted to the Bicaudal D2 (BicD2) and NudE/NudEL pathways, triggering Dynein loading to the NE. This recruitment of Dynein then ensures centrosome-NE tethering and correct centrosome positioning on the shortest nuclear axis, upon nuclear envelope permeabilization (NEP).
We propose that a mechanical signal triggered by mitotic chromosome condensation during the transition from G2 to mitosis ensures efficient spindle assembly and helps coordinate cytoplasmic and nuclear events.

B177/P2570

Over-accumulation of the ER blocks recruitment of centrosomal proteins resulting in mitotic failure

K. R. Rollins, J. T. Blankenship; Biological Sciences, UNIVERSITY OF DENVER, DEPARTMENT OF BIOLOGICAL SCIENCES, Denver, CO.

The Endoplasmic Reticulum makes contacts with organelles through the cell and has recently been shown to participate in processes beyond its canonical roles of calcium storage and protein synthesis. The ER also undergoes a remarkable transition in morphology during cleavage divisions in the syncytial *Drosophila* embryo, but whether this change in ER appearance modulates mitotic events is less clear. Mitotic divisions require massive remodeling of various membranous organelles such as the nuclear envelope and plasma membrane. Here, we examine the behaviors of the ER and its impact on

centrosomal and spindle function during the rapid cleavage divisions of the syncytial embryo. We see that the ER retracts and clusters around the spindle poles where it appears to form a coated structure around the spindle. As a means of perturbing this structure, we disrupted Rab1 function in the early embryo and observed an enhanced buildup of ER at the spindle poles. Interestingly, this led to large-scale defects in the division cycles. Mitotic spindle assembly is disrupted and displays a "mini-spindle" phenotype. These mini-spindles appear to be due to defects in centrosomal-based recruitment of microtubule nucleation factors. Our results suggest that the overaccumulation of ER can suppress the association of Centrosomin and Spd2 to the spindle piles leading to reduced γ-tubulin function. Finally, we show that the division failures and ER spindle-pole accumulation in *Rab1* knockdown embryos can be rescued with a Dynein inhibitor, demonstrating that Dynein motor activity is essential for the spindle association of the ER during mitosis. These results suggest that ER levels must be carefully tuned during mitotic processes to ensure proper assembly of the division machinery.

B178/P2571

miR-31 regulates actin dynamics to impact cell division during early development

M. Testa; University of Delaware, Newark, DE.

miR-31 is a highly conserved microRNA that has been shown to be important in various cancers, bone homeostasis, myogenesis, auto-immunity, lymphatic development, and embryonic development. Using the purple sea urchin as a model, we identified miR-31 and some of its targets to localize at the midzone of the mitotic spindle and perinuclearly in divided blastomeres. We also observed that miR-31 inhibitor-injected embryos display longer astral microtubules and increased interpolar and kinetochore microtubules, as well as increased filamentous actin compared to the control. These results lead to our hypothesis that miR-31 may regulate mitosis. We identified *Rab35*, *Fascin*, and *Gelsolin* to be directly regulated by miR-31. Rab35 is a member of the Rab family of small GTPases that plays essential roles in vesicular trafficking between the plasma membrane and endosomes as well as actin remodeling in eukaryotic cells. Rab35 localizes Fascin to the leading edge of the cell membrane, where Fascin bundles actin. Fascin has also been shown to regulate actin filaments for spindle migration and polar body extrusion in mice. Gelsolin is an actin-binding protein that is a key regulator of actin filament severance. Prior studies have shown that actin regulation is integral to mitosis and proper mitotic spindle assembly/disassembly. Since early cleavage stage embryonic development must be rapid, and strictly controlled to ensure the faithful segregation of chromosomes, miR-31 may regulate actin dynamics to mediate mitosis. Removal of miR-31's suppression of *Fascin* and *Rab35* lead to embryonic delay and arrest, phenocopying miR-31 inhibitor injected embryos. Identifying how miRNAs mediate spindle formation and mitosis is critical to the understanding of early embryonic development, and the predispositions to various diseases such as cancer.

B179/P2572

Monopolar spindles in RPE-1 cells are still bipolar, follow an intrinsic nematic order and exhibit a domain wall

G. Fabig[1], W. Conway[2,1], G. Ha[2], M. Basaran[2], R. Kiewisz[3], D. J. Needleman[2], T. Müller-Reichert[1]; [1]Medizinische Fakultät "Carl Gustav Carus", TU Dresden, Dresden, GERMANY, [2]Harvard University, Cambridge, MA, [3]Simons Machine Learning Center, New York Structural Biology Center, New York, NY.

The bipolar metaphase spindle is crucial in mitosis to reliably segregate chromosomes without errors to the newly forming daughter cells. An active liquid crystal theory, where microtubules locally align relative to one another, can quantitatively describe the steady state morphology of bipolar spindles, as well as the density and orientation fluctuations around the steady state. In this theory, microtubules grow along trajectories defined by the orientation field of the spindle, meaning that newly polymerizing microtubules grow along the trajectories of existing microtubules in the spindle. In prior work, we demonstrated that both kinetochore and non-kinetochore microtubules in mitotic human metaphase spindles are strongly aligned along the same orientation field. The trajectories defined by this field connect the kinetochores to the spindle poles. Here, we investigate this theory by perturbing the geometry of the spindle using a small molecule inhibitor of the plus-end directed motor kinesin-5, Monastrol (at a concentration of 200 µM). Upon treatment, the bipolar metaphase spindle changes to a monopolar spindle, with the condensed chromosomes surrounding the two centrosomes. Interestingly, the two centrosomes do not merge in the center of the monopole but instead stay at a defined steady-state distance and rotate around each other as observed by light microscopy. We used serial-section electron tomography after high-pressure freezing to reconstruct the trajectories of microtubules in these monopolar spindles and found that the two centrosomes in the center of the monopolar spindle are still arranged in a bipolar fashion with the two centrosomes organizing microtubules into a bipolar “mini spindle”. This “mini spindle” is surrounded by microtubules pointing radially outwards in an aster geometry. Kinetochore microtubules grow from chromosomes at the outer edge of the spindle towards the spindle center along the aster trajectories. The majority of the kinetochore microtubules stop at the boundary between the aster and the “mini spindle” domain wall and do not make direct contact with the centrosomes. Only kinetochore microtubules growing in the orientation of the pole-to-pole axis of this “mini- spindle” can make reliable contact to the centrosomes. This observation confirmed our previous finding that existing microtubules are crucial to guide kinetochore microtubules towards the centrosome and a disturbance of the nematic order will prevent them from growing further.

B180/P2573

NuMA can provide mechanical robustness to mitotic spindle poles independent of dynein

N. H. Cho[1,1], S. Dumont[1,1,2]; [1]University of California, San Francisco, San Francisco, CA, [2]Chan Zuckerberg Biohub, San Francisco, CA

Every cell division, the mitotic spindle builds itself to segregate chromosomes equally into two daughter cells. Together, both motor and non-motor proteins organize microtubules to give rise to spindle structure and function. One essential non-motor protein is NuMA, which recruits the motor dynein to microtubule minus-end cargos to assemble spindle poles and binds microtubules and oligomerizes independent of dynein *in vitro*. While it’s clear that spindles without NuMA or dynein have severely compromised function and mechanics, whether and how NuMA contributes to spindle mechanics independent of dynein is not known. To address this question, we generated NuMA mutants we

hypothesize cannot bind dynein based on sequence analysis. We show that overexpression of these mutants does not enrich dynein at spindle poles, unlike full-length NuMA overexpression. Further, these mutants are not sufficient to rescue spindle structure in NuMA-KO human RPE1 cells, consistent with them not recruiting dynein. However, we demonstrate that overexpressing these NuMA mutants is sufficient to mechanically reinforce spindles, preventing pole fracture under PDMS-based cell confinement while poles of control RPE1 spindles fracture. Thus, NuMA can reinforce the spindle independent of its motor partner dynein. We propose that NuMA not only recruits dynein to the spindle, but crosslinks spindle microtubules independent of dynein to increase spindle mechanical robustness. NuMA may as such play a unique physical role in the spindle, both targeting the motor dynein and crosslinking its microtubule cargos.

B181/P2574

Biophysical modeling of spindle dynamics inCaenorhabditis elegansembryos

Y. N. Young[1], R. Farhadifar[2], M. J. Shelley[3]; [1]NEW JERSEY Institute of Technology, Newark, NJ, [2]Flatironinstitute, New York, NY, [3]New York University/Flatironinstitute, New York, NY

In eukaryotic cells, the mitotic spindle forms during cell division and ultimately separates the chromosomes into the daughter cells. The position and orientation of the division plane, which is of fundamental importance for proper growth and development, are regulated by the spindle's position and orientation. Despite extensive knowledge about the molecular basis of spindle positioning and dynamics, the underlying force mechanisms remain elusive. Here, we developed a coarse-grained model of the spindle, which accounts for the dynamics of microtubules nucleating from centrosomes and their interactions with motor proteins localized on the cell cortex. We show that pulling forces from these motor proteins are enough to explain spindle positioning and elongation dynamics. Beyond a certain number of motors, the model exhibits an oscillatory behavior for the spindle, where the spindle axis rotates periodically. Our model quantitatively explains observed spindle dynamics in C. elegans embryos, such as elongation, asymmetric positioning, and oscillation. It also quantitatively predicts the scaling of these traits with cell size.

B182/P2575

Mechanism of spindle length control by the Tpxl-1AurA complex in the *C. elegans* embryo

J. L. Meaders, K. Oegema, A. Desai; University of California, San Diego, La Jolla, CA

A major regulator of spindle assembly is the conserved Aurora A Kinase (AurA) and its activator Tpx2. Tpx2-AurA is proposed to form an activity gradient that radiates out from the poles along spindle microtubules. Disrupting this gradient in the *C. elegans* embryo by depleting the Tpx2 ortholog TPXL-1 or preventing its interaction with AurA leads to abrupt spindle shortening immediately after nuclear envelope breakdown; the resulting spindles are extremely short and exhibit chromosome segregation defects. Importantly, spindle shortening is prevented by depletion of the conserved Ndc80 complex, the core kinetochore microtubule binding module, or by mutations that disrupt its ability to interact with microtubules. By contrast, loss of kinetochore dynein, removal of the Ska complex, or depletion of kinetochore-localized microtubule dynamics regulators did not suppress spindle shortening. These results suggest that the TPXL-1-AurA activity gradient serves as a molecular ruler that controls spindle length by determining whether end-on kinetochore-attached microtubules are in a polymerizing or depolymerizing state. How the TPXL-1-AurA activity gradient forms and regulates kinetochore-

microtubule attachments are open questions. Here, we tackle these questions using transgene-based replacements in the *C. elegans* embryo. Surprisingly, we find that disrupting the TPXL-1-AurA gradient by perturbing the TPXL-1-microtubule interaction does not result in the severe spindle shortening observed following TPXL-1 depletion. Unexpectedly, loss of the putative microtubule-interaction region led to a notable increase in the localization of TPXL-1-AurA to chromosomes. We hypothesize that this gain in chromosome localization may place TPXL-1-AurA in the vicinity of target substrates where it might cause kinetochores to assume a state conducive to polymerization of attached microtubules. We are currently testing this idea by identifying the region in TPXL-1 responsible for its chromosomal localization. In terms of targets of TPXL-1-AurA at the kinetochore, we are currently assessing whether TPXL-1-AurA could act directly on the Ndc80 complex. Overall, this effort is beginning to uncover the molecular mechanisms by which conserved Tpx2-AurA complexes control spindle assembly by regulating kinetochore-microtubule attachment dynamics.

B183/P2576

CAPS is required for chromosome attachment to the mitotic spindle

S. Spencer, B. Setu, S. Heiland, Q. Nie, P. Wombacher; Saint Louis University, Saint Louis, MO

In a screen for transcriptional targets of Epidermal Growth Factor Receptor signaling in Drosophila, our lab identified *CG10126*, a small calcium-binding protein orthologous to the human protein Calcyphosine. We find that both CG10126 and Calcyphosine show calcium-dependent binding to tubulin, and both are localized to kinetochore microtubules during mitosis. Knockdown of either gene (in *Drosophila* or human cells, respectively) produces defects in mitosis, most notably chromosomes that fail to congress during metaphase and the formation of multipolar spindles. In vitro assays suggest that CAPS cross-links and stabilizes microtubules to promote k-fiber formation. Further, coprecipitation assays suggest that CG10126 and CAPS associate with other proteins important for microtubule bundling. Our data suggests that CG10126 and CAPS play important and conserved roles in k-fiber formation and attachment of spindle microtubules to chromosomes during mitosis.

B184/P2577

Multiple Chromosome-Based Motors Regulate Spindle Length in *C. elegans* Oocytes

H. Horton, S. Wignall; Northwestern University, Evanston, IL

Female reproductive cells (oocytes) of most species form microtubule-based spindles that lack centrosomes, but the mechanisms by which these spindles assemble and mediate chromosome segregation are still poorly understood. Interestingly, *C. elegans* oocytes undergo chromosome congression and segregation independently of microtubule-kinetochore attachments; rather, microtubule bundles associate laterally with chromosomes to form “channels” that the chromosomes move through. Additionally, a ring-shaped protein complex made up of over fifteen known components forms around the center of each chromosome and is essential for spindle assembly as well as chromosome congression and segregation. How the ring complex (RC) interacts with the spindle in order to facilitate these microtubule-dependent functions is poorly understood.We previously found that KLP-19, a RC component and plus-end directed kinesin, is necessary for chromosome congression to the metaphase plate. Recent work using auxin-mediated depletion of KLP-19 indicates an additional role in spindle organization; specifically, we find that metaphase spindles become significantly longer when KLP-19 is depleted. Because other kinesin-4 family members have been proposed to pause microtubule

polymerization, we hypothesized that KLP-19 was performing a similar function on the meiotic spindle in order to regulate spindle length. To test this hypothesis, we generated an ATPase-defective KLP-19 mutant that binds to, but does not walk on, microtubules in order to generate an “over-paused” state. In this mutant, KLP-19 localizes broadly across the spindle microtubules and spindles are significantly shorter, indicating a role for KLP-19 in maintaining spindle length, possibly through microtubule pausing.Another kinesin, KLP-7, is a well-characterized microtubule depolymerase and RC protein that prevents spindle over-elongation; current investigation aims to identify the relationship between the spindle-length mediating functions of KLP-7 and KLP-19. Furthermore, we observe that depletion of KLP-7 on metaphase spindles results in the establishment of end-on kinetochore attachments. Altogether, we find that the RC components KLP-19 and KLP-7 each make unique mechanistic contributions to microtubule regulation at the chromosome, thus clarifying the function of this important protein complex in spindle architecture.

B185/P2578

Microtubule pivoting ensures passage of polar chromosomes across the centrosome required for timely alignment

V. Štimac, I. Koprivec, I. Tolić; Rudjer Boskovic Institute, Zagreb, Croatia

To ensure faithful mitosis, chromosomes located at different nuclear positions during nuclear envelope breakdown (NEB) need to properly congress to the metaphase plate. The most unfavorably positioned chromosomes are polar chromosomes, which are located behind the spindle pole at NEB and make up around 7 out 46 chromosomes in human cells. These chromosomes first need to approach the spindle pole and at a later point slide towards the metaphase plate in a motor-dependent manner. However, the mechanism of their passage across the polar region and its importance for faithful mitosis remain unknown. Here we show, by using live-cell confocal microscopy with high spatial and temporal resolution, that polar chromosomes are the last to reach the metaphase plate. They also stall behind the pole, indicating that the centrosome creates a physical barrier for their movement. To test this hypothesis, we removed one centrosome using centrinone, which indeed resolved the observed delay, suggesting the existence of an additional and distinct mechanism of congression in a polar region. We reveal that this mechanism is based on pivoting of chromosome-attached astral microtubules around the spindle pole, by imaging the microtubule plus end marker EB3. The angle that the kinetochores of these chromosomes form with the spindle axis changed during the period of rapid spindle elongation, indicating a role of centrosome separation in this process. By using different kinesin-5 (Eg5) inhibitors to stop or reverse spindle elongation, we confirmed that pivoting occurs due to a hydrodynamic drag force created by centrosome movement. Superresolution STED microscopy of astral microtubules and Mad2, a marker of unstable attachments, revealed that polar chromosomes are mostly laterally attached to one astral microtubule that emanates from the pole behind which they are situated, with other more complex attachments observed less frequently. Finally, we show that polar chromosomes cause aneuploidy in 62% of cases when the spindle checkpoint is weakened, highlighting their importance in faithful chromosome segregation. Altogether, we propose a model in which pivoting of microtubules around the spindle pole, driven by spindle elongation, promotes the movement of polar chromosomes towards the spindle body and consequently their proper congression to the spindle equator.

Centromeres

B186/P2579

Centromeric Chromatin Persists with No New Assembly in Oocytes over the Fertile Lifespan of Mice

A. Das[1], K. G. Boese[2], K. Tachibana[3], B. E. Black[4], M. A. Lampson[2]; [1]Biochemistry and Biophysics, Biology, University of Pennsylvania, Philadelphia, PA, [2]Biology, University of Pennsylvania, Philadelphia, PA, [3]Biochemistry, Max Planck Institute of Biochemistry, Munich, GERMANY, [4]Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA.

Centromeres direct genetic inheritance but are not themselves genetically encoded. Instead, centromeres are defined epigenetically by the presence of a histone H3 variant, CENP-A. New assembly of centromere chromatin is inhibited by cyclin dependent kinases and therefore restricted to G1 phase of the cell cycle. In somatic cells in culture, G1 assembly is sufficient to replenish centromere chromatin after dilution by partitioning between sister centromeres in S phase. Mammalian oocytes arrest in prophase I for months in mice or decades in humans without an opportunity for new assembly by the conventional G1 pathway. Two models have been proposed for maintaining centromere identity through this long arrest: (1) stability of CENP-A nucleosomes assembled before the arrest or (2) a specialized prophase assembly pathway that continually replenishes centromere chromatin during the arrest. To distinguish between these models, we inhibited assembly by conditional knockout of *Mis18a*, an essential assembly factor, early in prophase I. To confirm that genetic deletion of *Mis18a* fully removes the protein, we assessed the ability of zygotes from *Mis18a* knockout mothers compared to wild type mothers, to assemble tagged CENP-A at the first mitotic division where conventional CENP-A assembly would occur using maternal protein. We find that unlike wild type embryos, all *Mis18a* maternal knockout embryos fail to assemble CENP-A, indicating a complete absence of the protein. We find no effect of *Mis18a* deletion on either CENP-A chromatin levels in the oocyte or the ability of the oocyte to be fertilized by WT sperm, even after 6-8 months of aging. Thus, we conclude that centromere chromatin is maintained throughout the oocyte prophase arrest without new assembly consistent with the first model. This is in stark contrast to the other prominent H3 variant, H3.3, that requires continual replenishment. Our findings show that CENP-A nucleosomes assembled before meiosis are sufficient to support essential chromosome segregation functions in meiosis and centromere inheritance through the female germline.

B187/P2580

Modulating CENP-A Nucleosome Mobility Dictates Centromere Chromatin Accessibility

D. P. Melters[1], K. C. Neuman[2], T. Rakshit[3], Y. Dalal[1]; [1]National Cancer Institute, Bethesda, MD, [2]National Heart Lung and Blood Institute, Bethesda, MD, [3]Shiv Nadar University, Dadri, INDIA.

Histone variants fine-tune transcription, replication, DNA damage repair, and faithful chromosome segregation. Mechanical stress can induce transcription within seconds, yet whether and how nucleosome variants encode unique mechanical properties to their cognate chromatin structures remain elusive. One of the most divergent histone variants is the H3 variant CENP-A, which is functionally essential for faithful chromosome segregation and primarily localized to the centromere. Although CENP-A nucleosomes are buried in pericentric heterochromatin, centromeric chromatin remains transcriptionally active. This suggests that centromeric chromatin is readily accessible to the transcriptional machinery even when bound to kinetochore proteins. This puzzling dichotomy can be

explained either by intrinsic mechanical properties or by epigenetic alterations drive by chromatin effectors. We therefore first set out to determine the elasticity properties of CENP-A nucleosomes unbound or bound to inner kinetochore protein CENP-C. We found that CENP-A nucleosomes are twice as elastic compared to H3 nucleosomes. Upon binding of CENP-C, CENP-A nucleosomes rigidified three-fold. To understand how changing the mechanical properties of CENP-A nucleosomes impact chromatin dynamics, we used high-speed atomic force microscopy. With high-speed AFM, we can quantitatively track individual nucleosomes in the context of chromatin, similar to live cell imaging. A quantitative analysis of CENP-A chromatin showed that CENP-A nucleosomes are very mobile, with a diffusion coefficient consistent with numbers previously reported for H3 nucleosomes. Interestingly, CENP-C strongly restricted the diffusion coefficient of CENP-A nucleosomes, thus limiting nucleosome mobility. This observation correlated with clustering of CENP-A chromatin in vitro and in vivo. Furthermore, upon overexpressing CENP-C in vivo, we observed a loss of centromeric RNA polymerase 2 and centromeric transcription, as well as an almost 3-fold reduction in new CENP-A loading. Altogether, these data suggest a model in which the inner kinetochore proteins are critically involved in modulating material properties of CENP-A nucleosomes, which in turn fine-tunes chromatin accessibility and thus centromeric transcription required for new CENP-A loading. This model predicts that in cycling cells two populations of CENP-A exist: one population that binds the kinetochore and one population that creates an intrinsically open chromatin state permissive of transcription.

B188/P2581

Pairing of non-exchange centromeres in meiotic prophase promotes their subsequent segregation at anaphase I

B. Rapacz, J. Evatt, A. Sadli, D. Dawson; Department of Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK.

The homologous partners enter meiosis as separate chromosomes, but during meiotic prophase, the partners usually become connected by crossovers. These crossovers tether the partners together, which allows them, as a unit, to bi-orient on the meiotic spindle and segregate correctly in anaphase I. Nonetheless, in multiple organisms, when a single chromosome pair fails to experience a crossover (called non-exchange pair) the pair still segregates properly. This demonstrates that there are mechanisms, beyond crossing-over, that help homologous partners bi-orient on the spindle in meiosis. This study was undertaken to investigate one possible mechanism, the centromeric chromatin tether. Our previous work demonstrated that in budding yeast, the centromeres of non-exchange partners are paired in meiotic prophase. Here we show that this pairing is necessary to allow them to segregate from each other at anaphase I. Using live cell imaging experiments, we are exploring how meiotic prophase pairing promotes bi-orientation on the metaphase spindle. Our data suggest the model that in prophase the chromatin of the paired centromeres becomes tethered providing an inter-centromere bridge that allows cells to use a tension-sensing mechanism to bi-orient the partner centromeres. We hypothesize that cohesins link together chromatin loops from the paired centromeres in prophase. Our ongoing studies are testing this model.

B189/P2582

Evolutionary innovation in the CENP-T histone fold domain regulates centromere binding and kinetochore size

D. Dudka[1], K. Boese[1], A. L. Nguyen[2], B. Akins[1], B. E. Black[3,4,5], I. M. Cheeseman[2,6], M. A. Lampson[1,5]; [1]Department of Biology, School of Arts and Sciences, University of Pennsylvania, Phildalephia, PA, [2]Whitehead Institute for Biomedical Research, Cambridge, MA, [3]Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Phildalephia, PA, [4]Epigenetics Institute, University of Pennsylvania, Philadelphia, PA, [5]Penn Center for Genome Integrity, University of Pennsylvania, Philadelphia, PA, [6]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Centromeres direct chromosome segregation by building kinetochores to mediate attachments to spindle microtubules. Evolutionary theory suggests that centromere proteins innovate to reduce fitness costs associated with selfish centromere DNA sequences, which bias their segregation to the egg in female meiosis. There is experimental evidence for selfish centromeres, and molecular evolution analyses have identified recurrent amino acid changes in centromere proteins, but the functional impacts of these changes remain untested. Here, we show that the histone fold domain (HFD) of a key centromere scaffold protein, CENP-T, has evolved to reduce its binding to mouse centromeres. Reversing innovation by swapping a divergent HFD into mouse CENP-T increases recruitment to mouse centromeres in both mitosis and meiosis, dependent on rapidly evolving residues, and increases kinetochore size. A divergent CENP-T HFD also promotes localization to pericentromeres, suggesting innovation to restrict kinetochore assembly to specific centromere regions. We propose that microtubule attachment errors associated with larger kinetochores are a fitness cost of selfish centromeres, favoring innovation to limit kinetochore formation.

B190/P2583

FREEDA: a fully automated molecular evolution pipeline maps positive selection across rodent kinetochores and reveals functional divergence in CENP-O

D. Dudka[1], B. Akins[1], M. A. Lampson[1,2]; [1]Department of Biology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, [2]Penn Center for Genome Integrity, University of Pennsylvania, Philadelphia, PA.

Cell biology research typically focuses on functions of conserved protein domains. However, rapid protein divergence by positive selection often leads to crucial innovations whose detection requires specialized expertise in molecular evolution and computational biology. Here, we present a stand-alone, automated pipeline, FREEDA (Finder of Rapidly Evolving Exons in De novo Assemblies), for detecting positive selection in candidate proteins in frequently used vertebrate cell biology models: human, mouse and chicken. FREEDA takes advantage of the ever-growing number of non-annotated genome assemblies and streamlines the widely used molecular evolution and genomic tools, while providing a simple graphical user interface tailored for cell biologists without expertise in computational biology and molecular evolution. FREEDA displays regions under positive selection within the amino acid sequence and within 3D structural models predicted by AlphaFold. We provide a comprehensive map of positive selection across the rodent kinetochore including nearly 100 proteins. We further show that a key centromeric complex, CENP-OPQUR, evolves under positive selection across three different taxa: primates, rodents and birds, suggesting recurrent functional innovation. Leveraging natural variation, we

show that divergence of the CENP-O C-terminus regulates centromere binding. Overall, we demonstrate how molecular evolution analyses can reveal regulation of protein function, and we provide an accessible computational tool to guide cell biology research.

B191/P2584

A tale of two CENP-C's: Bridging the long prophase pause to support oocyte ploidy

J. Fellmeth[1], H. Sturm[2], J. K. Jang[3], N. Changela[3], K. S. McKim[3]; [1]Biology, Millersville University, Millersville, PA, [2]Rutgers University, Piscataway, NJ, [3]Biology, Rutgers University, Piscataway, NJ.

The centromere is an epigenetic mark that acts as a loading site for the kinetochore during meiosis and mitosis. This mark is characterized by an H3 variant known as CENP-A (CID in *Drosophila*) which replaces canonical H3 at the centromeres during G1 with the help of CENP-C and CAL1 (HJURP in mammals). Failure to re-establish a proper centromere after division, or failure to recruit an effective kinetochore during M-phase both can result in aneuploidy via chromosome segregation defects which is a hallmark of many cancers, infertility, and developmental disorders. CENP-C is a critical component of establishing both the centromere and kinetochore. During mitosis, these functions occur in quick succession. However, during female meiosis, these two functions are separated by a long prophase pause. This prophase pause is implicated in the increase in the occurrence of aneuploidies in women over the age of 34. Because CENP-C is one of the few components that links the centromere and kinetochore through the prophase pause, we have chosen to investigate this role using mutants and RNAi knockdown of CENP-C.
CENP-C that is incorporated into cells prior to onset of meiosis is involved in centromere establishment and CID recruitment, this population is also critical for establishing homolog synapsis via centromere pairing, establishment of SC and cohesins, and preventing centromeric crossovers. This population of CENP-C is stable and remains at the centromeres until diluted via chromosome division. We have identified another population of CENP-C that is dynamic during meiotic prophase and acts to recruit kinetochore proteins and promote chromosome segregation prior to metaphase I. This study shows that CENP-C function in meiosis provides kinetochore support and centromere support as it does in mitosis, but that these two functions are not linked. We propose that the dynamic nature of CENP-C during the long prophase pause is necessary to prevent errors in chromosome segregation

B192/P2585

HistoneH3/H4 chaperones prevent the mislocalization of CENP-A for chromosomal stability

R. Shrestha[1], V. Balachandran[1], A. Rossi[1], H. Kim[2], P. Vadlamani[3], C. Hammond[4], L. Ozbun[1], S. Lin[5], K. Cheng[5], R. Chari[6], G. Pegoraro[1], A. Groth[7], D. Foltz[3], M. Basrai[1]; [1]National Cancer Institute, NIH, Bethesda, MD, [2]Genetics Branch, NCI, NIH, Bethesda, MD, [3]Northwestern University, Chicago, IL, [4]Novo Nordisk Center for Protein Research, University of Copenhagen, Copenhegan, Denmark, [5]National Center for Advancing Translational Sciences, NIH, Bethesda, MD, [6]Genome Modification Core (GMC), Frederick National Lab for Cancer Research, NCI, Frederick, MD, [7]Novo Nordisk Center for Protein Research (CPR), University of Copenhagen, Copenhegan, Denmark

Unequal distribution of chromosomes into two daughter cells and/or structural rearrangements of the genome causes chromosomal instability (CIN) and aneuploidy that are observed in many cancers. One of the key determinants that prevents CIN is the structural integrity of centromeric chromatin that ensures faithful chromosome segregation. Restricting the localization of the evolutionarily conserved

centromeric histone H3 variant CENP-A to the centromeres is essential for chromosomal stability. Overexpression and mislocalization of CENP-A has been reported in many cancers and correlated with poor prognosis. Using cell biology assays and miFISH, we have previously shown that the mislocalization of overexpressed CENP-A to non- centromeric regions contributes to aneuploidy, CIN and karyotypic heterogeneity in cell lines and xenograft tissues. Therefore, it is important to define mechanisms that prevent mislocalization of CENP-A. Here we used an image based high-throughput genome-wide siRNA screen to identify gene depletions that increase nuclear CENP-A in HeLa cells. Amongst the top hits were components of NuA4 complex (EP400 and TRRAP), histone chaperones (CHAF1B, CHAF1A, and HIRA) and a member of the DnaJ family of heat shock proteins (DNAJC9). We focused on defining the roles of CHAF1B in preventing mislocalization of CENP-A. Our results showed an enrichment in protein levels of CENP-A, mislocalization of CENP-A and CIN phenotypes in CHAF1B depleted cells. Previous studies have shown that depletion of H3.3 chaperone DAXX suppresses mislocalization of overexpressed CENP-A. We observed increased expression of DAXX, but not other histone chaperones upon CHAF1B depletion. Consistent with these results, depletion of DAXX suppressed the mislocalization of CENP-A and CIN phenotypes in CHAF1B depleted cells. Based on our results, we propose that increased levels of DAXX promiscuously chaperone overexpressed CENP-A to non- centromeric regions, and this contributes to CIN upon CHAF1B depletion. In summary, we have identified factors that prevent CENP-A mislocalization and defined a role of CHAF1B in restricting the localization of CENP-A to centromeric chromatin to prevent CIN. These studies will advance our understanding for the causes and consequences of CIN in CENP-A overexpressing cancers.

B193/P2586

EWSR1 (Ewing Sarcoma Breakpoint Region 1) Maintains Centromere Identity

K. Kitagawa, R. Kitagawa, A. Becker, P. Houghton; Greehey Childrens Cancer Research Institute, UT Health San Antonio, San Antonio, TX.

The centromere is essential for ensuring high-fidelity transmission of chromosomes. CENP-A, the centromeric histone H3 variant, is thought to be the epigenetic mark of centromere identity. CENP-A deposition and maintenance at the centromere is crucial for proper centromere function and inheritance. Despite its importance, the precise mechanism of maintenance of CENP-A, i.e., the maintenance of centromere position, remains obscure. Here, we report a novel mechanism to maintain centromere identity. We found that CENP-A interacts with EWSR1 (Ewing Sarcoma Breakpoint Region 1) and EWSR1-FLI1 (the oncogenic fusion protein in Ewing sarcoma). EWSR1 is required for maintaining CENP-A at the centromere in interphase cells. EWSR1 and EWSR1-FLI1 bind to CENP-A through the SYGQ2 region within the prion-like domain, which is important for phase separation. EWSR1 binds to R-loops through its RNA-recognition motif (RRM) *in vitro*. Both domain/motif are required for maintaining CENP-A at the centromere. Therefore, we conclude that EWSR1 guards CENP-A in centromeric chromatins by binding to centromeric RNA. The fusion protein CENP-A-RRM localized at the centromere in EWSR1-depleted cells, supporting our conclusion.

B194/P2587

Constitutive methylation of budding yeast CENP-A contributes to chromosomal instability

P. K. Mishra[1], N. Ali[2], W. Au[1], L. Boeckmann[1], Y. Takahashi[1], P. Castineira[1], K. Bloom[3], P. Thorpe[2], M. Basrai[1]; [1]NCI, NIH, Bethesda, MD, [2]Queen Mary University, London, UNITED KINGDOM, [3]UNC, Chapel Hill, NC.

The centromere (CEN) is essential for high fidelity chromosome segregation as CEN dysfunction leads to chromosomal instability (CIN) and aneuploidy, the hallmark of cancer cells. CEN identity is specified epigenetically by specialized nucleosomes containing the evolutionarily conserved CEN-histone H3 variant (Cse4 in budding yeast, CENP-A in humans), which is essential for faithful chromosome segregation. However, the epigenetic mechanisms that regulate CENP-A function have not been clearly defined. We have recently identified post-translational modifications (PTMs) of budding yeast CENP-A such as phosphorylation, acetylation and methylation. In this study, we report that cell cycle dependent methylation of Cse4 regulates kinetochore structure-function and high fidelity chromosome segregation. We show that methylation of Cse4 is regulated by the cell cycle with the highest enrichment of methylated Cse4 observed in the G2/M but not in G1 cells. In agreement with these results, ChIP experiments uncovered high levels of methylated Cse4 at the CEN chromatin in the G2/M cells. A methyl-mimic cse4 mutant (cse4-R37F) shows reduced levels of CEN-associated kinetochore proteins, defects in structural integrity of the CEN chromatin, synthetic lethality with kinetochore mutants, and CIN phenotype suggesting that constitutive methylation of Cse4 is detrimental to faithful chromosome segregation. Our ongoing studies are focused on the identification and characterization of enzymes that contribute to methylation of Cse4. In summary, our results from budding yeast provide new insights into molecular mechanisms by which constitutive methylation of Cse4 contributes to CEN dysfunction and CIN.

B195/P2588

Probing centromere strength in the genesis of age-related oocyte aneuploidy

T. Lemonnier[1], M. Dumont[2], S. Redemann[3], D. Fachinetti[2], B. Mogessie[1]; [1]Yale University, New Haven, CT, [2]Institut Curie, Paris, France, [3]University of Virginia, Charlotteville, VA

Chromosome segregation errors are surprisingly common in mammalian oocyte meiosis. This generally results in egg aneuploidy, which is a leading cause of miscarriages, infertility and genetic disorders. Moreover, the incidence of egg aneuploidy increases dramatically with advancing maternal age. It has long been known that meiosis violates Mendel's law of random segregation as some chromosomes are more likely to be retained in the egg, and thus to be passed on to the progeny. Recent studies have shown that centromere strength (centromeric protein content) and spindle asymmetries in intrinsic spindle properties underpin this phenomenon of biased meiotic segregation. We are interested in understanding whether such bias also underlies egg aneuploidy, wherein some chromosomes are more predisposed to mis-segregation. We are therefore investigating existence of chromosome-specific aneuploidies and the molecular basis for biased chromosome mis-segregation in mammalian oocytes. By coupling techniques to specifically identify each chromosome with centromere protein content measurements and single cell sequencing, we seek to examine chromosome-specific segregation errors that contribute to high incidence of egg aneuploidy at advanced reproductive ages. Our preliminary data indicate that abundance of the centromeric protein CENP-A varies between chromosomes as well as between reproductively young and older female mice. Furthermore, targeted degradation of CENP-A in

oocytes of reproductively young animals induces chromosomal alignment defects. Our analyses of centromeric-DNA sequences such as minor satellites also revealed inter-chromosomal differences in young and aged mice. These initial data indicate existence of centromere strength discrepancies between oocyte chromosomes that could lead to chromosome-specific segregation errors in reproductively older females.

Cancer Therapy: Targeting Kinases 2

B197/P2589

ROR1 is negatively regulating CREB3L1 through activation of DNMT3A, in Triple-Negative breast cancer.

V. L. Reed, B. Peethambaran; Saint Joesph's University, Philadelphia, PA.

Triple Negative breast cancer (TNBC) is the most aggressive form of breast cancer. This disease lacks any cell-surface receptors used for therapeutic targets, such as estrogen, progesterone, or Human epidermal growth factor receptor 2 (HER2). Patients with TNBC have very limited therapeutic options besides chemotherapy, which often causes them undesirable side effects and creates a resistance to treatment. TNBC tumors are highly heterogeneous and contain many different types of CSCs, leading to increased epithelial to mesenchymal transition (EMT). Thus, CSCs are partially blamed for the high instance of chemotherapy resistance in TNBC patients. ROR1, a receptor tyrosine kinase, is thought to be associated with CSCs and to activate oncogenic pathways, such as PI3K/AKT. This gene is specifically expressed only during early fetal development and in cancerous tissues. TNBC patients that have high expression of ROR1 are found to have increased disease state and poor prognosis, compared to those with low ROR1 expression. Therefore, this protein is of high research interest to be investigated as a potential therapeutic target for TNBC. CREB3L1, a transcription factor that works as a tumor suppressor and is found to be associated with poor prognosis in TNBC patients, when expressed at low levels. This current study works to develop a novel molecular mechanism that explains how ROR1 is negatively regulating CREB3L1 in TNBC. We hypothesize that ROR1 is activating DNA methyltransferases, specifically DNMT3A, which epigenetically modifies CREB3L1 to reduce its expression in TNBC. Knocking down ROR1 shows that the protein level of CREB3L1 increases, while the protein level of DNMT3a decreases. This work will provide further understanding of the oncolytic properties associated with ROR1 in TNBC, helping to potentially form a basis for cell-targeted based therapies for TNBC patients.

B198/P2590

Novel ROR1 inhibitor CPD86 suppresses cell survival and migration in triple-negative breast cancer.

T. Ta, 1[1], S. R. R. Gupta[2], V. Reed[1], C. Scanlon[1], N. Fultang[1], B. Peethambaran[1]; [1]Biological Sciences, Saint Joseph's University, Philadelphia, PA, [2]Department of Zoology, University of Delhi, New Delhi, INDIA.

Breast cancer is the second most common cancer in women in the United States, with approximately 15-20% of new cases characterized as triple-negative breast cancer (TNBC), the most aggressive subtype. It is histologically characterized by the absence of hormone receptors that are typically targeted by hormone therapies such as Tamoxifen or Docetaxel, leading to difficulty in treatment. The standard of care for TNBC consists of traditional chemotherapy and radiation which are often highly toxic to both TNBC cells and normal cells. Therefore, it is critical to identify novel anti-cancer compounds that specifically target TNBC with minimal damage to normal tissues. It has been reported that receptor

orphan tyrosine kinase-like receptor 1 (ROR1) is an oncofetal protein overexpressed specifically in several human malignancies such as breast, lung, pancreatic and prostate cancers, but not in adult normal tissues. Blockade of ROR1 signaling has been shown to inhibit proliferation and induce apoptosis of cancer cells. Through *in silico* molecular docking, we characterized a novel compound that interacts strongly and inhibits ROR1 (hereafter referred to as **CPD86**). Our study aims to investigate the anti-TNBC effect of this compound both *in vitro* and *in vivo*. Preliminary evidence shows that **CPD86** suppresses cell migration and induces cell apoptosis at a half-maximal inhibitory concentration of approximately 2 μM toward MDA-MB-231 cells (TNBC cells with high ROR1 expression). We hypothesize that treatment with **CPD86** will increase apoptosis and decrease cell proliferation, migration, and invasion in TNBC cells, but not in MCF-10A cells (normal breast epithelial cells with no ROR1 expression). We will also test the efficacy of **CPD86** in inhibiting tumor growth in a cell-derived xenograft mice model of TNBC. ROR1 inhibitors could be of potential therapeutic approach for TNBC and the data would serve as proof-of-concept justification for their evaluation in other ROR1-upregulated cancers.

B199/P2591

Scamp3 Regulates EGFR and Modulates AKT and ERK Signaling Pathways

A. Acevedo-Díaz[1], B. Morales-Cabán[2], **I. J. Suarez-Arroyo**[2]; [1]Biology, University of Puerto Rico, Bayamon, PUERTO RICO, [2]Biochemistry, Universidad Central del Caribe-School of Medicine, Bayamon, PUERTO RICO.

Triple Negative Breast Cancer (TNBC) treatment development remains challenging due to disease heterogeneity and the absence of specific molecular targets. The secretory carrier membrane protein 3 (SCAMP3) is overexpressed and associated with poor prognosis in a variety of cancers and is identified as a regulator of epidermal growth factor receptor (EGFR). Accordingly, we aimed to investigate the association of SCAMP3 with the EGFR signaling pathway in TNBC. We hypothesize that SCAMP3 regulates EGFR degradation and induces EGFR signaling to promote TNBC. To assess how EGFR signaling is affected, we used our SUM-149 WT and SCAMP3 knockout models (SC3KO). Immunoblots showed that SCAMP3 knockout in SUM-149 abolished ERK1/2. Later, we inhibited EGFR activation with the tyrosine kinase inhibitor (TKI) erlotinib. EGFR inhibition reduced ERK phosphorylation in SCAMP3-expressing cells but was not affected in depleted cells. However, ERK phosphorylation increased compared to SCAMP3-depleted cells treated with vehicle. Interestingly, the treatment decreased AKT phosphorylation in the absence of SCAMP3. Furthermore, inhibition of EGFR did not affect STAT3. This result may suggest that SCAMP3 is involved in regulating the EGFR, AKT, and ERK pathways. Therefore, we sought to investigate the molecular mechanism behind the regulation of EGFR by SCAMP3. We examined the impact of SCAMP3 silencing on receptor degradation after EGF stimulation in a time-course experiment. Upon stimulation, the percentage of EGFR retained in the cell decreased in SCAMP3 knockout cells as early as 30 min showing an acceleration in the degradation kinetics of EGFR. In summary, depletion of SCAMP3 decreases ERK phosphorylation independently of its interaction with EGFR. Concurrently, the reduction of AKT phosphorylation depends on the downregulation of both. Furthermore, the absence of SCAMP3 accelerates the degradation of EGFR. We conclude that SCAMP3 contributes to cancer development through the regulation of multiple pathways and has the potential to be a target for breast cancer therapy.

B200/P2592

Silencing SCAMP3 Sensitizes TNBC Cells to EGFR Inhibitor

A. C. Acevedo-Diaz[1], **V. M. Hernandez-Cosme**[2], I. J. Suarez-Arroyo[2]; [1]Biochemistry, Universidad Central del Caribe, Bayamon, PUERTO RICO, [2]Biochemistry, Universidad Central De Caribe, Bayamon, PUERTO RICO.

Triple negative breast cancer (TNBC) is one of the most lethal subtype of breast cancer and patients with metastatic TNBC have a 5-year survival rate of 11%. These cancer cells are characterized by being negative for estrogen and progesterone receptors, and human epidermal growth factor receptor 2 (HER2). Nonetheless, there is a gap in knowledge regarding therapeutic agents that may work against TNBC. We have previously reported that secretory carrier-associated membrane protein 3 (SCAMP3) is highly expressed in TNBC cells and in breast cancer tissues. Preliminary studies showed that SCAMP3 is associated with epidermal growth factor receptor (EGFR) endocytosis. EGFR acts as a mechanism of transport of cancer-associated signaling proteins, like signal transducer and activator of transcription 3 (STAT3). EGFR and STAT3 have been associated with TNBC poor prognosis, metastasis, stem cell maintenance, and drug resistance. Our preliminary studies focus on identifying the effects of silencing SCAMP3 in association with the inhibition of EGFR or STAT3 using erlotinib and C188-9 inhibitors, respectively. To this end, we knocked out SCAMP3 in SUM-149 TN (inflammatory breast cancer (IBC) cells) and MBA-MB-468 (TN-non-IBC cells) using CRISPR-CAS9. MBA-MB-468 WT and SC3KO cells were treated with erlotinib or C188-9 with 1μM - 10μM for 72h. SUM-149 WT and SC3KO cells were treated with C188-9 for 72h. Then, cells were fixed with methanol and stained with propidium iodide, and fluorescent intensity was measured utilizing a spectrophotometer to assess cell viability after treatment. We found that MBA-MB-468 WT cells treated with C188-9 were sensitive (IC50 = 8.338 μM) to the treatment. While MBA-MB-468 SC3KO cells were resistant (IC50 = 13.62 μM) to C188-9. On the other hand, we confirmed, as stated in the literature, that MBA-MB-468 WT cells are resistant (IC50 = 10.14 μM) to erlotinib. Interestingly, SC3KO cells were sensitive (IC50 = 5.689 μM) to treatment. Furthermore, we found that SUM 149 WT and SC3KO cells were sensitive (WT: IC50 = 3.474 μM; SC3KO: IC50 = 3.477 μM) to C188-9. Altogether, our results suggest that knockout of SCAMP3 sensitizes cells to EGFR inhibitor and plays a key role in the cytotoxicity effect of STAT3 inhibitor. Interestingly, knockout of SCAMP3 in IBC cells had no effect on the toxicity associated with erlotinib in these cells. These findings could point out that targeting SCAMP3 is a promising treatment for those TNBC patients with resistance to EGFR inhibitors.

B201/P2593

Scamp3 knockout sensitizes triple negative breast cancer cells to erk inhibitory treatments

B. M. Morales-Cabán, I. Suarez-Arroyo; Biochemistry, University of Central Del Caribe, Bayamon, PUERTO RICO.

Triple-negative breast cancer (TNBC) is the most aggressive and deadly type of breast cancer and its lethality is due to the absence of estrogen and progesterone receptors and HER2). The absence of targeted therapies creates the need to identify molecular targets with the potential to serve as effective therapeutic agents. Increased expression of the secretory carrier membrane protein 3 (SCAMP3) was identified in breast cancer cells and tissues and it has been associated with the growth, progression, and survival of TNBC cells. Increased activation of the extracellular signal-regulated kinase (ERK) signaling pathway is closely related to tumor formation, progression, and metastasis in TNBC. We found that in

the absence of SCAMP3,ERK is inhibited and TNBC cells proliferation is reduced. Thus, we aim to elucidate the role of SCAMP3 in the regulation of ERK in TNBC development. To investigate how SCAMP3/ERK contributes to the TNBC cellular response, we knockout the expression of SCAMP3 (SC3-KO) in TNBC SUM-149 using the CRISPR/Cas9 technique and overexpressed (SC3-OE) SCAMP3 in non-tumorigenic epithelial cells MCF-10A. The cells were treated with MK-8353, an ERK inhibitor, at different concentrations (62nM - 64uM) to assess cell viability at 24, 48, and 72 hours. Our results show that SUM-149 SC3-KO were still sensitive to MK-8353 at 72h in comparison with WT. However, we observed the effect of treatment is time dependent in these cells (24h: WT: IC50=1.7µM, SC3-KO: 2.1µM; 48h: WT: IC50=1.3µM, SC3-KO: 1.5µM; 72h: WT: IC50=0.22µM, SC3-KO: 0.74 µM). On the other hand, MCF-10A SC3-OE showed a resistance to ERK inhibitor at early times (24h: control: IC50=11µM, SC3-OE=21µM; 48hcontrol: 0.170µM, SC3-OE=1.6µM) when compared to control cells. Interestingly, SC3-OE showed an increase cytotoxic effect at 72h control IC50=120nM, SC3-OE=600nM). To compare our results observed in TNBC cells, we used the HER2+ cell line, SUM-190 and its isogenic model of SCAMP3 knockout treated with MK-8353. Interestingly, we found similar results to SC3-OE cells 24h: WT: IC50=11.4µM, SC3-KO=27.9µM; 48h: WT: IC50=1µM, SC3-KO=2.8µM; 72h: WT: IC50=0.50 µM, SC3-KO=0.40 µM). Altogether, our results demonstrate that overexpression of SCAMP3 is associated with ERK inhibitor resistance as early times of exposure. Furthermore, is required longer exposure time to inhibitor to increase cytotoxic effects in HER2+ silenced cells in comparison to TNBC. Taken together, our study suggests that SCAMP3 plays a crucial role in the effectivity of ERK treatment

B202/P2594

Diminazene aceturate exhibits anti-tumor activities in human cervical carcinoma via inhibition of c-MYN oncogenic signaling

R. Gharbaran[1], L. Somenarain[1], S. Redenti[2]; [1]Biological Sciences, Bronx Community College/The City University of New York, Bronx, NY, [2]Biological Sciences, Lehman College/City University of New York, Bronx, NY.

The pro-protein convertase furin gain spotlight in the current COVID-19 pandemic. Recent structure-based analysis showed that diminazene (DIZE), an anti-parasitic drug, is a competitive inhibitor to furin. Here, we investigated anti-tumor activities of DIZE-induced inhibition of furin in human cervical carcinoma. Kaplan-Meier analyses on expression data retrieved from Human Genome Atlas showed that overexpression of furin mRNA expression is associated with shortened survival ($p<0.001$). Immunofluorescence studies confirmed the expression of furin in Hela cells, a well-studied cellular model of human cervical carcinoma. Water-soluble tetrazolium 1 (WST-1) cell proliferation assay showed DIZE caused dose-dependent decrease in growth of Hela cells after treatment for 48 h. The observe response was associated with transcriptional downregulation of cell proliferation markers Ki67 and PCNA. Acridine orange-ethidium bromide (AO-EtBr) dead-live assay revealed significant cell death associate DIZE. Staining with a caspase3/7-specific dye showed caspase activation and immunofluorescence showed increased caspase 3 activity in DIZE-treated cells. In addition, DIZE-treated cells showed transcriptional downregulation of furin and the oncogene c-MYC. These results suggest that DIZE maybe consider a candidate molecule for human cervical carcinoma therapeutics. However, further research is needed to evaluate its anti-tumor mechanisms.

B203/P2595

Phosphomevalonate kinase as novel target of radiosensitizer in lung cancer cells

S. Park, M. Kwon, S.-Y. Jeong; Asan Medical Center, Seoul, Korea, Republic of

Radiotherapy (RT) plays a crucial role in the local treatment of lung cancers. However, although RT locally targets and controls malignant lesions, due to RT-resistance, RT is still not an effective treatment for lung cancer. In this study, we identified the phosphomevalonate kinase (PMVK) as a novel radiosensitizing target and explored the underlying mechanism for the first time. Cell viability and survival fraction after RT were significantly decreased by PMVK knockdown in lung cancer cell lines. PMVK knockdown cells showed increased apoptosis, DNA damage and G2/M phase arrest after RT. DNA damage increased by siRNA PMVK plus RT was induce by inhibiting the DNA HR repair pathway via siRNA PMVK-induced RPA1 downregulation. Moreover, a stable shRNA PMVK mouse xenograft models verified *in vivo* radiosensitizing effects. Furthermore, PMVK expression was increased in lung cancer tissues and significantly correlated with patient survival and recurrence. Collectively, our results demonstrate that knockdown of PMVK enhances radiosensitivity in lung cancer through DNA damage repair pathway, suggesting that knockdown of PMVK may offer an effective therapeutic strategy to improve therapeutic efficacy of RT.

B204/P2596

Image-based phenotypic profiling of a chemogenomic screening library identifies novel druggable targets in the EGFR-pathway

K. Tanabe; Tokyo Women's Medical University, Tokyo, Japan

The gene encoding epidermal growth factor receptor (EGFR) is a major driver gene in cancer. Many drugs targeting EGFR-associated molecules have been developed, yet many have failed in clinical trials due to a lack of efficacy and/or unexpected side effects. In this study, I used image-based phenotypic profiling to screen a pharmacologically active compound library with the aim of identifying new druggable targets in the EGFR pathway. As anticipated, the phenotypic screen identified compounds that produce phenotypes resulting from targeting a known specific molecule or pathway. The assay also showed that compounds with diverse known mechanisms of action produced similar, EGFR-related cellular phenotypes. Biochemical assays revealed that those compounds share a previously unappreciated common target/pathway, showing that the image-based assay can identify new target molecules that are independent of the compound’s known target.

Cancer Therapy: Metabolism

B205/P2597

Hormetic effect of an Ethanolic Graviola Leaf Extract on HGF-1 cells viability.

C. Cassé; University of Puerto Rico, Mayagüez,PR, PR.

Plant-extracted compounds have been used for centuries in traditional pharmacopeia. Some of them have proven to be excellent drug alternatives for cancer treatment as they target metabolic pathways that are key to cancer cells such as apoptosis, energy-producing catabolic pathways, and the response to oxidative stress. Since some anticancer drugs have been shown to produce dose-dependent biologically opposite effects, it is crucial to determine the range of doses for which the compounds have maximum

therapeutical benefits. Annona muricata or Graviola is a tropical tree that is common in the Puerto Rican landscape. Although a plethora of studies conducted *in vitro* and *in vivo* studies have indeed reported that extracts prepared from the Graviola root, fruit, bark, and leaves possess antiproliferative activities in a large variety of cancer cells, the efficiency of Graviola extracts to curb the progression of head and neck cancers has been overlooked. Furthermore, the bioactivity of Graviola extracts on sane/non-cancerous cells has largely been ignored. The present work reports the *in vitro* antiproliferative/anticancer behavior of an ethanolic Graviola leaf extract on squamous cell carcinoma cell lines 9 and 25 vs. a sane/non-cancerous human gingival fibroblast cell line -1. Our results show that the Graviola extract induces cell death in the squamous cell carcinoma cell lines at all concentrations tested and a dose-dependent biphasic concentration-dependent/hormetic effect on the fibroblastic cells. This suggests that, at low doses, the phytochemicals present in the prepared Graviola extract could offer potential therapeutic avenues for curbing the progression of head and neck cancers.

B206/P2598

Versatile Metabolic Activities of Lupane-, Oleanane-, and Ursane- type Pentacyclic Triterpenes in Lung Cancer Cells

G. Torres[1], S. Estrada[2], D. Pérez[3], Y. Delgado[1]; [1]Biochemistry & Pharmacology Department, San Juan Bautista School of Medicine, Caguas, PR, [2]Biology Department, University of Puerto Rico-Cayey, Cayey, PR, [3]Neuroscience Department, Universidad Central del Caribe, Bayamón, PR.

In recent times, a great interest has been motivated on plant derived compounds known as phytochemicals. The pentacyclic oleanane-, ursane-, and lupane-type triterpenes are phytochemicals that exert significant activities against diseases like cancer. Lung cancer is the leading cause of cancer-related death worldwide. Although chemotherapy (eg. cisplatin) is the treatment of choice for lung cancer, its effectiveness is hampered by the dose-limiting toxic effects and chemoresistance. Herein, we investigated the metabolic activities of several pentacyclic triterpenes: oleanolic acid, ursolic acid, asiatic acid, betulinic acid, betulin, and lupeol on non-small cell lung carcinoma A549 cells. From our studies, we confirmed that all these triterpenes showed cytotoxic activities in the μM range after 24h of incubation. Then, we performed metabolic activity assays as DNA damage, cell cycle arrest, reactive oxygen species (ROS) production, caspase-3 activation, and induction of EGFR, MAPK, and Ki67 using flow cytometry. All the pentacyclic triterpenes showed to activate caspase-3 and ROS production. In addition, these triterpenes induced cell cycle arrest in S-phase and DNA double-strand breaks by activation of the histone H2A.X. Furthermore, the signaling of MAPK, EGFR, and Ki67 were activated in cells treated with the triterpenes confirming the cell cycle arrest and DNA damage. In summary, our results could change the way first- and second-line anticancer therapies are implemented and selected giving an important adjuvant role to these phytonutrients. These results demonstrate the important adjuvant role of these triterpenes and the great potential in the treatment of lung cancer.

B207/P2599

The metabolic activity of Deferasirox in combination with Doxorubicin chemotherapy against lung carcinoma

S. Estrada-Mojica[1], G. Torres[2], D. Pérez3[3], Y. Delgado[2]; [1]Biology Department, University of Puerto Rico-Cayey, Cayey, PR, [2]Biochemistry & Pharmacology Department, San Juan Bautista School of Medicine, Caguas, PR, [3]Neuroscience Department, Universidad Central del Caribe, Bayamón, PR.

In cancer cells, iron metabolism is commonly altered because of an increased dependence on iron compared with normal cells. Accumulating evidence suggests that iron overload is associated with lung cancer (LC). Doxorubicin (Doxo) is a naturally occurring anthracycline chemotherapy commonly used in LC. However, Doxo is not very effective in non-small cell lung carcinoma (NSCLC) type that represents approximately 85% of LC. Due to this, there is a need to develop alternatives to increase the efficacy of Doxo in NSCLC. Herein, we studied Deferasirox (Def), an FDA-approved iron chelator as a strategy for iron deprivation in combination with Doxo in NSCLC. Viability assay results showed that Def has synergistic cytotoxic effects in combination with low μM concentrations of Doxo after 24 h of incubation in A549 NSCLC cells. The metabolic activity of Doxo in combination with Def seems to involve downregulation of the PI3K, EGFR and MAPK. In addition, the combination of Doxo with Def induced cell cycle arrest in S-phase and DNA double-strand breaks. Iron deprivation induced by Def or other iron chelators in combination with chemotherapeutic drugs could thus help to improve the effectiveness of cancer therapy in NSCLC.

B208/P2600

Iron metabolism regulated via DMT1-dependent early endosome-mitochondria interactions is associated with metastatic capacity of invasive breast cancer cells

J. Barra, I. Crosbourne, J. M. Lamar, M. Barroso; Albany Medical College, Albany, NY

Iron translocation into mitochondria can be facilitated by transient and direct interactions between early endosome (EE) and mitochondria. We show that Divalent Metal Transporter 1 (DMT1) mediates mitochondrial iron translocation via EE-mitochondria interactions in invasive MDA-MB-231 breast cancer cells. DMT1 also regulates EE speed, labile iron pool (LIP) and mitophagy levels. Moreover, DMT1 silencing impaired both mitochondrial metabolism and invasion in MDA-MB-231. Re-expression of DMT1 in MDA-MB-231 DMT1-KO cells rescues all these phenotypes. Importantly in invasive breast carcinoma patient's tumor samples, DMT1 is upregulated and associated with lower overall free survival. Our results show that DMT1 bridges EE and mitochondria to support higher mitochondrial iron translocation and lower LIP levels, necessary for sustaining mitochondrial bioenergetics and invasive cancer cell migration. To explore DMT1's role in cancer related processes, we used both 3D multicellular spheroids and *in vivo* metastasis experiments. We found that DMT1 ablation disrupts the normal shape/morphology of MDA-MB-231 spheroids. Immunofluorescence showed a significant difference in EE-mitochondria association between periphery and center of spheroids upon DMT1 silencing. Surprisingly, DMT1 silencing does not affect the number of lung metastases but significantly increases their size. To characterize the role of DMT1 in metastatic growth, we are evaluating EE-mitochondria interactions in WT vs DMT1 KO metastatic tissue sections. Interestingly in metastatic cancer cell lines derived from MDA-MB-231, with tropism for brain (BrM2) and lung (LM2) tissues, DMT1 and transferrin receptor (TfR) decreases compared to parental cells. We observed that EE-mitochondria association is lower in BrM2 cells compared to its parental counterpart. Finally, spheroid morphology among the two

metastatic cell lines compared with the parental cells were drastically different, presenting some of them more cells at the periphery e.g., in BrM2, indicating a more invasive phenotype. YAP/TAZ are master regulators of metastatic growth. Silencing of YAP but not TAZ, positively regulates the levels of DMT1, ferroportin and ferritin. However, TfR was not affected by YAP/TAZ silencing. We hypothesize that the presence of DMT1 is critical for the invasive phenotype, but once metastatic cells reach their niche, DMT1 may need to be downregulated for efficient metastatic growth. The characterization of iron metabolism regulatory mechanisms and its difference between parental and metastatic cells using 3D cell culture and *in vivo* experiments, should provide important insights into both metastatic cellular mechanisms and new possibilities for therapies against metastatic growth.

B209/P2601

Modulating Arginine Metabolism in Breast Tumor Microenvironment via Sepiapterin - A Novel Immunotherapeutic Strategy for Breast Cancer

V. R. Fernando, V. Sharma, X. Zheng, S. Furuta; University of Toledo, Toledo, OH.

The immunosuppressive nature of the breast tumor microenvironment (TME) often renders immunotherapy moderately responsive. Current approaches employing cytokines to induce immunostimulation in breast TME fail to move towards clinical trials due to their systemic toxicity. Hence there is a critical need for a safe strategy that can improve the immunogenicity of breast TME. We aimed to address this by targeting the arginine metabolism to induce an immunogenic shift in breast TME. Tumor cells and tumor-associated macrophages (TAMs) utilize arginine metabolism to influence the immunosuppressive milieu by producing polyamines that promote tumor progression over nitric oxide (NO) that exert anti-tumor activities. Such differential arginine utilization is due to the reduced biosynthesis of tetrahydrobiopterin (BH4), the essential cofactor of NO synthase (NOS), in malignant tumors and immunosuppressive M2-TAMs. Our objective was to redirect the arginine metabolism in breast TME towards NO production to induce a strong immunostimulatory shift by supplementing sepiapterin, the endogenous precursor of BH4. Using *in vitro*, *ex vivo* and *in vivo* models of breast TME, we studied the effect of sepiapterin treatment on both tumor cells and M2-TAMs. Employing a combination of ELISA, flow cytometry and imaging techniques, we evaluated sepiapterin-induced changes in arginine metabolism, immune responses and tumor growth. Our *in vitro* and *ex vivo* results show that sepiapterin treatment significantly reduced polyamine levels while increasing NO levels in both tumor cells and M2-TAMs. The treatment also led to the reprogramming of M2-TAMs to immunostimulatory M1-TAMs. Furthermore, sepiapterin-treated *ex vivo* and *in vivo* tumors showed a significant reduction in tumor epithelia and tumor size. Interestingly the expression of immune checkpoint ligand, PD-L1 was abrogated in sepiapterin-treated tumors via inactivation of STAT3. In conclusion, our findings provide the first evidence that sepiapterin supplementation can be employed as a safe and potent immunotherapeutic strategy for breast cancer.

B210/P2602

The anti-tumorigenic mechanisms of 4R cembranoid in non-small cell lung carcinoma

J. G. Cirino, I. Suarez, A. Acevedo, N. Sabeva, Y. Ferrer; Universidad Central Del Caribe, Bayamon, Puerto Rico

Eighty-four percent of lung cancers are classified as non-small cell lung carcinomas (NSCLC), with an average 5-year survival rate of 26% due to less sensitivity to traditional treatments such as radiation and

chemotherapy. Chemotherapies such as cisplatin and carboplatin are platinum-based and cause adverse effects on non-cancerous healthy cells. Therefore, the need for non-toxic treatments is crucial for a better prognosis in patients. In our search for these anticancer agents, our group tested and demonstrated that (1*S*,2*E*,4R,6*R*,-7*E*,11*E*)-2,7,11- cembratriene-4,6-diol (4R), a cembranoid extracted from *Nicotiana tabacum*, is non-toxic and has anti-proliferative and anti-tumorigenic activity in NSCLC. 4R has been shown to negatively modulate the alpha 7 nicotinic acetylcholine receptor (α7nAChR), which induces signaling that promotes cellular proliferation and survival. Our studies aim to elucidate the specific mechanism of 4R's anti-tumorigenic effect in NSCLC. Based on our data, we hypothesize that 4R, as a negative modulator of nicotinic signaling, will help inhibit molecular cascades involved in survival and proliferation while also inducing apoptosis. To do this, we first detected the levels of the α7nAChR in NSCLC cell lines compared to primary normal lung cells (MRC5) by Western blot (WB). Results showed significant overexpression of the α7nAChR in A549 and Lewis Lung Carcinoma (LLC) lung cancer cell lines when compared to MRC5 ($P<0.0001$). We then evaluated the α7nAChR negative modulator LYNX-1, in LLC cells. Results demonstrated that when treated with 4R, LYNX-1 expression significantly increases compared to its vehicle (*$p<0.0383$). We also studied Akt expression and activation, a key protein kinase for cell survival downstream of α7nAChR, by WB in LLC. Results demonstrated that 4R-treated cells significantly decreased phospho-S473 and total Akt (**$p<0.0078$ and **$p<0.0061$, respectively). Furthermore, we studied the pro-apoptotic protein BAD and observed a significant decrease in phosphor-S112 when treated with 4R (*$p<0.0453$). In summary, our results suggest that in NSCLC, 4R negatively regulates α7nAChR signaling by upregulation of LYNX-1. This modulation decreases the activation of pro-survival pathways and promotes the activation of pro-apoptotic proteins such as BAD. Thus, we propose that 4R could potentially serve as a novel, non-toxic alternative or adjuvant treatment against NSCLC.

B211/P2603

Targeting of 7- or 24-dehydrocholesterol reductase decreased cancer progression in head and neck cancer.

B.-S. Lee, J. Padilla, K. D. Brock, S. Choi, J. Lee[,1]; The George Washington University, Washington, DC

The elevated expression of cholesterol metabolism has been associated with many cancer including head and neck squamous cell carcinoma (HNSCC). However, the molecular mechanisms between cholesterol biosynthesis and head and neck cancer have not yet been studied. In this study, we identified upregulation of dehydrocholesterol reductase such as DHCR7 or DHCR24 in HNSCC compared with adjacent normal tissues from the same patients using RNA sequencing data. We observed protein expression level of DHCR7 or DHCR24 in head and neck cancer cell lines through Western blotting. We also checked high expression of DHCR7 or DHCR24 in HNSCC tissues from patients using immunohistochemistry. Yet, when DHCR7 or DHCR24 was knocked down using siRNA, the viability of HNSCC cells was decreased in MTT assays. In parallel, we adapted small inhibitory drugs; AY9944 for DHCR7 inhibition and Triparanol or SH-42 for DHCR24 inhibition. Treatment of AY9944, Triparanol, or SH-42 also resulted in decreased viability of HNSCC cells. Moreover, when we used a cholesterol blocker, MCD, the cell viability was decreased, whereas the cells overcame cell death by adding the combination of cholesterol and LDL. Taken together, we concluded that head and neck cancer requires DHCR7 and DHCR24 activity involved in cholesterol metabolism for cancer growth. Our data suggest that targeting DHCR7 or DHCR24 using AY9944, Triparanol or SH-42 is one of the potential strategies that will definitely help with cancer treatment for head and neck cancer.

B212/P2604

Viperin-Induced Metabolic Reprogramming and Cancer Progression

J. J. Kim, K. M. Choi, J. H. Yoo, K. S. Kim, Y. Gu, J. Y. Seo; Yonsei University College of Medicine, Seoul, Korea, Republic of

Viperin is a multifunctional interferon (IFN)-inducible protein that possesses antiviral properties, mediates signaling pathways, and regulates cellular metabolism. Recent studies have shown that viperin increases lipogenesis and glycolysis *via* inhibition of fatty acid β-oxidation in mitochondria, suggesting that its function can be exploited to drive metabolic alteration of cancer cells. Here, we show that viperin plays a critical role in cancer metabolism. Analyses of human tissue microarray and data from the cancer genome atlas reveal that viperin is inversely correlated with the survival rate of cancer patients. We screened viperin expression in various cancer cell lines and generated viperin knockdown or stably expressing cell lines. Using these cell lines, we demonstrated that viperin promotes glycolysis and lipogenesis in cancer cells, resulting in enhancement of cancer progression. Additionally, viperin expression is upregulated by the deficiency of fatty acids and oxygen and by the production of IFNs *via* the PI3K/AKT/mTOR/HIF-1αand JAK/STAT pathways, respectively. Therefore, these results indicate that viperin drives metabolic reprogramming and cancer progression, suggesting that this protein might be a potential target in the development of anticancer therapeutics.

Extracellular Matrix and the Cytoskeleton in Cancer

B213/P2605

Depletion of Rnd3 in lung cancer decreases both invasion and migration in a ROCK1 independent manner

N. Garcia Garcia[1], D. Richey[1], C. Ashby[2], **K. R. Ryan**[1]; [1]Biochemistry and Molecular Biology, University of Arkansas Medical Sci, Little Rock, AR, [2]Biomedical Informatics, University of Arkansas Medical Sci, Little Rock, AR.

Background:Lung cancer is one of the most common cancer types, with a 5-year relative survival rate of ~21%. Most lung cancers metastasize resulting poor prognosis. Expression levels of Rnd3 have been linked to several cancers and Rnd3 is involved in the regulation of several cellular processes which are commonly dysregulated in cancer. Objective:Investigate the effect low Rnd3 expression has on lung adenocarcinoma patient survival and utilize a proteomic approach to study the effect of Rnd3 knockdown in the lung adenocarcinoma cell line, A549 cells. Methods:Analyze TCGA survival data of lung adenocarcinoma patients to generate Kaplan-Meier survival plots for patients expressing low Rnd3 compared to high Rnd3 expression. Perform tandem mass tag mass spectrometry on Rnd3 knockdown (low) compared to control Rnd3 expressing (high) A549 cells to generate global protein profiles. Use Ingenuity Pathway Analysis (IPA) to identify pathways and processes altered in the low Rnd3 expressing cells compared to control cells. Validate the predicted findings *in vitro* using transient Rnd3 knockdowns and identify signal pathways involved in the regulation of these processes using inhibitors and double knockdowns. Results:Our analysis of TCGA survival data of lung adenocarcinoma patients shows that patients expressing low levels of Rnd3 expression have significantly higher survival probability rates compared to patients expressing high levels of Rnd3. We utilized proteomics to generate global protein expression profiles of Rnd3 expressing (high) and Rnd3 knockdown (low) A549 cells, to gain insight into

how low Rnd3 expression maybe beneficial to patient survival. Then used IPA which predicted low Rnd3 expression decreases cell migration and invasion, two hallmarks of metastasis. We validated these predictions *in vitro* by knocking down Rnd3 expression in A549 cells, which significantly decreased both cell migration and invasion compared to control A549 cells. We also showed that Rnd3 regulation of both cell migration and invasion was independent of ROCK1 signaling, a known regulator of Rnd3, indicating a novel pathway. Summary:Our data has substantiated the role of Rnd3's involvement in lung cancer cell migration and invasion and identified a new pathway involved in Rnd3 regulation, which may lead to identifying key regulators and new therapeutic targets of lung cancer metastasis.

B214/P2606

Ezrin Asserts its Roles on Osteosarcoma Metastasis Through Interacting with RNAs

E. Deniz[1], T. Shaw[1], P. Tiwari[1], L. Penalva[2], J. Toretsky[1], A. Uren[1]; [1]Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, [2]UT Health San Antonio, Children's Cancer Research Institute, San Antonio, TX.

Osteosarcoma (OS) is the most frequent primary bone cancer of children and adolescents. Most patients exhibit microscopic pulmonary metastases during initial diagnosis. In cases of clinical metastases, prognosis is worse. Therefore, besides treating primary tumor, patients need interventions to cease metastasis. Ezrin is a cytoskeletal linker that bridges F-actin to membrane. It is essential in maintaining fundamental cellular processes including shape, polarity, adhesion and motility of cells. Its levels correlate with OS grades and homing of metastatic OS in lung depends on ezrin as shown by xenograph and allograph models.

We identified a compound, NSC305787, that directly binds to and interferes with the functions of ezrin. We investigated the underlying molecular mechanisms by multiple genome wide approaches. Principal component (PC) analysis on the transcriptomic patterns in ezrin high (K7M2) and low (K12) cells in response to NSC305787 treatment clearly segregated the two different cells by PC1 depending on their ezrin levels and only K7M2 cells by PC2 depending on the compound treatment, showing a striking selective effect of NSC305787 on ezrin. Proteomic analysis listed differential abundancy of proteins in response to ezrin levels and upon NSC305787 treatment. Work with surface plasmon resonance proved that ezrin has a tendency to directly bind to RNA, specifically towards RNAs containing G-quadruplexes. Hence, we immunoprecipitated ezrin from cells and identified co-precipitated RNAs. Integrated analysis of such data pointed out ezrin-bound RNAs, whose levels as RNA or as protein product changed in response to the inhibition of ezrin with NSC305787. The list includes genes previously associated with migration and cytoskeleton properties. Additionally, ezrin high cells resisted mechanically stressed growth conditions and proliferated at comparable rates with or without attachment to solid surface, whereas, ezrin low cells were dependent on solid attachment for proliferation. Lastly, we created ezrin null cells to study its molecular functions in detail.

In conclusion, we hypothesize that ezrin has diverse functions including binding to RNA in addition to its canonical roles on maintaining cytoskeleton. Precise definitions of molecular mechanisms of metastasis will led us develop better intervention modalities to cease metastasis in osteosarcoma.

B215/P2607

SNED1: A novel regulator of ECM organization

D. Pally, M. Davis, F. Lee, A. Naba; University of Illinois At Chicago, Chicago, IL.

The extracellular matrix (ECM) is a complex meshwork of proteins that plays critical roles in health and disease. In the context of cancer, the ECM acts either as a barrier or as a permissive microenvironmental cue to the growth and dissemination of cancer cells. Using a proteomic approach to profile the ECM composition of tumor microenvironment, my mentor, Dr. Naba, identified a novel ECM protein, SNED1 and established, for the first time, its role as breast cancer metastasis promoter. Histological characterization of *SNED1* knockdown tumors revealed the presence of a thick ECM layer surrounding the tumors and were less invasive. This suggests that SNED1 may regulate breast cancer metastasis by contributing to remodeling the tumor ECM and making the tumor microenvironment conducive for cancer cell invasion. Cell-derived matrices (CDMs) are an excellent model to study ECM organization since it recapitulates many biochemical and biophysical properties of *in vivo* ECMs. To test the role of SNED1 on ECM architecture, we have generated *Sned1*^{KO} mouse embryonic fibroblasts (MEFs) that can be engineered to re-express SNED1 and obtain CDMs. We observed that lack of SNED1 in CDMs resulted in poorly aligned ECM as quantified by fibronectin and collagen I fiber orientation, while overexpression of *SNED1* resulted in aligned fibronectin and collagen I fibers. We further observed that SNED1 co-localizes with fibronectin and collagen I fibers suggesting that SNED1 might mediate ECM organization by interacting with these two abundant structural ECM proteins. Further investigations to understand SNED1-collagen I interactions revealed that SNED1 does not affect collagen I fibril formation kinetics but it decreased collagen I fiber density as observed using confocal reflectance microscopy. Taken together, our results suggest that SNED1 plays an active role in shaping ECM architecture by interacting with other ECM proteins. Future work will focus on identifying the mechanisms by which SNED1-dependent ECM remodeling may affect cancer cell invasion, a key event during cancer metastasis.

B216/P2608

Cells in hybrid Epithelial-to-Mesenchymal Transition state can induce invasivity of surrounding epithelial cells

J. Geay[1,2], S. Seetharaman[3], L. Blanchoin[4,1], M. Théry[1,2,5]; [1]CytoMorpho Lab, CEA/CNRS/Unité de Thérapie Cellulaire, Hôpital Saint Louis, Paris, FRANCE, [2]Institut Pierre Gilles de Gennes, Paris, FRANCE, [3]James Frank Institute, Department of Physics, University of Chicago, Chicago, IL, [4]CytoMorpho Lab, Interdisciplinary Research Institute of Grenoble, CEA/CNRS/Université Grenoble Alpes, Grenoble, FRANCE, [5]CytoMorpho Lab, Laboratoire de Physiologie Cellulaire et Végétale, Interdisciplinary Research Institute of Grenoble, CEA/CNRS/Université Grenoble Alpes,, Grenoble, FRANCE.

The epithelial-to-mesenchymal transition (EMT) is an extensively studied phenomenon in the context of cancer and metastasis. Over the last few years, the existence of a hybrid intermediate state of EMT has been described, where cells keep some epithelial markers, but also gain some mesenchymal markers. This particular state has been suggested to promote invasiveness, tumor progression and metastasis. A recent study showed that the loss of FAT1, which is a highly mutated protocadherin in cancers, led to a hybrid state of EMT, and induced invasion and metastasis. However, the mechanisms of this hybrid state induction remain elusive. In skin squamous carcinoma cells, using a combination of cell biology and biophysical approaches, we show that the knockout of FAT1 displays reversed cell polarity, a main characteristic of an EMT induction. Moreover, the loss of FAT1 leads to drastic remodelling of the

actomyosin network and a reduction in traction forces. Surprisingly, we found that FAT1 KO did not display remarkable migration properties, but instead increases the invasion capacities of adjacent wild type cells. In addition, WT cells exposed to conditioned medium of FAT1 KO culture exhibit a less contractile phenotype, similar to that of KO cells and increased invasive capacities in 3D migration assay . Thus, we propose a mechanism by which FAT1 mutated cells in tumors, through their hybrid state of EMT, impart invasive properties to non-mutated cells, and thereby promote extensive metastasis.

B217/P2609

Invadopodia formation is spatially and functionally coupled to the nuclear envelope by septin 9

J. Okletey[1], T. Jones[1], C. Montagna[2], E. Spiliotis[1]; [1]Biology, Drexel University, Philadelphia, PA, [2]Radiation Oncology, Rutgers University, New Brunswick, NJ.

Metastatic cancer cells invade through the extracellular matrix (ECM) of tissues by forming invadopodia, actin-rich protrusions which secrete matrix metalloproteinases (MMPs). Invadopodia formation is stimulated by growth factors (e.g., epidermal growth factor; EGF) and requires membrane recruitment of the scaffold protein TKS5, cortactin and other actin regulators. Invadopodia formation takes place preferentially at plasma membrane regions around the nucleus, and previous work has indicated that invadopodia physically interact with the nuclear envelope. It is poorly understood, however, how invadopodia formation is spatially controlled. Here, we show that invadopodia formation requires septin 9 (SEPT9), a member of the septin family of GTPases that is overexpressed in breast cancer, and enhances cancer migration and invasion. In the highly invasive breast cancer MDA-MB-231 cells, SEPT9 isoform 1 (SEPT9_i1) localizes to domains of the nuclear rim and along the ventral surface of the nuclear envelope. SEPT9_i1 is also enriched at base and along invadopodia, which form in 2D gelatin degradation and 3D chemoinvasion assays. Depletion of SEPT9_i1 alters the morphology of nuclei, which become ruffled and indented. This phenotype is accompanied with a significant reduction in the number and length of invadopodia, and a loss in the spatial bias in the localization of invadopodia around the nucleus at steady state and upon stimulation with EGF. We show that depletion of SEPT9_i1, but not SEPT9_i2, results in dramatic reduction of TKS5, cortactin and pTyr421-cortactin clusters at the ventral cell membrane and impairs the degradative and invasive abilities of MDA-MB-231 cells in gelatin degradation and transwell chemoinvasion assays. In SEPT9-depleted cells, TKS5 and Cortactin are diffusely mislocalized about the nuclear envelope and/or abnormally localize to perinuclear aggregates, with MT1-MMP (MMP-14) shifting from perinuclear-cytoplasm to peripheral edges - suggesting enrichment in peripheral focal adhesion sites. Taken together, these data suggest that the machinery of invadopodia formation is coupled to the nuclear envelope by a septin-mediated mechanism that spatially biases ECM degradation to the perinuclear over peripheral regions of the cell membrane, which enables the cancer cells to thread the nucleus through the ECM during metastasis.

B218/P2610

Modulation of ASAP1 in FN-RMS cells accelerates tumor progression and metastasis as revealed by intravital microscopy

S. M. Hammoudeh, Y. Ng, M. P. Yadav, H. Yoon, M. E. Yohe, R. Weigert, P. A. Randazzo; National Cancer Institute, NIH, Bethesda, MD.

ASAP1 is a multidomain ADP-ribosylation factor (Arf) GTPase-activating protein (GAP) involved in the regulation of the actin cytoskeleton, focal adhesions dynamics and receptor tyrosine kinases trafficking.

ASAP1 expression levels correlate with progression of solid tumors (e.g. breast, colorectal, pancreatic, ovarian, gastric, and prostate cancer). The first evidence for a role of ASAP1 in cancer progression was the discovery that the amplification of ASAP1 gene on chromosome 8 correlates with poor prognosis in uveal melanoma. A similar amplification of chromosome 8 has been reported in ~72% of fusion negative rhabdomyosarcoma (FN-RMS) tumors, suggesting a potential role for ASAP1 signaling in the progression of FN-RMS. To determine the function of ASAP1 in tumor growth, invasion and metastasis, we used a tongue orthotopic xenograft of FN-RMS which recapitulates physiologically relevant tumor progression and metastasis to lymph nodes and lungs, two of the common metastatic sites in patients. Using intravital microscopy, we observed that injected ASAP1-expressing control FN-RMS cells fused into myofibril-like structures, exhibited dynamic branching, and invaded the local tongue muscle. On the other hand, ASAP1-depleted FN-RMS cells initially exhibited an accelerated growth of the primary tumor and a reduction in the intercellular fusion as compared to the control group. Notably, at later stages, ASAP1 depletion led to a decrease in the size of the primary tumors but increased the rate and growth of early metastases within the tongue and in locoregional lymph nodes resulting in a shorter survival of the mice in comparison to those with ASAP1-expressing FN-RMS tumors. While further investigation is required into the cellular and molecular mechanisms, these results suggest a role for ASAP1 in controlling cell behaviors that determine whether cells survive at the primary site with local invasion or intravasate and metastasize.

B219/P2611

Novel γ-Ionizing radiation-induced RIP1-IL1 β axis promotes migration and invasion of non-small cell lung cancer cells

A. Kang, J. Kwon, **J. Park**; KIRAMS, SEOUL, KOREA, REPUBLIC OF.

We demonstrated the involvement of a novel intracellular signaling mechanism in γ-ionizing radiation (IR)-induced migration/invasion of non small cell lung cancer (NSCLC) cells. Initially, we detected IR enhanced expressions of receptor-interacting protein (RIP) 1 in A549, one of NSCLC cell lines. Next, we found intracellular signaling of RIP1-including mechanism could induce epithelial-mesenchymal transition (EMT). IR-induced RIP1 promote invasion/migration of NSCLC cells by enhancing of the expression/activity of matrix metalloproteases (MMP-2 and MMP-9) and vimentin. We also found RIP1 is located downstream of EGFR and sequentially induces Src-STAT3-EMT signaling to promote invasion/migration. Blockage of RIP1 kinase activity and expression using specific pharmaceutical inhibitor and RIP1 siRNA suppressed the levels and activities of MMP-2, MMP-9, and vimentin followed by decrease of invasion/migration. Next, we found IR-induced RIP1 also induced IL-1β expression associated with stimulation of the transcriptional factor NF-κB. IR could trigger NF-κB activation, and inhibition of NF-κB suppressed IR-induced RIP1 expression and invasion/migration as well as EMT. Additionally, treatments of siRNA and specific pharmaceutical inhibitors of RIP1 and NF-κB suppressed protein expression and secreted concentration of IL-1β in condition of IR treatment. Treatment of IL-1Ra, an antagonist protein of IL-1β, suppressed IR-induced epithelial-mesenchymal transition (EMT) followed by increase of invasion/migration *in vitro*. We also demonstrated IR could induce IL-1β expression *in vivo* and, additionally, IL-1 Receptor (R) I/II, receptors of IL-1β , *in vitro/in vivo*. Taken together, we suggest novel IR-induced cell migration/invasion machinery that IR- EGFR/ RIP1-Src/STAT3-EMT and IR-NF-κB - RIP1 - IL-1β - IL-1RI/II - EMT pathways.

B220/P2612

Adhesion Strength of Disseminating Cells Predicts Severity of Metastatic Disease

K. G. Birmingham[1], B. Yeoman[1], M. Kane[1], P. Beri[1], J. Tuler[1], I. L. Williams[2], A. Kumar[1], S. Klein[1], P. Katira[1], **A. J. Engler**[1]; [1]University of California, San Diego, La Jolla, CA, [2]Rice University, Houston, TX

Despite better outcomes with early-stage detection, local invasion significantly reduces patient survival rates for many carcinomas. Heterogeneity within and between tumors has precluded identification of predictive biological markers, but adhesion strength has emerged as a potential biophysical marker. Here we demonstrate that cells disseminating from mammary tumors are weakly adherent, and when presorted by adhesion, primary tumors created from strongly adherent cells exhibit fewer lung metastases than weakly adherent cells or unsorted populations. Migratory ontologies from tumors correlate with freshly sorted cells, suggesting that cell intrinsic differences are maintained *in vivo*. We further demonstrate that admixed cancer lines can be separated by label-free adhesive signatures using a next-generation flow chamber. When applied to metastatic tumors, the device retrospectively predicted metastatic disease from stromal samples with 100% specificity, 85% sensitivity, and AUC of 0.94. Data from this device suggest that label-free adhesive signatures may effectively predict clinical outcomes in patients.

B221/P2613

Loss-of-function of AWP1 Induces the Epithelial-mesenchymal Transition of Prostate Cancer Cells

Y. Hwang, M. Lee, E. Kim, J. Kim, D. Yoon, H. Min, Y. Sung, E. Chang; University of Ulsan College Med, Seoul, KOREA, REPUBLIC OF.

Loss-of-function of AWP1 Induces the Epithelial-mesenchymal Transition of Prostate Cancer Cells

Yumi Hwang[1,2], Myungjin Lee[1,2], Eun-Young Kim[1,2], Ji-Eun Kim[1,2], Dohee Yoon[1,2], Hyo-Jin Min[1,2], Yoolim Sung[1,2], Eun-Ju Chang[1,2,3]*

[1]Department of Biomedical Sciences, AMIST, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea.[2]Stem cell Immunomodulation Research Center, AMISST University of Ulsan College of Medicine, Asan Medical Center, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 0550, Korea.[3]Department of Biochemistry and Molecular Biology, AMIST, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea.

EMT (Epithelial-Mesenchymal transition) has an essential role in tumor progression and metastatic dissemination. We here showed that zinc-finger protein AWP1 (zinc finger AN1 type 6, ZFAND6) regulates EMT in prostate cancer. The depletion of AWP1 using siRNA increased the proliferation of DU-145 cells and augmented migration ability as observed by wound healing assay. In addition, the mRNA expression of EMT markers were upregulated in DU-145 with AWP1 depletion. Especially, the protein level of α-SMA was increased in AWP1-depleted DU-145 cells. Moreover, knockdown of AWP1 in DU-145 showed increased tumor growth compared to control in subcutaneously xenografted *in vivo* model. Our results suggest that the loss-of-function of AWP1 induces EMT, contributing to prostate cancer progression.

B222/P2614

A Novel Relationship Between Small G Protein ARF1 and Arf GTPase-activating Protein ASAP1

A. Kim[1], K. E. Hebron[1,2,2], O. L. Perkins[2,2,3], X. Jian[2], S. A. Girald-Berlingeri[2,4], P. A. Randazzo[2], M. E. Yohe[1], M. E. Yohe[1,2,2]; [1]National Cancer Institute, NIH, Frederick, MD, [2]National Cancer Institute, NIH, Bethesda, MD, [3]Vanderbilt University, Nashville, TN, [4]Inova Fairfax Hospital, Falls Church, VA

Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. The overall survival rate for patients with advanced RMS has been less than 20% for the last 30 years. Investigating novel treatment strategies for RMS is necessary to improve prognosis. PAX/FOXO1 Fusion Negative RMS (FN-RMS) is commonly associated with alterations in the RAS/RAF/MEK/ERK pathway and arises due to a disruption of differentiation in skeletal muscle precursor cells. Trametinib, a MEK inhibitor, has been shown to induce myogenic differentiation in FN-RMS cell lines. However, it is imperative to note that trametinib monotherapy is not durable. Therefore, exploring additional viable targets for combination therapy is necessary. ASAP1, an Arf GTPase-activating protein (GAP), is a candidate due to its decisive role in osteogenic and adipogenic differentiation. Additionally, ASAP1 has been shown to be overexpressed in several cancers. Furthermore, we see an overexpression of ASAP1 in FN-RMS and lower survival rates for patients with ASAP1 overexpressed FN-RMS. We believe that ASAP1 plays a role in the differentiation of FN-RMS. Upon knockdown (KD) of ASAP1, trametinib-induced differentiation was reduced. Additionally, upon KD of ARF1, we see a block in trametinib-induced differentiation. Due to these conflicting observations, we hypothesize that there may be a novel relationship between ARF1 and ASAP1. To investigate this relationship, we exogenously rescued ARF1 (WT) and ARF1[I46D] mutant, a mutation that renders ARF1 insensitive to ASAP1's GAP activity, following KD of endogenous ARF1. Overall, we see that trametinib-induced differentiation was rescued in expression of either exogenous WT or ARF1[I46D]. This supports the observation that ASAP1 may be an effector of ARF1 in addition to being an ARF-GAP. To further examine ASAP1 and ARF1's interaction, we investigated trametinib-induced differentiation upon KD of known effectors of ARF1 (GGA1, GGA2, and GGA3). We observed that, upon KD of established ARF1 effectors, there was also a decrease in trametinib-induced differentiation, suggesting that multiple ARF1 effectors impact differentiation. In conclusion, in addition to being an ARF-GAP, ASAP1 may also function as an ARF1 effector that regulates differentiation of FN-RMS. Future work will consist of investigating the downstream signaling effects of this novel ASAP1 and ARF1 interaction in FN-RMS.

Regulation of the Cytoskeleton in Breast Cancer

B223/P2615

Tight junction proteins restrict cancer cell metastasis

J. West, K. Cho, I. Roberts, A. Ewald; Johns Hopkins University, Baltimore, MD.

Breast cancer is a leading cause of cancer deaths among women. Even though metastasis drives most breast cancer deaths, our understanding of this process remains limited. Metastasis starts with invasion and needs to overcome the constraints of several types of adhesive junctions. We focus on the role of the tight junction because decreased expression of tight junction proteins is a defining feature of the highly aggressive 'Claudin-low' subtype of breast cancer. In normal epithelial cells, the tight junction mediates intercellular adhesion,barrier function, and partitioning the plasma membrane into apical and basal domains. Wehypothesized that tight junctions suppress aggressive cell behaviors, and that their

loss could induce metastasis. Using genetically engineered mouse models (GEMMs) of breast cancer, we have observed a spontaneous decrease in mRNA expression of multiple TJ components during tumor growth in vitro and in vivo. Localization of tight junction proteins is also disrupted in cells found within hyperplastic lesions. Using 3D organotypic culture assays we demonstrated that knockdown oftight junction proteins generally results inincreased growth and invasion of mammary organoids. Specifically, we have observed 2-fold increases in organoid size and average organoid growth rate after depleting the tight junction proteins, ZO1, ZO2, or Cldn7. Furthermore, in vivoknockdown of Cldn7 in orthotopic breast cancer metastasis models caused increased cellular heterogeneity in the primary tumor and increased metastatic burden in distant organs. Taken together, our results support a model in which downregulation of tight junction proteins accelerates tumor progression and increases metastatic efficiency. Our data are consistent with the observation that breast tumors with a Claudin-low phenotype are more aggressive, independent of hormone receptor status.

B224/P2616

Collagen density primes the mammary tumor microenvironment for early dissemination by promoting macrophage infiltration and an EMT-associated transcriptional program in tumor cells.

E. J. Hoffmann[1], A. R. Nobre[2], A. E. Williams[3], L. M. Arendt[3], J. A. Aguirre-Ghiso[4], S. M. Ponik[1];
[1]Department of Cell & Regenerative Biology, University of Wisconsin-Madison, Madison, WI, [2]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, [3]Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI, [4]Department of Cell Biology, Cancer Dormancy and Tumor Microenvironment Institute, Albert Einstein College of Medicine, New York, NY.

High mammographic density due to increased collagen I deposition has long been recognized as a risk factor for breast cancer progression in women, however the biology underlying this risk factor remain largely unknown. The goal of this study is to identify the exact mechanisms by which collagen I affects breast cancer progression. In advanced tumors, stiff, aligned collagen fibers are known to promote epithelial to mesenchymal transition (EMT), invasion of cancer cells, and increase metastatic burden. Importantly, we observe key differences between normal and collagen-dense mammary glands before a tumor is present that may predispose the microenvironment to early tumor cell dissemination. One such factor is the expression of ZFP281, a transcription factor associated with stem cell primed pluripotency and recently identified as a regulator of early dissemination and dormancy in disseminated breast cancer cells. Using the MMTV-PyMT mammary carcinoma model crossed with a mutant collagen I gene, which prevents collagen proteolysis to mimic mammographic density, we identified an increase in periductal/intraepithelial macrophages and expression of the transcription factor ZFP281 in normal epithelial cells, similar to that reported for early MMTV-HER2 lesions in a WT collagen I gene background. However, this increase in $ZFP281^{+}$ cells is lost by the time a primary lesion progresses to an overt tumor. Using a 3D collagen gel cell culture model to investigate the effects of collagen I on ZFP281, we observed that increasing collagen density alone is sufficient to decrease protein levels of ZFP281 in late stage breast cancer cells, but not in normal epithelial cells. This affect appears to be primarily mediated by the increased stiffness of the matrix. This potential mechanical regulation of ZFP281 expression by collagen I, paired with the timing of fiber accumulation and reorganization, may be an important microenvironmental cue that determines the switch from early dissemination to high proliferation at the primary tumor, a common hallmark of collagen-dense mammary tumors

B225/P2617

Investigating the Role of Compressive Stress in Cellular Unjamming

G. Cai[1], A. Nguyen[2], Y. Bashirzadeh[1], S. Lin[1], D. Bi[2], A. Liu[1]; [1]University of Michigan, Ann Arbor, MI, [2]Northeastern University, Boston, MA.

Cellular unjamming is the collective fluidization of cell motion and has been linked to many biological processes, including development, wound repair and tumor growth. In tumor growth, the uncontrolled proliferation of cancer cells in a confined space generates mechanical compressive stress. However, because multiple cellular and molecular mechanisms may be operating simultaneously, the role of compressive stress in unjamming transitions during cancer progression remains unclear. Here we investigate which mechanism dominates in a mechanically stressed monolayer. We find that long-term mechanical compression triggers cell arrest in benign epithelial cells and enhances cancer cell migration in transitions correlated with cell shape, leading us to examine the contributions of cell-cell adhesion and substrate traction to unjamming transitions. We show that cadherin-mediated cell-cell adhesion regulates differential cellular responses to compressive stress and is an important driver of unjamming. Importantly, compressive stress does not induce epithelial—mesenchymal transition in unjammed cells. Traction force microscopy reveals the attenuation of traction stresses in compressed cells within the bulk monolayer during wound healing and on microcontact printed cell islands, regardless of cell type and motility. Interestingly, as traction within the bulk monolayer diminishes with compressive pressure, traction at the leading edge of cancer cell sheets is sustained. Seeking a theoretical explanation for the observed differential cellular responses to mechanical compression, we use the self-propelled Voronoi model to map the observed cell's condition to a phase diagram with two model parameters: cell motility and target cell shape index. Using a jammed—unjammed boundary and comparing experimentally measured cell shapes and traction forces with our simulation model reveal two distinct transition paths under mechanical compression. Together, increased intercellular adhesion and attenuated traction forces within the bulk cell sheet under compression drive fluidization of the cell layer and may impact collective cell motion in tumor development and breast cancer progression.

B226/P2618

Profilin-1/CCL2 is a novel signaling axis of tumor cell-directed migration of immune cells

I. Eder, V. Yu, M. Joy, P. Lucas, P. Roy; University of Pittsburgh, Pittsburgh, PA.

Profilin-1 (Pfn1) is an actin-binding protein that is downregulated in human breast cancer (BC). Pfn1 has been previously demonstrated to have tumor-intrinsic roles as a suppressor of tumorigenicity and dissemination in BC; however, whether Pfn1 has extrinsic immunological effects on the tumor microenvironment (TME) is unknown. We sought to investigate the effect of Pfn1 expression on the immune composition of the TME and the chemotaxis of immune cells. We performed multiplexed quantitative immunohistochemistry on a tissue microarray of clinical BC samples which revealed a significant positive correlation between tumor cell-specific Pfn1 expression and the percent of CD8+ T cells in the TME, more prominently in triple-negative breast cancer (TNBC) samples. Bioinformatics analyses of the METABRIC transcriptome dataset confirmed Pfn1 expression to be positively correlated with the CD8+ T cell fraction, in addition to, the pro-inflammatory IFN-gamma gene signature and the M1 to M2 macrophage ratio in TNBC. Co-culture studies demonstrated that elevating Pfn1 expression in TNBC cells enhances chemotactic migration of monocytes in a paracrine fashion, as well as, augments chemotactic migration of T-cells in a monocyte-dependent manner. To investigate the underlying

mechanism, we performed luminex analyses of the conditioned media of TNBC cells, and identified CCL2, a major chemoattractant of monocytes, to be dramatically upregulated and downregulated upon overexpression and knockdown of Pfn1, respectively. These data were further supported by real-time quantitative PCR-based confirmation of Pfn1-dependent changes in CCL2 transcription in TNBC cells as well as a significant positive association between Pfn1 and CCL2 mRNA expression in the clinical specimens of TNBC. Silencing CCL2 expression in TNBC cells abolished Pfn1-dependent changes in the chemotactic migration of monocytes, suggesting that CCL2 is a key mediator of Pfn1-stimulated migration of monocytes. Collectively, these findings provide the first evidence for extrinsic immunological effects of Pfn1 in BC. Since tumor infiltration of CD8+ T cells is associated with improved prognosis in BC and is a key determinant of immunotherapy success in TNBC, our work lays the conceptual foundation for future studies to explore whether Pfn1 modulation could be a novel strategy to enhance immunotherapy efficacy in BC.

B227/P2619

Hypusine Signaling Cooperates with SLC3A2 and Fibronectin to Support Cancer Cell Proliferation

C. Geller[1], J. Maddela[1], R. Tuplano[1], R. Güth[1], J. Cantor[2], **J. Kelber**[1]; [1]California State Univ-Northridge, Northridge, CA, [2]BD Biosciences, San Diego, CA.

Elevated fibronectin expression within the microenvironment of breast cancer correlates with poor outcomes. In this regard, we reported that the DHPS-/DOHH-dependent hypusination pathway, which activates eukaryotic initiation factor 5A 1/2 (eIF5A1/2), is necessary for PEAK1 pseudokinase translation and fibronectin/TGFβ-mediated breast cancer metastasis. Using a panel of eIF5A1/2-/PEAK1-related genes together with markers of intratumoral heterogeneity, we established a bioinformatics pipeline that led us to identify solute carrier 3A2 (SLC3A2 or CD98hc) as an indicator of poor prognosis in breast cancer patients. Inhibition of SLC3A2 or DHPS decreased tumor spheroid growth. Interestingly, spiking fibronectin into culture media partially rescued spheroid growth - an effect that was abrogated by dual DHPS/SLC3A2 inhibition. Additionally, dual DHPS/SLC3A2 inhibition in cancer cells plated onto fibronectin significantly reduced the percentage of cells in S phase. Intracellular trafficking of eIF5A1/2 between the nucleus and cytoplasm has been reported to mediate its mRNA transport and protein translation functions. In this regard, we further discovered that fibronectin induced a DHPS-/SLC3A2-dependent increase in cytoplasmic eIF5A1/2 levels. Finally, we profiled breast cancer cells using a panel of functional state markers at single-cell resolution and discovered that extracellular fibronectin decreased $GRP78^{hi}$ breast cancer cells. Notably, the remaining $GRP78^{hi}$ breast cancer cells are highly proliferative and can be eliminated by dual DHPS/SLC3A2 inhibition. Taken together, these data provide a mechanistic basis for a new treatment strategy that may improve outcomes in patients harboring solid tumors rich in fibronectin and with a high proliferative index.

B228/P2620

Scaffold Protein RACK1 Inhibitor Compounds Prevent the Focal Adhesion Kinase Mediated Breast Cancer Cell Migration and Invasion Potential

H. Ullah[1], N. Ahsan[2], S. Dakshanamurthy[3]; [1]Howard University, Washington, DC, [2]The University of Oklahoma, Norman, OK, [3]Georgetown University, Washington, DC

Scaffold protein RACK1 mediates cancer cell migration mostly through regulation of focal adhesion (FA) assembly by promoting a focal adhesion kinase (FAK) activation downstream of the integrin clustering

and adhesion at the extracellular matrix (ECM). Here we demonstrated the efficacy of our recently developed RACK1 Y246 phosphorylation inhibitor compounds (SD29 and SD29-14) to inhibit the migration and invasion of MCF7 and MDA-MB-231 breast cancer cell lines. Using multiple assays, our results confirmed that inhibitor compounds effectively prevent the filopodia/lamellipodia development and inhibits the migration of breast cancer cells. A mechanistic model of the inhibitor compounds has been developed. Migration and invasion capabilities of the cancer cells define the metastasis of cancer. Thus, our results suggest a potential therapeutic mechanism of the inhibitors to prevent metastasis in diverse cancers.

B229/P2621

Triple negative breast cancer metastasis involves complex EMT dynamics and requires vimentin

E. M. Grasset, M. Dunworth, G. Sharma, M. Loth, J. Tandurella, A. Cimino-Mathews, M. Gentz, S. Bracht, M. Haynes, E. J. Fertig, A. J. Ewald; Johns Hopkins Medicine, Baltimore, MD

Triple negative breast cancer (TNBC) is an aggressive subtype, associated with early metastatic recurrence and worse patient outcomes. TNBC patient tumors express molecular markers of the epithelial to mesenchymal transition (EMT) but its role during spontaneous TNBC metastasis *in vivo* remains incompletely understood. In this study, we demonstrate that spontaneous TNBC tumors from a genetically engineered mouse model (GEMM), multiple patient-derived xenografts (PDXs), and archival patient samples exhibit large populations of hybrid E/M cells *in vivo* that lead invasion *ex vivo*. We found that the mesenchymal marker vimentin promotes invasion and represses metastatic outgrowth. We next tested the requirement for five EMT transcription factors and observed distinct patterns of utilization during invasion and colony formation. These differences suggest a sequential activation of multiple EMT molecular programs during the metastatic cascade. Consistent with this model, our longitudinal single-cell RNA analysis detected 3 different EMT-related molecular patterns. We observed cancer cells progressing from epithelial to hybrid E/M and strongly mesenchymal patterns during invasion and from epithelial to a hybrid E/M pattern during colony formation. We next investigated the relative epithelial vs. mesenchymal state of cancer cells in both GEMM and patient metastases. In both contexts, we observed heterogeneity between and within metastases in the same individual. Strikingly, we observed a complex spectrum of epithelial, hybrid E/M, and mesenchymal cell states within metastases, suggesting there are multiple successful molecular strategies for distant organ colonization. Taken together, our results demonstrate an important and complex role for EMT programs during TNBC metastasis.

B230/P2622

Hypoxic Gradients Direct the Spatial Organization of Epithelial-to-Mesenchymal Transition in an *in vitro* Breast Cancer Tumor Microenvironment

S. Anmalsetty[1], S. J. Flaherty[1], A. J. Walke[2], D. A. Scheiblin[2], L. Ridnour[3], D. A. Wink[3], S. J. Lockett[2], W. F. Heinz[2]; [1]Optical Microscopy and Analysis Laboratory, Office of Science and Technology Resources, CCR, National Cancer Institute, National Institutes of Health, Frederick, MD, [2]Optical Microscopy and Analysis Laboratory, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD, [3]Laboratory of Cancer Immunometabolism, CCR, National Cancer Institute, National Institutes of Health, Frederick, MD

Poor prognosis in ER- breast cancer is strongly predicted by high expression of cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (NOS2). These proinflammatory proteins, alongside a hypoxic tumor microenvironment, promote cancer cell migration, metastasis, invasion, and survival. The objective of the study was to understand the spatial relationships of epithelial (E), mesenchymal (M), and hybrid (E/M) cells in relation to hypoxia, COX2, and NOS2. We hypothesize that E/M phenotypes - defined by E-cadherin (ECAD) and vimentin (VIM) positive cells - (1) organize along cell-generated hypoxic gradients and (2) are correlated with expression of COX2 and NOS2. Restricted exchange environment chambers (REECs) mimic the cell-generated gradients of oxygen (O_2) concentration and nutrients of solid tumors in 2D live cell culture and facilitate high resolution fluorescence microscopy. 4T1 cells, a murine model of human triple-negative breast cancer, were cultured in REECs for up to 168 hours. During this time, cells migrated toward the opening in the chamber supplying O_2 and nutrients to the cells and formed a disk centered on the O_2 source. The O_2 concentration decreases radially from the normoxic disk center to the hypoxic disk edge. Over a few days of cell growth in the REECs, the proportion of VIM+ cells increased steadily, with a maximum in normoxic regions like its distribution in 4T1 mouse tumors. In hypoxic regions, discrete VIM+ clusters emerged in a spoke-like pattern aligned along the cell-generated hypoxic gradient. ECAD had similar expression dynamics to VIM. E/M cells, which are associated with high metastatic potential, increased in all regions over time. COX2 and NOS2 expression also increased significantly in response to hypoxic gradients, with COX2 being present in the normoxic region and NOS2 forming a stable ring-like pattern in the hypoxic region and centered on the O_2 source. At early time points of growth, the majority of COX2+ cells were VIM-, but after 48 hours became VIM+. Inhibition of NOS2 or COX2 decreases the number of VIM+ clusters relative to untreated samples, but the number of cells per cluster increases. This supports the hypothesis that COX2 and NOS2 are associated with the organization of E/M phenotypes in a hypoxic gradient. These results provide insight into the mechanisms of COX2 and NOS2 on tumor metastasis and further support their potential as therapeutic targets. Supported in part by NCI contract: 75N91019D00024.

B231/P2623

Stromal PI3Kbeta regulates breast tumor cell invasion

R. Graff, C. Jakubik, A. J. Haimowitz, S. Sidoli, M. J. Gamble, A. R. Bresnick, **J. M. Backer**; Albert Einstein College of Medicine, Bronx, NY

The ability of breast cancer cells to metastasize depends on cell autonomous signaling within the tumor cells, as well as paracrine and juxtracrine interactions with stromal cells in the tumor microenvironment. We have previously shown that activation of the Class IA phosphoinositide 3- kinase PI3Kbeta in tumor cells is required for matrix degradation, invasion, and experimental metastasis. We now show the PI3Kbeta signaling is also critical for the pro-metastatic properties of two distinct stromal cell types: macrophages and platelets. We have developed a knock-in mouse expressing a mutant PI3Kbeta ($PI3Kbeta^{KKKDD}$) that has basal activity but is defective for activation. Bone marrow-derived macrophages (BMMs) from $PI3Kbeta^{KKKDD}$ mice have cell autonomous defects in invasive capacity, as they are defective for podosome-mediated matrix degradation and transendothelial migration. Importantly, they also show a reduced capacity to stimulate the invasive behavior of tumor cells. Whereas wild type BMMs stimulate matrix degradation and transendothelial migration by MDA-MB-231 breast cancer cells, these responses are significantly reduced during co-culture with $PI3Kbeta^{KKKDD}$ BMMs. RNAseq data from tumor cell-BMM co-cultures show that over 100 genes are differentially upregulated in wild type versus $PI3Kbeta^{KKKDD}$ BMMs; these include numerous chemokines and pro-metastatic factors. In parallel, we

have studied the stimulation of tumor cell invasion by interactions with platelets. Co-culture of wild type platelets with MCF-10A breast epithelial cells for 40h induced a mesenchymal morphology, up-regulated N-cadherin expression (consistent with EMT), and stimulated Matrigel invasion by 15-fold. In contrast, MCF-10A invasion was stimulated by only 8.7-fold after co-culture with PI3Kbeta^{KKKDD} platelets. PI3Kbeta^{KKKDD} platelets were defective for activation in response to ADP/collagen and showed a 2-fold decrease in binding to tumor cells in a FACS-based assay. Given that depleted thrombin-stimulated platelet membranes are capable of stimulating tumor cell invasion, we are analyzing changes in the expression of cell surface receptors and adhesion molecules in PI3Kbeta^{KKKDD} platelets. Taken together, our data suggest that PI3Kbeta plays important roles in tumor metastasis in at least 3 cell types - tumor cells, macrophages and platelets - and suggest that PI3Kbeta-selective inhibitors could be clinically useful in the treatment or prevention of metastasis.

B232/P2624

Metformin's effects on pro-metastatic gene expression in triple-negative breast cancer cells

M. Anaafi, D. Alton, B. C. Trask; Weber State University, Ogden, UT.

In recent years, metformin's use as an anti-cancer agent has been a topic of intense investigation. The anti-hyperglycemic drug has been shown to directly induce apoptosis of breast cancer cells, decrease their production of some pro-angiogenic factors, and reduce their ability to produce lung metastases in immunodeficient mice (Orecchioni, *et al.*, 2015). Although these results suggest that metformin might provide some clinical benefit, recent human studies in which breast cancer patients were treated with the drug suggest that disease-free survival is not improved (Goodwin, *et al.*, 2022). To explore why this might be the case, we investigated the effect of metformin treatment on the expression of several pro-metastatic genes, including monocyte-recruiting C-C chemokines and matrix-degrading metalloproteinases, in MDA-MB-436 triple-negative breast cancer cells using RT-qPCR. Our results support that, despite a reported mTOR-mediated decrease in global protein synthesis (Dowling, *et al.*, 2007), metformin treatment does not significantly decrease expression of several pro-metastatic genes. The ability of triple-negative breast cancer cells to produce pro-metastatic proteins even in the presence of metformin may explain the absence of an observable clinical benefit in metformin-treated breast cancer patients.

B233/P2625

Bioprospecting native*Ganoderma*spp. extracts with selective anti-triple-negative breast cancer potential.

G. M. Silverio[1], L. Arroyo-Cruz[1], S. Sagardia-Gonzalez[2], K. Miller[2], F. Rivas[3], T. Ling[3], M. Martinez-Montemayor[1]; [1]University of Central Del Caribe, Bayamon, Puerto Rico, [2]Huerto Rico, Carolina, Puerto Rico, [3]Louisiana State University, Baton Rouge, LA

Bioprospecting native *Ganoderma* spp. extracts with selective anti-triple-negative breast cancer potential.Silverio-Alvarado G.M.[1], Arroyo-Cruz L.V.[1], Sagardía-González S.[2], Miller K.[2], Ling T.[3], Rivas F.[3], Martínez-Montemayor M. M.[11]Department of Biochemistry and Cancer Research Unit, Universidad Central del Caribe-School of Medicine, P.O. Box 60327 Bayamón, PR 00960-6032; [2]Huerto Rico, Carolina, P.R., [3]Louisiana State University, Baton Rouge, LA; **Background**: Triple-negative breast cancer (TNBC) accounts for about 10-15% of all breast cancers and tends to be more common in younger women, who are Hispanics or African Americans. TNBC is characterized by the lack of hormone (ER & PR) and the

epidermal growth factor receptor 2 (HER2). Thus, currently there are no approved targeted therapies for TNBC, and typically these women are treated with a combination of non-targeted chemotherapy, radiation, and surgery. Therefore, the development of novel therapeutic strategies is necessary to combat TNBC. *Ganoderma* is a basidiomycete white rot fungus that has been used for medicinal purposes for centuries. Much is known about *Ganoderma lucidum* and its properties against cancer, but other *Ganoderma* species, including neotropical ones, are being studied in depth for this purpose. The objective of this study is to identify the effects of native *Ganoderma*species found in Puerto Rico, and test their efficacy. Native fractions extracted from *Ganoderma multiplicatum* (GMu; F1, F2, F3, F4, F6, F7) were tested against TNBC (SUM149) and non-cancerous (MCF10A) cell lines.**Methods**: Cell viability assays were performed using 48-well plates. Cells were treated with increasing concentrations of different fractions ranging from 0 to 75 µM for 72h. After treatment, cell viability was determined by fluorescence detection (GloMax, Promega).**Results**: Our results indicate that SUM149 cancer cells have a significantly reduced viability in fractions F1-F4, F6, and F7. A greater significant inhibitory concentration was detected for F7 (IC_{50} = 3.9 µM), F1 (IC_{50} = 8.4 µM), and F2 (IC_{50} = 11.3 µM). These fractions were effective at lower concentrations compared to the other tested fractions. On the other hand, the MCF10A non-cancerous cell line results indicated that fractions for F2-F4 and F6, we could not calculate an inhibiting concentration. These were calculated only F1 and F7 showed an IC_{50} of 497.9 µM and 1,116 µM, respectively. **Conclusions**: We conclude that fractions F1, F2 and F7 can be evaluated in more depth as fractions that might contain selective anti-TNBC bioactive compounds with great therapeutic potential.

B234/P2626

Endomembrane Localization of IFI6/G1P3 in Breast Cancer Cells

A. Davenport[1,2], M. Morris[2], D. Shakya[2], S. Sabti[2], F. Sabti[2], D. Hynds[1], V. Cheriyath[2]; [1]Texas Woman's University, Denton, TX, [2]Texas A&M University-Commerce, Commerce, TX.

Breast cancer remains the second leading cause of cancer-related deaths among U.S women. G1P3 (IFI6/ISG6-16) is an interferon-stimulated gene encoding a 13kD protein with pleotropic functions. Initial studies by us and others characterized G1P3 as an anti-apoptotic protein localized in mitochondria with a role in cancer progression and metastasis. Additionally, G1P3 is a mediator of the antiviral activity of interferons. Based on the pleiotropic functions of G1P3, we hypothesized the multi-organellar localization of G1P3. Using confocal microscopic and biochemical approaches, the subcellular localization of G1P3 was determined in breast cancer cells. Since a cell staining competent antibody for G1P3 was unavailable, confocal studies were carried out using MCF-7 cells expressing a FLAG-tagged G1P3 protein (MCF7/G1P3-Flag). In co-staining studies, ~40% of G1P3 co-occurred with the inner mitochondrial membrane protein COXIV. In agreement with this, protease digestion of affinity purified mitochondria identified that ~30% of G1P3 resides inside the mitochondria. Additionally, G1P3 was found to co-occur with organelles of endomembrane system such as endoplasmic reticulum (16%), *trans*-Golgi (22%), and lysosome (13%). Among endosomes, G1P3 preferentially co-occurred with Rab5 positive endosomes (55%) than with Appl1 endosomes (8.4%) or with EEA1 endosomes (6.9%). Considering the role of Rab5 endosomes in apoptosis resistance and cell migration, inhibiting the association of G1P3 with Rab5 endosomes may induce cancer cell death to improve clinical outcomes in breast cancer patients.

B235/P2627

Suppression of Breast Cancer Cell Proliferation by the Dietary Polyphenols Resveratrol, Ellagic Acid, Fisetin, Luteolin, and Chrysin

B. Clark, K. Whitinger, S. Phipps, H. Waters, E. Brandenburg, **K. Baker**; University of Indianapolis, Indianapolis, IN

Breast cancer is a worldwide health problem that continues to rank as one of the leading causes of death in women. Resveratrol, ellagic acid, fisetin, luteolin, and chrysin, dietary polyphenols found in a variety of fruits and vegetables, have been shown to exhibit anticancer activity; thus, the use of these dietary agents in combination may be effective for the chemoprevention and therapy of breast cancer. In this study we evaluated the antiproliferative effects of resveratrol, ellagic acid, fisetin, luteolin, and chrysin individually and in combination, using MCF-7 breast cancer cells. We found that each of the phytochemicals inhibited MCF-7 cell proliferation in a dose-dependent manner. Furthermore, we found that co-administration of lower doses of resveratrol and ellagic acid, resveratrol and fisetin, or resveratrol and luteolin, resulted in greater inhibition of cell proliferation than either agent alone at the same dose and indicate an additive effect. These results suggest that combinatorial treatments using resveratrol with ellagic acid, fisetin, or luteolin may be an effective chemotherapeutic strategy against breast cancer.

B236/P2628

Structural basis for trafficking and oligomerization defects of a breast cancer-associated mutation in caveolin-1

B. Han[1], A. Gulsevin[2], S. Connolly[3], T. Wang[1], **B. Meyer**[1], J. Porta[3], A. Tiwari[1], A. Deng[2], L. Chang[3], Y. Peskova[1], H. S. Mchaoraub[2], E. Karakas[2], M. D. Ohi[3], J. Meiler[2], A. K. Kenworthy[1]; [1]University of Virginia School of Medicine, Charlottesville, VA, [2]Vanderbilt University, Nashville, TN, [3]University of Michigan, Ann Arbor, MI

Flask-shaped pits in the plasma membrane known as caveolae regulate numerous cellular processes including intracellular trafficking, signaling, lipid homeostasis, and membrane buffering. Caveolae assembly is driven by caveolin-1 (CAV1), a multimeric integral membrane protein. Mutant forms of CAV1 are implicated in a number of diseases, including cancer and pulmonary arterial hypertension. However, the structural bases for how mutations in CAV1 impact caveolae assembly and function remain poorly understood. Here, we study the molecular basis for trafficking and oligomerization defects of a breast cancer-associated mutation of one of the most highly conserved residues in CAV1, P132L using a combination of computational, structural, biochemical, and cell biological approaches. We show P132 is positioned at a major site of protomer-protomer interactions within the CAV1 complex, explaining why the mutant protein fails to homo-oligomerize and traffic to the plasma membrane correctly. Despite its homo-oligomerization defects, P132L, is capable of forming hetero-oligomeric complexes with wild type CAV1 and can even become incorporated into caveolae in this form. These findings provide insights into the fundamental mechanisms that control the formation of homo- and hetero-oligomers of caveolins that are essential for caveolae biogenesis, as well as how these processes are disrupted in human disease.

Microenvironment

B237/P2629

LncRNA *LUCAT1* increases the oncogenic properties of gastric cancer cells and macrophages by regulating MIF expression in *H. pylori*-induced gastric cancer.

S. Kim[1,2], S. Lee[1]; [1]Yonsei University, Seoul, KOREA, REPUBLIC OF, [2]Institute of Gastroenterology, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, KOREA, REPUBLIC OF.

<META NAME="author" CONTENT="김서연(소화기내과)">Long non-coding RNAs (lncRNAs) are proven to play critical roles in cancer biology. Tumor-associated macrophages predominantly induced to be polarized into M2, a pro-tumorigenic type when recruited with the tumor tissue and thereby favoring the tumorigenesis. In our study, we aim the possible mechanisms of the regulatory link between lncRNAs and macrophages in gastric cancer caused by *H. pylori*.Differentially expressed lncRNAs between *H. pylori*-CagA+ infected and control AGS cells, and between cagA vector-transfected and control AGS cells were identified by RNA-seq and validated by qRT-PCR. Next, we built a co-culture system to explore the interaction between THP-1 macrophage cells and AGS cells in the presence of *H. pylori*-CagA+.We validated that the expression of LUCAT1 was significantly up-regulated after of both *H. pylori*-CagA+ infection and cagA transfection. The knock down of LUCAT1 significantly attenuated the proliferation, migration, and invasion of AGS and MKN74 cells. The infection of *H. pylori*-CagA+ increased secretions of inflammatory cytokines which produced a huge chemotactic effect on THP-1 cells. In our study, LUCAT1 up-regulated macrophage migration inhibitory factor (MIF), one of the crucial cytokines involved in cancer and inflammation in AGS and THP-1 cells. We found that LUCAT1 increased by *H. pylori*-CagA+ infection up-regulates MIF in AGS cells and subsequently MIF polarizes surrounding THP-1 macrophages into M2 macrophages. In turn, M2 macrophages activate carcinogenesis in gastric cancer.Our findings provide important insights for the potential development of lncRNA-based targeted approaches for the treatment of *H. pylori*-related gastric cancer.

B238/P2630

Expansion of myeloid-derived suppressor cells in pancreatic adenocarcinoma patients is associated with increased epithelial-mesenchymal transition in their tumor microenvironment

A. Aggarwal, V. Sharma, V. Gupta; Post Graduate Institute of Medical Education and Research Chandigarh India, Chandigarh, INDIA.

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer-related deaths worldwide and its incidence is rising continuously due to poor prognosis. An immunosuppressive microenvironment is the characteristic feature of PDAC. The dysregulated immune system leads to suppression of anti-tumor immunity by aberrant production of Myeloid-derived suppressor cells (MDSCs) but the underlying mechanism highlighting their role of tumorigenesis remains inconclusive. Therefore, we aimed to study the net expansion of MDSCs in PDAC patients and their role in promoting invasiveness. **Methods:** We assessed the relative frequencies of MDSCs and chemokines receptors (CCR4 and CCR5) present on their subsets, in the circulation and surgically resected local pancreatic tumor tissue of PDAC patients by flow cytometry. We investigated the status of epithelial-mesenchymal transition (EMT) related markers E-Cadherin, N-Cadherin, Snail, and ZEB1 by qRT-PCR and

immunohistochemistry. **Results:** We found a higher frequency of circulating and tumor infiltrated MDSCs in PDAC patients compared to healthy controls. MDSCs in peripheral blood showed a significant positive correlation with MDSCs burden in cancer tissue. In tumor tissue, expression of E-Cadherin was significantly reduced, while N-Cadherin, Snail, and ZEB1 were markedly raised. The frequency of CCR4+ Monocytic-MDSCs significantly correlated with the expression of mesenchymal transition markers N-Cadherin, Snail, and ZEB1. **Conclusion:** Our results suggest that CCR4+ Monocytic-MDSCs possess significant tumorigenic potential and their expansion might be responsible for higher invasiveness in pancreatic cancer cells. This study sheds light on the tumorigenic role of MDSCs, which can serve as a crucial target of future anti-cancer strategies to inhibit tumor cell invasiveness

B239/P2631

Osteoblast regulated autophagy and Wnt/b-catenin signaling in AML cells

K. Shivhare[1,2], N. Satija[3,4]; [1]Developmental Toxicology Division, CSIR-Indian Institute Of Toxicology Research, Lucknow, INDIA, [2]Molecular Neurotoxicology andcell integrity Lab, System toxicology and health risk assesment group, Academy of Scientific & Innovative Research, Lucknow, INDIA, [3]Molecular Neurotoxicology andcell integrity Lab, System toxicology and health risk assesment group, CSIR-Indian Institute Of Toxicology Research, Lucknow, INDIA, [4]Academy of Scientific & Innovative Research, Lucknow, INDIA.

Abnormal proliferation and differentiation is a characteristic feature of Acute Myeloid Leukemia (AML). Relapse is the prime phenomenon associated with the fatal outcomes of AML. These AML cells show metabolic changes in response to chemotherapy or specific treatments. Autophagy is the cellular adaptive response towards the stress and has demonstrated the distinct role in cellular metabolism, involved in the maintenance of cancer condition, resistance to chemotherapy. Autophagy and Wnt/β-catenin signaling are key players involved in regulation of cellular differentiation and proliferation. GSK-3β, one of the main mediator of Wnt/β-catenin signaling is reported to induce autophagy via phosphorylation of ULK-1. Current study explored osteoblast cells regulated autophagy/Wnt/β-catenin axis in AML cells. Different AML cells (KG-1a, U937, HL60) were co-cultured with Osteoblast cells (Saos-2) and were assessed for key autophagy and β-catenin proteins. The three cell lines belong to different subtypes of AML. HL60 and KG-1a in direct contact with Saos-2 consistently showed significant up-regulation in autophagy proteins including p62, LC3, Atg-5, Atg-7, Beclin-1. However, U937 cells showed no significant changes in these autophagy proteins under similar conditions. Upon placing transwell between AML cells and osteoblast cells to restrict their physical contact, expression of autophagy proteins remained unchanged in all 3 AML cell lines. Furthermore, direct contact of AML and osteoblast cells significantly up-regulated levels of β-catenin and GSK-3β in all AML cells which remained unaffected upon physically restricting cell contact. Altogether, the results reveal importance of osteoblast cells in regulation of autophagy and Wnt/β-catenin in AML cells and possible mechanistic insight towards relapse which can be intervened for therapeutics for future perspective.

B240/P2632

Spatial transcriptomics reveals mechanically-regulated cell state transitions at the tumor-microenvironment interface

M. V. Hunter[1], R. Moncada[2], I. Yanai[2], R. M. White[1]; [1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]NYU Langone Health, New York, NY.

As tumors grow, they interact with different cells and tissues neighboring the tumor, but it is unclear how these interactions influence tumor cell biology. To investigate this, we applied spatial transcriptomics, single-cell RNA-seq, and single-nucleus RNA-seq to a whole-animal zebrafish model of melanoma. Using spatial transcriptomics, we identified a unique "interface" cell state localized to where the tumor contacts neighboring tissues, suggesting that cell state transitions occur at the tumor boundary. We used single-cell and single-nucleus RNA-seq to find that the interface is composed of specialized tumor and microenvironment cells that upregulate a common gene set. We found evidence of an "interface" population in patient samples, suggesting it is a conserved feature of human melanoma. In both fish and human data, interface cells are characterized by significant upregulation of the chromatin modifier HMGB2, which is prognostic in human melanoma. Loss of HMGB2 impairs invasion of both human melanoma cells *in vitro* and in zebrafish tumors *in vivo*. Paired RNA-seq and ATAC-seq revealed that developmental/pluripotency pathways are enriched in cells overexpressing HMGB2, suggesting developmental gene programs may be hijacked to support tumor invasion. To investigate the mechanism by which HMGB2 may facilitate cell state transitions at the interface, we used confocal microscopy to discover that HMGB2 is preferentially enriched in confined tumor cells under high levels of mechanical force during invasion. When we subjected human melanoma cells to confinement *in vitro*, we discovered that confinement causes HMGB2 to become cytoplasmic, where previous work suggests it may be secreted to be taken up by neighbouring cells. We are currently investigating how mechanical force signals through HMGB2 at the tumor border to induce changes in gene expression, chromatin accessibility, and cell state in both the tumor and surrounding cells. Together, our work suggests that HMGB2 may be a novel mechanosensor of the mechanical microenvironment at the tumor boundary, and demonstrates the power of spatial and single-cell transcriptomics in uncovering the biology underlying tumor cell behavior *in vivo*.

B241/P2633

Vascular endothelial profilin-1 Inhibition suppresses tumor progression in renal cancer

D. Gau[1], A. Allen[1], A. Daoud[1], J. Kunkel[1], A. N. Paranjape[2], F. S. Villanueva[2], P. Roy[1]; [1]Bioengineering, University of Pittsburgh, Pittsburgh, PA, [2]Cardiology, UPMC, Pittsburgh, PA.

Renal cell carcinoma (RCC) is estimated to result in 79,000 new cases and 13,920 deaths in 2022. Clear cell renal cell carcinoma (ccRCC) is the most common subtype of RCC and is characterized by a high vascularized tumor microenvironment (TME). While anti-angiogenic and vascular normalization inducing therapies are initially effective, almost all patients develop resistance to these therapies. Therefore, there is a need to identify alternative fundamental therapies for ccRCC. The goal of the present work was to investigate whether profilin1 (Pfn1), an actin-binding protein that is dramatically upregulated in tumor-associated vascular endothelial cells (VEC) in human ccRCC and linked to advanced disease features and adverse clinical outcome, is a driver of disease progression in RCC. We found that triggering endothelial Pfn1 gene deletion, either globally or restricted to kidney only, dramatically inhibits tumor formation and metastatic dissemination from syngeneic transplants of RCC cells. Loss of endothelial

Pfn1 led to a major suppression of tumor angiogenesis ensuing massive tumor cell death. In a delayed induction setting, loss of endothelial Pfn1 also retarded progression of pre-established tumors. Through small molecule screening, we identified a novel inhibitor of the Pfn1-actin interaction (Pfn1i) and further showed its ability to inhibit aggressiveness of RCC cells *in vitro*, reduce tumor angiogenesis and tumor growth *in vivo*. Furthermore, toward the goal of targeted delivery of Pfn1i in TME, we have successfully encapsulated Pfn1i into lipid microbubbles, and demonstrated prominent anti-angiogenic action by Pfn1i in cell culture setting released by ultrasound-mediated disruption of microbubbles. These findings lay the groundwork for our ongoing efforts that are focused on localized ultrasound-guided delivery of Pfn1i in tumor microenvironment as a therapeutic strategy in mouse model of RCC. Collectively, these findings establish endothelial Pfn1 as a driver of tumor progression as well as a potentially novel therapeutic target in kidney cancer.

B242/P2634

Identifying novel INSM1-associated protein networks that drive neuroblastoma differentiation

D. Chakravorty, A. Kelly, A. Saxena; University of Illinois At Chicago, Chicago, IL.

Neuroblastoma (NB), the most common cancer in the first year of life, is sometimes seen to resolve spontaneously by differentiating into neurons. Chemotherapeutic treatments of NB can aid in differentiation but demonstrate varied efficacy and significant side effects. We recently identified several neurogenesis-promoting factors that are necessary but not sufficient for inducing NB differentiation *in vivo*. Meanwhile, we have observed that neuron-like NB cells express higher levels of the transcription factor INSM1, in comparison to progenitor-like NB cells. Although INSM1 has been previously shown to promote human NB cell survival/proliferation, its role in NB differentiation remains uncharacterized. Therefore, to delineate the role of INSM1 in NB survival versus differentiation, we have generated and transfected a 3xFLAG-tagged INSM1 construct to pull down protein complexes for identification via LC-MS/MS. With this approach, we will identify INSM1's interactome in experimental conditions ranging from non-differentiating to differentiating NB cells, with and without induction of differentiation. Furthermore, INSM1 and its candidate interactor proteins will be perturbed in NB cells that will then be injected into distinct zebrafish embryonic microenvironments to evaluate any shift in the balance between NB survival and differentiation. This approach may provide new insights into the mechanisms of NB differentiation *in vivo* and lay the groundwork for pursuing novel therapeutic targets.

B243/P2635

MRTF-SRF interaction promotes bone metastasis of breast cancer cells

P. Chawla[1], D. M. Gau[1], Q. Sun[2], D. Boone[3], D. Galson[2,4,5], P. Roy[1,4,5]; [1]Bioengineering, University of Pittsburgh, Pittsburgh, PA, [2]Medicine, Division of Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, [3]Biomedical Information, University of Pittsburgh, Pittsburgh, PA, [4]UPMC Hillman Cancer Center, Pittsburgh, PA, [5]McGowan Institute for Regenerative Medicine, Pittsburgh, PA.

Bone is a frequent site for breast cancer metastasis. Conditioning of local tumor microenvironment through crosstalk between tumor cells and stromal cells in the metastatic niche is a major driving force for bone colonization of cancer cells. The objective of the present study was to determine the role of Myocardin-related transcription factor (MRTF - a major cofactor for the transcription factor serum-response factor, SRF) in bone colonization of breast cancer cells. We performed experimental metastasis assay to demonstrate that MRTF elevation dramatically increases bone colonization ability of breast

cancer cells *in vivo*. Either genetic disruption of SRF's interaction or small molecule inhibition of MRTF dramatically inhibited bone colonization of breast cancer cells *in vivo*. To gain mechanistic understanding, we next performed RNA-sequencing analyses of 3D cultures of breast cancer cells under genetic and pharmacological perturbations of MRTF, and compared differentially expressed genes in these settings against a 21-gene signature specifically associated with bone metastasis of breast cancer cells. These studies followed by confirmatory qRT-PCR and immunoblot analyses identified connective tissue growth factor (CTGF), a cell-secreted factor known for promoting osteoclastic differentiation/activity and osteolytic lesions by tumor cells, to be transcriptionally upregulated by MRTF in an SRF-dependent manner. These findings were further supported by bioinformatics-based confirmation for MRTF's positive association with CTGF expression in human breast cancer samples, and differential expression of CTGF between isogenic pairs of bone-colonization-competent *vs* incompetent breast cancer cell lines. Inhibition of the MRTF/SRF pathway suppresses osteoclast differentiation of primary bone-marrow derived monocytes in both tumor-intrinsic and -extrinsic manners. Based on these findings, we conclude that MRTF inhibition could be a novel strategy to suppress osteoclastic activity and skeletal involvement in metastatic breast cancer.

B244/P2636

Reprogrammed Schwann cells organize into dynamic tracks that promote pancreatic cancer invasion

S. Deborde[1], L. Gusain[1], A. Powers[1], A. Marcadis[1], Y. Yu[1], C.-H. Chen[1], A. Frants[1], E. Kao[1], L. Tang[1], E. Vakiani[1], M. Amisaki[1], A. Calo[2], V. Balachandran[1], T. Omelchenko[3], B. Reva[4], K. R. Jessen[5], R. J. Wong[1]; [1]Memorial Sloan-Kettering Cancer Center, New York, NY, [2]Institute for Bioengineering of Catalonia, Barcelona, Spain, [3]Rockefeller University, Rockefeller University, NY, [4]Mount Sinai Medical Center, New York, NY, [5]University College London, London, United Kingdom

Nerves are a component of the tumor microenvironment contributing to cancer progression, but the role of cells from nerves in facilitating cancer invasion remains poorly understood. Here we show that Schwann cells (SCs) activated by cancer cells collectively function as Tumor Activated Schwann cell Tracks (TASTs) that promote cancer cell migration and invasion. Non-myelinating SCs form TASTs and have cell gene expression signatures that correlate with diminished survival in patients with pancreatic ductal adenocarcinoma. In TASTs, dynamic SCs form tracks that serve as cancer pathways and apply forces on cancer cells to enhance cancer motility. These SCs are activated by c-Jun, analogous to their reprogramming during nerve repair. This study reveals a mechanism of cancer cell invasion that co-opts a wound repair process and exploits the ability of SCs to collectively organize into tracks. These findings establish a novel paradigm of how cancer cells spread and reveal therapeutic opportunities.

B245/P2637

Study of the adipokine resistin as a mediator of migration, invasion and secretion of extracellular vesicles in PC3 prostate tumor cells

M. Oregel Cortez, O. Galindo Hernandez; Universidad Autonoma De Baja California, Mexicali, B.C., Mexico

Accumulating evidence has reported that physiological levels of resistin increase tumor aggressiveness in prostate cancer (1). On the other hand, the literature presents evidence that cells are capable of secreting extracellular vesicles with various cargo molecules (2). Even tumor cells can secrete extracellular vesicles related to tumor progression in processes such as degradation of the extracellular

matrix, inhibition of apoptosis, angiogenesis and escape from the immune system (3). In this research, we used PC3 prostate cancer cells as a model. The cells were incubated and stimulated with different concentrations of resistin (10, 25, 50, 100 ng/ml). The migration and secretion processes of metalloproteinases were analyzed by wound closure and zymography assays, respectively. The enriched fraction of extracellular vesicles was obtained by differential ultracentrifugation, to later use them as a stimulus in prostate cell cultures. Cell invasion was analyzed using the Boyden chamber technique coated with matrigel. Our results show that resistin induces an increase in the migration and MMP-2 secretion in conditioned media of the PC3 cell line. Additionally, cells that receive treatment with extracellular vesicles obtained from conditioned media of PC3 cells stimulated with resistin, increase the invasive capacity. Thus, the results show that extracellular vesicles from PC3 cells treated with resistin significantly increase prostatic tumor progression in vitro. In summary, our findings demonstrate that extracellular vesicles obtained from resistin-stimulated conditioned media increase cell migration and invasion processes in the PC3 prostate tumor line. These data strongly suggest that extracellular vesicles secreted by PC3 induce migration and invasion. **References**1. Kim, H. J., Lee, Y. S., Won, E. H., Chang, I. H., Kim, T. H., Park, E. S., Kim, M. K., Kim, W., & Myung, S. C. (2011). Expression of resistin in the prostate and its stimulatory effect on prostate cancer cell proliferation. *BJU international*, *108*(2 Pt 2), E77-E83. https://doi.org/10.1111/j.1464-410X.2010.09813.x2. Cocozza, F., Grisard, E., Martin-Jaular, L., Mathieu, M., & Théry, C. (2020). SnapShot: Extracellular Vesicles. *Cell*, *182*(1), 262-262.e1. https://doi.org/10.1016/j.cell.2020.04.0543. Turturici, G., Tinnirello, R., Sconzo, G., & Geraci, F. (2014). Extracellular membrane vesicles as a mechanism of cell-to-cell communication: advantages and disadvantages. *American journal of physiology. Cell physiology*, *306*(7), C621-C633. https://doi.org/10.1152/ajpcell.00228.2013

Oncogenes and Tumour Suppressors-Signaling

B247/P2638

Caveolin-1 organization and function drive functional plasticity in Ewing Sarcoma

D. Segal[1], D. Rajendran[1], K. Bhatt[1], J. F. Amatruda[2], G. Danuser[1]; [1]Bioinformatics, UT Southwestern Medical Ctr, Dallas, TX, [2]Cancer and Blood Institute, Children's Hospital Los Angeles, Dallas, TX.

While it is widely accepted that plasticity of tumor cells drives cancer disease progression in the form of drug resistance and metastasis, most cellular mechanisms that drive plasticity have yet to be discovered. We uncover the scaffolding protein Caveolin-1 as a putative plasticity factor driving two distinct functional cell states in Ewing Sarcoma, a pediatric cancer of bone and soft tissue. Specifically, single-cell transcriptomics uncovers two transcriptionally distinct cell populations associated with differential expression of the cell surface glycoprotein CD99. Exploiting this CD99 expression level difference for flow-cytometry based isolation of the two cell states, we find distinctions in morphology, proliferation, and gene expression profiles tightly associated with differences in endogenous Caveolin-1 expression levels and spatial organization. Ultrastructure analysis reveals distinctions between the two cell states in presence of caveolae, cholesterol-rich membrane invaginations driven by Caveolin-1. Differences in Caveolin-1 expression and organization are also associated with differences in the oncogenic Receptor Tyrosine Kinase/AKT signaling pathway. Taken together, this work uncovers a link between Caveolin-1 function and organization of oncogenic signaling driving pathogenesis. Since previous work has suggested that mechanical cues trigger changes in Caveolin-1 localization and activity, future

experiments will scrutinize Caveolin-1 as a potential integrator of environmental cues and cell signaling that can drive cellular plasticity in Ewing Sarcoma.

B248/P2639

Targeting MAPK/Hippo Pathway Interactions in Metastatic Melanoma

N. K. Aboud, L. García-Gutiérrez, W. Kolch, D. Matallanas; University College Dublin, Dublin, IRELAND.

Background: RAF-1 kinase independent functions were shown to inhibit apoptosis by binding to the Hippo core kinase MST2. We previously identified small compounds that disrupt the RAF-1/MST2 interaction to promote apoptosis. Our current work is aimed at exploring the therapeutic potential of targeting the RAF-1/MST2 interaction in NRAS and BRAF mutant melanoma. Methods: First, we downregulated RAF-1 using siRNAs in various human melanoma cell lines such as the NRAS mutant (SKMEL2), the BRAF mutant (A375) and the BRAF inhibitor resistant (A375R) and levels of apoptosis were measured using flow cytometry. We proceeded to determine the effects of disrupting the RAF-1/MST2 interaction by utilizing the previously identified disruptors, VCC923573:16 and VCC199189:07. Protein-protein interactions were assessed by performing co-immunoprecipitation experiments and western blots. The level of apoptosis induced by treatment was analysed by flow cytometry. Furthermore, to measure pathways activated by the treatment, a time-course experiment was performed and changes in protein phosphorylation levels indicative of activation were detected using western blots. Finally, to test the efficacy of these disruptors in vivo we generated a zebrafish melanoma model with induced skin tumours driven by NRASQ61L or BRAFV600E mutations. Histological analysis of the zebrafish sections was performed after H&E staining, and the proteome of the collected tumours were studied using mass spectrometry. Results: We have found that RAF-1 downregulation is lethal downstream NRASQ61 signalling, while mutations in BRAFV600 overcome this effect. Interestingly, the secondary mutations acquired during BRAF inhibitor resistance seem to resensitize the cells towards RAF-1 downregulation. Consistently, treatment of the NRAS-driven melanoma cell line with RAF-1/MST2 disruptors activates the Hippo pathway and promotes apoptosis in a dose dependent manner. Furthermore, our in vivo results have revealed differential tumour onset and progression in zebrafish dependent on the tumour driver mutation and the proficiency of the Hippo core kinase LATS1. Conclusions: Our work highlights the importance of RAF-1 downstream NRAS and reveals the potential of targeting RAF-1 kinase independent interactions in melanoma. Future work will focus on further deciphering the disruptors' mechanisms of action and testing the effect of disrupting the RAF-1/MST2 interaction in our in vivo model.

B249/P2640

Optogenetic investigation into altered signaling code of Oncogenic KRAS by membrane clustering

A. Chandra[1], A. Venkitaraman[2], S. Mayor[1]; [1]National Center for Biological Sciences, Bangalore, INDIA, [2]Cancer Science Institute of Singapore, Singapore, SINGAPORE.

Small GTPase - Kras is a vital mediator of receptor-initiated signal transduction in human cells. Heterozygous activating point mutations that lock KRAS protein in an "active" GTP-bound state are amongst the common oncogenic lesions found in human cancers. How KRAS protein couple to receptor-triggered signals, and how oncogenic KRAS escape this regulation, remains a central unresolved problem in the field. Emerging evidence suggests that the homo- and hetero-typic interaction in nanoscale protein clusters at the plasma membrane drives Ras-mediated signaling. The central aim of this work is

to elucidate the mechanisms that underlie KRAS protein clustering in the plasma membrane, and its role in physiological and oncogenic signaling. Relationship between KRAS membrane clustering and signal output was analyzed using optically activable fluorophore-coupled KRAS proteins. This enabled microscopic analysis of Ras membrane dynamics in oncogenic signaling. Next, we focused on testing the hypothesis that dynamic actin filaments embedded in a more stable cortical actin meshwork organize membrane KRAS to regulate this relationship, and test its implications for the mechanism underlying oncogenic KRAS signaling.

B250/P2641

Trafficking and signaling abnormalities of the epidermal growth factor receptor EGFR variant III

S. Subramanian[1], R. Dey[1], S. Ramadesikan[2], R. C. Aguilar[1]; [1]Department of Biological Sciences, Purdue University, West Lafayette, IN, [2]The Steve and Cindy Rasmussen Institute for Genomic Medicine, Nationwide Children's Hospital, Columbus, OH.

Epidermal Growth Factor Receptor variant III ($EGFR^{viii}$) is found in several cancers such as glioblastomas (GB) (40% cases), head and neck squamous cell carcinoma (30%), prostate cancer (20%), breast cancer (20%) and squamous cell carcinomas (10%). In comparison to wild-type EGFR ($EGFR^{wt}$), $EGFR^{viii}$ lacks amino acids 6-273 due to an in-frame deletion which creates a junction between amino acid 5 and 274 along with the introduction of a novel glycine at the junction. Although this deletion affects a major portion of the extra-cellular domain including the EGF-binding domain, $EGFR^{viii}$ is considered to be constitutively active by a not fully understood molecular mechanism. This EGFR variant is an excellent candidate for targeted therapy since it is found only in tumors and not in normal tissue. All this emphasizes the importance of understanding the basic biology behind the trafficking and signaling pertinent to $EGFR^{viii}$.Here we showed that cells expressing GFP-labeled $EGFR^{viii}$ (and very low levels of $EGFR^{WT}$) exhibited weak binding of tetramethyl-rhodamine labelled EGF. Nevertheless, $EGFR^{viii}$ was efficiently internalized and participated in signal transduction by combined action with other receptor tyrosine kinases (including $EGFR^{wt}$) presumably by forming heterodimers.
Furthermore, $EGFR^{viii}$ triggered the assembly of a unique and previously unnoticed large, intracellular compartment. Immunofluorescence with anti-EEA1 (early endosome marker) and anti PDIA3 (ER marker) revealed nearly 100% colocalization of these markers with GFP-$EGFR^{viii}$, indicating an ER and endosomal mixed nature of the $EGFR^{viii}$ compartment.
To test the potential role of ligand binding in compartment assembly, we treated cells expressing $EGFR^{viii}$ with different growth factors. Our studies showed that these compartments disassembled in the absence of serum/EGF ligand and reassembled when stimulated with growth factors as a function of time and dosage. We showed that as early as 4-8 of stimulation triggered the assembly of $EGFR^{viii}$ containing compartments. We speculate that these large, and unique intracellular $EGFR^{viii}$-positive compartments possessing an early endosomal nature could be sites for endosomal signaling. It has been proposed that $EGFR^{wt}$ can form heterodimers with $EGFR^{viii}$. Thus, it will be crucial to understand the role of co-expressing $EGFR^{wt}$ and characterize the compartment assembly and downstream signaling events.This project will help understanding the molecular mechanism by which the $EGFR^{viii}$ variant subverts intracellular physiology and lay the foundation for future novel cancer therapies.

B251/P2642

Serine/Threonine kinase STK25 negatively regulates mTORC1 activity to suppress tissue overgrowth and tumorigenesis

R. Ho[1], S. Lim[2], N. Ganem[3]; [1]Molecular and Translational Medicine, Boston University School of Medicine, Boston, MA, [2]Mayo Clinic Rochester, Rochester, MN, [3]Pharmacology, Boston University School of Medicine, Boston, MA.

The maintenance of cellular and tissue homeostasis relies on the tight regulation of cell growth and proliferation. These processes are predominantly regulated by two evolutionarily conserved pathways: the mammalian Target of Rapamycin (mTOR) pathway and the Hippo pathway. Dysregulation of both the mTOR and Hippo pathways promotes aberrant cell proliferation, tissue overgrowth and tumorigenesis. Elucidating how the mTOR and Hippo signaling pathways coordinate their activities is therefore a vitally important, yet poorly understood, area of cell cancer biology. Our lab previously demonstrated that STK25, a serine/threonine kinase, functions as an upstream activator of Hippo signaling to limit cell growth. We now demonstrate that STK25 also functions to regulate mTORC1 signaling. We observe that genetic deletion of *STK25* hyperactivates the mTOR pathway, both *in vitro* and *in vivo*. To elucidate the molecular mechanisms of STK25, we utilized the BioID2 proximity labeling system and comparative LC MS/MS analysis to identify a list of proteins that interact significantly with STK25. Based on those findings, we propose that STK25 disrupts the stability of the mTORC1 protein complex by interfering with the CCT/TRiC chaperone protein complex, ultimately attenuating mTOR signaling. Furthermore, using a conditional *STK25* knockout mouse model, we found that deletion of *STK25* alone is sufficient induce tumorigenesis. Taken together, our data suggests that STK25 plays a critical role in modulating cell growth and tumor suppression by negatively regulating the mTORC1 pathway. This would establish STK25 as an upstream regulator of both the Hippo and mTOR pathways, providing further insights to the coordinated regulation of these two crucial pathways.

B252/P2643

GOLPH3 protein controls organ growth by interacting with TOR signaling proteins in Drosophila.

A. Frappaolo, A. Karimpour-Ghahnavieh, S. Sechi, **M. Grazia Giansanti**; Istituto di Biologia e Patologia Molecolari, Italian National Research Council (CNR), Roma, ITALY.

GOLPH3 (Golgi phosphoprotein 3) is an evolutionarily conserved phosphatidylinositol 4-phosphate effector, mainly localized to the Golgi apparatus, where it supports organelle architecture and vesicular trafficking. Overexpression of GOLPH3 correlates with poor prognosis in several cancer types and is associated with enhanced signaling downstream of mTOR (mechanistic target of rapamycin). However, the molecular link between GOLPH3 and mTOR remains elusive. Studies in *Drosophila melanogaster* have shown that Translationally controlled tumor protein (Tctp) and 14-3-3 proteins are required for organ growth by supporting the function of the small GTPase Ras homolog enriched in the brain (Rheb) during mTORC1 (mTOR complex 1) signaling. We show that *Drosophila* GOLPH3 (dGOLPH3) physically interacts with Tctp and 14-3-3ζ. Knockdown of dGOLPH3 reduces wing and eye size and enhances the phenotypes of *Tctp* RNAi. Moreover, the enlargement of the wings caused by Rheb overexpression is suppressed by *dGOLPH3* RNAi. We also show that Golgi localization of Rheb in *Drosophila* cells depends on dGOLPH3. Consistent with dGOLPH3 involvement in mTORC1 activation, depletion of dGOLPH3 reduces levels of phosphorylated ribosomal S6 kinase, a downstream target of mTORC1. Overall, our

data provide the first *in vivo* demonstration that GOLPH3 regulates organ growth by directly associating with mTOR signaling proteins.

B253/P2644

RAI14 Drives Pancreatic Tumorigenesis and Supports Proliferative Mesenchymal Tumor Cells

F. Runa[1], L. Tomaneng[1], Y. Adamian[1], C. Nathan[2], C. Gonzalez[1], J. Hong[1], K. Bhakta[1], M. Boyce[2], J. Kelber[1]; [1]Biology, California State University Northridge, Northridge, CA, [2]Biochemistry, Duke University, Durham, NC.

Pancreatic Ductal Adenocarcinoma (PDAC) accounts for the vast number of malignant tumors in the pancreas. It is a highly lethal solid tumor estimated to be the second-leading cause of cancer-related death in the United States by 2030. Hallmarks of PDAC include post-metastasis diagnosis and therapy resistance. To identify new mechanisms underlying the aggressive nature of this cancer we previously identified roles and mechanisms of action for pseudopodium-enriched atypical kinase 1 (PEAK1) and integrin α1 (ITGA1) as pseudopodium-enriched (PDE) proteins during PDAC progression. Here, we identify a new PDE protein, retinoic acid induced 14 (RAI14, Ankycorbin or NORPEG), that is a constituent of the KRasG12D PDAC cell autonomous phosphoproteome, localizes to cytoskeleton/adhesion domains in PDAC cells and predicts poor patient outcomes. Knockdown or knockout of RAI14 in KRas mutant PDAC cells reduced adhesion-dependent proliferation/survival *in vitro* and abrogated the ability of PDAC cells to form tumors *in vivo.* To identify signaling pathways and potential vulnerabilities that depend upon RAI14, we used cyclic immunofluorescence (CycIF). Interestingly, we discovered that RAI14 is necessary to support a proliferative mesenchymal PDAC cell subpopulation. These data combined with the output of a RAI14-focused bioinformatics pipeline revealed a targetable vulnerability centered around PLK1-mediated mitosis that may be leveraged to sensitize PDAC cells to therapeutic intervention and improve patient outcomes.

B254/P2645

Characterization of gamma-glutamyltransferase 6 protein in breast cancer

G. Peguero Kushner, C. Smith, Z. Li, B. Papp; University of Florida, Gainesville, FL.

Objectives: We recently identified Gamma-glutamyltransferase 6 (GGT6) as a protein required for oncogenic virus lytic reactivation. Once activated, Gamma-Glutamyl-Transpeptidase family (GGT) proteins cleave gamma-glutamyl bonds to maintain intracellular cysteine and glutathione homeostasis. GGT6 was predicted to lack enzymatic activity, and it remains an uncharacterized member of the GGT family. Bioinformatics studies report decreased GGT6 in various subtypes of cancers and this loss is associated with poor survival and increased tumorigenesis markers. Since breast cancer (BC) is one of the leading causes of death in women and GGT6 has not been investigated in this context, we aim to characterize GGT6 and evaluate its potential dysregulation in BC progression by assessing expression levels and protein modifications as well as evaluate its potential as a biomarker. **Methods:** To characterize GGT6 protein we transfected 293T cells with mammalian expression vectors encoding full length GGT6 open reading frame or cDNA derived GGT6 isoforms. To study the half-life of GGT6, GGT6 positive cells were treated with the protein synthesis inhibitor cycloheximide. To evaluate our predicted N-linked glycosylation, enzymatic treatment was used for removal of N-linked oligosaccharide chains. We used bioinformatics, RT-qPCR, and immunoblot analysis to compare GGT6 expression with epithelial or mesenchymal markers across mammary tissues, breast cancer subtypes and BC lines. Kaplan-Meier

survival plots were generated to assess whether GGT6 expression correlates with survival in subtypes of BC. **Results:** GGT6 has a shorter half-life compared to the other GGT proteins and endogenous GGT6 is glycosylated in T47D and MDAMB468 BC cells. High GGT6 expression positively correlated with epithelial markers (such as EpCAM1) but negatively correlated with mesenchymal markers (such as ZEB1) at the mRNA and protein level, suggesting GGT6 was expressed in epithelial-like cells but decreased in mesenchymal subtypes. Immunoblots revealed a smaller GGT6 derived N-terminal fragment in the cells suggesting that GGT6 protein might be processed. Data mining revealed GGT6 to be significantly downregulated in the more aggressive mesenchymal subtypes compared to normal tissue. High GGT6 transcript levels correlate with increased survival in Luminal A and estrogen receptor positive subtypes which have better prognostic outcomes. **Conclusion:** GGT6 is a N-linked glycosylated protein with a short half-life and high expression in mammary cells with epithelial features, and associates with better prognosis in breast cancer.

B255/P2646

Optimization of M13 bacteriophage-mediated gene delivery for cancer therapy

C.-Y. Kao, Y.-C. Pan, Y.-H. Hsiao, K. Mou; Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

Gene therapy based on viral vectors is a promising modality to treat a variety of diseases, including cancers. Current limitations of viral vectors include pre-existing neutralization antibodies in patients; the high cost of clinical-grade production; and poor cell-type specificity. Here, we aim to engineer the M13 bacteriophage as an efficient gene delivery vehicle. We displayed various epidermal growth factor receptor (EGFR) ligands on the M13 pIII capsids and encapsulated the green fluorescent protein gene for the measurement of transduction efficiency in human cells with different levels of EGFR expression. The M13 bacteriophages displaying high-affinity EGFR ligands showed the binary transduction efficiency depending on the EGFR levels in cells. Additionally, we hypothesized that the human protein PrimPol might facilitate the conversion of single-stranded DNA to double-stranded DNA and enhance transgene expression. Indeed, the M13 bacteriophage resulted in higher transgene expression in the human cells over-expressing PrimPol. To investigate other possible cellular determinants for bacteriophage transduction, we performed the transcriptome analysis between two cell populations with high and low transduction efficiencies. The data revealed a skewed distribution with many transcripts down-regulated in high-transduction cells, including many viral defending proteins. Furthermore, we hypothesized that the M13 bacteriophage size might influence transduction efficiency. We generated a series of M13 bacteriophages from 300 nm to 1000 nm. We observed an anti-correlation between the M13 bacteriophage length and the transgene transduction efficiency. With properly re-structuring the M13 origin, we designed an optimal M13 bacteriophage, which dramatically improved the transduction efficiency to the levels comparable to the adeno-associated viral vectors. On the basis of our optimal bacteriophage design, we delivered several therapeutic genes, such as N-terminal gasdermin D (GSDMD) and membrane-anchored fragment crystallizable region (Fc), to test their anti-tumor activities. Delivery of the N-terminal GSDMD greatly induced pyroptosis in cancer cells. The membrane-anchored Fc gene delivered by the bacteriophage successfully enable the cell killing by natural killer cells. Overall, our engineered M13 bacteriophage achieved an unprecedented transduction efficiency in human cells and holds a great potential for the next-generation gene therapy.

B256/P2647

The Drosophila Accessory Gland: a model for the study of tumor-promoting features in non-dividing multinucleated polyploid cells.

S. Church, A. Box, L. Buttitta; University of Michigan, Ann Arbor, MI

Polyploid multinucleated tumor cells are believed to be a main source of tumor recurrence and resistance against chemotherapy and irradiation in numerous cancers. Here we describe a Drosophila-based model for the study of polyploid multinucleated cells. The accessory glands of male Drosophila are the fly functional analog of the human prostate. The cells of the accessory gland are post-mitotic, binucleated, and polyploid. Using this model, we examined how activating fly versions of oncogenes associated with prostate cancer, such as Myc and Yki, the fly homolog of YAP, affect the accessory gland epithelium. We find that overexpression of these oncogenes induces cellular hypertrophy, nuclear anaplasia, loss of proper epithelial polarity, and endocycling in the Drosophila accessory gland. Additionally, oncogene activation recapitulated features of cancer such as upregulation of beta-integrin, expression of matrix metalloproteinases 1 (MMP1), upregulation of phosphorylated MAPK, as well as apical and basal extrusions, and alterations to glandular morphology, all in the absence of mitosis. We also observe non-autonomous effects of oncogene activation in neighboring cells, demonstrating the power of this model for examining complex tumor vs. non-tumor heterogeneity in cancer development. Our work provides a novel model to study tumor-promoting features of non-dividing polyploid multinucleated cells under oncogenic conditions.

B257/P2648

GGPPS as a therapeutic target in cancer

S. Mitra, P. Devreotes; Johns Hopkins University Sch Med, Baltimore, MD

Cancer cells have unique metabolic demands that can be harnessed to provide novel therapeutic approaches. Recently, the mevalonate pathway has been implicated in supporting tumorigenesis. Statins target this pathway and are typically used for lowering cholesterol. But these drugs target the entire mevalonate pathway and the effects on cancer cells have been traced to depletion of GGPP, a downstream intermediate. GGPP is required to geranylgeranylate a network of signal transduction proteins, like small GTPases, which are essential for macropinocytosis and cell motility. These processes are upregulated in cells with oncogenic mutations, making such cells selectively sensitive to GGPP depletion. The goal of this proposal is to identify the enzyme that synthesizes GGPP in cells, geranylgeranyl transferase synthase (GGPPS), as a therapeutic target in cancer. Towards this goal, I will investigate the significance of the enzyme in cell survival and transformation, explore the structure-function relation of the enzyme to identify key residues for enzyme function, and explore the mechanism of its localization, regulation, and dynamics in cells

B258/P2649

“Altering FAK-paxillin interaction: a potential therapeutic strategy to target tumor growth and metastasis”

M. Christoforou, A. Charalambous, P. Skourides; University of Cyprus, Nicosia, Cyprus

Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that is implicated in several cellular functions including cellular adhesion, motility, proliferation and survival. Interestingly, FAK is

overexpressed and activated in primary or metastatic cancers and both its kinase-dependent and kinase-independent scaffolding functions facilitate cancer progression and metastasis. Therefore, FAK is considered an attractive drug target for cancer therapy. Several small FAK inhibitors are currently undergoing clinical trials, but so far these have been met with limited success and none of them has been approved and launched in the market. Our group has developed a new strategy for the inhibition of FAK, successfully targeting both kinase dependent and independent functions of FAK at the same time. The strategy relies on the displacement of FAK from Focal Adhesions (FAs), using a small peptide to compete with interactions taking place between the LD motifs of paxillin and the hydrophobic pockets of the FAK FAT domain, shown to be necessary and sufficient for FAK´s FA targeting. We have previously shown that a short peptide dimer containing the LD2 and LD4 motifs of paxillin is sufficient to mask interactions taking place at the HPs on FAK's FAT domain and lead to the elimination of endogenous FAK from FAs. *In vitro* experiments demonstrate that the LD2-LD4 peptide inhibits FAK/Src signaling at FAs and results in the dramatic reduction of tumor cell migration and invasion. We now show that LD2-LD4 also targets proline-rich tyrosine kinase 2 (PYK2), that is also overexpressed in cancerous tissues and stimulates multiple oncogenic signaling pathways. This further enhances its anti-metastatic potential as a dual FAK/PYK2 inhibitor. We have examined the efficacy of this newly developed strategy for the inhibition of FAK and PYK2 by exploring the impact of LD2-LD4 on tumor growth and progression in mice. Collectively, data from our pre-clinical experiments validate the *in vitro* results and indicate that LD2-LD4 expression can inhibit tumor growth. Most importantly, LD2-LD4 had a dramatic impact on tumor progression and metastasis nearly abolishing the appearance of metastatic tumors, highlighting the potential of this newly developed strategy as a therapeutic approach against cancer and metastasis.

B259/P2650

Characterizing Variants of Undetermined Significance in the BRCA Repair Pathway

K. Anglin; Prairie View A&M University, Prairie View, TX.

"Pathogenic" or "Likely Pathogenic" germline mutations in DNA repair genes such as Brca1, Brca2, and Palb2 in the BRCA repair pathway can lead to an increased risk of developing breast, pancreatic, and ovarian cancers. Other mutations may be found in the patient population, yet whether they play a role in increasing the risk of developing cancer is unknown; these are called Variants of Undetermined Significance (VUS). The impact of these variants' expression on cell proliferation, apoptosis, oxidative stress factors, and internalization in certain cell types are unclear. Further, several questions remain about the prevalence and impact of DNA repair gene variants in certain tissues. Characterizing the phenotypic impacts of these variants in different cell-based models will provide more information about relevant molecular targets. The aim of this project is to investigate the role of DNA repair gene variants using cell-based and functional assays. We hypothesize that knockdown of the DNA repair gene *Palb2* will lead to a decline in homologous recombination in a cervical cancer cell line. To test the hypothesis, DOTC2-4510 cells were grown in complete culture medium at 37°C. Following expansion, $2.5x10^5$ cells were plated onto culture well-plates. Brca2 and/or Palb2 were overexpressed using an expression vector, or Palb2 was silenced using siRNAs. Cells were maintained for 48 hours and extracted lysates were assessed for expression of wild type Palb2, Brca2, and gamma H_2ax. Knockdown of Palb2 in DOTC2-4510 cells will lead to a decrease in genomic stability, as observed with gamma H_2ax expression. We will carry out additional cell-based assays in the presence of clinical variants of DNA repair genes,

including *Brca1*, *Brca2* and *Palb2* in DOTC2-4510 cells. We will also perform co-immunoprecipitations to look for resultant binding partners of Palb2 in DOTC2-4510 cells.

B260/P2651

Expression of oxidative stress-related genes in triple negative breast cancer cell lines derived from African American and European American women

Z. Robinson, J. Francois, C. Jeun, R. Minter, A. Mazza, K. E. Miletti-Gonzalez; Delaware State University, Dover, DE

Breast cancer is the most frequently diagnosed cancer among African American (AA) women and among the different types of this cancer, triple negative breast cancer (TNBC) is an aggressive breast cancer with the worst prognosis due to the absence of three key therapeutic targets, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). TNBC, which comprises 15-20% of all breast cancers, is disproportionately higher in AA women compared to European American (EA) women. For this reason, we compared the gene expression of oxidative stress-related genes in two groups of TNBC cell lines, one group derived from AA (group AA, cell lines HCC70 and HCC1806) and the other group derived from EA women (group EA, cell lines HCC38 and HCC1143), before (control) and after treating the cells with hydrogen peroxide. We hypothesize that these two groups of TNBC cell lines will have a differential cellular response to oxidative stress caused by hydrogen peroxide as well as a differential gene expression in oxidative stress-related genes. These four cell lines were treated with hydrogen peroxide from 2 mM to 0.0078 mM. MTS cell viability assays were performed after 24 hours of treatment and total RNA was extracted, sequenced (RNA-seq) and bioinformatically analyzed. The group AA TNBC cell lines resulted to be more resistant to oxidative stress caused by hydrogen peroxide than the group EA TNBC cell lines. The RNA-seq generated gene expression profile revealed several differentially expressed genes involved in glutathione synthesis (e.g., GLS2, SLC7A11), reactive oxygen species scavenging (e.g., CAT, GPX2) and xenobiotics detoxification (e.g., ALDH3A1, UGT1A6). Among them, glutathione peroxidase 2 (GPX2) was the highest differentially expressed gene with a fold change of 274 times higher in untreated group AA compared to group EA TNBC cell lines. These results suggest a correlation between the origin of the TNBC cell lines used and their response to oxidative stress caused by hydrogen peroxide. Because CD44 is highly expressed in TNBC cells and has been reported to have a prognostic role in TNBC, we propose future experiments to investigate the expression of CD44 in these cell lines and a potential role in oxidative stress-related gene regulation.

B261/P2652

Annotation of BARD1, A Familial Ovarian Cancer Gene?

D. Holmes, G. Regisford; Prairie View A&M University, Prairie View, TX.

Familial ovarian cancer is a genetic phenomenon in which a family lineage is genetically predisposed to ovarian cancer. Ovarian cancer is the 4th most deadly cancer-related gynecological disease mainly because it is detected during the latter stages of disease progression. The main signifiers of ovarian cancer are the mutations of the BRCA1 and BRCA2 genes. However, a less characterized gene called BRCA1 Associated RING Domain 1 (BARD1) has been implicated to predict predisposition to hereditary forms of this deadly cancer. Therefore, the objective of this study was to annotate and characterize the BARD1 gene in silico. We hypothesized that the BARD1 gene is a functional protein-coding gene that

may lead to familial ovarian cancer when mutated. In testing this hypothesis, we used the GeneCards and National Center of Biotechnology Information (NCBI), University of California Santa Cruz (UCSC) and Ensembl bioinformatics databases, as well as the Basic Local Alignment Search Tool (BLAST) to investigate the location, structure, expression pattern, and function of the BARD1 gene. According to the GeneCards and UCSC bioinformatics database, the BARD1 gene is located on chromosome 2q35, is approximately 84kb in length, and has a minus-strand orientation. BARD1 is expressed in several tissues, including reproductive tissues such as the testis, prostate, breast, uterus, and ovaries. The NCBI and Ensembl databases and BLAST indicated that orthologs of BARD1 are found in mice (Mus musculus), chickens (Gallus gallus), lizards (Anolis carolinensis), African clawed frogs (Xenopus laevis), and Zebrafish (Danio rerio). However, there are no known paralogs of BARD1. Diseases associated with mutations of the BARD1 gene are breast cancer along with hereditary breast and ovarian cancer syndrome. These results indicate that BARD1 is a functional protein-coding gene, and its mutations may be associated with familial ovarian cancer. Future studies will determine the effect of photothermal therapy (PTT) and radiation on the expression of BARD1 in several ovarian cancer cell lines, in vitro.

B262/P2653

Assessing cancer cell senescence as a novel anti-cancer therapy for pancreatic cancer

K. Morris, S. Cole, P. Vaughan, K. Vaughan; University of Notre Dame, Notre Dame, IN

Pancreatic ductal adenocarcinoma (PDAC) has the lowest 5-year survival rate of any major cancer; just 10% in 2022. Gemcitabine (GEM) is the current standard of care for the treatment of PDAC, however, it is a cytotoxic chemotherapy that is relatively ineffective and comes with harsh side effects such as fever, nausea, and extreme fatigue due to the cytotoxic nature of the drug. **Alternative approaches are desperately needed to increase overall survival and reduce the suffering of PDAC patients**. In recent years, there has been a surge of interest in immune activation for the treatment of cancer, however, thus far no such treatments have been discovered for PDAC. Recently, our lab discovered a novel treatment pathway for PDAC that works through induction of nuclear blebbing and cellular senescence, opening the door to a completely new therapeutic approach. The combination (EpoAurorin) of EpoB and an Aurora B Kinase inhibitor, prevents phosphorylation of lamin A by Aurora B. When de-phosphorylated, lamin A can no longer bind to phospho-Barrier-to-autointegration factor (pBAF), causing to nuclear blebbing. As nuclear blebbing often leads to micronuclei and DNA in the cytosol, a treatment with this approach could activate the cGAS-cGAMP-STING pathway to bring the innate immune system to fight the cancer. Targeting cancer by inducing cellular senescence and activating the immune system provides many benefits over current treatment regimens including growth prevention without the harsh side effects common for cytotoxic chemotherapies. While further investigating the mechanism, we became interested in the BAF half of the pathway and identified vaccinia-related kinase 1 (VRK1) as the BAF kinase responsible for the association of phospho-BAF and phospho-lamin A. Luteolin, a natural flavonoid, has been identified as a VRK1 inhibitor, however, its potency and absorption are very low. Through the synthesis of a library of luteolin analogues, we have been searching for a novel compound that has the same effects as luteolin at lower dosages. This compound can then be used a lead compound for the therapeutic development of a VRK1 inhibitor for the treatment of PDAC.

Gene Structure and Transcription

B264/P2654

Single-molecule tracking reveals two low-mobility states for chromatin and transcriptional regulators within the nucleus

K. Wagh*[1,2], D. A. Stavreva*[1], R. A. M. Jensen[1,3], V. Paakinaho[1,4], G. Fettweis[1], R. Schiltz[1], D. Wüstner[3], S. Mandrup[3], D. M. Presman[1,5], A. Upadhyaya[2,6], G. L. Hager[1]; [1]Lab of Receptor Biology and Gene Expression, National Cancer Institute, NIH, Bethesda, MD, [2]Department of Physics, University of Maryland, College Park, MD, [3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, DENMARK, [4]Institute of Biomedicine, University of Eastern Finland, Kuopio, FINLAND, [5]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-Universidad de Buenos Aires, Buenos Aires, ARGENTINA, [6]Institute for Physical Science and Technology, University of Maryland, College Park, MD.

Transcription factors (TFs) scan the nucleus in search of their consensus binding motifs located within enhancers or promoter-proximal regions. The mechanism by which TFs navigate the complex nuclear environment to assemble the transcriptional machinery at specific genomic loci remains elusive. Using single-molecule tracking, coupled with machine learning, we examined the mobility of multiple transcription factors and coregulators. We show that chromatin (labeled by histone H2B), steroid hormone receptors, as well as other transcriptional coregulators, architectural proteins, and remodelers, all display two distinct low-mobility states. Our results indicate that both low-mobility states are intimately coupled with mobile chromatin. Ligand activation results in a dramatic increase in the proportion of steroid receptors in the lowest mobility state. Mutational analysis revealed that chromatin interactions in the lower mobility state require an intact DNA-binding domain as well as domains important for forming protein complexes with other binding partners. These domains are not necessary for engagement with the higher mobility fraction of chromatin. Importantly, these states are not spatially separated as previously believed but in fact, individual H2B and TF molecules can dynamically switch between them. Together, our results identify two unique and distinct low-mobility states of transcriptional regulators that appear to represent common pathways for transcription activation in mammalian cells.

* Equal contribution

B265/P2655

Dynamic mechanisms of the Notch transcriptional response

J. M. Rogers[1], B. Guo[1], E. D. Egan[1], J. C. Aster[2], K. Adelman[1], S. C. Blacklow[1]; [1]BCMP, Harvard Medical School, Boston, MA, [2]Department of Pathology, Brigham and Women's Hospital, Boston, MA.

Cells must enact specific gene expression programs in response to signal activation in order to establish and maintain identity. The transcriptional response to Notch signaling relies on co-activators of the MAML family, which are thought to recruit additional co-activators (e.g. p300, chromatin remodelers) to stimulate transcription. In previous work, I showed that different Notch responsive genes depend on different portions of MAML1 for transcription in Jurkat T-ALL cells (Rogers, Guo et al 2020). In particular, HES4 is dependent only on TAD1 of MAML1, which is thought to recruit p300, whereas DTX1 requires both TAD1 and TAD2. Additionally, Notch dependent HES4 expression can be supported by direct fusion of the p300 HAT domain to the NTC-binding portion of MAML1, but DTX1 expression cannot, indicating

the need for recruitment of other co-factors. The differences between these two model target genes motivate the importance of expanding mechanistic studies to a genome-wide context.
To that end, I used a recently developed cellular system for Notch activation, in which a mutated and truncated form of Notch1 (ΔEGF-L1596H) is expressed in a squamous cell carcinoma cell line that lacks endogenous Notch1 (Pan et al 2020). Because nuclear access of this Notch protein is ligand-independent and gamma-secretase dependent, the cells can be maintained in a Notch-naïve state with a gamma-secretase inhibitor (GSI) and activated by GSI washout, allowing for time resolved investigation of how the genome responds to the introduction of Notch. In this system, I have performed Precision Run-on sequencing (PRO-seq) over a fine time course to identify the transcriptional response to Notch activation. Analyses of these data reveal clusters of genes with distinct temporal responses to Notch activation - genes that respond quickly, those that respond slowly, genes that are transiently expressed, and even genes that are downregulated - highlighting the heterogeneity in the dynamic responses to signal induction. PRO-seq data also enables identification of active enhancers that regulate gene expression. Further integration of ChIP-seq and ATAC-seq datasets will enable a more high- resolution mechanistic understanding of the Notch transcriptional response.
Rogers JM, Guo B, Egan ED, Aster JC, Adelman K, Blacklow SC. MAML1-dependent Notch responsive genes exhibit differing co-factor requirements for transcriptional activation. Mol Cell Biol. 2020 Mar 16. pii: MCB.00014-20. doi: 10.1128/MCB.00014-20.
Pan L, Lemieux ME, Thomas T, Rogers JM, Lipper CH, Lee W, Johnson C, Sholl LM, South AP, Marto JA, Adelmant GO, Blacklow SC, Aster JC. *IER5*, a DNA damage response gene, is required for Notch-mediated induction of squamous cell differentiation. eLife 2020;9:e58081 DOI: 10.7554/eLife.58081

B266/P2656

Transcription is a key regulator of nuclear bleb formation, stabilization, and rupture, but not nuclear mechanics

I. K. Oder[1], M. L. Currey[2], S. Gupta[3], Y. Berrada[2], B. Nguyen[1], J. M. Schwarz[3,4], E. J. Banigan[5,6], **A. D. Stephens**[2,1]; [1]Molecular and Cellular Biology, University of Massachusetts Amherst, Amherst, MA, [2]Biology Department, University of Massachusetts Amherst, Amherst, MA, [3]Department of Physics and BioInspired Syracuse, Syracuse University, Syracuse, NY, [4]Indian Creek Farm, Ithaca, NY, [5]Department of Physics, Massachusetts Institute of Technology, Boston, MA, [6]Institute of Medical Engineering & Science, Massachusetts Institute of Technology, Boston, MA.

Chromatin is an essential component of nuclear mechanics and shape that maintains nuclear compartmentalization and function. It is well established that the biophysical properties of chromatin alter nuclear shape stability, but little is known about whether and how major genomic functions can impact the integrity of the nucleus. We hypothesized that transcription might affect the physical properties of the cell nucleus through its effects on chromatin structure and dynamics. To test the effects of transcription, we inhibited transcription with the RNA Pol II inhibitor alpha amanitin in wild-type cells and cells treated with valproic acid (VPA), which decompacts and softens chromatin and increases nuclear blebbing and rupture. We observed that transcription inhibition suppresses nuclear blebbing for several cell types, histone modification profiles (drug treatments), and transcription inhibitors. However, transcription inhibition does not alter chromatin histone modification state or nuclear rigidity, which are mechanisms for nuclear blebbing. Instead, transcription appears to be necessary for nuclear bleb formation, stabilization, and bleb-based nuclear ruptures. Our data reveal that active/phosphorylated RNA Pol II ser5, marking transcription initiation, is enriched in nuclear blebs

relative to DNA and the transcription elongation marker RNA Pol II phosphorylated at ser2 . Thus, transcription initiation is a structural hallmark of nuclear blebs, and it is vital to forming a nuclear bleb. Polymer simulations suggest that motor activity, such as that of RNA Pol II, within chromatin can generate active forces that deform the nuclear lamina, and that nuclear deformations depend on motor dynamics. Our data provide evidence that the genomic function of transcription impacts nuclear shape stability, and suggests a novel mechanism, separate and distinct from chromatin rigidity, for regulating large-scale nuclear shape and function.

B267/P2657

Enhancer and promoter coordination of transcription bursting and heterogeneity

F. Chapus[1,2], C. Day[3,2], J. Rodriguez[4,2]; [1]Fleur Chapus, Durham, NC, [2]Escbl, NIEHS, NIH, Durham, NC, [3]Christopher Day, Durham, NC, [4]Joseph Rodriguez, Durham, NC.

Intra-tumoral gene expression heterogeneity is a roadblock toward effective cancer treatment due to subpopulations acquiring resistance and metastatic ability. This expression heterogeneity can be explained by the stochastic nature of transcription. Transcriptional initiation is coordinated through distal elements known as enhancers. These elements function *via* proximity which aids in recruitment of cofactors and chromatin remodelers. During transcriptional activation, genes are transcribed in episodic bursts of nascent RNA synthesis. Moreover, heterogeneously expressed genes exhibit variably long periods of transcriptional inactivity. Given that enhancers are key elements of transcriptional activation, how they coordinate transcriptional bursting of target genes remains unclear. Here, we use single molecule RNA FISH and live cell imaging to decipher the coordination between enhancer activity and transcription burst initiation. We observe coupling between the transcription of the estrogen-responsive gene TFF1 and its enhancer transcription upon estrogen stimulation. Moreover, TFF1 enhancer RNA sporadically form condensates correlated with an increase of TFF1 mRNA content. To accurately dissect the temporal relationship between enhancer and promoter activity, we are using 2-color live cell RNA imaging to monitor endogenous enhancer and promoter activity of several enhancer-gene pairs. Our work highlights the dynamic coupling of enhancer and promoter regulation of estrogen responsive genes and its implications on expression heterogeneity.

B268/P2658

Defining nanoscale genetic interactions of bacteria with engineered nanoparticles

T. Noussi, D. Lajeunesse PhD; Nanoscience, UNC Greensboro, Greensboro, NC.

Engineered nanoparticles have distinct physicochemical properties which enable their utilization in a variety of sectors from Biomedicine to Environmental remediation. Engineered nanoparticles interact with biological systems potentially changing the behavior of these systems by inducing specific physiological and metabolic modifications within the exposed organisms; furthermore, these responses to engineered nanoparticles may also differ from one organism to another. While a great deal of work has been done to identify lethal/antimicrobial nanomaterials to control pathogenic microbial biofilm formation, little work has been done to study non-lethal impact of nanomaterials of microbes. Moreover, the genetic impact of this cell-nanoparticle interaction is not well understood. We have defined the transcriptional genetic response of the gram-negative bacterium Escherichia coli (E. coli) to three different engineered silica, gold, and polystyrene nanoparticles. Screening an E. coli reporter gene library that covers 70% of the E. coli genome, we have identified eights genes that are upregulated in

response to nanoparticle exposure and possibly represent a common nanoparticle response mechanism. These eight genes have been verified using qRTPCR and include previously identified stress response genes (rssB, evgA, sodC,) genes encoding transports (yhdY, yhhT) and several genes with unknown function (glcC, vacJ/MlaA, cysQ). The gene ontology of the eight genes shows that metabolic pathways, signal transduction pathways, oxidative stress and protein transport systems are significantly affected in response to the three nanoparticle exposures. These results demonstrate that there is specific common response of E. coli to round-shaped metal oxide, metal, and polymeric nanoparticles. Overall, this research will provide a better understanding of bacteria stress response as well as bacterial resistance to nanoparticle-based antibiotics and identify potential new targets for drugs given that the bacteria-nanoparticle interactions have crucial implications in public health and the environment.

B269/P2659

Each individual promoter has a small region with a distinctive physical property

K. UEMURA, I. Hattori, T. Ohyama; Waseda University, Tokyo, JAPAN.

We previously reported that TATA box and initiator (Inr) sequences comprise distinctively flexible and rigid sequences when compared with the other parts of the promoter region (Fukue *et al*., *Nucl. Acids Res*., 2004). Interestingly, the same physical property lies in the transcription start site (TSS) in the case of "core-less" promoters that do not contain any known promoter elements (Fukue *et al*., *Nucl. Acids Res*., 2005). In addition, recently we found that the same is true for non-coding RNA promoters (Uemura *et al*., ASCB/EMBO meeting 2019). However, these findings were based on the averaged physical properties of promoters. Thus, it remains unsolved whether each individual gene has and uses such a distinctive physical property for transcription. The current study examined this issue. Using the data set on "high-confidence TSSs" defined by the FANTOM5 consortium, we classified human pol. II promoters into several groups. Those were largely divided into *cis*-element-containing promoters and core-less promoters. The former was further sub-grouped into 67 according to the species of *cis*-element. Interestingly, core-less promoters accounted for more than 60% of the total. We confirmed that in most cases even each individual gene has some distinctive physical property on or adjacent vicinity of the TSS, irrespective of the promoter type. Thus, regarding physical properties of promoter DNA, we could finally succeed to link average profiles to each individual profile. Here, we discuss genetic information written in not DNA sequence but physical properties of DNA.

B270/P2660

RNA G-Quadruplexes in PE/PPE genes of *Mycobacterium* regulate transcription

A. Kumar, P. Vivekanandan; Kusuma School of Biological Sciences, Insitute Institute of Technology Delhi, New Delhi, INDIA.

G-quadruplex structures play a crucial role in many cellular processes like transcription, translation, and replication. The RNA G-quadruplexes are thermodynamically more stable and can readily form due to their single-stranded configuration. The role of RNA G-quadruplexes in the prokaryotes remains poorly understood. In this study, we analysed 1624 complete bacterial genomes and coding sequences for putative quadruplex sequences (PQS). We found that the mycobacteria have significantly higher PQS densities in their genomes and the CDS. Interestingly, PQS enriched in the CDS was observed only in slow-growing pathogenic mycobacteria but not fast-growing or non-pathogenic mycobacteria. Of note, a majority (over 50%) of the PQS in the CDS of pathogenic mycobacteria mapped to PE/PPE gene family,

an essential class of *Mycobacterium* genes involved in pathogenesis and host immunity evasion. This is particularly important as the PE/PPE gene family is unique to pathogenic mycobacteria, and it represents an expanding family of genes (~10% of the CDS) in mycobacterial genomes, which are well-known for their genomic down-sizing during evolution. The PQS in the CDS of the PE/PPE gene family will also be present in the mRNA of the PE/PPE genes. Using RNA oligos for a selected subset of PQS from PE/PPE genes, we demonstrate their ability to form RNA G-quadruplexes in vitro using standard biophysical methods. The addition of BRACO-19, a G-quadruplex binding ligand, stabilized the quadruplexes. Further, BRACO-19 significantly inhibits the growth of pathogenic mycobacteria at low micromolar concentrations. This effect was less pronounced on non-pathogenic mycobacteria, suggesting a role for RNA G-quadruplexes in regulating mycobacterial growth. To understand the biological role of RNA G-quadruplex in mycobacteria, we used BRACO-19 in log phase cultures of *Mycobacterium tuberculosis* H37Ra, in vitro transcription and heterologous expression in *E. coli*. Our results suggest that the stabilization of RNA G-quadruplexes in the PE/PPE genes of pathogenic mycobacteria inhibits transcription resulting in reduced levels of PE/PPE protein expression. Taken together, our findings suggest that RNA G-quadruplexes in mycobacterial genomes may regulate transcription and growth dynamics among pathogenic mycobacteria. In sum, this work highlights how RNA G-quadruplex containing genes may contribute to pathogenesis and virulence of mycobacteria and lays the groundwork for understanding the biological role of RNA G-quadruplexes in prokaryotes.

B271/P2661

Carnivory of the protocarnivorous plant *Roridula*

N. Arai[1], T. Ochiai[2], H. Doi[3], T. Ohyama[2]; [1]Kanagawa University, Yokohama, JAPAN, [2]Waseda University, Tokyo, JAPAN, [3]Hyogo Prefectural Flower Center, Hyogo, JAPAN.

Carnivorous plants have adapted themselves to the nutrient-deficient habitats. They can obtain nitrogen and phosphate from insects for amino acid and nucleotide syntheses. Many studies have been performed to clarify the mechanisms underlying formation of trap leaves and nutrient absorption, repertoire of the enzymes in the digestive fluid, and expression specificity of the corresponding genes. However, it is still enigma how carnivorous plants have evolved from non-carnivorous plants. We focused on a protocarnivorous plant *Roridula* to elucidate the evolutionary process of carnivorous plants. Most carnivorous plants use their own systems to break down their prey and absorb the degradation products. On the other hand, *Roridula* depends on symbiontic bugs of the genus *Pameridea* to get nutrients from the prey: *Pameridea* eats the prey trapped on the *Roridula* leaves and its feces dropped on the ground help the plant to grow. Using glandular tentacles on the trap leaves of *R*. *dentata* and *R. gorgonias* and mucus secreted from them, we performed transcriptome and proteome analyses. Contrary to the preceding reports, we found that the mucus of each plant contains a few digestive enzymes that are commonly found in the digestive fluid of carnivorous plants. Furthermore, the genes encoding hydrolytic enzymes and transporters were found to be highly expressed in the glandular tentacles. These results may strongly suggest that *Roridula* is a carnivorous plant, though further studies are still required to clearly substantiate its carnivory. In this meeting, we will compare *Roridula* with canonical carnivorous plants and discuss how plants have evolutionary acquired carnivory.

B272/P2662

Polyploidy is essential for high rates of biosynthesis in the *C. elegans* intestine

A. Lessenger, J. Feldman; Biology, Stanford University, Stanford, CA.

The maximum production of biological molecules in a system, called its biosynthetic capacity, increases as cells proliferate and organisms grow, but how this capacity is determined is poorly studied outside of unicellular contexts. RNA and protein become more dilute when cells expand in volume without proportional replication of DNA, indicating that the biosynthetic capacity of cells is ultimately limited by the number of genomes. Proliferating cells can maintain the ratio between DNA and cytoplasm by coupling cell cycle entry to an increase in cell volume. However, many cells types in multicellular organisms can increase their biosynthetic capacity without cell division by becoming polyploid, a condition where cells have many copies of the genome. Using the multicellular model, *C. elegans,* we show that **polyploidy is critical to maintaining normal levels of organ growth and biosynthesis.** Artificially lowering the DNA/cytoplasm ratio by reducing polyploidization in the intestine causes these cells to become smaller and have more dilute RNA. This limitation does not appear to affect the growth of other tissues, but severely impairs fitness by reducing brood size and causing a developmental delay in the F1 generation. We performed RNA sequencing to understand whether DNA content was equally limiting to the production of all transcripts. Instead, we found that the most highly expressed genes are most severely limited by reduced DNA content, suggesting that their promoters may be near maximum occupancy by polymerase under normal conditions. In contrast, most other genes increase the number of transcripts produced by each genome. Surprisingly, we did not find evidence for upregulated production of transcriptional machinery, but do observe a significant upregulation of the production of translational machinery. Together, these data support a model where total transcription of most genes is limited to maintain balance with the most highly expressed genes. Future work will investigate whether these transcriptional changes translate to the protein level, and how these changes mechanistically reduce fitness.

B273/P2663

Annotation of Genes and Genomic Elements in Contig 48 of *Drosophila kikkawai*

N. Connolly[1], T. Sadikot[2]; [1]Biology Department, Washburn University, Topeka, KS, [2]Biology, Washburn University, Topeka, KS.

The genome of *Drosophila melanogaster* has been a highly studied genome in biology for the past twenty years. *D. melanogaster* is a model organism for studying the developmental and cellular processes common in other eukaryotes. This project will use *D. melanogaster* genome as a reference for identifying genes and genomic elements in other *Drosophila* species. The analysis of the sequences and data collection will be done using open-source computational genomic tools for sequencing, gene-prediction, and genome browsing. The resources used during this project have been obtained through the Genomic Education Partnership (GEP). GEP is a bioinformatics program that is sponsored by Washington University, in St. Louis. The analysis and interpretation of the *Drosophila* genome requires screening of the sequence of interest, gene markers, and a comparison of predicted gene features using *D*. *melanogaster* as a reference. This project focuses on annotating an approximately 62,000 bp region of the *Drosophila kikkawai* genome which is predicated to have nine protein-coding genes. The validity of these predictions is tested using bioinformatics analysis.

B274/P2664

Transcriptional heterogeneity in the giant, multinucleated placenta syncytiotrophoblast cell

M. M. Keenen, A. Thulluru, A. Skinkle, A. S. Gladfelter; UNC Chapel Hill, Chapel Hill, NC

During pregnancy, the placenta facilitates the transport of nutrients, oxygen, and hormones between the pregnant person and fetus. The cellular interface between the maternal and fetal vasculature is the multinucleated syncytiotrophoblast (STB), a giant tissue-sized single cell. The STB wraps around the entire surface of the placenta encasing it with one common cytoplasm containing billions of nuclei. This giant cell must not only facilitate the exchange of nutrients, but is essential for fetal immunosuppression, is the primary producer of pregnancy peptide and steroid hormones and performs essential metabolic functions. To accomplish these diverse functions in a single cell, the STB appears to spatially organizes its cytoplasm for specific and distinct local functions. For instance, hormones are produced in cytoplasmic regions that exhibit high rates of translation and secretion and contain many transcriptionally active nuclei. In contrast, areas adjacent to the fetal vasculature have a narrower cytosol to facilitate diffusion and express vasculature growth factors. We hypothesize neighboring nuclei can express differential gene expression programs despite being bathed in a common cytoplasm, enabling distinct spatial organization of the STB cytosol. To test this, we utilize single molecule RNA FISH of primary human placenta tissue. We find that the RNA of pregnancy peptide hormones (hCG, hPL) and vasculature receptors (FLT, PLGF) are all heterogeneously expressed in syncytial nuclei, with high expressing nuclei adjacent to nuclei with little or no expression. Further, the transcription factors involved in STB hormone expression, including GCM1 and CREB, also exhibit differential enrichment in STB nuclei implicating their localization with heterogenous hormone transcription. Finally, we apply single nucleus RNA sequencing to measure the degree of transcriptional heterogeneity at a genomic scale within the STB. This work provides mechanistic, molecular insights into how this essential and massive multinucleate cell is functionally organized to support human life.

B275/P2665

Bioinformatic prediction revealed the potential effects of *THBS1* mutation on liver fibrosis and inflammation

Q. Lin[1], C. S. Tang[1], P. H. Chung[1], V. C. Lui[1], P. K. Tam[1,2]; [1]Department of Surgery, Li Ka Shing Faculty of Medicine, The University Of Hong Kong, Hong Kong, HONG KONG, [2]Faculty of Medicine, Macau University of Science and Technology, Macao, MACAO.

Biliary atresia (BA) is a rare, life-threatening inflammatory disease of the liver and bile ducts that occurs in newborns. Patients with BA may develop profound liver fibrosis, leading to liver transplantation. We performed whole genome sequencing on the patients with BA and their unaffected parents. Bioinformatic analysis, including protein-protein interaction network and hub genes analysis, identified *THBS1* (Thrombospondin 1) as the most vital hub gene of the candidate disease-causing genes of BA. Here, we identified a compound heterozygous variant in one BA trio. Protein structure perdition using AlphaFold and SWISS-MODEL suggested that the compound heterozygous variant on *THBS1* could affect the tertiary structure, stability, or calcium ions environment of THBS1. From the RNA-seq data, we observed differential expression between BA patients and normal controls of *THBS1* in the human liver (Luo et al. 2019). Moreover, we also observed abnormal expression of *THBS1* in the murine model of biliary atresia from a previously published paper (Bessho et al. 2014). To investigate the potential mechanism of cell activities on BA, we applied the gene deconvolution method to impute cell type-

specific expression from bulk RNA-seq data of BA and normal controls. Notably, *THBS1* showed dysregulation in BA samples in hepatocytes and inflammatory macrophages. Together with a literature review on *THBS1*, we hypothesized that dysregulation of *THBS1* and its associated pathways would promote liver fibrosis and inflammation, contributing to the etiology and progression of BA.

B276/P2666

Variability of Human rDNA and Transcription Activity of Ribosomal Genes

N. Chmurciakova[1], E. Smirnov[1], J. Kurfurst[2], F. Liska[1], D. Cmarko[1]; [1]Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, CZECH REPUBLIC, [2]Institute of Organic Chemistry and Biochemistry of the CAS, Prague, CZECH REPUBLIC.

Human ribosomal DNA (rDNA) is represented by multiple repeats in each cell. Every repeat consists of a 13 kb long part with genes encoding 18S, 5.8S and 28S rRNAs as well as a 30 kb long intergenic spacer (IGS). However, transcription does not take place in all repeats. The transcriptionally active genes are characterized by the epigenetic marks of the active chromatin, including hypomethylation of the promoter and adjacent IGS areas. But it is still unknown what causes the differentiation of the genes into active and silent. In this study we examine whether this differentiation is related to the nucleotide sequence of IGS. We isolated rDNA from nucleoli of human fibrosarcoma cells (HT1080) and separated methylated and non-methylated DNA by chromatin immunoprecipitation. Then we used PCR to amplify a 2 kb long region upstream of the transcription start and sequenced the product. We found a significant sequence variability represented by single nucleotide variations (SNV), short insertions and deletions. Six SNVs correlated with the DNA methylation status. Additionally, there was significant correlation between the methylation status and the number of certain simple (tetrameric) repeats, favourable for the formation of G-quadruplexes. These data indicate that IGS structure is involved in the differentiation of ribosomal genes into active and silent. Moreover, one of the discovered SNVs belongs to a known micro-RNA (miR) gene involved in cancer related pathways. Two other SNVs are situated in the regions well matched with miRs produced in other parts of genome. Thus, our data suggest a number of pathways that may connect variations of rDNA sequence with its transcription activity.
This work was supported by the Grant Agency of Czech Republic (19-21715S) and by the Charles University (program Cooperatio - Oncology and Haematologhy).

B277/P2667

Comparing Neuronal Splicing Events in Spinocerebellar Ataxias and Myotonic Dystrophy

C. E. Pritchard[1], A. Aliyeva[2,3], C. D. Lennon[2], E. E. Davey[2], C. C. DeMeo[2], J. M. Velez[2], A. Mascorro[2], J. D. Cleary[2], H. K. Shorrock[2], J. A. Berglund[2,3]; [1]Department of Biological Sciences, Lehigh University, Bethlehem, PA, [2]RNA Institute, University at Albany, Albany, NY, [3]Department of Biological Sciences, University at Albany, Albany, NY.

Spinocerebellar ataxias (SCA) are autosomal dominant neurodegenerative diseases defined by cerebellar degeneration and movement issues, notably balance and coordination, but also muscle spasticity, weakness and peripheral neuropathy. SCAs are genetically heterogeneous with several types caused by CAG repeat expansion mutations in different genes (e.g., SCAs 1-3, 6, 7 & 12). In many of these disorders, the CAG expansion is translated into polyglutamine (Q) expansion proteins, although the pathogenic role of these polyQ proteins is not well understood. In contrast, the mechanism of disease pathogenesis is relatively well understood for the CTG repeat expansion disease myotonic dystrophy

type 1 (DM1). DM1 is an autosomal dominant neurodegenerative disease defined by myotonia, muscle weakness and wasting as well as multisystemic complications. In DM1, CTG expansions within the *dystrophia myotonica protein kinase* (DMPK) gene are transcribed into CUG repeats, which sequester RNA binding proteins (RBPs), leading to mis-regulation of alternative splicing and other RNA processing defects. While widespread alternative splicing dysregulation has also recently been reported in Huntington's disease (another CAG repeat expansion disorder), similar alternative splicing defects have not been widely investigated in CAG expansion SCAs. In this study, we used conserved neuronal splicing events as a metric to assess potential interplay between these CAG/CTG repeat expansion disorders. Using data from both cellular and animal models of DM1 and CAG expansion SCAs, we identified widespread dysregulation of alternative splicing in each dataset. Skipped cassette exon events represented the majority of all alternative splicing events and accounted for over 50% of all event types in each dataset. Pairwise gene ontology analysis and interaction network visualization revealed interaction groups that shared enriched terms related to neuron development and function. Overall, this work supports neuronal spliceopathy as a common feature between these CAG/CTG repeat expansion disorders.

B278/P2668

Chromatin-mediated hypoxic stress response by Set4 in *S. cerevisiae*

W. Sun[1,2], Y. Jethmalani[1], M. Negesse[1], I. Justice[1], S. Amos[1], E. Green[1,2]; [1]Biological Sciences, University of Maryland-Baltimore County, Baltimore, MD, [2]Biochemistry and Molecular Biology, University of Maryland, Baltimore, Baltimore, MD.

Stress responses in yeast are often mediated by changes in gene expression that allow the cells to adapt to the new environment. This dynamic signaling occurs through changes in transcriptional regulation, often dependent on chromatin. Set4 is a chromatin-associated protein that is protective in oxidative and hypoxic stresses. Set4 protein is lowly expressed under normal conditions, but under hypoxic conditions its expression is induced. Set4 is part of the SET domain family of lysine methyltransferases but appears to lack catalytic activity. Besides its paralog Set3 in yeast, there are other orthologs that share this feature: Set-9 and Set-26 in *C. elegans*, UpSET in *D. melanogaster*, and MLL5 and SETD5 in mammals. The mechanism by which Set4 promotes survival under hypoxic stress has yet to be determined. Set4 regulates expression of subtelomeric genes, including stress response genes and cell wall components such as the *PAU* family, which are known to be highly induced under hypoxia. We have shown that loss of *SET4* leads to changes in local chromatin environment in hypoxia, including an increase in H3K9ac in regions where Set4 localizes during hypoxic stress. This correlates with our observation that loss of *SET4* affects the localization of the histone deacetylase Rpd3 and chromatin-associated component of the SIR complex, Sir3, at stress-induced genes. Set4 appears to work, at least in part, through regulating the activity of the transcription factor Upc2. Interestingly, Upc2 controls Set4 expression, suggesting a feedback loop regulating stress response gene expression. Here, we will present our current model on Set4 interactions with other chromatin modifiers and transcription regulators at stress response genes. Altogether, this work provides insight into potential players that may be involved in the role of Set4 during hypoxic stress and identifies new chromatin regulatory pathways that may be controlled by related proteins in other organisms.

B279/P2669

Analysis of Genes in the Initial Interaction of Global Genomic Nucleotide Excision Repairin Zebrafish

J. Sanchez, N. Goerner, E. E. Gestl; Biology, West Chester University, West Chester, PA.

DNA replication occurs in all living organisms and is essential for the passing down of genetic information. As a complex mechanism, it is prone to errors that the cell must be able to fix to stay alive. The Global Genome-Nucleotide Excision Repair (GG-NER) pathway works to fix bulky damage such as those caused by UV light. The GG-NER pathway consists of proteins that are recruited and interact with the damaged DNA site including DDB1, DDB2, CETN2, HR23B, XPA, and XPC the were studied in zebrafish. The zebrafish's genome contains all the NER genes, similar to humans that were studied. RNA samples were collected from 1 to 24 hours after treatment with UV light as well as untreated RNA. The highest amount of was used for further experiments utilizing zebrafish that were either treated with UV light, caffeine, or both in combination. Developmental expression of the genes was also analyzed throughout the zebrafish lifetime of 2 hours to 10 years of age.

B280/P2670

Analysis of Transcription-Coupled Nucleotide Excision Repair Genes in Zebrafish

B. Hanson, N. Detweiler, E. Gestl; Biology, West Chester University, West Chester, PA.

This project was designed to examine the expression of genes involved in the process of Transcribed-Coupled Nucleotide Excision Repair in zebrafish. The RNA levels of CSA, CSB, XAB2, MMS19, UVSSA, and USP were measured via qPCR at various time increments after the completion of the UV treatment up to 24 hours following treatment. Treatments involving both UV light and caffeine were also carried out. In addition, RNA was collected from zebrafish from 2 hours to 10 years of age and the expression levels analyzed to determine correlation with age. Zebrafish were utilized as a model system since they have all the counterparts to the human NER process. The embryos are transparent, making it easier to observe their internal structures and can be isolated in large numbers necessary for this type of experiment.

Chromatin and Chromosome Organization

B281/P2671

Oncogenic lncRNAs alter epigenetic memory at a fragile chromosomal site in human cancer cells

G. Arunkumar, S. Baek, D. Sturgill, M. Bui, Y. Dalal; Center for Cancer Research, National Cancer Institute/NIH, Bethesda, MD.

Chromosome instability is a key event in cancer progression. The essential histone H3 variant CENP-A plays a fundamental role in defining centromere identity, structure, and function, but is innately overexpressed in several types of solid cancers. In the cancer background, excess CENP-A is deposited ectopically on chromosome arms, including at the 8q24/*cMYC* locus, by invading transcription-coupled H3.3 chaperone pathways. Intriguingly, in many cancers, transcription of lncRNAs is upregulated and correlates with poor prognosis, therapeutic resistance, and cancer recurrence in patients. Here, we report that the transcription of chromosome 8q24-derived oncogenic lncRNAs plays an unanticipated role in altering the chromatin landscape of the 8q24 locus. We report that transcription of oncogenic ncRNAs and associated R-loop formation at the 8q24 genomic locus results in the recruitment of H3.3

chaperone-CENP-A histone variant complexes to 8q24. Finally, we demonstrate that a transgene cassette which encodes a specific oncogenic lncRNA from the 8q24 region integrated into a naïve chromosome locus, recruits CENP-A to the new location specifically in a *cis*-acting manner. These data provide a plausible mechanistic link between locus-specific oncogenic lncRNAs, aberrant local chromatin structure, and the generation of new epigenetic memory in human cancer cells.

B282/P2672

Super-silencers regulated by chromatin interactions control apoptotic genes

Y. Zhang[1], K. Chen[2], Y. Cai[1], A. Nambu[1], Y. See[2], C. Fu[3], A. Raju[4], M. Lakshmanan[4], M. Osato[1], V. Tergaonkar[4], **M. J. Fullwood**[2]; [1]Cancer Science Institute of Singapore, Singapore, SINGAPORE, [2]School of Biological Sciences, Nanyang Technological University, Singapore, SINGAPORE, [3]Mechanobiology Institute, Singapore, SINGAPORE, [4]Institute of Molecular and Cell Biology, Singapore, SINGAPORE.

Human silencers have been shown to exist and regulate developmental gene expression. However, the functional importance of human silencers needs to be elucidated such as the working mechanism and whether they can form “super-silencers”. Here, through interrogating two putative silencer components of *FGF18* gene, we found that two silencers can cooperate via compensated chromatin interactions to form a “super-silencer”. Furthermore, double knock-out of two silencers exhibited synergistic upregulation of *FGF18* expression and changes of cell identity. To disturb the “super-silencers”, we applied combinational treatment of an EZH2 inhibitor GSK343, and a REST inhibitor, X5050 (“GR”). We found that GR led to severe loss of TADs and loops, while the use of just one inhibitor by itself only showed mild changes. Such changes of TADs and loops may due to reduced CTCF protein level observed upon GR treatment. Moreover, GSK343 and X5050 worked together synergistically to upregulate the apoptotic genes controlled by super-silencers, and thus gave rise to antitumor effects including apoptosis, cell cycle arrest and tumor growth inhibition. Overall, our data demonstrated the first example of a “super-silencer” and showed that combinational usage of GSK343 and X5050 could potentially lead to cancer ablation through disruption of “super-silencers”. Taken together, our results suggest that the usage of combination treatment to target super-silencers and 3D genome organization should be further researched as a potential future cancer therapy.

B283/P2673

***In situ* 3-D analysis of transcriptionally repressed yeast nuclei by cryo-ET**

C. Y. Y. Chong, J. K. Chen, S. Cai, J. Shi, **L. Gan**; National University of Singapore, Singapore, SINGAPORE.

The relationship between 3-D chromatin structure and transcription is poorly understood *in situ*. Recent advances in both imaging and acute perturbation make it possible to study changes in transcription and large-scale changes in chromatin packing at the molecular (~ 4 nm) level inside cells. Namely, cryo-electron tomography (cryo-ET) of cryogenically thinned frozen-hydrated cells can reveal the 3-D organization of macromolecular complexes inside cell nuclei in a minimally perturbed life-like state without the artifacts associated with fixation and staining. Inducible knockdowns by the anchor-away and auxin-inducible-degron systems can rapidly perturb the function of essential genes of yeasts. Using cryo-ET, we recently found that in unperturbed budding yeast *S. cerevisiae*, less than 10% of the nucleosomes have the canonical form (146 base pairs of DNA wrapped in ~ 1.7 left-handed turns in a 10 x 6 nm particle) *in situ*. The majority of the nucleosome-like particles are so heterogeneous that they cannot be classified into a canonical structure. This heterogeneity is consistent with other studies and

with the notion that the yeast genome is largely “open” in a euchromatic state. In contrast, canonical nucleosomes are abundant and therefore easily located *ex vivo* in lysed nuclei. Here we use cryo-ET to test the hypothesis that transcription by the highly abundant RNA polymerase II (RNAPII) is responsible for the low abundance of canonical nucleosomes *in situ*. We imaged the 3-D organization chromatin in a temperature-sensitive strain, an anchor-away strain, and an auxin-inducible-degron strain - all of which allow rapid inactivation of RNAPII function. In all three strains, non-canonical nucleosomes remained the majority species after RNAPII knockdown. Furthermore, comparison with unperturbed cells shows that the 3-D packing of nucleosome-like particles does not undergo large changes after transcription shutoff. Our work shows that RNAPII transcription does not radically alter yeast chromatin organization at the mono- or oligonucleosome level *in situ*. Future work that combines genetic perturbation and *in situ* cryo-ET will be done systematically to reveal the factors responsible for the highly open state of yeast chromatin.

B284/P2674

SUMOylation-mediated phase separation drives APB formation and telomere clustering in parallel in ALT cancer cells

R. Zhao[1], D. Chenoweth[2], H. Zhang[1]; [1]Department of Biological Sciences, Mellon College of Science, Carnegie Mellon University, Pittsburgh, PA, [2]Department of Chemistry, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA.

In telomerase-free cancer cells that employ an alternative lengthening of telomeres (ALT) pathway for telomere maintenance, PML bodies are mislocalized to telomeres to form ALT telomere-associated PML bodies (APBs). APBs contain DNA repair factors and telomere clusters to promote homology-directed telomere DNA synthesis and are potential therapeutic targets. We previously reported that mimicking SUMOylation on ALT telomeres with a chemical dimerization system drives phase separation to form APBs and cluster telomeres. However, how APBs are formed by SUMOylation mediated phase separation and whether enrichment of DNA repair factors and telomere clustering depends on APB formation is not clear yet. To answer these questions, we followed PML bodies in live cells after recruiting SUMOs to telomeres with our dimerization system. We found APBs can form through two pathways: nucleation of de novo PML bodies on SUMOs coated telomeres or fusion of existing PML bodies with SUMO coated telomeres. In addition, we observed three types of telomere clustering: SUMO-coated telomeres fuse, SUMO-coated telomeres fuse with APBs, and APBs fuse. The ability of SUMO-coated telomeres to fuse without APB formation implies PML independent phase separation after SUMOylation. Indeed, recruiting SUMOs to telomeres in PML knock-out cells enriched DNA repair factors such as BLM to promote PML-independent phase separation and telomere clustering. In addition, telomere clustering is not affected by PML deletion when telomere-fused nuclease is used to trigger the ALT pathway. However, in the endogenous ALT cancer cells, when only a small number of telomeres are SUMOylated, PML is required for both enrichment of SUMOs on telomeres and telomere clustering. Our results show that SUMOylation-mediated phase separation drives APB formation and telomere clustering in parallel rather than sequentially, though the former can promote the latter. Supporting the importance of SUMOylation in ALT, we observe that SUMO inhibition leads to less telomere DNA synthesis, more telomere shortening, and less colony formation in ALT cells but not non-ALT cells, suggesting SUMOylation can be a potential target for ALT cancer therapy.

B285/P2675

Role of Hox Gene *Ultrabithorax* in Wing Differentiation in Butterflies and Moths

A. Tendolkar, K. van Horne, C. Heryanto, A. Mazo-Vargas, A. Martin; The George Washington University, Washington, DC.

The origin and evolution of *Hox* genes were key for the diversification of animal body plans.These deeply conserved developmental genes are organized in the genomes as serial homologs in bilateral animals. *Hox* genes trigger segment-specific signaling networks that establish the fate of the different regions of the embryo. Mutations in these genes result in homeosis - loss of one body identity and gain of another. Our research focuses on the *Hox* gene *Ultrabithorax* (*Ubx*)and its role in insect wing diversification. Fore and hind wings show morphological differences among insect orders - balancing halteres in flies, protective hardened shells in beetles and intricate patterns in butterflies. In insects, *Ubx* is expressed in the third thoracic segment and is responsible for the ontogenesis of the hindwing. Role of *Ubx* in hindwing differentiation has been studied in flies, beetles and planthoppers; however,description of its role in butterflies and moths is limited. We used CRISPR targeted mutagenesis to generate *Ubx* loss-of-function somatic mutations in two nymphalid butterflies (*Junonia coenia*,*Vanessa cardui*)and a pyralid moth (*Plodia interpunctella*). The resulting mosaic clones yielded hindwing-to-forewing transformations in scale shape and size, color patterns, and wing venation and structure. Additionally, in the moth *P. interpunctella*, loss of *Ubx* generated ectopic forewing secondary sexual traits on the hindwing, showing that *Ubx* is necessary for specifying many aspects of hindwing-specific identities. Our study establishes *Ubx* as the micromanager of hindwing identity, a result that parallels observations in other insects. To tease apart the different functions of *Ubx* in hindwing differentiation, we used ATAC-seq to identify putative regulators of *Ubx* and used CRISPR-Cas9 for functional testing. We are currently attempting to generate enhancer knock-out lines to characterize *Ubx* expression in mutants. Taken together, our study will provide insight into the different aspects of lepidopteran hindwing that Ubx controls and contribute knowledge to compare lepidopteran hindwing development with other insects.

B286/P2676

Computational predictions of 3D hubs of chromatin many-body interactions uncover physical scaffolds for regulation of gene expression

H. Farooq, L. Du, A. Perez-Rathke, K. Chronis, J. Liang; University of Illinois At Chicago, Chicago, IL.

Chromatin 3D organization plays important roles in the regulation of gene expression. However, Hi-C captures only population-averaged pairwise contact frequencies and does not detect higher-order many-body interactions. Here we use CHROMATIX, a computational method which reconstructs ensemble of single-cell chromatin conformations by deconvolving Hi-C data to detect enrichment of many-body interactions. We generate large ensembles (2x104) of independent 3D single-cell chromatin conformations using population Hi-C data. Our single-cell models reproduce imaging, Dip-C, and 3D FISH single cell measurements. In addition, the aggregations of single-cell chromatin models are also in strong agreement with population Hi-C measurements. The reconstructed ensembles of single-cell chromatin structures enable us to discover 3D hubs of higher-order many-body interactions across hundreds of loci in B-lymphocyte cells. We illustrate the functional roles of 3D hubs of many-body interactions in regulating gene expression using the example of eQTL associated genes. We show higher- order 3D hubs of gene and eQTLs provide the physical basis of differential gene expression, with examples of hubs of 1-gene-to-many-variants and many-genes-to-1-variant. Overall, our results show 3D chromatin many-body

hubs are important for gene expression, and they can be discovered through computational modeling of ensembles of single-cell 3D chromatin conformations using population Hi-C data

B287/P2677

Live cell quantification of telomere length in single cell using CRISPR-based imaging

K. Lanning, L. Bowling, K. Bellingham-Johnstun, B. Levesque, C. Laplante; MBS, North Carolina State University, Raleigh, NC.

Telomeres are heterochromatin regions of repetitive DNA sequences that protect the ends of chromosomes. Therefore their length and structure are critical for cellular functions. However, the local structural organization of telomeres remains mostly unknown owing to the lack of methods to measure chromatin organization in living cells. We developed a quantitative platform to measure the length of telomeres in live cells using a combination of fluorescence microscopy and a nuclease-deficient Cas9 (dCas9) chromatin labeling method. Our approach is based on a calibration method developed to measure the concentration of proteins in living fission yeast cells. We modified this method to measure the local concentration of dCas9-3xmEGFP bound to guide RNAs (gRNAs) that recognize repeats of the TTAGGG telomeric sequences of U2OS and HeLa cells. Binding of dCas9-3xmEGFP to gRNAs tiled along telomeres resulted in dozens of bright fluorescent foci across the nuclei of both types of cells. In single U2OS cell, the fluorescence intensity and the size of telomeric foci varied greatly. In contrast, in HeLa cells, the foci were smaller and their fluorescence intensity more consistent. Using our quantification method, we measured a broad rage in the local concentration of dCas9-3XmEGFP (0.1 to 5.1 μM, with a mean of 1.3 ± 0.9 μM) per telomere In U2OS cells. Consistent with our observations, we measured a narrower range of dCas9-3XmEGFP concentration (0.3 to 1.0 μM, with a mean of 0.5 ± 0.2 μM) per telomere in HeLa cells. We converted these local concentration values to number of dCas9 polypeptides per telomeric foci. Assuming non-overlapping binding of the gRNAs along the DNA sequences, these numbers of dCas9 per telomere represent a length of DNA of 5.8 to 296.1 Kb (mean of 72.5 ± 50.4 Kb) for telomeres in U2OS cells. These values agree with current estimations of telomere lengths of 5 to 150 kB for U2OS cells. In HeLa cells, our calculated numbers of dCas9 per telomere represent lengths of DNA ranging from 7.2 to 24.9 Kb (mean of 12.3 ± 5.6 Kb). Therefore, U2OS cells show a wide range in the length of telomeres while HeLa cells have more consistent and shorter telomeres. We applied our image analysis platform to the study of repetitive chromatin sequences in living cells. With appropriate adaptations, this system could also be used for investigating unique DNA sequences within the genome thus opening the door to understanding dynamics of chromatin state and compartmentalization in single cell.

B288/P2678

Functional Chromatin Spatial Interactions Provide 3D Basis for Gene Expression and Their Discovery through Integrated Analysis of 3D Single-cell Chromatin Ensemble Modeling and Epigenetic Marks

L. Du[1], H. Farooq[1], K. Chronis[2], J. Liang[1]; [1]Richard and Loan Hill Department of Biomedical Engineering, University of Illinois Chicago, Chicago, IL, [2]Department of Biochemistry and Molecular Genetics, University of Illinois Chicago, Chicago, IL.

Three-dimensional structures of chromatin play important roles in gene regulation and in nuclear function. Hi-C measurements have provided a wealth of information on chromatin interactions. Emerging evidence suggests functional chromatin interactions, including promoter-enhancer

interaction, are related to gene expression. However, gene promoters and their regulatory elements can be separated by 10^3-10^6 base pairs apart. Therefore, examining 3D chromatin conformations and identifying functionally important spatial long-range interactions are important for deciphering the structure-function relationship of 3D genome. Our recent work of the CHROMATIX deep sampling algorithms can identify non-random chromatin polymer interactions. Despite being 5-7% of total Hi-C counts, they can be used for 3D chromatin folding. The large ensembles (5.0×10^4) of 3D single-cell chromatin conformations deconvolved from population Hi-C heatmap accurately reproduce both single-cell capture and imaging studies, as well as population Hi-C measurements. In this study, we examine predicted ensembles of single-cell 3D chromatin conformations of loci containing differentially expressed genes between myelogenous leukemia cancer cells K562 and normal lymphoblastoid cells GM12878. We have identified subsets of non-random chromatin interactions based on large random ensembles of 3D chromatin chains folded in confined nuclear volume using publicly available Hi-C data. These non-random interactions are used to generate folded ensembles of 5.0×10^4 single-cell 3D conformations for individual loci. We have further quantified the heterogeneity of 3D chromatin conformations by clustering, with each spatial cluster and its proportion defined. In addition, we have carried out integrated analysis of patterns of RNA-seq, ATAC-seq, and epigenetic data of these loci in K562 and GM12878 cells. Results from analysis of 33 gene-active loci show that functional chromatin spatial interactions provide 3D basis for gene expression, and subpopulations 3D chromatin conformations with larger radius of gyration are generally more active. Overall, functional interactions can be identified through integrated analysis of 3D single-cell chromatin ensemble modeling and patterns of epigenetic marks. Our results show that there are well-defined signatures of 3D subpopulations of chromatin conformations involving distinctive patterns of epigenetic marks, and these signatures are strongly associated with differentially expressed genes.

B289/P2679

A targeted, transposase-mediated, RNA-DNA proximity-ligation sequencing technique to identify centromeric chromatin associated RNAs

K. A. Fryer, C. Limouse, A. F. Straight; Stanford University, Stanford, CA.

Establishing and maintaining chromatin organization is essential for genome stability and gene regulation. The tight control of this organization is mediated by interaction between proteins, the DNA sequence, and RNA. Mounting evidence suggests a crucial role for non-coding RNAs in regulating various aspects of chromatin architecture. To identify and map chromatin associated RNAs in a targeted manner, we have developed a transposase mediated RNA-DNA proximity ligation sequencing method. To link the genomic localization, and associated RNAs at a region of interest, we designed a biotinylated bridge sequence that allows for transposition into the genome at the 5' end and ligation of a nearby RNA to the 3' end. Transposition of the bridge is targeted via binding of the protein-A fused to the N-terminus of Tn5 transposase to an antibody bound to a protein of interest. Once transposed, nearby RNAs are then ligated to the 3' end of the bridge. Sequences containing the transposed bridge are selected via streptavidin bead binding and the RNA side is converted to cDNA resulting in a cDNA-bridge-DNA molecule that can then be sequenced using both NextGen sequencing and long read sequencing. We have implemented this method to identify RNAs associated with the centromere, a specialized chromatin domain that serves as the attachment site for microtubules during cell division. Centromeric chromatin is defined by the presence of nucleosomes containing the histone H3 variant, CENP-A. Assembly of CENP-A is orchestrated by several multi-protein complexes, many of which have been

shown to bind nucleic acid. CENP-A assembly is also influenced by neighboring heterochromatin environments. Previous studies have demonstrated a critical role for RNA binding by the histone methyltransferase, SUV39H1, which is responsible for depositing H3K9me3 at pericentromeric regions. However, the role of RNA at the centromere is not fully understood, in part due to the highly repetitive DNA sequence which precludes unique mapping of short read sequencing data. The method we have developed utilizes long read sequencing to allow for unique mapping of highly repetitive sequences and interrogation at the individual chromatin-RNA interaction level. Using this technique, we will be able to identify centromere associated RNAs and map their spatial organization within individual centromeres.

B290/P2680

Resolving Cellular Heterogeneity through 3D Ensemble Models of Single-Cell Chromatin Conformations for Understanding Genome Structure-Function Relationship

J. Liang, H. Farooq, L. Du, P. Delafrouze, A. Perez-Rathke; Richard and Loan Hill Department of Biomedical Engineering, University of Illinois at Chicago, Chicago, IL.

Computational modeling of 3D chromatin plays important roles in understanding the principles of genome organization. We discuss a minimalistic approach towards polymer modeling and how it can be used to invert population Hi-C into high-resolution, high-coverage single-cell chromatin conformations. Utilizing only basic physical properties such as nuclear volume and no adjustable parameters, this model reveals that a few specific Hi-C interactions (15-35 for enhancer-rich loci in human cells) can fold chromatin into individual conformations consistent with single-cell imaging, Dip-C, and FISH-measured genomic distance distributions. Aggregating individual conformations also reproduces population Hi-C frequencies. Furthermore, this single-cell modeling approach allows quantification of structural heterogeneity and discovery of specific higher-order many-body units of spatial physical interactions. This minimalistic 3D chromatin model has revealed a number additional insights: 1) chromatin scaling rules are a result of volume-confined polymers; 2) TADs form as a byproduct of 3D chromatin folding driven by a few specific interactions; 3) cell subpopulations equipped with different chromatin structural scaffolds are developmental stage-dependent; and 4) quantification of the functional landscape and epigenetic marks of many-body units which are simultaneously spatially co-interacting within enhancer-rich, euchromatic regions. We also discuss the implications of these findings in understanding the genome structure-function relationship.

B291/P2681

The SUN-family protein Sad1 interacts with the histone H2A-H2B dimer to mediate the spatial organization of heterochromatin through phase separation

Q. Dong[1], W. Sun[2], J. Gao[1], Y. Chen[3], **F. Li**[1]; [1]Department of Biology, New York University, New York, NY, [2]Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences,, Shanghai, China, [3]Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China

Heterochromatin generally associates with the nuclear periphery, but how the spatial organization of heterochromatin is regulated to ensure epigenetic silencing remains unclear. Heterochromatin in fission yeast preferentially assembles at peri-centromeres, telomeres, and the mating-type locus. Sad1, a conserved SUN (Sad1-UNC-84) family protein, associates with spindle pole body (SPB), responsible for tethering centromeres to SPB in fission yeast vegetative cells. Here, we demonstrated that Sad1 directly interacts with the histone H2A-H2B heterodimer. To our surprise, we found that in addition to its SPB

localization, Sad1 is also distributed throughout the nuclear envelope in a dynamic manner. The nuclear envelope distribution of Sad1 depends on its interactions with histone H2A-H2B. We further showed that the Sad1-H2A-H2B complex plays a critical role in the attachment of telomeres and the mating-type locus to the nuclear envelope by interacting with the inner membrane protein Bqt4. Our results demonstrated that Sad1 uses two different motifs at its N-terminus to regulate centromere clustering and the spatial positioning of telomeres and the *mat* locus. In addition, the complex is essential for heterochromatin silencing. We identified that Sad1-H2A-H2B interacts with HDACs, including Clr3 and Sir2, to mediate heterochromatin assembly. Interestingly, histone binding to Sad1 robustly induced the formation of liquid-like condensates, which facilitate recruitment of factors important for heterochromatin localization and assembly. We solved the crystal structure of the Sad1-histone complex, revealing the intimate contacts between H2B and an acidic motif of Sad1. Finally, we demonstrated that Sad1 can serve as a histone chaperone to promote nucleosome assembly. Our results revealed that the conserved SUN-domain protein Sad1 together with histone H2A-H2B serves as a previously unrecognized regulatory module to mediate the organization of heterochromatin at the nuclear periphery. Our results also indicated a nucleosome-independent role of histone H2A-H2B. Importantly, this study provides insight into how LLPS of proteins on the nuclear envelope impacts the spatial organization of chromatin domains.

B292/P2682

Nuclear Position Modulates Long-Range Chromatin Interactions

E. H. Finn[1], T. Misteli[2]; [1]Oklahoma Medical Research Foundation, Oklahoma City, OK, [2]National Cancer Institute, NIH, Bethesda, MD

The human genome is non-randomly organized within the cell nucleus. Spatial mapping of genome folding by biochemical methods and imaging has revealed extensive variation in locus interaction frequencies between cells in a population and between homologs within an individual cell. Commonly used mapping approaches typically examine either the relative position of genomic sites to each other or the position of individual loci relative to nuclear landmarks. Whether the frequency of specific chromatin-chromatin interactions is affected by where in the nuclear space a locus is located is unknown. We simultaneously mapped at the single cell level the interaction frequencies and radial position of more than a hundred locus pairs using high-throughput imaging to ask whether the location within the nucleus affects interactions frequency. We find strong enrichment of many interactions at specific radial positions. Position-dependency of interactions was cell-type specific, correlated with local chromatin type, and cell-type-specific enriched associations were marked by increased variability, sometimes without a significant decrease in mean spatial distance. These observations demonstrate that genome organization relative to itself and relative to nuclear landmarks are closely interwoven.

B293/P2683

Linker Histone condensates wet chromatid surface to modulate its nuclear amount

V. Murugesan[1,2], M. Rinaldin[1,2], T. Quail[1,2], M. Elsner[1,2], J. Brugues[1,2]; [1]Max Planck Institute for Molecular Cell biology and Genetics(MPI-CBG), Dresden, GERMANY, [2]Max Planck Institute for Physics and Complex Systems (MPI-PKS), Dresden, GERMANY.

Nuclear composition dictates nuclear function. However, controlling nuclear composition is challenging particularly in early development as the Nuclear-to-Cytoplasmic(N/C) ratio reduces exponentially. By

studying H1.8 incorporation in the nucleus in X. laevis extract, we uncovered an alternative mechanism to import that leads to nuclear enrichment. We observe that the linker histone H1.8 forms phase-separated condensates in the cytoplasm and the nucleus. Despite the enrichment inside the nucleus, we find that H1.8 is not imported across the nuclear membrane. Using live imaging, we demonstrate that H1.8 nucleates and forms condensates on the mitotic chromatids, prior to the formation of the nuclear envelope. These condensates wet the chromatids and are then sequestered into the interphase nucleus as the cell cycle progresses, resulting in the observed enrichment. We further show that cytoplasmic droplets help maintain a steady nucleation rate by buffering the soluble amount in the cytoplasm. Our findings show that the properties of droplet wetting on chromatid surfaces provide a robust mechanism to regulate nuclear composition independently of the N/C ratio.

B294/P2684

Coarsening of nuclear condensates is governed by the interplay between chromatin stiffness and condensate intermolecular interaction strength

D. Sankar Banerjee[1], **T. Chigumira**[1], J. Kratz[1], D. M. Chenoweth[2], S. Banerjee[1], H. Zhang[1]; [1]Carnegie Mellon University, Pittsburgh, PA, [2]University of Pennsylvania, Philadelphia, PA

Biomolecular condensates are implicated in many cellular processes. Growth dynamics and size control of condensates within the complex cellular environment and at time scales relevant to biological processes are therefore of great importance but are not well understood. To characterize the long-term growth dynamics of condensates in the nucleus, we used chemical tools to induce protein condensate formation and followed their growth for up to eight hours. Contrary to previous reports of mainly coalescence-based condensate coarsening due to suppression by the chromatin network, we observed ripening and continuous diffusion-based growth at long timescales. To explain these surprising observations, we developed a quantitative physical model and found that in addition to chromatin stiffness, condensate protein intermolecular interaction strength, which determines condensate properties such as fluidity, surface tension and partition coefficient, controls condensate growth. To test the model prediction that protein intermolecular interactions affect growth, we tracked the growth of condensates comprised of proteins with weaker intermolecular interaction strength. We observed a significant increase in the ripening fractions and rates, agreeing with model predictions. To test that condensate ripening is governed by the interplay between protein interaction strength and chromatin stiffness, we followed the growth dynamics of the two types of condensates in perturbed chromatin environments. We found that smaller variance in the chromatin stiffness within cells results in narrower condensate size distribution for both condensates and the effect is greater for condensates with weaker molecular interactions. This confirms that chromatin stiffness affects condensate growth, but that the extent of the effect depends on the strength of the protein interactions within the condensates. By combining theoretical modeling and experimental validation, our findings provide a new mechanism for condensate growth and size control in the nucleus to aid the understanding of condensate functionality and regulation.

B295/P2685

Condensation dynamics of sticky chromatin

A. R. Lamson[1], O. Troyanskya[1,2], M. Shelley[1,3]; [1]Flatiron Institute, New York, NY, [2]Princeton University, Princeton, NJ, [3]New York University, New York, NY

Multicellular organisms require cells to differentiate to perform specific tasks and functions even though every cell contains the information necessary to produce all cell types of that organism. Cells differentiate by regulating gene expression in various ways. One way is by forming DNA-protein condensates inside the nucleus. Certain condensates, such as heterochromatin, suppress gene expression while others, like transcription hubs, up-regulate gene expression in the surrounding nuclear region. Current biomolecular condensate models explain equilibrium properties, like size and stability, but lack dynamics. For example, rheological properties and collapse times for large lengths of chromatin remain poorly characterized or explained. To study such dynamics, we use a mixture of coarse-grained 3D Brownian dynamics and kinetic Monte-Carlo algorithms to model DNA and associated binding proteins. Our simulations reveal two ways 'sticky' filaments go from being uncondensed, to condensed at multiple locations, to having a single condensate populating the filament. The conformational path taken is determined by the protein-DNA binding kinetics, the protein density, and the filament slack. This work sheds light on DNA and chromatin fibers reorganization on time-scales physically relevant to the cell cycle and crucial for proper gene expression during events like cell division.

B296/P2686

Short-range chromatin structure in linker histone depleted cells

I. Duba, A. Clerkin, V. Risca; Rockefeller University, New York, NY

Chromatin structure is known to be a key regulator of gene expression and cellular function. Linker histone H1, a chromatin-binding protein known to condense chromatin in vitro, is essential in mammals and has profound effects on cellular function upon knockdown, ranging from compromised ability to differentiate in embryonic stem cells to an increase in malignant transformation of B cells. In embryonic stem cells, H1 depletion produces modest changes in gene expression, primarily at repeats. In B and T cells, it impacts genomic compartments and accessibility in heterochromatin. Extensive *in vitro* work shows that H1 causes nucleosome arrays to adopt more compact conformations, but how this translates to locus- and epigenetic state-specific regulation of sub-kilobase chromatin structure in cells has not been fully characterized. To probe the role of H1 in shaping short-range chromatin structure, we performed Micro-C, a nucleosome-resolution proximity ligation-based assay, in the human leukemia cell line K562 with and without depletion of H1. We find that, genome-wide, the contact probability curve of control (WT) cells exhibits enriched contacts between a nucleosome and its third nearest neighbor and fifth nearest neighbor. This pattern is lacking in the H1-depleted cells, which instead show a monotonic decrease of nucleosome contact probability with genomic distance. Within each sample, comparing heterochromatin (enriched for H1) and promoter regions (depleted of H1), a similar pattern of contact probability is observable. This H1-dependent contact probability pattern is likely indicative of an underlying chromatin folding motif promoted by H1 in which N/N+3 and N/N+5 contacts are favored. We are currently investigating how these changes relate to chromatin's accessibility to the nuclease and what contact patterns would be predicted from simulated chromatin structures. The finding that H1 promotes a chromatin fiber structure across multiple contexts represents a step toward understanding the structural impact H1 has on chromatin *in situ*.

B297/P2687

Imaging-based CRISPR Knockout Screen Identifies Regulators of Centromere Clustering

K. Guin, A. Keikhosravi, L. Ozbun, G. Pegoraro, T. Misteli; National Cancer Institute, National Institutes of Health, Bethesda, MD

Centromeres are specialized genomic loci that assemble the multi-protein kinetochore complex, which interacts with the spindle apparatus and ensures faithful chromosome segregation during cell division. Centromere function is highly conserved in most eukaryotes although centromere DNA sequences and the structure of kinetochores can be diverse across species. Centromeres appear non-randomly localized in the 3D space of the cell nucleus. While in most yeasts all centromeres form a single cluster at the nuclear periphery, multiple heterochromatin embedded clusters of centromeres, known as chromocenters, are observed in *Drosophila* and mice. In human cells clustering is less pronounced, but some centromeres tend to cluster near the nucleolus. The molecular mechanisms that determine the location and extent of clustering of centromeres in 3D space are largely unknown. By quantitatively measuring the degree of centromere clustering, we find cell-type specific differences in the localization patterns of human centromeres. For example, HCT116 colon cancer epithelial cells tend to have a central arrangement of centromeres while centromeres are well distributed in hTERT-RPE1 retinal epithelial cells. To address the molecular mechanisms of cell-type specific centromere clustering patterns, we performed an arrayed high-throughput CRISPR knockout screen for 1068 chromatin associated proteins in HCT116 and hTERT-RPE1 cells. Our data suggest cell-type specific as well as conserved role of chromatin-associated proteins regulating centromere clustering. These experiments are providing novel insights into mechanisms of higher order genome organization.

B298/P2688

Polymer modeling reveals interplay between physical property of DNA and the size and distribution of condensin-based chromatin loops

D. Kolbin[1], B. Walker[2], C. Hult[3], D. Adalsteinsson[1], G. Forest[1], K. Bloom[1]; [1]UNC Chapel Hill, Chapel Hill, NC, [2]UC Irvine, Irvine, CA, [3]Gettysburg College, Gettysburg, PA

Condensin and cohesin have profound effects on the higher order organization of chromatin. Through their ability to extrude loops, the genome can be compartmentalized into subdomains that are topologically isolated, condensed into a bottlebrush for tension sensing between sister kinetochores and packaging in cell division. How the size, distribution and duration of loops is regulated is an emerging question in the field. To understand this regulation, we have turned to bead-spring polymer models of the chromatin. Such polymer models have been critical in gaining insight into a number of DNA metabolic processes including nucleolar function, transcription and DNA repair. DNA loops are constantly formed and dissolved through thermal fluctuations of a highly flexible DNA polymer. Loop-extrusion provides a mechanism to regulate this process and mold specific regions of the genome, depending on cell cycle stage, cell type, or environmental perturbations. We have found that the **stiffness of the polymer** substrate (DNA chain), the **spring properties** of condensin, and the distribution of attachment sites (hard or soft, i.e. centromeres or nuclear pores) have disproportionate effects on the properties of chromatin loops. Constraining the ends of DNA will result in regulation of loops by types of condensin (strong, moderate, or weak) and diminishes the role of the chromatin type (stiff or floppy) forming the loops. Biologically such regulation can be achieved by swapping out members of the

condensin complex or by modifying its subunits by PTM. However, when the ends of DNA are unconstrained (free ends) chromatin's polymer property (stiffness) will overwhelmingly dictate loop sizes formed by condensins. In this regime the control over loop sizes is relegated toward the substrate itself and can be actuated by chromatin modifiers and nucleosome remodelers. Through modeling experiments and theory, this study formulates the critical physical inputs into how chromatin loops can be regulated in vivo. We show that regulation can be achieved via the complexes like condensin and dictated by local chromatin properties like tethering or its stiffness (persistence length), leading us to propose a **multimodal control** of higher order chromatin looping.

B299/P2689

Active hydrodynamic theory of chromatin dynamics

A. S. Rautu[1], D. Saintillan[2], M. J. Shelley[1,3]; [1]Simons Foundation Flatiron Institute, New York City, NY, [2]University of California San Diego, San Diego, CA, [3]New York University Courant Institute, New York City, NY

The organization of chromatin inside the cell nucleus is crucial for the proper functioning of many nuclear processes in eukaryotes. In differentiated cells, chromatin is spatially segregated into euchromatin and heterochromatin compartments. The former is loosely packed and transcriptionally active, while the latter is compacted and mostly consists of transcriptionally silent genes. Here we describe a hydrodynamic model of chromatin and nucleoplasm at micron scales. The chromatin is modeled as a viscous, compressible fluid, as informed by microrheology experiments and their response at long time. Heterochromatin is distinguished by the presence of contractile stresses due to HP1 crosslinking. This stress induces density instabilities and large-scale flows of the chromatin fluid and nucleoplasm. Simulations reveal coarsening of heterochromatic components, which in an open system lead to a finite-size droplet, whereas in a confining domain we observe a redistribution at the boundaries, resembling a wetting phenomenon. Hence these mechanical processes may play an important role in the spatial organization of heterochromatin which is usually enriched near the nuclear periphery.

B300/P2690

Targeted degradation of macroH2A to investigate its role in chromatin compaction and organization

A. Ou, J. Rendleman, J. Soroczynski, V. Risca; The Rockefeller University, New York, NY

Non-canonical histones play also an important role in regulating and organizing the genome. One such histone of interest is macroH2A, a variant of H2A with an unusually large molecular weight and tripartite structure consisting of the histone folding region, positively charged linker, and a macrodomain. The two subtypes of macroH2A (macroH2A1 and macroH2A2) are encoded by two genes; however, exon switching in macroH2A1 can give two isoforms (1.1 and 1.2) which seem to play opposing roles in gene activation.

The macroH2A family of histones can have a wide range of roles related to their regulation of gene expression including maintaining cell states, X-chromosome inactivation and senescence, as well as functioning in DNA damage repair. MacroH2A1.1 has been implicated in transcriptional activation through the regulation of nucleosome acetylation, while the other isoforms play a repressive role. Mechanistically, the function of macroH2A is not yet well understood, especially with respect to how they repress transcription and has been proposed to be maintaining and organizing higher-order

chromatin organization or interfering with transcription factor binding.To study macroH2A, we utilized a proteolysis targeting chimera (PROTAC) against an endogenously tagged macroH2A to allow for acute protein degradation. Our preliminary results showed that acute loss of macroH2A1 did not drastically alter chromatin accessibility.

B301/P2691

Cooperation of condensin, histone modifications, and nuclear architecture in dosage compensation

J. Trombley, A. Rakozy, L. E. Tushman, G. Csankovszki; University of Michigan, Ann Arbor, MI

In organisms with differing numbers of sex chromosomes between sexes, the process of dosage compensation balances X chromosome gene expression. In *C. elegans* hermaphrodites (XX), X chromosomes are bound by a dosage compensation complex (DCC) to down-regulate gene expression on both X's to cause expression levels comparable to males (XO). The DCC is a multi-subunit protein complex composed of Condensin I^{DC} and additional X chromosome-associated proteins. The DCC on the hermaphrodite X mediates three known mechanisms of dosage compensation. The binding of the DCC specifically to both of the hermaphrodite X chromosomes causes X-specific enrichment of histone 4 lysine 20 monomethylation (H4K20me1). The DCC also, via an unknown mechanism, influences the tethering of the X to the nuclear lamina. In addition, the *C. elegans* specific SMC complex, Condensin I^{DC} restructures the hermaphrodite X's to reduce transcription. However, it is unknown if Condensin I^{DC} is needed for the maintenance of dosage compensation in mature *C. elegans*. Previous studies in our lab showed that these processes individually make significant contributions to X chromosome structure and gene expression. Strong null mutations in the DCC lead to varying degrees of hermaphrodite-specific lethality. Yet, the hermaphrodite mutants with defects for both the H4K20me1 and nuclear lamina tethering pathways are viable. We are testing whether the combination of defects from the three known pathways will increase dosage compensation defects, or destabilize repression during the maintenance phase, to a greater degree than what was seen in previous studies.

B302/P2692

Heavy Ion Damage on Chromatin; Break Mapping Across Genomic Compartments

H. Canaj[1], A. Scortea[1], D. W. West[1], I. Plante[2], V. Risca[1]; [1]Rockefeller University, New York, NY, [2]KBR, NASA, Houston, TX

Space radiation, including gamma rays and galactic cosmic ray ions traveling at relativistic speeds, presents a challenge to the health of astronauts engaged in long-term space travel. Although chromatin is generally thought to protect DNA from a range of insults, including ionizing radiation, the relationship of chromatin local organization to the sensitivity of the underlying genomic locus to damage is not yet well understood. Breaks that span across genomic compartments could cause rearrangements that are highly mutagenic. Understanding how different kinds of radiation drive damage patterns across genomic compartments can help us understand the nature of the damage events and to what extent chromatin protects against deleterious outcomes. To investigate how chromatin structure shapes patterns of DNA damage caused by ionizing radiation, we measured the density of breaks in DNA across different functional genomic compartments after irradiation of human fibroblast and umbilical vein endothelial cells with gamma rays, X-rays and heavy ions. We irradiated cells with O_{18} and Fe_{56} at the NASA space radiation laboratory at Brookhaven National Lab, and measured cell proliferation and DNA repair dynamics by immunofluorescence at various timepoints after irradiation. We also used RICC-seq and

End-seq, two DNA sequencing-based methods, to measure genome-wide single stranded DNA and double stranded DNA breaks, respectively, in ice-chilled cells immediately after irradiation. By chilling the cells, we sought to reduce the impact of the DNA damage response on the DNA break landscape. We compared break densities across genomic compartments defined by histone marks, nuclear localization, or accessibility. Under X-ray irradiation, we see that heterochromatic regions, like lamina-associated domains and H3K9me3 marked chromatin, show a lower break density compared to a genomic control, whereas regions associated with euchromatic, ATAC-seq accessible regions or H3K27ac-marked chromatin have higher break density for both ssDNA and dsDNA breaks. These data will provide the basis for future chromatin state-specific nanodosimetry and evaluation of explicit simulations of DNA damage events by packages such as NASA'S RITRACKS.

B303/P2693

OpenTn5 Project: Open-source resource for robust and scalable Tn5 transposase purification and characterization.

J. Soroczynski, D. A. Oren, L. J. Anderson, J. Yeung, J. Rendleman, V. Risca; The Rockefeller University, New York, NY

Tn5 is a bacterial cut-and-paste transposase, which can simultaneously fragment target DNA and append its ends with custom DNA sequences, in a process referred to as tagmentation. This property of Tn5 has been leveraged over the last decade to develop an ever-increasing number of technologies for generating high-throughput sequencing DNA libraries. Despite the versatility and widespread adoption of tagmentation, a persistent challenge in the development of Tn5-based technologies has been lack of a recombinant expression and storage protocol that robustly maintains enzyme activity, and of standardized activity assays. We describe a robust protocol for high-yield Protein G-Tn5 fusion (pG-Tn5) expression and purification, as well as detailed quality control assays. We benchmarked our pG-Tn5 fusion against commercial enzymes across a variety of applications, including both bulk and single-cell ATAC-seq, as well as Cut&Tag, showing it is functionally identical. The protocol we developed is short, affordable and scalable, allowing one to obtain usable quantities of Tn5 from as little as 50 mL E. coli culture using only bench-top equipment. The pG-Tn5 produced is highly pure and stable in -20 °C for at least 1 year. We provide the expression plasmid and protocol as open-source resources, and as a platform for development of new applications of tagmentation.

B304/P2694

Optimization of the Trim-Away system for rapid degradation of endogenous proteins from *Xenopus* egg extract for the study of cellular dynamics

A. Jevitt, J. Chen, S. Rankin; OKLAHOMA MEDICAL RESEARCH FOUNDATION, Oklahoma City, OK

Extracts made from the eggs of *Xenopus laevis* have provided a valuable model for studying subcellular events, including chromosome dynamics, DNA replication, mitochondria assembly, cell cycle progression, and many others. In this system, protein depletions are often used to analyze the requirement for endogenous proteins, and rescue experiments with recombinant proteins are often used to confirm activities and map function. Here we show that the TRIM21 ubiquitin ligase can be effectively used to deplete proteins from egg extracts rapidly. The advantage of this approach is that it requires far less antibody than is typically required for immunodepletions, making it more cost-effective. We provide several examples of antibodies against *Xenopus* proteins that can be used in combination

with the TRIM21 ligase to cause efficient protein depletion, suggesting this approach's general usefulness and applicability in egg extracts. We demonstrate that TRIM-away depletions occur efficiently in both interphase and meiotically arrested extracts. The simplicity with which TRIM-away can be applied to *Xenopus* egg extracts suggests that it is uniquely powerful and useful in this system.

B305/P2695

Characterizing ESCO1's Interaction with Chromatin

J. Schoen[1,2], A. Poss[1], E. Lima da Silva[1], J. Chen[1], E. Floyd[1], S. Rankin[1,2]; [1]OKLAHOMA MEDICAL RESEARCH FOUNDATION, Oklahoma City, OK, [2]University of Oklahoma Health Sciences Center, Oklahoma City, OK

ESCO1 is an acetyltransferase enzyme that regulates chromosome organization and gene expression by modifying Cohesin, a key regulator of genome architecture. Cohesin organizes DNA into loops and is critical for normal chromosome structure and function. Acetylation of the SMC3 subunit of Cohesin by ESCO1 stabilizes Cohesin on DNA, promoting long residence time at functional sites. Factors that shape when, where, or how ESCO1 stabilizes Cohesin are not understood.

We have found that tethering ESCO1 to a specific location in the nucleus results in gross local rearrangement of chromatin. Strikingly, this local chromatin rearrangement occurs independently of ESCO1's acetyltransferase activity and does not occur through Cohesin. We have mapped this activity to a 35 amino acid motif within ESCO1 and shown that this region of ESCO1 binds directly to DNA, with a likely preference for single stranded DNA. Deletion of this DNA binding domain (DBD) leads to a reduction in chromatin-bound ESCO1.

We hypothesize that the ESCO1 DBD directs it to preferred binding sites where it regulates Cohesin and gene expression. Experiments are ongoing to complete the characterization of the DBD, define how it is regulated, and characterize its impact on Cohesin localization and gene expression. With these experiments, we will define the biological impact of ESCO1's DNA binding activity.

B306/P2696

The Effect of Nuclear Compartmentalization on DNA Repair

A. Laffitte, M. King; Yale University, New Haven, CT

DNA double-stranded breaks (DSBs) represent a danger to genome stability. DSBs are repaired primarily through canonical non-homologous end-joining (NHEJ) or homologous recombination (HR). Repair pathway choice is critical because failure to choose the most faithful repair mechanism can lead to loss of genome integrity. HR is the more error-free repair pathway, using the sister chromatid as a template for repair. In line with previous work, I found that nuclear compartmentalization impacts repair pathway choice, specifically in regulating the loading of repair factors onto resected ssDNA, which arises as a consequence of nucleolytic processing of the DSB. My results show that Rad52, which remodels RPA to Rad51 loading in fission yeast, is efficiently recruited to resected DSBs and is retained there during repair in the nuclear interior. By contrast, in DSBs tethered to the nuclear periphery, Rad52 loading is transient. The mechanism behind this observation is still unknown. I hypothesize that HR is disfavored at the nuclear periphery due to factors residing there that compete with Rad52 for loading onto resected DNA. It is possible that one of these factors is DNA-damage induced RNA (ddRNA) that hybridizes with resected ssDNA to form RNA-DNA hybrids. Consistent with such a model, RNaseH overexpression partially rescues persistent Rad52 loading at the nuclear periphery. This led me to investigate ddRNA as a possible source of RNA that could contribute to the RNA-DNA hybrids that antagonize Rad52 loading.

Accordingly, after a DSB, I observed a temporary upregulation of transcripts in the surrounding genomic region. This upregulation was only detectable in a Dicer-deficient background, where processing into small RNA is perturbed. One model to explain these observations is that damage-induced bidirectional transcription flanking a DSB produces dsRNA that is then a substrate for Dicer. This leaves open the possibility that these damage-induced transcripts are the form of RNA that hybridizes with resected ssDNA, thereby antagonizing Rad52 loading. These initial findings support the idea that repair by HR proceeds differently in the nuclear interior and periphery. Ongoing work will continue to shed light on the relationship between nuclear compartments and DNA repair mechanism choice and efficiency.

B307/P2697

Phosphorylation-Dependent Regulation of ESCO2

B. Rankin[1,2], J. Chen[1], D. Flavors[1], V. Moore[1], S. Rankin[1,2]; [1]Oklahoma Medical Research Foundation, Oklahoma City, OK, [2]University of Oklahoma Health Science Center, Oklahoma City, OK

Tethering of sister chromatids by the cohesin complex following DNA replication ensures accurate alignment and segregation of chromosomes during the subsequent cell division. Sister chromatid cohesion is promoted by acetylation of the SMC3 subunit of cohesin by the ESCO2 acetyltransferase. This is coordinated by interactions between ESCO2 and DNA replication machinery. Through conserved motifs in its intrinsically disordered N-terminal tail, ESCO2 interacts with PCNA and the MCM2-7 helicase complex.
Several proteomic reports suggest that ESCO2 is phosphorylated, though the impact of these modifications on ESCO2 function are not known. We have engineered mutations in potentially modified residues in ESCO2 and tested their impact on cohesion establishment and interactions of ESCO2 with the replication machinery. Using a knockdown and replacement strategy we have found that one phosphorylation site, strongly impacts sister chromatid cohesion. We also found that this mutation had significant impact on the ability of ESCO2 to interact with the replication machinery.
We conclude from these experiments that phosphorylation of ESCO2 plays a critical role in sister chromatid cohesion establishment during DNA replication. This regulation provides novel insight about the cell cycle-dependent regulation of this essential mediator of sister chromatid cohesion.

Chromatin and Nuclear Bodies

B309/P2698

Nuclear speckle integrity and function require TAO2 kinase

S. Gao[1], M. Esparza[1], I. Dehghan[2,3], V. Aksenova[4], K. Zhang[1], K. Batten[1], M. B. Ferretti[5], B. E. Begg[5], T. Cagatay[1], J. W. Shay[1], A. García-Sastre[6,7,8,9,10], E. J. Goldsmith[11], Z. J. Chen[2,3], M. Dasso[4], K. W. Lynch[5], M. H. Cobb[12], B. M. A. Fontoura[1]; [1]Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, [2]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, [3]Hhmi, University of Texas Southwestern Medical Center, Dallas, TX, [4]Division of Molecular and Cellular Biology, National Institute of Child Health and Human Development, NIH, Bethesda, MA, [5]Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, [6]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, [7]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY, [8]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, [9]The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, [10]Department of Pathology,

Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, [11]Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, TX, [12]Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX.

Nuclear speckles are non-membrane bound organelles known as storage sites for mRNA processing and splicing factors. In addition, nuclear speckles have also been implicated in splicing and export of a subset of mRNAs, including the influenza virus M mRNA that encodes proteins required for viral entry, trafficking, and budding. However, little is known about how nuclear speckles are assembled or regulated. Here we uncovered a role for the cellular protein kinase TAO2 as a constituent of nuclear speckles and as a factor required for the integrity of these nuclear bodies and for their functions in pre-mRNA splicing and trafficking. We found that a nuclear pool of TAO2 is localized at nuclear speckles and interacts with nuclear speckle factors involved in RNA splicing and nuclear export, including SRSF1 and Aly/Ref. Depletion of TAO2 or inhibition of its kinase activity disrupts nuclear speckle structure, decreasing the levels of several proteins involved in nuclear speckle assembly and splicing, including SC35 and SON. Consequently, splicing and nuclear export of influenza virus M mRNA were severely compromised and caused a disruption in the virus life cycle. In fact, low levels of TAO2 led to a decrease in viral protein levels and inhibited viral replication. Additionally, depletion or inhibition of TAO2 also resulted in abnormal expression of a subset of mRNAs with key roles in viral replication and immunity. More recently, we performed RNA-seq analysis of nuclear and cytoplasmic fractions of cells depleted of TAO2 and of control cells. We found that decreased levels of TAO2 inhibited nuclear export of a subset of cellular mRNAs whereas their total levels were not significantly altered. Among this group are mRNAs that encode key enzymes involved in post-translational modifications and transcription factors that regulate gene expression. Together, these findings uncovered a role for TAO2 in nuclear speckle formation and function and revealed host requirements and vulnerabilities for influenza infection.

B310/P2699

Novel role of Translesion DNA synthesis polymerase kappa in the nucleolus and in nucleolar stress response recovery

S. Paul[1], A. Heinbaugh[1], A. Paul[1], P. Cifani[2], D. Pappin[2], T. Spratt[1]; [1]Penn State University College of Medicine, Hershey, PA, [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Translesion DNA synthesis (TLS) polymerases evolved to tolerate DNA damage that bypasses DNA lesions. Multiple TLS polymerases exist with different damage tolerance capabilities. A major knowledge-gap in elucidating the roles of these polymerases is that it is difficult to identify which polymerase is active in a specific situation. We synthesized a novel nucleotide analog N^2-benzyl-2'-deoxyguanosine (EBndG) that is highly selective toward DNA polymerase kappa (PolK), a Y-family TLS polymerase. PolK can bypass bulky lesions generated by benzo[*a*]pyrene diolepoxide (BPDE), an environmental carcinogen. Although PolK has been identified to have multiple cellular roles such as protection of stalled replication forks and non-B DNA synthesis, the mechanisms regulating its different cellular activities are unknown. To interrogate the identity of proteins surrounding the PolK active sites, we performed an extensive study using modified aniPOND (accelerated native isolation of proteins on nascent DNA), called iPoKD (isolation of proteins on Pol kappa synthesized DNA). Human cell-lines were treated with BPDE, subsequently 5-ethynyl-2'-deoxyuridine (EdU) or EBndG was added and proteins bound to the DNA containing EdU and EBndG were analyzed by mass-spectrometry (MS). Our data identified DNA replication and repair proteins previously identified with EdU pull-downs; and

interestingly enrichment of ribosome biogenesis and nucleolar proteins associated with EBndG pull-downs. Using super-resolution confocal microscopy, our data suggest, that PolK is active in the nucleolus after BPDE damage, inserts EBndG in the nucleolar DNA, and it's activity is regulated by the canonical polycomb complex recruited by the PARylation by PARP1. We noted that BPDE lead to nucleolar stress and PolK is essential in recovering cells from nucleolar stress. Surprisingly, we also discovered that in PolK compromised cells 5-ethynyl uridine (EU) incorporation and transcription of pre-rRNA was significantly reduced. PolK active site interactome identified RNA polymerase I subunits and ribosome biogenesis proteins. We are now further exploring PolK's role in maintaining ribosomal biogenesis and ribosomal DNA integrity after BPDE damage. This study will provide first insight into the novel of TLS DNA synthesis polymerase in the nucleolus.

B311/P2700

Nuclear speckle proteins drive neurodegenerative tauopathy

R. Eck, **B. Kraemer**; University of Washington, Seattle, WA.

Pathological accumulation of the microtubule binding protein tau drives age-related neurodegeneration in a variety of disorders, collectively called tauopathies. In the most common tauopathy, Alzheimer's disease (AD), the accumulation of pathological tau strongly correlates with cognitive decline. The underlying molecular mechanisms that drive neurodegeneration in tauopathies remain unknown**.** We employed classical forward genetic approaches and identified multiple loss of function alleles in the *C. elegans spop-1 g*ene that ameliorate tauopathy, suggesting SPOP is required for tau mediated neurodegeneration. CRISPR based genome editing methodology enabled the generation of customized *SPOP-1* loss of function and null alleles. Molecular genetics, behavioral, neuronal reporter assays, and biochemical analyses were also employed to characterize the consequences of *spop-1* loss of function on tauopathy related phenotypes in model systems. Knockout of SPOP-1 rescues tau mediated behavioral deficits caused by neuronal dysfunction in tau transgenic *C. elegans.* Biochemical analysis revealed that SPOP-1 loss of function promotes clearance of phosphorylated and total tau species from *C. elegans* neurons, but no change in tau transgene mRNA levels. Tau transgenic animals exhibit obvious neurodegeneration of GABAergic neurons, but loss of *spop-1* rescues neurodegeneration. While SPOP functions as an CUL3 E3 ligase adaptor protein, CUL3 function is not required for SPOP loss of function rescue of tauopathy. Genetic epistasis analysis suggests the nuclear speckle resident poly(A) RNA binding protein *sut-2* and *spop-1* function in a parallel molecular pathway. SPOP is a novel modifier of tauopathy phenotypes. Combined with previous findings investigating ALYREF, PARN/TOE1, SUT-1 and SUT-2/MSUT2, this work suggests phase-separated nuclear speckles are an important cellular site controlling susceptibility to pathological tau. Recent work showing SPOP modification of PR dipeptides derived from C9orf72 expansion suggests common pathways may be at work in the neurodegenerative molecular mechanisms of tauopathy, repeat dipeptides, and perhaps other proteinopathy disorders.

B312/P2701

Zinc Finger 692 (ZNF692) regulates nucleolar shape and activity through its interaction with nucleophosmin 1 (NPM1)

I. N. Brown[1], M. Lafita Navarro[2], M. Conacci-Sorrell[2]; [1]UT Southwestern Medical Center, Dallas, TX, [2]UT Southwestern, Dallas, TX.

Nucleolar function and ribosome biogenesis are essential for protein synthesis and cellular growth. Therefore, aberrant increase in nucleolar size and number are hallmarks of many cancers and have been associated with poor prognosis; nucleolar size is one of the most frequently used parameters by pathologists to grade solid tumors. While basic components and general molecular functions of the nucleolus have been studied, fundamental information on the mechanisms and molecules involved in the increased nucleolar size and activity specifically in cancer cells is missing. In an unbiased screen designed to identify growth promoting pathways in MYC-transformed cells, our lab identified a novel and critical nucleolar regulator, the Zinc finger protein 692 (ZNF692) (manuscript submitted). Our data has shown that ZNF692 downregulation interferes with protein synthesis and nucleolar function, and overexpression promotes nucleolar activity and protein synthesis. Moreover, we found that ZNF692 expression is dramatically increased in nearly all solid tumors deposited in the TGCA and is correlated with poor patient outcome. On a molecular level, our lab has shown that ZNF692 facilitates small ribosome subunit assembly and the final steps of 18S rRNA processing in the nucleolus. Through this project, we have unexpectedly discovered that ZNF692 also interacts with nucleophosmin 1 (NPM1) to assemble the granular component of the nucleolus. In the absence of ZNF692, NPM1 aberrantly localizes as ring-like structures. This unexpected distribution of NPM1 resembles the liquid condensates formed *in vitro* by purified NPM1. Therefore, we propose a working model that ZNF692 regulates nucleolar architecture and activity to accommodate the demands for ribosome biogenesis, directly controlling the biosynthetic capabilities of hyper-proliferative cells.

B313/P2702

Sequence Determinants of 53BP1 Phase Separation

M. Garabedian, M. C. Good; University of Pennsylvania, Philadelphia, PA.

DNA double strand breaks (DSBs) are among the most deleterious events that cells encounter. As such, elegant and complex systems exist to address these breaks, primarily in one of two pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). High fidelity repair of breaks and proper balance between these pathways is essential for maintaining genomic integrity and preventing tumorigenesis. While many aspects and players involved in DNA repair and repair pathway selection have been characterized, our understanding of the complex interplay between different tumor suppressors, their ligands, and other downstream factors remains incomplete. Recently, 53BP1, along with many other disordered proteins found in the nucleus, was found to phase separate into biomolecular condensates during DNA damage response. We hypothesize that liquid-liquid phase separation (LLPS) of 53BP1 is driven by π-π and/or cation-π interactions and is required for stimulating NHEJ. Here, using in vitro condensation experiments and imaging in live cells, we show that specific disruption of π-π and/or cation-π interactions disrupt phase separation of 53BP1. Loss of these interactions leads to reduction of NHEJ components at break sites, consistent with the role of 53BP1 as a disordered protein that acts as a scaffold. Further, point mutants are unable to suppress HR at break

sites. Collectively, we propose that the degree of 53BP1 phase separation at DSBs can influence cellular decision making between NHEJ and HR to resolve broken DNA.

B314/P2703

Fission yeast nuclear pore protein nup211 regulates the transcriptome

D. Kamel[1,2], A. Sookdeo[1,2], W.-G. Qiu[1,2], H. Zhong[1,2]; [1]Hunter College, The City University of New York, New York, NY, [2]The Graduate Center, The City University of New York, New York, NY

While nuclear pore proteins primarily mediate nucleocytoplasmic transport, they are also involved in other activities like transcription, chromatin organization, and cell cycle regulation. The fission yeast nucleoporin nup211 localizes to the nuclear basket and is known to play important roles in regulating RNA quality and mRNA export. Interestingly, several orthologs of nup211 have been shown to regulate transcription in addition to their roles in nucleocytoplasmic transport. Although nup211 preferentially associates with heterochromatin, it is unclear whether it also modulates transcription. To better understand the full scope of nup211 function, we examined how cells are affected when nup211 protein level is reduced. Since *nup211* is an essential gene, we constructed a conditional mutant strain (*nup211-so*) in which *nup211* expression is controlled by a thiamine-repressible promoter. RNA-Seq analysis revealed that reducing *nup211* expression significantly affected the transcript abundance of ~1,600 genes. Among the upregulated genes are transcription factors (e.g., *atf1*, *pcr1* and *mbx1*) , cell cycle regulators (e.g., *wee1*), and genes involved in histone modification. Consistent with septation and cytokinesis defects observed by microscopy, several genes known to play important roles in septation and cell wall synthesis (e.g., *pom1*, *bgs1*, *pxl1* and *knh1*) were also upregulated. RT-qPCR results confirmed the upregulation of these genes. Furthermore, through domain analysis we determined that the first 655 residues of nup211 are sufficient for cell viability as exogenous expression of this domain in *nup211-so* cells was able to rescue cell growth and morphology. Finally, RT-qPCR results showed that ectopically expressing either full-length nup211 or nup211$_{1\text{-}655}$ was able to partially or fully restore the transcript levels of the subset of genes examined in *nup211-so* cells. Our findings reveal a novel function of nup211 in regulating the transcriptome in addition to its role in nuclear transport. Further studies will be carried out to determine the underlying mechanism(s) of nup211 in regulating gene expression.

Functions of Nuclear Envelope Proteins

B315/P2704

The Inner Nuclear Membrane Protein NEMP1 is Required for Proper Erythropoiesis and Supports Nuclear Envelope Openings in Erythroblasts

D. Hodzic; Washington University School of Medicine, St. Louis, MO.

Nuclear Envelope Membrane Proteins (NEMP) are a conserved family of nuclear envelope proteins that reside within the inner nuclear membrane. Even though *Nemp1* knockout (KO) mice are overtly normal, they display a pronounced splenomegaly. This phenotype and recent reports describing a requirement for nuclear envelope openings during erythroblasts terminal maturation led us to examine a potential role for Nemp1 in erythropoiesis. Here, we report that *Nemp1* knockout (KO) mice show peripheral blood defects, anemia in neonates, ineffective erythropoiesis, splenomegaly and stress erythropoiesis. The erythroid lineage of *Nemp1* KO mice is overrepresented until the pronounced apoptosis of polychromatophilic erythroblasts. We show that NEMP1 localizes to the nuclear envelope of

erythroblasts and their progenitors. Mechanistically, we discovered that NEMP1 accumulates into aggregates that localize near or at the edge of nuclear envelope openings and *Nemp1* deficiency leads to a marked decrease of both nuclear envelope openings and ensuing enucleation. Together, our results for the first time demonstrate that NEMP1 is essential for nuclear envelope openings and erythropoietic maturation *in vivo* and provide the first mouse model of defective erythropoiesis directly linked to the loss of an inner nuclear membrane protein.

B316/P2705

Chromatin-based membrane remodeling and bilayer lipid metabolism enable spindle disassembly to seal nuclear envelope holes

S. Barger, H. Yang, S. Lee, S. Bahmanyar; Yale University, New Haven, CT

After open mitosis, spindle microtubule disassembly coordinates with closure of nuclear envelope holes. The nuclear envelope protein, LEM-2, and CHMP-7 serve as adaptors for ESCRT membrane remodeling machinery to execute sealing. The transient accumulation of LEM-2/CHMP-7 to nuclear envelope holes is conserved across multiple organisms; however, loss of CHMP-7 gives rise to subtle or no defects in nuclear permeability suggesting redundant mechanisms are responsible for sealing holes. Using the stereotypical divisions of early *C. elegans* embryos, we found that CNEP-1, the nuclear envelope-associated regulator of the phosphatidic acid phosphatase lipin, contributes to nuclear closure by controlling metabolic changes in bilayer ER/nuclear envelope lipids. Here, we show that spindle disassembly requires membrane remodeling via CNEP-1 control of lipin and chromatin-LEM-2 binding to ensure sealing of holes. Using endogenous CRISPR-mediated mutagenesis, we show that the interaction between LEM-2 and the DNA crosslinking protein BAF prevents membranes from transiently enwrapping mitotic chromosomes, frequently at sites adjoining the mitotic spindle. Ectopic membranes around sealing sites can also result from loss of *cnep-1/*lipin suggesting a role for lipid metabolism in remodeling membranes around chromatin. Strikingly, enwrapped mitotic chromosomes persist when both *lem-2*-BAF binding and *cnep-1/*lipin are absent causing enhanced nuclear leakiness. In some cases, abnormal membrane enwrapping prevents disassembly of chromosome-associated spindle microtubules leading to severe spindle and nuclear morphology defects. Importantly, nuclear formation defects resulting from disrupted lipid metabolism are specific to loss of the LEM-2-BAF interaction - deletion of *chmp-7* or the domain of LEM-2 that binds CHMP-7 results in comparatively subtle defects. We also provide evidence suggesting regulation of lipid metabolism through the human CNEP-1 homologue, CTDNEP1, and lipin 1 is also coupled to chromatin-mediated nuclear envelope remodeling in U2OS cells. Together, our data suggest a conserved mechanism in which membrane remodeling mediated by chromatin-LEM-2 interactions and bilayer lipid metabolism prevent abnormal membrane enwrapping of chromosomes to allow timely spindle disassembly and proper sealing. We are currently testing whether global or local changes in lipid metabolism are required to cooperate with chromatin-based membrane remodeling for hole closure.

B317/P2706

A mechanosensitive checkpoint at the meiotic nuclear envelope revealed by chemically-induced proximity

C. Liu, A. F. Dernburg; University of California, Berkeley/HHMI, Berkeley, CA

A variety of surveillance mechanisms help to ensure accurate transmission of genetic information during mitosis and meiosis. While certain pathways respond to unrepaired DNA damage, additional checkpoints monitor meiosis-specific events such as synapsis, the assembly of the synaptonemal complex (SC) between homologous chromosomes. During *C. elegans* oogenesis, defects in synapsis lead to a cell cycle delay and eventually trigger apoptosis. Previous work revealed that this "synapsis checkpoint" depends on functional pairing centers (PCs), special regions on each chromosome that mediate nuclear envelope attachment and promote homolog pairing and synapsis. However, how cells monitor and respond to defects in SC assembly remains unclear. In early meiotic prophase, the Polo-like kinase PLK-2 normally relocalizes from PCs to the SC as chromosomes synapse. Synapsis defects result in prolonged association of PLK-2 with PCs and extended phosphorylation of nuclear envelope proteins. To test whether the presence of PLK-2 at PCs confers a signal that promotes apoptosis, I repurposed the auxin-inducible degradation (AID) into a chemically-induced proximity (CIP) system that enables inducible and ectopic targeting of TIR1-tagged PLK-2 to degron-tagged HIM-8, a protein that specifically binds to X-chromosome PCs through meiotic prophase.

I found that ectopic targeting of PLK-2 to X-chromosome PCs was sufficient to induce elevated germline apoptosis that is uncoupled from synapsis defects and does not require HUS-1, an essential component of the DNA damage checkpoint. Using a kinase-dead PLK-2::TIR1 fusion, I found that the kinase activity of PLK-2 is required for CIP-triggered apoptosis. Intriguingly, targeting PLK-2 to PCs also greatly extends the phosphorylation of Lamin/LMN-1 at Ser32, an established PLK-2 target associated with labile nuclear lamina at meiotic entry. Indeed, targeting PLK-2 directly to the nuclear lamina, or ectopic depletion of the nuclear lamina, also induces HUS-1 independent germline apoptosis, even in the absence of functional PCs. Live imaging shows elevated deformation of the meiotic nuclear envelope upon weakening the nuclear lamina, suggesting mechanical stress. Unexpectedly, I found that a mechanosensitive Piezo1/PEZO-1 channel localizes to the nuclear envelope of meiotic nuclei and is required for elevated germline apoptosis triggered by either ectopic PLK-2 recruitment to the nuclear lamina or asynapsis. To our knowledge, this is the first evidence of a role for mechanosensitive channels in detecting nuclear events or contributing to quality control during gamete production. Thus, in response to unsynapsed chromosomes, modification of the nuclear lamina by PLK-2 is likely a direct trigger for apoptosis through mechanosensation.

B318/P2707

Loss of SUN2 is protective against injury-induced lung fibrosis

E. Carley[1], X. Peng[2], E. Kocak[1], E. L. Herzog[2], M. C. King[1,3]; [1]Department of Cell Biology, Yale University, New Haven, CT, [2]Department of Pulmonary, Critical Care and Sleep Medicine, Yale University, New Haven, CT, [3]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT.

Fibrosis is the excessive excretion of extracellular matrix proteins and tissue stiffening that leads to a decrease in organ function. Both biochemical and mechanical signals are integrated into the progression of fibrosis, however the mechanisms by which the mechanical arm of this pathway exerts its effect

remain to be fully defined. The expression level of mechanosensitive A-type lamins, a key component of the nuclear lamina, is increased in Idiopathic Pulmonary Fibrosis across multiple cell types, suggesting that mechanotransduction to the nuclear envelope could play a role in fibrotic progression. Mechanical forces can be transmitted from the extracellular matrix, through the cytoskeleton, to the nucleus via Linker of Nucleoskeleton and Cytoskeleton (LINC) complexes, which mechanically couple the cytoskeleton to the nuclear lamina. To investigate the role of mechanotransduction via LINC complexes in the progression of fibrosis we turned to mouse models of LINC complex ablation. We discovered that loss of SUN2 leads to protection from injury-induced lung fibrosis, suggesting that force transduction through the LINC complex is a driver of extracellular matrix production in this context. We also observed an intriguing dependence on gender, with loss of SUN2 in male mice delaying, but not preventing ECM deposition and loss of SUN2 in female mice completely preventing ECM deposition after lung injury. To define the underlying mechanisms of these observations, we have probed the impact of SUN2 loss on the cell populations present in the lung at various time points after lung injury and found that while loss of SUN2 does not appear to impact the initial injury of the tissue, it does lead to higher levels of epithelial cells at 14 days post injury. These observations suggest that SUN2 may impact epithelial regeneration after injury, a function that could be mechanistically related to our recent work demonstrating that LINC complexes control epithelial differentiation in the epidermis. This work highlights the possibility that intervening in a SUN2-dependent mechanotransduction pathway could short-circuit fibrotic progression, in part by promoting greater regeneration of the epithelia.

B319/P2708

Functional and Domain Analysis of the Inner Nuclear Membrane Protein Csa1 in Budding Yeast

L. Carson, H. Yu; Florida State University, Tallahassee, FL.

The nucleus is surrounded by the nuclear envelope, a double membrane structure composed of the inner nuclear membrane (INM) and the outer nuclear membrane (ONM). Embedded within the nuclear envelope, the nuclear pore complex (NPC) controls trafficking between the nucleus and the cytoplasm. The nucleolus, a large crescent shaped membraneless suborganelle within the nucleus, forms around the ribosomal DNA (rDNA) and functions in the production of ribosomes. In budding yeast, rDNA, and therefore the nucleolus, is tethered to the nuclear periphery during interphase. Due to the highly repetitive nature of rDNA, recombination at the rDNA is suppressed. Aberrant rDNA recombination can result in the formation of extrachromosomal rDNA circles (ERCs). The accumulation of ERCs and aged NPCs have been shown to advance cellular senescence. Although these aging factors are known to decrease the replicative lifespan, the mechanism of how they arise inside the nucleus remains to be further elucidated. We show here that the INM protein Csa1 plays a critical role in regulating rDNA recombination, as well as potentially controlling ERC formation and NPC biogenesis. Using point and deletional yeast mutants, we have found that the N-terminus of Csa1 is essential for its localization to the INM. Using a gene reporter assay, we show that Csa1 suppresses rDNA recombination. We propose that Csa1 functions in the tethering of rDNA/nucleolus to the INM, thereby inhibiting rDNA recombination and ERC formation. Consistent with this idea, Csa1 interacts with the LEM domain protein Heh1, which is required for tethering rDNA/nucleolus to the nuclear periphery. We also show that Csa1 interacts genetically with the TREX-2 complex, known to function in the asymmetric retention of ERCs to the mother cell. This unexpected role of Csa1 as a regulator of rDNA recombination and nuclear envelope architecture provides a foundation for further studies on the mechanism of replicative cell aging in budding yeast.

B320/P2709

Emerin Interacts with Histone Methyltransferases to Regulate Repressive Chromatin at the Nuclear Periphery

N. Marano, J. M. Holaska; Cooper Medical School of Rowan University, Camden, NJ.

X-Linked Emery-Dreifuss muscular dystrophy is caused by mutations in the gene encoding emerin. Emerin is an inner nuclear membrane protein important for repressive chromatin organization at the nuclear periphery. Myogenic differentiation is a tightly regulated process characterized by genomic reorganization leading to coordinated temporal expression of key transcription factors, including MyoD, Pax7, and Myf5. Emerin was shown to interact with repressive histone modification machinery, including HDAC3 and EZH2. Using emerin-null myogenic progenitor cells we established several EDMD-causing emerin mutant lines in the effort to understand how the functional interaction of emerin with HDAC3 regulates histone methyltransferase localization or function to organize repressive chromatin at the nuclear periphery. We found that, in addition to its interaction with HDAC3, emerin interacts with the histone methyltransferases EZH2 and G9a in myogenic progenitor cells. Further, we show enhanced binding of emerin HDAC3-binding mutants S54F and Q133H to EZH2 and G9a. Treatment with small molecule inhibitors of EZH2 and G9a reduced H3K9me2 or H3K27me3 throughout differentiation. EZH2 and G9a inhibitors impaired cell cycle withdrawal, differentiation commitment, and myotube formation in wildtype progenitors, while they had no effect on emerin-null progenitors. Interestingly, these inhibitors exacerbated the impaired differentiation of emerin S54F and Q133H mutant progenitors. Collectively, these results suggest the functional interaction between emerin and HDAC3, EZH2, and G9a are important for myogenic differentiation.

B321/P2710

Characterization of 1700029J07RIK, a Novel Nuclear Membrane Associated Protein that is Induced During Skeletal Muscle Atrophy

D. Waddell, S. Lynch; Department of Biology, University of North Florida, Jacksonville, FL.

Skeletal muscle atrophy results from myriad physiological conditions including denervation, corticosteroid exposure, and aging. The objective of this study is to characterize the novel 1700029J07RIK gene, which is induced during neurogenic skeletal muscle atrophy and is differentially expressed in Muscle RING Finger 1 (MuRF1) knockout mice compared to wild-type mice. Muscle tissue isolated from wild-type control mice and MuRF1 knockout mice revealed that the expression of 1700029J07RIK is unchanged at 3 days post-denervation, while expression is significantly induced in wild-type mice at 14 days post-denervation but remains unchanged in the MuRF1-null animals. It has been well established that muscle cells go through a distinct developmental timeline in which they proliferate as single cell myoblasts and then differentiate and fuse into multinucleated myotubes. Quantitative PCR was used to determine that the 1700029J07RIK gene is expressed in proliferating cells and increases in expression as muscle cells differentiate. In addition, the 1700029J07RIK cDNA was cloned from muscle cells and fused with green fluorescent protein and visualized by confocal fluorescent microscopy revealing a cytoplasmic and nuclear membrane localization pattern of the 1700029J07RIK protein in proliferating muscle cells that is distinct from differentiated muscle cells. Utilizing cellular fractionation, it was determined that 1700029J07RIK is localized to both the cytoplasmic and nuclear fractions in proliferating cells, but then appears to translocate into the nucleus as muscle cells differentiate. Moreover, ectopic expression of 1700029J07RIK resulted in elevated levels of Nesprin-1

protein but did not affect Sun2 or Emerin protein levels. Interestingly, the 1700029J07RIK protein appears to undergo post-translational modification as relocation to the nuclear compartment progresses and overexpression leads to a decrease in intermediate filament proteins Desmin and Vimentin. Finally, it was observed that overexpression of 1700029J07RIK resulted in inhibition of muscle cell differentiation and attenuation of the ERK1/2 branch of the MAP Kinase signaling pathway. While there is virtually nothing known about this novel gene in the context of skeletal muscle, the results presented here suggest 1700029J07RIK may play a role in muscle cell differentiation and in skeletal muscle atrophy.

B322/P2711

The Nuclear Envelope Protein NEMP1 is Specifically Required for Female Mouse Fertility and Forms Novel Nuclear Envelope Structures in Maturing Oocytes

D. Hodzic[1], A. Ganguly[1], B. Hakim[1], C. Potter[1], W. Ha[2], Y. Tsatskis[2], A. Jurisicova[2], H. McNeill[1];
[1]Washington University School of Medicine, St. Louis, MO, [2]Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada

Nemp1 is an inner nuclear membrane protein of the nuclear envelope (NE) that is required for fertility of in worms, fishes and flies. Even though Nemp1 KO mice are overtly normal, females are subfertile with a significant decrease of the ovarian reserve and a loss of chromatin compaction during folliculogenesis. In ovary slices, endogenous Nemp1 expression levels are markedly higher in the germline by comparison to the somatic cells. As expected for a NE protein, Nemp1 formed homogenous nuclear rim-like pattern in primordial follicles. However, upon the transition from primordial to primary follicles, Nemp1 progressively accumulates into NE foci we named Nuclear Envelope Clusters (NECs). Remarkably, NECs numbers progressively increases during folliculogenesis from about ~5 to about ~150 in primary and antral follicles, respectively. With sizes up to 700 nm, NECs remain present at the NE of ovulated germinal vesicles. To get more insight into the dynamics of these novel NE structures, we developed a mouse model allowing for the conditional expression of Nemp1-GFP in the germline using Zp3-Cre. In this system, Nemp1-GFP faithfully reflects the localization of endogenous Nemp1 at the NE of the germline as well as its accumulation into NECs. Time-lapse video microscopy of Nemp1-GFP in GVs revealed an astoundingly dynamic NE with infolds and protrusions of the NE that form at a scale of seconds. The same dynamics was also observed in antral follicles within acute ovary slices. Our proteomic analyses of Nemp1-GFP fibroblasts have emphasized the interaction between Nemp1 and Ran. Accordingly, Ran colocalizes with Nemp1 in NECs. Finally, we also show that the conditional expression of Nemp1-GFP partially rescues the loss of fertility of Nemp1KO females. Taken together, our results show that Nemp1 is essential to mouse female fertility, that it is highly expressed at the NE of maturing oocytes where it progressively accumulates along with Ran into NECs. The latter correspond to novel structures of the female germline NE. Because the timing of NECs formation corresponds to the onset of chromatin condensation in the germline and that chromatin is decondensed in the Nemp1KO germline, we currently hypothesize that Nemp1 plays a central role in chromatin condensation during folliculogenesis.

B323/P2712

Lamin A/C proteomes from heart, muscle and brain in an IL10-KO model of human frailty

K. L. Wilson; Johns Hopkins School of Medicine, Baltimore, MD

Clinical frailty affects 5-11% of people over age 65 and involves significantly higher risk of morbidity and mortality. Frailty phenotypes include muscle weakening, inflammation and perturbed metabolism, which overlap the spectrum of phenotypes ('laminopathies') caused by mutations in *LMNA*. *LMNA* encodes nuclear intermediate filament proteins, lamin A and lamin C, with shared and distinct roles in mitosis and tissue-specific 3D genome organization and signaling. We hypothesized that wildtype lamins A & C are functionally perturbed in frailty, potentially contributing to tissue dysfunction. We tested this hypothesis by identifying native lamin A/C proteomes from skeletal muscle, heart and brain in an aged (21-22 month) chronic-inflammation (interleukin-10 knockout, IL10-KO) mouse model of frailty. We used lamin A/C antibodies to immunoprecipitate lamins and associated proteins from heart, skeletal muscle and brain lysates of ten female mice (five IL10-KO and five controls), then used Tandem Mass Tags to uniquely label each sample, and pooled each set of same-tissue samples for quantitative mass spectrometry analysis. We identified a native lamin A/C proteome in each tissue— novel for heart and brain— including two candidates (Perm1, Fam210a) previously linked to human frailty phenotypes in genome-wide studies. A subset of proteins in each tissue showed significantly increased or decreased association with lamin A/C in the IL10-KO. After filtering and considering known functions of 502 candidate lamin-associated proteins in muscle, 340 candidates in heart, and >2,200 candidates in brain, we selected twelve for further study. Candidate lamin-binding proteins were arrayed as a series of synthetic 20-mer peptides (staggered by 7 residues) and probed sequentially with either recombinant mature lamin A 'tail' (residues 385-646) or lamin C 'tail' (residues 385-572). As positive controls we included prelamin A, emerin and the peptide unique to lamin C. These results validated direct binding of one or both recombinant lamins to ten candidates. Plausibility was evaluated using PyMol, which showed that putative binding site(s) mapped to surface-exposed residues in novel partners including three affected in IL10-KO skeletal muscle (Ppme-1, Lmcd1, Fabp4) and two affected in heart (Gins3, AldoA). Since little-to-nothing is known about native lamin A/C proteomes in most tissues and organs, including brain, these proteomes provide foundations to study the mechanisms of human frailty and laminopathies based on understanding both conserved and tissue-specific functions of lamin A and lamin C.

B324/P2713

Differential interactomes of lamin A & lamin C in early G1

J. Sobo[1], A. Rendon-Jonguitud[1], X. Wong[2], K. Reddy[1]; [1]Johns Hopkins University Sch Med, Baltimore, MD, [2]A*STAR, Immunos, Singapore

The nuclear lamina comprises a network of type V intermediate filaments called lamins that form a meshwork that underlies the inner nuclear membrane (INM). Lamins have been implicated in numerous cellular functions including structural integrity and positioning as well as genome organization and regulation. Large regions of heterochromatin associate with the nuclear lamina in lamin associated domains (LADs). There are two general classes of somatic lamins: B-type (lamin B1 and B2; laB), which are expressed in all cell types and developmental stages, and A-type, which are developmentally regulated and display cell-type specific differences in expression levels. A-type lamins are comprised of two isotypes, lamin A (laA) and lamin C (laC), that are derived through alternative splicing of the *LMNA*

gene. We have recently shown that laC is uniquely important for proper LAD organization, with a potentially important role in reorganizing LADs to the lamina at mitotic exit. Intriguingly, both LADs and laC are in the nucleoplasm in early G1, while laB and most of laA are already polymerized at the nuclear periphery. Given the unique roles and disposition of laA and laC at mitotic exit, we have employed Turbo-ID, a derivative of the biotin-ligase proximity labeling method Bio-ID, to detect their differential proteomes during mitotic exit in synchronized cell populations. Using mass spectrometry analysis on biotin enriched peptides, we hope to identify dynamic differential proteome networks of laA and laC and uncover partners and regulators involved in LAD organization dynamics.

Endocytic Trafficking

B326/P2714

Phosphatidylinositol 4,5-Bisphosphate Is Required for Cargo Sorting of Epidermal Growth Factor Receptor to the Lysosome

S. Chu[1], J. Hsu[1,2]; [1]Institute of Biochemical Sciences, National Taiwan University, Taipei, TAIWAN, [2]Institute of Biological Chemistry, Academia Sinica, Taipei, TAIWAN.

Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) is a lipid messenger at the plasma membrane to control the endocytosis of several membrane receptors such as epidermal growth factor receptor (EGFR). Most of PI(4,5)P2 is generated by type I phosphatidylinositol phosphate kinase (PIPKI) from the substrate phosphatidylinositol-4-bisphosphate (PI4P) to support intracellular PI(4,5)P2 signaling. Recently, PI(4,5)P2 also has been found on endosomal and lysosomal membranes to facilitate endocytic protein transport. PIP5K1A, a type I phosphatidylinositol phosphate kinase, plays important roles in a variety of cellular functions including protein transport. How lipid kinase coordinates phospholipid signaling and membrane recruitment of downstream effector has been unclear. Here, we show that PIP5K1A regulates the lysosomal transport of EGFR for protein degradation. We also demonstrate that EGF stimulation promotes PIP5K1A to form a complex with EGFR for the cargo sorting to the lysosome. PI(4,5)P2-containing membrane compartment is also required to recruit cargo adaptors to promote EGFR lysosomal targeting for protein degradation. These findings identify a new aspect of endosome-to-lysosome trafficking by realizing how lipid membranes directly participate in regulating protein transport.

B327/P2715

UHRF1BP1 and UHRF1BP1L (SHIP164) are Chorein Motif Containing Lipid Transport Proteins that Control Membrane Dynamics in the endo-lysosome system

M. G. Hanna[1,1,2,1], P. De Camilli[1,1,2,1,3]; [1]Yale University, New Haven, CT, [2]Howard Hughes Medical Institute, Chevy Chase, MD, [3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD

Chorein motif proteins are a family of putative lipid transport proteins localized at intracellular membrane contacts sites. They have a rod-like shape and contain a hydrophobic groove running along their length which is thought to function as a conduit for lipid transfer between bilayers by a bridge-like mechanism. Recently, in collaboration with the Reinisch lab (Hanna, Suen, Wu, Reinisch & De Camilli; J. Cell Biol. 2022, PMID: 35499567), we reported a systematic structural and functional characterization of SHIP164 (UHRF1BP1L), another member of this family, previously shown to be a Rab5 effector. In such

study we have 1) confirmed lipid transport properties of this protein, 2) found that endogenous SHIP164 is localized to clusters of small early endocytic vesicles positive for the mannose-6-phosphate receptor (CI-MPR), 3) shown that SHIP164 over-expression expands the size of these clusters and 4) determined that loss of SHIP164 results in the abnormal traffic of CI-MPR. SHIP164 has a paralogue called UHRF1BP1, previously suggested to be a Rab7 effector. Accordingly, 5) we have also found that although exogenous UHRF1BP1 also induced large vesicle assemblies, such assemblies were associated with the lysosome marker LAMP1, rather than early endosomal markers, pointing to a role downstream of SHIP164. We have now further investigated the properties of UHRF1BP1 and SHIP164. We have corroborated evidence for their distinct subcellular localization along the endocytic pathway, in spite of their remarkable predicted structural similarity. We have investigated the physical properties of the vesicle assemblies where they accumulate, utilizing optogenetic and thermodynamic assays in live cells. We have found that both paralogues are enriched in brain, a finding of special interest as SHIP164 was reported to be a candidate Parkinson's disease susceptibility gene. In ongoing studies, we are exploring the impact of the combined loss-of-function of both proteins on the endocytic pathway. An attractive working hypothesis is that UHRF1BP1 and SHIP164, via their lipid transport properties, may help fine tune to protein-lipid ratio of membrane of the endocytic pathway.

B328/P2716

Apolipoprotein C3 facilitates endocytosis of cationic lipid nanoparticles via apolipoprotein receptor 2 and the peroxisome proliferator-activated receptor gamma pathway

M. Kulka[1], S. Alam[2], F. Wang[2], H. Qian[2]; [1]Medical Microbiology and Immunology, University of Alberta, Nanotechnology Research Center, NRC, Edmonton, AB, CANADA, [2]Nanotechnology Research Center, NRC, Edmonton, AB, CANADA.

Mast cells (MCs), are hematopoetically-derived secretory immune cells that release preformed as well as de novo synthesized inflammatory mediators in response to activation by several stimuli. Based on their role in inflammatory responses, particularly in the lung and skin, MCs provide a convenient and effective target for anti-inflammatory therapeutic strategies. Drug-delivery of lipophilic payloads to MCs can be challenging due to their functionally distinct intracellular structures. In the present study, pH-sensitive cationic lipid-based nanoparticles (LNPs) composed of DODMA, DODAP or DOTAP lipids that encapsulated a GFP or eGFP plasmid were constructed using non-turbulent microfluidic mixing. This approach achieved up to 75-92% encapsulation efficiency. Dynamic light scattering revealed a uniform and homogeneous size distribution of LNPs. To promote cellular internalization, LNPs were complexed with apolipoproteins, amphipathic proteins capable of binding lipids and facilitating their transport in the bloodstream and into cells. LNP preparations made up of DODMA or DODMA, DODAP and DOTAP lipids were coated with one of seven apolipoproteins (A1, B, C3, D, E2, E4 and H). Terminally differentiated bone-marrow derived mouse mast cells (BMMCs) were treated with Apo-LNP and internalization was measured using flow cytometry. Out of all the apolipoproteins tested, LNP coated with apolipoprotein C3 (ApoC3) most efficiently facilitated cellular internalization of the LNP into BMMCs as determined by GFP fluorescence using flow cytometry. These effects were confirmed in a less differentiated but also interleukin-3-dependent model of mouse mast cells, MC/9. ApoC3-LNP enhanced internalization by BMMC in a concentration-dependent manner and this was significantly increased when BMMC were pre-treated with inhibitors of actin polymerization, suggesting a dependence on intracellular shuttling. Activation of peroxisome proliferator-activated receptor gamma (PPARgamma) decreased ApoC3-LNP internalization and reduced the expression of apolipoprotein E receptor 2

(ApoER2) , suggesting that ApoC3-LNP binding to ApoER2 may be responsible for its enhanced internalization. Altogether, our studies reveal an important role of ApoC3 in facilitating internalization of cationic LNPs into MCs.

B329/P2717

Rab3 regulates microdomain-mediated plasma membrane recycling

K. R. Levental[1], B. Diaz-Rohrer[2], I. Castello-Serrano[1], H.-Y. Wang[1], I. Levental[1]; [1]University of Virginia, Charlottesville, VA, [2]University of Texas Health Science Center at Houston, Houston, TX

The composition of the plasma membrane (PM) must be tightly controlled despite constant, rapid endocytosis, requiring active, selective recycling of endocytosed membrane components. For most proteins, the mechanisms and pathways of recycling to the PM remain unknown. We report that association with ordered, lipid-driven membrane microdomains (i.e. rafts) is sufficient for PM localization of a subset of transmembrane proteins and that abrogation of raft association disrupts their trafficking and leads to their degradation in lysosomes. Using orthogonal, genetically encoded probes with defined, tunable raft affinity, we screened for trafficking mediators required for efficient recycling of microdomain-associated cargo from endosomes to the PM. Among a set of specific mediators, we identified Rab3 as an important mediator of plasma membrane localization of microdomain-associated proteins. Disruption of Rab3 reduced the localization of raft probes on the PM and led to their accumulation in Rab7-positive endosomes, suggesting inefficient recycling. Rab3 itself is palmitoylated and may associate with microdomains on Rab7-positive endosomes to facilitate sorting and subsequent recycling to the PM. Abrogation of Rab3 function mis-localized the endogenous raft-associated protein Linker for Activation of T-cells, leading to its intracellular accumulation and reduced T cell activation. These findings reveal a fundamental role for lipid-driven microdomains in endocytic traffic and suggest Rab3 as a central regulator of PM composition.

B330/P2718

P4-ATPases Require Retromer for their Localization

M. Jimenez, J. T. Best, C. Kyoung, T. R. Graham; Vanderbilt University, Nashville, TN.

The transport of proteins and lipids throughout the cell is crucial for diverse cellular processes including vesicular transport, signal transduction and apoptosis. One important aspect of cell functionality is an asymmetric membrane composition established by type-IV P-type ATPases (P4-ATPase), which uses the energy from ATP hydrolysis to transport lipids from the exofacial to the cytofacial leaflet of membranes. P4-ATPase deficiency disrupts vesicle-mediated protein transport from Golgi and endosomal membranes and can lead to a number of severe neurological defects. For proper P4-ATPase function, correct localization to appropriate membranes is essential. Of the five P4-ATPases in *Saccharomyces cerevisiae*, Dnf1 and Dnf2 are known to localize to the plasma membrane (PM) while Neo1, Drs2, and Dnf3 localize to the Golgi and each travels through the endocytic pathway as part of their trafficking itineraries. In this study, I seek to determine the recycling and retrograde trafficking pathways traveled by P4-ATPases, and how these flippases interact with components of those pathways. Key components of four major trafficking pathways between endosomes and the Golgi including Drs2/Rcy1/COPI, Snx4, retromer and AP-1/Clathrin, were deleted to determine the routes required for Dnf1/Dnf2 PM localization and Drs2 Golgi localization. Deletion of retromer components including Vps35, Vps5, Vps17, and Snx3 led to mislocalization of Dnf1, Dnf2, and Drs2 to the vacuole, as similarly described previously

for Neo1. These data suggest a primary role for retromer in proper localization of P4-ATPases although a minor role for Rcy1 and Snx4 was detected. In dissecting the interactions between retromer and the P4-ATPases, it was found that Dnf1 contains retromer recognition motifs in the N-terminal tail and both Dnf1 and Dnf2 have a novel C-terminal tail motif. **Together these results would suggest the loss of retromer leads to a substantial loss of P4-ATPases at their primary membrane and should therefore cause major changes in membrane organization.** Deficiencies in the human orthologs of Dnf1/Dnf2 and retromer have been linked to endosomal dysfunction leading to Parkinson's Disease. This study could help elucidate why mutations in these proteins cause the disease.

B331/P2719

Acute and reversible modulation of kidney proximal tubule endocytic capacity by fluid shear stress

E. Lackner[1], K. E. Shipman[1], K. R. Long[1], I. A. Cowan[1], O. A. Weisz[1,2]; [1]Department of Medicine Renal-Electrolyte Division, University of Pittsburgh, Pittsburgh, PA, [2]School of Medicine Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA.

The proximal tubule (PT) of the kidney is comprised of cells that are uniquely specialized for efficient apical uptake of albumin and other proteins that escape the glomerular filtration barrier. These proteins bind to the multiligand receptors megalin and cubilin and are internalized via clathrin-dependent endocytosis into small apical early endosomes that rapidly mature into larger apical vacuoles. Dissociated ligands are delivered from vacuoles to lysosomes for degradation, while receptors are collected into dense apical tubules for recycling to the cell surface. We have optimized a proximal tubule cell model cultured under orbital shear stress that recapitulates the essential morphologic and functional features of the PT *in vivo*, including high megalin and cubilin expression and robust apical endocytic capacity. Using this model, we found that acute changes in orbital speed trigger rapid changes in the endocytosis of albumin. We hypothesize that *in vivo*, this modulation of endocytic capacity enables PT cells to preserve uptake efficiency in response to changes in glomerular filtration rate. We are using biochemical and imaging approaches combined with mathematical modeling to identify the trafficking step(s) modulated by fluid shear stress. Using cell surface biotinylation to quantify the fraction of receptors at the apical membrane, we consistently observed an increase in surface megalin and cubilin at the apical membrane upon removal of shear stress. To determine the contributions of endocytic vs recycling rates to this altered profile, we are quantifying the endocytic rate of megalin and also measuring the colocalization with known markers of apical endocytic compartments. These data will be used to adapt our recently developed kinetic model of megalin trafficking in PT cells to determine effects shear stress changes on individual trafficking rates. This approach allows us to integrate effects of small changes in a single round of trafficking over multiple iterations, and will enable us to identify potential signaling pathways impacted by shear stress that modulate alterations in membrane traffic.

B332/P2720

Insulin Signaling Attenuates GLUT4 Endocytosis in Muscle Cells via GSK3α-Dyn2-Bin1 Interplay

J. Laiman[1], Y. Hsu[1], J. Loh[1], W. Tang[2], M. Chuang[1], H. Liu[3,4], W. Yang[5], B. Chen[6], L. Chuang[1,7], Y. Chang[7,8,9], Y. Liu[1,10]; [1]Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taipei, TAIWAN, [2]ResearchCenter for Applied Sciences, Academia Sinica, Taipei, TAIWAN, [3]National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taipei, TAIWAN, [4]Program in the Clinical Drug Development of Herbal Medicine, Taipei Medical University, Taipei, TAIWAN, [5]Division of Nephrology, Department of Internal Medicine, National Taiwan University Hospital, Hsin-Chu Branch,

Hsin-Chu, TAIWAN, [6]2ResearchCenter for Applied Sciences, Academia Sinica, Taipei, TAIWAN, [7]Department of Internal Medicine, National Taiwan University Hospital, Taipei, TAIWAN, [8]Institute of Medical Genomics and Proteomics, College of Medicine, National Taiwan University, Taipei, TAIWAN, [9]Institute of Biomedical Sciences, Academia Sinica, Taipei, TAIWAN, [10]Center of Precision Medicine, College of Medicine, National Taiwan University, Taipei, TAIWAN.

Skeletal muscle is the main tissue where insulin-stimulated glucose uptake occurs to maintain blood glucose. Glucose transporter 4 (GLUT4), is translocated from intracellular membrane compartments to muscle surface upon insulin signaling to facilitate glucose uptake and internalized back in the absence of insulin. Despite its role in promoting GLUT4 exocytosis, little is known about the role of the insulin signaling pathway in GLUT4 endocytosis. Here, we showed that the mechanoenzyme catalyzing GLUT4 internalization, dynamin-2 (Dyn2), is negatively regulated by insulin signaling in muscle cells. Bin1, a membrane remodeling protein highly expressed in muscle, inhibits Dyn2 via direct interaction of its SRC Homology 3 (SH3) domain with the proline-rich domain (PRD) of Dyn2. GSK3α, but not GSK3β, in turn phosphorylates Dyn2 to relieve its inhibition by Bin1. Conversely, insulin signaling inactivates GSK3α, suppressing Dyn2-mediated GLUT4 endocytosis to boost glucose uptake. In addition, the pharmacological inhibition of GSK3α in diet-induced obese mice leads to enhanced insulin sensitivity and glucose tolerance. Collectively, we demonstrated the attenuation of GLUT4 endocytosis by insulin signaling through GSK3α-Dyn2-Bin1 interplay in muscle cells.

B333/P2721

The Fragile X Messenger Ribonucleoprotein (FMRP) Negatively Regulates Clathrin-associated Adaptor Complex Protein 2 (AP2) and Endocytosis

L. Shi, A. Valdez-Sinon, N. Raj, A. Kosti, V. Faundez, G. Bassell; Department of Cell Biology, Emory University School of Medicine, Atlanta, GA.

FXS is a neurodevelopmental brain disorder caused by the loss of FMRP, known to regulate mRNA translation and local protein synthesis important for synapse development and function. A number of studies have shown that FMRP deficient neurons have impaired trafficking of receptors and ion channels, but the role of specific FMRP targets and their translational dysregulation are not well understood. Using RIP, we confirmed that FMRP was associated with *Ap2a1* and *Ap2b1* mRNAs. Increased expression of AP2 subunits were detected in synaptoneurosome fractions of *Fmr1* KO mouse, although no significant change in mRNA levels were found. Increased rate of newly synthesized AP2B1 was observed in *Fmr1* KO neurons. DHPG stimulation increased AP2 levels at postsynaptic sites in WT but not *Fmr1* KO cortical neurons. Importantly, smiFISH showed AP2 subunit mRNAs localized to dendrites. Taken together, these results suggest that FMRP regulates the postsynaptic expression of AP2 subunits, perhaps via local translation. To obtain further insight into how FMRP may regulate components involved in membrane trafficking, we performed a proteomics study of FMRP interactome in N2A cells. While major gene ontology categories were linked to translation and posttranscriptional regulation, three subunits of AP2 were identified. Co-IP and immunofluorescence further demonstrated that AP2 subunits were associated and colocalized with FMRP in dendrites. Because of the important functions of AP2 in membrane trafficking, transferrin was used as a reporter to study clathrin-mediated endocytosis and endosomal trafficking. Quantitative imaging analysis revealed that *Fmr1* KO neurons showed a delayed, but significant increase of transferrin positive puncta numbers, which suggests that *Fmr1* depletion may lead to increased rate of endocytosis. Compared to WT neurons, the early

endosomes in *Fmr1* KO neurons showed increased numbers and enlarged size after transferrin feeding. In this study, we uncovered impairments in the expression and synaptic localization of AP2 subunits that may be due to loss of FMRP mediated local translation regulation as well as FMRP interacting with AP2 subunits at synapses. Further work is underway to determine if dysregulated AP2 is responsible for the observed abnormal neuronal endocytosis and to identify the specific surface proteins adversely affected in FXS.

B334/P2722

Evolutionary cell biology of clathrin-mediated endocytosis in yeasts

A. Picco, C. P. Toret, A. Rivier-Cordey, M. Kaksonen; University of Geneva, Geneva, SWITZERLAND.

Clathrin-mediated endocytosis is a core, eukaryotic membrane trafficking pathway at the interface between the cell and its environment. Endocytosis requires biophysical events to occur sequentially: first, the endocytic coat proteins define the site of endocytosis and recruit cargo on the plasma membrane, then the membrane is deformed to create an invagination, and lastly, a vesicle is released into the cytoplasm. Much of the endocytic protein machinery is conserved across eukaryotes. However, there are a significant number of proteins that vary. Furthermore, the physical parameters of invagination and vesicle vary across species. Little is understood about how the endocytic processes diverged, and the large evolutionary gap between yeast and animals makes this divergence challenging to study. We aim to understand interspecies differences to identify the conserved and variable molecular mechanisms of endocytosis and ultimately reveal the evolutionary pathways that have led to variation of clathrin-mediated endocytosis.

We chose three distantly related fungal yeast species as model organisms: *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Ustilago maydis*. These species diverged around 600MYA. They are amenable to genetic manipulations and live-cell imaging and thus provide a powerful model clade to begin to understand the evolutionary cell biology of endocytosis.

We used quantitative live-cell imaging to compare the assembly and disassembly dynamics, and movements of seven groups of orthologous endocytic proteins tagged with fluorophores. Our comparative data across the three species revealed an unexpected diversity in protein dynamics. This variability was most prominent during the early phase and the last steps of endocytosis. Between these variable periods, we found that the growth rate of the membrane invagination was strikingly identical for the first 100 nm. Afterwards, the invaginations grew at variable rates and reached different total lengths in the different species. This reveals a previously unknown evolutionary constraint on membrane shaping.

This study quantitatively establishes that different modules of endocytosis evolved independently as a mosaic. Defining the variabilities, in addition to the conservation across species, is necessary for understanding the mechanisms that underly membrane shaping in a variety of cellular contexts. Furthermore, our comparative data lay the foundation to estimate ancestral states of endocytosis.

B335/P2723

Flat Clathrin Lattices Nucleate Reticular Adhesions

L. Hakanpää[1], A. Abouelezz[1], A. Lenaerts[1], M. Algie[1], J. Bärlund[1], P. katajisto[1], H. McMahon[2], **L. Almeida-Souza**[1]; [1]HiLIFE, University of Helsinki, Helsinki, FINLAND, [2]MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

The molecular machinery involved in clathrin mediated endocytosis (CME) forms two distinct structures at the plasma membrane: clathrin coated pits (CCPs) and flat clathrin lattices (FCLs). CCPs are the canonical endocytic carriers for CME and have been extensively studied. In contrast, the cellular role of FCLs, characterised by their stability and localisation to reticular adhesions, are not well understood. Reticular adhesions are a recently described type of cellular adhesion composed of integrin αvβ5 and devoid of typical focal adhesion markers.
Here we show that FCLs mediate the formation of reticular adhesions and that this process is controlled by the composition of the extracellular matrix.
We observed that cells plated on fibronectin displayed few FCLs and reticular adhesions. Notably, this effect occurred locally in cells, in an extracellular matrix-contact dependent manner. Pointing to a close relationship between these structures, inhibition of the CME machinery, using knockdowns or dominant negatives, led to complete disappearance of reticular adhesions. In agreement, using live-cell imaging we could visualise that FCL assembly is required for the establishment of reticular adhesions.
Manipulation of the fibronectin receptor integrin β1 revealed that the formation of FCLs - and consequently reticular adhesions - are controlled by the activity of this receptor.
CME and other endocytic routes have conventionally been linked to the disassembly of cellular adhesions by mediating the internalisation of their components. Our results present a novel paradigm in the relationship between these two processes by showing that endocytic proteins can also play a role in the assembly of cellular adhesions.

B336/P2724

Superresolution microscopy reveals partial preassembly and subsequent bending of the clathrin coat during endocytosis

A. Tschanz[1], M. Mund[2,1], Y. Wu[1], F. Frey[3], J. L. Mehl[1,4], M. Kaksonen[2], O. Avinoam[1,5], U. S. Schwarz[6,7], J. Ries[1]; [1]EMBL, Heidelberg, GERMANY, [2]University of Geneva, Geneva, SWITZERLAND, [3]Delft University of Technology, Delft, NETHERLANDS, [4]ETH, Zürich, SWITZERLAND, [5]Weizmann Institute of Science, Rehovot, ISRAEL, [6]Institute for Theoretical Physics and Bioquant, Heidelberg, GERMANY, [7]Bioquant, Heidelberg, GERMANY.

Eukaryotic cells use clathrin-mediated endocytosis to take up a large range of extracellular cargos. During endocytosis, a clathrin coat forms on the plasma membrane, but it remains controversial when and how it is remodeled into a spherical vesicle. Here, we use 3D superresolution microscopy to determine the precise geometry of the clathrin coat at large numbers of endocytic sites. Through pseudo-temporal sorting, we determine the average trajectory of clathrin remodeling during endocytosis. We find that clathrin coats assemble first on flat membranes to 50% of the coat area, before they become rapidly and continuously bent, and confirm this mechanism in three cell lines. We introduce the *cooperative curvature model*, which is based on positive feedback for curvature generation. It accurately describes the measured shapes and dynamics of the clathrin coat and could represent a general mechanism for clathrin coat remodeling on the plasma membrane.

B337/P2725

clathrin dynamics in endothelial cells are impacted by shear stress

T. Nawara, E. Sztul, A. Mattheyses; UNIVERSITY OF ALABAMA AT BIRMINGHAM, Birmingham, AL.

The environmental conditions present in the vasculature, including osmotic changes and cell squeezing, inhibit clathrin-mediated endocytosis (CME) in non-endothelial cells, such as fibroblast-like Cos-7 cells. How endothelial cells overcome physiological stresses such as cyclic stretch and shear stress, and preserve CME is not understood. To provide insight into this critically important physiological enigma, we expressed the clathrin light chain a dual-tagged with iRFP713 and EGFP (CLCa-STAR) in vascular model human umbilical vein endothelial cells (HUVECs) and probed clathrin dynamics using simultaneous two-wavelength axial ratiometry (STAR) microscopy. We show that clathrin dynamics, specifically the timing of clathrin accumulation and initiation of curvature in vesicle formation and the ratio of curved to flat clathrin structures in HUVECs grown under static conditions are similar to those in Cos-7 cells. We next examined if clathrin dynamics in HUVECs are altered by sheer stress. When HUVECs were cultured under static or flow conditions, sheer stress led to a characteristic cell alignment and actin organization. We found sheer stress also led to an increase in clathrin dynamics. Surprisingly, we found this was due to an increase of flat clathrin accumulations in flow-stimulated cells, while the number of curved events remained consistent between groups. The curvature positive events had significantly delayed curvature initiation in flow-stimulated cells, highlighting a shift toward flat-to-curved clathrin transitions in vesicle formation. We plan to investigate whether sheer stress changes the response of HUVECs to changes in osmotic pressure. Overall, our findings indicate that clathrin dynamics and endosomal vesicle formation can be modulated by local environment and represents an important regulatory mechanism.

B338/P2726

Clathrin-mediated trafficking of phospholipid flippases is required for local plasma membrane/cell wall damage repair in budding yeast

Y. Yamazaki, K. Kono; Okinawa Institute of Science and Technology, Okinawa, JAPAN.

Plasma membrane damage and repair frequently happen in cells. A critical process underlying plasma membrane repair is to redirect repair factors, such as protein kinase C and the exocyst complex, from the polarized site to the damage site. However, the mechanism underlying the repair factor delivery to the damage site remains unknown. Here, we demonstrate that clathrin-mediated trafficking of repair factors is involved in plasma membrane/cell wall repair in budding yeast. Using laser-induced plasma membrane/cell wall damage assay, we identified phospholipid flippases, Lem3-Dnf1/Dnf2 and Cdc50-Drs2, as essential clathrin cargos for plasma membrane/cell wall repair. We found that flippase impairment significantly compromised the recruitment of exocyst Exo70 to the damage site. In contrast, the recruitment of protein kinase C (Pkc1) was only mildly compromised. Taken together, clathrin-mediated trafficking of the phospholipid flippases is critical for the recruitment of exocyst to the damage site. Mechanisms to redirect exocyst via the clathrin and flippase-mediated pathways may be a general feature of effective plasma membrane repair in polarized cells.

B339/P2727

Measuring conformational changes in clathrin light chain at single sites of endocytosis with FRET-CLEM

K. Obashi, K. A. Sochacki, M. Strub, J. W. Taraska; National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Clathrin mediated endocytosis is the primary internalization mechanism of mammalian cells. Over 50 proteins have been shown to be involved in this process. The nanoscale localizations, interactions, and conformations of endocytic proteins are key regulators of clathrin-mediated endocytosis. Although super-resolution microscopy has revealed the detailed organization of endocytic proteins, the resolution of super-resolution imaging cannot assess molecular interaction and conformational changes. Fluorescence resonance energy transfer (FRET) occurs when dyes are separated by less than 10 nm for most fluorescent proteins pairs. Thus, it can be used to map close-range molecular complexes and dynamics. Yet, to understand how endocytosis works, measurements from FRET must be correlated to the distinct stages of endocytosis. To accomplish this, we developed a new correlative lifetime-based FRET (FLIM-FRET) and platinum replica transmission electron microscopy (PREM) method, named FRET-CLEM. Here, FRET-based atomic distances can be mapped directly to individual cellular structures visualized in EM at the plasma membrane. We used this method to measure the conformational changes in clathrin light chain (CLC), a component of the clathrin triskelion and assembled clathrin lattice. CLC conformational changes have been proposed to regulate the assembly of clathrin in solution. However, CLC conformational changes and their effects on clathrin lattice growth, curvature, and endocytosis at the membrane of living cells are unknown. Using FRET-CLEM, we discovered that CLC undergoes a conformational switch as clathrin lattices curve. Preventing this conformational switch with acute chemical tools increased clathrin lattice sizes and inhibited endocytosis. Therefore, a specific conformational switch in CLC regulates lattice curvature and endocytosis in mammalian cells. These new correlative light and EM data will help develop a complete mechanistic model of endocytosis. More generally, FRET-CLEM can map molecular interactions and conformational changes at targeted membrane-associated proteins at identified cellular compartments including exocytic sites and neuronal synapses.

B340/P2728

Clathrin light chain regulates neural development and function in *Drosophila melanogaster*

J. Das[1,2], A. Majumdar[1], D. Subramanyam[1]; [1]National Center for Cell Science, Pune, INDIA, [2]Savitribai Phule Pune University, Pune, INDIA.

Clathrin is a cytosolic protein involved in the intracellular trafficking of a wide range of cargo. It is composed of three molecules of the clathrin heavy chains and three molecules of the clathrin light chains that together form a triskelion, the subunit that polymerizes to form a clathrin coated vesicle. Although the role of the heavy chains in regulating important physiological processes has been well documented, we still lack a complete understanding of how the clathrin light chains regulate membrane traffic and cell signaling. In this study, we use *Drosophila melanogaster* as a model system to understand the role of clathrin light chains (CLC) in neural development and function. Using CRISPR -Cas9, we show that adult flies exhibit a severe locomotion defect upon ubiquitous depletion of the light chain. This phenotype is also seen upon neuron-specific loss of the protein indicating that a neuron specific function of the light chain, presumably it's role in synaptic vesicle recycling, is essential for locomotion in

adult flies. Interestingly, this behavioural defect is not seen at the larval stage. We also observe additional behavioural changes in flies lacking the clathrin light chain. These are accompanied by altered mushroom body structures, leading us to believe that the light chain may play an important role in brain development and function.

B341/P2729

Exploitation of components of the mammalian ESCRT-III machinery by the parasite *Toxoplasma*for the sequestration of host organelles into the parasitophorous vacuole

J. D. Romano[1], J. Mayoral[2], R. B. Guevara[2], Y. Rivera-Cuevas[3], V. B. Carruthers[3], L. M. Weiss[2], I. Coppens[1]; [1]Johns Hopkins University School of Public Health, Baltimore, MD, [2]Albert Einstein College of Medicine, Bronx, NY, [3]University of Michigan Medical School, Ann Arbor, MI.

The intracellular parasite *Toxoplasma gondii* multiplies in mammalian cells within a self-made niche - the parasitophorous vacuole (PV). Parasite proteins are incorporated into the PV membrane (PVM) creating a specialized compartment that avoids fusion with host cellular organelles. However, *Toxoplasma* proficiently salvages lipids from host organelles by diverting endosomal recycling pathways to the PV. The parasite sequesters host endo-lysosomes and Rab vesicles into the PV using an IntraVacuolar Network (IVN) of parasite-derived membranous tubules that can fuse to the PVM, creating open gates; in a mutant lacking an IVN, a significant decrease of host organelle internalization is observed. Host organelles accumulate in the PV center, suggesting dissociation from the PVM. We hypothesize that the parasite recruits host ESCRT components to mediate this scission. In HeLa cells, stably expressed GFP-CHMP4B localizes to the host cytosol/nucleus, and upon infection, associates with the PV and IVN as puncta, suggesting recruitment to the PV. To analyze host CHMP4B-PV association, we overexpressed host CHMP4B tagged C-terminally with mEmerald to induce dominant negative (DN) effects. CHMP4B-mEmerald largely distributes on the PVM; its more uniform distribution and absence on the IVN suggests the C-terminal tag may interfere with later scission steps. EM observations illustrate PVM invaginations forming long tubules containing CHMP4B polymers organized in spirals within the tubules, leading to tubule constriction to a diameter of 105 nm. These invaginations are significantly shorter in the IVN-deficient mutant, suggesting IVN tubule contribution to the elongation of PVM invaginations triggered by DN CHMP4B. In infected cells overexpressing $VPS4^{EQ}$, we observe abnormally enlarged intra-PV structures containing host endo-lysosomes, reflecting defects in PVM tubule scission. Knock-out mutants of two parasite proteins (TgGRA14, TgGRA64), that interact with ESCRT motifs, exhibit defects in CHMP4B-mEmerald-induced invagination formation and host organelle internalization, with more pronounced defects observed in the PV of *Δgra14Δgra64*, suggesting a synergistic role at the PVM/IVN via interactions with host ESCRT components.

B342/P2730

Quantitative fluorescence analysis of the endocytosis of GPCR Ste3

K. G. Oppenheimer[1], N. A. Hager[1], C. K. McAtee[1], M. P. Bruchez[2], A. F. O'Donnell[1]; [1]Biological Sciences, University of Pittsburgh, Pittsburgh, PA, [2]Biological Sciences and Chemistry, Carnegie Mellon University, Pittsburgh, PA.

The ability to sense and respond to extracellular signals is essential for cellular function and an organism's health. G-protein coupled receptors (GPCRs) enable specific and rapid response to extracellular signals through their ligand-induced activation of associated heterotrimeric G-proteins. Our

previous work demonstrated that the constitutive endocytosis of the yeast mating pathway GPCR, Ste3, is regulated by α-arrestins Ldb19, Aly1 and Aly2, but the contribution of these α-arrestins' to ligand-induced Ste3 endocytosis has yet to be explored. We have developed a new quantitative microscopy approach aimed at defining Ste3 endocytic trafficking dynamics in response to ligand. We developed the fluorogen activated protein (FAP) imaging technology for use in quantitative trafficking studies in yeast. We have calibrated the FAP-tag for yeast cell biology studies by codon optimization and have refined imaging conditions to optimize FAP's utility in this organism. Using this system, we determined that the α-arrestins Aly1, Aly2, and Ldb19 mediate the ligand-dependent endocytosis of Ste3, with a significant retention of the receptor at the plasma membrane in the absence of these three α-arrestins. We further re-assess regions of the Ste3 C-terminal tail that were identified as important for constitutive or ligand-induced internalization of Ste3. We found that the PEST-like domain, previously thought to be important for only constitutive internalization, also impacts ligand-dependent Ste3 endocytosis. Additionally, we find that a single lysine mutation in the C-tail of Ste3 impedes its endocytosis and posit that this may be an important site of receptor ubiquitination. We perform compared analyses between the FAP imaging technology to the pH sensitive fluorescent marker pHluorin, confirming that both FAP and pHluorin tagged Ste3 show similar internalization dynamics. This comparison also illustrates the advantage of FAP application in endocytic assays, as we can spatially and temporally visualize select Ste3 populations with FAP. Together, this work expands the role of α-arrestins in GPCR trafficking and demonstrates the utility of the FAP technology in yeast cell biology studies.

B343/P2731

Upregulated-flotillins derail endocytosis and vesicular traffic to induce cellular invasion

C. Gauthier-Rouviere, S. Bodin; CNRS-CRBM, Montpellier, FRANCE.

Altered endocytosis and vesicular trafficking are major players during tumorigenesis.Flotillins 1 and 2 are two ubiquitous, highly conserved homologous proteins that assemble to form heterotetramers at the cytoplasmic face of the plasma membrane (PM) in microdomains enriched in cholesterol and sphingolipids. They scaffold membrane microdomains - where different proteins concentrate - thanks to their oligomerization property. Flotillin 1 and 2 are upregulated in a large number of cancers, which is associated with a poor prognosis. Flotillin upregulation is necessary and sufficient for the acquisition of invasive cell properties. The upregulation of flotillins favors their oligomerization and induces the Upregulated Flotillin-Induced Trafficking (UFIT) pathway, an endocytosis pathway that targets cargo proteins in late endosomes positive for CD63 and LAMP-1, where flotillins accumulate. These flotillin-enriched late endosomes are not degradative and contain numerous intraluminal vesicles, their fusion with the PM allows the recycling of the proteins present in their outer membrane and the release of the exosomes/extracellular vesicles (EVs) they contain in the extracellular medium.We found that, in non-tumoral mammary epithelial cells, induction of the UFIT pathway promotes epithelial-to-mesenchymal transition (EMT) and accelerates the endocytosis of several transmembrane receptors towards flotillin-positive non-degradative late endosomes. The receptor tyrosine kinase AXL, the overexpression of which, frequently observed in cancer cells, is linked to EMT and metastasis formation, is stabilized and consequently overexpressed by the UFIT pathway. We also showed that flotillin upregulation in epithelial mammary (MCF10A) and mesenchymal myoblastic (C2C12) cells increases the number of secreted EVs. To identify the mechanisms, we are performing a siRNA-based screen with the Nluc-CD63 reporter in flotillin-overexpressing non-tumoral MCF10A cell line. The UFIT-pathway requires sphingosine-kinase 2 which is recruited in flotillin-rich membrane-domains at endocytic sites and in late

endosomes. This lipid kinase phosphorylates sphingosine to generate sphingosine-1 phosphate, a lipid frequently overproduced in cancer cells. We propose that in cancer cells, flotillin-upregulation promotes the formation of sphingosine-rich membrane microdomains where sphingosine kinase-2 locally generates sphingosine-1-phosphate to induce membrane remodeling events involved in the UFIT-pathway.Thus, flotillins along with sphingosine-kinase 2 emerge as a new machinery involved in the deregulation of endocytosis and vesicular trafficking that impacts on the level and activity of cargos involved in tumoral invasive progression.

B344/P2732

Loss of DLG1 Leads to Profound Changes in the Ciliary Proteome of Kidney Epithelial Cell Lines

C. K. Rezi[1], Z. Anvarian[1], M. Aslanyan[2], G. Diwan[3], K. Junger[4], K. B. Pauly[1], Y. Afshar-Bahadori[1], M. Georgieva[5], P. M. H. Olesen[1], S. T. Christensen[1], R. Russell[3], K. Boldt[4], R. Roepman[2], L. B. Pedersen[1]; [1]Department of Biology, University of Copenhagen, Copenhagen Ø, DENMARK, [2]Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, NETHERLANDS, [3]Bioquant and Biochemistry Center (BZH), Heidelberg University, Heidelberg, GERMANY, [4]Institute for Ophthalmic Research, Eberhard Karl University of Tübingen, Tübingen, GERMANY, [5]Bioquant, Heidelberg University, Heidelberg, GERMANY.

Background and objective: Polarized protein trafficking is essential for many cellular functions, such as the transport of polycystins and other transmembrane proteins to the primary cilium. However, for many ciliary proteins, the specific intracellular pathways that mediate their targeting to the cilium remain unknown. Disks Large MAGUK Scaffold Protein 1 (DLG1) is a core component of the Scribble complex implicated in regulating epithelial cell polarity. In addition to this well-described function of DLG1, several proteomics studies indicated that a pool of DLG1 may be present at the primary cilium. Here we used an unbiased, proteomics-based approach to address possible ciliary functions for DLG1 in kidney epithelial cells.

Methods and results: Using quantitative cilia-targeted BioID2-based proximity-labeling proteomics in wild-type and *Dlg1* knockout IMCD3 cells, we found that loss of DLG1 leads to significant and profound changes in the ciliary proteome. Specifically, ciliary levels of several proteins known to function in vesicle-mediated trafficking, cytoskeleton organization, or signaling were reduced in the *Dlg1* knockout cells. These included the retromer component Serologically Defined Colon Cancer Antigen-3 (SDCCAG3) and Intraflagellar Transport 20 (IFT20), previously shown to facilitate ciliary transport of Polycystin-2 (PC2), as well as Transforming Growth Factor β (TGF-β)-Activated Kinase 1 (TAK1; aka MAP3K7), which plays a crucial role in many cellular processes. Immunofluorescence microscopy analysis confirmed that loss of DLG1 impairs ciliary targeting of these proteins, and also revealed reduced ciliary levels of PC2 in DLG1-depleted cells. Results from live cell imaging experiments of cells expressing fluorescently tagged versions of DLG1 suggest that DLG1 primarily localizes to cell junctions, and localizes to the cilium only transiently or when overexpressed.

Conclusion: We propose that DLG1 regulates the transport of several ciliary membrane and signaling proteins by mediating their internalization from cell junctions prior to further transport via recycling endosomes to the cilium.

Funding: Supported by grant No. NNF18SA0032928 from the Novo Nordisk Foundation and the European Union's Horizon 2020 research and innovation program Marie Sklodowska-CurieInnovative Training Networks (ITN) under grant No. 861329.

B345/P2733

Phosphatidylinositide-3-Kinase Regulated Trafficking of C-C Chemokine Receptor 7 to Endosomal Compartments in Human T-cells

M. A. Brissette, C. A. Bill, C. I. Cardona, C. M. Vines; The University of Texas of El Paso, EL PASO, TX

The G-protein coupled receptor, C-C Chemokine Receptor 7 (CCR7), is important for chemotactic migration of immune cells to lymphoid organs and subsequent immune responses. We have shown in human and mouse T-cells that signaling via CCR7/C-C Chemokine Ligand 19 (CCL19) promotes rapid receptor internalization mediated by arrestin-3. In the human T cell line HuT78, we showed that ~20% of CCR7 re-expresses or recycles to the cell membrane. We hypothesized that internalized CCR7/CCL19 is recycled and/or degraded. Therefore, to better understand the pathway of CCR7 intracellular trafficking, we transduced human CEM T-cell acute lymphoblastic leukemia cells, which endogenously expresses CCR7, with Rab4-mCherry, Rab5-mCherry, Rab7-mCherry, and Rab11-mCherry to track CCR7 from the cell membrane through endosomal compartments. Following stimulation with 200nM CCL19 we examined colocalization at 0 min, 30 s, 1 min, 10 min, 30 min, 1 hr and 2 hr timepoints using confocal microscopy. Interestingly, even at 0 minutes, ~40% of CCR7 localized to the Rab11 compartment, suggesting that CCR7 may be stored in this compartment prior to expression on the cell membrane. Within 30s of stimulation, colocalization of CCR7 was observed with Rab4/5 proteins, indicating the presence of CCR7 in early endosomes. At 30 minutes ~78% of CCR7 was localized to late endosomes (Rab11). Upon continued incubation for 2 hrs, ~43% of CCR7 colocalized within the Rab7 compartment (lysosomes) indicating that CCR7 is also trafficked for destruction. Phosphatidylinositol-3-kinase (PI3K) family members regulate membrane composition within specific endosomes to control vesicle trafficking. To determine the role of PI3K in the recycling/degradation of CCR7 via Rab11 or Rab7, we used 5μM wortmannin to block class I and class II PI3K's and 0.5μM to block class I PI3K during CCR7 recycling using flow cytometry. We found that under both conditions, receptor recycling was reduced by 20% suggesting a role for class I or class II PI3K family members in recycling of CCR7 to the cell membrane via Rab11. In addition, following CCR7-eGFP internalization with CCL19 and 2 hrs of recycling, CCR7 remained within the trans-Golgi network in the presence of 0.5μM wortmannin, in contrast CCR7-eGFP, recycled to the cell membrane in the absence of wortmannin further demonstrating a potential role for class II PI3K in CCR7 recycling. We are currently confirming a role for class II PI3K in CCR7/CCL19 recycling using PI3K Class II shRNA (PI3KC2A), since PI3KC2A is a Rab11 effector protein and has been shown to regulate recycling of various GPCRs.

B346/P2734

Uncovering the role of actin assembly in fast-endophilin mediated endocytosis

S. L. Smith; University of California, Berkeley, Berkeley, CA

Force transmission through the actin cytoskeleton is fundamental for cells to sense the geometric and mechanical constraints of their environments, move through tissues, remodel the extracellular matrix, and regulate signaling receptors at the plasma membrane (PM) to determine cell fate. In cell systems, such as the leading edge of migrating cells or the endocytic machinery, polymerizing actin generates forces on the membrane. The dynamics, structure, and force generation of actin filaments are regulated by actin binding proteins (ABPs). In mammalian cells, actin assembly is not required at every endocytic site, but the dependency on actin assembly forces increases as membrane tension increases. The mechanisms responsible for actin assembly and load adaptation have been investigated for clathrin-

mediated endocytosis, but less so for other endocytic pathways such as fast-endophilin mediated endocytosis (FEME). While pathogen-hijacked FEME shows a requirement for actin assembly to facilitate proper internalization, how actin adaptation is triggered and the proteins responsible for this signaling cascade both have yet to be identified. I propose that, under high membrane tension, FEME sites require a more robust actin meshwork to aid in membrane deformation and promote successful cargo internalization. I hypothesize that actin binding proteins, which bind to both the plasma membrane and actin filaments, facilitate this adaptation. One particularly attractive candidate to provide this function is Myosin1E because it can transduce force and link the cytoskeleton to the membrane. High-resolution fluorescence microscopy of genome-edited human induced pluripotent stem cells (hiPSCs) expressing fluorescent protein fusions of Myosin1E demonstrated localization of Myosin1E at sites of FEME. Additionally, under increased membrane tension in response to osmotic shock, there is increased co-localization between Myosin1E and Dynamin2, a marker of endocytic scission. This endocytic pathway also appears to be conserved in hiPSC differentiated fibroblasts. From this work, I hope to build an understanding of how the actin network adapts to increased load to ensure successful vesicle internalization.

B347/P2735

Endosome recycling of guidance receptor regulates dendrite morphogenesis in *C. elegans.*

C. E. Richardson[1], C. Yee[2], K. Shen[2]; [1]University of Wisconsin Madison, Madison, WI, [2]Stanford University, Stanford, CA

In dendrite morphogenesis, guidance receptors on the neuron's plasma membrane instruct local actin dynamics to mediate attraction, stabilization, and repulsion. How these movements are coordinated temporally and spatially at the molecular level is incompletely understood. The *C. elegans* PVD neuron grows an elaborate dendritic arbor over the course of larval development that can be easily interrogated with genetic approaches. Such studies have identified PVD dendrite guidance receptors and downstream actin regulators that are required for morphogenesis, including DMA-1 and TIAM-1 (Tumor Invasion And Metastasis), respectively. Here, we used the *C. elegans* alternate physiological state, dauer, as an orthogonal tool to interrogate dendrite morphogenesis. Whereas the PVD dendrite gets progressively larger and more complex throughout normal larval development, dendrite morphogenesis is reversibly arrested in the dauer state. We found that this arrested morphogenesis is accompanied by an enrichment of the PVD dendrite guidance receptor DMA-1 on the plasma membrane. By developing a novel tool to distinguish between recycled endosomes and biosynthetic vesicles *in vivo,* we determined that this plasma membrane DMA-1 enrichment is accompanied by a corresponding depletion of DMA-1 from recycling endosomes. Using a combination of live imaging of dendrite growth dynamics and mis-targeting the TIAM-1 to different subcellular compartments in the dendrite, our data support the model that recycling of DMA-1-containing endosomes regulates the balance between dendrite stabilization and growth. We aimed to identify the mechanism whereby the dauer state inhibits DMA-1 endosomal recycling. Surprisingly, we found that constitutive endocytosis is broadly suppressed in dauer across multiple tissues. We envision that this would simultaneously arrest the many cell growth and development pathways that involve endosome recycling to suspend development in the dauer state.

B348/P2736

Roles and regulation of endocytic recycling at the postsynapse

T. Uygun[1,2], P. Rivero Rios[1,2], T. Tsukahara[1], G. Chavis[1], L. S. Weisman[1], M. A. Sutton[1]; [1]University of Michigan, Ann Arbor, MI, [2]Life Sciences Institute, Ann Arbor, MI

The levels of many cell surface proteins are regulated by trafficking from endosomes to the plasma membrane. In HeLa cells, the majority of cell surface proteins traffic via either the SNX27-Retromer-WASH pathway, or the SNX17-Retriever-CCC-WASH pathway. Endocytic recycling also plays important roles in synaptic function. Indeed, the SNX27-Retromer pathway regulates the recycling of several key neuronal receptors, including GLUT1, AMPA receptors, and Kir3. In addition, our recent data using rat hippocampal neurons shows that the SNX17-Retriever pathway is critical for the regulation of postsynaptic function and plasticity, and acts in part by regulating the recycling of β1-integrin, a well characterized SNX17 cargo in HeLa cells. Both Retromer and Retriever have been shown to localize to the same microdomain on endosomes in HeLa cells. This raises the question of whether these distinct pathways emerge from the same endosomes at postsynaptic sites. Here, I present the development of tools that will enable me to test whether the SNX17-Retriever, and SNX27-Retromer pathways colocalize at post-synaptic sites. I identified commercially available antibodies suitable for immunofluorescence that specifically recognize SNX17 and SNX27. I tested their specificity using shRNA mediated knockdown. In addition, wild-type SNX17, SNX27, VPS26B, and VPS26C proteins (which are subunits of the Retromer and Retriever complexes, respectively) have been fused with mScarlet and mNeonGreen fluorescent proteins. Using these tools I will test the subcellular localization of each complex via immunofluorescence and live cell imaging, both under basal conditions and upon long-term potentiation. Additionally, shRNAs which specifically target SNX17, SNX27, VPS26B, and VPS26C have been generated to investigate the effects of depletion of these complexes on synaptic function, synaptic plasticity and the architecture of dendritic spines. Elucidating the mechanisms regulating Retromer- and Retriever- dependent recycling in neurons will likely provide new insights into the regulation of surface-exposed proteins at postsynaptic sites, as well as their contribution to altered receptor recycling in diseases of the nervous system.

B349/P2737

Pathogenetic mechanism of a novel neurodevelopmental disorder caused by mutations in EIPR1

S. Ghosh[1], A. Sawafta[2], N. Damseh[3], S. Edvardson[4], O. Elpeleg[5], J. Bonifacino[1]; [1]National Institutes of Health, Bethesda, MD, [2]Faculty of science, An Najah National University Nablus Palestine, Nablus, Palestinian Territory, [3]Faculty of Medicine, Al-Quds University, Abu Deis, Palestinian Territory, [4]Department of pediatrics, neuropediatric unit, Hadassah-Hebrew University Medical Center, Jerusalem, Israel, [5]Monique and Jacques Roboh Department of Genetic Research, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Endosomes are a major hub of protein sorting that receive proteins from the plasma membrane (i.e., endocytosis) and the *trans*-Golgi network (TGN) (i.e., biosynthetic transport), and recycle them back to the plasma membrane (i.e., endocytic recycling) and TGN (i.e., retrograde transport). Proteins that are not retrieved by these pathways remain in endosomes for subsequent delivery to lysosomes. Two related multisubunit tethering complexes named endosome-associated recycling protein (EARP) and Golgi-associated retrograde protein (GARP) complex participate in endocytic recycling and retrograde transport, respectively. EARP associates with endosomes and supports recycling of proteins such as

transferrin receptor to the plasma membrane. GARP associates with the TGN to enable retrograde trafficking of proteins like the cation-independent mannose 6-phosphate receptor which recycles after delivering acid hydrolase precursors to lysosomes. Dysfunction of GARP leads to missorting of lysosomal acid hydrolases, as well as impaired trafficking of lipids and sterols, and compromise retrograde trafficking of bacterial toxins. In previous work, our lab identified a WD40-domain protein named EIPR1 that cooperates with EARP and GARP in these processes. However, the role of EIPR1 in human physiology remained unclear. Recently, we have identified the patients with biallelic mutations in the *EIPR1* gene. The three patients were children with severe global developmental delay, microcephaly, and spasticity. Throughout childhood there was hardly any acquisition of developmental milestones. To examine the effect of the mutations on EIPR1 properties, I expressed the mutant proteins in heterologous cell lines and found that the mutant proteins have lower expression levels and are less stable compared to wild-type form. The mutations also affected the interaction and co-localization of EIPR1 with the EARP and GARP complexes. Furthermore, analysis of patient fibroblasts showed a strong defect in retrograde transport from endosomes to the TGN and swollen lysosomes. Electron microscopy with immunofluorescence study revealed accumulation of autophagic vesicles and increased level of lipid droplets inside cells. Therefore, mutations in EIPR1 lead to defective retrograde trafficking, dysfunctional lysosomes, and altered autophagy. Taken together, these findings demonstrate a critical role for EIPR1 in human physiology and shed light on the molecular and cellular basis of a novel neurodevelopmental disorder.

B350/P2738

Rab Coupling Protein (RCP) function upon EGFR trafficking from perinuclear recycling endosomes is regulated by PKA

J. Venegas[1], J. Barra[1,2], C. López[1], C. Retamal[1], A. González[1,2,3]; [1]Universidad San Sebastian, Santiago, Chile, [2]Centro Científico y Tecnológico de Excelencia Ciencia & Vida, Santiago, Chile, [3]Centro de Envejecimiento y Regeneración (CARE-UC), Santiago, Chile

Introduction: RCP (Rab11-FIP1C) is an effector of Rab11 involved in the endocytic recycling pathway at the level of the Endocytic Recycling Compartment (ERC), a series of perinuclear tubular and vesicular membrane mediating the slow recycling pathway. Endocytic recycling of nutritional and signaling cell surface receptors is crucial in a variety of physiological and pathological conditions including cancer. However, its regulation by cell surface receptor signaling pathways remain unknown. We recently described that protein kinase A (PKA) basal activity is required for the recycling of epidermal growth factor receptor (EGFR), transferrin receptor (TfnR) and low-density lipoprotein receptor (LDLR) from ERC. Based on bioinformatic data, here we explored whether RCP has a role in endocytic recycling as a potential PKA substrate. **Material and Methods:** We evaluated PKA-mediated phosphorylation of RCP by immunoblot and compared the effects of PKA inhibitors (Myr-PKI) and TGF-α stimulation that decrease or promote EGFR recycling, respectively. We also compared trafficking effects of RCP phospho-inert and phospho-mimetic mutants. Analysis included confocal microscopy images of fluorescently tagged markers in live cells, cell surface EGF binding and biotinylation, coimmunoprecipitation (co-IP) assays and PKA activity assessed by a fluorescent sensor and immunoblot against PKA substrates. **Results:** TGF-α treatment known to promote EGFR recycling increased the PKA activity, resulting in higher RCP phosphorylation, whereas PKI arrested EGFR recycling and decreased the phosphorylation of RCP, leading to EGFR concentration at ERC in a complex with RCP and Rab11. Both TGF-α and PKI increased the co-IP of RCP with EGFR and Rab11. Phospho-inert RCP^{S435A} inhibited EGFR exiting from ERC,

colocalizing there with TfnR and Rab11, while the phospho-mimetic RCP^{S435D} showed opposite effects. **Conclusions:** RCP is a PKA substrate sensitive to EGFR recycling stimulators such as TGF-α and its PKA phosphorylation status impacts upon its interactions with EGFR and Rab11, as well as upon its role in EGFR recycling from ERC. **Acknowledgements:** National Agency for Research and Development (ANID)/Scholarship Program/DOCTORADO BECAS CHILE/2021-21110001, Basal Project ACE210009, and Centro Ciencia & Vida, Basal FB 210008. Jim Norman (Beatson Institute, Glasgow, UK) kindly provided anti-Phospho-RCP-Ser435 antibodies and the phosphoinert RCP^{S435A}-mCherry plasmid.

B351/P2739

Caveolin-1 Endocytosis upon Hypotonic Shock

T. H. Wong, L. Li, P. Torigny, B. Joshi, I. R. Nabi; University of British Columbia, Vancouver, BC, CANADA.

Caveolin-1 (Cav1) oligomerizes to form stable caveolar coats at the plasma membrane. Under hypotonic shock, caveolae disassemble in response to the mechanical stress providing a membrane buffer and protecting the membrane from mechanical stress. Using STED super resolution microscopy, we observe that large Cav1 positive spherical structures form at 15 minutes of hypotonic shock and accumulate over time in MDA-MB-231 cells. After hypotonic shock, Cav1 spherical structures are endocytic structures as they are labeled by uptake of antibodies to β1-integrin and CD44 after acid wash of the cell surface and their formation is prevented by dynasore inhibition of dynamin-dependent endocytosis. Cav1-positive spherical structures do not colocalize with endosomal markers Rab5 and Rab7 and show only partial colocalization with the lysosomal marker Lamp1. In PC3 prostate cells, that express Cav1 but not the coat protein Cavin1 required for caveolae formation, Cav1 spheres also form under hypotonic shock but do not accumulate over time as observed to the MDA-MB-231 cells. These findings suggest that Cav1 is internalized via a caveolae-independent, dynamin-dependent endocytic pathway in response to hypotonic shock.

B352/P2740

Endocytic dynamics are tissue dependent in developing embryos

C. Shirazinejad, I. Swinburne, D. Drubin; University of California, Berkeley, Berkeley, CA

Clathrin-mediated endocytosis (CME) is a receptor internalization pathway used in diverse cell types. While much of its parts list is known, how these parts are recycled for specialization in differentiated cells is less characterized. Here, we establish a vertebrate system that expresses fluorescent proteins fused to endogenous enzymes important for detecting CME. Following circulating blood during development, we have begun characterizing when and where blood cells internalize iron-bound receptors. Additionally, we are interested in how cells adapt to their local environments during CME with the assembly of branched actin, a force-generating cytoskeletal arrangement of dendritic actin created with the ARP2/3 complex. Our preliminary results indicate epithelial cells as well as non-epithelial cells may use ARP2/3 during CME.

B353/P2741

Identifying Curvature-Sensitive Features in the Early Stages of Clathrin-Mediated Endocytosis

P. Marchando, R. Cail, D. Drubin; University of California, Berkeley, Berkeley, CA

The plasma membrane (PM) is a highly dynamic structure that can be deformed, such as during cell locomotion and trafficking. In clathrin-mediated endocytosis (CME), the primary pathway through which extracellular cargo and transmembrane receptors are internalized, dozens of proteins work in concert to deform the PM into a deep pit that can undergo scission into the cytosol. Many of these components have been shown to be strongly sensitive to changes in membrane geometry. Recent advances in nanofabrication have enabled the creation of "nanoshapes," nanometer-scale structures that can reproducibly deform the PM of cells cultured on them, facilitating research into the relationship between membrane shape and protein recruitment. At small radii of positive curvature, the entire CME machinery has been shown to strongly relocate to nanoshape-induced PM bending. This nanoshape-induced curvature has even been shown to bypass the curvature-generating role of clathrin in clathrin knockdown cells. Many questions remain about the relationship between membrane curvature and CME. Since localization to induced curvature appears to start very early in CME, I have employed a screening strategy of knocking down several early CME components to identify proteins necessary for robust curvature-localized CME. Using homemade nanoshapes in concert with genome-edited human cells to study endocytic dynamics and pulse-chase assays to assess bulk endocytic defects, I have identified a condition that impairs CME differentially upon inducing curvature. Cells with early-arriving adaptor proteins EPS15 and EPS15R knocked down in concert with clathrin lose their ability to cluster the endocytic cargo transferrin, but upon inducing curvature, transferrin clusters once again along nanostructures in a way that depends on adaptor protein AP2's strong enrichment at sites of curvature. These results suggest a central role for AP2 in curvature-sensitive CME localization, as its localization to curvature is largely unperturbed by removal of its interacting partners and clathrin. Further studies are necessary to fully understand how early CME proteins and membrane curvature work in concert to initiate the process.

B354/P2742

Investigating the Role of Clathrin-Mediated Endocytosis in T-cell Activation

Y. Iwamoto, A. Ye, C. Shirazinejad, J. Hurley, D. Drubin; UC Berkeley, Berkeley, CA

Decades of research has established clathrin-mediated endocytosis (CME) as a major pathway through which mammalian cells internalize surface proteins. Recent studies have began to show that the function of the CME pathway goes well beyond its canonical role of internalization. For example, the pathway has been linked to functions such as scaffolding the cytoskeleton, wrapping around the extra-cellular matrix, and serving as hubs for organizing signaling factors. Furthermore, distinct CME structures and dynamics have been observed in different cell types, suggesting further exploration of CME in different cellular contexts will lead to novel insights. To further our understanding of the CME pathway, we chose to investigate its role in the context of T-cells. This is because, although defects in the CME pathway has linked to impaired stimulation of T-cells during an immune response, the exact role of the pathway is not understood. In this study, we utilize genome-edited Jurkat cells fluorescently expressing CME markers AP2 and DNM2 to visualize live dynamics of CME in a T-cell like system for the first time. We show that CME is a constitutively active pathway in unstimulated Jurkat cells but that the dynamics of the pathway change when the cells are activated. Activation with both PMA/Ionomycin and

CD3/CD28 bound dynabeads induce a transient stalling of CME, indicated by long lived AP2 puncta at the plasma membrane. Interestingly, this transient stalling is observed in the middle focal plane of the Jurkat cells, distinguishing them from previously observed stable CME structures such as plaques that form at the basal surface of the cell. Currently we are investigating the functional significance of this transient CME stalling upon Jurkat cell stimulation. We hypothesize that the stalling could be important for prioritizing other clathrin-independent endocytic pathways or that these sites are serving as hubs for organizing signaling factors involved in T-cell activation. In conclusion, we have observed that the CME pathway is constitutively active in unstimulated Jurkat cells but stalls in response to stimulation. Investigating the significance of this phenomenon will elucidate the molecular details of CME pathway's important connection to T-cell activation.

Membrane Fission and Coat Proteins

B355/P2743

Dynamin is primed at endocytic sites for ultrafast endocytosis

Y. Imoto[1], S. Raychaudhuri[1], Y. Ma[1], P. Fenske[2], E. Sandoval[1], K. Itoh[1], E. Blumrich[3], H. T. Matsubayashi[1], L. Mamer[2], F. Zarebidaki[2], B. Söhl-Kielczynski[2], T. Trimbuch[2], S. Nayak[4], J. H. Iwasa[4], J. Liu[1], B. Wu[1], T. Ha[1], T. Inoue[1], E. M. Jorgensen[4], M. A. Cousin[3], C. Rosenmund[2], S. Watanabe[1]; [1]Johns Hopkins University, Baltimore, MD, [2]Charité Universitätsmedizin Berlin, Berlin, GERMANY, [3]University of Edinburgh, Edinburgh, UNITED KINGDOM, [4]University of Utah, Salt Lake City, UT.

Dynamin mediates fission of vesicles during endocytosis. During clathrin-mediated endocytosis, recruitment of dynamin from the cytosol to endocytic pits takes tens of seconds. For synaptic vesicle endocytosis, dynamin must be recruited to the site of endocytosis on the order of milliseconds to mediate ultrafast endocytosis. However, the mechanism underlying such rapid recruitment is unknown. Here, we demonstrate that Dynamin 1 is enriched at endocytic sites prior to the initiation of ultrafast endocytosis. Specifically, Dynamin 1xA, a splice variant of Dynamin 1, interacts with Syndapin 1 to form molecular condensates on the plasma membrane. Single-particle tracking of Dynamin 1xA molecules confirms the liquid-like property of condensates with a small immobile fraction of molecules. Time-resolved electron microscopy by flash-and-freeze suggests that loss of Dynamin 1 causes an accumulation of endocytic pits on the plasma membrane; the base of these pits is wide-open, suggesting that Dynamin 1 is potentially involved from the early stage of ultrafast endocytosis. Over expression of Dynamin 1xA, but not another splice variant xB, rescues this defect. When binding of Dynamin 1xA to Syndapin 1 is dirupted, Dynamin 1xA cannot form condensates. Likewise, when Syndapin 1 binding to Dynamin 1 is disrupted, Dynamin 1xA becomes diffuse. Interestingly, disrupting Syndapin 1 interaction with the plasma membrane also leads to diffuse localization of Dynamin 1xA, suggesting that Syndapin 1 likely acts as a hub. When Dynamin 1xA is diffusely localized, ultrafast endocytosis is slowed. Thus, Dynamin 1xA condensates concentrate Dynamin 1xA at the sites of endocytosis to bypass the recruitment phase and accelerate.

B356/P2744

A Mechanism for Sculping Endosomal Tubular Carriers

C. Lopez-Robles[1], S. Scaramuzza[2], **E. N. Astorga-Simon**[1], M. Ishida[3], C. D. Williamson[3], S. Baños-Mateos[1], D. Gil-Carton[4], M. Romero[5], A. Vidaurrazaga[1], J. Fernandez-Recio[6], A. L. Rojas[1], J. S. Bonifacino[3], D. Castaño-Díez[4], A. Hierro[1]; [1]CICbioGUNE, Derio, SPAIN, [2]Biozentrum, Universtity of Basel, Basel, SWITZERLAND, [3]National Institutes of Health, Bethesda, MD, [4]Instituto Biofisika (UPV/EHU, CSIC), University of Basque Country, Leioa, SPAIN, [5]Barcelona Supercomputing Center (BSC), Barcelona, SPAIN, [6]Instituto de Ciencias de la Vid y del Vino (ICVV), CSIC, Logroño, SPAIN.

Just as cargoes enter the endosomal network, various protein machineries contribute to their recycling by either bulk flow or selective processes. The SNX-BAR subfamily of sorting nexins, which contain a phox-homology (PX) domain and a Bin/Amphiphysin/Rvs (BAR) domain, play a central role as coat proteins in multiple endocitic retrograde/recycling pathways. Generation of tubular carriers by SNX-BAR proteins combines membrane association, cargo capture, coat assembly and lipid bilayer deformation mechanisms that remain unresolved. Using X-ray crystallography, cryo-electron tomography and cell-based analysis we reveal an unprecedented coat architecture for tubular-based cargo sorting.

B357/P2745

Dynamically tuning the membrane lipid composition can control macromolecular assembly

J. A. Fischer[1], M. E. Johnson[2]; [1]Department of Biology, Johns Hopkins University, Baltimore, MD, [2]Department of Biophysics, Johns Hopkins University, Baltimore, MD.

Clathrin-mediated endocytosis (CME) is an essential process for transport into the cell that requires cytoplasmic proteins like clathrin to assemble into large macromolecular assemblies on the cell membrane. The protein components specifically bind to the lipid phosphotidylinositol-4,5-bisphosphate (PI(4,5)P2), and thus assemblies form at areas of the membrane enriched with PI(4,5)P2. The kinase PI(5)K and phosphatase synaptojanin can dynamically tune the local abundance of lipid PI(4,5)P2 in opposing ways**,** and both enzymes localize to sites of CME via interactions with adaptor proteins. The complexity and relatively fast time scales of CME make it difficult to resolve the specific roles of individual proteins *in vivo,* and thus it is not known whether these lipid enzymes impact the nucleation or success of clathrin-coated vesicle formation. Here, we use computational modeling to quantify and classify how macromolecular self-assembly depends on the composition of the plasma membrane as it is dynamically remodeled by lipid-modifying enzymes. Our models solve differential equations implemented in the structure-resolved reaction-diffusion software NERDSS, as well as non-spatial ordinary differential equations that are amenable to efficient parameter optimization and sensitivity analysis. We found a novel mechanism for biochemical feedback loops that allowed for a synchronous and rapid transition to and from a membrane with high PI(4,5)P2 density; because both PI(5)K and synaptojanin localize to the membrane surface via adaptor protein binding, they can exploit dimensional reduction to amplify their activity rate as their effective concentration is higher on a 2D membrane. This effect allows for switch-like lipid remodeling, which we found could be achieved within the time-scales of CME nucleation and using physiological concentrations. Our model can be pushed into an oscillatory regime, demonstrating how positive and negative feedback can couple to trigger protein recruitment or dissociation from the membrane, with a significant dependence on the ratio of cytosol volume to membrane area**.** We discuss how the principles established here for controlling recruitment,

dissociation, and oscillations of protein copy numbers on and off the membrane via enzymatic activity are informative for a broader range of membrane remodeling processes across cell biology.

B358/P2746

Impact of zip8 and fabp5 in liver diseases an iron trafficking related genes signature in metabolic dysfunction associated fatty liver disease

S. PRAKASH[1], P. Priyatma[1], S. Shalimar[2], N. K. Vikram[3], A. Priya[1], M. Saroj[1], R. Aasarey[1]; [1]Laboratory Medicine, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, ANSARI NAGAR, NEW DELHI, INDIA-110029, New Delhi, INDIA, [2]Gastroenterology and Human Nutrition, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, ANSARI NAGAR, NEW DELHI, INDIA-110029, New Delhi, INDIA, [3]Medicine, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, ANSARI NAGAR, NEW DELHI, INDIA-110029, New Delhi, INDIA.

Nonalcoholic fatty liver diseases (NAFLD) is the most common chronic liver disease worldwide and related to comorbid with metabolic syndrome, obesity, type 2 diabetes, and insulin resistance. Instability in mitochondrial architecture and mitochondrial fusion-related genes are involved in situations of type 2 diabetes, metabolic dysfunction of fatty liver, hypertension and obesity, leading to enormously fissioned mitochondrial network. As zinc deficiency is common worldwide and triggering a variety of transcription factors i.e. variants in metal ion transporters via ZIP8 which suppress mitofusin proteins with the association of fatty acid binding protein 5 (FABP5) and triggering to IL-6 and IL-10 mRNA in metabolic syndrome. Despite extensive research efforts, there are no treatments of metabolic fatty liver disorders. Iron trafficking proteins (ZIP8/ZIP14), FABP-5, mitofusin/s and dysregulation of Nrf-2 genes may have potential role in MAFLD, we explored whether FABP-5, iron proteins and mitofusin/s (MFN1and MFN2) are involved in NAFLD, NASH, cirrhosis or hepatocellular carcinoma (HCC). Liver tissues and blood samples were collected from Gastroenterology, AIIMS, Delhi. Liver biopsy was done only in NAFLD patients. Fibroscan was done in NAFLD (n=25), diabetes mellitus (DM without Mets) (n=35), Metabolic syndrome (Mets without DM) (n=30), and Healthy subjects (n=25). ZIP8, Zip14, FABP5, Nrf2, MFN1and MFN2 expressions were determined in all groups. FABP5 enhances ZIP8 activity by promoting the ZIP14 synthesis while disrupting the MFN2 integration in NAFLD. Though, increased expression of FABP5 mRNA was associated with the poor prognosis with NASH and cirrhosis. On contrary FABP5 and ZIP14 association was decreased in diabetic patients. ZIP-8 and MFN-2 expressions were highly associated with metabolic syndrome without diabetes and MFN-1 was suboptimal. Mitofusin2 levels may have pleotropic relations in respect to waist hip ratio, BMI and HOMA IR. Nrf-2 was 5folds downregulated in NASH, cirrhosis and HCC. Zip8 and FABP5 mRNA expressions were upregulated in NASH and HCC patients. MFN-2 levels and FABP-5 are positively correlated with the stages of liver fibrosis (>F2 to F4). No correlations to Zip14 expressions with MFN-1 and IL-10 were found. Our study potentially identifies ZIP-8, a new class of route may be interlinked with fatty acid induced FABP-5 upregulation that drives NASH, cirrhosis and HCC progression through mitochondrial fusion proteins especially MFN-2 in circulation. Dysregulation of iron trafficking proteins and FABP-5 expressions could be novel emerging blood biomarkers of hepatocytes injury. Zip8 may possible therapeutic option to prevent liver diseases in at risk individuals and open the door to understand the pathogenesis of MAFLD..

B359/P2747

Dynamin regulates synaptic actin organization and dynamics at endocytic sites

A. M. Silveira, S. J. Del Signore, A. A. Rodal; Brandeis University, Waltham, MA.

Endocytosis relies on F-actin and dynamin GTPases to deform membranes and release vesicles. Recently, dynamin was shown to bind and bundle actin filaments in a manner that is dependent on its GTPase activity and proline-rich domain (PRD), but independent of membrane-binding. These dynamin-actin interactions likely contribute to the function of diverse actin-rich structures. Yet, their role in endocytosis at neuronal synapses, where efficient membrane trafficking is particularly important, is unknown. To begin to answer this question, we investigated how Drosophila dynamin mutants affect the organization and dynamics of synaptic actin patches, which are functionally correlated to endocytosis. Temperature sensitive GTPase mutants showed an overall increase in the number of actin patches and a reduction in the frequency of actin patch initiation at the neuromuscular junction (NMJ), which suggests that dynamin's GTPase activity is important for actin structure initiation and turnover. Interestingly, distinct neuronally-expressed isoforms of dynamin are predicted to differentially affect an actin-binding site in the PRD, which raises the hypothesis that the isoforms may have unique actin-related functions. These isoforms can also be distinguished by their localization patterns at the NMJ, where the long isoform is more punctate and the short isoform exhibits a reticulum-like pattern. Thus, the dynamin isoforms and their interaction with F-actin may add an unexpected layer of regulation to actin dynamics, protein organization, and endocytic efficiency at synapses.

B360/P2748

Reconstitution of membrane scission by ESCRT-III (Endosomal sorting complexes required for transport)

P. Aditya[1], N. Miguet[2], W. Weissenhorn[2], P. Bassereau[1]; [1]Institut Curie, Paris, FRANCE, [2]Institut de Biologie Structurale, Grenoble, FRANCE.

Endosomal sorting complexes required for transport (ESCRT) proteins are involved in severing of narrow membrane necks in several processes like HIV virus release, exosomal and intra-endosomal budding (Multi-vesicular bodies formation) from membranes and cytokinesis (cell division), among others. It is an evolutionarily conserved protein machinery composed of ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III. ESCRT-III complexes and VPS4, an accessory protein, have been proposed to constitute the membrane fission machinery in these processes. The proteins of these complexes tend to assemble into polymer filaments. Recent findings point towards an affinity of ESCRT-III to positively curved membranes or with a **negative Gaussian curvature**, like necks or horse saddle. The geometry for this process is complimentary to the process of endocytosis using Dynamin and is referred to as inverse geometry. The underlying mechanisms involving scission by ESCRT-III are still unclear. Different physical models have been proposed to account for membrane constriction and cleavage but no hard-experimental proof of any of the models has yet been confirmed. The objective of my work is to understand the mechanism of **human ESCRT-III** and VPS4-catalyzed membrane remodeling processes leading to membrane fission. Since ESCRTs are involved in plethora of processes, them along with some accessory proteins are associated with deformation of the membrane, formation of neck, constriction, scission and finally dispersal of the machinery. Our focus is more on the scission process and to that end, we do experiments using an **in-vitro system** of **Giant Unilamellar Vesicles** (GUVs) and polystyrene beads to reproducibly make the neck like shape. The scission event has been shown in yeasts with a large set of

proteins. In other set of experiments with human ESCRTs, the scission event took minutes. Some viruses like HIV-1 uses only a subset of these proteins to perform scission in seconds, and we are trying to reproduce this event. The attempt is to find the **minimum set of proteins** needed for the scission step and identify the affinity of the respective proteins on the **geometry and the effect of shape**, in general, on the process.

B361/P2749

An activating *de novo* variant in ARF1 causing a neurodevelopmental disorder

M. Ishida[1], M. G. Otero[2], C. Freeman[2], P. A. Sánchez-Lara[2], C. M. Guardia[1], T. M. Pierson[2], J. S. Bonifacino[1]; [1]NIH, Bethesda, MD, [2]Cedars-Sinai Medical Center, Los Angeles, CA

ARF1 is a small GTPase that regulates protein sorting from the Golgi apparatus to the ER, endosomes and plasma membrane by recruiting effectors. Although ARF1 is one of the most studied small GTPases, the functional importance of ARF1 in human physiology is still poorly understood. In this presentation, we report a child with a monoallelic *de novo* missense mutation (c.296 G>A; p.R99H) in the *ARF1* gene, associated with developmental delay, hypotonia, intellectual disability, and motor stereotypies. Neuroimaging revealed a hypoplastic corpus callosum and subcortical white-matter abnormalities. Functional studies of the ARF1-R99H variant protein revealed that it was expressed at normal levels and properly localized to the Golgi apparatus; however, expression of this variant caused swelling of the Golgi apparatus, increased the recruitment of coat proteins such as COPI, AP-1, and GGA3, and altered the morphology of recycling endosomes. In addition, we observed that expression of ARF1-R99H prevented dispersal of the Golgi apparatus caused by the ARF1-inhibitor brefeldin A (BFA). Finally, protein-interaction analyses showed that ARF1-R99H bound more tightly to the ARF1-effector GGA3 relative to wild-type ARF1. These properties resemble those of the well-characterized constitutively-active ARF1-Q71L mutant, indicating that the pathogenetic mechanism of the ARF1-R99H variant involves constitutive activation with resultant Golgi and endosomal alterations. These findings suggest that the neurodevelopmental disorder in the patient is caused by a gain-of-function mutation in ARF1, opening up the possibility of treatment using ARF1 inhibitors.

B362/P2750

High-resolution interaction map between Rab1 and secretory cargo using Photo-switching FRET reveals Cis-Golgi domainsassociated with carrier fusion.

Y. Malis[1], G. M. Hirschberg[1], E. H. Sklan[1], G. H. Patterson[2], **K. Hirschberg**[1]; [1]Tel Aviv University, Tel Aviv, Israel, [2]NIH, National Institute of Biomedical Imaging and Bioengineering, Bethesda, MD

FRET is a powerful tool for biologists asking to simultaneously establish and localize interactions between fluorescently tagged proteins with high spatial resolution. Photo-switching FRET (psFRET) using the Dronpa Photo-switching fluorescent protein was recently introduced by Rainey K.H. and Patterson G. H.(Rainey and Patterson, PNAS, 2019). Here we formulated a comprehensive experimental approach and provide a powerful software tool to perform the extensive data analysis involved. This allows a straightforward assimilation of psFRET to diverse experimental systems. Image stacks, recording the decay of the Dronpa donor serve as input to the software utility that calculates exponential decay coefficients at the single pixel level allowing to create a highly detailed interaction map. Moreover, image preprocessing options increase the adaptability and versatility of the psFRET method. psFRET analysis demonstrated that the interaction between the GTPase Rab1 and the VSVG cargo protein or the

tethering protein GRASP65 was detected during ER to Golgi transport yet did not proceed within the Golgi apparatus. Additionally, Micro domains associated with transport carrier fusion decorating the cis side of the Golgi exhibited high FRET efficiency between above interactors. Lastly, we employ the psFRET method in live cell imaging to document interactions at different time points throughout ER to Golgi transport.

Post-Golgi Trafficking

B363/P2751

Liquid-liquid phase separation in the TGN mediates delivery of proinsulin to secretory storage granules

A. PARCHURE[1], M. Tian[1], D. Stalder[2], S. K. Mahata[3], S. B. Stephens[4], D. Gershlick[2], J. V. Blume[1]; [1]Cell Biology, Yale University, New Haven, CT, [2]University of Cambridge, Cambridge, UNITED KINGDOM, [3]University of California San Diego, La Jolla, CA, [4]University of Iowa, Iowa City, IA.

Insulin secretion helps regulate blood glucose levels and is thus a critical metabolic regulator in mammals. Insulin is made by pancreatic β-cells and stored in their secretory granules (SGs). Responding to nutrient stimuli, mature SGs are mobilized to fuse with the plasma membrane and deliver insulin to the bloodstream. The mechanism of how proinsulin and its processing enzymes are sorted and targeted from the *trans*-Golgi network (TGN) to SGs remains largely mysterious. Remarkably, no cargo receptor for proinsulin has been identified in the last three decades. Chromogranin proteins (CGs) are central regulators of granule biogenesis in many cell types. Here, we show that CGs organize in condensate-like structures in the lumen of the TGN of rat insulinoma INS-1 cells. Furthermore, purified CGs undergo liquid-liquid phase separation (LLPS) at a mildly acidic pH independently of divalent cations, such as calcium and recruit clients like proinsulin to the condensates. Similarly, in INS-1 cells, the elevation of the TGN pH or downregulation of CG proteins leads to a redistribution of CG clients from a punctate to a diffuse pattern. Intriguingly, material properties of CG assemblies govern client recruitment as liquid-like CG condensates, but not aggregates, recruit and sort granule-destined cargo molecules. Strikingly, our work shows that cargo selectivity of CGs is independent of conserved sequence but is based on the relative abundance of the client molecules at the TGN. In conclusion, we propose that the TGN provides the milieu in which soluble CGs convert into a “cargo sponge,” gathering soluble client proteins into the condensate. Client molecules can partition into the condensate driven by weak intermolecular interactions, a feature of IDR-containing proteins, thus, facilitating receptor-independent sorting. These findings challenge the canonical TGN sorting models in insulin-secreting β-cells and potentially many other cell types. They are therefore of central relevance for cell biology and diabetes research.

B364/P2752

The insulin inhibitory receptor regulates insulin turnover in pancreatic beta cells

J. Siehler[1], **P. Chapouton**[1], S. Bilekova[1], F. Ribaudo[1], S. bhattacharya[1], P. Xu[1], C. Malhotra[1], H. Lickert[1,2,3]; [1]Institute of Diabetes and Regeneration Research, Helmholtz Zentrum Muenchen, Neuherberg, GERMANY, [2]German Center for Diabetes Research (DZD), Neuherberg, GERMANY, [3]Technical University Munich, School of Medicine, Munich, GERMANY.

We are aiming at understanding the dynamics of proinsulin processing and turnover in beta cells of the pancreas, an important process that controls the biosynthesis of the insulin hormone. Among the

players involved, the insulin inhibitory receptor (inceptor) is a newly characterized single pass transmembrane protein described recently in our lab for its role in the internalization of the insulin receptor via clathrin-coated endocytosis at the plasmamembrane of beta cells (Ansarullah et al., 2021). Our new results reveal the biochemical binding of inceptor to insulin and proinsulin and the subcellular localization of inceptor to the trans-Golgi network as well as a to nascent secretory granules, where it co-localizes with proinsulin. The confined localization in the trans-Golgi network is increased under starvation. In human stem cell-derived beta cells, an absence of inceptor leads to a massive increase in insulin content. We are currently testing two hypotheses to understand the mode of action of inceptor in proinsulin turnover: inceptor might route (pro)-insulin or nascent granules directly to the lysosome for degradation, or it might as well maintain proinsulin for longer times in the trans-Golgi network and nascent granules. To test these hypotheses, we use biochemical and imaging approaches on inceptor knock-outs insulinoma cell lines as well as antisense oligonucleotides in primary islets. We are further analysing in human donor samples how the colocalization of inceptor to proinsulin is altered under diabetic conditions. Together, inceptor plays a key function in regulating insulin turnover in beta cells.

B365/P2753

Uncovering the Role of VPS33B/16B in Megakaryocyte A Granule Formation

H. H. Y. Yao; University of Toronto-47731885, Toronto, ON, CANADA.

Platelets are small and numerous anucleate blood cells that play a primary role in recognizing vascular wound sites, where they adhere, aggregate, and activate to facilitate fibrin generation during hemostasis to limit blood loss. Platelets also play roles in wound healing and bone regeneration, and they have been implicated in atherogenesis and cancer progression. Many platelet functions are linked to vesicles known as alpha granules, which contain an assortment of proteins that can be secreted upon platelet activation. The development of platelet secretory vesicles is poorly understood. Our lab has established that the vacuolar protein sorting-associated proteins VPS33B and VPS16B form a complex, VPS33B/16B, that is essential for alpha granule biogenesis. This role was initially detected in studies of patients afflicted by the rare autosomal recessive neonatal lethal multisystem disorder Arthrogryposis, renal dysfunction, and cholestasis (ARC) syndrome, which is associated with gene variants that cause loss of VPS33B or VPS16B expression/function. The goal of this project is to define the specific role of VPS33B/16B for alpha granule formation using a VPS33B KO mouse model. Mouse models can provide megakaryocytes in their bone marrow niche to evaluate morphology and maturation. I have indicated the potential of using a tamoxifen-inducible hematopoietic lineage-specific Vps33b knockout mouse by validating the deletion of VPS33B, absent VPS16B and showing reduced or absent cargo protein levels, and absent platelet alpha granules, abnormal endosomal morphology, and abnormal trafficking of cargo proteins. Using immunofluorescence microscopy, I showed altered localization of alpha granule cargo proteins using antibodies, thus providing further evidence for their roles in alpha granule formation. These data suggest that VPS33B/16B is involved in the trafficking of synthesized cargo proteins from the Golgi and along the endosomal vesicular transport pathway - specifically, to the late endosomes/multi-vesicular bodies. This project will identify functions of VPS33B/16B during megakaryocyte maturation and alpha granule formation, thus advancing our understanding of platelet development and processes affecting other cells and physiological systems. This could potentially facilitate efforts to develop new therapies for a range of health problems, such as bleeding and thrombotic disorders.

B366/P2754

Quaternary Structure Dynamics Regulate Lipoprotein Lipase Activity During Trafficking

K. H. Gunn[1], B. S. Roberts[1], F. Wang[2], J. D. Strauss[3], M. J. Borgnia[3], E. H. Egelman[2], S. B. Neher[1]; [1]Biochemistry and Biophysics, UNC, Chapel Hill, NC, [2]Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA, [3]Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, Durham, NC.

Lipoprotein lipase (LPL) is the enzyme responsible for hydrolyzing triglycerides from chylomicrons and very low-density lipoproteins (VLDL) in the capillaries to release free fatty acids. LPL adopts at least three different oligomeric states and these changes to LPL's oligomeric state ensure precise spatiotemporal regulation of its activity during trafficking. We uncovered an inactive helical LPL oligomer and used cryogenic electron microscopy (cryoEM) to solve the structure of this LPL filament. We showed that LPL stored inside of adipocyte vesicles forms a filament-like structure in association with heparan sulfate proteoglycan (HSPG) syndecan-1 (SDC1). We have purified LPL containing vesicles and are using cryo-electron tomography to visualize the LPL filament *in situ.* To further explore the role of oligomeric state in regulating LPLs activity, we used cryoEM to determine the structure of an active LPL dimer at 3.9 Å resolution. The structure of dimeric LPL reveals an unexpected dimerization interface that bears significant overlap with the interface for LPL binding to glycosylphosphatidylinositol anchored high density binding protein 1 (GPIHBP1). GPIHBP1 is a vascular endothelium membrane protein that forms a heterodimer with monomeric LPL and facilitates its transfer to the capillary. LPL is active while bound to GPIHBP1 and following dissociation from GPIHBP1, LPL associates with free-floating lipoproteins in the capillary. This is likely where LPL adopts an active dimeric state. Thus, LPL engages in quaternary structure dynamics as it transitions from storage in adipocytes as a helical oligomer, to secretion out of cells and into the capillary as a hetero- and homodimer. These changes constitute an intricate dance in which changes to LPL's oligomeric state regulate its activity.

B367/P2755

Arf-GAP Gcs1 phosphorylation modulates SNARE Snc1 recycling transport in response to ER stress

P. Cai[1], Y. Wu[1], W. Chiu[1], C. Yu[2], F. Lee[1,3]; [1]Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taipei, TAIWAN, [2]Department of Cell and Molecular Biology, Chang Gung University, Tao-Yuan, TAIWAN, [3]Department of Medical Research, National Taiwan University Hospital, Taipei, TAIWAN.

ADP-ribosylation factor (Arf) and Arf-like (Arl) small GTPases are known to play important roles in membrane trafficking. In *Saccharomyces cerevisiae*, the activity of Arl1 is regulated by guanine nucleotide exchange factor (GEF) Syt1 and GTPase activating protein (GAP) Gcs1. Previously, we demonstrated that unfolded protein response (UPR) induces phosphorylation of Syt1 GEF, which is critical for Arl1 activation and the recruitment of golgin protein Imh1 to the late-Golgi (1). We recently reported that Slt2/Erk2-dependent phosphorylation of Imh1 is critical for SNARE recycling under ER stress (2). However, the mechanisms of how Gcs1 is involved in tuning late-Golgi protein transports in response to UPR remain unclear. Gcs1 possesses several phosphor-residues according to prior large-scale screening analysis. Here, we showed that Gcs1 phosphorylation was induced after the treatment of UPR inducer tunicamycin (TM). We identified Slt2/Erk2 as the responsible kinase to dictate this phosphorylation. Deletion of Gcs1 leads to the defects in the recycling transports of SNARE Snc1 to the plasma membrane (PM). The phosphor-deficient Gcs1, but not phosphor-mimetic Gcs1, restore the Snc1

PM distribution in the TM-treated *gcs1*-deletion cells. However, Arl1 and Imh1 remain proper Golgi localization in cells expressing phosphor-deficient or phosphor-mimetic Gcs1 upon ER stress. Together, we reveal a novel role of Gcs1 phosphorylation in regulating proper Snc1 recycling transport under ER stress, which is independent of Arl1-Imh1pathway.

References:
1. Hsu J.-W. et al. (2016) "Unfolded protein response regulates yeast Arl1p activation at late-Golgi via phosphorylation of Arf GEF Syt1p" **Proc. Natl. Acad. Sci. U S A.** 113: E1683-E1690.
2. Wang Y.-H. et al. "Golgin Imh1 and GARP complex cooperate to restore the impaired SNARE recycling transport induced by ER stress" **Cell Reports** 2022, 38: 110488

B368/P2756

Golgin Imh1 and GARP complex cooperate to restore the impaired SNARE recycling transport induced by ER stress

Y.-H. Wang[1], W.-Y. Chiu[1], Y.-T. Chen[1], P.-J. Cai[1], Y.-C. Wu[1], J.-L. Wu[1], B.-H. Chen[1], Y.-W. Liu[1,2], C.-J. Yu[3,4], F.-J. Lee[1,2,5]; [1]Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan, [2]Center of Precision Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan, [3]Department of Cell and Molecular Biology, College of Medicine, Chang Gung University, Taoyuan, Taiwan, [4]Chang Gung Memorial Hospital, Taoyuan, Taiwan, [5]Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan

The accumulation of misfolded proteins in the endoplasmic reticulum (ER) induces the unfolded protein response (UPR), which acts through various mechanisms to reduce ER stress. Whereas the UPR has been well studied for its effects on the ER, less understood has been its effects on the Golgi. The Golgi complex receives transport vesicles from the endosome through two types of tethering factors: long coiled-coil golgin and the multisubunit Golgi-associated retrograde protein (GARP) complex. Whether these two types of tethers cooperate in endosome-to-Golgi recycling remains unclear. Here, we report that ER stress increases the phosphorylation of golgin Imh1 to maintain the GARP-mediated recycling of the t-SNARE Tlg1 to the Golgi and the exocytic transport of the v-SNARE Snc1 from the Golgi to the plasma membrane. We also identify a specific function of the Golgi affected by ER stress and elucidate a homeostatic response to restore this function, which involves both an Ire1-dependent and a MAP kinase Slt2/ERK2-dependent mechanism. Furthermore, our findings advance a general understanding of how two different types of tethers act cooperatively to mediate a transport pathway.

References:
1. Hsu J.-W. *et al*. (2016). "Unfolded protein response regulates yeast Arl1p activation at late-Golgi via phosphorylation of Arf GEF Syt1p" ***Proc. Natl. Acad. Sci. U S A.*** *113*: E1683-E1690.
2. Wang Y.-H. *et al*. (2022). "Golgin Imh1 and GARP complex cooperate to restore the impaired SNARE recycling transport induced by ER stress" ***Cell Reports*** *38*: 110488

B369/P2757

A new function of caveolin in sphingolipid trafficking

J. Podkalicka, C. Viaris De Lesegno, J. Manzi, C. Lamaze, P. Bassereau; Institut Curie, Paris, France

Various lipid and protein composition gives identity to membrane compartments and is essential to their unique biophysical and biological properties hence organelle functioning. In that context efficient lipid and protein sorting cannot be underestimated. Mechanisms of protein sorting are well established, yet

still little is known about the sorting mechanisms of lipids thus, in **this study we aim to understand one mechanism of sphingolipids trafficking within the cells.** Sphingolipids (SLs) are a particular class of lipids, that are synthesized only at trans-Golgi network (TGN) and transported to the plasma membrane (PM) where they are highly enriched. Moreover, to date there is no PM-Golgi contact site reported suggesting that SLs are transported by a classical vesicular secretory pathway. However, SLs enrichment in curved, vesicular carriers cannot occur spontaneously due to increased SLs bending energy and propensity to form stiff membranes, comparing to bulk phospholipids, and to the very weak effect related to intrinsic curvature for lipids. Therefore, for SLs a mechanism of protein-assisted lipid sorting has been proposed that, takes advantage of protein's affinity for curved membranes and specific lipids. One of the proteins enriched in the same secretory pathway as SLs is caveolin (Cav1), which shuttles from the Golgi to the PM where it forms cup-shape, SLs-cholesterol-enriched domains called caveolae. Although the abundance of SLs within caveolae is well documented, it is not known whether SLs are enriched and co-transported with Cav1 after assembling at the TGN or sequestered only at the PM level. Our study demonstrates, by live and fixed cells imaging, that cells lacking Cav1 show a major distributional change in SLs with significantly reduced PM level and SLs sorting redirected towards lysosomes, indicating that Cav1 plays a role in SLs trafficking. Using RUSH technology, which enables synchronized release and trafficking of desired molecules, we demonstrate that SLs-Golgi-exit is significantly slowed down in the absence of Cav1. Moreover, feeding Cav1-deficient cells with fluorescent glyco-SLs results in SLs accumulation in lysosomes by failing incorporation into recycling tubule at the level of recycling endosome. All together suggesting that interaction with Cav1 is the limiting factor for proper SLs sorting and distribution within the cell.

B370/P2758

Live Cell Imaging of PI4P During Osh-Dependent Polarized Secretion

L. A. Heckle, K. G. Kozminski; University of Virginia, Charlottesville, VA

To support polarized cell growth in *S. cerevisiae*, secretory vesicles traffic to the presumptive bud site, bud tip, and bud-neck. While trafficking, vesicles undergo a process known as maturation during which specific proteins associate and dissociate with the vesicle surface. Several models (Mizuno-Yamasaki *et al.*, 2010; Ling *et al.*, 2014; Smindak *et al.*, 2017) posit that vesicle maturation also requires an Osh protein-dependent loss of vesicle-associated PI4P, creating a PI4P gradient between the Golgi and plasma membrane. However, there is a lack of direct data supporting the existence of this gradient. To test these models, we used live cell imaging with fluorescent proteins to track secretory vesicles and quantify vesicle-associated PI4P. We validated the specificity of the PI4P-probe P4M in *S. cerevisiae* by demonstrating P4M localizes to membranes enriched in PI4P and P4M distribution reflects known changes in PI4P localization. These data demonstrated P4M is specific to PI4P in *S. cerevisiae,* and can be used to detect changes in PI4P levels. To quantify vesicle-associated PI4P, we co-expressed mCherry-P4M and GFP-Ypt32p, a Rab GTPase reported to dissociate from secretory vesicles as the PI4P gradient is established. Using live cell imaging, we quantified the percent change in the intensity of mCherry-P4M and GFP-Ypt32p as vesicles trafficked to sites of polarized growth. Consistent with current models, we found mCherry-P4M and GFP-Ypt32p co-localize on secretory vesicles and at sites of polarized exocytosis. However, the intensity of vesicle-associated mCherry-P4M did not consistently decrease over time and distance as expected. Rather, mCherry-P4M and GFP-Ypt32p intensity fluctuated as vesicles trafficked, with no correlation between mCherry-P4M and GFP-Ypt32p. These data suggest the PI4P gradient may not be steep or may manifest only when vesicles are in very close proximity to the

plasma membrane. To determine whether the Osh protein family effects PI4P distribution, we expressed Clover-P4M in strains lacking one of the seven members of the Osh protein family. Loss of any member of the Osh protein family resulted in altered PI4P distribution relative to wildtype cells. Although the Osh protein family shares a yet unknown common function, these data indicate individual Osh proteins serve unique roles in maintaining PI4P distribution.

B371/P2759

Golgi localized Arl15 regulates cargo transport, cell adhesion and motility

P. Sharma[1], P. Hoovina Venkatesh[1], N. Paddillaya[1], N. Shah[2], R. BR[2], A. Dakua[1], A. Penmatsa[1], N. Balasubramanian[2], N. Gundiah[1], S. Gangi Setty[1]; [1]IISc, bangalore, India, [2]IISER, Pune, India

Small G-proteins of Arf-like (Arl) GTPase subfamily are shown to regulate several cellular processes including intracellular trafficking, cytoskeletal organization, organelle biogenesis, cell adhesion and migration. Around 21 genes belong to this family have been identified in human. However the role of Arl15 in cargo transport was unclear. In our study we observed that Arl15-GFP localizes to Golgi, plasma membrane (PM) including filopodia, and a cohort to recycling endosomes. The dual localization of Arl15 to Golgi and PM is independent of the actin cytoskeleton, but it is dependent on Golgi integrity. The dissociation of Golgi using small molecular inhibitors or the expression of Arf1 dominant-negative mutant completely mislocalizes Arl15 to the cytosol. We identified a novel V80A mutation in the GTP-binding domain that turns the Arl15 into a dominant-negative form and results in a reduced number of filopodia. Depletion of Arl15 in HeLa cells causes mislocalization of cargo such as caveolin-2, STX6 from Golgi and accumulation of lipid droplets. Further, Arl15 knockdown cells display reduced filopodial number, altered focal adhesion kinase organization, and enhanced soluble and receptor-mediated cargo uptake without affecting the recycling kinetics. In addition, Arl15 knockdown decreases cell migration and increases cell adhesion, and displays enhanced cell spreading. Traction force microscopy studies revealed that Arl15 depleted cells exert higher traction force and generate multiple focal adhesion points. These studies demonstrated a function to Arl15 in Golgi, which regulates cargo transport to organize membrane domains at the cell surface to control cell migration, spreading and adhesion, including filopodial biogenesis.

Establishment and Maintenance of Polarity

B373/P2760

Cytokinesis Drives Apical Migration of Centrosomes to Promote Polarized Vesicle Trafficking and Epithelial Polarization

P. Wang[1], K. Lin[2], T. Tang[3]; [1]Program in Molecular Medicine, National Yang Ming Chiao Tung University and Academia Sinica, Taipei, TAIWAN, [2]Institute of Physics, Academia Sinica, Taipei, TAIWAN, [3]Institute of Biomedical Sciences, Academia Sinica, Taipei, TAIWAN.

The centrosome has been observed to be always located near apical surface in polarized epithelial cells. However, the detail mechanisms of how centrosomes migrate to the precise position and how the centrosome position affects the cell polarity remain elusive. Using 3D cultures of renal MDCK cysts, we show that the apical membrane components clustering at the pericentrosomal region follow the path of centrosome migration during cytokinesis and transport to apical membrane initiation site (AMIS) for *de novo* epithelial polarization. Unexpectedly, we found that the centrosome is not essential for apical

lumen formation, but its migration can promote the efficiency of polarized vesicle trafficking and the targeting of apical membrane components to the AMIS. Interestingly, blocking cytokinesis in aggregated MDCK cells shows immovable centrosomes and pauses the polarized membrane trafficking. While, dividing MDCK cells that are cultured in suspension without extracellular matrix (ECM) could induce centrosome migration toward the cytokinetic bridge but result in inverted apical membrane. Our findings suggest that cytokinesis is sufficient to drive centrosome migration, but the decision of apical-basal orientation indeed relies on ECM signaling. Furthermore, Par3, which associates with dynein, first emerges at cytokinetic bridge before centrosome positioning and apical membrane formation. Depletion of Par3 results in a defects of centrosome migration. We thus speculate that the Par3-dynein complex at the cytokinetic bridge creates a pulling force for placing centrosomes near the site which will become apical region. Then the recruitment of apical membrane components to the centrosomes accompanied with centrosome migration during cytokinesis promotes the efficient transportation of apical membrane components to form the apical membrane. Our study provides an in-depth understanding the mechanism of centrosome migration and its roles in the promoting cell polarization during the development of epithelial-like tissues.

B374/P2761

Differential Regulation of Cdc42 GTPase for polarity patch dynamics during mating in *S. pombe*

S. Saha, L. Merlini, S. G. Martin; Department of Fundamental Microbiology, University of Lausanne, Lausanne, SWITZERLAND.

Eukaryotic cells such as neurons, epithelial cells, developing embryo, yeasts etc. display a wide variety of polarized morphologies necessary for their particular functions. Despite the large diversity in cell types, establishment of polarity is often governed by highly conserved small GTPases like the Rho family GTPase Cdc42. In fission yeast *S. pombe*, active Cdc42 establishes cell polarity in mitotic cells. Cdc42-GTP zones are stabilized by a scaffold-mediated positive feedback and trigger cytoskeletal remodeling through effectors, such as formin and exocyst, to induce bipolar growth imparting a characteristic cylindrical shapes to the cells. Upon nitrogen starvation *S. pombe* cells of distinct P and M mating types secrete pheromones, P and M-factors respectively, to mate and produce dormant spores. During the mitotic to mating transition, Cdc42-GTP zones at cells tips become smaller and form dynamic patches that aid in partner searching by appearing and disappearing at different locations on cell's periphery. These dynamic patches function in both pheromone secretion, releasing a local signal, and perception, probing for strong pheromone gradients from the partner cell, a phenomenon called "cellular speed-dating". When dynamic patches of opposite mates come in contact, they stabilize and the cells extend a mating projection or "shmoo" towards their partner. Although Cdc42 GTPase governs cell polarization in both mitosis and mating, our knowledge of its regulation is largely limited to mitotic growth and we do not know what mediates its dynamics or stabilization during mating. Using genetic and imaging techniques, we are investigating if scaffold-mediated Cdc42 positive feedback also operates in mating and how negative regulation of Cdc42 promotes patch dynamics. Unexpectedly, we discovered that a scaffold mutant that blocks positive feedback during mitotic growth remains mating competent. We also observed that deletions of GTPase Activating Proteins (GAPs) and Guanosine Dissociation Inhibitor (GDI), which lead to vastly extended Cdc42-GTP zones during mitotic growth, do not prevent formation of patches of restricted size in mating cells. These findings indicate that Cdc42 regulation is significantly different in mating.

B375/P2762

Semaphorin Secretion by Endothelial Cells Stimulates Epithelial Polarization

P. Caceres; Hypertension and Vascular Research, Henry Ford Hospital, Detroit, MI.

The microvasculature has emerged as a local determinant of tissue homeostasis *via* secretion of paracrine factors. This notion is relevant for diseases characterized by microvascular alterations like cancer and diabetes, among others. How microvasculature dysfunction affects differentiation and homeostasis of surrounding tissues has received little attention. Given the close association between epithelia and microvascular beds throughout the body, the objective of this study is to determine whether two cell-cell communication proteins of the Semaphorin family, Sema3E and Sema3F, are secreted by endothelial cells and target epithelial cells, therefore stimulating epithelial polarization. Two epithelial cell lines, Calu-3 (lung) and RPTEC (kidney) were cultured in permeable support in monoculture or co-culture with Human Umbilical Vein Endothelial Cells (HUVEC) in the lower chamber. We measured tight junction maturation via trans-epithelial electrical resistance (TEER) and basolateral polarization of E-cadherin via domain-selective surface biotinylation. First, we determined by ELISA that HUVEC endothelial cells secreted Sema3E (7.8 ±1.8 pg/sq.cm/day) and Sema3F (205.8 ± 2.9 pg/sq.cm/day). High glucose (25mM) had no effect on Sema3F but increased Sema3E secretion 6-fold ($p<0.01$). Osmolality control 15mM mannitol had no effect. This suggests that high glucose, like in diabetes, could affect the endothelial-determined microenvironment. Next, we determined whether semaphorins either exogenous or from co-cultured endothelial cells, stimulated epithelial polarization. We found that exogenous recombinant Sema3E or 3F (300 ng/mL) accelerated TEER over two weeks ($p<0.01$) in Calu-3 and RPTEC cells. Similarly, co-culture with HUVEC cells stimulated TEER ($p<0.01$). However, silencing Sema3E or 3F in HUVEC cells prevented them from stimulating TEER in co-cultured epithelial cells. We observed the same when measuring progression of E-cadherin basolateral distribution. Equivalent co-culture experiments silencing semaphorin receptors Plexin-B1 or B2 in epithelial cells indicated that HUVEC cells could not promote epithelial polarization. We conclude that HUVEC endothelial cells secrete Sema3E and 3F, which may act as signals through Plexin receptors in epithelial cells to stimulate epithelial maturation and polarization.

B376/P2763

Asymmetric cell division in human hematopoietic stem cells is regulated by cell-cell interaction

A. Candelas[1,2], B. Vianay[1,2], L. Blanchoin[2,1], S. Brunet[1,2], M. Thery[1,2]; [1]CytoMorpho Lab, Institut Pierre-Gilles de Gennes, PSL, Paris, FRANCE, [2]CytoMorpho Lab, LPCV, CEA, Grenoble, FRANCE.

The centrosome is pointed at the core of unique cellular structures such as the immune synapse, the cilia, the flagella or the mitotic spindle. These structures involve specific functions: communication, sensing, motility and division, respectively. In hematopoietic stem and progenitor cells (HSPC)s, the multipotent cells that generate all the blood cell lineages, centrosome has been described at the rear of the migratory cells. However, its behaviour and role in interaction remain unknown, despite its key presence in the cell-cell interaction of some differentiated hematopoietic lineages i.e. immune synapse of B-/T-cells.

To address this question, we co-culture human HSPCs with different cell types of the bone marrow, that form the niche of the HSPC. Using microwells, we confine both cell types in a restrictive space and maintain a continuous cell-cell interaction. We find that HSPCs polarize toward the interaction with the bone marrow cells, forming a single cellular protrusion called magnupodium. This protrusion presents a

dense actin network enriched in receptors and adhesion molecules. Remarkably, the centrosome localizes also at the core of this structure and together with it, the Golgi and proteins involved in cell-cell communication. In other stem cells, polarization can precede asymmetric cell division. This type of division drives a differentiation process by producing two daughter cells with different cell fates. To further investigate this hypothesis, we combine our microdevice setup with live staining and imaging to track consecutive (a)symmetric inheritance of fate determinants and the progeny identities. We find that HSPC polarization conditions increase perpendicular mitotic spindle orientation and the levels of asymmetric inheritance in the progeny, both indicators of asymmetric cell division.
Our data determine a novel centrosome-based structure of polarization upon specific cell-cell interaction and suggests that it is involved in the regulation of the asymmetric cell division of hematopoietic stem cells.

B377/P2764

How Do Cells Determine the Timing and Rate of Polarized Growth?

W. Vickers, M. Das; Biology, Boston College, Chestnut Hill, MA.

Cells must be able to regulate the specific timing and position of growth to form the wide diversity of shapes and structures we see throughout biology. While the shape of the cell tends to be more important to the function of multicellular organisms, the basic pathways for the regulation of growth and polarity are also present in single-celled eukaryotes. After division, fission yeast initially grows only from the old end that was inherited from the previous generation. Later, in a process known as new end take-off (NETO) the cell transitions to bipolar growth. However, the rate of growth at the new end is slower than that of the old end. It is not fully understood what establishes this old end dominance or delayed new end growth. To answer this question, we investigated Rax1 as a potential regulator of NETO. *rax1Δ* cells begin new end growth prematurely and have a more symmetric distribution of Cdc42 activity between the two ends, as well as overall higher levels of active Cdc42. However, the growth rate of the new end after NETO remains similar to wildtype and the old end retains its dominance. Our findings suggest that the old end retains an advantage even when the new end grows earlier and that growth rate is not simply a function of the level of Cdc42 activation. We propose that the timing and rate of growth are regulated by at least two distinct regulatory systems.

B378/P2765

Role of the Galpha-MAPK interaction in mating in *Saccharomyces cerevisiae*

C. Robertson, D. Lew; Pharmacology And Cancer Biology, Duke University, Durham, NC.

Many cells direct their movement (chemotaxis) or growth (chemotropism) in response to chemical cues in their environment. This behavior involves translating spatial information from binding of cell surface receptors into cytoskeletal activity that directs movement or growth in a direction influenced by extracellular chemical gradients. The best understood model for gradient sensing is the budding yeast *Saccharomyces cerevisiae*, where the simple biology, lack of redundancy, and ease of manipulation has allowed investigators to identify molecular mechanisms whereby spatial information is conveyed to the cytoskeleton. Budding yeast cells are immobile and rely on chemotropism to mate with a nearby partner cell. Mating cells secrete pheromones that are sensed by cell surface G-protein coupled receptors (GPCRs) on potential partner cells. When these receptors bind pheromone, Gα is activated and dissociates from Gβγ, which then triggers a signaling cascade that activates MAPK to promote cell cycle

arrest, changes in gene expression, and polarization. Polarization is directed towards the partner via a scaffold protein, Far1, that connects Gβγ to activation of the polarity regulator Cdc42, which orients actin to promote directed growth. However, there are additional requirements for effective chemotropism whose mechanism has not been elucidated. The Gα subunit binds to the MAPK and disrupting the Gα-MAPK interaction (*gpa1*^{DSD} mutant) results in a mating defect (Metodiev et al., 2002). We found that the directionality of polarity site movement during partner search was partially defective in *gpa1*^{DSD} mutants, and that a subset of cells failed to recognize when polarity sites were aligned, leading to "kiss-and-run" encounters. One hypothesis to explain the role of the Gα-MAPK interaction is that it localizes active MAPK at the polarity site to phosphorylate its substrate Bni1, a formin that regulates actin (Matheos et al., 2004). Using bni1 temperature-sensitive mutants, we found that polarity site movement still occurred and allowed alignment of partner polarity sites in the absence of Bni1, although subsequent fusion was delayed. However, epistasis analysis showed that *gpa1*^{DSD} mutants still caused defects in polarity site directionality even in cells lacking Bni1. We conclude that the Gα-MAPK interaction regulates mating through Bni1-independent pathways. Other MAPK substrates enriched at polarity sites include Gβγ (Ste4 and Ste18, respectively) and Far1, and we are testing whether their phosphorylation may explain the need for Gα-MAPK interaction in mating yeast.

B379/P2766

Control of cell polarity and exploratory Cdc42 dynamics by conserved NDR kinase Orb6 and Cdc42 GAP Rga3

L. Doyle, V. Mariani, D. D'Urso, F. Verde; University of Miami Miller School of Medicine, Miami, FL.

Cell polarization is crucial to cell function, and changes dynamically during the cell cycle and in response to environmental stress. Our work focuses on the conserved NDR kinase, which regulates cell morphogenesis from yeast to man, and has a role in the onset of disease in humans. In fission yeast cells, the NDR kinase Orb6 spatially controls the key morphology regulator Cdc42 GTPase. During interphase and in the presence of nutrition, Orb6 promotes Cdc42 function at the cell tips by increasing the activity of the upstream regulator Ras1. This pattern of Cdc42 activation during vegetative growth displays anticorrelated Cdc42 oscillations at the cell tips. Conversely, loss of Orb6 kinase activity leads to the emergence of an alternative, "exploratory" pattern of Cdc42 activation, where ectopic foci of Cdc42 activity dynamically appears and disappear throughout the cell membrane. We previously reported that this effect depends, at least in part, on the Cdc42 GEF Gef1, a substrate of Orb6, that moves to the cell membrane upon Orb6 inhibition. To better understand the emergence of these alternative Cdc42 dynamics, we undertook a mass spectrometry screen to identify novel Orb6 substrates. In this screen, we identified a Cdc42 GAP Rga3, as a substrate of Orb6. We found that loss of Orb6 kinase activity leads to a significant increase of Rga3 at the cell membrane, a phenotype mimicked by expression of Rga3-S683A, mutated in a single Orb6 phosphorylation site. The *rga3-S683A* mutation alters cell dimensions and decreases the rate of bipolar growth activation. Consistent with the idea that Rga3 has a role in promoting the emergence of alternative states of cell polarization, we found that *rga3* deletion prevents the ectopic Cdc42 activation observed during Orb6 inhibition. Further, we find that the *rga3-S683A* mutation promotes "exploratory" Cdc42 dynamics when introduced in a cellular background where the vegetative Cdc42 control axis has been attenuated, and the *gef1-S112A* mutation (mimicking Orb6 inhibition) has been expressed. In conclusion, we report that decreased Orb6 activity promotes the function of an alternative Cdc42 regulatory module, where Cdc42 GAP Rga3 functions in cooperation with Cdc42 GEF Gef1 to foster an exploratory pattern of Cdc42 dynamics. Since Orb6 kinase activity is

downregulated in response to nutritional and environmental cell stress, we discuss the function of this Cdc42 module in cell adaptation to stress.

B380/P2767

Cdc42 Mobility and Membrane Flows Regulate Fission Yeast Cell Shape and Survival

D. M. Rutkowski[1], V. Vincenzetti[2], D. Vavylonis[1], S. G. Martin[2]; [1]Lehigh University, Bethlehem, PA, [2]University of Lausanne, Lausanne, SWITZERLAND.

Cdc42 activation directs cell growth by forming membrane associated patches which promote localized exocytosis. Secretion-driven in-plane membrane flow depletes peripheral proteins with low mobilities, including Cdc42 GAPs, away from the region of localized membrane addition/removal. To investigate the self-organizing properties of the Cdc42 secretion-polarization system, we developed a stochastic particle model implementing the reaction-diffusion of Cdc42. The model includes positive feedback by discretely modeled GEFs, hydrolysis by discrete GAPs, and flow-induced displacement by exo/endocytosis targeted towards regions of Cdc42 activity. Our simulations show how stable polarization of wild-type cells relies on flow-induced depletion of low mobility GAP when using Cdc42-GDP and Cdc42-GTP diffusion and membrane dissociation rates estimated from FRAP experiments. To probe the role of Cdc42 mobility, which is fast enough to not be strongly affected by membrane flow in WT cells, we changed its membrane binding properties by replacing its prenylation site with NxRitC, where N is the number of repeats of the Rit1 C terminal membrane binding domain. While Cdc42-3xRitC is inviable, surprisingly, Cdc42-1xRitC and -2xRitC cells survive and polarize, even though the unbinding and diffusion rates for these constructs (as measured from diploid cells also expressing WT Cdc42) are significantly lower than WT-Cdc42. By systematically varying Cdc42 mobility in our model, we find that GEF positive feedback and GAP flow-displacement allows polarization even when membrane flows are strong enough to displace Cdc42 itself away from its activation region. However, lower Cdc42 mobility in the model results in lower Cdc42 activation level and wider patches, in agreement with our experimental observations in haploid and diploid cells. The model predicts that GAP depletion increases Cdc42 activity at the expense of loss of polarization. Experiments confirm this prediction, as deletion of Cdc42 GAPs restores viability to Cdc42-3xRitC cells. Overall, our combined experimental and modeling studies demonstrate how membrane flows are an integral part of Cdc42-driven pattern formation.

B381/P2768

Endocytosis Regulates Negative Feedback to Promote Bipolar Growth in Fission Yeast

M. Harrell[1], Z. Liu[2], T. Hong[2], M. Das[1]; [1]Biology, Boston College, Chestnut Hill, MA, [2]University of Tennessee Knoxville, Knoxville, TN.

Precise regulation of polarized growth promotes specific cell shapes for optimal function. The Rho GTPase Cdc42 is highly conserved and is the major regulator of polarized growth in most eukaryotes. Cdc42 is localized to inner membranes but is only activated at specific sites for growth. Resources that activate Cdc42 are limited and must be properly shared among multiple sites of growth. In the bipolar yeast *S. pombe*, Cdc42 periodically cycles between active and inactive states at sites of growth. Periodic activation and inactivation of Cdc42 is regulated by positive and negative feedback loops. Thus, periodic Cdc42 activity at both cell ends allows sharing of limited polarity machinery resulting in bipolar growth. Positive feedback loops are established when active Cdc42 recruits Scd2 (a scaffold) which then recruits Scd1 (primary Cdc42 activator) to activate more Cdc42. This is the positive feedback loop. Active Cdc42

also recruits Pak1 kinase (inhibitor of Scd1 recruitment) to dismantle the complex via negative feedback. It is unclear how Cdc42 regulators are properly shared between both ends. To understand this, we built a mathematical model based on the positive feedback loop between Cdc42 & Scd1 at both ends of the cell. The model shows the essential roles of this feedback loop and the optimal removal rate of these molecules for bipolarity in *S. pombe*. We find, using genetic and chemical perturbations of only branched actin (required for endocytosis) but not actin cables (needed for vesicle transport) that growth sites no longer share resources. Instead, they accumulate at one end. This is consistent with the model which suggests that sharing of Cdc42 activity depends on endocytosis. Since endocytosis is known to recycle proteins from the membrane, we asked if a Cdc42 regulator accumulates in the absence of endocytosis. Contrary to our expectations and the model's prediction, Scd1 (Cdc42 activator) and Scd2 (scaffold) levels decrease at the cortex upon loss of branched actin. Therefore, we explicitly added Pak1 into the model then examined if altering Pak1 dynamics would explain the loss of sharing between sites of growth upon loss of branched actin. Our model indicates that the reduced removal rate of Pak1 (Scd1 inhibitor) from the cortex leads to loss of bipolarity and sharing as well as decreased Scd1 at the cortex. We then performed in vivo experiments and found that Pak1 did stabilize at a growth site when cells lost branched actin (endocytosis). Taken together, we propose that endocytosis promotes growth from multiple sites by mediating the sharing of polarity machinery via the removal of negative feedback proteins. Optimal removal of negative feedback proteins allows growth to re-establish at that site allowing for periodic activation and inactivation at each site.

B382/P2769

Loss of mechanical homeostasis drives epithelial acinar eversion

V. Narayanan[1], P. Purkayastha[2], B. Yu[3], R. Dickinson[3], **D. E. Conway**[4], T. P. Lele[2]; [1]Virginia Commonwealth University, Richmond, VA, [2]Texas A&M University, College Station, TX, [3]University of Florida, Gainseville, FL, [4]The Ohio State University, Columbus, OH.

Epithelial cells lining a gland and cells grown in a soft extracellular matrix polarize with apical proteins exposed to the lumen and basal proteins in contact with the extracellular matrix. Alterations to polarity, including an apical-out polarity, occur in human cancers. While some aberrant polarity states may result from altered protein trafficking, recent observations of an extraordinary tissue-level inside-out unfolding suggest an alternate pathway for altered polarity. Because mechanical alterations are common in human cancer including an upregulation of RhoA mediated actomyosin tension in acinar epithelia, we explored whether perturbing mechanical homeostasis could cause apical-out eversion. Acinar eversion was robustly induced by direct activation of RhoA or indirect activation of RhoA through blockage of β1 integrins, disruption of the LINC complex, oncogenic Ras activation, or Rac1 inhibition. Furthermore, laser ablation of a portion of the untreated acinus was sufficient to induce eversion. Analyses of acini revealed high curvature and low phosphorylated myosin in the apical cell surfaces relative to the basal surfaces. A vertex-based mathematical model which balances tension at cell-cell interfaces revealed a five-fold greater basal cell surface tension relative to the apical cell surface tension. The model suggests that the difference in surface energy between the apical and basal surfaces is the driving force for acinar eversion. Our findings raise the possibility that a loss of mechanical homeostasis may cause apical-out polarity states in human cancers.

B383/P2770

Cell-Cycle-Dependent Polarized Growth Inhibition in Fission Yeast

J. C. McDuffie, M. E. Das; Morrissey College of Arts & Sciences - Biology Department, Boston College, Chestnut Hill, MA.

Cells are defined by periods of division, interspersed by periods of growth. Precise coordination of the timing and position of this growth ensures proper cell division and is fundamental to the establishment of diverse cellular shapes and specialized functions. Highly conserved amongst eukaryotic organisms is the major regulator of polarized cell growth, the Rho-GTPase Cdc42. When activated by the binding of GTP, Cdc42 establishes sites of polarized growth via a positive feedback loop. The single-celled fission yeast employs a simple yet highly conserved Cdc42 regulatory system to maintain a bipolar rod-like growth pattern and to coordinate arrest of this growth during mitosis. Within this system, the membrane-associated Rga4 is a potent inhibitor of Cdc42. Rga4 localizes to the cortex of fission yeast cells in a punctate pattern during interphase, confined to the cell sides where it inhibits Cdc42 activity everywhere but the growing cell ends. Upon entrance into mitosis, Rga4 localization shifts to a more homogeneous distribution along the cortex and creeps up onto the cell tips, blocking growth until completion of division. How Rga4 localization is being regulated in a cell-cycle-dependent manner is currently unknown. To answer this question, we investigated Septins (Spns) as potential regulators of Rga4 localization. This filament-forming family of cytoskeletal proteins share a punctate cortical localization pattern with Rga4 during interphase. During mitosis, these Septins move off of the cell sides to form a ring at the middle of the cell. Our findings demonstrate that disruption of Septin filament formation via *spn1Δ* results in a more homogeneous distribution of Rga4 along the cell cortex, along with its mis-localization to the cell ends during interphase, a localization pattern normally associated with mitosis. Along with this mis-localization of Rga4, we observe changes in Cdc42 activity at the growing cell ends, disruptions in the feedback loop cycle of Cdc42 activity and changes in cell morphology. We propose that Septins regulate Rga4 localization in a cell-cycle-dependent manner by confining Rga4 to the cell sides during interphase, during mitosis the Septin filaments recede toward the division plane thus freeing Rga4 to spread out along the cell cortex.

B384/P2771

Defining the septin interactome and its role in appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

I. Eisermann, A. Foster, P. Derbyshire, F. Menke, N. Talbot; The Sainsbury Laboratory, Norwich, UNITED KINGDOM.

Rice blast disease, caused by the plant pathogenic fungus *Magnaporthe oryzae*, destroys rice to feed more than 60 million people every year. The disease is initiated by formation of a specialized dome-shaped infection cell called the appressorium. Turgor pressure of up to 8MPa is generated within the appressorium and enables the fungus to develop a rigid penetration peg to breach the rice cuticle. Cytoskeletal proteins called septins play a major role during appressorium-mediated plant infection by rigidifying the cortex of cells, scaffolding F-actin and localizing proteins, such as polarity determinants at the plasma membrane. *M. oryzae* possesses six septin GTPases. The four core septins, Sep3, Sep4, Sep5 and Sep6 form a large hetero-oligomeric ring structure at the base of the appressorium, while the two non-core septins, Sep7 and Sep8 are responsible for the formation of a range of membrane and cytoskeleton-associated structures in the cell. Interestingly the non-core septins Sep7 and Sep8 are

absent in mammals and belong to a unique class 5 group of septins. We have used a combination of approaches to determine the function of each septin during appressorium-mediated plant infection to define the dynamics of the septin interactome in so far unparalleled detail. We carried out Ultra-High-Throughput Yeast Two Hybrid analysis and combined the results with those of an *in vivo* immunoprecipitation tandem mass spectrometry (IP-MS-MS) approach using GFP-tagged septins. For each septins we have identified a wide range of interaction partners during appressorium development, including polarity determinants, cytoskeletal components, and a range of regulatory proteins. Interestingly we observed that Sep7 interacts with Sep3, Sep4, Sep5 and Sep6 specifically during early appressorium formation, 4h after conidial germination, forming a plasma membrane-associated complex. Sep8, which contains a transmembrane helix, also interacts with each septin and may link septins to the plasma membrane. Additional to the determination of the septin interactome, we gained knowledge about the regulation of the septins, by caring out a phosphoproteomic analysis. Our approaches define the septin interactome in unparalleled detail and determine how *M. oryzae* septin complexes organise the appressorium pore and deploy polarity determinants to facilitate cuticle rupture and invasive fungal growth.

B385/P2772

Spatiotemporal regulation of the Rho GTPase Cdc42

A. DAlessandro, K. Moran, D. Lew; Duke University, Durham, NC.

In *S. cerevisiae*, polarization of the actin cytoskeleton is regulated during the cell cycle by the main cell cycle regulator, cyclin dependent kinase (CDK). CDK activation promotes local clustering of the polarity regulator Cdc42, leading to actin orientation and bud emergence. How exactly CDK activity impacts Cdc42 behavior is not yet clear, but various studies have suggested that CDK controls polarity by regulating Cdc42-directed regulators (GEF, GAPs) or effectors.With respect to GEF regulation, CDK has been proposed to promote binding of the GEF Cdc24 to the scaffold protein Bem1 (Witte et al., 2009). This interaction would promote positive feedback by enabling recruitment of GEF to sites with active Cdc42. Using synchronized cell populations, we assessed whether Bem1-Cdc24 interaction (measured by co-immunoprecipitation) varied through the cell cycle. However, we detected a constitutive and unvarying interaction, inconsistent with the model.With respect to GAP regulation, CDK was shown to directly phosphorylate GAPs, and indirect assays suggested that phosphorylation might reduce GAP activity (Knaus et al., 2007; Sopko et al., 2007). To test this hypothesis, we probed Cdc42-GAP interaction by incubating extracts from synchronized cells with beads coated with GTP-locked $Cdc42^{Q61L}$. Epitope-tagged GAP proteins bound to the beads, and we are quantifying the degree of binding through the cell cycle. With respect to effectors, two related PAK-family kinases, Ste20 and Cla4, displayed differential localization through the cell cycle, with Ste20 localizing earlier than Cla4 (Moran et al., 2018). Effector localization requires both Cdc42 and membrane binding (Takahashi and Pryciak, 2007). As both effectors bind Cdc42 via a CRIB motif, but contain different membrane-binding domains (basic-rich amphipathic helix in Ste20 and PH domain in Cla4), we speculated that CDK activation might regulate membrane composition, enabling differential recruitment of these effectors. We constructed probes containing dimerized membrane-binding domains (without CRIB) from each effector and tested whether membrane localization varied during the cell cycle. Quantification showed no differences, suggesting that both domains bind membranes constitutively.In conclusion, two of the proposed mechanisms for cell cycle regulation of polarity (Bem1-Cdc24 association and effector-membrane interaction) are not

supported by our findings. Investigation of GAP regulation is ongoing. For now, the mechanism(s) by which CDK regulates Cdc42 remain mysterious.

B386/P2773

SGEF, a RhoG-specific GEF, Regulates Lumen Formation and Collective Cell Migration in 3D Epithelial Cysts

M. Lovejoy, A. Rabino, G. Kreider-Letterman, V. Gangasani, R. Garcia-Mata; University of Toledo, Toledo, OH.

Most internal organs consist of a polarized epithelium surrounding a central lumen, which separates the interior of cells from the external environment. The establishment of polarity is essential for epithelial cells' function, and abnormalities in this process are a hallmark of many diseases, such as cancer. Polarization is initiated and maintained by the coordinated action of three protein complexes: Crumbs, Par, and Scribble. The Scribble complex is comprised of Scribble, Dlg1, and Lgl, and was primarily identified as a critical regulator for polarity. However, it was later shown to be involved in other related cellular processes, including cell-cell adhesion, asymmetric cell division, and vesicular trafficking. We previously showed that Scribble and Dlg1 can form a ternary complex with SGEF, which is a RhoG-specific guanine-nucleotide exchange factor (GEF) that also functions as a scaffolding protein. We also showed that SGEF is important in junctional stability and E-cadherin expression in a two-dimensional monolayer. Here we used single epithelial MDCK cells embedded in Matrigel, which develop into multicellular, polarized 3D cysts over time, to study the role of SGEF in regulating polarity and lumen formation in 3D. Using this model, we found that mature SGEF knockdown cysts have multiple lumens, which are often closed, decreased E-cadherin levels, and compromised tight junction barrier function. Multiple lumens can result from defects in proper mitotic spindle orientation, barrier function, or vesicle transport, whereas closed lumens may result from leakage due to the instability of tight junctions. Interestingly, when we followed cyst formation using live 4D spinning disc microscopy, SGEF KD cysts showed a surprising increase in collective motility. This was further confirmed in two-dimensional monolayers with a wound healing assay, where SGEF KD cells migrated much further at a higher velocity during migration to close the wound. Our results suggest that, because E-cadherin expression is reduced, there is a decrease in the strength of cell-cell adhesions. This phenotype has been observed in most cases of metastasis, suggesting that SGEF could play a role in maintaining epithelial stability in MDCK cells. We plan to grow cysts from several SGEF mutant rescue cell lines that isolate the catalytic and scaffolding role to further characterize the molecular mechanisms that control lumen phenotype, E-cadherin expression, junctional stability, and collective cell migration.

B387/P2774

Mechanism of the transition from a Par-3- to Cdc42-bound Par complex

E. Vargas, K. Prehoda; BIology, University of Oregon, Eugene, OR.

The polarization of cell membranes into discrete domains is critical for the function of diverse animal cells. The Par complex (Par-6/aPKC), a central regulator of animal cell polarity, must localize in a spatially and temporally defined manner in order for proper polarization to occur. Two regulators of the Par complex, Par-3 and Cdc42, are responsible for the precise control of Par complex polarization and activity. Numerous *in vivo* studies propose that a key step in polarization is the transition from a Par-3- to a Cdc42-bound Par complex, as each regulator is thought to induce a distinct activity, yet limited

biochemical evidence exists to support these models. Utilizing a biochemical reconstitution approach with all purified components, we show that Par-3 and Cdc42 exhibit strong negative cooperativity for binding to the Par complex. We find that displacement of Cdc42 from the Par complex is mediated by the interactions between the second and third PDZ domains of Par-3 and the aPKC Kinase-PBM (PDZ binding motif). We also show that the epithelial specific protein, Crumbs, displaces Par-3 in a similar manner to Cdc42. Furthermore, Cdc42 and Crumbs work synergistically to displace Par-3 from the Par complex. These results provide a mechanistic framework for understanding how the Par complex can transition between different regulators and function in diverse polarized cell types.

B388/P2775

Polarized cell plasma membrane compartment composition and protein sorting

C. Shurer, I. Levental; University of Virginia, Charlottesville, VA

Epithelial cell polarization is an essential biological process, serving many physiological roles including tissue morphogenesis and wound healing. A defining feature of polarization is the separation of cell plasma membrane lipids and proteins into apical and basolateral compartments between which molecular exchange is restricted. Decades ago, the apical membrane was found to be enriched in saturated lipids, glycolipids, and cholesterol. Mechanistic hypotheses to explain the biogenesis and unique composition of the apical membrane include self-assembling membrane domains (i.e., lipid rafts), specific protein sorting motifs, and post-translational modifications mediating protein sorting. However, neither the detailed composition nor the mechanisms of protein and lipid sorting between plasma membrane domains in epithelial cells have been resolved. Particularly, the lipid profile of the basolateral membrane remains unstudied, leaving doubts about the differentiation and lipid separation of the apical and basolateral membrane. We use advanced lipidomics and imaging techniques to characterize the changes in lipid organization, membrane composition, and membrane properties during the cellular polarization process. We observe that the apical membrane is enriched in highly saturated lipids and glycolipids relative to the basolateral membrane, with apical membrane biophysical properties reflecting a raft-enriched environment. Similar to lipids, the determinants of protein sorting between apical and basolateral domains are poorly understood, with past studies focusing on either specific proteins or trafficking machinery. Importantly, the apical membrane hosts an extensive extracellular glycocalyx consisting of glycolipids, glycoproteins, and polysaccharides. The role of these glycocalyx molecules on protein sorting has not been revealed, despite a major fraction of apical proteins being glycosylated. We systematically evaluate the structural determinants of apical versus basolateral sorting with a focus on transmembrane domain features, protein raft affinity, and glycosylation. We find that these protein features cooperatively direct protein sorting to the apical membrane.

B389/P2776

Optimal inhibition by RasGAPs: the key for directed cell migration

Y. Lin, P. N. Devreotes; Johns Hopkins School of Medicine, Baltimore, MD

Directed cell migration, a process that cells migrate directional in response to various environmental cues, is critical to majority of biological processes, including embryogenesis, would healing, immune response and cancer metastasis. It can be dissected into three processes: directional sensing, motility and polarity, among which whether the cell can build the polarity memory is the key for long-term

directional migration. We addressed the issue by looking at Ras activity in social amoeba *Dictyostelium discoideum* and uncovered that two C2-domain containing RasGAPs have distinct functions. C2GAPA inhibits cell migration while C2GAPB polarizes cell and promotes directed migration. More strikingly, starved amoeba usually undergoes development and builds polarity within hours by the coordination of signal transduction and cytoskeletal events. However, we show that using optogenetics to manipulate Ras activities, we can either induce or break polarity in seconds. This study might provide new insights on how polarity is established and emphasize the importance of having optimal inhibitory regulation.

B390/P2777

An Unexpected Function of P-cadherin is Required for Single Lumen Formation in Epithelial Cystogenesis

K. Fong-Ngern[1], S. Chang[1], A. S. Vijayakumar[1], R. Zaidel-Bar[2], P. Kanchanawong[3]; [1]Mechanobiology Institute, National University of Singapore, Singapore, SINGAPORE, [2]Department of Cell and Developmental Biology, Faculty of Medicine, Tel Aviv University, Tel Aviv, ISRAEL, [3]Mechanobiology Institute and Department of Biomedical Engineering, National University of Singapore, Singapore, SINGAPORE.

Cadherin-mediated adherens junctions are known to be essential for epithelial morphogenesis during development. While E-cadherin is by far the best characterized cadherin, other cadherin subtypes, such as P-cadherin, are often co-expressed. E-cadherin and P-cadherin are highly homologous type I classical cadherins with juxtaposed chromosomal loci in vertebrates. However, their specific and differential roles in epithelial morphogenesis are less clear. We used Madin Darby Canine Kidney II (MDCK II) cells, which express both E-cadherin and P-cadherin, to investigate the distinct contribution of each cadherin in self-organized cystogenesis in Matrigel after knocking out either E- or P-cadherin by CRISPR/Cas9. We found that E-cadherin KO MDCK cysts successfully generate a stereotypical single lumen, whereas P-cadherin KO MDCK cysts gave rise to multiple lumens, implicating P-cadherin in proper lumenogenesis. At the two-cell stage, P-cadherin KO doublets exhibited defects in both podocalyxin transcytosis and mitotic spindle orientation during the formation of the Apical Membrane Initiation Site (AMIS), a structure required for lumen positioning and lumen generation. During mitosis in cell doublet, E-cadherin remains on the cell surface and at the interface between adjacent cells, whereas P-cadherin localization is dynamically modulated. We found the accumulation of P-cadherin on the condensed chromosomes during prometaphase and at the spindle poles during metaphase, suggestive of a potential role of P-cadherin in mitosis and mitotic spindle orientation. In 2D monolayers, we observed both mitotic spindle misorientation as well as aberrant chromosome alignment. By probing various types of intracellular vesicles, including clathrin-, Rab5-, Rab11-, and Rab35-possitive vesicles, we identified Rab5-possitive vesicle as the major P-cadherin carrier mediating P-cadherin localization and function during mitosis. In conclusion, our findings reveal a distinct function and localization of P-cadherin in the morphogenesis of epithelial tissues by controlling the formation of the AMIS and the alignment of mitotic spindle, via a Rab5-vesicle mediated mechanism.

B391/P2778

The *pam1* Suppressor *spam3* Rescues Polarity and Mitotic Defects in *C. elegans*

A. Durkan, B. Adams, K. Hryniszak, I. Marsh, R. Lyczak; Ursinus College, Collegeville, PA

Puromycin-sensitive aminopeptidase (PSA) is highly conserved across the animal kingdom. Moreover, PSAs are implicated in cell cycle regulation and seem to play a protective role in neurological disorders such as Alzheimer's. *Caenorhabditis elegans* have one PSA homolog called PAM-1 (puromycin-sensitive aminopeptidase), and PAM-1 is known to play a role in cell cycle regulation, especially in fertility, meiosis, and mitosis. PAM-1 plays a role in maintaining polarity by keeping centrosomes close to the cortex of the embryo. The first cell division in *C. elegans* is asymmetrical and is controlled by cortical flows and polarity proteins of the PAR class, localizing to the anterior and posterior domains of the cortex. When PAM-1 is inactivated, it disrupts the cortical flows and movement of PAR proteins by affecting the centrosome cortex contact. Though PAM-1 is highly conserved, little is known about its targets/interacting partners. Here, we identified one potential target/interaction of PAM-1 through a suppressor screen. This suppressor brings embryonic viability from ~12 % of a nonsense mutation in the *pam-1* gene to ~70% with the suppressor mutation dubbed *spam-3.* Through whole genome sequencing, we hope to identify the *spam-3* gene. For now, we are analyzing the phenotypes that *spam-3* mutant may resolve and why there is a significant increase in embryonic viability, by looking at mitosis and polarity. Looking at landmark phases in mitosis we have found that *pam-1* mutants lead to DNA segregation errors, such as DNA bridges or failed anaphase, which can lead to a deformed nuclear envelope. The *spam-3* suppressor mutant seems to resolve these issues in *pam-1* embryos. To analyze the polarity of the embryo PAR-1 protein localization and non-muscular myosin (NMY-2) puncta at the cortex are being analyzed. We have found that the *spam-3* mutant decreases the symmetrical cell division caused by mutant *pam-1.* Analyzing the PAR proteins will shed light on why there is a decrease in symmetrical cell division. NMY-2 is required for cytokinesis as well as for the establishment of asymmetry during embryogenesis. Analyzing NMY-2 will add to our understanding of why *pam-1* mutants undergo symmetrical cell division. A better understanding of how PAM-1 is functioning will broaden our understanding of cell cycle regulation and hopefully give insight into some neurological disorders.

B392/P2779

3D reference map of retinal pigment epithelium during apical-basal polarization

D. Ortolan[1], P. Sathe[2], A. Volkov[1], D. Reichert[1], S. Sebastian[1], J. Ferrari Escoto[1], N. Lin[1], N. Schaub[3], G. Pegoraro[4], A. Maminishkis[1], R. Sharma[1], P. Bajcsy[2], K. Bharti[1]; [1]National Eye Institute, Bethesda, MD, [2]National Institute of Standards and Technology, Bethesda, MD, [3]National Center for Advancing Translational Sciences, Bethesda, MD, [4]National Cancer Institute, Bethesda, MD

The retinal pigment epithelium (RPE) is a monolayer of cells that faces two different environments - the retinal photoreceptors apically and the choroid basally. To perform different functions on each side, mature RPE cells polarize along the apical-basal axis. Failure to acquire a polarized phenotype leads to retinal degeneration, as seen in some ciliopathies.

In this study, we generated a three-dimensional reference map of RPE organelles and cytoskeletal structures during the process of cell polarization, using high-content imaging and object segmentation. RPE cells were derived from induced pluripotent stem cells reporter lines (iPSC-RPE), each expressing a fluorescently tagged protein to highlight specific intracellular structures. About 10,000 images of iPSC-

RPE were taken at different timepoints during polarization of each reporter line. iPSC-RPE cells treated with HPI4, a compound known to disrupt apical-basal polarity, were included as control. A machine-learning algorithm was trained to identify and segment cells and nuclei borders. These were used as reference to calculate organelle location. Each organelle or structure was segmented using a combination of classic segmentation algorithms and the quality of segmentation was verified by an imaging expert. Three rounds of segmentation with the human in the loop were carried out to ensure the accuracy of the results. The average organelle location and morphological properties were calculated for each condition. Cells became more compact horizontally and elongated on the vertical axis during polarization. At the same time, the nuclei became rounder and developed invaginations of the nuclear envelope. The volume of endoplasmic reticulum increased, while becoming more compact on the x and y axis, when compared to the HPI4 control. On the other hand, the volume of Golgi apparatus didn't change while becoming more compact horizontally. Similarly, lysosomes increased in volume and localized toward the center of the cell, while endosomes didn't appear to change volume or location. This work provides a three-dimensional reference map of organelle morphology and distribution during normal development of RPE apical-basal polarity. Abnormal RPE phenotypes occurring in different diseases can now be compared to this reference map to understand the underlying mechanisms of action.

B393/P2780

Polarity in *Saccharomyces cerevisiae* spores

B. Cooperman, M. McMurray; University of Colorado Sch Med, Aurora, CO

The mechanisms responsible for establishing polarity have been studied in the budding yeast *Saccharomyces cerevisiae* for many decades. In mitotically dividing (vegetative) yeast cells the bud site selection process both requires and directs the placement of stable landmark proteins that mark the site of future buds. The cytoplasmic polarity machinery is recruited to these landmarks via direct interactions with components of the Ras-family GTPase Rsr1 module. This module is itself coupled to the Cdc42 GTPase module, linking downstream effector machinery to the previously marked bud site. While much progress has been made towards untangling the complicated web of molecular interactions underlying these pathways, many unknowns remain. Unlike vegetative cells, spores form when a diploid undergoes meiosis upon nutrient limitation. Four spores are created with *de novo* membranes and cell walls all contained within the original diploid cell wall, creating an ascus. When spores are exposed to nutrients and germinate, there is no previous bud site landmark to direct the placement of their future buds. We recently showed that despite the lack of traditionally placed landmarks, spores are indeed born polarized: they bud away from the center of the ascus. This finding provides a unique opportunity to improve our understanding of polarity by investigating how the molecular machinery functions in a system with different rules. We found that spores form a stable, polarized site and identified a variety of proteins that localize there, including the landmark protein Bud8 and Bud5, a GEF for Rsr1. The corresponding GAP for Rsr1, Bud2, is absent, suggesting that cycling of Rsr1 is not required. Upon germination, the site marks both the direction of budding and of early morphogenesis prior to reorienting towards a mating partner. Intriguingly, while Bud2 and Bud5 are both required to direct the budding machinery to the polarity site, loss of Rsr1 destabilizes the site, causing it to relocalize, together with the budding machinery. Unlike in vegetative cells, this occurs multiple times within the same bud, rather than stabilizing at a single, random site. Future experiments aim to further characterize this

system as well as the molecular interactions that occur upon germination in the hopes of understanding how, when, and why this site is marked.

B394/P2781

Visualizing the remodeling of planar cell polarity during cell division

D. Ouyang, L. Basta, D. Devenport; Princeton University, Princeton, NJ

To preserve tissue structure and function throughout life, stem cells must continuously divide and differentiate to replenish and replace aged or damaged tissue. The regenerative process of cell division requires elaborate morphological and internal reorganization whereby dividing cells round up, reorganize their cytoskeleton and organelles, and rearrange with their neighbors to incorporate daughter cells into the tissue. How epithelial stem cells maintain cell polarity, an essential architectural feature of all epithelial organs, during such elaborate cellular reorganization remains poorly understood. Polarity organizes along both the apicobasal and planar axes of epithelial tissues through the asymmetric partitioning of polarity proteins along the plasma membrane. In the skin epidermis, the core PCP proteins Vangl and Frizzled localize to the anterior and posterior sides of intercellular junctions where they partner with the atypical cadherin Celsr1. Our lab previously showed that when epidermal progenitors divide, PCP proteins are removed from the cell surface via bulk endocytosis resulting in a temporary loss of PCP. After division, PCP is restored but the mechanisms that direct PCP protein transport and repolarization are poorly understood. Here, using live-imaging and super-resolution microscopy of endogenously-tagged *Celsr1-3xGFP, Fz6-3xGFP*, and *tdTomato-Vangl2* proteins in murine embryonic epidermis, we characterize the differential internalization, partitioning, transport and delivery of PCP protein-containing vesicles over the course of mitosis and through developmental time. Our data suggest that mitotic internalization and repolarization of PCP proteins serves not only to preserve PCP asymmetry once it is established but may also function to enhance PCP asymmetry during its initial emergence.

B395/P2782

Galectin-8 induces Epithelial-Mesenchymal Transition in Cysts of Fully Polarized MDCK cells

R. Santander[1], J. Cancino[1], F. Perez-Molina[1], C. Herrera-Cid[1], A. González[1], **A. Soza**[1,2,3]; [1]Universidad San Sebastián, Santiago, Chile, [2]P. Universidad Católica de Chile., Santiago, Chile, [3]Centro Científico y Tecnológico de Excelencia Ciencia & Vida., Santiago, Chile

Introduction: Epithelial cells undergo changes towards a more mesenchymal migratory phenotype during processes of organogenesis, tissue repair and cancer development and progression. Tumor cells co-exist with fully differentiated cells within their originating epithelia and therefore their secreted factors able to induce this epithelial-mesenchymal transition (EMT) entail great interest. Galectin-8 (Gal-8) is a carbohydrate binding protein that is overexpressed in several tumors and upon secretion by a nonconventional mechanism elicits a variety of cellular responses, interacting with different cell surface glycoproteins. Our previous studies show that Gal-8 promotes EMT in sub-confluent Madin-Darby Canine Kidney (MDCK) epithelial cells by trans-activating the epidermal growth factor receptor (EGFR) through integrin-mediated FAK activation. However, whether fully differentiated epithelial cells also undergo similar Gal-8-induced changes remain unknown. **Methods:** MDCK cells were grown in matrigel for 10 days to form three-dimensional (3D) cysts with their apical surfaces facing a central lumen and their basal surfaces contacting the extra cellular matrix and exposed to added recombinant Gal-8. Apical

(GP135) and basolateral (E-Cadherine) markers, cyst structure, and EMT marker Snail transcription factor were analyzed by confocal immunofluorescent microscopy. **Results:** Gal-8 promoted lumen loss and three-dimensional disorganization in 80% of cysts. Cysts that did not lose their lumen under these conditions were depolarized, according to the localization of E-cadherin and GP135. FAK activity was required to induce three-dimensional disorganization and polarity loss of the cysts. In contrast, EGFR activity was only required to induce three-dimensional disorganization. Lactose, which inhibits galectin binding to carbohydrates, prevented cyst three-dimensional disorganization and polarity loss. Gal-8 promoted the expression of Snail in the nucleus. **Conclusion:** Gal-8 has the potential to induce EGFR-mediated EMT in fully polarized epithelial cells and therefore might contribute to induce this process in epithelial cells adjacent to tumor cells overexpressing Gal-8. **Acknowledgements:** FONDECYT 1211829 and 1221374, ANID Centro de Envejecimiento y Regeneración, ACE210009, and Centro Ciencia & Vida, Basal FB 210008.

Neuronal Morphogenesis and the Cytoskeleton

B397/P2783

Coro1A role in TRIM67-regulated neuronal morphogenesis

C. T. Ho, E. B. Evans, S. Menon, S. L. Gupton; University of North Carolina At Chapel Hill, Chapel Hill, NC.

To establish functional neuronal circuitry, neurons progress through several developmental stages and shape changes, including neurite initiation, neuron polarization, axon branching and guidance, and synaptogenesis. The dramatic neuronal shape change during these morphological events depends on the cytoskeleton remodeling machinery. Previously, our lab discovered that the neuronally expressed E3 ubiquitin ligase TRIM67 is essential for appropriate neuronal morphogenesis. We found that TRIM67 localizes to the growth cone periphery and actin-rich filopodia structures, where it regulates filopodia number and stability. Interestingly, TRIM67 lacking neurons exhibit aberrant growth cone morphology, and have defects in netrin-dependent axon turning and branching. As E3 ubiquitin ligases typically have multiple substrates, and to further understand how TRIM67 regulates cytoskeleton dynamics during development, we conducted an unbiased proximity-dependent biotin identification (BioID) assay to identify putative TRIM67 substrates. **Coronin 1A**, which is a conserved actin binding protein crucial for the regulation of actin dynamics, was identified as a potential TRIM67 interactor/substrate from our proteomic study. Although Coronin 1A is neuronally expressed, and the gene lies in a chromosome locus where copy number variants are associated with various neurodevelopmental disorders, its role in neuronal development remains elusive. Through live-cell imaging and immunofluorescence staining, we discovered that Coronin 1A is enriched in the growth cones of developing neurons and localizes to the filopodia structures, particularly at the base region. In addition, using neurons from *Coro1A*$^{+/+}$ and *Coro1A*$^{-/-}$ littermates we discovered that Coronin 1A is crucial for netrin-dependent growth cone morphology and axon branching, which phenocopies neurons lacking TRIM67. Here we utilized microscopy and biochemical assays to show Coronin 1A and TRIM67 are binding partners, and have mapped out the domains that are required for TRIM67:Coronin 1A interaction. In addition, we discovered that Coronin 1A is ubiquitinated by immunoprecipitating Coronin 1A under denaturing conditions. Ongoing work is examining the impact of Coronin 1A knockout in axon turning *in vitro* and axon guidance *in vivo*, as well as testing whether Coronin 1A is ubiquitinated in a TRIM67-dependent or sensitive manner. These findings suggest a *novel role for Coronin 1A in regulating neuronal morphogenesis, and possibly functioning downstream of TRIM67-mediated pathway.*

B398/P2784

CKAP5 enables seeding of actin bundles by exploratory microtubules

J. Sabó, **M. Ždímalová**, Z. Lánský, M. Braun; Institute of Biotechnology of the Czech Academy of Sciences, Prague, CZECH REPUBLIC.

Neuronal growth cones are cellular structures responsible for neuronal guidance in a response to the cues presented to the cell from its surroundings. The function of the growth cones is heavily dependent on the cytoskeleton. Actin filaments in the form of networks and bundles are present at the periphery of the growth cone. Microtubule bundles are located in the central domain from which individual exploratory microtubules grow out towards the cell periphery. Recently, CKAP5 (in human, or its *Xenopus* and *Drosophila* equivalent, XMAP215 and MSMP, respectively) has been reported to play a role in mediating a cross-talk between microtubules and actin filaments in growth cones. However, the molecular mechanism of this process is unknown. Here, we found in an *in vitro* reconstituted system that CKAP5 enables seeding of prevailing actin bundles by dynamic microtubules. We observed that CKAP5 binds to microtubules with higher affinity than to single actin filaments. CKAP5 bound to the microtubule lattice can recruit actin filaments, which form bundles around the microtubules at concentrations insufficient for microtubule-independent actin bundle formation. When the microtubules depolymerize, the actin bundles prevail at the positions and orientations predetermined by the microtubules. This readily suggests a mechanism explaining how exploratory microtubules in filo- and lamellipodia set the positions of actin bundles, for example in neuronal growth cones.

B399/P2785

Biophysical regulation of axon morphology and plasticity

J. Griswold[1], C. Zhu[2], P. Rangamani[2], S. Watanabe[1]; [1]Johns Hopkins University Sch Med, BALTIMORE, MD, [2]University of California, San Diego, San Diego, CA.

Neurons are known for their intricate cellular morphology. Axons in particular are exceptionally long (100-1000 mm) and ultrathin (100 nm). Their cable-like morphology is essential for conduction of electrical signals, or action potentials, throughout the brain and body. Thus, it has been long assumed that axons are tubular structures with occasional synaptic varicosities. However, our work has challenged this assumption. Using high-pressure freezing and plunge freezing to preserves tissue morphology in a more native state, we performed ultrastructural analysis of axons in Caenorhabditis elegans motor neurons, mouse hippocampal neurons, and human cortical neurons. We discovered that axons are not simple tubes but rather exhibit a pearls-on-a-string morphology through their entire length, with the pearls being ~250 nm and the strings ~70 nm in diameter. This morphology is reminiscent of membrane tubes undergoing tension-driven instability. Consistent with this notion, the pearled area becomes smaller when hyperosmotic solution is applied and larger when hypoosmotic solution is applied. Interestingly, pharmacological perturbation of the cytoskeleton did not greatly alter axon morphology, suggesting that membrane mechanics drives axon morphology. In further support of this, increasing the membrane fluidity by cholesterol depletion from the plasma membrane led to a shrinking of the pearled membrane regions. Functionally, when axon morphology is altered with pharmacological cholesterol depletion, action potential velocity increases. Similarly, neuronal stimulation that induces plasticity increases action potential velocity by altering the pearled axon morphology. Our *in silico* modeling further supports the experimental data that membrane mechanics

can cause pearled axon morphology and that pearled morphology greatly impacts action potential conductance. These data have revealed for the first time that axons are pearled not tubular, and that pearled axon morphology has an important functional role in neuronal activity and plasticity.

B400/P2786

Investigating the role of netrin receptors in Trim9 and Trim67 dependent morphogenesis

S. Mutalik, S. L. Gupton; Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC.

Accurate axon guidance is crucial to form functional connections in the developing brain. The motile tip of axons, called growth cones sense various guidance cues in their surroundings through the surface receptors. Netrin-1 is a classic guidance cue essential for axonal guidance and neuronal development. Netrin-1 induces a diversity of responses through the regulation of multiple different receptors. In the current study, we investigate the role of netrin receptors DCC and UNC5C, which regulate attraction and repulsion, respectively. Over the years, our lab has established functions of neuronally enriched E3 ubiquitin ligases of the Tripartite motif (TRIM) family, TRIM9 and TRIM67 in neuronal morphogenesis and development. Consistent with previous work, we show that netrin-1 induces a concentration-dependent biphasic response in murine cortical neurons, attraction at lower concentrations and repulsion at higher concentrations. Previous work from the lab has established functions of TRIM9 and TRIM67 in attractive response. In the current study, we show that repulsive response is abolished in *Trim9*$^{-/-}$ and *TRIM67*$^{-/-}$cortical growth cones. Using TIRF-based live imaging, we show that TRIM9 ΔRING and TRIM67 ΔRING that lack the ligase activity colocalize with UNC5C in cortical growth cones, highlighting TRIM-dependent regulation of UNC5C in cortical growth cones. Using immunocytochemistry, we investigated netrin-dependent dynamic changes in receptor levels in cortical growth cones. We show that the surface level of DCC is increased and the total available pool of UNC5C is decreased in *Trim9*$^{-/-}$ growth cones. consistent with this, our preliminary results from the surface biotinylation assay show that the surface UNC5C levels are reduced while DCC levels are increased in Trim9$^{-/-}$ cortical neurons. The Previously published work has established TRIM9 and TRIM67 interaction with DCC. Further, TRIM9-dependent ubiquitination of DCC in the cytoplasmic domain including the UNC5C interacting domain has been predicted. Hence, we hypothesized that TRIM9 regulates DCC-UNC5C interaction. Using coimmunoprecipitation assay we showed that DCC-UNC5C interaction is not regulated by TRIM9. Collectively, the current work shows that altered baseline levels of DCC and UNC5C are likely to contribute to netrin unresponsiveness Trim9$^{-/-}$ and *Trim67*$^{-/-}$ cortical neurons.

B401/P2787

Shootin1-DCC Interaction Mediates Netrin-1-induced Axonal Haptotaxis

Z. Qiu, K. Abe, D. Aoki, T. Minegishi, N. Inagaki; Nara Institute of Science and Technology, Ikoma city, JAPAN.

During the development of the brain, neuronal axons are navigated to right destinations and connected with appropriate targets, the process called axon guidance. There are two types of axon guidance mediated by chemical cues, chemotaxis and haptotaxis. Chemotaxis is the movement directed by spatial gradients of soluble chemicals; haptotaxis is regulated by chemicals presented on adhesive substrates. Netrin-1 is one of the best-characterized axon guidance molecules; it is thought to attract commissural axons by chemotaxis. Recent studies reported that netrin-1 also functions as a haptotactic attractive

axon guidance molecule in hindbrain and spinal cord, but the mechanism underlying it is unknown yet. We previously reported that shootin1a is involved in neuronal polarization, axon outgrowth and guidance. In addition, shootin1a functions as 'clutch' molecule in laminin-induced axonal haptotaxis. However, involvement of shootin1a in netrin-1-induced axonal haptotaxis is not clear. Here, we analysed the molecular mechanism for netrin-1-induced axonal haptotaxis. First, we found that shootin1a was colocalized with the netrin-1 receptor DCC in axonal growth cones by immunostaining. Further co-immunoprecipitation analysis demonstrated the interaction between shootin1a and the intercellular domain of DCC. Next, we confirmed that neurons cultured on netrin-1-coated coverslips extended significantly longer axons than on polylysine, suggesting that axon outgrowth is promoted by substrate-bound netrin-1. Moreover, we compared shootin1 KO and WT neurons cultured on netrin-1-coated coverslips and microscale patterns. Shootin1 KO neurons extended shorter axons and did not undergo haptotaxis on microscale patterns. Those results demonstrate that shootin1a interacts with DCC-intercellular domain and mediates netrin-1-induced axonal haptotaxis. To further analyse the mechanism, we preformed speckle imaging using HaloTag-F-actin to determine the change of F-actin retrograde flow; the F-actin flow speed on netrin-1 was significantly slower than that on polylysine, indicating that the clutch linkage is promoted on netrin-1. In addition, traction force produced by growth cones was higher on netrin-1 than on polylysine, and shootin1 KO reduced the traction force on netrin-1. These data indicate that shootin1a-mediated clutch coupling is promoted by netrin-1 on the substrate. Furthermore, we preformed speckle imaging using DCC-HaloTag. Retrograde speed of DCC was slower on netrin-1 compared to that on polylysine; DCC-HaloTag underwent more stopped state and less flowing state on netrin-1 substrate, indicating netrin-1 promotes DCC-substrate clutch linkage. All our data suggest that shootin1-DCC interaction drives netrin-1 induced axonal haptotaxis.

B402/P2788

How evolution diversifies specific protein recognition

G. Wiseglass, R. Rubinstein; School of Neurobiology, Biochemistry and Biophysics, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, ISRAEL.

There has been great interest in studying the co-evolution of specific receptor-ligand interactions, as these interactions mediate the identification and incorporation of extracellular signaling, cell-cell recognition and adhesion. In some cases, and due to disruptive mutations, some co-evolving proteins undergo a non-interacting intermediate evolutionary stage, while others become promiscuous and form novel interactions with new partners. The Down syndrome cell adhesion molecule (Dscam1) provides an extreme example of receptors evolving strict homophilic interactions. In insects, exon duplications have led to the expansion of Dscam1 to tens of thousands of isoforms. Stochastic alternative splicing of Dscam1 results in a unique expression pattern in each neuron. These properties allow neurons to distinguish between interactions with neurites from the same cell and neurites from other cells, which is crucial for the correct formation of complex nerve architectures. The current study focuses on the evolutionary paths leading to the strict homophilic specificity exhibited by Dscam1. We use phylogenetics to analyze sequences of Dscam1 isoforms across species and identify the origins of exon duplication. Then, using ancestral reconstruction, we predict candidate intermediate sequences. We experimentally tested the binding specificities of these intermediates. Specifically, we tested whether their interactions are strictly homophilic, promiscuous, or whether they harbor disruptive mutations that prevent them from binding. Our results provide a detailed evolutionary pathway by which the Dscam family of proteins gains new recognition specificities. Our results indicate an evolutionary

pathway via “intermediate” proteins that form promiscuous interactions rather than non-interacting “intermediates”. Altogether, this work provides insights into protein families evolution and co-evolution of protein interfaces.

B403/P2789

Neurexin and frizzled intercept axonal transport at microtubule minus ends via VAB-8 to control synapse formation

J. Park[1], S. Balseiro-Gomez[1], Y. Yue[2], C. Ding[1], L. Shao[1], S. Çetinkaya[1], C. Kuzoian[1], M. Hammarlund[3], K. Verhey[2], S. Yogev[1]; [1]Neuroscience, Yale, New Haven, CT, [2]Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, [3]Neuroscience/Genetics, Yale, New Haven, CT.

Neurons depend on the general polarity of microtubule population for the compartmentalization of dendrites and axons. Recent evidence has suggested that each end, plus (+) or minus (-) end, of a microtubule polymer plays an important role in determining how individual transport event terminates and thereby sculpting the precise synaptic connectivity of each neuron. Here, we report that VAB-8/KIF26, an immotile kinesin, as the downstream effector of anti- and pro-synaptogenic activities of frizzled and neurexin in *C. elegans*. We find that endogenous VAB-8 localizes to microtubule minus ends *in vivo* and its levels at synaptic microtubules are controlled by frizzled and neurexin. Loss of VAB-8 mimics frizzled hyperactivation or loss of neurexin, where posterior half of synapses in the tail of the worm are lost. VAB-8 is required for the synaptic localization of other minus-end binding proteins such as PTRN-1 and NOCA-1 although its effect on general microtubule dynamics and steady-state synaptic microtubule organization is minimal. Consistently with the possible role of VAB-8 at minus ends, we find that *vab-8* mutants show significantly shorter retrograde pause duration during synaptic vesicle precursor transport, which may affect synaptic cargo delivery at minus ends. Reducing retrograde transport, but not anterograde, rescues synapse loss in *vab-8* and neurexin mutants. Collectively, VAB-8 functions downstream of two separate signaling pathways to regulate retrograde transport and determine precise synaptic length of different neurons.

B404/P2790

The kinesin-1 adaptor UNC-14 is required for synapse formation and microtubule polarity

M. Lyu; Yale University - New Haven, CT, New Haven, CT.

Synapse formation depends on long-range axonal transport on microtubule tracks. We identified the kinesin-1 binding protein UNC-14/RUSC2 as a regulator of microtubule organization and synapse formation in *C. elegans*. In *unc-14* mutants, we observe the loss of synaptic vesicle clustering and an ectopic accumulation of synaptic material in the dendrite. We speculate that these defects are due to misdirected transport of synaptic proteins, because *unc-14* mutants also show defects in microtubule polarity, with dendritic microtubules assuming an axonal-like organization and axonal microtubules showing dendritic features. We also hypothesize that microtubule defects underlie the previously observed axon guidance and animal locomotion defects in the mutant animals. We are currently working to elucidate how this pair of proteins control microtubules and synapses.

B405/P2791

Coordination of ion channel delivery and dendrite growth in Drosophila sensory neurons

J. Mitchell[1], I. Midillioglu[2], B. Wang[3], C. Han[3], **J. Wildonger**[2]; [1]University of Wisconsin-Madison, Madison, WI, [2]University of California, San Diego, La Jolla, CA, [3]Cornell University, Ithaca, NY.

Sensory neurons enable an organism to perceive external stimuli, which is essential for survival. The sensory capacity of a neuron depends on the elaboration of its dendritic arbor and the delivery of ion channels to the dendritic membrane. However, it is not well understood how ion channels are trafficked to morphologically complex sensory dendrites and whether their delivery is coordinated with dendrite growth. We investigated the trafficking of the DEG/ENaC/ASIC ion channel Pickpocket (Ppk) in peripheral sensory neurons in fruit fly larvae. We used CRISPR-Cas9 genome engineering to tag endogenous Ppk1 and visualize it live, including monitoring Ppk1 membrane localization via a novel secreted split-GFP approach. Strikingly, Ppk1 is present throughout the membrane of actively growing dendrites, and Ppk1 density scales in proportion to the dendritic membrane, even when dynein-mediated transport to dendrites is disrupted. Our data suggest that Ppk1 is integral to the membrane of growing dendrites and implicate the recycling endosome GTPase Rab11 in the forward trafficking of Ppk1 to dendrites. Together, our results suggest that Ppk channel transport is coordinated with dendrite morphogenesis, thus ensuring proper ion channel levels and distribution in sensory dendrites.

B406/P2792

Investigating the underlying mechanisms mediating dendrite pruning in *Caenorhabditis elegans*

P. Figueroa-Delgado[1], S. Yogev[2]; [1]Department of Cell Biology, Yale University, New Haven, CT, [2]Department of Neuroscience, Yale University, New Haven, CT.

Neurite remodeling is a highly conserved process that refines and establishes a mature nervous system. A failure in neurite remodeling leads to neurological and neurodevelopmental disorders. While developmental dendritic pruning, a means of neurite remodeling, has been extensively studied, the cell-biological mechanisms that control pruning remain poorly understood. Specifically, there is a *fundamental gap* in understanding the cell-biological mechanisms that mediate dendrite branch-specific pruning. The nematode *Caenorhabditis elegans* inner labial 2 (IL2) neurons, upon entering a developmental diapause, extend a stereotypical dendritic arbor that is pruned when development is resumed - leaving primary dendrites intact. The stereotypic remodeling of IL2 neurons allows experimental access to elucidate the cell-biological mechanisms that confer selective pruning. To identify IL2 dendrite pruning regulators, I conducted an unbiased forward genetic screen to isolate genes by selecting mutants that exhibited a pruning defect, such as loss of pruning or loss of pruning specificity. Among the nine (9) isolated mutants, mutant *shy87* showed a severe loss of pruning defect characterized by the remanence of the dendritic arbor into adulthood. Rescue experiments robustly show *sax-1*, a gene that encodes a highly conserved serine/threonine kinase, is the causal gene for the defective pruning observed in *shy87* mutants. In addition, to address if SAX-1 acts with known conserved scaffolding protein SAX-2 to mediate IL2 higher-order dendrite pruning, I tested a loss-of-function allele for *sax-2*, which phenocopies *shy87* mutants post-dauer exit. These findings suggest that SAX-1 and SAX-2 are important regulators of neuronal remodeling in IL2 neurons.

B407/P2793

Regulation of Human Neurofilament-light Assembly State by O-GlcNAc and Potential Hypoglycosylation of Charcot-Marie-Tooth Disease Mutants

D. T. Huynh[1], J. Hu[1], K. Tsolova[1], M. Zorawski[1], C. Lin[2], E. Soderblom[3], N. Linhart[1], D. Li[1], J. Chi[2], M. Boyce[1]; [1]Biochemistry, Duke University, Durham, NC, [2]Molecular Genetic and Microbiology, Duke University, Durham, NC, [3]Proteomics and Metabolomics Core Facility, Center for Genomic and Computational Biology, Duke University, Durham, NC.

Neurofilaments (NF) are cytoskeletal proteins composed of light, medium and heavy subunits that structurally support neurons. These proteins assemble in discrete states, from low-order oligomers to fully assembled filaments, and require the light subunit (NF-L) to form filamentous networks. NF-L dysregulation, such as mutants in a subtype of Charcot-Marie-Tooth (CMT), a neuromuscular disease, causes aggregations that also occur in various neurodegenerative disorders. In rodent brains, NFs are modified post-translationally by O-GlcNAc, an intracellular form of glycosylation on serine/threonine residues. However, how O-GlcNAc regulates human NF-L is unclear. In order to determine the impact of O-GlcNAc on NF-L functions, we first used immunoprecipitation to show dynamic O-GlcNAcylation of human NF-L. Then, we combined CRISPR-tagged endogenous human NF-L with mass spectrometry to find novel glycosites (S48, S431). To examine the effect of O-GlcNAc on NF-L assembly, we expressed wild type (WT) NF-L in NF-L$^{-/-}$ cells and biochemically fractionated discrete NF-L assembly states. Most of the total NF-L was in fully assembled filaments. However, elevating O-GlcNAc levels by expression of O-GlcNAc-transferase (OGT), which adds O-GlcNAc to substrates, significantly reduced this proportion, indicating a shift towards lower-order oligomers. Expression of WT NF-L in NF-L$^{-/-}$ neuroblastoma cells and immunofluorescence imaging revealed that OGT expression reduced NF-L full-length filaments and increased the prevalence of puncta, representing lower-order states. An unglycosylatable (S/T-to-A) NF-L mutant partially suppressed these phenotypes, demonstrating that site-specific glycosylation of NF-L itself is required for these effects. Interestingly, among CMT NF-L mutants, those near glycosites abolished NF-L O-GlcNAcylation. In immunofluorescence and fractionation assays, these mutants formed aggregates that were unaffected by increased O-GlcNAc levels. Therefore, our data indicate that increasing O-GlcNAcylation drives WT NF-L to lower-order assembly states, and NF-L may be hypoglycosylated in disease. We hypothesize that NF dysregulation found in other neurodegenerative diseases may involve perturbed NF-L O-GlcNAcylation, which we will test in future studies. This work advances our knowledge of the neuronal cytoskeleton and may inspire future efforts to rationally manipulate O-GlcNAc on NFs and other neuronal proteins for therapeutic benefit.

B408/P2794

Neuron Isolation on the Cyto R1 Platform: A Customized Workflow for Subpopulation Enrichment

K. E. Degen[1], J. D. Joyce[2], T. Harrell[2], A. S. Bertke[2], A. R. Hyler[1]; [1]CytoRecovery, Inc., Blacksburg, VA, [2]Department of Population Health Sciences, VA-MD College of Veterinary Medicine, Blacksburg, VA.

The isolation of primary neurons from peripheral ganglia of adult animals results in a dense mixture of axon debris, satellite glial cells (SGCs), and neurons. Conventional clean up techniques utilize density gradients, which achieve roughly 50-75% removal of SGCs and debris. Here, a novel workflow was developed using the Cyto R1 Platform to deplete debris and satellite glial cells to achieve more purified downstream neuron cultures. The Cyto R1 employs label-free, biophysical enrichment (dielectrophoresis) to separate live cells from debris by trapping cells with intact membranes. Using a

disposable microfluidic cartridge, Cyto Chip, neurons can be enriched in a sterile system in which trapping occurs at stationary pillars in the Chip. In order to generate the appropriate electrical fields for enrichment, we transferred samples into an ultra-low conductivity buffer, Cyto Buffer. For this workflow development, a customized buffer formulation was needed to support neuron viability. Murine trigeminal ganglia (TG) neurons were exposed to 6 novel buffers in suspension for 30 min at room temperature, plated on Matrigel and maintained in Neurobasal A medium with neurotrophic factors. Neuron viability was assessed immediately prior to plating, then 24 hours, 5 days and 14 days after plating. One buffer out-performed the others, maintaining neuronal viability on par with the control neurons in supplemented Neurobasal A media under the same conditions. Neurons exposed to this buffer were able to reestablish normal biophysical architecture once plated and remained viable for at least 14 days in post-exposure culture. With the adjusted formulation of buffer, additional TG suspensions were then enriched on the Cyto R1 Platform at 400 kHz and 640 Vpp in 5-minute batches. After enrichment, 85% recovery of purified neurons was achieved. Additionally, we observed variable trapping efficiencies as different flow rates, voltages, and frequencies suggesting that even finer sorts of cell subpopulations will be possible. The Cyto R1 provides a versatile platform for isolation of purified primary neurons from adult animal peripheral nervous system tissues for downstream culture or assays.

B409/P2795

Dual Roles of RBM8A in Neural Progenitors and Astrocytes

J. Mott, C. McSweeney, S. Asthana, Z. Pei, Y. Mao; Penn State University, State College, PA.

RBM8A, a core component of the exon junction complex, is located in the distal region of 1q21 (a prominent copy number variation associated with neurodevelopmental disorders). Studies have shown that *Rbm8a* modulates the balance between proliferation and differentiation of neural progenitors (NPs). Intricate balance between proliferation, differentiation, and migration of NPs is critical in cerebral cortical development. Here, we investigated the role of *Rbm8a* in interneuron development using a conditional knockout (cKO) mouse model. Additionally, RBM8A has been hypothesized to play regulatory roles in the radial glia. Dysfunction in the radial glia development may impair the development of the astrocytes, thus affecting synapse formation, leading to neurological disease. What role do astrocytes play in neurodevelopmental disorders? We explored the role of astrocytes in *Rbm8a*-deficient mouse phenotype.

We generated *Rbm8a* cKO mice by crossing homozygous floxed mice with *nestin-cre* mice to achieve heterozygous *Rbm8a* cKO selectively in NPs. Additionally, *Rbm8a*$^{fl/fl}$ mice were crossed with *Nkx2.1-cre* mice to selectively delete *Rbm8a* in interneuron progenitors. Then, *Nkx2.1-cre; Rbm8a*$^{fl/+}$ mice were crossed with *cre*-dependent *Ai9* reporter line to specifically trace progenies of *NKX2.1+* progenitors when *Rbm8a* expression is reduced in vivo. Furthermore, we generated astrocyte-specific *Rbm8a* cKO mice by crossing *Rbm8a* KO mice with B6.Cg-Tg(*Gfap-cre*)73.12Mvs/J mice, which features transgenic glial fibrillary acidic promoter driven expression of *Cre* Recombinase to selectively delete *Rbm8a* in the mouse astrocyte cell. We performed various behavioral tests to investigate motor and anxiety behavior in *GFAPCre; Rbm8a*$^{f/+}$ mice.

Our data reveal that *Rbm8a* haploinsufficiency in neural stem cells results in smaller body and brain size, as well as abnormal migration and cell size of cortical interneurons. This haploinsufficiency in *NKX2.1*-positive interneuron progenitor decreases cell proliferation and affects interneuron differentiation. Moreover, *GFAPCre; Rbm8a*$^{f/+}$ mice show significantly increased locomotor and mobility function and

altered anxiety state. Taken together, our study shows that *Rbm8a* plays a critical role in interneuron development and suggests that *Rbm8a* in astrocytes affects motor function and anxiety state.

B410/P2796

Exposure of SHSY5Y cells with Tideglusib, Gallein, and Okadaic acid reveal that microtubule disruption but not Tau phosphorylation at ser 396 (ps396Tau) is key event in neurodegeneration.

D. Olivas, S. Roychowdhury; The University Of Texas At El Paso, El Paso, TX

BACKGROUND: As our elderly global population increases, so does the diagnosis of neurodegenerative diseases (ND), such as Alzheimer's disease (AD). The pathology of AD is characterized by extensive loss of neurons. Hyperphosphorylation of Tau (a microtubule-associated protein) leading to Tau aggregation and neurofibrillary tangles (NFT) formation has emerged as a major feature in the pathogenesis of AD and other taupathies. Due to this hypothesis, many inhibitors that decrease tau phosphorylation, such as Tideglusib (an inhibitor of GSK3β) have been targeted in clinical trial to treat AD and have failed suggesting that Tau phosphorylation may not be a key event in the pathogenesis of AD. Previously, we have shown that Gallein, an inhibitor of Gβγ decreased the expression of phosphorylated tau at the serine 396 sites (ps396 tau). However, it disrupted microtubules (MTs) and inhibited neuronal differentiation of human neuronal cells SHSY5Y suggesting that hypo-phosphorylation of Tau could also be linked to neurodegeneration similar to that observed with hyperphosphorylation of Tau. (doi: 10.1091/mbc.E21-11-0545). **GOAL:** In the current study, we used Tideglusib, Gallein, as well as Okadaic acid (a phosphatase inhibitor) to further understand the role of Tau phosphorylation in the pathogenesis of AD. **METHODS: To conduct the study, Retinoic acid (RA)-differentiated** SHSY5Y cells were treated with Tideglusib for 1 hr. To **induce a hyperphosphorylated Tau state,** cells were treated with OKA overnight. For comparison cells were also treated with Gallein. The samples were then subjected to confocal microscopy and whole cell lysis (WCL). **RESULTS: Confocal microscopic analysis showed that Tideglusib caused disruption of MTs and inhibited neuronal differentiation However, Tideglusib was more effective in disrupting structure of neurons compared to Gallein. In addition, neuronal cell numbers were decreased significantly in the presence of Tideglusib. As expected, immunoblot revealed a decrease in the expression of ps396 tau in the presence of Tideglusib. We found that OKA did not disrupt neuronal morphology. However, OKA treated cells had more short neurite projections than control. CONCLUSION: Although OKA induces the hyperphosphorylation of tau, OKA treatment did not disrupt the neuronal cytoskeleton. In the contrary, both Tideglusib and Gallein disrupted the neuronal structure but decreased the expression of ps396. Our result suggests that that the hyperphosphorylation of tau may not be the key event in the pathogenesis of AD, and therefore, the potential drugs against AD should be evaluated in neuronal cells for structural and morphological alterations which is reflective of neurodegeneration.**

B411/P2797

NeuroPAL: A "PAL" for Identifying hot Gene Neuron Identities within the Ly6 Family

S. Seth, K. Mali, R. Sung, **A. Petropoulos**; Carleton College, Northfield, MN.

The Ly6 protein family is highly conserved among multiple species including humans, mice, Drosophila, zebrafish, and C. elegans. Surprisingly, these endogenously expressed proteins also share significant structural similarities to alpha-neurotoxins (such as bungarotoxin) found in snake venom. Ly6 proteins have been observed to be involved in processes such as odor sensing, cell adhesion, and regulation of

receptor trafficking; however, despite this knowledge, little is known about the function of these proteins. In order to investigate the function of these proteins as a family, we are using C. elegans as a model organism, which has a manageable 10-member Ly6 family in comparison to larger Ly6 families in mammalian systems. Given that previous work has identified roles for Ly6 protein function in a variety of cellular processes related to neuronal activity, we hypothesize that Ly6 protein expression in C. elegans would occur in neuronal cell types. In order to deduce the expression of the Ly6 proteins, we are utilizing a tool called NeuroPAL in conjunction with transcriptional GFP reporters for each Ly6 gene of interest (known as the hot genes in C. elegans). NeuroPAL is a transgene that creates a color-coded atlas of the hermaphrodite C. elegans nervous system, and we have created a transgenic C. elegans model that contains both the NeuroPAL transgene and the GFP transcriptional reporter for hot-1 gene. Using confocal microscopy, we have begun examining hot-1 gene expression in hermaphrodites. We have tentatively identified hot-1 gene expression in the DD, RMDV, and AVE neurons.We plan to confirm our identification using additional reporter-lines for those specific cells. Lastly, we are in the process of generating additional transgenic lines containing NeuroPAL and other GFP transcriptional reporters for the other hot genes. Employing confocal microscopy, we are able to use this model to visualize the exact location of each GFP-tagged neuron for each hot gene. Moving forward, we hope to use this anatomical information to develop behavioral assays to assay the function of each hot gene in those specific tissues/cells.

B412/P2798

Co-exposure of Alcohol and Nicotine in Human Neuronal Cells SHSY5Y on Tau Phosphorylation and Microtubule alteration suggest a possible explanation of comorbidity of drug use

T. Poeschel, D. Olivas, S. Roychowdhury; University of Texas At El Paso, El Paso, TX

BACKGROUND: The use of alcohol and nicotine is common and show the most serious comorbidity. It is a major public health concern. Research has established that alcohol and nicotine addiction alter the ability for neurons to send and receive signals. Normal neuronal activities also heavily depend on microtubules (MTs), a major component of neuronal cytoskeleton. Recently, alcohol/nicotine was shown to alter microtubules (MTs). Tau is a MT-associated protein and is known to stabilize MTs, and critical for neuronal development. Tau proteins were found to be abnormally phosphorylated in neurodegenerative diseases. RESEARCH GOAL: The goal of this project is to understand the effect of alcohol/nicotine on the phosphorylation of several Tau sites that are pathologically relevant including Ser 396, Thr181, Ser404, and how MT organization and neuronal morphology is affected by alcohol/nicotine treatment. METHODS: To conduct the study, human neuronal cells SHSY5Y were exposed to nicotine [5uM] and ethanol [100mM] separately, and together for 24 hours. The samples were then subjected to immunoblotting and confocal microscopy using antibodies for tau, phosphorylated tau, and tubulin. RESULTS: The immunoblot analysis showed that ethanol caused an increase in phosphorylation of ps396Tau (~50%). While nicotine itself did not have any significance on tau phosphorylation, when co-treated with ethanol it reduced the ethanol effect by ~23% which could explain the cause behind the severe comorbidity. There was no significant difference in expression of pTau, Thr181 and Ser404 suggesting that the effect is site-specific. The results from confocal microscopy showed that when exposed to nicotine, no difference in neurite length was observed except that tubulin labeling appeared to be segmented along some neurites, indicating MT structure is disrupted by nicotine treatment. In the presence of ethanol, most neurites are significantly longer compared to control. In the presence of both nicotine and ethanol, the neurites were similar in length and morphology as seen in

the control. DISCUSSION: Overall, our result suggests that the exposure of alcohol and nicotine in neuronal cells alter Tau phosphorylation and microtubule organization. However, when co-exposed with nicotine and ethanol together, the individual effects were reduced which may explain the comorbidity among users.

B413/P2799
The Impact of DHA Supplementation on Neurogenesis in the Developing Embryo
G. Okros, M. DeLano-Taylor; Grand Valley State University, Allendale, MI

Differentiation and the rate of division of neural stem cells (NSCs) are regulated by various factors, including our diet. The impact of prenatal supplements, such as the omega-3 fatty acid docosahexaenoic acid (DHA) -a long chain polyunsaturated fatty acid (LCPUFA)- have shown to be important for the development of neuronal cell membranes. The impact of DHA and other LCPUFAs on neuronal stem cell differentiation in-vivo has not yet been tested. In this study DHA and other LCPUFAs were introduced into the developing neural tube of chick embryos to determine their effects on NSC development. By examining the number of neurons in LCPUFA treated embryonic NSCs compared to the vehicle control during different time periods of development, we found that DHA treated embryos had an increased number of neurons that persisted through development up to 72 hr after DHA injection. The persistent increase in neurons over the course of development suggests DHA is promoting neurogenesis without depletion of the stem cell pool, likely by promoting proliferation of stem cells or neuronal progenitors. The impact of this prenatal supplement on the developing nervous system in vivo provides some insight of how it could shape neurogenesis in the course of development.

Synaptogenesis

B414/P2800
Structural Analysis of Glutamategic Synapse Auxiliary Protein Neto Isoform
S. Chen; NIH, Bethesda, MD.

The synapse is a fundamental communication unit of the nervous system that connects neurons and other non-neuronal target cells to govern complex cognitive processes and behavior. A functional synapse requires the assembly of the pre-and postsynaptic structures and continuous trans-synaptic communication between them. *Drosophila melanogaster* neuromuscular junction (NMJ) is a powerful genetic model system to study glutamatergic synapse development. Previous studies established that Neto, an auxiliary protein for glutamate receptors, is essential for the recruitment of postsynaptic receptors and proper NMJ functionality. *Drosophila* neto encodes two isoforms, Neto-α and Neto-β, with the same extracellular domains but distinct cytoplasmic domains. Mutations in Neto-β result in failure to accumulate and stabilize a particular subtype of glutamate receptors (Type A) at the synapses. To determine which specific domains of Neto-β are essential for its function, we have embarked on a series of structural function analyses in which we removed specific domains and conducted loss of function studies. The current project utilizes the ALFA system, a novel epitope/nanobody pair, for the in vivo detection of tagged Neto-β. CRISPR-Cas9 was used to insert ALFA-tag in all Neto-β variants to standardize the detection of various truncated Neto-β proteins. Immunohistochemistry showed modified Neto-β and tagged β-AT with the same synaptic distribution when stained with a previously described Neto-β antibody. This work will inform us of the molecular mechanisms underlying the

recruitment and organization of the pre-and postsynaptic components crucial in gaining insights into neural circuit formation and plasticity necessary for normal brain development and function.

B415/P2801

Engineered *in vitro* models based on selective neuronal adhesion to study synapse formation

N. Piette, P. Strale, M. Munier, M. Sainlos, I. Chamma, O. Thoumine, V. Studer; IINS -CNRS Bordeaux, Bordeaux, FRANCE.

Information transfer in the brain is ensured by a complex network of specialized interconnected neurons responding to environmental cues. The proper functioning of this network relies on the establishment and maintenance of synaptic connections, nanoscale organization of their molecular components, and plasticity of their architecture. It is well known that these mechanisms are regulated by specific trans-synaptic adhesion proteins linked to membrane receptors or scaffolding proteins.
We present here two cell based *in vitro* models that we have specifically engineered to study the biophysical properties of trans-synaptic adhesion proteins and their role in the nanoscale organization of synapses during development. These models rely on micropatterns of synaptic proteins printed on glass coverslips. They were fabricated with a commercially available contactless and maskless UV projection system which allows us to reproducibly control the shape and the protein density of the microcontact areas.
In the first model, recombinant Fc-tagged neuronal protein (e.g. Neurexin1β) are anchored to 5µm wide patterned lines and presented to COS-7 cells expressing fluorescent tagged paired protein (e.g. Neuroligin1) providing highly selective recognition. By using fluorescence recovery after photobleaching and single particle tracking we are able to measure the diffusion coefficient and the interaction rate of trans-synaptic adhesion complexes, respectively. We also show how to use this model to measure the interaction between trans-membrane synaptic proteins and cytosolic partners (e.g. PSD95).
The second model consists in surfaces micropatterned with arrays of small dots (5µm diameter) coated with Fc-tagged neuronal ligands. When cultured on these substrates, primary neurons form thousands of standardized hemi-synapses after 14 days *in vitro*. We show that we can precisely locate patches of pre or post synaptic markers as well as the cytoskeleton within micropatterned areas. By averaging the super-resolved STORM (Stochastic Optical Reconstruction Microscopy) image of hundreds of these standardized hemi synapses for each developmental stage, we hope to get a new insight on the sequence of events leading from the creation of an initial contact to the formation of stable synapses. Together, these two *in vitro* models will allow us to screen and characterize the role of synaptic proteins in the context of synaptogenesis. We also hope to get a better understanding of the role of synaptic adhesion proteins in the nanoscale organization of the synapse during development.

B416/P2802

Multilayered control for subcellular distribution and functional properties of KaiR1D autoreceptor

W. Hsieh, T. Han, R. Vicidomini, P. Nguyen, Z. Li, M. Serpe; National Institutes of Health, HHS, Bethesda, MD.

At excitatory synapses, autoreceptors provide a feedback mechanism that modulate neurotransmitter release and ensure stable neuronal networks. Disruptions to this feedback have been linked to various neuronal disorders. Autoreceptors localize on presynaptic membranes and respond to neurotransmitter released by the cell on which they sit. However, detection of these low abundant proteins has been

challenging. At *Drosophila* NMJ, an autoreceptor containing the KaiR1D glutamate receptor has been implicated in the control of glutamate release; *KaiR1D* mutants have basal neurotransmission reduced to half of the normal levels. We and others previously showed that KaiR1D requires at least two auxiliary proteins, Neto-α and Sol1. Here, we focus on the roles of Neto-α in modulating KaiR1D properties and distribution. Neto-α limits KaiR1D *in vivo* activities: Basal neurotransmission is reduced by 50% in *neto-α^{null}*. Neuronal overexpression of KaiR1D cannot rescue this defect. Using fast perfusion on outside-out patches of HEK cells transfected with KaiR1D alone or with Neto-α, we found that Neto-α modulates the gating properties of KaiR1D channels, decreasing the desensitization and deactivation rates. Neto proteins have conserved extracellular domains, including two CUB domains and a LDL motif, and variable intracellular domains (CTD). We found that CUB1 is required for modulation of KaiR1D gating properties as well as *in vivo* autoreceptor activities. The CTD contributes to modulation of KaiR1D gating properties but is dispensable *in vivo*. This difference may be reconciled by the presence of Sol1 *in vivo* but not in reconstituted systems.To search for a role for Neto-α in the subcellular distribution of KaiR1D, we examined KaiR1D localization in primary rat hippocampal neurons. KaiR1D localizes to dendrites, with or without Neto-α, but cannot efficiently enter the axon by itself even when overexpressed. Neto-α or ΔCUB1, but not ΔCTD, promote KaiR1D axonal localization. Neto variants distributed at both neurites and formed puncta largely colocalizing with KaiR1D. Finally, Neto-α and KaiR1D colocalize in the proximity of the active zones suggesting that Neto-α may stabilize KaiR1D at the site of autoreceptor function. Our data indicate that multiple layers of modulation ensure proper autoreceptor activities and reveal potential new targets for pharmacological interventions.

B417/P2803

The fly Neuropilin, Neto, mediates presynaptic homeostasis through Plexin-dependent and Plexin-independent mechanisms

R. Vicidomini[1], T. Han[1], W. Hsieh[1], E. Giniger[2], M. Serpe[1]; [1]NIH/NICHD, Bethesda, MD, [2]NIH/NINDS, Bethesda, MD.

Homeostatic mechanisms ensure stable neuronal circuits function by maintaining constant synaptic strength during development and learning. *Drosophila* neuromuscular junction is a powerful genetic system to study molecular mechanisms underlying homeostasis at glutamatergic synapses: Chronic depletion of postsynaptic receptors or acute inactivation with philanthotoxin trigger a robust compensatory increases of neurotransmitter release, bringing the evoked response to normal levels. This presynaptic homeostatic potentiation (PHP) requires active zone cytomatrix remodeling to increase release probability. Previous studies implicated the flavoprotein monooxygenase (Redox) enzyme Mical, an actin regulator, in PHP. Mical is activated by binding of muscle secreted Semaphorin2b (Sema2b) to PlexinB (PlexB) on the motor neurons. Our previous work demonstrated that the Neuropillin and Tolloid-like protein, Neto, functions as an effector of PHP. More specifically, genetic null mutants lacking the presynaptic Neto-α isoform show no chronic or acute PHP response; this phenotype is rescued by neuronal overexpression of full length Neto-α but not Neto-ΔCTD, a Neto variant lacking the intracellular domain. Here we examine how Neto-α controls the PHP response. Using genetics and electrophysiology approaches, we found that *neto-α* interacts genetically with the *Sema2b/PlexB* pathway components, including *Mical*. Our epistasis analyses indicate that Neto-α functions together with Sema2b to relieve PlexB autoinhibition and thus enabling signaling downstream PlexB. These results suggest that *Drosophila* Neto-α resembles the vertebrate Neuropilins which promote binding of secreted Semas to Plexin receptors and activate Sema/Plex signaling. *Drosophila* genome does not contain a *neuropilin*

gene; instead, Neto-α seems to be the functional fly orthologue. Surprisingly, excess Neto-α activates the PHP response in the absence of PlexB but not in the absence of Mical, suggesting that Neto-α could activate Mical independently of PlexB. Interestingly, a Neto-α variant lacking the putative SH3 binding motif can restore the PHP response in *neto-α^{null}* but not in *PlexB* mutants. The SH3 binding motifs have been implicated in the activation of Abelson kinase (Abl) which phosphorylates and hyperactivates Mical in other contexts. We found that Abl is also required for the PHP response and its kinase activity is critical for this function. Neuronal overexpression of *neto-α* can only partly rescue the PHP deficits of *abl* mutants, highlighting the key role of Abl in PHP. Our data support a model whereby Neto-α engages both the the Sema/Plex pathway as well as its amplifier, Abl, to deploy a rapid and robust compensatory response and buffer fluctuations in synaptic activity.

B418/P2804

Endocytic machinery are primed at neuronal synapses through enrichment of PI4P and PS at endocytic zones

B. Goulden, Y. Imoto, T. Ogunmowo; Johns Hopkins, Baltimore, MD.

Synaptic vesicle proteins used in neurotransmitter release are retrieved within 50 milliseconds. This rapid membrane internalization, called ultrafast endocytosis is mediated by a splice variant of dynamin 1 (Dyn1xA) and a BAR domain containing protein Syndapin 1. Dyn1xA and Syndapin 1 are primed at endocytic zones in synapses to accelerate the kinetics needed for ultrafast endocytosis. However, it is currently unclear how they are targeted to the endocytic zone membrane. Here we show that plasma membrane lipids, phosphatidylinositol-4-phosphate (PI4P) and phosphatidylserine (PS), are enriched at endocytic zones and mediate recruitment of endocytic protein machinery Dyn1xA and Syndapin 1. Using genetically encoded lipid biosensors and super-resolution imaging with STED microscopy, we analyzed nano-scale organization of membrane lipids. We demonstrate that PI4P and PS are colocalized with Dyn1xA at endocytic zones, which is devoid of other membrane signaling lipids such as PIP2. To keep endocytic zones devoid of PIP2, synapses utilize the endocytic 5-phosphatase synatojanin-1 to maintain its enrichment of PI4P. The lipid localization of PI4P and PS at endocytic zones are used by the lipid interacting domains in endocytic machinery. We showed that the BAR domain of Syndapin 1 recognizes PS at the endocytic zone, whereas Dyn1xA does not require its Pleckstrin Homology (PH) domain, but instead is localized to endocytic regions through protein-protein interactions in a phase condensate by interreacting with Syndapin 1. Together, these data indicate an entirely novel method of lipid regulation for endocytosis of synaptic vesicle proteins, and allowing for the high temporal and spatial precision needed for endocytic protein machinery recruitment in synapses.

B419/P2805

Formation of neuronal synapses through phase separation is activated by the SAD-1 kinase

N. McDonald[1], L. Tao[1], M. Dong[2], K. Shen[1]; [1]Stanford University, Stanford, CA, [2]National Institute of Biological Sciences, Beijing, CHINA.

The formation of synapses by neurons is essential to establish nervous systems. Molecularly, a conserved core “active zone” structure is assembled in all synapses. We recently found that two core active zone scaffold proteins, SYD-2/Liprin-α and ELKS, form liquid phase-separated condensates during synapse formation. These condensates are required for the assembly of active zone components, clustering of synaptic vesicles, and synaptic function - suggesting phase separation is a critical

mechanism of synapse formation.
Here we investigate how neurons initiate active zone phase separation in order to build synapses at the appropriate place and time. We find that the neuronal kinase SAD-1 phosphorylates C. elegans SYD-2/Liprin-α in order to activate its phase separation and presynaptic active zone formation. The SAD-1 kinase localizes to presynaptic sites and has been previously implicated in the functional maturation of synapses and neuronal polarity, but its substrates and mechanisms of action are unknown. We identified phosphorylation sites in SYD-2 *in vivo* with mass spectrometry and confirmed direct phosphorylation by SAD-1 on specific residues within SYD-2's C-terminal SAM domains with *in vitro* kinase assays. *In vivo*, we introduced an endogenous SYD-2 mutant that blocked SAD-1 phosphorylation and imaged individual synapses at single-cell resolution. This phosphomutant inhibited the assembly of active zone proteins and resulted in animal-wide synaptic transmission defects. Furthermore, at developing synapses, the SAD-1 phosphomutant lost the ability to form dynamic condensates, indicating SAD-1 phosphorylation may directly regulate SYD-2's phase separation activity. Supporting this notion *in vitro*, we find SYD-2's SAM domains inhibit the protein's phase separation when unphosphorylated. We find the unphosphorylated SAM domains directly bind SYD-2's N-terminal coiled-coil domains, revealing an autoinhibitory mechanism where the interaction of C- and N-terminal folded domains inhibit the phase separation of an intervening intrinsically disordered region. As SAD-1 localizes to presynaptic sites, this mechanism may constrain SYD-2's phase separation elsewhere in the neuron until it is properly located to build an active zone. We conclude that SAD-1 phosphorylation of SYD-2/Liprin-α activates its phase separation to build presynaptic active zones in neurons.

B420/P2806

The Effects of alpha-Synuclein on the Phase Separation at Synapses

D. Milovanovic[1], R. Sansevrino[1], **D. Milovanovic**[2], C. Hoffmann[1,2], B. Rankovic[1], A. Condric[1], G. Aguilar Perez[1], J. Tromm[1]; [1]Molecular Neuroscience, German Center for Neurodegenerative Diseases (DZNE), Berlin, GERMANY, [2]Marine Biological Laboratory (MBL), Woods Hole, MA.

To sustain neurotransmission, synapses must maintain a proper supply of neurotransmitter-filled synaptic vesicles (SVs). Hundreds of SVs form biomolecular condensates through the interaction with synapsins, the highly abundant family of synaptic phosphoproteins. Another major family of disordered proteins at the presynapse includes synucleins, most notably alpha-synuclein. The precise physiological role of alpha-synuclein in synaptic physiology remains elusive, albeit its role has been implicated in nearly all steps of the SV cycle. To determine the effect of α-synuclein on the synapsin phase, we employ the reconstitution approaches using natively purified SVs from rat brains and the heterologous cell system to generate synapsin condensates. We demonstrate that synapsin condensates recruit alpha-synuclein, and while enriched into these synapsin condensates, alpha-synuclein still maintains its high mobility. The presence of SVs enhances the rate of synapsin/ alpha-synuclein condensation, suggesting that SVs act as catalyzers for the formation of synapsin condensates. Notably, at physiological salt and protein concentrations, alpha-synuclein alone cannot trigger the phase separation of SVs. The excess of alpha-synuclein attenuates the kinetics of synapsin/SV condensate formation, indicating that the molar ratio between synapsin and alpha-synuclein is important in assembling the functional condensates of SVs. alpha-Synuclein can be depleted from synapsin condensates by synphilin 1, another intrinsically disordered, scaffold protein at the presynapse implicated in Parkinson's Disease. Interestingly, synphilin 1 can form fluid condensates by itself, and alpha-synuclein shows the ability to fully wet synphilin condensates in a salt-dependent manner. Understanding the molecular mechanism of alpha-synuclein

interactions at the nerve terminals is crucial for clarifying the pathogenesis of synucleinopathies, where alpha-synuclein, synaptic proteins, and lipid organelles all accumulate as insoluble intracellular inclusions.

B421/P2807

Synaptic vesicle proteins and ATG9A self-organize in distinct vesicle phases within synapsin condensates

D. Park[1,2], Y. Wu[1,2], A. Baublis[3], P. De Camilli[1,2]; [1]Departments of Neuroscience and of Cell Biology, Yale University, New Haven, CT, [2]Howard Hughes Medical Institute, Yale University, New Haven, CT, [3]Harvard Chan Advanced Multi-omics Platform, Harvard T.H. Chan School of Public Health, Boston, MA.

A defining feature of presynaptic nerve terminals is the presence of tight clusters of synaptic vesicles anchored to active zones of the presynaptic plasma membrane. We have provided evidence that principles of liquid-liquid phase separation (LLPS) may explain the formation of these vesicle clusters (PMID: 28279363 and 29976799). Recently, we have shown that ectopic expression in fibroblasts of synapsin1, a peripheral synaptic vesicle-associated protein, and just an integral synaptic vesicle membrane protein, synaptophysin, is sufficient to generate clusters of small vesicles (a separate cytosolic phase or condensate) highly reminiscent of synaptic vesicle clusters at presynapses and with liquid like properties (PMID:33431828). We have now followed-up these studies by investigating the fate of other synaptic vesicle proteins when expressed in fibroblasts. We found that, unlike synaptophysin, other major integral synaptic vesicle membrane proteins, such as synaptobrevin2 (VAMP2), synaptotagmin1, SCAMP5, vGlut and vGAT fail to form condensates with synapsin but can coassemble into the vesicle clusters formed by synaptophysin and synapsin. Another vesicle protein present in nerve terminal, ATG9 (the only transmembrane protein of the core autophagy machineries), was recently shown to undergo activity-dependent exo-endocytosis at synapses in parallel with synaptic vesicle proteins (PMID: 35065714), raising questions about the overlap of the traffic of this protein with the traffic of synaptic vesicles. We have found that ATG9 does not coassemble into the synaptophysin and synapsin condensates but assembles with synapsin into a distinct phase. Our findings suggest that ATG9 undergoes differential sorting relative to synaptic vesicle proteins and also point to a dual role of synapsin in controlling the clustering at synapses of bona fide synaptic vesicles and ATG9 vesicles.

B422/P2808

The E3 ubiquitin ligase TRIM9 regulates actin dynamics and synapse formation

L. E. McCormick, L. Herring, N. G. Brown, G. H. Diering, S. L. Gupton; University of North Carolina At Chapel Hill, Chapel Hill, NC.

In neurons, actin-rich filopodia are critical at many stages of morphogenesis, including neuritogenesis, axon guidance, and dendritic spine formation. Defects in these critical developmental processes can result in improper synaptic connectivity, neurodevelopmental disorders, and psychiatric syndromes. Previously, we have demonstrated the E3 ubiquitin ligase TRIM9 localizes to growth cone filopodia and regulates axon pathfinding downstream of the guidance cue netrin. *Trim9*$^{-/-}$ mice have overt spatial learning memory deficits, yet the role of TRIM9 in synapse formation and maintenance is unknown. Here we show TRIM9 is enriched in the post-synaptic density following differential centrifugation, suggesting a role for TRIM9 in dendritic spines. We find Netrin-dependent increases in dendritic spine number, synapse maturation, and neuronal firing in vitro, and all these responses are abrogated in

Trim9$^{-/-}$ neurons. Furthermore, neurons over-expressing TRIM9 show defects in spine maturation but not dendritic spine number. In vivo, we demonstrate that loss of *Trim9* alters the proteome of the post-synaptic density. In particular, we observe changes in numerous cytoskeletal proteins, including the Arp2/3 complex. Ongoing work is investigating the functional consequence of these changes to Arp2/3 accumulation in dendritic spines and neurotransmitter receptors on the neuronal surface.

B423/P2809

Presynaptic dTACC controls NMJ growth through regulation of microtubule dynamics in *D. melanogaster*

S. A. Johnson[1], V. Chou[2], J. Long[2], D. Van Vactor[2]; [1]Simmons University, Boston, MA, [2]Harvard Medical School, Boston, MA

Growth of the neuromuscular junction (NMJ) is a tightly controlled process that requires many separate factors working together. Errors in synaptic development in which growth is either to great or small are linked to neurological disorders including autism spectrum disorder (ASD) and Parkinson's disease. We have identified a new protein which regulates NMJ growth in *Drosophila melanogaster*, dTACC, previously established as a microtubule associated protein (MAP) responsible for stabilizing microtubules (MTs) particularly during mitosis. dTACC is found decorating MTs within the presynaptic neuron at the NMJ as well as in a punctate pattern that closely juxtaposes the active zone marker BRP. Loss of dTACC within motoneurons produces an overgrowth phenotype characterized by a loss of presynaptic tubulin and an abundance of diffuse MT within synaptic boutons, a pattern consistent with boutons that are actively growing. Taken together, our results suggest that regulation of presynaptic MT dynamics as controlled by dTACC represent an important mechanism to control NMJ growth.

Mitochondrial Homeostasis and Quality Control

B427/P2810

Mitochondrial RNA and RNA biogenesis factors are nucleoid components linked to mitochondrial genome replication

J. Smolka, S. Lewis; Department of Molecular and Cellular Biology, University of California, Berkeley, Berkeley, CA.

Human mitochondria contain a genome (mtDNA) coding for 13 mitochondrial inner membrane proteins, as well as ribosomal and transfer RNAs (tRNAs), that support cellular respiration and ATP production. There can be hundreds to thousands of mtDNA copies per cell, and each genome can form supramolecular complexes, or nucleoids, with nuclearly encoded proteins imported into mitochondria. These proteins organize, propagate, and express mtDNA, and nucleoids heterogeneously and asynchronously engage in replication and transcription throughout a cell cycle. However, how replication and transcription of a population of mitochondrial genomes could be regulated to attain homeostatic genome copy numbers and gene expression levels remains elusive. This gap in knowledge stems from an incomplete understanding of the steady state protein composition of nucleoids and how discrete, variable, or dynamic that composition can be. To address this gap in knowledge, we bioinformatically inferred a human mitochondrial nucleoid protein network using publicly available proteomic and genetic interaction data from proliferative cell culture models. This analysis suggests that mitochondrial nucleoids are an amalgam of DNA, RNA, and proteins that bind both, and that

mitochondrial tRNA synthetases, which aminoacylate mitochondrial tRNAs, associate with proteins that replicate mtDNA. Using untransformed human fibroblasts, live imaging of mitochondrial RNA and a well-established nucleoid protein, TFAM, indicates nucleoids do contain, and physically associate with, RNA. Furthermore, treatment of cells with compounds targeting the mitochondrial RNA polymerase and a mitochondrial tRNA synthetase acutely perturb mtDNA replication. These data suggest that mitochondrial RNA and RNA biogenesis factors are constituents of nucleoids, and that mtDNA replication is potentially integrated with both mitochondrial transcription and translation via regulation by tRNA metabolism.

B428/P2811

Mitochondrial phospholipids regulate mitochondrial-derived compartment biogenesis

T. Xiao, A. M. English, A. L. Hughes; Department of Biochemistry, School of Medicine, University of Utah, Salt Lake City, UT.

Mitochondrial protein homeostasis is necessary for cellular health during pathogenic cellular stresses. In recent studies, our lab identified a pathway that regulates the mitochondrial proteome in response to amino acid overabundance stress, called the Mitochondria-Derived Compartment (MDC) pathway. MDCs are membrane structures generated from mitochondria that selectively sequester a subset of mitochondrial proteins upon formation. Importantly, in cells with elevated intracellular amino acid levels, failure to form MDCs compromises cell health and impacts amino acid metabolism. Both in yeasts and mammalian cells, MDCs form at ER-mitochondria contacts, and proteins at the contact sites are required for this process. However, the mechanism(s) that regulate MDC biogenesis remains poorly understood. To uncover factors that control MDC formation, we conducted a microscopy-based genome-wide screen of yeast non-essential deletion collection. We identified 977 individual gene deletions that impair MDC biogenesis, and 68 gene deletions that enhance MDC formation. Among these gene hits, *UPS1* and *UPS2*, which encode for two mitochondrial proteins that transfer substrates for cardiolipin (CL) or phosphatidylethanolamine (PE) synthesis from the mitochondrial outer membrane to inner membrane, respectively, have opposing effects on MDC biogenesis. In the absence of Ups1, MDC formation in response to amino acid stress is blocked. In contrast, cells with *UPS2* deletion constitutively form MDCs. We further assessed MDC biogenesis in mutants lacking other components of the CL and PE synthesis pathways. Similar to *UPS1*, genes that govern CL synthesis, including *GEP4* and *CRD1*, are required for MDC formation, and consistent with *UPS2*, deletion of *PSD1*, the decarboxylase that catalyzes mitochondrial PE synthesis, leads to constitutive MDC formation. These results suggest that mitochondrial CL and PE levels regulates MDC biogenesis opposingly. In addition, we investigated the lipid profiles of cells treated various agents that increase cellular amino acid pools and trigger MDC formation. Interestingly, under numerous known MDC-inducing conditions, total cellular PE levels were decreased. Preventing MDC formation by the removal of amino acids from the medium attenuates the drop in PE level caused by MDC activating agents, and depleting mitochondrial PE by *PSD1* deletion re-activates the MDC pathway. Our data indicate a critical role of mitochondrial phospholipids in MDC biogenesis, potentially as a signal to initiate MDC formation downstream of amino acid stress. We are currently characterizing how mitochondrial phospholipids are regulated in cells experiencing MDC-inducing stresses, and how this contributes to MDC formation.

B429/P2812

A spatial map of hepatic mitochondria uncovers functional heterogeneity and plasticity at the tissue scale

S. Kang[1], R. P. Cunningham[1], C. Cultraro[1], A. Lobanov[2], M. Cam[2], N. Porat-Shlion[1]; [1]Thoracic and GI Malignancies Branch, NCI, National Instituts of Health, Bethesda, MD, [2]CCR Collaborative Bioinformatics Resource (CCBR), National Instituts of Health, Bethesda, MD.

Hepatocytes in the liver are organized in hexagonal units called lobules. Blood flows directionally within each lobule, resulting in gradients of nutrients along the periportal (PP) - pericentral (PC) axis. These gradients, in turn, drive spatially distinct gene expression and metabolic functions, a phenomenon that is known as liver zonation. At the cellular level, mitochondria continuously adjust their metabolic output to match nutrient supply. However, how liver zonation impacts mitochondrial functions was never investigated. Here, we used a combination of protein mass spectrometry and light microscopy to spatially characterize hepatic mitochondria. Our analysis reveals great diversity between PP and PC mitochondria in protein composition, respiration, morphology, and turnover. In response to acute fasting, PC mitochondria undergo substantial remodeling. This adaptive response is prominent in PC hepatocytes and conferred by upregulation of translation and phosphorylation to simultaneously increase mitochondrial oxidative capacity and decrease pericentral pathways that may not be immediately required during fasting. Collectively, our data provide tissue scale insights into mitochondrial spatial diversification and their phospho-proteome remodeling in response to changes in nutrient supply. This work begins to capture the complexity of mitochondria heterogeneity and plasticity in intact tissue and during physiological metabolic transitioning.

B430/P2813

Quantitative mitochondrial proteome analysis differentiates the specific vs general mitochondrial effects caused by Drosophila mutants affecting three different critical pathways for mitochondrial function

A. Sen[1], R. T. Cox[2]; [1]Uniformed Services University/Henry M. Jackson Foundation, Bethesda, MD, [2]Uniformed Services University, Bethesda, MD.

Dysfunctional mitochondria affect almost all major organs in the body. About 1 in 4300 people in the United States are affected by mitochondrial diseases and they are often hard to diagnose. Although mitochondria have their own genome, they need hundreds of proteins encoded by nuclear genome for proper functioning. Mutations in one of these genes can cause a wide spectrum of abnormalities in animals. Despite the challenges in studying the mitochondrial proteome due to the dynamic nature of mitochondria, it holds the potential to identify both the specific and general mitochondrial damages caused by single gene mutations. In this study we have analyzed and compared the effects of three Drosophila mutants, *clueless* (*clu*), *Superoxide dismutase 2* (*Sod2*) and *PTEN-induced putative kinase 1* (*Pink1*), which are associated with mitochondrial dysfunction and changes to the mitochondrial proteome. Clueless (Clu) is a nucleus-encoded ribonucleoprotein that forms mitochondrion-associated particles. Sod2 scavenges mitochondrial free radicals and mutants have increased oxidative damages. Pink1 is a component of the mitophagy pathway used to cull damaged mitochondria. After performing quantitative tandem mass tag mass spectrometry analysis from mitochondrial extracts from these mutants, we identified changes to protein classes that were unique for each mutant as well as shared between them. Our analysis indicates that some changes in the mitochondrial proteome are due to

general mitochondrial damage whereas others are gene-specific. While all three mutants induce a general mitochondrial stress response, *clueless* mutants predominantly affect mitochondrial respiratory chain protein. For the first time, we have directly compared the in vivo steady-state levels of mitochondrial proteins in mutants affecting three pathways critical for mitochondrial function. These data could be useful to better understand the etiology of mitochondrial diseases, and articulate better therapeutic aspects based on specific proteomic changes as opposed to changes due to generalized mitochondrial damage.

B431/P2814

Genome-wide screen reveals novel regulators of PINK1-Parkin pathway and Mitofusin-2 expression

J. Thayer[1], X. Huang[1], J. Hawrot[1], D. Ramos[2], A. Subramaniam[1], M. Ward[1], D. Narendra[1]; [1]National Institute of Neurological Disorders and Stroke, NIH, BETHESDA, MD, [2]National Institute of Aging, BETHESDA, MD.

We sought to identify novel regulators of the PINK1-Parkin mitochondrial quality control (MQC) pathway and Mitofusin-2 (MFN2) expression using a novel flow cytometry reporter in a genome-wide CRISPRi (GWCi) screen. Mitochondrial dynamics and quality control are critical for neuronal health. Loss of the PINK1-Parkin MQC pathway is the leading cause of recessive Parkinson's disease. Also, dominant mutations in the mitochondrial fusion protein MFN2 are the leading cause of Charcot-Marie Tooth 2A. MFN2 is the preferred substrate of Parkin's E3 ubiquitin ligase activity, linking the PINK1-Parkin MQC pathway and mitochondrial dynamics. MFN2 in HeLa cells co-expressing mCherry-Parkin and dCas9-KRAB was endogenously tagged (HaloTag). Cells were transduced with a pooled dual guide GWCi library. Oxidative phosphorylation (OXPHOS) inhibitors were used to activate the PINK1-Parkin pathway. Treated and untreated cells were sorted into high and low MFN2-Halo populations by fluorescence activated cell sorting (FACS). Guide counts from each population were calculated after next generation sequencing of extracted genomic DNA. We verified by microscopy that endogenous MFN2-Halo was expressed on mitochondria. Also, we demonstrated that activation of the PINK1-Parkin pathway by OXPHOS inhibition causes degradation of MFN2-Halo by flow cytometry and immunoblotting. Next, we completed two FACS-based GWCi screens of MFN2-Halo in duplicate: one under basal conditions and one with OXPHOS inhibition. Under basal conditions, we identified genes increasing or decreasing MFN2 expression (with 795 reaching genome-wide significance). The top mitochondrial hit increasing MFN2 levels was SLC25A46, a known negative regulator of MFN2, validating this approach. Additionally, we identified four components of a transcriptional complex not previously linked to MFN2 regulation, ranked 1, 5, 6, and 11, respectively. Under OXPHOS inhibited conditions, PINK1 was the top hit opposing MFN2-Halo degradation, validating this approach. Novel hits (among 330 reaching genome-wide significance) have suspected roles in promoting PINK1 expression, facilitating autophagic capture of impaired mitochondria, mediating fusion of the mito-autophagosome with the lysosome, and promoting mitochondrial degradation in the lysosome. Surprisingly, the HOPS complex and the V-ATPase were among the factors most limiting for MFN2 degradation by the PINK1-Parkin pathway. In a GWCi screen of a MFN2-Halo FACS reporter, we identified novel regulators of the PINK1-Parkin MQC pathway and MFN2 expression. While requiring additional study, these regulators may have far-reaching implications for modulating mitochondrial quality control and dynamics for the treatment of neurodegenerative disorders.

B432/P2815

Mitochondrial fission and fusion maintain mitochondrial genome integrity by different mechanisms

B. T. Wisniewski, L. L. Lackner, First; Molecular Biosciences, Northwestern University, Evanston, IL.

Mitochondria exist as an interconnected and dynamic network of membrane tubules within cells. Mitochondrial network morphology contributes to proper organelle function and cell health. Two opposing processes: mitochondrial fission and mitochondrial fusion maintain mitochondrial morphology. Mitochondrial fission and fusion are also required to maintain the integrity of mitochondrial DNA (mtDNA)—chromosomes within mitochondria that encode components of the respiratory chain. Intact mitochondrial DNA is required for proper mitochondrial function and defects are associated with multiple disease states. The mechanism by which fission and fusion maintain mitochondrial DNA integrity is not fully understood. We use live-cell imaging to localize mtDNA to mitochondrial fission and fusion sites. We find that a minimal, but balanced, amount of fission and fusion are required to maintain functional mtDNA. Finally, we introduce a model in which increased mtDNA copy number buffers the loss of functional mtDNA in the absence of both fission and fusion. Current work is expanding our model to include the molecular mechanism by which mtDNA mutation is caused by the simultaneous disruption of fission and fusion.

B433/P2816

Identification of a novel negative regulator of BNIP3/BNIP3L-dependent receptor-mediated mitophagy

M. Gok, J. Friedman; UT Southwestern Medical Center, Dallas, TX.

Mitochondria play critical roles in cellular metabolism and are functionally maintained by several quality control pathways. During hypoxia, the outer mitochondrial membrane proteins BNIP3 and BNIP3L are upregulated and promote receptor-mediated mitophagy, whereby autophagy machinery is directly recruited to facilitate the selective turnover of a subset of mitochondria. Constitutive low levels of BNIP3/BNIP3L are found under normoxic conditions, but it is unclear how they are regulated and whether they contribute to low levels of basal mitophagy that occur independently of the well-characterized PINK1/Parkin pathway. In exploring the role of the poorly characterized mitochondrial morphology regulator TMEM11, we unexpectedly find that it localizes to the outer membrane and forms a complex with BNIP3 and BNIP3L. We find that the mitochondrial morphology defects that occur in the absence of TMEM11 are caused in a BNIP3/3L-dependent manner. Using a fluorescence-based assay to monitor mitophagy in live cells, we find that BNIP3/3L-dependent mitophagy is hyper-active in both normoxic and hypoxic conditions in the absence of TMEM11. Additionally, our data reveal that BNIP3 is indeed required for basal mitophagy in certain cell types. In addition to interacting with BNIP3/3L, TMEM11 interacts with the mitochondrial inner membrane cristae-organizing MICOS complex. We observe that TMEM11 bridges the BNIP3/3L and MICOS complexes and that depletion of MICOS triggers BNIP3/3L-mediated mitophagy during normoxia. Our data are consistent with a model that TMEM11 serves as a “brake” against mitophagy by directly binding and inhibiting BNIP3 and that a MICOS/TMEM11/BNIP3/3L axis coordinately regulates mitochondrial quality control by sensing and responding to mitochondrial dysfunction. We thus uncover a non-canonical role for TMEM11 as a novel regulator of BNIP3/3L-mediated mitophagy.

B434/P2817

Mechanism of mitochondrial cholesterol import is via an intermembrane space cholesterol shuttle: STAR

P. P. Koganti, A. H. Zhao, M. C. Kern, A. Guo, S. Wasti, V. Selvaraj; Cornell University, Ithaca, NY

Cholesterol import into mitochondria is an essential first step in the production of all steroid hormones. CYP11A1 localized to the matrix-side of the inner mitochondrial membrane (IMM) converts cholesterol to pregnenolone, the first steroid in the biosynthetic pathway. Acute regulation of IMM cholesterol availability via the synthesis, function and degradation of the steroidogenic acute regulatory protein (STAR) has been well documented. Nevertheless, the precise mechanism of STAR action remains undefined. We recently refuted the two-step model that purported cooperation of STAR and the mitochondrial translocator protein/TSPO. The fallback was another model that portrayed STAR as a molten globule at the outer mitochondrial membrane (OMM) apposing the IMM. However, this model has remained at odds with the need for a cholesterol-binding domain that is present in STAR. By generating STAR-deleted Leydig cells we evaluated functional reconstitution using different STAR mutants and fusion proteins with ultrastructural localization as means to uncover its mechanism of action. Truncation of the STAR mitochondrial targeting sequence (MTS; ΔN36, ΔN42, ΔN50, ΔN54 and ΔN62) or anchoring STAR to the OMM using a TOM20 fusion (TOM20-ΔN62.STAR), resulted in a complete loss of steroidogenic function; a result that was in direct contrast to that previously reported to support the molten globule model. Use of the MTS of cytochrome C1 (CYC1) that is known to target proteins into the intermembrane space (IMS) in the place of the STAR MTS (CYC1.MTS-ΔN62.STAR) could completely restore import indicating the resident site of STAR function as the IMS. Ultrastructural localization using ascorbate peroxidase (APEX2) fusion proteins to evaluate both STAR (STAR.APEX2) and CYC1 targeting (CYC1.MTS-APEX2) confirmed an IMS localization in steroidogenic mitochondria. Structural analysis revealed a C-terminal amphipathic α4-helix, truncation of which (STAR.ΔC261) or site-directed mutagenesis of residues that formed the hydrophobic face to hydrophilic amino acids (I264T, F266W, A267S and L270T) resulted in complete loss of function. These results indicated STAR actions in membrane docking, cholesterol extraction, and diffusion, as an IMS cholesterol shuttle. As such, our findings resolve a core steroidogenic regulatory mechanism that has baffled the field of steroid endocrinology for decades.

B435/P2818

A novel cytoskeleton-based pathway regulates mitochondrial dynamics and energetics in skeletal muscle

K. Voos[1], J. Tzeng[2], T. Pharr[2], G. Choi[2], D. Lorenzo[1]; [1]University of Pennsylvania - Philadelphia, PA, Philadelphia, PA, [2]University of North Carolina- Chapel Hill, Chapel Hill, NC.

Regulation of mitochondrial fission-fusion dynamics is key for maintaining bioenergetic homeostasis, especially in cell types with high energy demand. Changes in the balance between mitochondria fission and fusion events in skeletal muscle (SKM) promote metabolic deficits associated with impaired energetic capacity, muscle atrophy and diabetes. Previous studies have identified the actin cytoskeleton as a key modulator of mitochondria fission-fusion events. However, the cytoskeletal factors and mechanisms that regulate mitochondria dynamics are poorly understood. Ankryin-B (AnkB) is a ubiquitously expressed actin cytoskeleton-associated protein that scaffolds and stabilizes submembrane protein complexes. Human variants in 220-kDa AnkB, the most abundant isoform in SKM, have been

identified as risk factors for cardio-metabolic disease, and shown to cause age-dependent metabolic syndrome in mice. In SKM, AnkB stabilizes the dystrophin-dystroglycan axis to provide structural support. However, the metabolic role of AnkB in SKM has not been characterized. Using a conditional knock-out mouse model that selectively lacks 220-kDa AnkB in SKM (SKM-AnkB-KO mice), we identified a novel role of AnkB in regulating SKM energetics through its association with mitochondria. SKM-AnkB-KO mice exhibit deficits in glucose metabolism that worsens with age, impaired activity and exercise capacity, and reduced levels of oxidative phosphorylation proteins in mitochondria-rich muscle. In addition, Transmission Electron Microscopy (TEM) evaluations of muscle sections show the presence of enlarged mitochondria in SKM-AnkB-KO mice, which suggests alterations in mitochondria dynamics that likely contribute to energetic stress. In support of this idea, we found that critical regulators of mitochondria fission-fusion, including dynamin-like protein 1 (DRP1) and Optic Atrophy 1 (OPA1) were reduced at the protein and transcript levels. Importantly, AnkB co-fractionates with mitochondria in control SKM. Also, interactome and biochemical analysis identified the DRP1-receptor Mitochondrial Fission Factor (MFF) as an AnkB-interactor in SKM. Here, we will discuss our findings that support a model in which AnkB transiently interacts with DRP1 and scaffolds the DRP1-MFF to promote mitochondrial fission and homeostasis. We propose that this pathway is critical for bioenergetic balance in SKM.

B436/P2819

NME3 and PLD6 Mediate Mitochondrial Tethering for Selective Fusion

C. Sung, Y. Su, H. Chiu, Y. Chang, Z. Chang, Y. Liu; National Taiwan University, Taipei City, TAIWAN.

Mitochondria are dynamic organelles regulated by the fission and fusion processes. The fusion of biomembranes requires elaborative coordination of proteins and lipids, and is crucial for mitochondrial quality control. It is known that phosphatidic acid (PA) generated from cardiolipin (CL) by PLD6 is essential for the fusion of mitochondria. However, how PA promotes the fusion process of mitochondrial outer membrane remains unclear. Here, we show a mitochondrial outer membrane protein, NME3, is required for PLD6-induced mitochondrial tethering. Live-cell microscopy reveals that NME3 is enriched at the contact interface of two closely positioned mitochondria by activation of PLD6. Biochemical analysis indicates that NME3 is capable of binding directly to PA-exposed lipid packing defects via its N-terminal amphipathic helix. The PA binding function and the hexamerization confer NME3 tethering the PLD6-remodeled mitochondrial membrane. Given externalized CL is a signal of unhealthy mitochondria, our findings suggest that the membrane tethering function of NME3 has the selectivity to the damaged mitochondria targeted by PLD6 that promotes fusion process for the quality control. To explore this hypothesis, we utilized UV or mitochondrial damaging agent to induce mild mitochondrial stress and observed higher enrichment of NME3 to the mitochondria contact increased. In conclusion, we discover that NME3 and PLD6 play critical roles in mitochondrial fusion via promoting tether between damaged mitochondria.

B437/P2820

Interrogating neurodegeneration-linked CHCHD2 and CHCHD10 and their mitochondrial interaction network

T. Huang, N. Randolph, X. Huang, B. Wu, D. Narendra; NIH-NINDS, Bethesda, MD.

Objective: To identify endogenous protein-protein interactions (PPIs) with CHCHD2 and CHCHD10 in cultured cells and mouse tissues by affinity purification mass spectrometry (AP-MS).
Introduction: Dominant mutations in the paralogs *CHCHD2* (*C2*) and *CHCHD10* (*C10*) cause neurodegenerative disease. These nuclear genes code small proteins localized to the intermembrane space side of mitochondrial cristae. Their functions are crucial for maintaining cristae structure and oxidative phosphorylation (OXPHOS). However, the detailed molecular mechanism for their action has not been fully characterized.
Methods: Using a novel antibody and C2/C10 double knockout cell lines and mouse models, we will establish authentic endogenous interactors of C2 and C10 in cell culture and *in vivo* by AP-MS.
Results: In the first comprehensive study of C2/C10 endogenous PPIs, we confirm several previously published PPIs and establish that OXPHOS subunits are major interactors. Using KO cell lines for individual PPIs, we find that most PPIs are independent of one another, suggesting transient association of C2/C10 with multiple inner mitochondrial membrane complexes. Unexpectedly, we find substantially increased C2/C10 PPIs following cristae disruption (due to KO of TAZ, MIC60, and STOML2 KO), suggesting a role for C2 and C10 in maintaining cristae proteostasis. Finally, we find that a myopathy/cardiomyopathy-causing mutation in C10, which causes C10 misfolding, strongly increases C2/C10 binding to an intermembrane space protease, HTRA2/OMI, in the affected mouse heart. This suggests that HTRA2/OMI may protect against pathogenic C10 misfolding.
Conclusion: In the first comprehensive study of C2/C10 endogenous interactors we identify dynamic changes in PPIs following cristae disruption and a protease that may selectively associates with mutant C10 *in vivo*.

B438/P2821

YME1L1 Knockdown Induces Mitochondrial Fragmentation and Dysfunction during Porcine Embryonic Development

D. ZHOU, X. Cui; Aminal Science, Chungbuk National University, Cheongju, KOREA, REPUBLIC OF.

YME1L1, a member of the AAA family of ATPases, is a nuclear genome-encoded ATP-dependent metalloprotease embedded in the IMM. Its protease domain faces the IMS (also termed i-AAA protease). YME1L1 is also a mitochondrial metalloprotease responsible for the cleavage of the mitochondrial GTPase OPA1. However, how YME1L1 regulates mitochondrial function in porcine embryos is still obscure. In the present study, we knock down the mRNA level of *YME1L1* by the microinjection with dsRNA of *YME1L1* at the 1-cell stage. Immunofluorescence and western blot were used for detecting the localization and expression of YME1L1 and related proteins. Embryo cleavage rates, blastocyst diameter, and total cell number were measured to assess the YME1L1 knock-down effect. Firstly, we found that endogenous YME1L1 locates at punctate structures of mitochondria and is highly expressed in the BL stage. Knock-down of YME1L1 in porcine embryos results in mitochondrial fragmentation and leads to significantly decreased quality of blastocyst. Knock-down of YME1L1 induced an increase in ROS and reduced mitochondrial membrane potential and ATP level. In addition, YME1L1 knock-down also caused a significant release of cytochrome c from mitochondria, which is a pro-

apoptotic signal. Taken together, YME1L1 plays a critical role in embryo development by regulating mitochondrial morphology, function, and apoptosis in pigs.

B439/P2822

Mitochondrial-derived compartments are stress-induced multilamellar whorls that sequester mitochondrial outer membrane cargo

Z. N. Wilson[1], A. M. English[1], M. West[2], G. Odorizzi[2], A. L. Hughes[1]; [1]University of Utah, Salt Lake City, UT, [2]University of Colorado Boulder, Boulder, CO.

Preserving the functional health of the mitochondrial network is critical to cell viability and longevity. To do so, cells employ mitophagy to remove dysfunctional mitochondria or remove select portions of the organelle through a variety of mechanisms, including mitochondrial-derived vesicles, structures positive for the outer membrane, or mitochondrial-derived compartments (MDCs). For these latter mechanisms, it remains unclear what features they share in common or the characteristics and functional roles that distinguish them. To further define MDCs, we performed electron tomography on MDCs in the budding yeast *Saccharomyces cerevisiae.* We show that MDCs consist of multilamellar whorls derived from the mitochondrial outer membrane that form concentric spherical compartments with up to eight membrane bilayers. Time-lapse super-resolution microscopy of MDC biogenesis revealed that the mitochondrial outer membrane repeatedly extends and fuses to form these multilamellar compartments. As such, MDCs strongly enrich and sequester portions of the mitochondrial outer membrane and encase cytosolic material within the MDC lumen. Remarkably, subunits of the translocase of the outer membrane (TOM) complex are excluded from MDCs unless assembly of the TOM complex is inhibited, suggesting that criteria also exists within the mitochondrial outer membrane for selecting cargo that incorporate into MDCs. Considering that overloading the outer membrane with membrane proteins constitutively induces MDC formation, we propose that one functional role of MDCs is to segregate and sequester excess hydrophobic cargo through mitochondrial outer membrane expansion and enclosure to maintain mitochondrial homeostasis during stress.

B440/P2823

The Role of *C. elegans* Metaxins in Mitochondrial Homeostasis

J. Dietz, E. Park, N. Salazar-Vasquez, N. Kane, C. Nowlen, C. Rongo; Rutgers University, Piscataway, NJ.

Mitochondria are critical for neuronal function and health, as they are the primary supplier of energy and calcium storage for neurons. Mitochondria are also dynamic organelles - undergoing fusion and fission in response to various stimuli. In neurons, which have elaborate morphology, mitochondrial dynamics also includes transport to specific sub-compartments, where they supply energy and buffer calcium for the local cellular environment. Disturbances in mitochondrial function or dynamics contribute to various neurological neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease, and Leigh syndrome. Using a forward genetics screen in *C. elegans* searching for novel mutants defective in neuronal mitochondrial dynamics, we found that mutations in metaxin 1 (MTX-1), metaxin 2 (MTX-2), and VDAC-1 resulted in fewer mitochondria in *C. elegans* interneuron dendrites. Homologs of MTX-1 and MTX-2 (the metaxins) in *S. cerevisiae* and mammals interact with SAM50 to form the sorting and assembly machinery (SAM) complex, which mediates β-barrel protein assembly in the mitochondrial outer membrane (MOM). VDAC-1 is a highly conserved SAM complex substrate that acts as a channel for metabolites (ATP/ADP, Ca^{2+}, NAD^{+}/NADH)

to move across the MOM. We are testing the hypothesis that the metaxins promote mitochondrial motility along *C. elegans* interneuron dendrites by mediating β-barrel assembly of VDAC-1 in the MOM. MTX-1, MTX-2, and VDAC-1 mutants are viable; however, their lifespans are reduced compared to WT. To determine the impact of the metaxins and VDAC-1 on mitochondrial homeostasis in *C. elegans*, we measured mitochondrial unfolded protein response (UPRmt) activation using a fluorescent reporter (HSP-6::GFP) in strains lacking MTX-1, MTX-2, and VDAC-1. We found that HSP-6::GFP expression was increased in *mtx-2* and *vdac-1* mutants, suggesting basal UPRmt activation and mitohormesis, and resulting in heat stress resistance. We are currently investigating the role of *C. elegans* MTX-1 and MTX-2 in mitochondrial outer membrane β-barrel protein (VDAC-1) assembly and how that impacts neuron integrity. Our results will provide key insights towards the impact of mitochondria on neurodegeneration.

B441/P2824

Zebrafish TMEM11 Localizes to Mitochondria and Interacts with Rhot2

S. S. Tadesse[1], P. Collado Cordon[2], S. Walsh[1]; [1]Life Sciences, Soka University of America, Aliso Viejo, CA, [2]Biology, Trinity College of Arts and Sciences, Duke University, Durham, NC.

Mitochondria are capable of moving around the cell, and this function is critical for cell function and viability. In addition, damaged mitochondria can produce toxic reactive oxygen species and therefore, must be removed from the cell through the protein-mediated process of mitophagy. These processes of motility and mitophagy are linked by common proteins, and failure of either can result in the cell death associated with neurodegenerative diseases. One important protein is Miro, and we sought to identify novel zebrafish proteins that might interact with the zebrafish orthologs of Miro using a zebrafish yeast two hybrid library. We identified 13 proteins that strongly interact with zebrafish Miro2/Rhot2 in this heterologous system, and one interactor was transmembrane protein 11 (TMEM11), also known as Pantagruelian Mitochondrion I (PMI) in fruit flies. We initially dismissed this interaction, given Miro's localization to the mitochondrial outer membrane (OM), and previous data demonstrating that PMI localized to the mitochondrial inner membrane. However, a more recent preprint stated that the human ortholog of TMEM11 localized to the OM, suggesting that Miro and TMEM11 might be more likely to interact. Thus far, we have tagged the zebrafish ortholog of TMEM11 on either the C-terminus or the N-terminus with Cherry or GFP, respectively. Based on structure predictions which indicate that the C-terminus is likely part of a transmembrane helix, the C-terminally tagged TMEM11-Cherry does not localize to mitochondria, but the N-terminally GFP-tagged protein does colocalize with mitochondrial markers when overexpressed in HeLa cells. Initial studies isolating mitochondria followed by treatment with Proteinase K further suggest that zebrafish TMEM11, like its human counterpart, may localize to the mitochondrial OM. Overall, these studies will provide new insights into the complex protein network that mediates mitochondrial physiology.

B442/P2825

Identification of a mitochondrial intermembrane space protein required for the completion of organelle fission

O. Connor, S. Matta, **J. Friedman**; UT Southwestern, Dallas, TX

Mitochondria are highly dynamic organelles that maintain their shape through the conserved processes of organelle fission and fusion. These mitochondrial dynamics are required for numerous functions,

including controlling the spatial positioning of the organelle, maintenance of the mitochondrial genome, regulation of organelle quality control, and regulation of cell death. Mitochondrial fission is performed by the dynamin-related protein Drp1 (Dnm1 in yeast), a large GTPase that constricts and divides the mitochondria in a GTP hydrolysis-dependent manner. Despite over 20 years of research into the mechanisms of mitochondria fission, it has remained controversial whether factors inside mitochondria help coordinate the process and if Dnm1/Drp1 activity alone is sufficient to complete fission of both mitochondrial membranes. In the process of studying poorly characterized mitochondrial intermembrane space proteins, we have identified a new factor required for mitochondrial division in yeast, which we name Mitochondrial Division Intermembrane Space 1 (Mdi1). We find that deletion of Mdi1 leads to hyperconnected mitochondria networks, similar to loss of Dnm1. Using a combination of temperature-dependent inhibition of mitochondrial fusion and pharmacological approaches, we find that Mdi1 is required for promoting mitochondrial division. Then, using live cell fluorescence microscopy, we spatially link Mdi1 to sites of mitochondrial constriction and fission. Unlike all other known division machinery, loss of Mdi1 does not inhibit Dnm1 recruitment to mitochondria. Even under conditions that promote fragmentation of mitochondria, we find Dnm1-mediated mitochondrial constriction is not sufficient to complete division of the organelle in the absence of Mdi1. Finally, we show that the requirement of Mdi1 for Dnm1 to complete mitochondrial division is conserved in fission yeast. Thus, we have identified the first internal mitochondrial factor absolutely required for mitochondrial division and provide evidence suggesting it works after Dnm1-mediated mitochondrial constriction to complete fission of the organelle.

B443/P2826

Targeted fission as a quality control mechanism for asymmetric inheritance of mitochondrial protein aggregates

G. Sun, C. Hwang, T. Jung, J. Liu, R. Li; Johns Hopkins University, Baltimore, MD

In budding yeast, mitochondria form intricate reticulated structures capable of undergoing fission and fusion. This process is thought to aid in efficient distribution throughout the cytoplasm. During the cell budding cycle, prior research indicates mother yeast preferentially pass more reductive parts of their mitochondria network to their daughters through asymmetric inheritance. While expressing a mitochondria targeted misfolded protein capable of aggregation, we similarly observe mother cells retaining aggregate containing mitochondria at a high probability while preferentially passing down non-aggregate containing mitochondria to their buds. Using this aggregate system, we identify the characteristics of mitochondrial fission/fusion and motion as a quality control mechanism for enforcing asymmetric inheritance. In aggregate-expressing cells, we observe the presence of a two-class system of mitochondria characterized by differential fission/fusion probabilities, fission site placement, and motility. Using experimentally measured physical and mitochondrial dynamic attributes, we develop a computational model of mitochondrial network dynamics and inheritance to identify targeted fission as a mechanism for accelerating consolidation of multiple aggregates into one mass while simultaneously reducing the probability of inheriting aggregate containing mitochondria. Experimental examination of mitochondrial fission machinery furthermore indicates a localization preference of fission machinery close to aggregate inclusions and suggests a possible positive feedback loop driving fission machinery accumulation. Finally, we demonstrate knockout of mitochondrial fission machinery results in aggregate buildup in specifically mitochondrial nets.

B444/P2827

Insulin-like Growth Factor 1 Signaling in Mitochondrial protection

J. Murray, S. Riis, **R. O'Connor**; University College Cork, Cork, Ireland

IGF-1 is essential for normal cell, organ and organismal growth but also supports oncogenic pathways that mediate initiation and progression of cancer. Although IGF-1signalling promotes mitochondrial metabolism and oxidative phosphorylation, this is modulated by nutrient availability, hypoxia, or cellular stresses that can lead to dysfunction and release of reactive oxygen species or DNA. We have previously described a mitochondrial protective role for IGF-1 through the induction of a PGC1beta-activated mitochondrial biogenesis programme that is coordinated with induction of the mitophagy receptor BNIP3 through Nrf2 and HIF-1α activation. Inhibition of IGF-1 signalling impairs this mitochondrial protection, suggesting that it is amenable for therapeutic co-targeting in cancer.In this study we sought to investigate whether IGF-1-induced BNIP3 is engaged in basal homeostatic mitophagy, and under which metabolic conditions, considering that IGF-1-activated mTOR is a potent suppressor of global cellular autophagy, including mitophagy. To address this we investigated BNIP-3 mediated mitophagy and autophagy flux in cells that were cultured in complete medium, serum starved or stimulated with IGF-1 and either exposed to bafilomycin or not. The results demonstrate that in serum-starved cells, although IGF-1 induced BNIP3, it did not promote association of BNIP3 with LC3 and overall suppressed mitophagy. In these serum-starved cell cultures the PINK1-Parkin axis was observed to engage in mitophagy. However, BNIP3 was associated with mitophagy flux in cells that were cultured in complete medium. Furthermore, in cells where the IGF-1 receptor was knocked out, autophagy flux was lower than in complete medium than in serum-depleted cultures, suggesting that IGF-1 signalling is required for maintaining basal mitophagy/autophagy. These findings support a mito- protective function for IGF-1 signalling in cancer cells that may be mediated by Hif-1a in normoxic conditions. The data further suggest that targeting this mito- protective function would augment current cancer therapies that target the IGF signalling pathway.

B445/P2828

A balanced distribution of mitochondria is required to maintain mtDNA integrity over generations

M. Ray, W. Chen, K. Krueger, A. Jacob, E. Rosario, L. L. Lackner; Northwestern University, Evanston, IL

A conserved feature of Eukarya's defining organelle, the mitochondrion, is a reticular network that dynamically rearranges. The cell maintains this network through mechanisms that are also conserved—division/fusion, tethering, and motility. Given that mitochondria cannot form *de novo*, these mechanisms ensure that an adequate amount of mitochondria is partitioned during mitosis. In the absence of such inheritance, cells die. To maintain viability over generations, the inherited compartment must also be expanded by biogenesis. Budding yeast serves as a historical and tractable model to study these processes; many of the molecular players and phenotypes of mitochondrial inheritance and biogenesis are well described in this species. Studies have shown that mitochondrial volume scales with cell size in yeast. Interestingly, the mitochondrial genome (mtDNA), required for the role of mitochondria in oxidative phosphorylation, is packaged into protein-DNA complexes called nucleoids that themselves scale with and are evenly distributed along the mitochondrial network. Our work supports a model in which the proper distribution of mitochondrial volume during mitosis is required to maintain the integrity of mtDNA. We find that a fundamental requirement for the maintenance of mtDNA is the inheritance of an adequate volume of mitochondria. Synthetically altering the partitioning

of mitochondria during mitosis to varying degrees results in gradated defects in mtDNA maintenance that are predicted by our model. Current work is addressing the specific mechanism by which inheritance of mitochondrial volume impacts mtDNA maintenance. One hypothesis is that network expansion and mtDNA replication compete for a limiting factor, such as phosphate. In this hypothesis, the more essential of the two—compartment propagation—wins at the cost of mtDNA integrity. Given the conserved nature of mtDNA spacing and mitochondrial positioning, we predict that our work will describe universal features of mtDNA regulation.

B446/P2829

The effects of *SHE9* deletion on *S.cerevisiae* mitochondrial function.

S. Kowaleski, N. A. Najor; University of Detroit Mercy, Detroit, MI

The model yeast organism Saccharomyces cerevisiae has roughly 10% of genes that remain as uncharacterized. To attempt to understand these genes of unknown function, genomic databases were mined to attempt to provide a potential function to these unknown genes. Data from Phobius and GeneMania databases, provided putative information suggesting the gene of unknown function SHE9 is a potential transmembrane protein in the inner membrane of the mitochondrion and is expressed with genes involving DNA Synthesis, DNA transcription/translation, and mitochondrial fission. While some data exists to support its expression and localization, the molecular function for the She9 protein is unknown. We ran a series of fitness tests on various yeast media or conditions with *she9* deletion mutants. The results indicate that the she9 deletion mutant was viable and resistant to both NaCl and Sorbitol osmotic stressors and was less viable under ultraviolet exposure compared to the wild-type Saccharomyces Cerevisiae strain. We hypothesize that She9 could be involved in maintaining mitochondrial DNA during mitochondrial fission. Since the genomic homology between yeast and humans is significant, uncovering these yeast genes of unknown functions, such as She9, have the potential to reveal undescribed pathways that could be implicated in human diseases.

B447/P2830

Eliminating mitochondrial import stress is independent of ATAD1 function

M.-C. Hsu[1], R. J. Youle[2]; [1]National Taiwan University, Taipei, Taiwan, [2]National Institutes of Health, Bethesda, MD

In eukaryotes, most mitochondrial proteins are encoded in nuclear genome; these proteins are synthesized in the cytosol and further imported into mitochondria across single or double organelle membranes. Maintaining the capability of mitochondrial protein import is a key for mitochondrial protein renewal and mitochondrial biogenesis, and also indirectly affects cellular physiological functions. Msp1, a yeast mitochondrial outer membrane AAA ATPase, has been shown to eliminate the excess precursor proteins from the mitochondrial translocases when the cells encounter the import stress by overexpressing bipartite signal-containing proteins. ATAD1 is a predicted human homologue of Msp1 and is shown to extract the mislocalized peroxisomal proteins from the outer mitochondrial membrane, however, the role of ATAD1 in protecting the mammalian cells from import stress has not been established. We aimed to determine whether ATAD1 is involved in eliminating the proteins that plugged on the mitochondrial import channel in the human cells. To test the hypothesis, we first developed a doxycycline-inducible mitochondrial import blocking system using dihydrofolate reductase (DHFR) fused to the N-terminus of human MIC60 in the HeLa cell. Upon adding methotrexate (MTX) which stabilizes

DHFR domain, this chimeric protein could clog translocase of outer membrane (TOM) channel and the part of N-terminus would be anchored on the mitochondrial inner membrane. Localization of MTX-DHFR domain complex on the mitochondrial outer membrane had been confirmed in both proteinase K protection assay and the immunofluorescent stain. In addition, the un-cleaved precursor of endogenous COX5A was accumulated in the cells, which clearly indicated the decrease in import efficiency. Based on this blocking approach, we further generated the cells with ATAD1 knockdown or CMV-driven overexpression. Manipulating ATAD1 expression level did not affect the removal of blocking chimeric protein, the clearance of COX5A precursor, and the cell viabilities. This work demonstrates that human ATAD1, unlike the protective role of Msp1 in yeast, couldn't ameliorate the mitochondrial import stress. However, the import blocking approach provides a platform for better understanding of other machinery in mitochondrial quality control.

B448/P2831

Identifying Domains of the Drosophila Ribonucleoprotein Clueless Critical for Clueless Particle Dynamics and Assemb

H. Hwang, R. T. Cox; USUHS, Bethesda, MD

Clueless (Clu) is a highly conserved ribonucleoprotein whose loss causes abnormal mitochondrial morphology and malfunction. Previous studies from our lab and others have shown that Clu is required for physiological functions such as energy production, metabolic pathways, stress responses, and mitochondrial quality control in various species. Drosophila *clu* mutants live less than a week, are sterile, and have mislocalized mitochondria. Remarkably, Clu forms highly dynamic cytoplasmic particles juxtaposed to mitochondria. Clu particles are formed or dispersed depending on the nutrient state of the cell or in response to various stressors. As we showed that Clu associates with ribosomes in mitochondrial fractions and others showed it appears to be involved in mitochondrial co-translational import in Arabidopsis, these particles could be involved in mRNA regulation. Clu has multiple domains: DUF727, CLU, and tetratricopeptide repeats (TPR). We previously demonstrated that Clu can self-associate. However, whether this happens as a dimer or multimer and which domains of Clu contribute to forming Clu particles is not known. To answer this question, we utilized confocal live-imaging with *Drosophila* expressing Clu deletion mutant constructs. We generated fly stocks expressing ectopic full-length or domain-deleted Clu tagged with mScarlet-i at the C-terminus under the control of the *UASp-Gal4* system. We then generated flies ectopically expressing mScarlet-I constructs in germ cells in the background of Clu GFP trap. We will present data from live-imaging demonstrating which domains are critical for interacting with endogenous Clu and which domains are important for forming particles. As mitochondrial co-translational import is poorly understood in metazoans, and Clu appears to play a role regulating transcripts of mitochondrial proteins encoded in the nucleus, these finding will shed light on how mitochondria regulate protein import during metabolic shifts in the cell. Understanding this important process is relevant to many different cell types, including germ cells and neurons.

B449/P2832

Mitophagy in heteroplasmic bivalve mollusks with DUI

H. Hosseini Khorami, S. Breton, A. Angers; University of Montreal, Montreal, QC, Canada

Mitochondrial heteroplasmy, the presence of more than one mitochondrial DNA in a cell or an individual is considered as a major factor in mitochondrial diseases. Strict maternal inheritance (SMI), by which

only the mother's mitochondria are passed to the offspring is regarded as an important mechanism to insure the maintenance of homoplasmy. In species with SMI, mitophagy is thought to play a major role in limiting heteroplasmy by elimination of paternal mitochondria in embryos and degradation of defective mitochondria in adults. Unlike most animal species, several bivalve mollusks pass the paternal mitochondrial genome to the offspring through a system called double uniparental inheritance (DUI). The molecular mechanisms behind this peculiar transmission that implies high degrees of heteroplasmy are largely unresolved. To gain insight into this phenomenon, we have established a system of primary cell culture from *Mytilus edulis* tissue dissociation that allow us to keep proliferating cells in vitro for several weeks. We showed that cultures established for dissociation of male mantle cells maintain a high degree of heteroplasmy, with germline progenitors showing the highest proliferation rate. Cells obtained from female tissue show a similar degree of germ cell proliferation but remained largely homoplasmic whereas male and female gill cells showed low heteroplasmy and only modest proliferation of unknown cell types. We induced mitophagy in these cultures by treatment with 100 uM CCCP (Carbonyl cyanide m-chlorophenyl hydrazone) for different times. We examined response to CCCP by probing Beclin1, a core component of autophagosome nucleation. We found that cells of male and female origin responded differently to the treatment. Female cells showed increased mitophagy after 2h of treatment, whereas male cells dose not respond or showed decrease beclin1 fluorescence. Further investigation into the nature of this compensation mechanism is likely to shed light in a possible involvement of mitophagy in the maintenance of heteroplasmy in species with DUI.

Receptors, Transporters, and Channels

B450/P2833

A novel ammonium transporter functions as a lysosomal detoxifier

R. Levin Konigsberg[1], A. Nigam[2], K. Mitra[3], K. Spees[1], P. Hivare[3], Y. Krishnan[3], M. C. Bassik[1]; [1]Genetics, STANFORD SCHOOL OF MEDICINE, Stanford, CA, [2]Computer Science, STANFORD UNIVERSITY, Stanford, CA, [3]Chemistry, University of Chicago, Chicago, IL.

Ammonia is a ubiquitous toxic by-product of cell metabolism. While systemic ammonia detoxifying mechanisms are well understood, only a small subset of cell types —mainly in the liver— express the necessary machinery to perform these reactions. Thus, how ammonia affects cell physiology and the molecular mechanisms whereby most cells tolerate and detoxify physiological levels of ammonia are incompletely understood. Our work describes a lysosomal co-transport mechanism whereby lysosomes export "ion-trapped" ammonium —along with chloride— to the cytosol, preserving the physiology and function of these organelles. Through a genome-wide CRISPR screen for regulators of macropinocytosis, we found SLC12A9 to be critical for membrane trafficking homeostasis. We show that SLC12A9 —previously reported as a plasmalemmal protein without ion transport capacity— is a lysosomal ammonium transporter. SLC12A9 KO cells showed a vacuolation phenotype with abnormally large lysosomes and increased levels of cellular ammonia. These phenotypes were rescued upon removal of a metabolic source of ammonium, inactivation of the lysosomal proton pump (vATPase) or exogenous expression of WT-SLC12A9. SLC12A9-mediated ammonium transport was dependent on the presence of chloride and on conserved chloride-binding residues among the SLC12A family of transporters. This was further supported by molecular dynamics simulations using the predicted structure of SLC12A9. Our findings suggest that SLC12A9 is an ammonium - chloride co-transporter that functions at the endo-

lysosomal level. Functionally, SLC12A9 inactivation impaired lysosomal osmolarity, cargo delivery, and fission, highlighting its critical role in the fundamental physiology of this organelle

B451/P2834

Investigating the C-terminal of KCNT1 to see its regulatory effects on K^+ and Ca^{2+} homeostasis, inspired from a patient with Brugada syndrome

P. Tsai[1], C. Pan[2,3], Y. Liao[4], J. Juang[5,6], F. Tsai[1,6]; [1]Department and Graduate Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, TAIWAN, [2]Department of Life Science, College of Life Science, National Taiwan University, Taipei, TAIWAN, [3]Graduate Institute of Brain and Mind Sciences, College of Medicine, National Taiwan University, Taipei, TAIWAN, [4]Department of Life Sciences, College of Health Sciences and Technology, National Central University, Taoyuan, TAIWAN, [5]Department of Medical Genetics, National Taiwan University Hospital, Taipei, TAIWAN, [6]Department of Internal Medicine, National Taiwan University Hospital, Taipei, TAIWAN.

Potassium sodium activated channel subfamily T member 1 (KCNT1) is a Na^+-activated K^+ channel known to carry an enormous cytoplasmic tail. In 2014, Dr. Jyh-Ming Juang and his colleagues identified a mutation, *KCNT1:R1106Q*, from a patient diagnosed with Brugada syndrome (BrS), which is an inheritable sudden cardiac death disease. Besides KCNT1's ability of K^+ current modulation, we found that the store-operated Ca^{2+} entry (SOCE) is also enhanced, which can be further increased with the point mutation of R1106Q. On the other hand, with the truncation of its C-terminus, KCNT1 lost its capability of SOCE promotion, suggesting C-terminus' importance in maintaining Ca^{2+} homeostasis. By performing patch clamp, the result shows that the change in SOCE intensity cannot simply be attributed to the difference in K^+ outward current. Since the amplified K^+ current may not be the only reason for SOCE increase, we are seeking the underlying mechanism. Stromal interaction molecule 1 (STIM1) resides on the endoplasmic reticulum (ER), which is crucial for SOCE assembly, we found that its puncta density is increased with KCNT1 overexpression, this may be another proof that the KCNT1 tail can regulate SOCE intensity independently. The aforementioned results suggested that besides electrostatic balance, KCNT1 might alter cellular Ca^{2+} homeostasis with a distinct, K^+-independent mechanism, thus may bring up some innovative treating strategies of KCNT1-related disease.

B452/P2835

The effects of Phosphatidylinositol 3,5-bisphosphate on the interdependent transport of $Ca^{2+}$and H^+on yeast vacuoles

C. Zhang; Biochemistry, University of Illinois At Urbana–Champaign, Urbana, IL.

Yeast vacuoles are acidified by the vacuolar type H+-ATPase (V-ATPase), a protein complex comprised of the membrane embedded VO complex and the soluble cytoplasmic V1 complex. The assembly of the V1-VO holoenzyme on the vacuole is stabilized in part through interactions between the VO a-subunit ortholog Vph1 and the lipid phosphatidylinositol 3,5-bisphospate (PI(3,5)P2). PI(3,5)P2 also affects vacuolar Ca2+ export through the channel Yvc1, and the Ca2+ importing pump Pmc1. Here we asked if H+ and Ca2+ transport activities were connected through PI(3,5)P2. We found that overproduction of PI(3,5)P2 by the hyperactive fab1^{T2250A} mutant led to augmented vacuole acidification, whereas the kinase inactive fab1^{EEE} mutant attenuated the formation a H+ gradient. Separately, we tested the effects of extraluminal Ca2+ on vacuole acidification. Adding Ca2+ blocked vacuole acidification, whereas chelating Ca2+ accelerated acidification, illustrating that affecting Ca2+ transport alters vacuole

acidification. The effect of adding Ca2+ on acidification was eliminated when the Ca2+/H+ antiporter Vcx1 was absent. Together this shows that the H+ gradient across the vacuole membrane can collapse during Ca2+ stress is Ca2+ through Vcx1 activity. To see if the link between Ca2+ and H+ transport was bidirectional we examined Ca2+ transport when V-ATPase activity was inhibited with Bafilomycin. When added at the start of the experiment Bafilomycin blocked Ca2+ uptake whereas adding the inhibitor after establishing a H+ gradient caused a rapid release of Ca2+. Together, these data illustrate that Ca2+ transport and V-ATPase efficacy are connected.

B453/P2836

Structural and functional insights into a peroxisomal fatty-acid transporter

M. Gupta[1], M. Dickinson[2], A. Myasnikov[3], R. M. Stroud[4]; [1]Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, [2]University of Washington, Seattle, WA, [3]École polytechnique fédérale de Lausanne, Lausanne, SWITZERLAND, [4]Department of Biochemistry and Biophysics, University of California, San Francisco, CA.

Objective: To elucidate mechanism of fatty-acid transport using biochemistry and structural biology Methods: We expressed the membrane protein in insect cells (SF), extracted the protein using detergent and purified using affinity tags. We performed biochemical characterization of the protein and used cryoEM to determine high-resolution structures in various conditions. Summary: Peroxisomes are ubiquitous eukaryotic organelles that form a focal point for multiple metabolic pathways. Lipid metabolism, and in particular, fatty acid transport related to it, depend heavily on peroxisomal membrane proteins that have specifically evolved for such purposes. The ATP-dependent cassette (ABC) transporters of 'D' subfamily' reside in the peroxisomal membrane and are responsible for fatty acid import into the peroxisomes, defects in which process are related to various metabolic disorders. Mutations in ABCD1 cause X-linked adrenoleukodystrophy (X-ALD) which manifests as mild to severe central nervous system (CNS) demyelination. Dysfunction of ABCD3 and/or peroxisome biogenesis factors (PEXs) may cause Zellweger syndrome (ZS), a heterogeneous group of peroxisome assembly disorders. In addition to inherited diseases, reduced peroxisomal function is associated with aging and pathogenesis of age-related acquired diseases like diabetes, neurodegenerative disorders. Key gaps in understanding the function of ABCDs in metabolism and disease are due to lack of structural details, particularly of their conformational plasticity during substrate transport, and how this is compromised during aging and disease. Using CryoEM, we determined the first high-resolution structures of any human peroxisomal ABC transporters (ABCD3). We show that the purified protein in the detergent is active in vitro. With the help of the structures and biochemical assays, we propose a mechanism of fatty-acid transport through these transporters and identify a potentially new substrate. We are also exploring interaction among peroxins and PMPs (peroxisomal membrane proteins) and have noteworthy results which help us comprehend PMP recruitment on peroxisomal membranes and peroxisome biogenesis. Conclusion: ABCD3 is a homodimer with fatty-acid (phytanoyl-CoA) as a primary substrate. The protein possibly splits fatty-acid and CoA moiety of the fatty-acyl ester using thioesterase activity as part of the transport mechanism. ABCD3 associates with Pex19 at the N-terminus and may serve as the initial step of membrane protein recruitment of the membrane proteins.

B454/P2837

Cryo-EM structures of the Inward Rectifier K+ channel Kir7.1 .

A. Peisley, first, C. Hernandez, M. Su, R. Cone; Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Inward rectifier potassium (Kir) channels are a class of K+ channels responsible for maintaining membrane potential and extracellular K+ concentrations. Kir7.1 is localized to a variety of ion-transporting epithelia including the apical membranes of retinal pigmented epithelium (RPE). Functional studies have established the involvement of Kir7.1 in maintaining subretinal K+ concentration and the RPE monolayer integrity. Patients diagnosed with adult onset retinitis pigmentosa, a progressive loss of photoreceptors, have been found to carry heterozygous missense mutations resulting in Arg-to-Gln (R162Q) and Glu-to-Ala (E276A) mutants of Kir7.1. We sought to understand the impact of these mutations on the structure of Kir7.1 channels using cryo-electron microscopy (cryo-EM).
Kir7.1 is the most divergent inward rectifying K+ channel whose structure has yet to be determined. Here we present the first high-resolution cryo-EM structures of full-length human Kir7.1 with phosphatidylinositol 4,5-bisphosphate (PIP2) and that of the retinopathy associated mutants R162Q and E276A. Each subunit of the Kir7.1 tetrameric channel consists of two transmembrane domains, a pore-forming loop containing the selectivity filter, and two cytoplasmic polar tails. To gain insight into the structural organization that governs the gating mechanism of Kir7.1 channels, we complexed Kir7.1 with PIP2 known to stabilize the open conformation of Kir channels. The structures of Kir7.1-PIP2 showed that PIP2 faces hydrophobic pockets of lipids of the transmembrane domain on the cytoplasmic side, and favors the rotation of the cytosolic domain that is relatively independent of the pore-forming domain, to adopt a conductive conformation of the channel. To understand mechanistically how the retinopathy associated mutations affect channel function we also determined their structures by cryo-EM. The R162Q mutant is located at the interface of the cytosolic and transmembrane domains in the PIP2 binding site. The channel harboring this mutation was found to be in a subconductance state and stabilized in an extended confirmation with a constricted pore. The E276A mutation is located in the highly conserved G-loop gate. We observed a rotation of the cytosolic domains relative to the transmembrane domains of the channel to a state reminiscent of the polyamine blocked conformation. Our results provide evidence of stabilization of channel conformations by Kir7.1 mutants as an underlying cause of disease.

B455/P2838

A common mechanism of Sec61 translocon inhibition by small molecules

S. Itskanov[1], **L. Wang*[2]**, T. Junne[3], R. Sheriff[2], L. Xiao[4], N. Blanchard[5], W. Q. Shi[6], C. Forsyth[4], D. Hoepfner[7], M. Spiess[3], E. Park[2,8]; [1]Biophysics Graduate Program, University of California, Berkeley, Berkeley, CA, [2]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, [3]Biozentrum, University of Basel, Basel, SWITZERLAND, [4]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH, [5]CNRS, LIMA, UMR 7042, Université de Haute-Alsace, Université de Strasbourg, Mulhouse, FRANCE, [6]Department of Chemistry, Ball State University, Muncie, IN, [7]Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, SWITZERLAND, [8]California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, CA.

*The first two authors contributed equally to this work.
The Sec61 complex forms a protein-conducting channel in the endoplasmic reticulum (ER) membrane

that is required for secretion of soluble proteins and production of many membrane proteins. Several natural and synthetic small molecules specifically inhibit the Sec61 channel, generating cellular effects that are potentially useful for therapeutic purposes, but their inhibitory mechanisms remain unclear. Here we present near-atomic-resolution structures by cryo-EM of the human Sec61 channel inhibited by a comprehensive panel of structurally distinct small molecules—cotransin, decatransin, apratoxinF, ipomoeassinF, mycolactone, cyclotriazadisulfonamide (CADA)and eeyarestatin I (ESI). All inhibitors bind to a common lipid-exposed pocket formed by the lateral gate and plug domain of the channel. Mutations conferring resistance to the inhibitors are clustered at this binding pocket. The structures indicate that Sec61 inhibitors stabilize the plug domain of Sec61 in a closed state, thereby preventing the protein-translocation pore from opening. Our study reveals molecular interactions between Sec61 and its inhibitors in detail and offers the structural framework for further pharmacological studies and drug design.
We have uploaded thepre-print for our work to biorxv at this link:
https://www.biorxiv.org/content/10.1101/2022.08.11.503542v1.

B456/P2839

PALM combined with ExM and advanced analysis show novel spatiotemporal organization of the AT1 receptor

C. Andersen[1], Y. M. Zulueta Díaz[1], C. Lagerholm[2], P. Wiseman[3], **E. C. Arnspang**[1]; [1]University of Southern Denmark, Odense, DENMARK, [2]University of Oxford, Oxford, UNITED KINGDOM, [3]McGill Unversity, Monteral, QC, CANADA.

Investigation of spatiotemporal interactions between a ligand and a receptor in the plasma membrane (PM) at the nanoscale, is a challenge [1]. Specially, the mechanisms by which Angiotensin II type 1 Receptor (AT1R) is distributed and diffused in the PM remains unclear, despite their crucial role in cardiovascular homeostasis [2,3]. Super-resolution techniques and images analysis are powerful tools to investigate protein dynamics in complex cellular environment [4,5]; however, there is only a limited number of approaches that combine super-resolution imaging and measurement of dynamics in the presence of spatially and temporally heterogeneous structures [6,7]. The aim of this study is to obtain quantitative information about dynamics and organization of AT1R, and their corresponding receptor-ligand complexes in living cells. For this purpose, Photoactivated localization microscopy (PALM) combined with image spatial-temporal correlation analysis were used. To study the organization of the receptor at the nanoscale, Expansion Microscopy (ExM) combined with PALM was performed.
This study revealed that AT1R lateral diffusion increased after binding to the agonist, Angiotensin II (Ang II) while no change for either the biased agonist, SII-Ang II or the antagonist, Losartan, was observed. The receptor diffusion was found to be transiently confined in the PM. Additionally, ExM showed that AT1R form nano clusters at the PM, and the cluster size significantly decreased after Ang II treatment. These results show that Ang II interaction cause reorganization and changes in the dynamics of AT1R at the PM. We here present quantitative data that helps explain AT1R distribution and dynamics in mammalian cell and contributes to a better understanding of receptor mechanism.

B457/P2840

Lysosomal-dependent remodeling of endoplasmic reticulum-plasma membrane contacts drives neurodegeneration

M. Casas[1], K. Hino[2], N. Vierra[1], S. Simó[2], J. Trimmer[1], R. Dixon[1], E. Dickson[1]; [1]Department of Physiology and Membrane Biology, University of California, Davis, Davis, CA, [2]Department of Cell Biology and Human Anatomy, University of California, Davis, Davis, CA.

Lysosome dysfunction is associated with the pathogenesis of a variety of neurodegenerative disorders. Thus, linking lysosome abnormal mechanisms to disruption of neuronal fidelity offers opportunities to understand the molecular basis of neurodegeneration and to potentially identify novel therapeutic targets. One mechanism that lysosomes use to communicate and receive instruction is via transfer of cholesterol at ER-lysosome membrane contact sites (MCSs) where the Niemann Pick C1 cholesterol transporter (NPC1) is a key gatekeeper that facilitates cholesterol flux from the lysosome to the ER, where it is further distributed to other cellular membranes. Briefly, loss of NPC1 function leads to the fatal neurodegenerative disorder, NPC disease, which is characterized by the progressive neurodegeneration of several brain regions. Despite altered ER contacts with lysosome, Golgi and Mitochondria membranes, in addition to a gross Ca^{2+} handling defect being reported in NPC1 deficient cells, the molecular mechanism(s) linking NPC1 loss of function to NPC disease associated neuropathology are still poorly understood. Giving the importance of ER MCSs, we wanted to study if molecular contents of other ER junctions are also altered and contribute to neurodegeneration observed in the NPC disorder. In neurons, a prominent ER-PM contact site forming protein is the $K_V2.1$ ion channel, which tunes lipid and Ca^{2+} nanodomains. Using super-resolution TIRF nanoscopy, high-speed fluorescence imaging, biochemistry, and animal models of NPC disease, we determined that NPC1 loss of function increases ER-PM MCSs and the area, density, and number of PM $K_V2.1$ channels in neurons. Moreover, interactions between $K_V2.1$ and Ca_V1 voltage-gated Ca^{2+} channels are elevated and result in significantly increased Ca_V1 clustering and activity, leading to elevated neuronal Ca^{2+} entry. A critical consequence of such enhanced $K_V2.1$-dependent Ca_V1 Ca^{2+} entry is neurotoxic elevations in mitochondrial Ca^{2+} concentrations. Noteworthy, reducing $K_V2.1$ phosphorylation with a Cyclin-dependent kinase 5 inhibitor or disrupting $K_V2.1$-Ca_V1 interactions using a synthetic peptide rescues the increases in Ca_V1 clustering, mitochondrial Ca^{2+} and neurotoxicity seen in NPC neurons. Collectively, these data demonstrate that NPC is a nanostructural ion channel clustering disease with altered ion channel distribution/activity and ER-PM remodeling that contribute to neurodegeneration.

B458/P2841

Characterization of zebrafish ABCG2 homologs

J. R. Thomas[1], R. Robey[1], W. Frye[1], C. Inglut[1], A. Warner[2], D. Butcher[2], J. Matta[2], E. Edmondson[2], M. Gottesman[1]; [1]Center for Cancer Research, National Cancer Institute, Bethesda, MD, [2]National Cancer Institute, Frederick, MD.

A principal component of the blood-brain barrier (BBB) is the expression of high levels of the ATP-binding cassette (ABC) transporters P-glycoprotein (P-gp, encoded by ABCB1) and ABCG2 (encoded by ABCG2) on the apical surface of brain microvascular endothelial cells (BMECs). ABC transporters at the BBB efflux toxins and a wide range of therapeutic drugs back into the bloodstream, preventing brain access. Zebrafish have emerged as an advantageous model for studying the BBB, however little information exists regarding zebrafish ABC transporters which comprise the BBB. We recently identified

and characterized the zebrafish homologs of P-gp (Abcb4, Abcb5), and now seek to advance this by characterizing the zebrafish homologs of ABCG2 (Abcg2a, Abcg2b, Abcg2c, Abcg2d). In situ hybridization with RNAscope™ probes was used to selectively detect expression of *abcg2a-d* in adult zebrafish tissues. The most widely expressed homolog was *abcg2a* which was detected in BMECs of the BBB, the intestine/GI tract, liver and ovaries. We noted *abcg2b* expression in the intestine/GI tract, *abcb2d* was detected in the liver, and all four homologs were detected in the ovaries. We stably expressed human ABCG2 and zebrafish *abcg2a-d* homologs in HEK293 cells to determine substrate overlap using known ABCG2 substrates in efflux and cytotoxicity assays. Abcg2a, -b, and -c, transported pheophorbide a, and only Abcg2a transported mitoxantrone. In agreement, only Abcg2a also conferred resistance to mitoxantrone and the additional cytotoxic substrates CUDC-101 and NMS1286937. Abcg2a has the greatest and Abcg2d has the least substrate overlap with ABCG2. In conclusion, our data indicate *abcg2a* is most phenotypically similar to ABCG2 and is likely the relevant homolog for BBB studies in zebrafish. We are developing a transgenic zebrafish model which expressing luciferase in the brain parenchyma. If the BBB is perturbed for example by ABC transporter inhibition or physical disruption, BBB impermeable luciferase substrates will be able to cross the BBB, resulting in a bioluminescent signal. We tested the substrates coelenterazine native, coelenterazine h and furimazine, and found they are substrates of both ABCG2 and Abcg2a. This indicates our transgenic model will be capable of detecting Abcg2a-mediated BBB disruption, allowing for a high-throughput method of screening zebrafish BBB integrity.

B459/P2842

Electric stress opens a transmembrane route in Na,K-ATPase alpha subunit

G. Silkuniene, U. Mangalanathan, A. G. Pakhomov, O. N. Pakhomova; Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA.

Several studies reported that electric fields could form a pore in a lipidic portion of the membrane and could suppress the function of ion channels, possibly by electroconformational changes in the channel proteins. The structural alterations in channel proteins likely impact membrane permeability, providing pathways for transporting ions and molecules across the plasma membrane. Therefore, this work aimed to identify ion channels and pumps affecting membrane permeabilization after cell injury by electric pulses using high-throughput screening (HTS). We generated gene knock-outs (KOs) in human cell monocytes U937 using CRISPR/Cas9 gene editing approach and obtained a total of 328 derivatives with KO for a single gene. Each KO was exposed to 20 electric pulses of 300 ns duration, all delivered at 7 kV/cm and 20 Hz. In 50 min, cells were imaged for YoPro-1 (YP) uptake on an Olympus IX83 microscope configured for HTS. It was found that KO of the ATP1A1 gene coding for the alpha-1 subunit (ATP1A1) of Na,K-ATPase (NKA) had reduced YP uptake by up to 30%. The effect of lower YP uptake was confirmed under the different conditions. YP uptake was measured within 5 min after application of electric field (10 pulses of 300ns, 7 kV/cm, at 5Hz) by timelapse imaging on a scanning confocal microscope. YP fluorescence in ATP1A1 KO cells was ~20% lower than in control cells, thus confirming the results of HTS. Next, ATP1A1 expression was suppressed using shRNA lentiviral particles. The YP uptake reduction in ATP1A1 knock-down (KD) cells was up to 25% in HTS and confocal setups. Additionally, it was noticed that the initially strong reduction effect in YP uptake tended to weaken with time after transduction. It was hypothesized that the function of the ATP1A1 gene was compensated by paralog genes typically low expressed in U937 cells. The expression levels of other NKA α subunits were determined. Results revealed that ATP1A4 expression was upregulated in ATP1A1 KD cells. Therefore, upregulation of NKA paralog genes can compensate for inhibited ATP1A1. Finally, it was tested if inhibition of NKA with

ouabain affects the YP uptake after electric pulses. It was found that the presence of ouabain did not reduce YP uptake but increased it by 21%. This result showed that reduced YP uptake after electric pulse injury in ATP1A1 KOs is not related to NKA physiological function as an ion pump but instead linked to modification in protein conformation. Overall, the study identified ATP1A1 as a potential protein target (or one of the protein targets) for cell membrane permeabilization by electric fields. Support: AFOSR MURI grant FA9550-15-1-0517 to AGP.

B460/P2843

A structural and functional analysis of Nematostella vectensis major intrinsic proteins in Saccharomyces cerevisiae

J. Grissom, J. Taylor, O. Mikula, M. Shahani, A. M. Reitzel, R. Chi; Biological Sciences, The University of North Carolina At Charlotte, Charlotte, NC.

There is currently a major gap in understanding tissue grade organisms from early diverging phyla, particularly within the context of understanding placatory responses to osmotic variabilities that can result from salinity fluctuation due to global climate change. A key protein family essential for the regulation of intracellular water and solute concentrations are the Major Intrinsic Proteins (MIPs), these include aquaporins (Aqps) and aquaglyceroporins (Glps). Here, we report the identification and functional analysis of MIPs in Nematostella vectensis, a member of the early diverging phyla that represents a unique group of tissue grade organisms. Using a molecular evolution approach, we successfully identified each MIP node in *N. vectensis*. Putative MIPs were cloned and recombinantly expressed in budding yeast, *Saccharomyces cerevisiae* and tested for functionality. *N. vectensis* MIPs displayed varying degrees of functionality, ranging from no effect to lethality, suggesting a phylogenetic approach paired with an in vivo functionality assay is critical for their characterization. Total N. vectensis MIP transcripts were also quantified during acute and long-term salinity exposure which also support our functional yeast results. Furthermore, based on high confidence protein structure homology modeling, we demonstrate a significant correlation between biophysical pore properties that can accurately predict functionality.

B461/P2844

Host Nucleotide Manipulation StrategiesImplemented by an Intracellular Bacterium

L. Blanchet, A. Subtil; Institut Pasteur, Paris, FRANCE.

The obligate intracellular bacteria *Chlamydia trachomatis* are auxotrophic for nucleotides. The expression of unique transporters enables capture of nucleotides from the host cell. However, to reach the bacteria, host-derived nucleotides need to cross the membrane of the vacuole in which the bacteria multiply, called the inclusion. We investigated the mechanism(s) by which nucleotides reached the inclusion lumen, and whether *Chlamydia* modulated nucleotide metabolism in the host.Overexpression of tagged forms of host nucleosides/nucleotides transporters indicate that two such transporters, ENT1 and AXER, are relocated to the inclusion membrane upon infection. Furthermore, silencing of the expression of either one of the corresponding genes using siRNA leads to an about 2-fold decrease in the generation of infectious bacteria, bringing additional support for their implication in nucleoside/nucleotide transport to the *Chlamydia* inclusion. We implemented click-chemistry coupled to quantitative microscopy to directly measure the transport of two nucleotides, ATP and CTP, to the inclusion. Preliminary data support the hypothesis that ATP transport to the *Chlamydia* vacuole uses

AXER, while CTP transport uses a different mechanism. Finally, we identified a post-translational modification triggered by infection on the enzyme that engages purine biosynthesis in the host. Biochemical assays are ongoing to test its incidence.In conclusion, completion of this work will answer the longstanding question of nucleotide transport to the *Chlamydia* inclusion and bring mechanistic insight on the modulation of nucleotide biosynthesis in the host. By doing so, it might identify novel targets for therapeutic intervention against *Chlamydia* infection. It will also shed light on the regulation of purine biosynthesis, and thereby help improving nucleoside-based chemotherapeutic strategies.

B462/P2845

Membrane stretching activates putative ion channel Pkd2 to promote calcium spikes during cytokinesis

A. Poddar[1], Y. Hsu[2], F. Zhang[1], A. Shamma[3], Z. Kreais[3], C. Muller[1], M. Malla[1], A. Ray[3], A. Liu[2], Q. Chen[1]; [1]Department of Biological Sciences, The University of Toledo, Toledo, OH, [2]Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, [3]Department of Physics and Astronomy, The University of Toledo, Toledo, OH.

During cytokinesis of many animal embryos, calcium transients accompany two crucial events, the initiation of cleavage furrow ingression and the separation of daughter cells. We have recently discovered similar transients, termed cytokinetic calcium spikes, in the unicellular model organism fission yeast. These spikes promote cleavage furrow ingression and maintain the integrity of separating cells in yeast. However, as in the embryonic cells, it remains unclear how such calcium spikes are generated. Here, we focused on the likely role of an essential fission yeast channel Pkd2. This plasma membrane protein translocates to the equatorial plane during the cleavage furrow ingression and remains there throughout the cell separation. It promotes cell separation but modulates the furrow ingression through unknown mechanisms. In this study, we first employed a genetically encoded calcium indicator GCaMP to measure the intracellular calcium level throughout the cytokinesis of the *pkd2* mutants. Both *pkd2-81KD* and *pkd2-B42* reduced the separation calcium spikes by 60%, compared to the wild-type. Surprisingly, they also increased the constriction spikes by more than two-fold. Next, to determine how Pkd2 is activated in cytokinesis, we reconstituted this putative channel in vitro. Pkd2 was translated in a cell-free expression system and inserted into giant unilamellar vesicles. It permeated calcium when the membrane was mechanically stretched. Consistent with this result, depletion of Pkd2 in vivo reduced the calcium spike triggered by the hypoosmotic shock by 59%, compared to the wild type. We concluded that membrane stretching activates Pkd2 to promote the calcium influx during cell separation, suggesting that the cytokinetic calcium transients are partially contributed by mechanosensitive channels.

B463/P2846

SLC-30A9 is required for zinc homeostasis, zinc mobilization, and mitochondrial health

H. Deng[1], X. Qiao[2], C. Chen[2], X. Wang[2], K. Shen[1]; [1]HHMI-Stanford, Stanford, CA, [2]Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

The trace element zinc is essential for many aspects of physiology. The mitochondrion is a major Zn^{2+} store, and excessive mitochondrial Zn^{2+} is linked to neurodegeneration. How mitochondria maintain their Zn^{2+} homeostasis is unknown. Here, we find that the SLC-30A9 transporter localizes on mitochondria and is required for export of Zn^{2+} from mitochondria in both *Caenorhabditis elegans* and

human cells. Loss of *slc-30a9* leads to elevated Zn^{2+} levels in mitochondria, a severely swollen mitochondrial matrix in many tissues, compromised mitochondrial metabolic function, reductive stress, and induction of the mitochondrial stress response. SLC-30A9 is also essential for organismal fertility and sperm activation in *C. elegans*, during which Zn^{2+} exits from mitochondria and acts as an activation signal. In *slc-30a9*-deficient neurons, misshapen mitochondria show reduced distribution in axons and dendrites, providing a potential mechanism for the Birk-Landau-Perez cerebrorenal syndrome where an SLC30A9 mutation was found.

B464/P2847

A "Hot" Date with Capsaicinoids: Molecular Networking meets TRPV1

J. Smith, H. Gaustad, P. Sacha, T. Pluskal; UOCHB, Praha, Czech Republic

Chili plants are known for their production of capsaicinoids, specialized metabolites defined by their vanilloid head and the C9-C11 carbon long tail (acyl moiety). The most well studied capsaicinoid, capsaicin, is a well-characterized agonist of the human pain and heat receptor, TRPV1, and has been used in a wide range of medicinal applications. Despite capsaicin's significant role in medicine, other capsaicinoids remain relatively uncharacterized, with about only 20 capsaicinoid structures roughly reported throughout the literature. Furthermore, most studies exploring capsaicin's biological relevance have not been expanded to capsaicinoids. Utilizing UPLC-HRMS and modern MS computational tools for the analysis of different Capsicum sp. varieties; I have already identified capsaicinoids with an undescribed structure by utilizing feature-based molecular networking. Over 30 different varieties of chilis have been collected for supercritical CO2 extraction and subsequent capsaicinoid library building. In addition to, capsaicinoids biological effects on glucose metabolism are being investigated using a stably transfected cell line expressing rTRPV1. Current experiments to investigate the effects of capsaicinoids are a mixture of calcium influx, western blot, cytotoxicity, and glucose uptake assays. The goal is to create the most complete library of capsaicinoids with corresponding biological information on its activation of TRPV1 and subsequent effects on glucose metabolism. Current experiments show that capsaicin cytotoxic effects trump any therapeutic benefit during excess exposure. Experiments are still being completed regarding the effects of capsaicin on glucose metabolism.

B465/P2848

Analysis of endocytic structures and B cell receptor clustering in Diffuse Large B cell Lymphoma Cell lines

A. Roberts[1], A. Arnold[1], S. Scheich[2], X. Zhuang[2], L. Staudt[2], J. Taraska[1]; [1]National Heart Lung and Blood Institute, Bethesda, MD, [2]National Cancer Institute, Bethesda, MD

The B cell receptor (BCR) is the primary receptor on the B cell plasma membrane that interacts with foreign antigens and transmits downstream signaling events that lead to cell activation and antibody production. A necessary first step in the production of antigen specific antibodies is the endocytosis of the receptor-antigen complex. The BCR may be endocytosed through the classical clathrin-mediated pathway or less well characterized clathrin independent mechanisms. Our previous work identified smooth raised membrane structures at the plasma membrane that co-localize with BCR clusters upon activation and mediate endocytosis of large membrane clusters through an actin-dependent hybrid endocytosis pathway. Here, we have extended our analysis of endocytic structures at the plasma membrane in four human B cell lymphoma cell lines: HBL1, TMD8, FL318, and WSU-FSCCL. Using

platinum replica electron microscopy, we identified an increased number of clathrin and smooth raised membrane structures in Diffuse Large B Cell Lymphoma (DLBCL) cell lines compared to control B cells. We also observed differences in density of different types (flat, domed, or sphere) of clathrin structures present on the plasma membrane. Finally we found differences in the rate of BCR recycling from the plasma membrane in germinal center and activated B cell like DLBCL cell lines.

B466/P2849

The role of salt bridge networks in the stability and function of the Hxt1 glucose transporter

L. Mailloux, D. Bloor, B. Maddox, J. Humble, J.-H. Kim; Liberty University, Lynchburg, VA

Glucose transport across the plasma membrane is tightly controlled by hexose (glucose) transporters. The yeast *S. cerevisiae* possesses 17 different hexose transporters that belong to the sugar porter subfamily of the major facilitator superfamily (MFS) of transporters. In this study, using homology modeling and site-directed mutagenesis, we identified conserved salt bridge networks within and between the two 6-transmembrane (TM) spanning segments of the yeast glucose transporter 1 (Hxt1) and constructed 15 derivatives of the transporter. Genetic and biochemical analyses show that the disruption of these bridges causes destabilization of the Hxt1 transporter and consequently abolishes transport activity. Of note, the Hxt1 transporters predicted to be locked in either an inward-facing or an outward-facing conformation are unable to complete the transport cycle and are targeted for degradation by cellular quality control machinery. Interestingly, the corresponding mutations in the Rgt2 glucose-sensing receptor are also predicted to arrest the receptor in inward-facing conformations, but do not reduce the stability of the protein; rather, they render Rgt2 constitutively active. This suggests that the salt bridge-forming residues in Hxt1 and Rgt2 may have different structural and functional roles. Taken together, our results provide insight into the role of salt bridge networks in the stability and function of Hxt1 as well as a framework for studying the structure-function relationship of yeast and other fungal glucose transporters, as high-resolution crystal structures are not currently available for them.

B467/P2850

Elucidating transport mechanisms of membrane protein variants that confer Alzheimer's risk in African Americans

A. Sekhar[1], K. Stepler[1], R. Robinson[1], **J. Davis**[2]; [1]Vanderbilt University, Nashville, TN, [2]Meharry Medical College, Nashville, TN

Alzheimer's disease (AD), the most common cause of dementia in older adults, disproportionally affects African Americans with an incidence rate as much as three times higher, compared to other racial/ethnic groups. Multiple factors contribute to this racial disparity however, an in-depth understanding of the biological or genetic contributions does not exist. Compelling evidence indicate that genetic variants of the lipid transport protein, ABCA7, is more strongly associated with AD in African Americans. To understand how ABCA7 contributes to AD on the molecular level, we used a combination of structural and cell biology techniques. We have found that the ABCA7 T319A variant, that confers risk in African Americans, is expressed, localizes to the plasma membrane and has reduced ATPase activity when expressed in human cell lines. Proteomic studies indicate reduced levels of the phospholipase C eta (PLCH1) protein in cells that expressed ABCA7 T319A compared to wild-type. PLCH1 plays a key role in the metabolism of phosphoinositol bisphosphate, PIP_2. The ABCA7 T319A protein variant displays

reduced binding with PIP_2. Our results suggest that this ABCA7 variant may contribute to AD by reducing the levels of PIP_2, a phospholipid reported to be decreased in the AD brain. These results provide a framework for targeting mechanisms that can increase PIP_2 levels as an effective strategy mitigating AD disparities.

B468/P2851

Tetraspanin CD82 regulates Epidermal Growth Factor Receptor signaling by modulating molecular organization and dynamics

S. Restrepo Cruz, E. A. Burns, K. A. Lidke, D. S. Lidke, J. M. Gillette; University of New Mexico, Albuquerque, NM

Molecular interactions on the plasma membrane significantly influence the outcomes of signaling events fundamental to cell function in both normal and disease states. Tetraspanins, a superfamily of transmembrane proteins, play a vital role as scaffolding proteins on the membrane, forming structural platforms termed Tetraspanin-Enriched Microdomains (TEMs). By facilitating compartmentalization, tetraspanins have been shown to act as regulators of cellular signaling, enhancing the recruitment of intracellular signaling proteins to the membrane, and modulating the organization of transmembrane proteins. Signaling by the Epidermal Growth Factor Receptor (EGFR), a receptor tyrosine kinase, has been shown to depend on the dimerization, auto- and cross-phosphorylation, and internalization of the receptor; these processes are dependent on the dynamics and landscape of the plasma membrane. While disruption of EGFR signaling has been implicated in the development and progression of several cancers, the efficacy of EGFR-targeting drugs remains limited. Genetic and biochemical studies have previously shown that specific tetraspanins can directly and indirectly influence EGFR signaling and internalization. However, the molecular mechanisms by which tetraspanins modulate EGFR signaling are not fully understood. Here, we assess the role of tetraspanin CD82 in the regulation of EGFR signaling. Using multiple Super Resolution (SR) microscopy techniques to achieve sub-diffraction limit resolution, we evaluate the molecular organization of EGFR and CD82 on the plasma membrane of cells under basal and EGF-activated conditions. Clustering analysis of reconstructed SR images reveals that expression of CD82 enhances ligand-independent clustering of EGFR but restricts ligand-dependent changes in EGFR organization. Moreover, EGF treatment is also found to alter the molecular organization of the CD82 scaffold. Additional biochemical analyses show that while CD82 can attenuate EGFR signaling, this attenuation is specific to discrete EGFR phosphorylation sites. Furthermore, single-particle tracking and receptor internalization analyses show that CD82 expression modulates EGFR dynamics, resulting in altered receptor mobility and enhanced internalization. Ongoing mutational studies of CD82 provide mechanistic insight into key residues responsible for the CD82-mediated regulation of EGFR signaling. Collectively, these studies suggest that the CD82 scaffold regulates EGFR signaling through modulation of molecular organization and receptor dynamics and implicate CD82 as a potential target for improving EGFR-directed therapies.

B469/P2852

UNIQUE responses of lens epithelial cells induced by nuclear cataract formation in AQP0 mutant mice

W.-K. Lo, S. Biswas; Morehouse School of Medicine, Atlanta, GA

Objective: A single epithelial layer is located anteriorly over the entire underlying fiber-cell mass of the lens. AQP0 water channel protein is distributed in the fiber cell membrane whereas AQP1 is in the

epithelial cell membrane. In the AQP0-KO mice, a distinct cataract is consistently formed in the fiber cells of the lens core. This study investigated unique responses in the lens epithelium induced by cataractogenesis in lens fibers of AQP0-KO mice. Methods: Immunolabeling of AQP1, Cx43, and EMT markers, and cell biology techniques were used to examine lenses from WT and AQP0-KO mice (P4-12wks old). Results: In WT, strongly labeled AQP1 was mainly observed in the equatorial epithelial cells (~250 μm along the epithelial layer) on frozen sections and wholemount samples. Only a trace or absence of AQP1 labeling was seen in the central epithelium. In contrast, the AQP0-KO lenses showed significant upregulation of strong AQP1 labeling in the entire anterior epithelial cells at various ages. Furthermore, epithelial-mesenchymal transition (EMT) was also induced by AQP0 deficiency. Using EMT markers, all WT lenses showed negative labeling for α-actin (α-SMA), fibronectin, and vimentin antibodies. In contrast, α-actin was strongly labeled in the entire epithelial layer in AQP0-KO lenses at P8 and older. Several distinct clusters of labeled epithelial cells in multiple layers were seen overlying the anterior lens fiber sutures. Strongly labeled fibronectin was regularly seen in the clusters of migrating EMT cells in the fiber-cell mass. Interestingly, at 2 weeks old, the migrated clusters of labeled mesenchymal cells had reached the lens core, seemingly passing through the enlarged anterior lens suture spaces. These EMT cells showed positively labeled α-actin, fibronectin, Cx43, and AQP1 in the disorganized lens nuclear cell region. By TEM analysis, 10 nm vimentin filaments were seen extensively distributed in EMT cells in the epithelium. Conclusions: AQP1 was significantly overexpressed in the anterior lens epithelium of AQP0-KO mice. This would cause a large increase in water influx from the epithelium into underlying fibers and result in further damage to the cataractous fiber cells. Due to the concomitant induction of EMT by AQP0 deficiency, some epithelial-mesenchymal cells eventually migrate into damaged nuclear fiber cell regions to form a large cataractous mixture containing both epithelial and fiber cell materials.

Mitochondrial Structure and Morphology

B470/P2853

Uncovering the mysterious role cardiolipin plays in shaping mitochondrial structure

K. Venkatraman, I. Budin; Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA.

Cardiolipin (CL) is a unique phospholipid exclusively localized and synthesized in the inner mitochondrial membrane (IMM). In mammalian systems, CL plays numerous mitochondrial roles in optimizing ATP synthesis and shaping cristae formation. However in yeast, it has been a long-standing mystery as to why knockouts of cardiolipin synthase (Crd1p) or CL remodelling enzymes (Taz1p and Cld1p) do not result in any differences in mitochondrial morphology or function. Here we show that CL becomes essential for mitochondrial structure in more saturated lipid environments, as shown via genetic modification of the lipid desaturase, Ole1p, and through microaerobic growth conditions, the latter of which is a hallmark for natural yeast environments. Our results also demonstrate the importance of CL remodelling in increasing the intrinsic curvature and membrane bending capabilities of CL molecules, and thus accentuates the mechanical role CL plays in bending of stiffer membranes. Together, our findings demonstrate that mitochondrial structure is dependent on physical contributions of CL and its interaction with the lipid environment.

B471/P2854

Molecular mechanism of TIM23-mediated mitochondrial protein import revealed by cryo-EM structure and crosslinking analysis

S. I. Sim, Y. Chen, E. Park; Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

The identity of membrane-bound organelles in eukaryotic cells depends on their protein composition. The targeting and transport of proteins across membranes is thus critical for organelle function and cellular homeostasis. While protein translocation at the endoplasmic reticulum is relatively well understood, the mechanisms of protein import into mitochondria are poorly defined. Mitochondria are essential organelles that have important roles in oxidative respiration, metabolism, and cell signaling. Approximately 99% of mitochondrial proteins are synthesized as precursors in the cytosol and must be imported into the organelle. Mitochondrial protein import is enabled by membrane-bound translocases, which thread hydrophilic polypeptides through the hydrophobic lipid bilayer during transport. The TIM23 (Translocase of the Inner Membrane 23) complex recognizes presequence-containing precursor proteins (preproteins) and mediates their translocation into the mitochondrial matrix and inner membrane. Though the subunits of TIM23 have been identified through biochemical and genetic studies, the organization of the complex and the molecular basis of its activity remained unclear due to a lack of structural information. Here, we present a 2.7 Å cryo-EM structure of the core TIM23 complex (Tim17-Tim23-Tim44) from *Saccharomyces cerevisiae* accompanied by in-depth biochemical analysis to define the preprotein translocation path in TIM23. Contrary to prevailing models, the membrane protein subunits Tim17 and Tim23 do not form an aqueous pore, but instead have separate, lipid-exposed cavities that face in opposite directions. Using mutational analysis and site-specific crosslinking, we show that the cavity of Tim17 forms the translocation path, while Tim23 plays more of a structural role. In addition, the non-essential subunit Mgr2 acts in coordination with Tim17 to seal the lateral opening of this cavity during active preprotein translocation. Our work forms the structural foundation to understand the mechanism of TIM23-mediated protein import in atomic detail.

B472/P2855

Linkage of Myosin 19 to the Mitochondrial Intermembrane space Bridging (MIB) complex

S. Shembekar, A. Attia, P. Nikolaus, U. Honnert, K. Topp, B. Lohmann, M. Bähler; Institute of Integrative Cell Biology and Physiology, WWU Muenster, Muenster, GERMANY.

The actin-based motor myosin 19 (Myo19) localizes to mitochondria and regulates the intracellular distribution of mitochondria in interphase and their inheritance during cell division. The outer mitochondrial membrane (OMM) proteins Miro1/2 that serve as receptors for microtubule-based motors interact directly with Myo19 and stabilize it. In absence of Myo19, mitochondrial inner membrane cristae architecture is disturbed along with impaired OXPHOS and enhanced ROS production. Cristae structure is regulated by the MICOS complex that is essential for cristae junction formation. The MICOS complex is part of a larger Mitochondrial Intermembrane Bridging (MIB) complex that connects the outer and inner membranes of mitochondria. A Turbo-ID screen with full-length Myo19 demonstrated that in addition to Miro1/2, metaxin-3 (Mtx3), a MIB component, and Mic60, a MICOS component, are in close contact to Myo19 *in vivo*. Immunoprecipitation experiments showed that Mtx3 and Mic60 co-precipitated with Myo19, supporting the notion that Myo19 interacts with the MIB complex to maintain structural stability of cristae. To elucidate further how Myo19 couples to the inner mitochondrial membrane (IMM) and regulates cristae structure, we generated Miro1KO, Miro2KO and

Miro1/2DKO cells. Because the lack of Miro1/2 leads to the degradation of Myo19 as mentioned above, we will supplement these cells with mutated Miro1/2 constructs to assess their potential involvement in the linkage of Myo19 to the MIB/MICOS complex. As stated previously, Mtx3 is a potential interactor of Myo19, whose role in MIB/MICOS complex is largely unknown. We will determine the functional significance of Mtx3, especially the role it might have in linking Myo19 to the MIB complex, by using Mtx3KO cells. To understand the formation of cristae architecture, a detailed characterization will be needed of how Myo19 transduces its directed force produced along actin filaments collectively to the outer and inner membranes of mitochondria.

B473/P2856

Identification of a functional metazoan ortholog of a fungal mitochondrial morphology regulator

A. R. Edington, J. Friedman; University of Texas Southwestern Medical Center, Dallas, TX.

Mitochondria are elaborately shaped and highly dynamic organelles whose function is dependent upon their ability to maintain proper morphology. Mitochondrial morphology is maintained in part through the conserved processes of division and fusion. Aberrant mitochondrial morphology is associated with organelle dysfunction and an underlying feature of numerous diseases, including neurodegeneration. While unbiased screens in budding yeast have identified many proteins that are required for normal mitochondrial morphology, many of these factors are not conserved in metazoans and/or have not been functionally characterized. One such protein, which we rename Mitochondria Division Regulatory Protein 1 (Mdp1), has been suggested to regulate mitochondrial morphology by facilitating phospholipid homeostasis and aiding in efficient mitochondrial division. However, Mdp1 lacks a sequence-conserved ortholog in metazoans and its specific functional role is poorly understood. Here, we use Alphafold2 to identify a potential structural analog in humans, which we name MIDP. Depletion of MIDP leads to the formation of lamellar mitochondrial structures, similar to those found in yeast Mdp1 deletion cells. Furthermore, we found that overexpression of human MIDP fragments the mitochondria in a DRP1-dependent manner, akin to Mdp1 in budding yeast, suggestive of a conserved role in mitochondrial division. Consistent with this, using live cell confocal microscopy, we find both MIDP in humans and Mdp1 in yeast can be spatially linked to sites of mitochondrial division. Interestingly, we find that acute depletion of MIDP in human cells causes the mitochondria network to become hyperfused prior to lamellae formation, further implicating the proteins in the regulation of mitochondrial division. Finally, we find that MIDP can rescue the mitochondrial morphology defects of yeast Mdp1 deletion cells, indicating these proteins are indeed functionally conserved. Together, our data suggests that Mpd1 and its structural ortholog MIDP play analogous roles in the regulation of mitochondrial division.

B474/P2857

Novel role for HSPE1 in OPA1 cleavage independent of co-chaperonin HSPD1

N. Yeung, D. Murata, M. Iijima, H. Sesaki; Johns Hopkins University School of Medicine, Baltimore, MD

The majority of the proteins in the mitochondria are imported from the cytoplasm, which requires the protein to be in an unfolded state. A series of chaperones/chaperonins are needed to ensure these proteins are correctly refolded within the mitochondria. The chaperonin, HSPD1, and co-chaperonin, HSPE1, are responsible for ensuring correct folding of proteins in the mitochondrial matrix and defects in this complex are implicated in cancer and neurodegenerative disorders. Our study aims to address how disruption to the HSPD1 and HSPE1 complex results in dysfunctional mitochondria. The depletion of

HSPD1 or HSPE1 leads to mitochondrial fragmentation indicating that these chaperonins could be important in mitochondrial fusion. We observe that only the depletion of HSPE1 results in the inactivation of OPA1, a dynamin related GTPase that is critical for mitochondrial fusion. We demonstrate that this inactivation is mediated by OMA1 which is a stress-activated metalloprotease. The data here suggests a novel role for HSPE1 in regulating mitochondrial morphology that is independent of its chaperonin activity with HSPD1.

Kinases and Phosphatases 2

B476/P2858

The mechanism of Cdc14-regulated Rim4-mRNA complex formation during meiosis

W. Feng[1], **R. Zhang**[2], S. Qian[2], F. Wang[2]; [1]Department of Cell Biology, Harvard Medical School, Boston, MA, [2]Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX.

Meiotic gene expression was regulated posttranscriptionally by RNA binding proteins (RBP), e.g., yeast Rim4, that bind meiotic transcripts to ensure their timely translation during meiotic divisions. At the same time, the mechanism that controls general RBP-mRNA dynamics during meiosis remains unclear. Using genetic, biochemical, and cell imaging approaches, we identified a *bona fide* Cdc14 docking site in Rim4's C-terminal low complexity domain (LCD) region, near Rim4's RNA recognition motif. Mutating this site (Rim4[PxLm]) alone causes severe nuclear retention of Rim4 due to impaired nuclear Rim4(PxLm)-mRNA assembly that consequentially drives Rim4 nuclear export. Using a systematic mutagenesis approach, we identified several Cdc14-targeted phosphorylation sites (P-sites) on Rim4. Upon alanine replacement mimicking dephosphorylation on these P-sites, the nuclear retention of Rim4(PxLm) was rescued, indicating that Cdc14 dephosphorylate Rim4 to facilitate Rim4-mRNA assembly in the nucleus. In addition, we discovered that phosphorylated (Rim4-p) forms a complex with Bmh1/2 (14-3-3 family proteins) heterodimer in a phosphorylation-dependent manner, while Bmh1/2-Rim4 excludes mRNAs. Thus, dephosphorylation facilitates Rim4-mRNA assembly with a dual mechanism, i.e., by modulating RRM-mRNA interface with site-specific negative charges and freeing Rim4 from Bmh1/2 to stimulate the Rim4-mRNA complex. Our data further show that Bmh1/2 protects Rim4 from autophagic degradation, as mRNA binding does. The mRNA- and Bmh1/2-free Rim4 stimulates Atg1, resulting in its selective degradation by autophagy during meiotic divisions. Thus, we demonstrate that the cell cycle phosphatase Cdc14 is a master regulator of Rim4's subcellular localization, meiotic functions (Rim4-mRNA complex formation), and stability, and thereby a modulator of meiotic translation.

B477/P2859

***Ocrl* mutation leads to reduced neuronal excitability and astrocyte dysfunction in an induced pluripotent stem cell model of Lowe Syndrome**

Y. Sharma, R. Padinjat; National Center for Biological Sciences NCBS-BLR-20121272, Bangalore, INDIA.

Objective: Mammalian brain development is a complex process that involves generation of neural stem cells, followed by their timely maturation into neurons, astrocytes and oligodendrocytes. Perturbations in any of these key events can result in neurodevelopmental disorders. Lowe Syndrome (LS) is an X-linked recessive disorder that presents in patients with brain defects such as delayed cognitive milestones, seizures, hypotonia and gliosis. LS is caused by mutations arising in *OCRL* that encodes for a

phosphoinositide 5-phosphatase enzyme that catalyzes the removal of the 5' phosphate from PI(4,5)P2 to generate PI4P. However, the mechanism by which absence of *OCRL* results in alterations in the brain remains to be elucidated.
Methodology: To address this, we identified a south-Indian family, consisting of monozygotic twins clinically diagnosed with LS and thereby reprogrammed their peripheral blood mononuclear cells into induced pluripotent stem cells (iPSCs). The LS patient and neurotypical control iPSCs were further patterned into dorsal forebrain neural progenitors, that were terminally differentiated into mature neurons and maintained for 30 days in vitro (DIV).
Results: We found that LS neural cultures maintained for 30 DIV, had increased expression of mature astrocytic marker, glial fibrillary acidic protein (GFAP) relative to the control. This finding is in line with the MRI scans of LS patients that have been reported to exhibit hyperechoic periventricular lesions that are indicative of gliosis. Thus, our iPSC-derived neural model system is able to recapitulate a feature of LS pathology. Further, to understand if there are any defects at functional level, we performed calcium imaging and observed that 30 DIV LS neurons exhibited reduced excitability compared to the control neurons. Lastly, we also verified these phenotypes by generating a protein-truncating mutation in *OCRL* using CRISPR-Cas9 technology in the background of another control iPSC line NCRM5.
Conclusion: Overall, the above findings suggest that *OCRL* is indeed instrumental in regulating neuronal differentiation and spontaneous neuronal activity. Ongoing efforts are focused on understanding the mechanism by which OCRL exerts its functions during early stages of neurogenesis.

B478/P2860

New genetically-encodable tools to probe the nuanced regulation of the Extracellular signal-regulated kinase (ERK) pathway

J. Keyes[1,2], K. Slye[1], T. Romania[1], A. Kotau[1], A. Kaib[1], C. Scott[1]; [1]Biology, Pennsylvania State University Erie, Erie, PA, [2]Allied Member, Magee Womens Research Institute, Pittsburgh, PA.

The Extracellular signal-regulated kinase (ERK) signaling axis is activated by a host of divergent signaling molecules which each induce their own specific responses. These cellular responses include fundamental processes such as proliferation, differentiation, migration, and, under certain conditions, even apoptosis. Thus, the ubiquitous utilization of ERK signaling in variable and contrasting cellular processes makes it difficult to inhibit the pathway without causing adverse side effects. Furthermore, dysregulation of the Ras/Raf/MEK/ERK signaling pathway is directly implicated in a variety of disorders ranging from cancer to diabetes. Here, we report new tools to elucidate the nuanced mechanisms of differential regulation of the ERK pathway. First, we report a new genetically-encodable tool to inhibit ERK at specific subcellular locations, allowing us to explore the specific function and mechanisms of regulation of ERK with extremely precise subcellular resolution. Additionally, we report a new genetically-encodable fluorescent biosensor based on previous generations of the ERK-kinase activity reporter (EKAR). Our new biosensor, which we have termed reDox-EKAR1, allows us to probe into the spatiotemporal activity of ERK towards a different class of substrates than substrates probed by previous generations of EKAR. Utilizing these new tools, we report how specific pools of ERK regulate particular cellular functions previously underappreciated in the field, and we present our preliminary results detailing how these specific subcellular pools of ERK are differentially regulated.

B479/P2861

Proximity proteomics identifies network of lipid metabolism regulators in JAK2 V617F interactome

M. Petersen, A. Chorzalska, A. Rodriguez, D. Bonal, A. Olszewski, P. Dubielecka; Brown University, Providence, RI.

Background: Janus kinase 2 (JAK2) is a non-receptor tyrosine kinase that activates cell survival/proliferation transcriptional programs in response to growth factors and cytokines, through signal transducers and activators of transcription (STAT) family of transcription factors. JAK/STAT upregulation is commonly seen in myeloproliferative neoplasms (MPN), and associated JAK2 V617F mutation is seen in a majority of MPN. JAK2-targeted therapeutics improve symptoms but do not alter underlying disease.
Objective: To understand JAK2 V617F signaling patterns in malignant transformation, we measured JAK2 WT and JAK2 V617F cellular interactomes by proximity dependent biotin labeling and mass spectrometry (MS).
Methods: Retroviral (MSCV-IRES-GFP) vectors expressing JAK2 WT or JAK2 V617F fused with biotin ligase (TurboID, PMID: 30125270) were used to transduce murine bone marrow FDC-P1 cells. FDC-P1 cell lines stably expressing TurboID, TurboID-JAK2 WT, or TurboID-JAK2 V617F were stimulated with 50 μM biotin for 3 hours. Cells were lysed and biotinylated proteins enriched using streptavidin-conjugated beads. On-bead trypsin digestion followed by MS determined a pool of biotinylated proteins representing proximal interactors of TurboID, TurboID-JAK2 WT, or TurboID-JAK2 V617F. Significantly enriched TurboID-JAK2 V617F or TurboID-JAK2 WT proximal interacting proteins were designated as 1.5-fold more enrichment (FDR ≤ 0.05) over TurboID, and 1.5-fold difference in enrichment (FDR ≤ 0.05) between TurboID-JAK2 V617F and TurboID-JAK2 WT.
Results: 441 proteins were significantly enriched in TurboID-JAK2 WT over TurboID control and TurboID-JAK2 V617F. 330 proteins were significantly enriched in TurboID-JAK2 V617F over TurboID control and TurboID-JAK2 WT. Top 10 Gene Ontology biological process (GO BP) terms enriched exclusively in TurboID-JAK2 WT included “Positive regulation of establishment of protein localization to telomere”, and “Glucose catabolic process”. GO BP terms exclusively enriched in TurboID-JAK2 V617F included “Cellular response to interleukin-3”, “Fatty acid beta-oxidation”, and “Mitochondrial gene expression”.
Conclusions: Our data indicate that JAK2 V617F is associated with changes in cellular energy metabolism, characterized by increased association with proteins involved in fatty acid beta-oxidation, and decreased association with proteins involved in glucose metabolism. These data are consistent with the report of Skoda group showing that expression of JAK2 V617F resulted in systemic metabolic changes including hypoglycemia, adipose tissue atrophy, and early mortality (PMID: 31511238). Further studies are warranted to determine druggable interactors to revert JAK2 V617F-dependent pathologies.

B480/P2862

Prolonged inhibition of Androgen Receptor Signaling induces expression of Nuclear ErbB3 which renders Prostate Cancer cells susceptible to targeted inhibitors

M. Jathal[1], P. M. Ghosh[2], M. M. Mudryj[1]; [1]Medical Microbiology and Immunology, University of California - Davis, Davis, CA, [2]Urologic Surgery, University of California - Davis, Davis, CA.

Objective: Nuclear expression of the receptor tyrosine kinase (RTK) ErbB3/HER3 increases inhighly aggressive prostate cancer cells, but its nuclear transport mechanism is currently unknown.Prostate tumors rely on the androgen receptor (AR), whose activation transcriptionally controlsErbB3 expression,

but its role in ErbB3 nuclear localization had not previously been reported.Here, we investigated the mechanism by which subcellular localization of ErbB3 was altered inprostate cancer.Methods: ErbB3 localization was investigated in the human prostate cancer tumor progressionmodel LNCaP, C4, C4-2 and C4-2B. ErbB3 was stimulated with heregulin-1β (HRG). Nucleartranslocation was tested with a nucleocytoplasmic transport inhibitor panel (chlorpromazine,filipin III, amiloride and Leptomycin B) LNCaP and C4 cells were continuously cultured with theAR activation inhibitor abiraterone acetate (AbiAc) or ethanol (VEH) or treated with the ARinhibitors enzalutamide, darolutamide and apalutamide. ErbB3 activity and subcellularlocalization were analyzed using confocal microscopy/subcellular fractionation/immunoblot. Cellviability was determined by MTT assay. Proliferation and apoptosis were determined by flowcytometry. Invasive potential was investigated by crystal violet staining of cell colonies. ErbB3expression was reduced by siRNA technology.Results: The ratio of nuclear to cytoplasmic ErbB3 increased in untreated cells from LNCaP<C4-2<C4<C4-2B. In all four lines, nuclear localization of ErbB3 peaked at 30 minutes after HRGtreatment, with a rapid return to the cytoplasm in LNCaP cells (at 1 hour), slower in C4 cells (at 4hours), and continued accumulation in the nucleus in C4-2 and C4-2B 8 hours following HRGtreatment. Treatment with the transport inhibitor panel showed that nuclear accumulation of ErbB3was prevented by the clathrin-dependent endocytosis inhibitor Chlorpromazine (CPZ) but not bythe others. In LN-VEH and C4-VEH cells, HRG induced nuclear translocation of ErbB3 but notin LN-AbiAc or C4-AbiAc cells. Accordingly, HRG increased ErbB3 phosphorylation at Y1328and nuclear Akt phosphorylation at S473 in VEH but not AbiAc cells. C4 cells, which had highbaseline nuclear ErbB3, were more sensitive to the AR inhibitors and showed decreased viabilityand invasive potential compared to LNCaP cells.Conclusions: These results indicate that (1) ErbB3 nuclear localization required clathrin-dependentendocytosis (2) ligand binding of ErbB3 as well as the presence of an active AR is necessary forErbB3 nuclear localization and (3) the presence of nuclear ErbB3 increases sensitivity to ARinhibitors.

B481/P2863

Role of ras in regulating nuclear dynamics

H. Baek, S. Arur; University of Texas/MD Anderson Cancer Ctr-52168088, Houston, TX.

Role of RAS in regulating nuclear dynamics

Han Bit Baek and Swathi Arur

Genetics and Epigenetics Program, Department of Genetics, The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, Houston, TX

The Rat Sarcoma protein (RAS) is a small GTPase that transmits extracellular growth factor signals through a downstream kinase cascade to regulate genes that promote proliferation, differentiation, and survival. Thus, mutations in RAS that render it constitutively active lead to oncogenesis and promote tumor onset and metastasis. In addition to somatic mutations in RAS that lead to cancer onset, germline mutations in RAS have been implicated to cause birth defects in humans, known as RASopathies. Whether the mechanisms through which activating mutations in RAS drive cancer progression and cause birth defects are convergent, remain unknown. To determine these mechanisms, I use the *C. elegans* model system to track the impact of activating mutations in RAS on embryonic development. The L19F oncogenic mutation in the GTPase domain of RAS is found in human cancers, yet how it leads to cancer onset remains unknown. Using a *C. elegans* mutant line with the L19F mutation, I discovered for the first time that an oncogenic RAS mutation leads to defects in nuclear dynamics and the formation of two nuclei per cell in the developing embryo. Specifically, I observed that the two nuclei arise during the

transition from the one to two cell stage of the embryo. This is a critical period in early embryo development because the maternal and paternal nucleus, contributed by the oocyte and sperm, must fuse to create the zygotic nucleus prior to the first cell division event. I hypothesize that the failure to fuse the maternal and paternal nucleus, followed by cell division leads to the dual nuclei phenotype in RAS L19F embryos. To test this hypothesis, I used live imaging to investigate a critical step in nuclear fusion: the ability for existing nuclear envelopes between the two nuclei to break down, a process known as nuclear envelope breakdown (NEBD). Thus, I assayed for nuclear dynamics during the first cell division and observed that there was a significant delay in NEBD in the RAS L19F embryos. In the most extreme cases, NEBD failed to occur between the maternal and paternal nucleus leading to the dual nuclei phenotype in the activated RAS mutant embryos. This is the first observation that oncogenic mutations in RAS may lead to a delay in NEBD during mitosis, setting the stage to uncover a novel role of RAS during cell division.

B482/P2864

Phosphorylation of Tyrosine 841 Play a Significant Role in JAK3 Kinase's Functions.

S. Sun[1], L. Li[2]; [1]THE UNIVERSITY OF TEXAS AT EL PASO, El Paso, TX, [2]THE UNIVERSITY OF TEXAS AT EL PASO, El paso, TX.

Phosphorylation of Tyrosine 841 Play a Significant Role in JAK3 Kinase's Functions.

AbstractJanus Kinase 3 (JAK3) plays a key role in the development, proliferation, and differentiation of various cells. It regulates gene expression by phosphorylation of Signal Transducer and Activators of Transcriptions (STATs). Recently, we found a new JAK3 kinase domain phosphorylation site, tyrosine-841 (Y841). The effects of phosphorylated tyrosine-841 (pY841) on ATP/ADP binding affinities of the JAK3 kinase domain were systematically studied and reported here. The results show that pY841 reduces the size of the cleft between the N-lobe and C-lobe of the JAK3 kinase domain. However, pY841 was found to enlarge the cleft when an ATP/ADP is bound to the kinase. For unphosphorylated JAK3 (JAK3-Y841), the binding forces between the kinase domain and ATP or ADP are similar. After phosphorylation of Y841, JAK3-pY841 exhibits more salt bridges and hydrogen bonds between ATP and kinase than ADP and kinase. Consequently, the electrostatic binding force between ATP and kinase is higher than that between ADP and kinase. The result is that compared to ADP, ATP is more attractive to JAK3 when Y841 is phosphorylated. Therefore, JAK3-pY841 tends to bind ATP rather than ADP.

B483/P2865

Characterizing the relationship of LGL1 and AKT in neural stem cells

A. Sprowles[1], N. Pedicino[1], S. Yang[1], J. Trzeciak[1], M. King[1], B. Light[1], Y. Hirata[2], L. Colegrove[1], S. Otis[1], M. Lacourse[1], H. Collins[1], C. Petritsch[2]; [1]Biological Sciences, Cal Poly Humboldt, Arcata, CA, [2]Neurosurgery, Stanford University, Palo Alto, CA.

Asymmetric cell division and migration are critical for neural stem cell differentiation and brain development. When these processes are dysregulated in neural progenitor cells (NPCs), developmental defects and diseases like glioblastoma multiforme (GBM) can result. *Lgl1* is a tumor suppressor gene first characterized in *Drosophila* neuroblasts that is best known for its regulation of apical-basal cellular polarity through its association with the Par complex. The PI3K/AKT signaling cascade is also regulated by Par signaling and involved in cellular migration. To investigate the role of LGL1 on the regulation of NPC migration, NPCs were cultured from genetically matched $Lgl1^{-/-}$ and $Lgl1^{+/+}$ primary cell lines

isolated from the subventricular zone (SVZ) and corpus collosum (CC) of P30 mice. Spheroid and scratch test migration assays revealed that loss of *Lgl1* increased migration rates by up to two-fold, demonstrating that LGL1 regulates NPC migration. To see if loss of *Lgl1* could be influencing migration through PI3K/AKT signaling, we used targeted drugs to inhibit mTOR and PI3K in the same cellular migration assays and evaluated the phosphorylation states of AKT in the same genetically matched cell lines and in the subventricular zone of GFAP CRE Lgl1 knock out vs. wild type mice. Our findings suggest that changes in Akt phosphorylation through PDK/PI3K sites are a key link between LGL1 and the migratory defects we observed. Our results are also consistent with findings that loss of *Lgl1* increases rates of cellular migration. Our results contribute to the understanding how loss of cell polarity affects neural cell differentiation, cancer cell properties, and GBM progression.

B484/P2866

Cip1 tunes cell cycle arrest duration upon calcineurin activation

M. J. Flynn, J. A. Benanti; University of Massachusetts Chan Medical School, Worcester, MA.

Cells exposed to environmental stress arrest the cell cycle until they have adapted to their new environment. Cells adjust the length of the arrest for each unique stressor, but how they do this is not known. Here, we investigate the role of the stress-activated phosphatase calcineurin (CN) in controlling cell cycle arrest in *Saccharomyces cerevisiae*. We find that CN controls arrest duration through activation of the G1 cyclin-dependent kinase inhibitor Cip1. Our results demonstrate that multiple stressors trigger a G1/S arrest through Hog1-dependent downregulation of G1 cyclin transcription. When a stressor also activates CN, this arrest is lengthened as CN prolongs Hog1-dependent multisite phosphorylation of Cip1. Cip1 plays no role in response to stressors that activate Hog1 but not CN. These findings illustrate how stress response pathways cooperate to tailor the stress response and suggest that Cip1 functions to prolong cell cycle arrest when a cell requires additional time for adaptation.

B485/P2867

A Subset of Lowe Syndrome-Causative Mutations Lead to a Conformational Disease Scenario Counteracted by Allosteric Activator Candidate Drugs

J. J. Lee[1], S. Ramadesikan[2], S. Mundre[1], A. F. Pacheco[1], L. E. Skiba[1], R. C. Aguilar[1]; [1]Biological Sciences, Purdue University, West Lafayette, IN, [2]The Steve and Cindy Rasmussen Institute for Genomic Medicine, Nationwide Children's Hospital, Columbus, OH.

Lowe syndrome (LS) is a lethal, X-linked, multisystem lethal disease with no available cure. LS is caused by mutations in the *OCRL1* gene that result in an altered inositol 5-phosphatase, Ocrl1. Many patient missense mutations have been identified in the catalytic domain of this enzyme, but half of these changes do not affect residues essential for lipid binding or catalysis. We hypothesize that this subset of mutated proteins present an altered conformational equilibrium dominated by a conformer lacking biological function. Therefore, we speculate that allosteric activators able to stabilize the active conformer could restore the function of several variants. In this study, we aimed to characterize the functional consequences of six mutants and evaluate the rescue of these abnormalities by four candidate drugs. We purified recombinant, bacterially produced wildtype ($Ocrl1^{WT}$) and mutated variants of Ocrl1 ($Ocrl1^{MUT}$) for *in vitro* testing of their enzymatic activity. These studies indicated that that $Ocrl1^{MUT}$ could be segregated in 3 different groups with respect to $Ocrl1^{WT}$ activity: comparable (S256N, D431N), altered (M299I), and no (I393F, D451G, V508D) activity. We also observed a similar

trend in Ocrl1^{MUT}-expressing, *OCRL1*-null HK2 cells for previously described Golgi complex fragmentation LS phenotype and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) accumulation, where inactive variants showed more severe abnormalities compared to those with measurable catalytic ability. From several large-scale biochemical drug screens, we identified potential activators of Ocrl1 exhibiting partial rescue of otherwise absent D451G activity. As these tests were carried out with the isolated phosphatase domain, changes seen with drug treatment can be correlated to direct interaction with the catalytic domain. Follow up studies confirmed this rescue for other Ocrl1^{MUT}, with candidate LC9 increasing activity for all tested Ocrl1^{MUT}, even those with unaltered catalysis. Our study sheds light on the pathogenicity and heterogeneity of protein dysfunction seen in various LS causing mutations; in fact, it indicates that some patients exhibit characteristics of a conformational disease. Additionally, candidate drugs tested here present novel therapeutics for LS mediating allosteric activation of patient's Ocrl1^{MUT} *i.e.,* the disease causative as opposed to treatments aimed to alleviate downstream consequences of *OCRL1* mutations.

B486/P2868

Dsk1 and Kic1 kinases regulate cellular localization and protein expression of poly(A)-binding protein Pabp

A. Vyas[1], A. Freitas[2], M. Nurimba[3], C. Ylagan[3], A. Sugarman[3], Z. Tang[1]; [1]Claremont McKenna College, Claremont, CA, [2]Pitzer College, Claremont, CA, [3]Scripps College, Claremont, CA.

Dsk1 and Kic1/Lkh1 are orthologs of human SRPK1 and mammalian Clk/Sty kinases, respectively, in the fission yeast *Schizosaccharomyces pombe*. These two proteins belong to a superfamily of LAMMER-related kinases that have crucial roles in diverse cellular processes such as control of cellular viability, differentiation, as well as development. Dsk1 and Kic1 serve as model molecules to decipher the mechanisms by which the LAMMER-related kinases may exert on various cellular processes comment to eukaryotes. We previously discovered the effects of these kinases on pre-mRNA splicing, reported a temperature-sensitive (*ts*) phenotype of partial retention of poly(A)$^+$ RNA in the absence of Dsk1 and Kic1, and illustrated a biochemical association in a complex between Dsk1 and a poly(A)-binding protein, Pabp. These results led us to propose a role of these two kinases in nuclear export of mRNA in addition to their functions in pre-mRNA splicing, possibly through their modulation of the Pabp protein activity. Consistent with this model, using the "Green RNA" system we observed that transcripts of the intron-less *lid1*$^+$/*apc4*$^+$ gene (mRNA) are retained in the nucleus in *Δdsk1* or *Δkic1* mutants. This result excludes improper mRNA splicing as the cause of the defective mRNA export observed in the absence of these kinases and supports their distinct roles in both mRNA export and pre-mRNA splicing. To test this model further, we tagged the *pabp* gene with GFP at its genomic locus for characterizing the cytological and biochemical influence of the kinases on Pabp. Using fluorescence microscopy, we have shown that the cellular localization of Pabp is altered from a diffuse pattern throughout *wild-type* cells to a pattern of partial nuclear retention in the kinase deletion mutants. Based on our preliminary results from FISH assays, the changes in Pabp cellular localization also overlaps to a certain degree with the nuclear retention of poly(A)$^+$ RNA phenotype in the kinase-deletion mutants. Additionally, preliminary results from untreated and cycloheximide treated cultures, indicate reduced levels of full-length Pabp protein in the *dsk1*- and *kic1*- deletion strains when compared to *wild-type* strains. Our findings indicate Dsk1 and Kic1 may regulate Pabp function, thereby affecting multiple steps of gene expression including mRNA processing.

B487/P2869

Activating the cAMP pathway promotes NF-κB and TNF-α expression in LPS-treated Schwann Cells

C. Henry, P. Kimmel, M. Wilcox, A. Asirvatham; Misericordia University, Dallas, PA.

Schwann cells have been found to play a critical role in neuron repair following peripheral nerve injury. During nerve injury, the myelin sheath becomes damaged, stimulating Schwann cells to secrete cytokines and initiate an inflammatory response. This recruits immune cells to the site of injury so that the myelin debris can be cleared. Neuronal growth is then facilitated by heregulin and an unknown growth factor that stimulates the cAMP pathway. Although it is clear Schwann cells play a role in nerve repair, there is still yet to be known regarding the exact mechanisms by which they do so. One potential mechanism is via the nuclear factor kappa B (NF-κB) pathway. Lipopolysaccharide (LPS), a cell wall immunostimulatory component of Gram-negative bacteria, can be used to activate the NF-κB pathway and stimulate the production of inflammatory mediators, like tumor necrosis factor alpha (TNF-α). Preliminary studies on Schwann cells treated with different concentrations of LPS at various time points revealed that lower doses promoted cell growth compared to higher concentrations. To further explore the effects of LPS on cell proliferation, the role of NF-κB and TNF-α secretion were examined. It was hypothesized that cells treated with LPS and growth factors will express less NF-κB and TNF-α than cells treated with LPS only. Cells from the immortalized S16 cell line were treated with or without 1 μg/mL of LPS for 3 hours without growth factors (control media, N_2), 12.5 ng/mL heregulin (H), 2 μM forskolin (F), or H+F. Using immunoblotting, NF-κB expression, as measured by densitometry analysis and expressed as a percent control, was higher in LPS-treated cells than in unstimulated cells. Surprisingly, NF-κB expression was higher in LPS-treated cells with H, F, or H+F (1500%, 1243%, and 1557%, respectively) than without growth factors (526%). The increase in NF-κB expression for the LPS-treated cells was accompanied by an increase in TNF-α expression. As expected, TNF-α expression was highest in LPS-treated cells without growth factors (444%) and lowest in untreated cells with F or H+F (34% and 70%, respectively). In summary, it appears as though 1 ug/mL of LPS and growth factors may act synergistically to upregulate NF-κB while downregulating TNF-α expression. These findings suggest that, during nerve injury, there may be some crosstalk between the cAMP and NF-κB pathways in Schwann cells. Therefore, a better understanding of these pathways may reveal a therapeutic target for the treatment of nerve injury and inflammation.

B488/P2870

The critical role of Smad H2 helix region in BMP signaling

D. Sinha, T. H. Nguyen, M. Serpe; NIH/NICHD, Bethesda, MD

The critical role of Smad H2 helix region in BMP signaling

Debabrata Sinha, Tho Huu Nguyen and Mihaela Serpe
Section on Cellular Communication, *Eunice Kennedy Shiver* National Institutes of Child Health and Human Development, NIH, Bethesda, MD, 20892, USA.

The highly conserved BMPs control many aspects of neural development, including synapse development and plasticity. The *Drosophila* NMJ, a powerful system to study synapse development, relies on BMP signals for NMJ growth and for synapse maturation and function. BMPs trigger signaling by binding to hetero-tetrameric complexes of type I and type II receptors, BMPRIs and BMPRIIs; activated BMPRIs bind and phosphorylate the intracellular transducers, Smads (Mad in *Drosophila*). In flies, phosphorylated Mad (pMad) accumulates at two locations: (1) in the motoneuron nuclei (nuclear

pMad), where it modulates gene expression in response to canonical BMP signaling, and (2) at the NMJ synapses (synaptic/local pMad), where it shapes synapse composition as a function of synaptic activity. Genetic studies indicate that local pMad remains associated with its own kinase, the BMPRI, and that the conserved H2 helix (within the MH2 domain of Mad) may modulate the Mad-BMPRI interaction. Here we examine how Mad H2 helix influences the Mad-receptor interface.
Using Alpha fold, we modeled the BMPRI and the MH2 domain of Mad with and without the H2 helix and found that H2 removal changes the Mad-BMPRI interface without disrupting the overall MH2 organization. Several human genetic variants map to the H2 helix, including Smad1 $Y^{362}C$ mutation, identified in a patient with colonic atresia, and Smad5 $N^{361}D$ substitution and N^{361}-G^{365} deletion, found in patients with malformation of the heart and epileptic encephalopathy respectively. We generated the equivalent *Drosophila* Mad variants and found that the N^{361}-G^{365} deletion, but not the single point mutations, destroyed the H2 helix. However, none of these mutations affect the overall conformation of the MH2 domain. We expressed these Mad variants in S2 insect cells and noted they have mildly reduced protein stability compared to control Mad. Using an *in vitro* signaling assay, we found that Mad mutants induce significantly reduced levels of pMad in response to BMP signaling (Mad-$N^{361}D$<Mad-(N^{361}-G^{365})<Mad-$Y^{362}C$ < Mad wt). Our current efforts are focused on testing how Mad variants associate with the activated BMPRI at cell membranes. We are also using CRISPR/Cas9 methodologies to edit the *Mad* locus and examine the impact on H2 human variants on BMP signaling *in vivo*, in the context of a whole animal. Specifically, we will employ the powerful *Drosophila* NMJ system to examine the impact of these mutations on canonical and local BMP signaling *in vivo*.

B489/P2871

Live-cell Analysis of Akt Activity Using a Genetically Encoded, Fluorescent Kinase Translocation Reporter

S. Foltin[1], J. Rauch[1], L. Oupicka[1], M. Dilsaver[1], G. Filonov[1], G. Lovell[2], J. Trigg[2], C. Schramm[1]; [1]Sartorius, Ann Arbor, MI, [2]Sartorius, Royston, United Kingdom

The PI3K/Akt signaling pathway has been extensively investigated as a therapeutic target due to its mechanistic association with several hallmarks of cancer. Studying kinase activity in live cells over time can be challenging, as standard approaches are limited to end point assays. By using the Incucyte® Kinase Akt Lentivirus Reagent, we were able to monitor dynamic changes in Akt activity in live cells maintained in a physiologically relevant environment throughout the experiment. The reagent encodes a kinase translocation reporter based on a green fluorescent protein-tagged Akt substrate whose subcellular localization is phosphorylation-dependent. In addition, a red fluorescent nuclear marker is utilized to denote the nuclear/cytoplasmic boundary. When Akt is active, the fluorescent reporter is driven to the cytoplasm, while fluorescence remains localized in the nucleus when Akt is inactive. Responses to compounds targeting the PI3K/Akt kinase pathway, including allosteric Akt inhibitors MK2206 and API-1, competitive Akt inhibitors AZD5363 and Ipatasertib, and upstream PI3K kinase inhibitors LY294002 and PI-103 were evaluated. Quantification of treatment effects using the Incucyte® Live Cell Analysis System showed concentration-dependent inhibition of Akt activity for all compounds with varying kinetic profiles over 24 hours. To study activation of Akt, HeLa cells were cultured in the absence of serum to reduce Akt activity prior to stimulation with either epidermal growth factor (EGF) or recombinant insulin-like growth factor (R3-IGF-1). An increase in Akt activity was observed for both treatments, but kinetic measurements demonstrated a more sustained response to R3-IGF-1. Cell lines with mutations in the tumor suppressor PTEN showed no response to serum starvation or activation

with EGF or R3-IGF-1. Differential sensitivity to Akt inhibition between cell lines was also demonstrated. MK2206 decreased Akt activity in a similar concentration-dependent manner in T-47D and MDA-MB-231 cell lines. In contrast, measurements of red object count from the same cells revealed concentration-dependent inhibition of T-47D cell growth but little effect on MDA-MB-231 proliferation. Overall, these data highlight the utility of the Incucyte® Kinase Akt Lentivirus Reagent to provide valuable kinetic measurements of Akt activity using live cells.

B490/P2872

Luteinizing hormone stimulation of meiotic resumption in ovarian follicles by protein kinase A and a PPP-family phosphatase

J. R. Egbert[1], I. Silbern[2,3], K. M. Lowther[1], T. F. Uliasz[1], S.-P. Yee[1], H. Urlaub[2,3], L. A. Jaffe[1]; [1]UConn Health, Farmington, CT, [2]Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany, [3]University Medical Center Göttingen, Göttingen, Germany

In mammalian preovulatory ovarian follicles, meiotic arrest of fully-grown oocytes is maintained by cyclic guanosine monophosphate (cGMP) that is produced by the membrane guanylyl cyclase natriuretic peptide receptor 2 (NPR2) in the follicle's somatic cells and diffuses through gap junctions into the oocyte. Phosphorylation of several juxtamembrane serines and threonines is essential for full NPR2 activity. The cyclic surge of luteinizing hormone (LH) acts on receptors in the outermost somatic cell layers to rapidly dephosphorylate and inactivate NPR2, lowering cGMP levels in the follicle and oocyte to trigger meiotic resumption. Our goals are to determine whether protein kinase A (PKA) signaling mediates this process, and which LH-activated PPP-family phosphatase dephosphorylates NPR2. Application of the specific PKA inhibitor Rp-8-CPT-cAMPS (Rp) to isolated mouse follicles prior to LH treatment (30 min) prevented NPR2 dephosphorylation, showing that phosphatase activation is mediated by PKA. To identify PPP-family regulatory subunits that undergo a rapid LH-stimulated increase in phosphorylation, lysates of rat follicles treated with or without LH were enriched for phosphorylated peptides and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) utilizing chemical labeling with tandem mass tags (TMT) for quantification. PPP1R12A and PPP2R5D emerged as two primary candidates. In PPP1R12A, peptides containing phosphorylated S507 residue had ~5-fold higher intensity in LH-treated follicles. PPP2R5D had four sites with elevated intensity with LH: S53 and S566 (10-fold and 4-fold, respectively), and S81/S82 (~2-fold). Quantitative western blotting confirmed these results and showed that inhibition of PKA activity with Rp prevented the LH-induced phosphorylation changes. To determine whether phosphorylation of one or both proteins mediates NPR2 dephosphorylation after LH, we have made mice in which S507 of PPP1R12A or S53/S81/S82/S566 of PPP2R5D are replaced with alanines, and are testing their role in this process. Thus, we have identified two PPP-family regulatory subunits that are rapidly phosphorylated in response to LH-PKA signaling as candidates for effecting NPR2 dephosphorylation, an event required for timely oocyte meiotic resumption.

B491/P2873

Investigating the role of Phosphoinositide-3 Kinases PikF, PikG, and PikH in multicellular development in *Dictyostelium Discoideum.*

H. Drebin, S. Farinas, D. Ali, J. Shen, **M. Edwards**; Amherst College, Amherst, MA

Phosphoinositide-3-kinases (PI3Ks) play crucial roles in multiple signaling networks and are strongly conserved across eukaryotes. Seven PI3Ks have been identified in the genome of the slime mold model *Dictyostelium discoideum*. However, none have been classified as homologs to human Class II PI3Ks . This work suggests that as many as three *Dictyostelium* PI3Ks-PikF, PikG, and PikH-may be functionally analogous to human Class II PI3Ks based on apparent substrate specificity. Furthermore, we demonstrate that two of these three PI3Ks are physiologically vital for *Dictyostelium* to proceed properly through multicellular development.Multiple sequence alignments of human and *Dictyostelium* PI3Ks reveal nearly identical homology in the PI3K catalytic domain region that, in humans, has been biochemically determined to confer substrate specificity. PikF, PikG, and PikH have uncharged residues in place of one of the two basic residues that is necessary for Human Class I PI3Ks to synthesize $PI(3,4,5)P_3$. Class II PI3Ks possess an identical apparent substrate specificity to that of PikF, PikG, and PikH, and suggests that, like human Class II PI3Ks, these *Dictyostelium* PI3Ks may synthesize PI(3)P and $PI(3,4)P_2$ in vivo. Alphafold structural predictions of the catalytic domain further substantiate the conserved homology between human and *Dictyostelium* PI3Ks. In addition, we present evidence for a prominent role for PikF, PikG in multicellular development in *Dictyostelium*. *pikf-* cells display a profound defect in streaming and aggregation during development and additional defects in stalk development. Similarly, *pikg- fail to initiate development altogether.* Our results demonstrate previously undescribed roles for putative Class II PI3K homologs and strengthen our understanding of the conserved homology between PI3Ks in Humans and *Dictyostelium*.

B492/P2874

Calcium modulates Rho-kinase activity during collective cell migration

A. E. Mancini, D. P. Isaacs, M. A. Rizzo; University of Maryland, Baltimore, Baltimore, MD

Collective cell migration (CCM) drives physiological processes such as embryogenesis, vascular sprouting, and wound healing, but also drives cancer metastasis. Ras homolog family member A (RhoA) drives CCM through modulation of the actomyosin cytoskeleton. Because of the tight spatiotemporal control of RhoA during CCM, we decided to investigate the role of RhoA's downstream effector, Rho-associated kinase (ROCK), in collectively migrating fibroblasts. We have observed calcium-dependent activation of ROCK with a new FRET-based ROCK sensor. Using a modified scratch assay, we have quantitated ROCK activity in parallel with CCM. Scratch wounding caused an increase in ROCK activity. We have also observed blunting of CCM in response to pharmacological inhibition of ROCK or depletion of intracellular calcium with 100 µM EGTA. To study the role of calcium from different sources in driving ROCK activity during CCM, carbenoxolone, a gap junction blocker, was applied to collectively migrating fibroblasts. Carbenoxolone decreased ROCK activity and intracellular calcium at corresponding time points and slowed CCM. Following treatment with PQ7, a gap junction activator, modest increases in CCM were observed. These results indicate that gap junction intercellular calcium signaling drives ROCK activity during collective cell migration.

B493/P2875

The role of Shank3 and zinc in CaMKII translocation by NMDA receptor stimulation

A. D. Nayak, L. E. Guzmán Clavel, G. A. Crescencio, S. A. Kulik, S. A. Kim; Amherst College, Amherst, MA

Synaptic transmission is transduced by postsynaptic proteins that coordinate activity-dependent changes in synaptic structure. It has been previously shown that glutamate binding to NMDA receptors triggers the translocation of Ca2+/calmodulin kinase II alpha (CaMKIIα) to dendritic spines where it acts to decode synaptic Ca2+ oscillations. Although this translocation is critical for synaptic plasticity, the molecular mechanisms underlying recruitment of CaMKIIα to the postsynaptic density (PSD) remain unknown. Recently found to bind directly to CaMKIIα, Shank3 is a zinc-dependent, postsynaptic protein that anchors many key functional molecules in the PSD. Here we show that both Shank3 and zinc play a role in the recruitment of CaMKIIα to dendritic spines. CaMKIIα levels at the spine were found to be dependent on Shank3 concentrations. Similarly, glutamate-induced translocation of CaMKIIα increased with Shank3 concentration. Since Shank3 activation and function requires zinc, we next examined whether zinc also regulates CaMKIIα translocation. Zinc was shown to enhance glutamate-induced recruitment of CaMKIIα, whereas zinc chelation decreased CaMKIIα concentration in spines. Even with Shank3 overexpression, zinc chelation prior to glutamate application led to decreased CaMKIIα translocation. Interestingly, zinc supplementation in Shank3 knockdown neurons rescued the diminished levels of CaMKIIα translocation. Thus, this role of Shank3 in recruiting CaMKIIα to the PSD points to a concerted mechanism between Shank3 and zinc in regulating CaMKIIα. Given that CaMKIIα is known to transduce calcium signaling, this novel interplay of zinc and CaMKIIα suggests that this enzyme may integrate both calcium and zinc signaling to coordinate structural postsynaptic changes.

Post Translational Modification in Signaling

B494/P2876

Flagellar cAMP signaling in the eukaryotic pathogen *Trypanosoma brucei*, lessons from phosphoproteomics and fluorescence spectroscopy

D. Velez-Ramirez, M. Shimogawa, A. Wijono, P. Limsuwannarot, A. Leung, K. Hill; University of California, Los Angeles, Los Angeles, CA.

Trypanosoma brucei is a flagellated protozoan parasite and causative agent of sleeping sickness. *T. brucei* flagellum plays critical roles in transmission and pathogenesis. It is surrounded by a phospholipidic bilayer and is laterally connected to the cell body along almost its entire length. We implemented, for the first time in this organism, APEX2-proximity proteomics to examine protein composition of *T. brucei* flagellum, using Dynein Regulatory Complex subunit 1 (DRC1) as bait. The resulting DRC1-proximity proteome gave good overlap with known flagellum proteomes, validating the APEX2 system in trypanosomes. In addition to its canonical role in cell motility, the flagellum is also a signaling platform. Adenylate cyclases located at specific flagellum domains produce cAMP that controls motility and parasite transmission, then cAMP is removed by the flagellar-restricted phosphodiesterase B1 (PDEB1). To investigate the flagellar cAMP cell signaling pathway, we sought to identify cAMP-dependent protein phosphorylation in the flagellum and assess its role in cell motility. By performing APEX2-proximity labeling in tandem with TiO_2-mediated phosphoenrichment, we have been able to define the flagellum phosphoproteome. We next obtained the flagellum phosphoproteome of a PDEB1-knockout, to identify cAMP-dependent phosphorylation. Principal component analysis of control and

PDEB1-knockout samples revealed that the flagellum phosphoproteomes are indeed different, with several proteins differentially phosphorylated in the PDEB1-knockout. Gene ontology analysis of the biological process of this group of proteins, retrieved terms of cell division, mRNA catabolic process and microtubule-based movement, among others. We are now working on obtaining the cAMP-dependent flagellar phosphoproteome of a PDEB1 inducible knockdown. These results will complement our previously obtained steady state cAMP-dependent flagellar phosphoproteome. We are also interested in visualizing cAMP fluctuations inside the flagellum and the cell body. Our working hypothesis is that on PDEB1 mutants, cAMP locally produced at the flagellum tip, freely diffuses along the flagellum and inside the cell body. To test this hypothesis we are implementing a FRET-based cAMP sensor. Altogether, our findings provide evidence on the ways eukaryotic pathogens sense and respond to their environment.

B495/P2877

Sheddase-dependent release of membrane-anchored FGF at the cytoneme contact sites

Y. Li, L. Du, A. Sohr, M. Rao, S. Roy; University of Maryland, College Park, MD.

During tissue morphogenesis, cells can precisely coordinate their functions over distance by communicating with secreted paracrine signaling proteins such as fibroblast growth factors (FGFs). The spatiotemporal regulations of FGF signaling have been shown to be critical for development and homeostasis, but the mechanisms that control the target-specific FGF communication over distance are not well-understood. In *Drosophila*, FGF produced in a small group of wing-disc cells is known to regulate the development of the disc-associated air-sac-primordium/ASP, which is functionally analogous to a growing vertebrate lung bud. Although FGF is known to be a secreted signal, previous work has shown that the inter-organ dispersion of FGF from the source to the ASP is highly polarized, target-specific, and is mediated over distance via long polarized actin-based filopodia, named cytonemes. The contact-dependent long-distance FGF exchange via cytonemes is programmed by the post-translational modifications of FGF, which is modified to be glycosylphosphatidylinositol (GPI)-anchored to the outer leaflet of the source cell membrane. The GPI anchor inhibits free/random secretion of FGF but also facilitates the release of FGF only at the source-recipient cytoneme contact sites. This work explores the molecular mechanisms that can control the contact-dependent FGF release at the cytoneme contact sites. By using cell biological, biochemical, and genetic analyses, we found that FGF is shed from the source cell membrane by proteolytic cleavage. We identified a specific region in the FGF backbone required for the cleavage. Phenotypic analyses of deletion FGF mutants, generated by genome editing, suggest that the proteolytic FGF release is essential for signaling and morphogenesis. We identified multiple cell surface proteases that can specifically shed GPI-anchored FGF from the producing cell surface. These sheddases are membrane-tethered and, except for one, are expressed only in the recipient ASP. Despite the lack of expression in the source, these sheddases can be delivered to the source-recipient contact sites and cleave FGF in trans to facilitate contact-dependent FGF release. These results, for the first time, provide new insights into how contact-mediated signal dispersion via cytonemes can be modulated by cell surface sheddases. This work is funded by NIH R35GM124878 to S.Roy.

B496/P2878

Understanding Hsp70 phosphorylation using the Hsp70 Chaperone Code array

A. Rysbayeva, S. Omkar, A. Truman; Department of Biological Sciences, University of North Carolina At Charlotte, Charlotte, NC.

Hsp70 (Heat Shock Protein 70) is a well-conserved molecular chaperone involved in the folding and stabilization of a large proportion of the proteome. Global mass spectrometry studies have identified over 80 post-translational modifications (PTMs) on Hsp70 which regulate its activity and which we collectively term the "Chaperone Code". Despite the complexity of the chaperone code, less than 20 PTMs have been fully explored in terms of regulation and function. To study the impact of phosphorylation on Hsp70 we mutated all 73 known phosphorylation sites on budding yeast Hsp70 (Ssa1) to either alanine (phospho-mutant) or aspartate/glutamate (phospho-mimic) and then expressed these 146 single mutants in a *ssa1-4Δ* background. Although nine mutations did not support cell viability, the remaining 137 mutants (Hsp70 Chaperone Code Array) were screened against a variety of stressors allowing us to generate a phenotypic fingerprint for each mutant. We are currently examining the impact of these chaperone code mutants on the toxicity of neurodegenerative proteins that cause ALS and Huntington's disease in humans. Taken together, we hope that the array will be a useful tool for the chaperone field to probe the intricacies of the chaperone code. We are creating equivalent arrays for other kinds of PTMs and the other major chaperones and co-chaperones in an effort to "crack the Chaperone Code". **Keywords: Hsp70, PTMs, Chaperone Code, phosphorylation, yeast**

B497/P2879

Betaglycan Glycosaminoglycan Chain Modifications impact Ectodomain Shedding and Endothelial Cell Function in Ovarian Cancer

A. Choi[1], L. Jenkins[2], Y. Liu[3], A. Nixon[3], N. Hempel[4], R. Arend[5], M. Karthikeyan[6]; [1]UNIVERSITY OF ALABAMA AT BIRMINGHAM - Birmingham, AL, Birmingham, AL, [2]University of South Carolina, Columbia, SC, [3]Department of Medicine and Duke Cancer Institute,, Duke University Medical Center, Durham, NC, [4]Department of Medicine, Division of Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, [5]Gynecologic Oncology, Obstetrics and Gynecology, UNIVERSITY OF ALABAMA AT BIRMINGHAM - Birmingham, AL, Birmingham, AL, [6]Department of Pathology, Molecular Cellular, UNIVERSITY OF ALABAMA AT BIRMINGHAM - Birmingham, AL, Birmingham, AL.

Betaglycan (BG) is a ubiquitously expressed transmembrane PG containing a core protein domain with glycosaminoglycan (GAG) attachments sites at S534/S545 residues on its extracellular domain, to which heparan sulfate (HS) and chondroitin sulfate (CS) chains are covalently attached. BG is commonly referred to as a "part-time proteoglycan" since BG can be expressed on the cell surface with or without GAG chain modifications. BG's ectodomain can also be shed (shed-BG) from the cell surface to release a soluble form. BG is an established co-receptor for the TGF-β superfamily that modulates signaling of TGF-β members both at the cell surface and via the shed form as has been previously reported in breast cancer. Although majority of the prior work has focused on BG's core protein interactions and functions, a thorough understanding of the effects of the BG GAG chains remains lacking. Upon, analysis of the GAG chains of shed-BG in OVCA patient ascites fluid, we find that majority of shed-BG is heavily glycosylated. We thus hypothesize that the GAG chains of BG may impact BG ectodomain shedding and impart autocrine and paracrine responses. To examine the effect of GAG modifications on BG ectodomain shedding, we constructed point mutations at S534/S545 to abrogate GAG chain attachment

sites on BG and expressed them in a panel of cancer cell lines . Cellular expression of BG constructs confirmed the alterations in BG GAG chains. We find that using autoradiography and ELISA a significant reduction in BG shedding in cells expressing unmodified BG (BG-ΔGAG) compared to cells expressing wild type BG (BG-FL), despite no change in cell surface expression. Next, we evaluated autocrine signaling effects of BG GAG mutant expression. We find that BG-ΔGAG mutant shows increased invasion and activation of the TGF-β signaling pathway compared to glycosylated BG mutant (BG-FL). Paracrine effects of Soluble-BG was tested on other cell types found in tumors particularly endothelial cells. Soluble-BG was able to abrogate the effects of growth factors on endothelial cell invasion. Lastly, RNA-SEQ of the BG-GAG mutants identified tissue inhibitor of metallopeptidase-3 (TIMP-3) as a possible regulator of BG ectodomain shedding. Knockdown of TIMP-3 expression through siRNA increased shedding of BG in BG-ΔGAG mutants. Through these findings, we report novel roles and mechanism of ectodomain shedding of the GAG modified and unmodified forms of BG, impacting cell biology, signaling, and endothelial cell functions in ovarian cancer cell systems.

B498/P2880

Regulation of Nucleotide-Sugar Levels is Required for Mammalian Glycoconjugate Biosynthesis *in vivo*

N. Linhart[1], A. Florwick[2], A. Tata[3], L. Macadlo[3], P. Tata[3], M. Boyce[1]; [1]Biochemistry, Duke University, Durham, NC, [2]Duke University, Durham, NC, [3]Cell Biology, Duke University, Durham, NC.

Mammalian nucleotide-sugar biosynthetic pathways are well-characterized biochemically, but how cells regulate flux through these pathways in response to normal biological processes or stresses remains poorly understood. To begin to understand how and why nucleotide-sugar levels are regulated, we chose galactose-4-epimerase (GALE) as a model enzyme. GALE directly balances four nucleotide-sugar pools and is implicated in human genetic diseases, such as subtypes of galactosemia and thrombocytopenia. We previously discovered that GALE is essential for the regulation of nucleotide-sugar metabolism and for downstream glycoconjugate biosynthesis in cultured human cell lines. In particular, mucin-type O-glycans, which are commonly found on mucins, rely on GALE for their production. Mucins are highly O-glycosylated and are a main component of mucus, an essential hydrogel that coats a variety of epithelial tissues. In current work, we are determining the role of GALE in mucin biosynthesis and function *in vivo*. mRNA and immunofluorescence data indicate that mucin-secreting club cells of the epithelium and mucous cells of the submucosal glands (SMGs) express high levels of GALE in the human airway. Immunofluorescence (IF) staining on human airway tissue revealed high levels of GALE expression in discrete cell types of the airway epithelium. GALE expression colocalizes with the club cell marker club cell secretory protein (CC10) on the epithelium and the SMG mucous cell marker mucin 5B (MUC5B). These results suggest that GALE may be required in specific mucin-secreting airway cell populations. Ongoing experiments are also defining GALE expression differences in the airways of healthy and muco-obstructive disorder patients (e.g., chronic obstructive pulmonary disease, cystic fibrosis). To assess the functional significance of GALE and nucleotide-sugar regulation in tissue physiology, we have created conditional knockout models in the total epithelial and mucin-secreting cell populations of the murine airway. Tissue characterization to deduce the effects of GALE loss is underway. In addition, we have established GALE$^{-/-}$ *in vitro* airway epithelial culture systems to determine GALE's role in the biosynthesis of airway mucin glycoproteins. Our work supports the hypothesis that GALE is important in the mammalian airway, especially in cell populations known to secret mucins.

B499/P2881

Exploring the molecular mechanisms to connect metabolism, DNA damage response and aging

J. Park, H. Lee, Y. Lee, **I. LEE**; Ewha Womans University, SEOUL, KOREA, REPUBLIC OF.

Exploring the molecular mechanisms to connect metabolism, DNA damage response, and Aging

Jingyeong Park*, Haein Lee*, Yelin Lee* and In Hye Lee
Department of Life Science, EWHA Womans University, Seoul, South Korea * These authors equally contributed.

Growing evidence indicates that metabolic signaling pathways are interconnected to DNA damage response (DDR). However, factors that link metabolism to DDR remain incompletely understood. SIRT1, an NAD+-dependent deacetylase that regulates metabolism and aging, has been shown to protect cells from DDR. Here we demonstrate that SIRT1 protects cells from oxidative stress-dependent DDR by binding and deacetylating Checkpoint Kinase 2 (CHK2). We first showed that essential proteins in DDR were hyper-acetylated in Sirt1-deficient cells and that among them the level of acetylated CHK2 was highly increased. We found that Sirt1 formed molecular complexes with BRCA1/BRCA2-associated helicase 1 (BACH1), H2AX, Tumor suppressor p53-binding protein 1 (53BP1), and CHK2, which are key factors of DDR. We then demonstrated that CHK2 was normally inhibited by SIRT1 via deacetylation but dissociated with SIRT1 under oxidative stress conditions. This led to acetylation and activation of CHK2, which increased cell death under oxidative stress conditions. Our data also indicated that SIRT1 deacetylated K235 and K249 residues of CHK2, whose acetylation increased cell death in response to oxidative stress. Thus, SIRT1, a metabolic sensor, protects cells from oxidative stress-dependent DDR by deacetylation of CHK2. Our finding suggests a crucial function of SIRT1 that inhibits CHK2 as a potential therapeutic target for cancer treatment.

* This work was supported by grants for the National Research Foundation of Korea (NRF 2021R1A2C1091259

B500/P2882

The role of docking site modulation of Extracellular Signal-Regulated Kinase on cell migration

T. Romania[1], A. Kaib[1], A. Kotau[1], J. D. Keyes[2]; [1]Pennsylvania State University Erie, Erie, PA, [2]Penn State University Erie, Erie, PA.

Extracellular signal-regulated kinase (ERK) is part of a vital kinase cascade that facilitates cellular communication. ERK has been heavily studied due to its hyperactivity in 90% of cancers but, not much is known above the basic knowledge that ERK is turned on and off in response to a variety of extracellular signals. Preliminary data suggests that ERK oxidation increases ERK activity towards migration-related substrates and decreases activity toward proliferation-related substrates. Initial results between transfected WT and C159S ERK2 in established NIH-3T3 cells provided evidence that ERK2 oxidation on C159 modulated ERK activity influences cell responses to extracellular stimuli. This project continues to explore the complexities of ERK regulation by investigating the oxidation of ERK on C159 substrate selectively.

B501/P2883

Impaired phosphorylation of CBP disturbed intestinal homeostasis to lead to colitis

C. Chen, Y. Lin; DEPARTMENT OF Pharmacology, College of Medicine, NTU, Taipei, TAIWAN.

Inflammatory bowel diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis (UC), are multifactorial disorders characterized by chronic inflammation of intestine. CBP is a transcriptional coactivator modulating DNA accessibility to transcription factors. We previously revealed that phosphorylation of CBP at Ser1382/1386 by IKKα plays a critical role in regulating cell fate by suppressing p53-mediated gene expression. CBP-AA-knockin mice were generated by replacing CBP Ser1383/1387 with alanines (AA), and CBP-AA mouse models are found to exhibit a specifically spontaneous colitis phenotype. Bone marrow chimera experiments reveal that the spontaneous colitis is driven by impaired phosphorylation of CBP in non-hematopoietic cells. We explored that a genetic defect of CBP phosphorylation in colonic IECs orchestrated by intestinal stem cells (ISCs) contributed to the spontaneous colitis in a CBP/p53-dependent manner. CBP phosphorylation at Ser1383/1387 (human: Ser1382/1386) plays a critical role in maintaining intestinal homeostasis *in vivo*. A molecularly basic mechanism translating into a clinically important human disease was demonstrated, and more light on the management of bowel disorders was shed.

B502/P2884

The Interaction of Gam1 and SAE1/SAE2 Through Structural and Functional Studies

K. Silva, R. Dong, B. Moreno, G. Avila, Y. Xian, C. Xiao; University of Texas At El Paso, El Paso, TX.

Gam1 is an early gene product of the chicken embryo lethal orphan virus. Up to today, it is the only viral protein that globally inhibits host cell SUMOylation. SUMOylation is a post-translational modification system that is essential for various cell functions including against viral infections. Gam1 achieves its function by the elimination of key enzymes SAE1/ SAE2 in the SUMOylation pathway. Understanding the molecular mechanism of the inhibition necessitates the structural studies of the Gam1/SAE1/SAE2 complex. Gam1 will be purified using published protocols. In addition, a recombinant SAE1/SAE2 plasmid was transformed into Rosetta 2 (DE3)pLysS cells to express these eukaryotic genes in bacteria cells. The cells were grown to a certain optical density prior to the induction with Isopropyl ß-D-1-thiogalactopyranoside. After expressing for an optimal length of time, the cells were harvested using centrifugations. SAE1/SAE2 will undergo various chromatographic techniques to isolate the complex that allows for in-vitro analyses of its interactions with Gam1. Purified Gam1/SAE1/SAE2 complexes will be analyzed by structural techniques such as cryogenic electron microscopy. These structural investigations will deepen our understanding of how Gam1 interacts with SAE1/SAE2 to inhibit host cell SUMOylation favoring viral replications. Results from the project will facilitate future development of antiviral strategies.

B503/P2885

Prediction and characterization of putative UBA domains in human NEK10.

I. Semchenkov Pastukhov[1], A. Silva Dos Santos[1,2], S. Singh[1,2,3]; [1]Biology, Brooklyn College, CUNY, Brooklyn, NY, [2]The Biochemistry PhD Program, The Graduate Center of the City University of New York, New York, NY, [3]The Biology PhD program, The Graduate Center of the City University of New York, New York, NY.

Ubiquitination is a form of post-translational modification of a protein by attachment of a ubiquitin protein (UBC) to it, varying in the number of chains attached e.g., monoubiquitylation, polyubiquitination, and multiple monoubiquitylation. Depending on the type of ubiquitination, different functions may be carried out by the modified protein ranging from tagging for degradation and recycling by the proteasome pathway to cell signaling, DNA repair, apoptosis, immune response, and protein processing. Ubiquitin forms an isopeptide bond between its terminal glycine and a lysine residue within a ubiquitin associated domain **(**UBA) on the surface of the target protein. Although there is no established motif for UBA domain as of now, researchers have suggested a few common key features present in the areas of interaction that have been experimentally established and analyzed. Using protein structure prediction software and predicted model assessment and analysis, we have identified two potential unreported sites of interaction of ubiquitin within a full length model of NEK10. Of two found sites, one fits the structural and biophysical criteria described in the literature well, while the second site meets the criteria less optimally, especially the net charge around the target lysine. Prediction of the NEK10 UBA-UBC interaction using docking software identifies six possible scenarios that put NEK10's lysine and UBC's glycine within a desirable distance that would allow interaction to happen. The identification of these putative UBA domains within NEK10 opens a complete new aspect of NEK10 biology and will allow new investigations of the role and function of ubiquitination in NEK10 signaling.

B504/P2886

Investigating Protein-Protein Interactions In The Purinosome

J. He[1], L. Zou[2], S. Benkovic[1]; [1]Department of Chemistry, Pennsylvania State University, University Park, PA, [2]Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA.

There is compelling evidence that the enzymes in the de novo purine biosynthesis (DNPB) pathway form a multienzyme complex — the purinosome — to facilitate substrate channeling and enhance pathway flux. However, biochemical evidence of protein-protein interactions (PPIs) between DNPB enzymes has been limited. Here, we employ two orthogonal approaches, bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation (co-IP), to investigate PPIs of the human bifunctional phosphoribosylaminoimidazole carboxylase/succinocarboxamide synthetase (PAICS), a central member of the DNPB pathway. We found evidence for PAICS interaction with all the other DNPB enzymes, with the single exception of amidophosphoribosyltransferase (PPAT), the first enzyme of the pathway. We also found PAICS interacts with methylenetetrahydrofolate dehydrogenase/cyclohydrolase/formyltetrahydrofolate synthetase 1 (MTHFD1), which produces folate co-factors essential for DNPB. These interactions were observed in cells cultured in both purine-rich conditions, where DNPB activity is low, and in purine-depleted conditions, where DNPB activity is high. This suggests a partial purinosome complex can exist inside the cell regardless of the cell's DNPB activity. We also found that the tagging of PAICS on its carboxyl-terminus disrupts its heteromeric interactions,

but not its homo-oligomerization. To further investigate the structure and composition of the purinosome, we are now using the split-TurboID biotin ligase proximity labeling system to probe higher order PPIs in this multienzyme complex. We found distinct DNPB enzymes, when fused to complementary TurboID fragments, and co-expressed in live cells, can reconstitute functional biotin ligase activity, thereby re-confirming PPIs between DNPB enzymes in vivo. We are now in the process of proteomic analysis of novel split-TurboID labelled products, with the aim of uncovering higher-order interactors within the purinosome. This knowledge will facilitate a better understanding of the composition and the regulation of this important metabolon.

B505/P2887

Obesity Increases Stress Granules-Associated Proteins in the First Trimester Placenta

J. Bandres-Meriz; Medical Universiversity of Graz, Graz, Austria

Background: Obesity, a strong inducer of cellular stress, is associated with reduced insulin sensitivity (IS_{HOMA}) and elevated C-peptide (a proxy of insulin serum concentration) in the first trimester of pregnancy. In this period, the human placenta is especially sensitive to changes in the intrauterine environment. We hypothesized that increased C-peptide alters placental proteome and function and, specifically, aimed to identify pathways differently regulated in placentas from obese pregnant women. **Methods:** Placental tissue (gestational age 5^{+0} - 6^{+6} weeks, n=19) from women with high- and low-insulin sensitivity (based on above/below median; median IS_{HOMA}= 0.61) were used for untargeted proteomics (LC-MS/MS). Signal intensities (LFQ values) of G3BP1 and FUS/TLS were correlated with maternal C-peptide and IS_{HOMA}. Immunohistochemistry was performed on placenta sections (3.5µm; n=19) and formation of G3BP1 foci assessed with a scan microscope (Olympus VS200). ACH-3P cells (first trimester trophoblast cell line) were challenged with 10nM insulin for 1h at physiological oxygen tension (6.5% O_2; hypoxia bench) and formation of G3BP1 foci assessed by immunohistochemistry (Zeiss Axio Z1 microscope). Sodium arsenite (500µM) was used as positive control. **Results:** Proteins that localize to stress granules (EIF4B, RPL7A, RPL23, RPS7, RPL10, DNR, HNRNPK, HNRNPD, HNRNPAB, HNRNPA2B1, SF3A1, TRA2B, YBX1, KHSRP, NPM1) were enriched ($p<0.05$) in placentas from low IS_{HOMA} mothers. Protein level of G3BP1, the key structural component of stress granules, correlated with maternal serum C-peptide ($r=0.495$, $p<0.05$), but not with IS_{HOMA}. The protein level of FUS/TLS, a marker of chronic stress granules, also correlated with C-peptide ($r=502$, $p<0.05$). In placenta sections, G3BP1 localized to the villous and extravillous cytotrophoblast. G3BP1 foci were mainly located on the villous cytotrophoblast. *In vitro*, challenging ACH-3P cells with insulin resulted in the formation of G3BP1 foci. **Conclusion:** G3BP1 foci are visible in the cytotrophoblast of the first trimester placenta, the main proliferative cell type early in pregnancy. Increased G3BP1 and FUS protein level suggest increased assembly of stress granules in placentas chronically exposed to hyperinsulinemia. Assembly of G3BP1 foci in ACH-3P cells after insulin treatment suggests that high insulin concentrations activate stress-response pathways in the placenta. In the first trimester placenta, chronic stress, as in obesity, is associated with increased level of proteins characteristics of stress granules.

B506/P2888

Investigation into prolific small GTPase ubiquitination by *Legionella pneumophila*

V. Bhadkamkar[1], A. Steinbach[1], D. Jimenez-Morales[2], E. Stevenson[1], G. Jang[1], N. Krogan[1], D. Swaney[1], S. Mukherjee[1]; [1]University of California, San Francisco, San Francisco, CA, [2]Stanford University, Palo Alto, CA

The intracellular bacterial pathogen *Legionella pneumophila* (*L.p.*) usurps eukaryotic host ubiquitination machinery in order to form its replicative vacuole. Among its arsenal of ~330 secreted bacterial proteins (effectors), a growing number have been biochemically characterized as ubiquitin ligases or deubiquitinases, although the targets of these effectors remain elusive. To better understand how *L.p.* hijacks host cell ubiquitination machinery and the targets of these effectors, we performed a global proteomics analysis that measured changes in protein abundance and ubiquitination during *L.p.* infection. Strikingly, we discovered that 79 of 163 mammalian small GTPases of the Ras superfamily are ubiquitinated, but not degraded, during infection in a process dependent upon bacterial effector secretion. Ubiquitinated GTPases include critical regulators of cell proliferation (e.g., Ras), intracellular membrane traffic (e.g., Rab, Arf), and cytoskeletal structure (e.g., Rho, Rac). Since a growing body of work has shown that ubiquitination is a potent regulator of small GTPase activity, we decided to better characterize pan-Ras family small GTPase ubiquitination during infection. Using the small GTPases Rab1, Rab5, and Rab10 as a test cases, we have determined that robust recruitment and retention of these GTPases into the *Legionella*-containing vacuole (LCV) membrane is a requirement for their ubiquitination. In conjunction with an analysis showing strong overlap between LCV-recruited small GTPases and small GTPases ubiquitinated in our mass spectrometry, our data suggest a model in which all LCV-recruited small GTPases are ubiquitinated during infection. Our data also show that two paralogous effectors, SidC and SdcA, promote, but are not required for small GTPase ubiquitination. SidC and SdcA are linked to timely LCV formation and recruitment of ER-membranes to the LCV, suggesting a link between ER-membrane recruitment and pan-small GTPase ubiquitination. Altogether, our data suggest that *L.p.* hijacks small GTPase activity during infection with prolific, pan-family ubiquitinations, and position *L.p.* as a tool to better understand how small GTPases can be regulated by ubiquitination in uninfected contexts.

B507/P2889

Progranulin processing in the epigenetically distinct SW13 cell subtypes

P. Biparva[1], A. S. Pascual[1], K. J. Leyva[1], M. R. Montgomery[2], **E. E. Hull**[1]; [1]Midwestern University, Glendale, AZ, [2]Oklahoma State University, Stillwater, OK

Progranulin (PGRN) is a pleiotropic signaling molecule composed of 7.5 granulin repeats, regulating many diverse biological processes including cellular proliferation, inflammation, tumorigenesis, and lysosome function. A variety of intracellular and extracellular proteases differentially process PGRN and their combined actions determine the biological activity of the processed PGRN products. Utilizing the two epigenetically distinct subtypes of the SW13 human adrenal carcinoma cell line, we focus on the extracellular and intracellular proteases involved in PGRN processing and characterize the resulting PGRN cleavage products. Results suggest that differential processing of PGRN occurring within each SW13 subtype is correlated to both differential growth and lysosomal pH. Specifically, the slow-growing, metastatic subtype produces higher levels of PGRN, increased levels of matrix metalloproteinases (MMP) 2/9, and has a higher lysosomal pH than the more proliferative subtype. Interestingly, when

PGRN is exogenously added to the culture medium, the proteases expressed by each subtype influences whether PGRN will be processed into a product(s) promoting growth or a product(s) promoting inflammation. We also show that the pH of lysosomes in each subtype differs, with the slowly growing, metastatic subtype having a significantly higher pH than the proliferative subtype. This result suggests that lysosomal proteases, which are more functional at a lower pH, may be responsible for producing a PGRN product(s) that promotes growth in the proliferative subtype, while the higher lysosomal pH of the slowly growing subtype does not. Ongoing experiments address the possibility that decreasing lysosomal pH may shift the processing of PGRN, influencing the biological activities associated with this versatile signaling molecule. As mutations in the PGRN gene are implicated both in dysfunctional lysosomal pH and a variety of neurodegenerative diseases, these results may provide a novel approach to regulating inflammation and growth by intracellular mechanisms.

B508/P2890

The polySUMOylation-dependent pathway promotes nucleolar release of Tof2 for mitotic exit

E. Gutierrez-Morton[1], L. Collins[1], S. Vanga[2], Y. Wang[1]; [1]Florida State University, Tallahassee, FL, [2]Lincoln High School, Tallahassee, FL.

SUMOylation involves the covalent attachment of SUMO (small ubiquitin-like modifier) to target proteins. This posttranslational modification is essential and impacts numerous cellular processes by altering protein localization and abundance. SUMOylation is a highly dynamic and reversible process. In *Saccharomyces cerevisiae*, SUMO proteases Ulp1 and Ulp2 readily reverse SUMOylation to prevent polySUMOylation. Perturbations to SUMO homeostasis are linked with aberrant cell cycle progression and tumor proliferation such as breast cancer. Recent work has shown that several yeast nucleolar proteins, including Tof2 and Net1, are SUMOylated. Tof2 and Net1 are required for nucleolar sequestration of Cdc14, a phosphatase that plays an essential role in mitotic exit by dephosphorylating the substrates of cyclin-dependent kinases. However, it is currently unknown if Tof2/Net1 SUMOylation regulates Cdc14 release and mitotic exit. We found that the protein level and the nucleolar localization of Tof2, but not Net1, were cell cycle regulated. Depletion of SUMO protease Ulp2 caused Tof2 polySUMOylation, nucleolar delocalization, and degradation.Interestingly, Tof2 delocalization and degradation depend on polySUMO-triggered ubiquitination by SUMO-targeted ubiquitin ligase and the subsequent extraction by Cdc48 segregase. In addition, the absence of Tof2 or induction of polySUMOylation leads to nucleolar release and activation of Cdc14 phosphatase. Overall, these results demonstrate a polySUMO-dependent mechanism for Tof2 delocalization from the nucleolus during anaphase, which frees Cdc14 to promote mitotic exit.

Signaling Networks Governing Cell Migration

B510/P2891

Phosphorylation of Arl4A/D promotes their binding by the HYPK chaperone for their stable recruitment to the plasma membrane

M. Lin[1], C. Yu[2,3], F. S. Lee[1,4,5]; [1]Institute of Molecular Medicine, National Taiwan University, Taipei, TAIWAN, [2]Department of Cell and Molecular Biology, Chang Gung University, Taoyuan, TAIWAN, [3]Department of Thoracic Medicine, Chang Gung Memorial Hospital, Taoyuan, TAIWAN, [4]Center of Precision Medicine, National Taiwan University, Taipei, TAIWAN, [5]Department of Medical Research, National Taiwan University Hospital, Taipei, TAIWAN.

The Arl4 small GTPases participate in a variety of cellular events, including cytoskeleton remodeling, vesicle trafficking, cell migration, and neuronal development. Whereas small GTPases are typically regulated by their GTPase cycle, Arl4 proteins have been found to act independent of this canonical regulatory mechanism. Here, we show that Arl4A and Arl4D (Arl4A/D) are unstable due to proteasomal degradation, but stimulation of cells by fibronectin (FN) inhibits this degradation to promote Arl4A/D stability. Proteomic analysis reveals that FN stimulation induces phosphorylation at S143 of Arl4A and at S144 of Arl4D. We identify Pak1 as the responsible kinase for these phosphorylations. Moreover, these phosphorylations promote the chaperone protein HYPK to bind Arl4A/D, which stabilizes their recruitment to the plasma membrane to promote cell migration. These findings not only advance a major mechanistic understanding of how Arl4 proteins act in cell migration, but also achieve a fundamental understanding of how these small GTPases are modulated by revealing that protein stability rather than the GTPase cycle acts as a key regulatory mechanism.

B511/P2892

Oligomerization of Arl4 small GTPases promotes their function in cell migration

Y. Lai[1,2], S. Wu[1,2], F. S. Lee[1,2,3]; [1]Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taipei, TAIWAN, [2]Department of Medical Research, National Taiwan University Hospital, Taipei, TAIWAN, [3]Center of Precision Medicine, College of Medicine, National Taiwan University, Taipei, TAIWAN.

Protein dimerization or oligomerization participates in regulating various of cellular signaling transduction. Oligomerization of Ras small G proteins has been reported to regulate effectors binding and downstream pathway, which is important for their oncogenic activity and merits further research. Three isoforms Arl4 (Arl4A, Arl4C and Arl4D) small G proteins belong to the Arf family of Ras superfamily and each of them has been reported to promote cell migration via specific downstream effectors. Here, we identified Arl4A and Arl4D could form homo- and hetero-oligomerization through its C-terminal tail. By co-immunoprecipitation, *in vitro* binding assay, and size exclusion gel filtration, we showed that Arl4A and Arl4D formed homo- or hetero-dimerization and/or oligomerization. By immunofluorescence image analysis, we observed that Arl4A and Arl4D were cooperatively recruited to the plasma membrane. Moreover, the oligomerization of Arl4A and Arl4D contributes to their plasma membrane localization and promotes cell migration. Thus, we infer that homo- and/or hetero-oligomerization Arl4A and Arl4D play pivotal roles in regulating plasma membrane localization and their downstream functions.

B512/P2893

A Local Ca^{2+} and RhoA Signaling Crosstalk Facilitates Cell Migration by Reinforcing Actin Network in the Lamellipodia

E. Iu[1], A. Bogatch[1], W. Deng[2], J. Humphries[3], M. Humphries[4], G. Tanentzapf[2], S. Plotnikov[1]; [1]University of Toronto, Toronto, ON, Canada, [2]University of British Columbia, Vancouver, BC, Canada, [3]Manchester Metropolitan University, Manchester, United Kingdom, [4]University of Manchester, Manchester, United Kingdom

Cell migration underlies a wide range of physiological processes from embryonic development to tissue regeneration. The process of cell migration begins by the extension of membrane protrusions, such as lamellipodia. Such protrusive structure is driven by actin polymerization, which is tightly regulated by a complex of signaling pathways, including Rho GTPases and Ca^{2+}-dependent signaling. While the key role of Ca^{2+} signaling in lamellipodia protrusions has been demonstrated, the molecular mechanisms by which Ca^{2+} facilitates lamellipodia assembly is largely unknown. We first identified a mechanosensitive Ca^{2+}-permeant channel, TRPV4, as a positive regulator of lamellipodia protrusion by screening a panel of siRNAs targeting ion channels. Suppression of TRPV4 reduces the Ca^{2+} influx in protruding lamellipodia, and subsequently, decreases the density of F-actin in lamellipodia. To understand how the TRPV4-mediated Ca^{2+} influx facilitates lamellipodia protrusion, we interrogated the involvement of small GTPases by using FRET biosensors. To our surprise, we found that RhoA activity peaks in protruding lamellipodia, which coincides spatially with the Ca^{2+} influx. Furthermore, we showed that local upregulation of RhoA activity in lamellipodia requires TRPV4 activity. Suppressing RhoA or its downstream effector, formins, also decreased F-actin density, suggesting that TRPV4-mediated Ca^{2+} signaling promotes actin assembly by maintaining a high RhoA activity. By immunostaining, we showed that suppression of TPRV4 decreased the amount of active Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII), a key Ca^{2+} signaling hub, in lamellipodia. Furthermore, we showed that local inhibition of CaMKII halted cell protrusions, suppressed RhoA activity and decreased F-actin density in lamellipodia, suggesting that CaMKII transduces local Ca^{2+} signals from TRPV4 to RhoA. To identify the RhoA regulator downstream of TRPV4 and CaMKII, we compared the phospho-proteome of isolated lamellipodia fractions purified from TRPV4- and CaMKII-inhibited cells. Using FRET and cell spreading assays, we functionally tested 14 potential RhoGEFs/GAPs, whose phosphorylation depends on TRPV4 and CaMKII. We found that depleting RhoGEF TEM4 decreased density of F-actin and RhoA activity independent of TRPV4 or CaMKII activation, suggesting that TEM4 is a Ca^{2+}-regulated RhoGEF that regulates lamellipodia protrusions. Finally, we showed that suppression of this signaling axis suppresses the protrusive activity and migration of melanoblasts in mouse skin explant. Together these data elucidate a novel Ca^{2+} signaling axis composed of TRPV4/CaMKII/RhoA that reinforces lamellipodial actin network, facilitates lamellipodial protrusions, and promotes cell migration.

B513/P2894

Exploring the Functional Roles of PI5K in Regulation of PI(4,5)P2 during Cell Migration

Y. Deng[1], P. N. Devreotes[2]; [1]Johns Hopkins University, Baltimore, MD, [2]Johns Hopkins University School of Medicine, Baltimore, MD.

Cell migration is involved in a lot of important physiological activities during development, such as wound healing and cancer metastasis. It is emerging that the amoeboid migration is organized by a coupled excitable network whose minimal topology consists of an autocatalytic positive feedback loop

and a delayed negative feedback loop. This gives rise to a defined threshold of activation either by stochastic noise or external input, causing a firing of signaling and cytoskeletal networks which is manifested by a protrusion or macropinosome formation. *Dictyostelium* cells display such excitability through signal transduction (STEN) and cytoskeletal (CEN) networks, but the mechanisms of regulation of some signaling components are still poorly studied. We are studying how PI(4,5)P2 is regulated and the relations between PI(4,5)P2 and Ras. Here we proposed that PI(4,5)P2 is primarily regulated by PI5K, an enzyme that converts PI4P to PI(4,5)P2. The piki- cells which have a significant PI(4,5)P2 level decrease, indicate that there is a strong correction between PI5K and PI(4,5)P2. We showed that PI5K colocalizes with PI(4,5)P2 at the rear of cells and shares complementary wave patterns with Ras and other front proteins. We identified the membrane binding region within PI5K using truncation studies. We found that deleting this membrane binding region will make PI5K fall to cytosol and potentially lower PI(4,5)P2 levels on plasma membrane. We recruited cytosolic PI5K to the membrane using iLiD optogenetic approach to determine if PI5K function and PI(4,5)P2 level can be restored upon recruitment. We performed co-immunoprecipitation assay and proved that PI5K can be pulled down from plasma membrane. Our hypothesis is Ras inhibits PI5K directly or relocates PI5K to further achieve the goal of inhibiting PIP2 levels and this forms a rapid autocatalytic loop. PKB slowly phosphorylates PI5K to form a delayed negative feedback loop. To test this hypothesis, we made PI5K point mutation T262A (nonphosphorylatable) and T262E (phosphomimetic) to study how PKB phosphorylation will restore PI5K and further inhibit Ras. We did cAMP global simulation assay on PI5K WT/T262A/T262E cells using PHcrac-RFP as the marker to look at the time difference for PKB phosphorylation. Taken these results together, we hope to find a new mechanism to explain Ras-PI5K-PI(4,5)P2 relations.

B514/P2895

Diffusion-limited Dynamic Partitioning Can Define Cortical Asymmetry and Control Wave-like Spatiotemporal Patterning of Multiple Membrane Proteins

T. Banerjee[1,2], S. Matsuoka[3,4], Y. Miao[1], Y. Kamimura[4], D. Biswas[5], D. S. Pal[1], M. Ueda[3,4], P. A. Iglesias[5], P. N. Devreotes[1]; [1]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, [2]Deaprtment of Chemical and Biomolecular Engineeering, Johns Hopkins University, Baltimore, MD, [3]Graduate School of Frontier Biosciences, Osaka University, Osaka, JAPAN, [4]Laboratory for Cell Signaling Dynamics, RIKEN Center for Biosystems Dynamics Research, Osaka, JAPAN, [5]Department of Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD.

Dynamic cortical patterning of different signal transduction and cytoskeletal components has recently emerged as an underlying phenomenon that organizes the spatial and temporal scale of pseudopodia formation, macropinocytosis, cytokinesis, and cell polarity. These propagating waves and flashes have been theoretically modeled using different biomechanochemical excitable or oscillatory networks consisting of multiple interacting autocatalytic and feedback loops. All these patterns, that orchestrate information flow between membrane and cytoplasm, are conventionally considered to propagate via a “trigger wave” or “stadium wave” mechanism. Typically, network activating proteins, such as PI3K, Akt, and SCAR/WAVE are thought to be transiently and asymmetrically recruited from cytosol to membrane, whereas deactivating components such as PTEN and myosin II dissociates from the membrane in those particular domains. The recruitment and release process is spatially coordinated and repeated multiple times to maintain asymmetric patterns. However, this model excludes the possibly of symmetry breaking of any integral or lipid-anchored membrane proteins which remain associated with the membrane. Here, we report that, multiple lipid-anchored membrane proteins, such as PKBR1, Gβγ, R-

Pre, and RasG, in contrary to various previous reports, exhibits highly asymmetric dynamics in the plasma membrane during cortical patterning and protrusion formation in migrating *Dictyostelium* cells. We found that the symmetry breaking processes of these membrane proteins could be successfully organized in absence of actin polymerization. Combining receptor activation and photoconvertible-protein based translocation assays, we demonstrated that these patterns are generated by dynamic partitioning or phase separation of different lipid-anchored proteins to specific membrane compartments. Using single-molecule imaging based measurement of membrane-dissociation and diffusion kinetics, we discovered that, instead of commonly accepted recurrent recruitment-release mediated "trigger wave" or "stadium wave" machinery, differential diffusion coefficient of these lipid-anchored proteins in different domains of the membrane drive this symmetry breaking and wave propagation processes. Computational simulation of excitable network involving stochastic reaction-diffusion master equation established that, while there are characteristic differences, either periodic shuttling or dynamic partitioning based self-organization can yield similar cortical wave patterns with the same length and time scale, as observed experimentally.

B515/P2896

Front rear polarity establishment during bleb based migration under confinement

A. Jha[1], A. Chandra[2], J. Haugh[3], C. Waterman[1]; [1]National Institutes of Health, Bethesda, MD, [2]North Carolina State University, Raleigh, MD, [3]North Carolina State University, Raleigh, NC.

Cancer cells with high contractility are prone to blebbing and when confined in the absence of integrin based cell-substrate adhesion, exhibit "leader bleb-based" migration characterized by a highly polarized morphology with a long, stable leader bleb leading the direction of migration especially during metastasis. However, the mechanisms mediating the establishment and maintenance of the extreme cell polarization are not known. We sought to test the hypothesis, that polarization is mediated by the segregation of signaling components on the plasma membrane especially growth factor receptors. To test this, I performed time-lapse imaging of highly metastatic melanoma cells under 3µm vertical confinement. In the absence of integrin signaling, Epidermal Growth Factor Receptor (EGFR) signaling is required for bleb stability and hence polarity during bleb-based migration. EGFR exhibited a strong gradient with accumulation at the base of the bleb compared to the bleb tip. Other transmembrane proteins (TM) also exhibit strong gradient however proteins on the outer and inner leaflet of the PM are distributed uniformly showing lack of any membrane flow from tip to the base of the bleb. With super resolution live imaging of actin and manipulations, we show TM gradients are established in the blebs due to strong corralling by membrane proximal actin, accumulation of large picket proteins like CD44 and rapid protein turnover on the back of the bleb compared to the tip of the bleb not by advection due to fast actin retrograde flow. The back of the bleb has stronger membrane-cortex link that enhances the actin corralling. With fluorescence anisotropy measurement we show that EGFR activity is high in the back of the bleb. This asymmetry results surprisingly in a reverse front-rear Rho-GTPase and phosphoinositide lipid polarity signature compared to adhesion based cell migration with RhoA/PIP2 in the front and Rac/PIP3 in the back of the bleb. With this work, I elucidate novel mechanisms of protrusion formation and front-rear polarity establishment during less understood bleb-based migration and front-rear polarity establishment.

B516/P2897

Mapping variation in the morphological landscape of human U2OS cells with optical pooled CRISPRi screening

R. D. Labitigan[1,2], A. L. Sanborn[3,4], C. V. Hao[4], C. K. Chan[5], N. M. Belliveau[2], E. M. Brown[5], M. Mehrotra[2], J. A. Theriot[1,6]; [1]Department of Biochemistry, Stanford University, Stanford, CA, [2]Department of Biology, University of Washington, Seattle, WA, [3]Department of Computer Science, Stanford University, Stanford, CA, [4]Department of Structural Biology, Stanford University, Stanford, CA, [5]Allen Institute for Cell Science, Seattle, WA, [6]Howard Hughes Medical Institute, University of Washington, Seattle, WA.

Image-based screening is a powerful platform to identify genetic regulators of spatial and organizational cell phenotypes. However, in the case of phenotypes relating to cell-scale cytoskeletal organization and morphology, the contributions of individual gene products are challenging to define due to the complexity present in natural phenotypic variation. While image-based screens are typically arrayed (i.e. perturbations are kept in separate wells), recent technologies have enabled a pooled format that promises to increase scalability and reduce technical variability, thus allowing for more precise phenotypic encoding and greater potential for biological discovery.

We leveraged the controlled nature of image-based pooled screening to assess the impact of CRISPRi knockdown of 366 genes that had been shown or proposed to alter cell morphology or actin filament architecture in human U2OS osteosarcoma cells. The large, widely varied shapes and intricate internal organization of U2OS cells provide an ideal system to study genetic determinants of complex morphological states. To determine the identity of the guide RNA in each cell, we optimized in situ sequencing technologies to maximize the efficiency and confidence of DNA barcode readout from fixed cells. We captured the phenotype and genotype of 1.5 million cells by imaging the actin cytoskeleton, nucleus, and DNA barcodes, and successfully assigned guide RNAs to 85% of cells.

A persistent challenge in image-based screens is how to rigorously describe high-dimensional phenotypes. Using a deep learning algorithm, the β-variational autoencoder, we generated a phenotypic space that quantitatively maps the range of single-cell phenotypes across the most important modes of variation in the full dataset. This phenotypic mapping was distinct from a mapping generated via a more conventional approach employing morphological profiling-based dimensionality reduction, but still detected similar gene hits while requiring minimal design decisions. We also developed a constrained sampling method to generate interpretable visualizations of morphological variation within this high-dimensional phenotypic space, enabling us to define the effects of specific gene knockdowns even in the presence of substantial underlying phenotypic variability. By relating these phenotypic shifts to each other, we construct a quantitative and interpretable landscape of monogenically tunable modes of variation in U2OS morphological structure. The approach we develop for mapping and visualizing phenotypic variation in a high-dimensional space is generalizable and can be applied to diverse biological image datasets.

B517/P2898

Investigating Intrinsic and Extrinsic Mechanisms of GBM Invasion

M. J. Barraza[1], A. Banisadr[1], F. B. Furnari[2], A. J. Engler[3,4]; [1]Biomedical Sciences Graduate Program, University of California, San Diego, La Jolla, CA, [2]Department of Pathology, University of California, San Diego, La Jolla, CA, [3]Department of Bioengineering, University of California, San Diego, La Jolla, CA, [4]Sanford Consortium for Regenerative Medicine, La Jolla, CA.

Glioblastoma Multiforme (GBM) is the most lethal and common primary brain tumor in adults, with a median patient survival of 12-15 months following current standard of care. This poor prognosis is largely due to highly invasive tumor cells that evade surgical resection and develop multiple mechanisms of therapeutic resistance, leading to patient relapse with recurrent GBM. Adding to this aggressive character, GBM is heterogeneous, with 25% of cases containing cells expressing a constitutively active epidermal growth factor receptor (EGFR) truncation of exons 2-7 known as variant III, i.e., EGFRvIII. These cells have enhanced oncogenic properties and can activate neighboring glioma cells overexpressing wtEGFR through an extrinsic mechanism, leading to cooperative invasion. It remains unclear how different EGFR mutation combinations, in conjunction with hallmark GBM mutations (i.e., *PTEN* deletion), impact tumor aggressiveness. Thus, we hypothesize that intrinsic signaling initiates invasion mechanisms, while extrinsic signaling in genotypically diverse tumor populations enhance glioma cell migration into brain parenchyma. To model GBM heterogeneity, we utilized an isogenic mouse astrocyte model of high-grade glioma (mHGG) containing combinations of common GBM mutations (i.e., *Ink4a/Arf* deletion, *PTEN* deletion, and wtEGFR or EGFRvIII overexpression). A spinning disc assay, which applies shear stress to cells seeded on an ECM-coated coverslip by spinning at defined angular velocities in a PBS buffer, was used to measure average adhesion strength of these cell populations. 2D and 3D migration assays were used to measure both intrinsic and extrinsic migratory properties to further characterize invasion phenotypes. We found that cells expressing EGFRvIII have intrinsically lower adhesion strength compared to wtEGFR and parental cells. Correspondingly, EGFRvIII cells traveled a greater distance and at a faster speed than wtEGFR and parental cells in the 2D migration assay. Interestingly, wtEGFR cells exposed to *Ink*$^{-/-}$EGFRvIII conditioned media (CM) for 24 hours had reduced adhesion strength and enhanced migration; however, wtEGFR cells exposed to *Ink*$^{-/-}$ *Pten*$^{-/-}$EGFRvIII CM remained unchanged in terms of adhesion and migration, suggesting a role for PTEN in regulating EGFRvIII extrinsic signaling. Furthermore, tumor spheroids composed of varying ratios of two cell populations (i.e., wtEGFR and EGFRvIII) exhibited a spectrum of migratory patterns when embedded in a collagen matrix, ranging from independent to collective migration. Overall, these studies provide insight into the role intratumoral heterogeneity plays in glioma cell migration and possibly suggest new targets for therapeutic intervention.

B518/P2899

Extracellular Matrix Composition Tunes Macrophage Morphology, Motility, and Adhesion through Distinct Integrin-Mediated Signaling Pathways

M. Stinson, J. D. Rotty, A. Laurenson; Uniformed Services University of the Health Sciences, Bethesda, MD.

The microenvironment of tissue-resident macrophages is complex and mutable, containing a variety of signaling molecules, glycoproteins, and fibrous proteins that are tissue and context dependent. Macrophages must be capable of rapidly navigating and responding to these 3D environments in order

to maximize their functions as first responders to pathogens, wounds, and sites of inflammation. Some of the most well characterized fibrous glycoproteins constituting the extracellular matrix (ECM) are collagen, fibronectin, and laminin. Substantial work has been done to understand the interactions between macrophages and fibronectin, a substrate that induces spreading and persistent migration. However, often-cited studies suggest that macrophages do not adhere to laminin. Our work has demonstrated that ECM components differentially elicit morphological and migratory phenotypes that depend on distinct integrin binding and activation of downstream cytoskeleton-regulating pathways. The same population of bone marrow derived macrophages (BMDM) plated on equal concentrations of fibronectin and laminin 111 demonstrate distinct morphological features and migration characteristics. Laminin 111 elicits faster, less persistent myosin II-dependent migration, lower adhesion, and smaller spread cell area. Conversely, BMDMs plated on fibronectin are slower, more persistent, adhere strongly and have a large spread cell area. We next demonstrate that each of these ECM sensing pathways are distinct. Lower concentrations of laminin 111 cause cells to slow down and spread out, and less fibronectin causes cells to speed up. Additionally, with a series of inhibitor experiments, we determined that laminin 111 appears to increase migration velocity and dynamic shape changes during motility by increasing myosin II activity, while decreasing persistence and inducing a smaller cell shape through ROCK activity. Furthermore, αvβ3 and αvβ5 inhibition was shown to increase macrophage velocity on fibronectin, while α6 inhibition blocked binding to laminin 111 completely. To summarize, α6 binds to laminin 111 and increases ROCK and Myosin II activity to a higher degree than the RGD-binding integrins that recognize fibronectin. The resulting laminin 111 phenotype overrides cell spreading, causing macrophages to ball-up, migrate less persistently and at a higher velocity. These data suggest that macrophages sense ECM differences through differential integrin binding, leading to altered adhesion, activation of specific cytoskeletal regulators, and altered dynamic macrophage behaviors. It is also possible that ECM sensing may be a critical modulator of additional innate immune functions beyond motility.

B519/P2900

Quantifying Correlation of Cell Migration, Membrane Remodeling, and Cortical Localization of Actomyosin Cytoskeletal Components in Live Animals

D. Chen[1], N. Melis[1], W. Losert[2], R. Weigert[1]; [1]National Institutes of Health, Bethesda, MD, [2]University of Maryland, College Park, MD.

Cell migration requires local membrane remodeling at the leading edge and the rear of the cell (e.g., uropod), a process which involves dynamic protrusions and retractions driven by cytoskeletal rearrangements. State of the art 2-photon intravital subcellular microscopy (ISMic) enables the acquisition of 4-D movies of interstitial cell migration *in vivo* with a resolution of a few hundred nanometers and a frame rate of 6 seconds. Here, we describe a quantitative tool to investigate how local levels of non-muscle myosin IIA (NMIIA) and cell membrane remodeling dynamics coordinate to drive the cell migration. The proposed method is customized with MATLAB. The cell shape is quantified through snake algorithm. The cell boundary at each frame is defined with consecutive boundary points at subpixel precision for local boundary curvature calculation. The local boundary motion is identified by tracking the boundary points with minimum displacement criterion. The onset, dynamics, and lifetime of each local membrane protrusion at the cell leading edge are monitored, as well. For each boundary point, a cortical box of a selected depth is defined, and the local average fluorescence intensity of cell voxels in that box is measured for the NMIIA-GFP, expressed in the migrating neutrophils. NMIIA

distribution in the leading edge and uropod of each migrating cell are defined as the fraction of time that a cell maintains a defined level (low, medium, high) of NMIIA during migration. At the subcellular level, this method provides the correlation between the cortical NMIIA and local cell membrane remodeling. At the cell level, it provides the correlation between persistence of cell migration direction, NMIIA distribution, and local membrane protrusions at the leading edge (or the uropod). Neutrophil migration *in vivo* and in 3-D collagen gel mimicking the *in vivo* ECM were quantified using this tool. We found that NMIIA was recruited not only to the retracting rear of migrating neutrophils but also at the leading edge, where it positively correlated with the local cell boundary curvature. Notably, this quantitatively defined phenotype was not observed in 2-D under agarose in vitro assays where NMIIA is mostly concentrated at the retracting uropod. Finally, NMIIA recruited at the leading edge of the cell facilitated the persistence of cell migration direction, possibly through stabilization of the lifetime of the local protrusions at the leading edge.

B520/P2901

Defining the role of CD56/NCAM in human natural killer cell polarization and migration

A. L. Martinez, M. J. Shannon, E. M. Mace; Columbia University Irving Medical Center, New York, NY.

CD56/neural cell adhesion molecule (NCAM) is a glycoprotein that plays a poorly defined role in lymphocyte functions including cell migration and natural killer (NK) cell cytotoxic function. While we have previously described localization of CD56 to the uropod of migrating NK cells, the role that it plays in cell migration and cell polarization remains undefined. The intracellular domain of the 140 kDa isoform of NCAM can play a role in mediating signaling interactions through the Src kinase Fyn and focal adhesion kinase in neurons, however the mechanism of CD56/NCAM function in immune cells has not been described.

To investigate the role of CD56 in NK cell migration, we deleted CD56/NCAM from human NK cell lines. We found that CD56-knockout (CD56-KO) NK cells had impaired spontaneous cell migration on the LFA-1 integrin ligand ICAM-1 compared to wild-type (WT). Automated detection of cell tracks and morphology from confocal microscopy data identified specific features of CD56 deficient cells, namely abnormal cell eccentricity and decreased speed, persistence, and track length. Live cell confocal imaging of LifeAct mScarlet WT and CD56-KO NK cells demonstrated that actin ruffling was intact in CD56-KO but cells formed multidirectional lamellipodia-like structures, suggesting that a lack of cell polarity contributed to the cell migration defect. Actin flow speed and percent immobile (non-flowing) actin was unchanged, suggesting that actin polymerization is unaffected by the absence of CD56. This imaging further identified the presence of unusual actin structures in CD56-KO NK cells, specifically large actin foci on the basal surface of migrating cells. Structured illumination microscopy (SIM) confirmed the presence of these structures, which were also enriched for vinculin and Pyk2, in migrating cells. Additional SIM imaging identified an increased frequency, but decreased area, of activated integrin foci in CD56-KO NK cells, further suggesting that CD56 plays a role in promoting integrin turnover and actin homeostasis. Reconstitution of CD56-KO cells with full-length CD56/NCAM, but not CD56/NCAM missing the intracellular domain, restored the actin phenotype, demonstrating that the intracellular domain of CD56/NCAM plays a role in integrin-mediated signaling in human NK cells. Together, our data identify a novel role for CD56/NCAM in NK cell adhesion and migration.

B521/P2902

Rap1 coordinates cell-cell adhesion and cytoskeletal reorganization to drive collective cell migration during rapid wound healing

K. E. Rothenberg, R. Fernandez-Gonzalez; University of Toronto, Toronto, ON, Canada

Coordinated cell movements contribute to tissue development and repair and to the spread of metastatic disease. We investigate collective cell migration during wound healing in the *Drosophila* embryonic epidermis. Upon wounding, the cells immediately adjacent to the wound become polarized: cell-cell adhesion molecules are internalized from the wound edge, and actin and the molecular motor non-muscle myosin II accumulate at the interface with the wounded cells, forming a supracellular cable around the wound that coordinates cell movements. The cable is thought to assemble from and anchor at former tricellular junctions (TCJs) along the wound edge, which are reinforced during wound closure through the accumulation of adherens junction components. However, the mechanisms that coordinate the adhesive and cytoskeletal rearrangements required for rapid wound closure are unclear. We found that reducing the activity of the small GTPase Rap1 using a dominant-negative form of the protein (Rap1DN) slowed wound repair by 45%. The slower wound closure was accompanied by defective actomyosin polarization to the wound edge and a loss of E-cadherin at TCJs. Embryos expressing a mutant form of the Rap1 effector Canoe/Afadin that cannot bind Rap1 displayed a defect in E-cadherin accumulation at TCJs similar to the effects of Rap1DN, but with normal myosin polarization. This suggests that Rap1 signals through Canoe to drive TCJ reinforcement, but not to assemble the supracellular cable. To understand how Rap1 may be affecting actomyosin cable assembly, we measured Rho1/RhoA activity in Rap1DN embryos and showed that Rho1 activity was reduced by 72% in Rap1DN embryos, which was accompanied by a 54% reduction in tension at the wound edge. We found that the RhoGEF Ephexin, which can be activated by Rap1, was necessary for myosin polarization to the wound edge and rapid wound repair, with no significant effects on E-cadherin redistribution. Our data support a model in which Rap1 simultaneously drives actomyosin cable assembly via activation of Rho1 through Ephexin and reinforces adhesion at TCJs via Canoe to enable rapid migration to heal embryonic wounds.

B522/P2903

Investigating Mechanisms of FGF Dispersal and Spatial Organization of Downstream Signaling in Migrating Cells

T. V. Gibney, L. Latifi, A. M. Pani; Biology, University of Virginia, Charlottesville, VA.

Cell signaling dynamics are tightly regulated in space and time to orchestrate cellular activities such as migration, proliferation, and differentiation. We are using *C. elegans* postembryonic muscle progenitors as a tractable model to dissect signaling mechanisms *in vivo*. One muscle progenitor type, sex myoblasts (SMs) migrate from near the tail of the worm to the center during larval development. Fibroblast Growth Factor (FGF) signaling is required for SM migration, and FGF has been hypothesized to act as a chemoattractant for migrating cells. FGFs are often thought of as soluble signaling proteins that can diffuse to form gradients. However, extracellular diffusion has not been observed with endogenous FGFs, and in several contexts FGFs are membrane anchored proteins that signal at cell contacts. To investigate how FGFs guide SM migration, we tagged the endogenous FGF (EGL-17) involved in SM migration and imaged its dynamics along with membranes of FGF-expressing cells and migrating SMs. Unexpectedly, we did not observe an extracellular FGF gradient from the center of the worm, but

instead observed FGF expressed by ventral cells along the route taken by migrating SMs. However, live imaging of FGF along with ligand and receptor expressing cells, in their natural context and in misexpression experiments, demonstrated that FGF/EGL-17 is diffusible *in vivo* and spreads between cells that are not in contact. We then anchored the endogenous FGF to source cell membranes, which disrupted SM migration and confirmed that free FGF dispersal is required despite the absence of a visible extracellular gradient. Misexpression experiments are also consistent with diffusible FGF acting as a directional cue for SMs. To investigate how extracellular FGF is translated into directed cell migration, we characterized and manipulated localization of downstream components that link activated FGF receptors to cytoskeletal regulators and/or Ras-MEK-ERK signaling. We found that the Ras GEF SOS-1 localizes to the leading and ventral edges of migrating SMs. Redistributing a SOS catalytic domain around the entire cell disrupted SM migration similar to SOS-1 degradation. This work provides direct evidence that an endogenous FGF acts as a diffusible signaling protein and provides new insights into mechanisms that translate extracellular signals into directed cell behaviors.

B523/P2904

CED-12/ELMO/ELMOD can switch from promoting to inhibiting F-actin formation in the same tissue

M. Soto, T. Venkatachalam, L. Cordova Burgos; Rutgers - Robert Wood Johnson Medical Sch, Piscataway, NJ.

ELMO (engulfment and cell migration) proteins were first discovered in *C. elegans*, and are conserved from humans to plants, fungi and amoebae. Comparisons in other species have identified two families within the ancient ELMO family. Shorter ELMOD proteins, primarily composed of an ELMO domain, typically encode a GAP with hydrolysis activity towards Arf and Arl GTPases. Longer ELMO family proteins, with additional domains, support the GEF function of partner CED-5/DOCK-180 proteins, to activate Rac/Rho family GTPases. The original ELMO, CED-12, is longer, includes a PH membrane binding domain, and was thought to act, together with CED-5/DOCK180, to activate CED-10/Rac1 during engulfment and cell migrations. We therefore expected CED-12/CED-5 to behave as a GEF for CED-10/Rac1, and activate F-actin during a cell migration essential for *C. elegans* embryogenesis, ventral enclosure. During ventral enclosure, a sheet of epidermal cells migrates to enclose all other tissues. Instead, we discovered that loss of CED-12, CED-5 or their partner protein CED-2/CRKII, all lead to embryonic lethality due to excess F-actin formation, increased dynamics in the migrating cells, and faster migrations. However, corpses that are engulfed by these same migrating epidermal cells show lower F-actin and slower corpse disappearance, as would be expected for loss of a CED-10/Rac1 GEF. To address how loss of CED-12/CED-5 can have two opposing effects in the same cells as they undergo corpse engulfment and cell migration, we investigated if CED-12 harbors both ELMOD and ELMO functions. Aligning CED-12 to other *C. elegans* GAP proteins and to ELMODs identified a GAP region in CED-12. Mutating a candidate catalytic Arginine in this region (R537A) altered the epidermal cell migration function, and not the corpse engulfment function. Thus, a single ELMO/ELMOD protein can use distinct domains to carry out two opposite functions. Additional studies, including those to identify the signals that activate the GEF or GAP functions of CED-12, and to identify the GTPase targets of the GAP function, will further our understanding for how GEF and GAP functions can be coordinated, even within the same cells.

B524/P2905

Glioblastoma cell migration requires membrane tension and endocytosis regulated by Plexin-B2

C. Junqueira Alves[1], T. Hannah[1], R. Wiener[1], M. Tipping[2], R. Alves Dias[3], J. Rodrigues Furtado de Mendonça[3], H. Zou[1], R. Friedel[1]; [1]Icahn School of Medicine At Mount Sinai, New York City, NY, [2]Memorial Sloan Kettering Cancer Center, New York City, NY, [3]Federal University of Juiz de Fora, Juiz de Fora, Brazil

Background: Infiltrative growth is a major cause of high lethality for the malignant brain tumor glioblastoma (GBM). To initiate invasion, GBM cells face the challenge of negotiating through tight interstitial space inside the brain. The mechanisms of how GBM cells regulate invasiveness are unclear. As tumor cells frequently usurp developmental pathways, we suggest that the Plexin axon guidance receptors may regulate GBM invasiveness.**Method:** We have established a novel *in vitro* paradigm that utilizes a 3D-printed microchannel device to investigate the capability of GBM cells to migrate through constrictions of different sizes. We speculated that the GBM cell migration may be through endocytosis, which is a major regulator of signaling events and has been shown to be regulated by membrane tension. We interfered with GBM cell migration using drugs known to inhibit endocytosis. Next, we deleted Plexin-B2 in GBM cells by lentiviral CRISPR-Cas9. We probed membrane tension using live fluorescent membrane tension probe and optical tweezers. We also investigated the endocytosis dynamics using fluorescent labeled dextran, plasma membrane-targeting dyes, and membrane-attached intracellular fluorescent proteins in live GBM cells. **Results:** Wild-type GBM cells showed active locomotion, high velocity, and forward motility through the constrictions. In contrast, Plexin-B2 knockout cells extend long cellular processes, displayed plasma membrane rupture, and failed to 'infiltrate' through narrow constrictions. It was associated with low uptake of cytoplasmic dextran, reduced endocytosis of membrane-attached intracellular proteins, and low membrane tension. Similar phenotypes were observed after treating WT GBM cells with endocytosis inhibitors. **Conclusion:** Plexin-B2 regulation of membrane tension and endocytosis provides biomechanical plasticity for GBMs facing the challenge of negotiating through tight spaces during cancer invasiveness.

B525/P2906

The molecular basis for rational targeting of FGFR-driven proliferation and motility

J. Migliavacca[1], K. Santhana Kumar[1], L. L. Kopp[1], A. Gries[1], S. Yan[1], C. Brunner[2], M. Schuster[3], O. Zerbe[3], G. Schneider[2,4], M. A. Grotzer[1], **M. Baumgartner**[1]; [1]University Children's Hospital Zurich, Zurich, Switzerland, [2]ETH Zurich, Zurich, Switzerland, [3]University of Zurich, Zurich, Switzerland, [4]ETH Singapore SEC Ltd, Singapore, Singapore

Objective: Receptor tyrosine kinases (RTK) promote growth and dissemination in pediatric tumors including medulloblastoma (MB). We hypothesized that repression of protein-protein interactions downstream of activated RTKs could constitute a novel means to inhibit oncogenic signal transmission. Therefore, we delineated molecular interactions downstream of fibroblast growth factor receptor (FGFR) signaling in MB and identified and validated small bioactive molecules that repress FGFR-driven growth and invasiveness.

Methods: Using 3D in vitro, in vivo and cerebellar slice culture models, we identified the FGFR signaling pathway as a driver of growth and invasiveness in MB. Using proteomics and functional assays, we determined key molecular components of oncogenic FGFR signaling in MB. By computational screening combined with functional, biophysical, and structural binding analyses, we discovered and validated novel small molecule compound inhibitors of FGFR-driven growth and invasiveness.

Results and discussion: We identified the FRS2 protein as an essential immediate downstream transmitter of FGFR signaling in MB and demonstrate a central role for STRN3 to further integrate these signals toward growth and invasiveness. Mechanistically, STRN3 interacts with the kinase MAP4K4, couples it to the protein phosphatase 2A and thereby inactivates growth repressing activities of MAP4K4. In parallel, STRN3 enables MAP4K4-mediated phosphorylation of PKC delta and VASP, which combined are necessary to promote tissue invasion. To selectively repress pro-invasive FGFR functions, we identified and functionally validated small molecule ligands of FRS2 that prevent FRS2 activation and downstream signaling. We demonstrate efficacy of these ligands in inhibiting invasion and growth promoting activities, confirmed on-target activity and identified potential off-targets.
Conclusion: The cooperation of STRN3 and MAP4K4 represses tumor suppressive hippo signaling and promotes invasiveness through PKC delta and VASP. These tumor promoting functions of the activated FGFR can be blocked by a small molecule ligand of the FRS2 adaptor protein. Thus, the here identified FRS2 ligand bears promise for further pre-clinical evaluation as an anti-growth promoting and anti-metastatic therapeutic applicable to FGFR-driven tumors.

B526/P2907

Lamellipodin drives stiffness-dependent cell cycling through YAP

J. A. Brazzo, Y. Heo, Y. Bae; Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY

The stiffness of the extracellular matrix (ECM) regulates cell proliferation via mechanotransduction. In this process, the fibrous ECM directly engages integrins in the cell membrane that activate FAK (focal adhesion kinase), p130Cas, and Rac GTPase. This signaling cascade triggers cyclin D1 for progression to S phase of the cell cycle and cell proliferation. We recently showed that lamellipodin is critical for this progression, and that lamellipodin itself is regulated by ECM stiffness through the FAK-p130Cas-Rac signaling axis. How stiffness-sensitive lamellipodin mechanistically drives cell cycling inside the nucleus is unclear. YAP (Yes-associated protein) is a proto-oncogene that translocates to the nucleus and induces cyclin D1 and cell cycle progression and also is critical for the mechanotransduction of ECM stiffness into proliferative gene expression. We hypothesize that lamellipodin controls stiffness-dependent cell cycle progression via YAP nuclear translocation. To test this, we cultured serum-starved wild-type and lamellipodin-overexpressing ($Lpd^{o/x}$) mouse embryonic fibroblasts (MEFs) on low-stiffness (physiologically relevant, 2-4 kPa) bis-polyacrylamide hydrogels functionalized with fibronectin in the presence of 10% serum for 24 h. The expression, phosphorylation, and nuclear translocation of YAP were assessed via immunoblotting and epifluorescence microscopy. The percentage of YAP in the nuclei of $Lpd^{o/x}$ cells was nearly 2-fold that in wild-type cells, but the total protein and S127 phosphorylation levels were not different. We then treated $Lpd^{o/x}$ cells on low-stiffness hydrogels with verteporfin, an inhibitor of YAP nuclear translocation, and assessed cyclin induction via immunoblotting and S phase entry via EdU incorporation. Treatment with verteporfin drastically reduced cyclin A and B induction and S phase entry. Our findings show that (i) mechanosensitive regulation of lamellipodin increases nuclear accumulation of YAP, (ii) nuclear YAP is critical for lamellipodin-mediated stiffness-dependent cell cycling, and (iii) the regulation of YAP by lamellipodin is independent of the phosphorylation of YAP and activation of MST1/2, which are mediators of the canonical Hippo signaling pathway that regulates YAP nuclear translocation. Further research will explore how lamellipodin directly regulates the nuclear translocation of YAP.

B527/P2908

Emergent actin flows explain distinct modes of gliding motility

C. L. Hueschen[1], L.-a. Segev Zarko[1], J.-H. Chen[2], M. A. LeGros[2], C. A. Larabell[2], J. C. Boothroyd[1], R. Phillips[3], A. R. Dunn[1]; [1]Stanford University, Stanford, CA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, [3]Caltech, Pasadena, CA

During host infection, single-celled apicomplexan parasites like *Plasmodium* and *Toxoplasma* use a motility mechanism called gliding, which differs fundamentally from other known mechanisms of eukaryotic cell motility. Gliding is thought to be powered by a thin layer of flowing filamentous (F)-actin sandwiched between the plasma membrane and a myosin-coated inner membrane complex. How this surface actin layer drives the diverse apicomplexan gliding modes observed experimentally - helical, circular, and twirling, and patch, pendulum, or rolling - presents a rich biophysical puzzle. Here, we use single-molecule imaging to track individual actin filaments and myosin complexes in live *Toxoplasma gondii.* Based on these data, we hypothesize that F-actin flows arise by self-organization, rather than following a microtubule-based template as previously believed. We develop a continuum model of emergent F-actin flow within the unusual confines provided by parasite geometry. In the presence of F-actin turnover, our model predicts the emergence of a steady-state mode in which actin transport is largely rearward. Removing actin turnover leads to actin patches that recirculate up and down the cell, a "cyclosis" that we observe experimentally for drug-stabilized actin bundles in live parasites. These findings provide a mechanism by which actin turnover governs a transition between distinct self-organized F-actin states, whose properties can account for the diverse gliding modes known to occur. More broadly, we illustrate how different forms of gliding motility can emerge as an intrinsic consequence of the self-organizing properties of F-actin flow in a confined geometry.

Single Cell Chemotaxis and Directed Migration

B528/P2909

Cortical actomyosin suppresses directed migration signaling networks

J. Kuhn, P. Devreotes; Johns Hopkins University, Baltimore, MD.

Many modes of eukaryotic migration require cells to separate the newly polymerizing actin meshwork at the cell front from actomyosin-based contraction at the cell back. The location and configuration of cytoskeletal molecules are controlled by upstream signal transduction events. The terms, Signal Transduction and Cytoskeletal Excitable Networks, STEN and CEN, reflect the biochemically excitable properties displayed by molecules in each of these systems. Much is understood about how the STEN regulates the CEN in random and directed migration. To examine how changes in cytoskeletal mechanics and organization might feedback to influence STEN, we developed methods to rapidly and locally alter myosin activity and cortical actin organization in migrating vegetative and developed cells. First, we show that *reducing* myosin assembly leads to an *increase* in activated Ras and an increased sensitivity to chemotactic stimulus. Second, *activation* of cortical actin regulator RacE *decreases* STEN activation. Surprisingly, Dictyostelium Rho homolog RacE activation leads to elevated actin polymerization, but this formation is relatively resistant to Arp 2/3 inhibition. Concomitantly, RacE activation causes a reduction in Ras activation and PtdIns(3,4,5)P3 levels. Taken together, our data suggest that cortical actomyosin suppresses cell front signaling molecules and suggests a novel mechanism for how cells can maintain front-back segregation during migration.

B529/P2910

PD-LI promotes rear retraction during persistent cell migration by altering integrin β4 dynamics

M. Wang, C. Xiong, A. Mercurio; University of Massachusetts Chan Medical School, Worcester, MA.

Although the immune checkpoint function of PD-L1 has dominated its study, we report that PD-L1 has an unanticipated intrinsic function in promoting the dynamics of persistent cell migration. PD-L1 concentrates at the rear of migrating carcinoma cells where it facilitates retraction, resulting in the formation of PD-L1-containing retraction fibers and migrasomes. PD-L1 promotes retraction by interacting with and localizing the β4 integrin to the rear enabling this integrin to stimulate contractility. This mechanism involves the ability of PD-L1 to maintain cell polarity and lower membrane tension at the cell rear compared with the leading edge that promotes the localized interaction of PD-L1 and the β4 integrin. This interaction enables the β4 integrin to engage the actin cytoskeleton and promote RhoA-mediated contractility. The implications of these findings with respect to cell-autonomous functions of PD-L1 and cancer biology are significant.

B530/P2911

An external, long-lived memory in cell migration based on retroactions with the substrate.

J. D'Alessandro[1], A. Barbier-Chebbah[2], V. Cellerin[1], O. Benichou[2], R. Mège[1], R. Voituriez[3,2], B. Ladoux[1]; [1]Université Paris Cité, CNRS, Institut Jacques Monod, Paris, FRANCE, [2]Sorbonne Université, CNRS, LPTMC, Paris, FRANCE, [3]Sorbonne Université, CNRS, Laboratoire Jean Perrin, Paris, FRANCE.

Cell migration is controlled by the integration of intracellular dynamics and extracellular cues. Meanwhile, it is known that cells do not leave their substrate virgin but instead they can modify its properties on their tracks. Although this bidirectional flow of information between the cell and its environment is well accepted, whether the two retroact onto each other had not been investigated so far. By studying the migration of isolated cells, either confined on 1d-tracks or moving freely in 2d, we evidenced such a crosstalk effect: cells leave a footprint on their path and they show a strong tendency to retrace their steps. This relies on modifications of the substrate by the cells, which in turn change the cell-substrate interaction when cells reach the edge of their footprint and eventually push them to repolarise backwards. To analyse the consequences of this self-attraction on the properties of cell motion, we designed a model of persistent self-attracting walk. We demonstrated that this entails ageing of the cell trajectories, but also changes dramatically the way cells explore their environment. Finally, our findings suggest that this “stigmergy” effect may also affect cell migration in situations where it was not suspected before.

B531/P2912

Piezo1 and ROCK2 Promote Fast Amoeboid Migration in Confined Environments

A. Caruso, N. Kar, J. Logue; Albany Medical College, Albany, NY

Cell migration through confined environments may induce a phenotypic transition to fast amoeboid (leader bleb-based) migration. However, the molecular mechanism(s) controlling this phenotypic transition remain poorly understood. Here, we show that regulation of intracellular calcium levels by the plasma membrane tension sensor, Piezo1, promotes the Leader Bleb-Based Migration (LBBM) of melanoma cells. Using a ratiometric assay, intracellular calcium is shown to rise with increasing levels of

confinement. Chelation of extracellular and intracellular calcium by BAPTA and BAPTA-AM, respectively, inhibits LBBM. Moreover, in highly motile cells, we found intracellular calcium levels to be dramatically increased at the cell rear. Using the Piezo1 inhibitor, GsMTx4, and RNAi, we can inhibit the phenotypic transition to fast amoeboid (leader bleb-based) migration. Therefore, we wondered if Piezo1 through calcium/calmodulin activates Myosin Light Chain Kinase (MLCK) to promote actomyosin contractility and amoeboid migration. Using a microchannel based assay, we find that ROCK2 and not MLCK, promotes amoeboid migration. Altogether, our work reveals an unanticipated collaboration between Piezo1 and ROCK2 in amoeboid migrating melanoma cells.

B532/P2913

Cortical turnover required for adhesion-independent pressure-driven migration

C. Copos[1], W. Strychalski[2]; [1]Northeastern University, Boston, MA, [2]Case Western Reserve University, Cleveland, OH.

Directed cell migration accompanies us from conception to death. Experiments have highlighted the diversity in migration strategies employed by cells in physiologically relevant environments. In three-dimensional fibrous matrices and confinement between two surfaces, some cells migrate using pressure-driven round membrane protrusions. In this form of migration, the role of substrate adhesion is thought to be minimal, and it remains unknown if and how a cell can migrate without any adhesion complexes. We build a biomechanical computational model of a cell moving in a channel using pressure-driven adhesion-independent motility mechanism. Simulations show that the cell cannot effectively migrate when the actin cortex is modeled as a purely elastic material, even with asymmetric channel geometry. Cells do migrate effectively if actin turnover is included with a viscoelastic description for the cortex. We report that locomotion occurs even when cells are placed in suspension, and locomotion displacement is enhanced with physical confinement and importantly, cortical turnover.

B533/P2914

An AMPK phosphoregulated RhoGEF feedback loop drives shape invariant amoeboid migration *in vivo*

B. Lin[1,2], J. Luo[1,2], R. Lehmann[1,2,3]; [1]Skirball Institute and Department of Cell Biology, New York University School of Medicine, New York, NY, [2]Whitehead Institute for Biomedical Research, Cambridge, MA, [3]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

The 'textbook' image of a migrating cell features a dynamic, actin-filled protrusion at its leading edge that orients and propels movement. However, cells can migrate in 3D/confined environments through alternative, shape invariant amoeboid modes powered by cell-scale, retrograde cortical actin flow. It remains unclear whether these amoeboid migration modes are natively employed *in vivo.* Moreover, how upstream signaling networks control such forms of motility without a defined leading edge remains undefined. Here, we develop cell type-specific imaging and perturbation techniques to show that inherently spherical *Drosophila* primordial germ cells (PGCs) employ global, retrograde cortical actin flows for orientation and propulsion during guided developmental homing. Extracted PGCs maintain constant cortical actin flows in the absence of confinement or contractile stimuli, suggesting this flow-based engine runs cell autonomously. These cortical actin flows are largely dependent on formin mediated actin polymerization rather than canonical branched actin polymerization associated with mesenchymal motility. PGCs use RhoGEF2, a RhoA-specific RGS-RhoGEF, as a dose dependent regulator of cortical flow through a feedback loop requiring its conserved PDZ and PH domains for membrane

anchoring and local RhoA activation. This feedback loop is regulated by RhoGEF2 availability and surprisingly requires AMPK rather than canonical $G\alpha_{12/13}$ signaling. AMPK multisite phosphorylation of RhoGEF2 near a conserved EB1 microtubule-binding SxIP motif releases RhoGEF2 from microtubule-dependent inhibition, providing a novel steering mechanism for shape invariant amoeboid motility. Thus, we establish the mechanism by which global cortical flow and polarized RhoA activation can be dynamically adapted during natural cell navigation in a changing environment.

B534/P2915

Neuronal growth cone turning evoked by chemotropic 5-HT gradients

X. Zhang, A. W. Schaefer, J. L. Burton, P. Forscher; MCDB, Yale University, New Haven, CT.

Bath application of serotonin (5-HT) increases rates of growth cone advance via a mechanism involving: 1) a Ca^{2+}-calcineurin-cofilin dependent increase in peripheral domain (P-domain) retrograde F-actin flow and 2) a parallel increase in PKC dependent nonmuscle myosin II activity. Here we report on how 5-HT chemical gradients affect the above behaviors. We used flash photolysis of caged 5-HT to generate soluble gradients and analyzed growth cone responses. Exposure to 5-HT gradients first triggered central domain (C-domain) reorientation and advance towards the source, and subsequently the peripheral domain (P-domain) turned and extended to the source. After calcineurin inhibition: C-domain advance was observed but significantly delayed while P-domain protrusion towards the source was inhibited. In contrast, Myosin II inhibition completely abolished both C-domain advance and P-domain protrusion. Quantitative analysis of actin filament dynamics during 5-HT evoked turning responses revealed that C-domain advance was guided by contractile actin arcs in the transition zone (T-zone) while P-domain retrograde F-actin flow rates remained constant. With calcineurin inhibition, actin arcs still formed in spite of the delayed C-domain advance. With non-muscle myosin II inhibition, actin arc activity and growth cone turning were both inhibited. A possible role for microtubule (MT) dynamics during chemotropic responses was also investigated. Preliminary results suggest MT growth trajectories preferentially aligned with actin arcs towards 5-HT sources. In summary, our results suggest a key role for contractile actin arc structures in coordinating these chemotropic growth cone turning responses. Our results also continue to cast the role of P-domain retrograde F-actin flow and the classical molecular clutch model of growth cone turning in a new light.

B535/P2916

Exploring the effect of extracellular matrix composition on the leading edge dynamics of dendritic cells migrating under confinement

N. M. Badillo, A. Gasilina, J. A. Hammer; National Institutes of Health, Bethesda, MD.

Dendritic cells play a critical role in initiating the adaptive immune response. Their function depends on their ability to take up antigens and migrate to lymph nodes, where they then present the antigens to naive T lymphocytes. During migration to lymph nodes, dendritic cells are guided by a gradient of the chemokine CCL19/CCL21 and must navigate environments of varying extracellular matrix composition. Efficient navigation through these microenvironments involves coordinated movements of the cell's nucleus, actin-rich extensions, and microtubule cytoskeleton, which together regulate space exploration, cell edge coordination and path finding. Like other leukocytes, dendritic cells can switch between non-adhesive and integrin adhesion-based modes of cell migration. Although the parameters required for leading edge coordination in the absence of adhesive substrates are well defined, much less

is known about leading edge coordination in dendritic cells migrating on adhesive substrates under confinement. To investigate this, we differentiated dendritic cells from murine bone marrow, stimulated them with lipopolysaccharide, and imaged their directed migration towards a CCL19 gradient on surfaces coated with BSA (blocked), collagen I, fibronectin, or laminin in an under-agarose assay. The resulting movies were analyzed for total distance traveled, velocity, directionality, space exploration, and leading edge formation using manual tracking. The organization of the actin and microtubule cytoskeletons was also determined by immunofluorescence staining for filamentous actin and α-tubulin. We observed that, unlike dendritic cells migrating on BSA-blocked surfaces or surfaces coated with collagen I or fibronectin, cells migrating on laminin-coated surfaces exhibited multiple, continuously protruding and retracting leading edges. Surprisingly, dendritic cells exhibited similar velocities, directionality and persistence regardless of matrix composition. Current efforts are directed at identifying the mechanisms by which dendritic cells maintain their migratory capacity while responding to varying extracellular matrix compositions.

B536/P2917

Understanding 3D Neutrophil Migration using PCA-based cell morphological analysis

A. van Loon[1], N. Belliveau[1,2], J. Theriot[1,2]; [1]University of Washington, Seattle, WA, [2]Howard Hughes Medical Institute, Chevy Chase, MD

Neutrophils are fast-moving innate immune cells that play a variety of important roles in the response to infection and inflammation. Although the ability of neutrophils to migrate in 3D is essential to mediate their immunological activity, relatively little is known about the mechanics of this process. Neutrophils rapidly deform and contort themselves to navigate the fibrous extracellular matrix, resulting in complex and dynamic morphology. Cellular morphology encodes information about the cell intrinsic mechanisms and environmental factors that define it. Recent analytical advances using spherical harmonic transformation and principal component analysis (PCA) have improved our ability to quantitate 3D cellular morphology. This new 3D shape analysis allows us to identify and visualize the primary ways in which 3D cell shape varies by reconstructing the shapes of average cells that represent each mode of variation. I probed the limitations of this new methodology by performing simulations using simple shapes with known dimensions of shape variation and determined that the method is highly sensitive to object alignment and resolution. To capture the shapes of migrating neutrophils, I fluorescently labeled the membranes and nuclei of dHL-60 neutrophil-like cells and imaged both labels at high resolution during 3D cell migration in collagen gels with instant structured illumination microscopy. In this data, I found that basic metrics of cell and nuclear shape, including surface area, volume, and elongation, are highly correlated with one another. To determine the primary modes in which dHL-60 shape varies, I performed PCA-based 3D shape analysis using dHL-60s cells aligned to their trajectories. Migration speed was most correlated with the top PC, which represented cell elongation along the axis of trajectory and alignment of the cell's long axis to the axis of trajectory. Collective shape analysis of CRIPSRi knockdown HL-60 cell lines for two candidate genes from a 3D motility screen, FMNL1 and CORO1A, along with control cells, revealed significant differences in a number of PCs that represented cell elongation as well as cell and nuclear volume. This work demonstrates that 3D shape analysis can be a useful tool in understanding the mechanics of neutrophil migration in 3D, but also has great potential as a platform for phenotypic comparison.

B537/P2918

Model of how septin ring compartmentalization aids T-cell circumnavigation in extracellular matrices

S. Alawadhi[1], D. M. Rutkowski[1], A. S. Zhovmer[2], E. D. Tabdanov[3], D. Vavylonis[1]; [1]Lehigh University, BETHLEHEM, PA, [2]U.S. Food and Drug Administration, Silver Spring, MD, [3]Pennsylvania State University, Hershey-Hummelstown, PA

In order to efficiently migrate through complex environments, T cells adopt a variety of migration modes including amoeboid and mesenchymal motions. Their ability to push the nucleus through narrow passages is critical for their migration through the extracellular matrix. Zhovmer et al. recently discovered that T cells in collagen matrices move with the aid of septin rings, which form around the nucleus at locations where extracellular matrix obstacles create high negative cell curvature. The resulting septin/F-actin rings subdivide the volume of the cell into separate compartments, with potentially different microenvironments. We developed a 2D computational model to test how such compartmentalization aids cell motility. In the model, beads representing the plasma membrane and nucleus move according to forces of bending rigidity, tension, contraction, fluctuating protrusions, and excluded volume interactions from encountered obstacles. Cell and nuclear volume conservation are implemented as area conservation forces. A weak nuclear centering force is implemented to represent cytoskeleton- and organelle-mediated nuclear centering. We assume that septin ring formation leads to compartment boundaries at sites where obstacle enforce proximity between the cell membrane and nucleus. We show that formation of these boundaries leads to a nuclear piston mechanism that enhances motility at high obstacle density.

B538/P2919

The role of acetylated microtubules in directed cell migration.

C. J. Saez Gonzalez, A. Deb Roy, T. Inoue; Johns Hopkins University Sch Med, Baltimore, MD

Cell migration is essential for many physiological processes including embryonic development, wound healing, and immune responses. Cells achieve efficient directional migration through two major steps: front-and-back polarization and re-arrangement of interactions between the cell and extracellular matrix (ECM). Cells adhere to the ECM using actin-based multiprotein complexes called focal adhesions (FAs) with which the cells exert forces to push or pull themselves to migrate, while long-term polarity is maintained by another cytoskeletal components, microtubules (MTs) Therefore, efficient cell polarity and migration are achieved by coordinated regulation of actin, MTs, and FAs. However, it remains unknown if there is a central regulator that orchestrates these seemingly distinct subcellular organizations. Our lab has recently found that cells lacking α-tubulin acetyltransferase 1 (αTAT1), the sole mediator of MT acetylation, display defects in FAs as well as in front-and-back polarity. To further characterize molecular mechanisms of αTAT1 functions as a potential master regulator of cell migration, live-cell, time-lapse fluorescence microscopy combined with pharmacological and genetic perturbations was performed in cell migration assays. As a result, cells with an αTAT1 knockout (KO) generally moved faster in random migration, in addition to interestingly exhibiting poor chemotactic performance with reduced directionality. In addition, while immunostaining of vinculin, a marker for FAs, showed a decreased signal in cells lacking αTAT1, western blot showed a similar expression of other FA proteins between our WT and αTAT1 KO cells, suggesting that it is molecular organization that contributes to FA defects, rather than changes in expression level of molecular constituents. Collectively, our data

illuminate a role of acetylated MTs as a regulator of directed cell migration by concertedly regulating front-and-back polarity and FAs dynamics.

B539/P2920

Non-canonical Wnt signaling promotes stem cell migration during regeneration of fly and mouse barrier epithelia

D. J. Hu, J. Simons, H. Jasper; Genentech, Inc., South San Francisco, CA

Somatic stem cells (SCs) are tightly regulated to preserve tissue homeostasis and repair damaged tissue. During regeneration, accurate stem cell positioning is critical for proper integration of newly-generated cells into damaged epithelia. However, the extent in which stem cells can migrate in different adult tissue, and the mechanisms regulating this behavior is poorly understood. We have previously demonstrated that, in the *Drosophila* intestine, intestinal stem cells (ISCs) undergo actin-dependent migration towards sites of injury. ISC migration is regulated by activation of the non-canonical Wnt pathway in ISCs by matrix metalloproteinase-dependent cleavage of Otk, the fly orthologue of the Wnt co-receptor Ptk7, in neighboring enteroendocrine (EE) cells. We have now demonstrated that stem cells in the mouse trachea, known as basal cells (BCs), share a similar capacity to migrate after tissue damage. We observe that BCs undergo actin-dependent migration towards sites of injury after the mouse trachea is damaged by polidocanol or laser ablation. Similar to *Drosophila* ISCs, migration of BCs is regulated by non-canonical Wnt signaling. Genetically knocking out Ptk7 inhibits BC migration, and results in impaired regeneration. Our findings identify a key role of SC migration during mammalian airway repair, and provide insight into the molecular mechanisms governing tissue regeneration.

Cell-cell Junctions

B541/P2921

The *Caenorhabditis elegans* ASPP homolog APE-1 is a junctional protein phosphatase 1 modulator

G. M. Beacham[1], **D. T. Wei**[1], E. Beyrent[1], Y. Zhang[2], J. Zheng[1], M. M. K. Camacho[1], L. Florens[2], G. Hollopeter[1]; [1]Molecular Medicine, Cornell University, Ithaca, NY, [2]Stowers Institute for Medical Research, Kansas City, MO.

How serine/threonine phosphatases are spatially and temporally tuned by regulatory subunits is a fundamental question in cell biology. Ankyrin repeat, SH3 domain, proline-rich-region-containing proteins are protein phosphatase 1 catalytic subunit binding partners associated with cardiocutaneous diseases. Ankyrin repeat, SH3 domain, proline-rich-region-containing proteins localize protein phosphatase 1 catalytic subunit to cell-cell junctions, but how ankyrin repeat, SH3 domain, proline-rich-region-containing proteins localize and whether they regulate protein phosphatase 1 catalytic subunit activity in vivo is unclear. Through a *Caenorhabditis elegans* genetic screen, we find that loss of the ankyrin repeat, SH3 domain, proline-rich-region-containing protein homolog, APE-1, suppresses a pathology called “jowls,” providing us with an in vivo assay for APE-1 activity. Using immunoprecipitations and mass spectrometry, we find that APE-1 binds the protein phosphatase 1 catalytic subunit called GSP-2. Through structure-function analysis, we discover that APE-1’s N-terminal half directs the APE-1-GSP-2 complex to intercellular junctions. Additionally, we isolated mutations in highly conserved residues of APE-1’s ankyrin repeats that suppress jowls yet do not preclude GSP-2 binding, implying APE-1 does more than simply localize GSP-2. Indeed, in vivo reconstitution of APE-1

suggests the ankyrin repeats modulate phosphatase output, a function we find to be conserved among vertebrate homologs.

B542/P2922

Mechanical Cell Model of Epithelialization

C. Cammarota[1], N. Dawney[2], M. Jüng[3], A. G. Fletcher[4,5], D. T. Bergstralh[1,2]; [1]Physics & Astronomy, University of Rochester, Rochester, NY, [2]Biology, University of Rochester, Rochester, NY, [3]Biomedical Engineering, University of Rochester, Rochester, NY, [4]School of Mathematics and Statistics, University of Sheffield, Sheffield, UNITED KINGDOM, [5]Bateson Centre, University of Sheffield, Sheffield, UNITED KINGDOM.

Monolayered epithelia are sheets of polarized cells that are one-cell thick; this arrangement is vital to tissue functions like filtration, secretion, or absorption. Our work aims to understand how physical constraints, including cell density, cell stiffness, cell-cell, and cell-substrate connections, affect the development of a polarized tissue architecture. While computational modeling can be used to address these problems, existing models generally focus on the arrangement of cells in the tissue plane rather than tissue depth. We therefore developed a new model to explore this axis. We find that a spatial constraint holding the cells in close proximity is required for cells to develop lateral connections. We have validated this in culture; Madin Darby Canine Kidney (MDCK) cells will spread along their substrate rather than grow lateral connections given space. Additionally, the model predicts that cells will form into a polarized architecture even at reduced cell-cell adhesion strength, which we have confirmed in culture using calcium depletion. Together, these results suggest that cell-cell adhesion is subordinate to tissue density in the development of the polarized architecture. We are currently working to model cell division to probe how densification influences development.

B543/P2923

Actin-dependent recruitment of AGO2 to the zonula adherens

M. Bridges, A. Daulagala, A. Risner, C. Kingsley, M. E. Davis, J. Nair-Menon, **A. Kourtidis**; Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC.

The adherens junctions (AJs) are cadherin-catenin - based structures that are essential architectural components of epithelial tissues. E-cadherin junctions in mature epithelial cell monolayers tether to an apical actomyosin ring to form the zonula adherens (ZA). In addition to their critical architectural role, epithelial AJs mediate numerous signaling pathways directing cell behavior. Along these lines, we have previously demonstrated that the E-cadherin - p120 catenin partner PLEKHA7 recruits and regulates core RNA interference (RNAi) complexes, such as the microprocessor and RNA-induced silencing complex (RISC), specifically at the ZA. However, the mechanism mediating recruitment of these RNAi components to the ZA remained unexplored. Analysis of the PLEKHA7 proteome reveals that the top group of its interactors are actin-binding proteins. This group includes LIM-domain containing protein family members, some of which have been associated in the past with the key enzymatic component of RISC, namely Argonaute 2 (AGO2), in cytoplasmic p-bodies. We now show that depletion not only of PLEKHA7, but also of either one of three of its interacting LIM-domain containing proteins, namely LMO7, LIMCH1, and PDLIM1, results in loss of AGO2 junctional localization. Depletion of either of PLEKHA7, LMO7, LIMCH1, PDLIM1, also results in defects on actomyosin organization at the ZA, including disruption both of structural integrity and of tension of the apical actin ring. Using

pharmacological disruption of actomyosin and calcium-switch assays, we demonstrate that junctional recruitment of AGO2 indeed depends on both the structural and the tensile integrity of the actomyosin cytoskeleton at the ZA. Taken together with our previous findings, showing that junctional recruitment of AGO2 is critical for regulation of a set of homeostatic miRNAs, our results portray a mechanosensitive RNAi machinery at the ZA, responsive to actomyosin perturbations, with potential implications in tissue remodeling and in disease.

B544/P2924

Architecture and dynamics of a novel desmosome-endoplasmic reticulum organelle

N. Bharathan[1], W. Giang[1], J. S. Aaron[2], S. Khuon[2], T. Chew[2], S. Preibisch[2], E. T. Trautman[2], L. Heinrich[2], J. Bogovic[2], D. Bennett[2], D. Ackerman[2], W. Park[2], A. Petruncio[2], A. V. Weigel[2], S. Saalfeld[2], C. Project Team[2], A. W. Vogl[3], S. N. Stahley[1], A. P. Kowalczyk[1]; [1]Dermatology, Pennsylvania State College of Medicine, Hershey, PA, [2]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, [3]Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, CANADA.

Desmosomes are adhesive intercellular junctions that provide strength and integrity to the epidermis and other epithelia. Dysfunction of the endoplasmic reticulum (ER) resident calcium pump, SERCA2, causes Darier's disease (DD), an epidermal disorder characterized by compromised desmosomal adhesion and abnormal keratinocyte differentiation. However, the mechanism by which ER dysfunction impairs desmosome formation is unclear. Using electron microscopy and live-cell fluorescence imaging, we found that peripheral ER tubules are in close proximity to desmosomes and form mirror image arrangements at desmosomes. Close to 100% of desmosomes made contact with ER tubules within a 2-minute duration. At desmosomal regions, 66% of ER membranes are stable compared to only 39% at non-desmosomal regions, suggesting that desmosomes stabilize ER associations. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) and 3D reconstructions revealed intricate nanoscale associations of ER tubules with keratin filaments and the desmosome outer dense plaque. Knockout of a key desmosomal cadherin, desmoglein 2, resulted in altered peripheral ER morphology at cell-cell contacts, indicating that desmosomes regulate peripheral ER tubule organization. Keratin intermediate filaments, which anchor to desmosomes, aligned and intertwined with ER tubules on either side of the desmosome plaque. To determine how alterations in keratin filaments influence ER organization, we expressed a keratin 14 mutant that forms aggregates and causes the epidermal blistering disease, epidermolysis bullosa simplex. Virtually all keratin aggregates exhibited persistent ER association and shifted ER morphology from tubular to sheet-like. Finally, we tested associations between ER, keratin, and desmosomes during dynamic cellular processes such as desmosome fusion and formation of nascent cell-cell contacts. We observed that ER tubules maintained contact with desmosome puncta immediately prior to and after fusion. Furthermore, we observed that ER tubules extend to the cell periphery at sites of de novo desmoplakin puncta formation and keratin filament assembly at sites of new cell-cell contact, suggesting that ER tubules pattern regions of the plasma membrane for desmosome formation. Our results reveal a unique tripartite structural complex comprising the ER, desmosomes, and keratin filaments. Furthermore, this architectural arrangement of the ER is altered when desmosomes or keratins are perturbed, thus revealing desmosomes and intermediate filaments as a previously unappreciated modulator of ER tubule morphology and dynamics.

B545/P2925

Syncytin-mediated membrane tubular connections facilitate the intercellular transfer of cargos including Cas9 protein

C. Zhang, R. Schekman; HHMI/UC Berkeley, Berkeley, CA.

Intercellular communication is an essential process for continuity of life in diverse organisms. Many modes of communication have been reported including extracellular vesicles (EVs) and tunneling nanotube structures. Here, we set up two delivery systems, Cas9-based dual-luciferase system and tri-fluorescent split-GFP system, to study cargo transfer between cells. We found that the EVs are inefficient to deliver Cas9 protein into reporter cells containing an integrated copy of nano-luciferase behind a site which when edited would allow the expression of nano-luciferase. In contrast, donor and acceptor cells cocultured to near confluence showed a 60-fold increase in luciferase expression. Among our tested donor & acceptor cell pairs, HEK293T & MDA-MB-231 is the best for intercellular transfer. However, Cas9 was not transferred among HEK293T cells. The tri-fluorescent split-GFP based system further confirmed that cargos were transferred through cell-cell contact from HEK293T to MDA-MB-231, but not among HEK293 cells. Next, we found that the intercellular transfer is actin and actin-related protein dependent rather than through the endocytosis pathway. Live-cell imaging results suggest that Cas9 transfer is mediated by open-ended membrane tubular connections with several microns in diameter. The structure of tubular connection between cells was further confirmed by correlative light and electron microscope. The human endogenous fusogen, syncytin, in recipient cells appears to control membrane fusion at the point of cell-cell junction. The depletion of syncytin, especially syncytin 2 in recipient cells significantly reduced the Cas9 transfer and blocked the formation of open-ended membrane tube. Full-length syncytin, but not truncated mutants, rescued the effect of depletion of syncytins on Cas9 transfer. Syncytin overexpression in HEK293T cells partially facilitated Cas9 transfer among HEK293T cells. Additionally, dynamin-mediated transport of plasma membrane proteins including syncytin to cell surface in recipient cells, appeared necessary for membrane tube formation and cargo transfer. These findings uncover a molecular basis for the formation of open-ended membrane tubular connections between cells, which may also facilitate to further understand the cell-cell communication in multicellular organisms and permit the development of an efficient means for targeted delivery of Cas9/gRNA.

B546/P2926

The Cell Cycle Modulates Active Stresses in Epithelial Tissue via Regulation of Junctional Mechanics

S. Mukherjee[1], J. Devany[2], M. L. Gardel[1]; [1]James Franck Institute, University of Chicago, Chicago, IL, [2]Physics Department, University of Chicago, Chicago, IL.

Epithelial tissue is comprised of cells which form adhesive, junctional contacts with each other. Epithelia are highly dynamic, with cellular level processes such as cell division, cellular shape changes and junctional remodeling leading to generation of forces that are transmitted across junctions. Tissue level active stresses derived from these cellular level processes underly complex behavior such as morphogenesis, collective migration, and wound healing. While some of the underlying cellular processes contributing to active stresses have been described in some detail, the extent to which active stresses are modulated by the cell cycle in proliferative epithelia is largely unknown. To investigate the effects of the cell cycle on active stresses, we cultured model MDCK monolayers on soft collagen substrates. Under these conditions, focal adhesion area and stress fiber abundance are greatly reduced,

and cellular motion is derived from active stresses transmitted across cellular junctions. We abrogated the cell cycle via pharmacological inhibition of CDK1, or via overexpression of $p27^{kip1}$, a multifunctional cyclin dependent kinase inhibitor. We observed a marked reduction in active stresses, as indicated by a reduction in cellular motion, when the cell cycle was abrogated with either method. We further found that CDK1 inhibition led to a reduction in junctional E-cadherin, and phosphorylated p120-catenin levels, but no change in total p120-catenin levels. Junctional dynamics have been implicated in modulation of active stresses in epithelia, and we found that CDK1 inhibition led to a marked reduction in E-cadherin surface mobility, likely as a result of impaired E-cadherin endocytosis. Taken together, our results support a model where the cell cycle regulates junctional dynamics to modulate active stresses in epithelia. We postulate that active stresses arise from differential regulation of junctional dynamics in cells at different stages of the cell cycle in a proliferative epithelial monolayer, and we are working to test this hypothesis. We are also working to elucidate the mechanism through which the cell cycle regulates junctional composition and dynamics and investigating if the cell cycle regulates cortical Rho-GTPase signaling to modulate active stresses.

B547/P2927

Coactosin-Like Protein 1 (COTL1) is a Novel Regulator of the Intestinal Epithelial Barrier Integrity and Repair

S. Lechuga, A. Marino-Melendez, A. I. Ivanov; Inflammation and Immunity, Cleveland Clinic Foundation, Cleveland, OH.

The actin cytoskeleton plays critical roles in regulating integrity and repair of epithelial barriers by mediating assembly of adherens junctions (AJs) and tight junctions (TJs) and controlling epithelial wound healing. Actin filaments associated with epithelial junctions and driving cell migration are highly dynamic and have a turnover that is controlled by numerous actin-binding proteins. The mechanisms underlying actin filament turnover in healthy and injured intestinal epithelium remains poorly understood. Coactosin-like protein 1 (COTL1) is an essential regulator of actin filament assembly, yet its functions in mucosal epithelia have not been previously investigated. In this study, we investigate the roles of COTL1 in regulating the structure and remodeling of AJs and TJs in model human intestinal epithelial cells (IEC). COTL1 was found to be enriched at apical junctions in polarized SK-CO15 IEC monolayers. The knockdown of COTL1 by RNA interference significantly increased paracellular permeability of cell monolayers, altered the recruitment of AJ/TJ proteins to steady-state apical junctions, attenuated junctional reassembly in a calcium-switch model, and inhibited formation of polarized epithelial cysts in 3-D Matrigel. Furthermore, COTL1 depletion attenuated collective IEC migration in a wound healing model. Our findings highlight COTL1 as a novel regulator of the intestinal epithelial barrier integrity and repair.

B548/P2928

Oral pathogens regulate epithelial cell behavior though the adherens junction-associated RNAi machinery

C. R. Kingsley, J. Nair-Menon, M. Engevik, A. Kourtidis; Regenerative Medicine and Cell Biology, MUSC, Charleston, SC.

Increasing evidence portrays key roles of host-microbial interactions in the progression of epithelial diseases, including tumorigenesis. A pathogen that has been linked with tumor progression is oral

microbe *Fusobacterium nucleatum* (*F. nucleatum*). However, the mechanisms by which *F. nucleatum* may influence epithelial cell behavior are not fully known. Notably, *F. nucleatum* has been associated with loss of epithelial integrity. The adherens junction (AJ) is an essential, E-cadherin-based, cell-cell adhesion complex that is key for epithelial tissue integrity but has also been implicated in regulation of cell behavior. Further, we have previously shown that epithelial AJs through the E-cadherin-p120 catenin partner PLEKHA7, recruit and regulate core components of the RNA interference (RNAi) machinery, such as DROSHA and AGO2, to suppress oncogene expression and pro-tumorigenic cell transformation. Thus, we sought to interrogate the AJ-associated RNAi machinery as a potential mechanism mediating the effects of *F. nucleatum* in epithelial integrity and pro-tumorigenic transformation. We used a well-differentiated colon epithelial cell line model (Caco2), and assessed effects of *F. nucleatum* on AJs and other pro-tumorigenic markers. Caco2 cells were exposed to either heat-inactivated bacteria, or to bacterial supernatant from *F. nucleatum spp. nucleatum*,*Streptococcus salivarius* and *Escherichia coli spp. Nissile*. Cells were fixed after 8, 16, and 24 hours of incubation, and examined by immunofluorescence and confocal microscopy for changes in the junctional localization of E-cadherin, p120 catenin, PLEKHA7, AGO2, and DROSHA. Results showed that localization of PLEKHA7, AGO2, and DROSHA was disrupted by *F. nucleatum*, but not by *Streptococcus salivarius* or *Escherichia coli.* Also, these changes in localization were accompanied by upregulation of oncogenes such as JUN and SNAIL, as shown by western blot of cells treated with *F. nucleatum* supernatant for 24 hours. Oncogenes JUN and SNAIL were previously found to be suppressed by the junctional RNAi. This data supports that *F. nucleatum* may be promoting pro-tumorigenic transformation through disruption of the AJ-associated RNAi machinery leading to increased expression of oncogenes, and this can help deepen our understanding of the mechanisms mediating host-pathogen interactions in epithelial homeostasis and disease.

B549/P2929

The blood-brain barrier responds to inflammation differently in juvenile and adult brains

J. D. Ochrietor, A. Gonzalez, F. Laveus, J. Gelsleichter; Biology, University of North Florida, Jacksonville, FL.

The blood-brain barrier (BBB) is essential for protecting the central nervous system from foreign insults. Because of this barrier, which restricts the free passage of most bacteria, viruses, and other pathogens, the brain maintains a sense of immune priviledge. However, diseases resulting from or contributing to inflammation, especially chronic inflammation, may alter the integrity of the BBB and allow pathogen-containing fluids to leak into the central nervous system. Basigin, a member of the immunoglobulin superfamily, is a cellular marker of blood vessel endothelial cells and hence is a component of the BBB. Reports indicate that Basigin may be responsible for reduced integrity of the BBB in the neural retina in response to chronic inflammation associated with diabetes. Therefore, the purpose of the present study was to evaluate Basigin expression at the BBB in the mouse brain in response to an inflammatory stimulus. Because Basigin serves as a chaperone for plasma membrane expression of monocarboxylate transporters (MCTs), which are expressed in the central nervous system, expression of MCT1 was also evaluated. Mouse brains were isolated from animals at several post-natal ages and incubated in the presence of lipopolysaccharide (LPS) or saline, for various times. Protein expression was measured via quantitative ELISA and immunohistochemistry. In juvenile animals, Basigin expression decreased with increasing LPS treatment time. Conversely, in adult animals, Basigin expression increased with increasing LPS treatment time. The change in expression was observed on blood vessel endothelial cells within the

brain samples. In contrast, MCT1 expression in response to LPS was like that of the saline-treated samples at all ages tested. The data suggest that the integrity of the BBB is influenced differently at different ages. As Basigin increases in response to an inflammatory stimulus in the adult brain, barrier integrity may concomitantly diminish, leading to the potential for neurodegenerative diseases. Young brains appear to prevent this degenerative process via the decreased expression of Basigin in response to chronic inflammation. It is not yet known whether intracellular signaling via TLR4, which is the receptor for LPS, or via subsequent cytokine production accounts for the changes in Basigin expression. Future studies will examine if Basigin expression, and hence BBB integrity, is similarly influenced by SARS-CoV-2 spike protein, for which Basigin is a known receptor.

B550/P2930

The RhoG guanine nucleotide exchange factor SGEF controls junctional localization of desmosomal proteins.

H. C. Lee[1], A. Rabino[2], R. Garcia-Mata[2], A. Dubash[1]; [1]Biology, Furman University, Greenville, SC, [2]Biological Sciences, University of Toledo, Toledo, OH.

The desmosome is a cell-cell adhesion complex which facilitates the mechanical stability of tissues. Desmosome function depends upon a tripartite organizational structure wherein transmembrane cadherins (Desmoglein and Desmocollin) link adjacent cells in the extracellular space, armadillo proteins (Plakophilin and Plakoglobin) stabilize the intracellular plaque, and the cytolinker Desmoplakin (DP) connects the plaque to the intermediate filament network. Disruption of the desmosome adhesion complex is predominantly observed in a range of different cutaneous syndromes and several types of cardiomyopathy, as skin and heart tissues undergo continuous mechanical stress. Loss of desmosomal integrity is also observed in cancer, where loss of desmosomal proteins accompany epithelial to mesenchymal transition (EMT). Determining the signaling mechanisms which control desmosome structure is therefore relevant for a wide range of different pathologies. Recent work has uncovered an important role for the protein Src Homology 3 (SH3)-containing GEF (SGEF) in maintaining the structure of both adherens junctions and tight junctions in epithelial cells. SGEF is a guanine nucleotide exchange factor specific for the RhoG GTPase, as it enhances the activity of the GTPase via promotion of GDP-GTP exchange. In our study, we analyzed a potential role for SGEF in regulation of the desmosome adhesion complex. Compared to control MDCKs, SGEF knockdown cells demonstrate a significant reduction in the junctional localization of several different desmosomal proteins, such as Desmoplakin, Desmoglein-2 and Plakoglobin. Importantly, these changes are not due to alteration of gene expression, as total protein levels of desmosomal proteins (assessed by western blot) are not changed upon SGEF knockdown. Interestingly, we observe a similar reduction in localization (but not expression) of desmosomal proteins upon knockdown of RhoG. Taken together, these studies have uncovered an important role for SGEF and RhoG in maintaining the junctional localization of desmosomal proteins. Future work will focus on whether regulation of desmosomal structure by SGEF requires its guanine nucleotide exchange function and whether SGEF-mediated changes in desmosome protein localization affect junctional strength and integrity.

B551/P2931

Kinetics study of hydration and skin barriers markers in a novel calcium differentiated normal human keratinocyte model

M. Juan, A. Guillemain, T. Naiken, **E. Leblanc-Noblesse**, K. Pays, C. Nizard; LVMH Recherche, Saint Jean de Braye, FRANCE.

INTRODUCTION

Skin is the largest and outermost organ of the human body, which makes it an important barrier against external aggressions and water loss. Due to its stratified epithelium formed by layers of keratinocytes and the lipophilic film on its surface, skin is impermeable to most molecules and microorganisms. Skin hydration, claimed by cosmetic products, involves many markers such as aquaporins for water transport, proteins of the cornified envelope, adherens and tight junctions as well as transport of lipids or nutrients. This study permits us to study the expression of several markers in a new model of normal human keratinocytes cultured to confluence in the presence of calcium to mimic the different cell layers of epidermal differentiation.

MATERIALS AND METHODS

A cell culture of normal human keratinocytes is carried out in 96 plate systems dedicated to microscopy. When the cells reach the confluent stage, the culture is treated with calcium to promote epidermal differentiation. On day 1, 2,3,4, 6 and 7 cells are fixed, and immunostaining performed. The expression of 12 hydration and skin barrier markers is measured by immunofluorescence in a High Content Screening system (Cellomics). A specific program image analysis to each marker has been specially developed on the Cellomics system.

RESULTS

The expressions of each studied marker are quantified at every time of calcium treatment, taking into account the cellular localization specific to each marker. This has been verified by bibliographical data and immunostaining on normal skin sections. For example, it should be noted that the membrane expression of Aquaporin 3, a protein involved in the epidermal transport of water and glycerol, reaches its maximum expression after 4 days of calcium treatment and then declines. On the contrary, the expression of involucrin, a major protein of the cornified envelope, is optimally expressed after 7 days of Calcium.The results obtained in this study allowed us to better understand the kinetics of appearance of its markers in a model of normal human keratinocytes in culture. The cellular localization specific to each marker, as well as the associated image analysis method, allow us to obtain a very good in vitro model to test the cosmetic efficacy of active ingredients on cutaneous phenomena linked to hydration, nutrition and the barrier function.

B552/P2932

Gap junction transendocytosis in glia modifies brain-derived extracellular vesicle formation

L. Udo-Bellner, E. Khodra, S. Khan, **R. Stout**; New York Institute of Technology College of Osteopathic Medicine, Old Westbury, NY

Gap junctions are required for normal brain function through their roles in neurons, brain endothelial cells, and very prominent functions in glia. Gap junctions made up of connexin proteins serve several functions in addition to forming direct routes for intercellular communication of metabolites, signaling molecules, and RNA. Among numerous non-channel functions, Cx43 gap junctions regulate autophagy and mediate intercellular transfer of protein and membrane through a process called transendocytosis.

Internalization of gap junctions by endocytosis has long been known to act via transendocytosis of protein, membrane, signaling molecules, RNA, and even organelles such as mitochondria. Other research groups have shown that downstream processing of endocytosed gap junctions (called connexosomes) can take varied paths; one prominent path was shown by electron microscopy to be multivesicular bodies. We hypothesize that brain derived extracellular vesicles (EVs) found in the blood are derived from glia (astrocytes and tanycytes) located in brain areas proximal to fenestrated vessels without blood brain barrier. We broke the investigation into subparts tested them using a variety of super-resolution microscopy tools and custom image analysis methods that we developed for this research. We found: 1) EVs derived from Cx43-expressing astrocyte-condition culture media contain Cx43. 2) EVs derived from serum of mice contain Cx43 and characteristics of EVs are radically altered in a mouse model of neurodevelopmental disease. 3) *In situ,* Cx43 and CD63 (marker for both multivesicular bodies and EVs) colocalized frequently in glial cell bodies and rarely at specialized cell extensions in the brain. It will be critical to understand the steps downstream of gap junction transendocytosis and connexosome processing in the hypothalamus because gap junctions and transcytosis in the glial cells of this brain region have recently been shown to be critical in systemic metabolism regulation and the effects of anti-obesity drugs. We will present our most recent unpublished findings with expected major significance for health conditions ranging from obesity, to metabolic disorder, to neurodegenerative disease.

B553/P2933

Regulation of Heterotypic Endothelial-Osteoblast interactions via Connexin-43

S. Alimperti; Georgetown University, Washington, DC

Objectives: Glucocorticoids (GCs) are key treatments of inflammatory and immune disorders. Due to prolong use, most patients are at high risk of developing osteopenia or osteoporosis. Although GCs are closely associated with bone loss, their effect in bone microvasculature is incompletely understood. Here, we engineered a 3D biomimetic vascularized bone platform, named bone on a chip, to investigate molecular mechanisms of osteoblast-endothelial interaction in response to glucocorticoids. **Methods:** This bicellular microfluidic system consists of 3D perusable blood vessels embedded within a mixture of green fluorescent protein (GFP) labelled human osteoblasts (hOBs) and collagen I/fibrinogen/gelatin matrix. By using this platform, we microscopically captured the connexin-43 (labeled with red fluorescent Alexa Fluor® 568) heterotypic endo-bone interaction in presence of GCs, such as hydrocortisone, prednisone, prednisolone, and dexamethasone. Under these conditions, vascular leakiness were measured by the particle/ FITC-dextran assay and osteogenic potential was evaluated by calcium deposition, osteoimage assay, and quantitative RT-PCR analysis. In addition, we knockdown connexin-43 with siRNA compared to scrambled siRNA and assay for angiogenesis and osteogenic potential. We also used on overexpression viral vector with mutant inactive Connexin-43 (Con-43SD, Con-43SA) to rescue the effect of GCs on angiogenesis and osteogenic potential to confirm the role of Connexin-43. **Results:** We engineered and characterized a 3D biomimetic glucocorticoid-induced osteoporosis models to investigate osteoblast-endothelial interaction. Our results showed that the presence of glucocorticoids, 200nM hydrocortisone, 100nM prednisone, 100nM prednisolone, and 100nM dexamethasone, decreased the formation of neovessels and bone compared to the untreated group by 5-fold and 7-fold, respectively. GCs inhibited early, intermediate, and late bone markers, such as RUNX2, COLA1 and OPN and decreased the barrier integrity. We identified that GCs controlled angiogenesis via MAPK signaling pathway by phosphorylating a gap junction molecule, named connexin-

43. To this end, connexin-43 knockout endothelial cells showed inhibition of angiogenesis and loss of their interaction with bone cells, indicating that connexin-43 is necessary for their intracellular communication. **Conclusions:** Collectively, this study permits us to capture dynamic changes in endothelial-bone interactions under inflammatory conditions by dissecting the connexin-43/MAPK mechanism. Our findings may shed light on microvascular barrier function related to inflammatory bone diseases and may utilize it as potential therapeutic target to better maintain bone homeostasis.

B554/P2934

Dissecting the roles of claudins and JAM-A in providing tight junction-dependent mechanical resistance

T. Nguyen; School of Life Science, SOKENDAI, Okazaki, Japan

Epithelial cell monolayer is constantly exposed to mechanical stress. Epithelial cells resist mechanical stress to maintain monolayer integrity, which is essential to maintain body homeostasis. Unlike adherens junctions and desmosomes whose functions in withstanding mechanical stress are well established, little has been known about the roles of tight junctions (TJs) in epithelial mechanical resistance. Here we report that claudins and JAMs, the two major classes of membrane proteins of TJs, play essential roles in maintaining epithelial integrity upon mechanical stress. MDCK II cells that lost claudins and JAM-A (Claudin/JAM-A KO cells) showed the local disruption of the apical junctional complex. Time-lapse imaging revealed that junction breakages occur when the cells are exposed to mechanical stress. Intriguingly, these local junction breakages were accompanied by the local disorganization of circumferential actin bundles. Perturbation of F-actin polymerization in Claudin/JAM-A KO cells enhanced the junction breakage phenotype, supporting the idea that the cell junction integrity in Claudin/JAM-A KO cells critically depends on the F-actin arrangement. Taken together, these results reveal a novel function of TJs in the mechanical resistance of epithelial cell junctions.

B555/P2935

Terminating Structures of Tunneling Nanotubes

V. Ayres[1], V. Tiryaki[2], I. Ahmed[3], D. Shreiber[4]; [1]Michigan State University, East Lansing, MI, [2]Siirt University, Siirt, Turkey, [3]CUNY Medgar Evers, New York, NY, [4]Rutgers, The State University of New Jersey, Piscataway, NJ

Tunneling nanotubes (TNTs) are a recently recognized mechanism for cell-cell communication involving direct exchange of molecular through organelle materials along microns-long nanoscale-diameter pathways. Reported investigations have elucidated TNT actin, or actin-tubulin, along-length structures, and their associated transporters for molecules and/or organelles. Recent work has demonstrated specialized initiating and terminating structures. While TNTs are demonstrated as triggered by environmental stressors such as hypoxia and/or disease situations such as cancer(s) [1], they are also observed under non-pathological conditions. In this work, morphological and protein investigations of terminating structures triggered by environmental conditions ranging from mild (quiescent-like) to stressful (hypoxic) are presented for purity-enhanced cultures of primary astrocytes. Nanoscale “hand” terminating structures are investigated using gSTED super-resolution microscopy, atomic force microscopy and immunocytochemistry. Actin and intermediate filament components are observed, while tubulin may be secondary, if present. A previously reported actin gap is observed with an upper limit of 50 nm diameter or less. Similarities and differences of terminating structures across mild

through stressful environments are correlated with differing levels of RhoGTPase activity [2,3]. 1. Giulia Pinto, Inés Saenz-de-Santa-Maria, Patricia Chastagner, Emeline Perthame, Caroline Delmas, Christine Toulas, Elizabeth Moyal-Jonathan-Cohen, Christel Brou and Chiara Zurzolo. Patient-derived glioblastoma stem cells transfer mitochondria through tunneling nanotubes intumor organoids. Biochemical Journal (2021) 478 21-39. https://doi.org/10.1042/BCJ20200710 2. Volkan M. Tiryaki, Virgnia M. Ayres, Ijaz Ahmed, David I. Shreiber. Differentiation of reactive-like astrocytes cultured on nanofibrillar and comparative culture surfaces. Nanomedicine, 2015 10(4): 529-545. https://doi.org/10.2217/NNM.14.33 3. J. Michael Henderson, Nina Ljubojevic, Thibault Chaze, Daryl Castaneda, Aude Battistella, Quentin Giai Gianetto, Mariette Matondo, Stéphanie Descroix, Patricia Bassereau and Chiara Zurzolo. Arp2/3 inhibition switches Eps8's network associations to favour longer actin filament formation 6 necessary for tunneling nanotubes. bioRxiv preprint doi: https://doi.org/10.1101/2022.08.24.504515

B556/P2936

Investigating the Role of Claudins in Epithelial Cell Shape

J. N. Warshaw[1], C. P. Chaney[2], D. K. Marciano[1]; [1]Cell Biology/Internal Medicine, Nephrology, UT Southwestern Medical Center, Dallas, TX, [2]Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX.

Epithelial cells may have specialized shapes that allow them to execute diverse functions. These include transient plasma membrane protrusions, such as filopodia and lamellipodia, as well as longer-lived protrusions. The mechanisms that underly cell shape specializations and their maintenance are not well understood. Using a novel fluorescent reporter, our lab identified a tubular cell type in the kidney medulla with branched, finger-like cell protrusions. It is unknown how these protrusions form and what function they serve. To better understand how these cells develop protrusions, we conducted a time-course of single nuclei RNA sequencing in the postnatal mouse kidney. Differential gene expression analysis revealed that Claudin-10 transcript is highly enriched in these cells throughout development. Claudins are components of tight junctions that regulate paracellular permeability. Several tight junction proteins, including claudins, have been implicated in "waviness" at the plasma membrane. To interrogate the role of Claudin-10 in cell protrusion formation, we generated a *Cldn10* conditional knockout mouse (*Cldn10* cKO). Our preliminary findings demonstrate that neonatal deletion of *Cldn10* in the kidney abrogates the formation of cell protrusions, which appear truncated and irregular. These changes in cell shape are accompanied by abnormalities in renal function. In summary, our group has identified a novel cell type in the kidney that forms complex, interdigitating cell protrusions whose formation is dependent on Claudin-10. Future studies will continue to characterize the *Cldn10* cKO phenotype and establish a mechanistic link between tight junctions, cell shape, and function.

B557/P2937

Contact Stimulation of Collective Migration as a Novel Driver in Tissue Morphogenesis

M. C. Bischoff[1], J. E. Norton[1], S. Bogdan[2], M. Peifer[1]; [1]University of North Carolina At Chapel Hill, Chapel Hill, NC, [2]University of Marburg, Marburg, Germany

Cells migrate collectively to form tissues and organs during morphogenesis and tissue homeostasis, and many metastatic cancer cells adopt similar behaviors. This process requires the directed, coherent movement of cell groups. We developed a new model to explore the underlying mechanisms: *Drosophila* muscle cell precursors migrating to ensheath the testis. They use a new type of collective cell

behavior, lacking lamellipodial protrusions, but instead, using numerous filopodia to move as a cohesive cluster in a formin-dependent manner. During migration, these cells are loosely interconnected via filopodia containing N-Cadherin. Loss of N-Cadherin triggers individualized cell behavior at the expense of overall directionality. Genetic, pharmacological and mechanical perturbation analyses reveal an essential role for the Rho family GTPases, Rac2, Cdc42 and Rho1. They differentially control both protrusion dynamics and focal adhesion formation and stability. Our data uncover a cell contact-dependent asymmetry in matrix adhesion dynamics, as matrix adhesions have a much shorter live time at the cell-cell edge. This appears to allow cells to transduce intracellular force into locomotion towards the free space, and thus enables highly coordinated collective motility without any differentiation into leader and follower cells. We propose a model in which Rho family GTPase mediated contact stimulation of migration drives the directional movement of a cell sheet that behaves like a supracellular unit. Currently, we are addressing which specific signal(s) activate/deactivate Rho family GTPases and thus trigger the cell-cell contact-dependent changes in adhesion dynamics. To identify potential candidates, we are using bulk RNA-seq to compare migratory and post-migratory stages. We are then testing candidate regulators from this list and the curated literature via an RNAi-screen. To determine if candidates regulate Cdc42-activity, we are developing a specific Cdc42-GTP live-sensor.

Focal Adhesions and Invadosomes

B558/P2938

Evolutionary Analysis of Cell-Substrate Adhesion Composition and Dynamics During Migration

J. Fierro Morales, M. Roh-Johnson; University of Utah, Salt Lake City, UT.

In Metazoans, cells use cell-substrate adhesions called focal adhesions (FAs) - a multilayered protein complex physically linking the extracellular matrix to the intracellular actin cytoskeleton - for efficient migration. Though this machinery was believed to be Metazoan-specific, genomic sequencing of organisms across Eukaryotes suggests organisms as evolutionarily distant as Amoebozoa possess putative homologues of core FA components. It is unclear, however, if cell-substrate adhesions in organisms evolutionarily distant from Metazoans are conserved in function and composition to Metazoan FAs. An ideal organism for investigating cell-substrate adhesions across evolutionary time is the Amoebozoan, *Dictyostelium discoideum*. Our evolutionary analyses suggest *Dictyostelium* can form cell-substrate adhesions despite lacking homologues of known core FA components. It is contentious, however, if *Dictyostelium* form cell-substrate adhesions and, if so, what the composition of these adhesions is. Time-lapse fluorescent microscopy of actin and paxillinB (PaxB), the *Dictyostelium* homologue of the core FA component Paxillin, shows the two components co-localize at punctate structures on the cell ventral surface. Inhibition of actin ablates PaxB punctae formation, and the integrin beta-like protein, SibC, also localizes to ventral surface punctate structures. These data suggest that *Dictyostelium* form cell-substrate adhesions at the ventral surface, and quantitative analysis reveals that *Dictyostelium* cell-substrate adhesions are highly dynamic and are smaller in size and shorter in duration than Metazoan FAs. Furthermore, preliminary imaging of cells expressing truncated versions of PaxB indicate conservation of Paxillin domain specific functions. We are currently studying the localization of additional *Dictyostelium* homologues of core FA components to cell-substrate adhesions with the goal of **elucidating how cell-substrate adhesions have evolved. This knowledge will subsequently aid research into the evolution of adhesion-dependent mechanisms such as migration, cytokinesis, and pathogenesis**.

B559/P2939

CASK, a Novel Mediator of Adhesion Cytoskeletons

Y. Tseng, Y. Lin, F. Tsai; Department of Pharmacology, National Taiwan University College of Medicine, Taipei, TAIWAN.

Calcium/calmodulin dependent serine protein kinase (CASK) is a scaffolding protein with multiple domains, and its multi-domain property provides the possibilities for CASK to interact with various proteins. Previous studies have shown that CASK plays an important role in the function of certain calcium channels, and that cell migration could be affected by decreased expression of CASK, suggesting that CASK may be critical for cytoskeletal remodeling. Therefore, the aim of this study is to investigate whether and how CASK regulates cytoskeletal remodeling, with specific focus on adhesion cytoskeletons.To investigate the effect of CASK perturbation on adhesion cytoskeleton, short-hairpin RNA (shRNA) lentiviruses were used on cultured human keratinocytes (HaCaT) to knockdown CASK.We first studied CASK-mediated effects on cell-matrix adhesion, using immunofluorescence (IF) to label paxillin, which is the key component of focal adhesion (FA) complexes. Interestingly, FA signals in CASK knockdown (shCASK) HaCaT cells were increased compared with the control group, indicating that CASK reduces FA structures. To further elucidate whether CASK reduces FA by suppressing its formation or enhancing its degradation, a ROCK inhibitor (Y27632) and calpain knockdown (shCalpain) HaCaT cells were used together with CASK knockdown to determine how FA would be influenced. Increased FA can be observed after treating cells with either shCASK or shCalpain. However, double knockdown of CASK and calpain did not lead to further increase in FA signals, indicating that CASK may be involved in calpain-mediated FA degradation. In contrast, under the suppressive effect of Y27632 on FA, shCASK treatment could still enhance focal adhesion signals, suggesting that the ROCK signaling pathway may not contribute to the shCASK-mediated alterations in FA signals. We also studied CASK effects on cell-cell adhesion by labeling E-cadherin. The distribution of E-cadherin was altered in CASK-knockdown HaCaT cells. Compared with the control group, E-cadherin was mostly presented in the membrane of shCASK treated cells, indicating that CASK may regulate the dynamics of cell-cell adhesion structures.We are currently working on the molecular mechanisms of how CASK regulates cell-matrix and cell-cell adhesions, with the hope to elucidate CASK functions on adhesion cytoskeletons.

B560/P2940

Signal transducer and activator of transcription 3 (STAT3) and Talin-1 colocalize at focal adhesions

A. E. Temple, A. M. McCarthy, F. Chu, S. Walker; Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, NH.

STAT3 activation is important for cell proliferation, migration, and angiogenesis, and elevated phosphorylated STAT3 generally correlates with poor prognosis in many human cancers. In addition to its nuclear transcriptional activity, phosphorylated STAT3 has been shown to localize to focal adhesions where it interacts with phosphorylated focal adhesion kinase. However, the role of STAT3 at focal adhesions is still not completely understood. To better understand the interacting partners of STAT3 in the cytoplasm, we used a phosphoserine 727 STAT3 antibody to pulldown STAT3 and interacting proteins from whole cell lysates of breast cancer cells with LIF stimulation to activate STAT3. Samples were subjected to mass spectrometry analysis and Talin-1 was identified with LIF stimulation, indicating a potential interaction. Talin-1 has an important function in cell adhesion and is essential in integrin

activation and focal adhesion assembly. To assess the interaction of STAT3 and Talin-1, we overexpressed fluorescently labeled constitutively active STAT3 and Talin-1 in breast cancer cells and utilized confocal microscopy to demonstrate an overlap between STAT3 and Talin-1 at focal adhesions. This supports our hypothesis that STAT3 and Talin-1 colocalize and interact at focal adhesions. To assess the effects of Talin-1 on STAT3 activity, Talin-1 was overexpressed and STAT3 transcriptional activity was measured using a STAT3-responsive luciferase reporter. We found that STAT3 transcriptional activity was reduced with Talin-1 overexpression, suggesting that Talin-1 may sequester STAT3 at focal adhesions, thereby reducing its transcriptional activity. Our data indicates a potential interaction between STAT3 and Talin-1 at focal adhesions. Additional coimmunoprecipitation experiments and colocalization studies are being completed to further validate the mass spectrometry findings and siRNA studies are being utilized to determine if the interaction of STAT3 and Talin-1 is necessary for STAT3 localization at focal adhesions. This STAT3-Talin-1 interaction could implicate a novel STAT3 function at focal adhesions, thus providing an alternative approach to targeting STAT3 in cancer.

B561/P2941

Regulation of focal adhesions during single cell migration *in vivo*

Q. Xue, S. Varady, T. Waddell, J. Carrington, M. Roh-Johnson; University of Utah, Salt Lake City, UT

The majority of focal adhesion research over the last 40 years has been on 2D substrates. Research using 3D *in vitro* models initially led to controversies regarding whether cells even formed focal adhesions when migrating in a 3D environment. Follow up work has shown that cells do indeed form focal adhesions when in this environment - These focal adhesion structures are difficult to resolve in a more complex 3D environment, and thus, much less is known about their regulation. Currently, it is also entirely unknown what molecules make up a focal adhesion, or how these focal adhesions form and are regulated, during single cell migration in a native *in vivo* environment. To address this critical aspect of cell migration, we developed an *in vivo* zebrafish system in which we can directly visualize the formation of focal adhesion structures of highly migratory cells with high resolution. By comparing focal adhesions between this *in vivo* system and the traditional *in vitro* cell culture model, we find that a key site of phosphoregulation on Paxillin, tyrosine 118 (Y118), exhibits reduced phosphorylation in migrating cells *in vivo* in both zebrafish and mouse melanoma models, contrary to the critical role for this phosphorylation event in cell culture studies. Furthermore, direct modulation of this residue by site-directed mutagenesis leads to opposite cell migration phenotypes *in vivo* versus *in vitro*: Expression of a non-phosphorylatable version of Y118-Paxillin promotes cell migration in both migrating cancer cells and macrophages *in vivo*, despite inhibiting cell migration in the *in vitro* cell culture conditions. To further understand the mechanism of this regulation, we find that the upstream kinase, focal adhesion kinase (FAK), is downregulated in cells *in vivo*, and that cells expressing non-phosphorylatable Y118-Paxillin exhibit increased activation in CRKII-DOCK180-Rac pathway, leading to increased cell migration. This work reveals an altogether new mechanism for how focal adhesions are regulated during single cell migration, and potentially generates alternative therapeutic approaches in the treatment of cancer.

B562/P2942

The Rac-GAP alpha2-Chimaerin interacts with paxillin and regulates invadopodia turnover

G. Kreider-Letterman[1], **R. Garcia-Mata**[2], K. Snyder[1], A. Rabino[1], F. Aftab[1], S. Goicoechea[1]; [1]University of Toledo, Toledo, OH, [2]University of Toledo, TOLEDO, OH.

Metastatic cells escape the tumor and enter the bloodstream by developing actin-rich membrane protrusions called invadopodia that degrade the extracellular matrix to allow invasion of surrounding tissues. Invadopodia formation is regulated by Rho GTPases, a family of proteins that regulates the actin cytoskeleton; however, the molecular mechanisms that control their turnover are still poorly understood. Rho GTPases are regulated by the opposing actions of guanine nucleotide exchange factors or GEFs, which regulate the activation, and GTPase-activating proteins or GAPs, which catalyze the inactivation. Here, we identify the Rac1-specific GAP α2-Chimaerin (α2-CHN), as a key regulator of invadopodia dynamics in human breast cancer cells in a process that involves the binding of α2-CHN to the tyrosine-phosphorylated form of paxillin. Our results show that when the expression of α2-CHN is silenced, invadopodia are more stable and have a longer lifetime than control cells. This phenotype is rescued by re-expressing an shRNA-resistant α2-CHN. Furthermore, overexpression of α2-CHN inhibited the formation of invadopodia. Silencing α2-CHN promoted matrix degradation and invasive behavior, consistent with the increased invadopodia formation. Using a combination of fixed and live cell imaging, and a FRET-based Rac1 sensor, we show that α2-CHN localizes to invadopodia and that silencing α2-CHN promotes Rac1 activation at invadopodia. The molecular mechanisms by which α2-CHN regulates invadopodia dynamics are still under investigation, but we believe that paxillin phosphorylation is playing a role. Paxillin is a key component of invadopodia, and its phosphorylation plays a role in the disassembly of invadopodia. Here we show that α2-CHN knockdown induces an increase of phospho-paxillin at invadopodia compared to control cells. We also demonstrate that phospho-deficient paxillin (Y31-118F) has a lower affinity for α2-CHN than WT and phospho-mimetic (Y31-118E) paxillin. We have previously shown that the Rho GTPase RhoG was important for invadopodia disassembly in a process that involved paxillin phosphorylation. Our new results suggest that RhoG and Rac1 may have opposite roles in the regulation of invadopodia turnover. While RhoG activity needs to be high during invadopodia disassembly, our new data suggests that RhoG-mediated phosphorylation of paxillin may mediate the recruitment and activation of α2-CHN to inactivate Rac1 at invadopodia during disassembly. In summary, we have identified a novel signaling pathway involving α2-CHN, Rac1, and paxillin phosphorylation, which functions in the regulation of invadopodia turnover in human breast cancer cells.

B563/P2943

Focal adhesion kinase phase separation regulates autophosphorylation

N. E. Lea, L. B. Case; Massachusetts Institute of Technology, Cambridge, MA

Focal adhesion kinase (FAK) is a 120 kDa cytosolic tyrosine kinase which localizes to focal adhesions, where it is activated and involved in signaling to promote cell migration, proliferation and anchorage-dependent survival. FAK is often overexpressed or overactive in cancers where it contributes to tumor angiogenesis, metastasis, invasion and anchorage-independent survival. FAK activation is initiated by autophosphorylation of tyrosine 397 *in trans*, however the mechanism of FAK activation is not fully understood. Our lab has recently shown purified FAK can undergo phase separation alone *in vitro.* We are currently investigating how FAK phase separation influences its autophosphorylation on tyrosine

Y397 using biochemical assays with purified FAK. We measured the rate of autophosphorylation using western blot analysis as well as a newly developed fluorescence microscopy assay. We found that phase separation consistently correlates with a significant increase in the rate of autophosphorylation. Conditions or mutations that reduce phase separation result in slower autophosphorylation while conditions that increase phase separation result in faster autophosphorylation. We conclude that phase separation is sufficient to increase the rate of FAK autophosphorylation *in vitro*. Future experiments will determine if FAK phase separation is necessary for normal FAK signaling in cells or if aberrant FAK phase separation contributes to phenotypes of FAK overexpressing cancers.

B564/P2944

Understanding the mechanism behind inhibition of Focal Adhesion Kinase (FAK)

D. E. Akwa, V. Yang, N. Lea, L. B. Case; Massachusetts Institute of Technology, Cambridge, MA

Focal adhesion kinase (FAK) is a 125 kDa cytosolic tyrosine kinase that regulates cellular migration, proliferation, and survival. In cells, it is localized and activated at focal adhesions where it acts as a scaffold and promotes the phosphorylation of several substrates. FAK signaling can be regulated through an alternative promoter in the FAK gene, which results in expression of FAK related non kinase (FRNK). FRNK lacks the N-terminal FERM domain and kinase domain, but contains the C-terminal intrinsically disordered linker and the focal adhesion targeting domain (FAT). FRNK expression in cells is sufficient to inhibit FAK autophosphorylation and downstream signaling, but the mechanism of inhibition is unclear. Our preliminary data shows that FAK undergoes phase separation *in vitro*, but adding FRNK is sufficient to inhibit FAK phase separation. We hypothesize that FRNK could inhibit FAK signaling by disrupting the oligomerization and subsequent phase separation of full length FAK protein. Using our *in vitro* system, we have characterized mutants of FRNK that differentially affect FAK phase separation. We assess phase separation by measuring solution turbidity (which increases as droplets form and scatter light) and by visualizing droplets with DIC or fluorescence microscopy. Changing the intrinsically disordered linker results in FRNK proteins that have different effects on phase separation. We also find that dimerizing or oligomerizing FRNK is sufficient to restore phase separation, likely through restoring the oligomerization of full-length FAK. We conclude that FRNK acts as a monovalent ligand that destabilizes FAK phase separation *in vitro*. Now, we are testing the ability of these FRNK mutants to inhibit FAK signaling in mouse embryonic fibroblasts (MEFs) to determine if FAK phase separation is important for FAK signaling in cells.

Stress Response, Chaperones and Quality Control

B566/P2945

Dissecting the synergistic role of the unfolded protein response in wound infections

S. Lee, C. Celik, A. Tan, M. Veleba, K. A. Kline, **G. Thibault**; Nanyang Technological University, Singapore, SINGAPORE.

Infected wounds affect 7-15% of hospitalized patients and significantly increase the duration of hospitalization and treatment cost per day. Biofilm-forming *Enterococcus faecalis*, has been isolated from wounds associated with diabetic foot ulcers, burns and surgical sites. Therapeutic efforts have been concentrated on addressing microbial factors. However, this is inadequate in the presence of biofilm-forming microorganisms which renders antimicrobials sub-effective. The arrest in the

inflammatory phase during wound healing, due to incomplete elimination of pathogens, has been underpinning the basis for wound chronicity. Recent evidence from us and others has shown that the mammalian unfolded protein response (UPR) promotes the development of bacterial skin and soft tissue infection and impairs wound healing. Therefore, we hypothesized that UPR activation serves as a synergistic node to propagate wound infection. To investigate, we use *in vitro* and *in vivo* infection and wound healing models to delineate the relationship between the bacteria, UPR and wound healing. We found that the infection of C57BL/6 mice, NIH 3T3 mouse fibroblasts and HaCaT human keratinocytes with *E. faecalis* infection result in significant UPR activation, specifically through the IRE1 and PERK branches. The UPR activation translated to a reduced rate of wound migration in our *in vitro* cell migration assay. We established and optimized a high-throughput screen pipeline to reveal *E. faecalis* factors that induce UPR activation. In parallel, we are performing single cell RNA-sequencing of infected wound to reveal the stress responses in different cell types. These findings suggest that the UPR may serve as a target for combinational therapy to reduce the recurrence of wound infections, eliminating the bottleneck of wound care.

B567/P2946

Regulation of nuclear morphology under stress conditions in *Xenopus laevis* extracts

S. Sengupta, D. Levy; University of Wyoming, Laramie, WY.

Size is critical to function at all levels of biological organization. For example, there is significant variation in cell size between species, cell types, and developmental stages. The size of the nucleus tends to scale with cell size. Normal cells have a nuclear size within a defined range, while aberrations in nuclear morphology are often associated with disease, for instance cancers and laminopathies. The exact mechanisms by which a cell maintains nuclear size, shape, and structure are not well understood, although nuclear lamins have been implicated. We are addressing how nuclear shape and size change in response to various stress conditions, specifically osmotic stress (mannitol), oxidative stress (hydrogen peroxide), and proteotoxic stress (proteasome inhibitor MG132). Because treating cells with stressors can elicit complicated and pleiotropic effects, we chose to use a simplified system, de novo nuclear assembly in *Xenopus* egg extract. The extract is transcriptionally inert, allowing us to investigate transcription-independent stress responses, and the biochemical composition of the extract is easily manipulated, for instance through addition of molecular stressors. We first examined how stress conditions impact nuclear assembly. Nuclei assembled in MG132-treated extracts showed reduced stability with frequent ruptures, while osmotic and oxidative stress had little effect on assembly. Next, we treated pre-assembled nuclei, finding that all stress conditions induced changes in nuclear morphology, typically a reduction in nuclear size and wrinkling of the nuclear envelope. Concomitant with these nuclear morphology changes we observed a delocalization of lamin B3 away from the nuclear envelope and appearance of ring-like structures within the nucleus that stained for Aquaporin 2. Our future work will elucidate the underlying mechanisms responsible for these stress-induced changes in nuclear morphology.

B568/P2947

Cracking the Hsp70 chaperone code

A. Truman; Biological Sciences, University of North Carolina At Charlotte, Charlotte, NC.

The Hsp70 molecular chaperone has been extensively studied for the last 50 years; it is highly conserved and is essential for many cellular processes. Research has primarily focused on how Hsp70 function is regulated via transcription, expression of highly related paralogs and the suite of co-chaperone binding partners. Despite this, we still know very little about the post-translational modifications (PTMs) of Hsp70 and how these modifications may affect its cellular function. Powerful technologies such as affinity purification of proteins coupled with tandem mass spectrometry (AP-MS/MS) have uncovered a substantial number of modified sites on Hsp70. These PTMs can be added and removed rapidly, allowing fine-tuning chaperone function when required. The large number of detected modifications such as phosphorylation, ubiquitination, SUMOylation and acetylation) suggest a “chaperone code” similar in nature to the combinatory PTM code that exists on histones. We are currently examining the role of 73 Hsp70 phosphorylation sites on a range of chaperone-mediated processes that include the heat shock response, glucose utilization, the DNA damage response and human pathologies such as cancer and neurodegenerative disease. We hope that understanding of the reciprocal interplay between chaperones and key signal transductions system will lead to an unprecedented ability to manipulate chaperone function for experimental and translational purposes.

B569/P2948

Rapid heat-induced Hsp70 phosphorylation is critical for proteostasis in yeast

S. Omkar, A. Truman; Department of Biological Sciences, UNCC, Charlotte, NC.

Heat shock protein 70 (Hsp70) is a central component of protein folding and signal transduction pathways. Recent studies have identified roles for post-translational modifications (the Chaperone Code) in fine-tuning chaperone function. The role of post-translational modifications (PTMs) on Hsp70 under heat shock are poorly understood. To understand how heat shock impacts Hsp70 PTMs, we performed quantitative mass spectrometry on yeast Hsp70 isolated from untreated and heat shocked cells. We identified a novel heat-induced Hsp70 phosphorylation site on the substrate binding domain of Hsp70 that was activated rapidly (<5mins) by heat and other agents that challenged cell integrity. Surprisingly, phosphorylation of this site was induced in all the cytosolic paralogs of Hsp70 (Ssa1-4). Using genetic and molecular techniques, we identified key cell integrity proteins necessary for activation of Hsp70 phosphorylation. Mutation of Hsp70 to prevent heat-induced phosphorylation resulted in yeast that were viable but were sensitized to heat stress and cell wall damaging agents. Phospho-mutant yeast were unable to mount a significant transcriptional response to heat shock, suggesting Hsp70 phosphorylation is needed for correct formation of core heat shock transcriptional complexes. Taken together we believe that rapid heat-induced phosphorylation of Hsp70 is an initiating event in the heat shock response, a process which may be universally conserved in all organisms. **Key words: Hsp70, phosphorylation, heat shock, yeast, chaperone code**

B570/P2949

Tardigrade small heat shock proteins can limit desiccation-induced protein aggregation

J. D. Hibshman[1], S. Carra[2], B. Goldstein[1]; [1]Biology Department, The University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]Department of Biomedical, Metabolic, and Neural Sciences, University of Modena and Reggio Emilia, Modena, ITALY.

Small heat shock proteins (sHSPs) are chaperones known for their response to heat stress. While sHSPs have well-characterized roles in heat tolerance, potential roles for sHSPs in desiccation tolerance have not been as thoroughly explored. We identified nine sHSPs from the genome of the tardigrade *Hypsibius exemplaris,* each containing a conserved alpha-crystallin domain flanked by disordered regions. Many of these sHSPs are highly expressed and in some cases are upregulated during desiccation. We found that tardigrade sHSPs were sufficient to improve desiccation tolerance when expressed in *E. coli*. Purification and subsequent analysis of two sHSPs, HSP21 and HSP24.6, revealed that these proteins can form large complexes *in vitro*, similar to oligomeric assemblies documented for other sHSPs. These proteins limited heat-induced aggregation of the model enzyme citrate synthase. Heterologous expression of HSP24.6 improved bacterial heat shock survival, and the protein significantly reduced heat-induced aggregation of soluble bacterial protein. Thus, HSP24.6 likely chaperones against protein aggregation to promote survival of heat stress. Furthermore, HSP21 and HSP24.6 also limited desiccation-induced aggregation and loss of function of citrate synthase. This suggests a mechanism by which tardigrade sHSPs promote desiccation tolerance: by limiting desiccation-induced protein aggregation, thereby maintaining proteostasis and supporting survival. These results suggest that sHSPs, classical chaperones, provide a mechanism of general stress resistance that can also be deployed to support survival during anhydrobiosis.

B571/P2950

Novel stress granule proteins identified by Microscoop, a microscope-guided hypothesis-free subcellular protein discovery platform

C. H. Y. Cheung, W. Chong, H. Chang, C. Chung, C. Huang, Y. Chen, C. Chang, **J. Liao**; Syncell Inc., Taipei, TAIWAN.

Stress granules (SGs) are dynamic, non-membrane-bound assemblies of protein and RNA formed in response to cellular stress. Their pathological implications in cancer and neurodegenerative disease render it crucial to study SG composition for therapeutic applications. Previous SG proteomic studies have been achieved by biochemical fractionation or proximity labeling prior to mass spectrometric (LC-MS/MS) analysis; however, the procedures are usually labor intensive and technically challenging due to the small-sized and membrane-less nature of SGs. Moreover, the essence that SG markers mostly have multiple sub-cellular localizations, further complicates the studies. In this work, we took advantage of Microscoop, an automatic microscope system integrated with a machine learning-based algorithm, to explore the SG proteome. Microscoop enables site-specific protein identification by inducing photo-labeling precisely at the selected region-of-interest. SGs were first induced in U2-OS cells by arsenite and stained for G3BP1, a common SG marker. Immunofluorescence images of G3BP1 were applied to generate a computer vision (CV) based algorithm to differentiate G3BP1 in stress granules from that in the cytoplasm. A sequential process of fluorescence imaging, CV-driven pattern generation, and photochemical labeling was automatically implemented to achieve SG protein-specific biotinylation until sufficient cells was labeled for subsequent protein enrichment and LC-MS/MS analysis. In total, 2,614

proteins were found in three biological replicates. 77% of the triplicate-overlapped proteins were identified as SG proteins, showing a high specificity of the enrichment. STRING analysis of the proteins with a stringent threshold further disclosed 8 highly confident proteins as the core interactors in the SG interaction network. More importantly, these 8 proteins have no prior annotation as SGs. Immunofluorescence staining confirmed that 6 of them, i.e. EIF3CL, DDX17, PPIA, RPSA, YWHAE, and RPLP0 showed SG pattern and co-localized with G3BP1 in stressed cells but not in normal cells, confirming the discovery capability of our method. Collectively, our study unravels novel SG proteins, which possibly serve as core SG components, and demonstrates the capability of the Microscoop technology to achieve subcellular in situ protein discovery.

B572/P2951

RRM of eIF4B affects stress granule assembly and disassembly

J. Bolivar, N. Decuzzi, E. Kofke, K. Beglinger, J. Albeck, C. Fraser; UC Davis, Davis, CA.

Cells must respond to environmental stress to ensure survival and recovery. One stress response mechanism involves forming cytoplasmic structures called stress granules (SGs). SGs are non-membrane bound organelles composed of mRNA transcripts, RNA binding proteins, and 40S ribosomal subunits bound by eukaryotic initiation factors (eIFs). Rapid formation of SGs (within ~30 minutes of oxidative stress) serves to protect mRNAs and the translation initiation machinery during survival and recovery. However, the precise mechanism by which SGs assemble during stress and disassemble after stress is poorly defined. Specifically, we do not understand how interactions between the translation machinery and other SG components regulate the kinetics of SG assembly and disassembly. To overcome this barrier and move the field forward, I have generated a real-time single-cell kinetic assay to monitor stress granule assembly and disassembly. I found that the RNA recognition motif (RRM) domain of eIF4B protein can change the rate at which stress granules assemble and disassemble. Our findings could provide insight to other applications such as the effect of chemotherapeutic drugs on SG assembly and disassembly, or to determine if changes in the translation initiation machinery could speed up SG disassembly in a neurodegenerative model.

B573/P2952

A new role for the p24 family in antitrypsin Z clearance from the endoplasmic reticulum

B. Roberts, D. Mitra, **P. Satpute-Krishnan**; Biochemistry and Molecular BIology, Uniformed Services University, Bethesda, MD.

We report a novel role forp24-family proteins as protein quality control (PQC) factors that associatewith and facilitate the clearance of the misfolding alpha1-antitrypsin Z mutant(ATZ) from the endoplasmic reticulum (ER). ATZ has been well established to becleared from the ER to ER-derived vesicles for direct delivery to lysosomes viaone or more ER-to-lysosome-associated degradation (ERLAD) pathways that requirethe involvement of lipidated LC3 (LC3-II) molecules and are inhibited withautophagy inhibitor, 3-methyladenine. We discovered that ATZco-immunoprecipitated with p24-family members including Tmp21 and TMED9, inaddition to the ERLAD components calnexin and FAM134B that were previouslyreported by the Molinari lab (Fregno et al. 2018). This contrasts with wildtype alpha1-antitrypsin (AAT), which did not co-immunoprecipitate with FAM134B,calnexin or the p24-family members. Live cell imaging experiments revealed thatATZ and the p24-family members trafficked together from the ER to lysosomes butwere trapped together at the ER-exit sites marked by Sec24C

upon treatment withbrefeldin A and nocodazole. Similarly, ATZ and p24-family members were trappedtogether in ER-derived compartments upon treatment with 3-methyladenine.Importantly, depletion of Tmp21 or TMED9 significantly increased theintracellular levels of ATZ and prevented the trafficking of ATZ to lysosomes.Conversely, overexpression of these p24-family members promoted the degradationof ATZ. These and additional preliminary co-immunoprecipitation andcolocalization data and p24-family depletion experiments suggest a model inwhich the p24-family plays a key role in ATZ-clearance by bridging ATZ togetherwith ERLAD machinery and COPII coat proteins for the formation of ER-derivedERLAD vesicles. Taken together, our work reveals a new role for thep24-family in clearing misfolded proteins by-way-of the ERLAD pathway.

B574/P2953

Elucidating CHCHD10 Phenotypes Using Inducible Cellular Models

C. I. Lantz; NINDS, Bethesda, MD.

Mutations in the mitochondrial protein CHCHD10 cause autosomal dominant neuromuscular disorders including frontotemporal dementia (FTD)/ALS, mitochondrial myopathy, and lower motor neuronopathy (SMAJ, spinal muscular atrophy Jokela type). Most of these CHCHD10 disease phenotypes result from mutations in a conserved hydrophobic α-helical region in the middle of the protein and have a strict genotype-phenotype relationship, with the G58R mutation causing a pure myopathy and the G66V mutation causing a pure lower motor neuronopathy. The molecular basis for this genotype-phenotype relationship is not well understood. The current model of pathogenicity is toxic gain-of-function, as CHCHD10 knock-in mice but not CHCHD10 KO mice recapitulate the myopathy phenotype seen in patients with the G58R or S59L variants, as demonstrated by our lab and others. Work from our laboratory also identifies OMA1-dependent mitochondrial fragmentation and activation of the integrated stress response, as adaptive responses to mutant CHCHD10 misfolding. Preliminary evidence from our lab demonstrates that the G66V variant but not the myopathy-causing G58R variant decreases the solubility of the protein. We hypothesize that differences in misfolding of the G58R and G66V variants underlie their selective toxicity on myocytes and lower motor neurons, respectively. Using iPS cells, we have developed cellular models of both neuropathy and motor neuron disease causing mutations. We are comparing their phenotypes when differentiated into both induced myocytes and induced motor neurons. As a reference, we have also compared transcriptional responses of induced myocytes and induced motor neurons to mitochondrial toxins. We anticipate study of these cells will provide insight into the genotype-phenotype combinations observed in patients.

B575/P2954

Possible involvement of a heat-dependent myokine CCL5 on adipogenesis in mice

K. Murata, Y. Sato-Ishiuchi, T. Nedachi; Toyo University, Gunma, JAPAN.

Recently, skeletal muscle is recognized as an endocrine organ, producing proteins and peptides, defined as myokines. It has been established that contraction of skeletal muscle and/or nutritional states regulate the secretion of some of these myokines. Although the temperature of skeletal muscles can be changed during exercise and/or by changes in ambient temperature, its impact on myokine regulation has not been completely understood. In this study, we focused on skeletal muscle-derived C-C chemokine ligand 5 (CCL5), which we recently identified as a heat-dependent myokine and investigated its precise regulation by heat stress. In addition, we also investigated the potential involvement of the

heat-dependent myokines on adipogenesis.First, comprehensive analysis of myokines secreted from mouse C2C12 myotube revealed that CCL5 secretion is significantly reduced by heat stimulus. This heat-dependent CCL5 reduction was further confirmed by ELISA, western blotting, and quantitative PCR analysis. We next examined whether the acute heat stress in mice affects serum CCL5 concentration, as well as CCL5 gene expression in skeletal muscle. Male C57B6/J mice, maintained at 23 C, were exposed to 45 C for 1 hour, followed by back to 23 C chamber (the recovering time). CCL5 gene expression in skeletal muscle was significantly reduced by approximately 0.1- to 0.5-folds after 3 hours of the recovering time. Serum CCL5 concentration was acutely elevated by 1 hour of the heat exposure; however, importantly, was significantly reduced by approximately 0.5-fold after 23 hours of the recovering time. Moreover, we explored the physiological significance of the heat-dependent reduction of the myokine CCL5. We found that the addition of heat-stimulated C2C12 myotube cell culture supernatant to 3T3-L1 adipocytes causes an increase in cell size and large lipid droplet, whereas this change is abolished by the addition of recombinant CCL5.Overall, these suggest that heat stimulus decreases secretion of CCL5 from skeletal muscle both in vivo and invitro, and this CCL5 reduction by heat stimulus potentially altered the adipocyte metabolism. Although further studies are required to elucidate the physiological significance of the heat-dependent decreases in CCL5, it can be hypothesized that these heat-dependent myokines regulate lipid homeostasis which was disrupted by heat stimulus.

B576/P2955

Characterization of the unfolded protein response in normal human skin fibroblasts

L. Sobilo, L. Maclart Marsal, K. Lazou, M. Moreau, P. Schaeffer, M. Juan, E. Leblanc-Noblesse, A. Bulteau, K. Pays, C. Nizard; LVMH Recherche, Saint Jean de Braye, FRANCE.

Introduction: By ensuring protein synthesis and folding, endoplasmic reticulum (ER) is essential for the maintenance of proteostasis. Numerous perturbations (inflammation, oxidative stress) can lead to an accumulation of misfolded proteins in the ER leading to its engorgement and thus to an ER stress. Response to this stressful situation, the cell has developed an adaptive mechanism to repair the misfolded proteins: the UPR (Unfolded Protein Response). This response is induced by the recruitment of chaperon protein GRP78/BiP which leads an activation of three distinct signaling pathways coming from the ER transmembrane proteins: IRE1, PERK ATF6. Dermal matrix which is essential for skin elasticity and thickness is composed of protein such as collagens with unique and specific structure which function rely on their folding. Therefore, ER is essential for the maintenance of the biological functions of the skin, so the purpose of this study was, first to study le ER Stress in dermal Fibroblasts et then characterize the expression of aims targets of the UPR. **Materials and Methods:** Firstly, NHF were cultured with a specific pharmacological ER stressor, thapsigargin (a SERCA2 Ca^{2+} pump inhibitor that depletes ER Ca^{2+}) and the cells were collected at different times to study the gene expression (RT-qPCR by TLDA Technology) and the proteins expression (Western Blot). Afterward, the targets of UPR were observed in NHF stressed by immunostaining with confocal microscopy. Finally, the impact of stress induced by thapsigargin on NHF were studied on activity of proteasome by spectrophotometry. **Results:** Our results showed that the Ca^{2+} depletion in ER with thapsigargin causes a strong cytotoxicity on skin fibroblasts. The ER stress were detected with the significative increase of le GRP78/BiP target which leads to the activation of certain UPR pathway. In fact, we constated that the three UPR pathways didn't responded of the same manner in the time and with different concentrations of the thapsigargin. ATF6 pathway seems to be more sensitive then IRE1a pathway and that PERK which require a strong ER stress to be activate. The different response of three UPR pathways describe that dermal fibroblasts cells can

activate some defense strategies to rebalance the right conformed proteins production to maintain homeostasis and avoid cell death.

B577/P2956

Aspirin metabolite sodium salicylate downregulates the unfolded protein response and cell migration in colorectal cancer cells

N. Rauth, D. Bhandari; Chemistry and Biochemistry, California State University Long Beach, Long Beach, CA.

A daily regimen of low dose aspirin has been epidemiologically linked to reduced occurrence of many cancers particularly colorectal cancer. However, the cellular mechanism(s) for this protection is not well understood. Cancer cells have altered metabolic demands and rapid proliferation rate that can induce endoplasmic reticulum (ER) stress. During ER stress, cells activate an evolutionarily conserved signaling pathway known as unfolded protein response (UPR). The adaptive UPR signaling has been correlated to acquisition of malignancy and chemoresistance in cancer cells. Therefore, we investigated if aspirin affects the ability of cancer cells to activate UPR upon induction of ER stress. We treated colorectal cancer cell line DLD1 with sodium salicylate (NaSal), a metabolic derivative of aspirin and induced ER stress with tunicamycin, which halts N-linked glycosylation - an importance posttranslational modification of secretory proteins in the ER. We then analyzed the expression of various proteins involved in UPR signaling via western blotting. Our data indicate that NaSal treatment downregulates UPR signaling. Specifically, we observed significantly reduced levels of ER chaperone glucose-regulated protein of 78kDa (GRP78) whose overexpression has been implicated in chemoresistance. Using flow cytometry based annexin V assay, we observed a greater percentage of apoptosis in cells treated with NaSal-tunicamycin as compared to the control sets. Finally, using a wound healing assay, we also observed reduced cell migration upon NaSal treatment. Together, this work sheds light on a potential mechanistic basis for the chemopreventive action of aspirin. Our current and future experiments are directed toward understanding the molecular mechanism by which NaSal regulates UPR and cell migration.

B578/P2957

The G protein-coupled receptor (GPCR) FSHR-1 and the SPHK-1 lipid kinase regulate *C. elegans* life- and healthspans via a common pathway

R. Adkins, J. R. Kowalski; Biological Sciences, Butler University, Indianapolis, IN.

Later years of life are often characterized by reduced cognitive function and mobility. One contributor is the cellular accumulation of reactive oxygen species (ROS), but our understanding of how cells respond to ROS to promote health and survival is incomplete. Follicle stimulating hormone receptor-1 (FSHR-1) and sphingosine kinase-1 (SPHK-1) mediate responses to oxidative stress and regulate life history traits in the roundworm *Caenorhabditis elegans*. The genes encoding both proteins are conserved in humans where they are implicated in stress responses, cell survival and neuronal function. *fshr-1* and *sphk-1* work together to mediate responses to intestinal oxidative stress in *C. elegans*. Whether these genes work in a common pathway to control lifespan or healthspan is unknown. Previous studies indicated potential phenotypic differences in life history traits based on the worms' bacterial food source. The typical source, OP50 *E. coli,* is pathogenic compared to the HB101 strain. We hypothesized differences in pathogenicity between the two strains cause decreased lifespan and locomotion through oxidative

stress effects that may differentially affect the mutants. Here we show *sphk-1* and *fshr-1* are required for normal life- and healthspan in *C. elegans*. We found significant reductions in mutant lifespans on both lawns versus wild type worms, but no differences within genotype across food. *fshr-1, sphk-1* and double mutants had decreased swimming rates compared to wild type worms on HB101 at all timepoints, but no significant difference in motor decline over time between the genotypes. This suggests the genes may not act in a timed manner to regulate accumulation of oxidative stress. We also saw non-additive effects for the genes on both lifespan and healthspan, as double mutants showed phenotypes equal to single mutants, indicating *fshr-1* and *sphk-1* work in a single pathway to mediate life- and healthspan. Lawn pathogenicity levels do not affect lifespan in the mutants, suggesting the potential decrease in innate immunity does not make the worms susceptible to increased OP50 pathogenicity. These data provide evidence of a common genetic pathway between *sphk-1* and *fshr-1* during aging, consistent with their known roles in oxidative stress mediation. Future studies will explore details of this relationship via tissue-specific *fshr-1* rescue and depletion experiments.

B579/P2958

Unveiling 'musica universalis' of the cell: central dogma riding the 12-h tides

B. Zhu; University of Pittsburgh, Pittsburgh, PA.

Biological oscillations often cycle at the harmonic frequencies of the 24h circadian rhythm, a phenomenon coined "Musica Universalis". Like the circadian rhythm, the 12h ultradian rhythms are also evolutionarily conserved, prevalent and robust. Originally thought to be regulated by the circadian clock and/or environmental cues, recent new evidence support the notion that the majority of 12h rhythms are regulated by a distinct and cell-autonomous oscillator that is separate from both the 24-h circadian clock and the cell cycle oscillator. Mechanistically, 12h oscillator is transcriptionally regulated at multiple steps. 12h rhythmic chromatin occupancy of Spliced form of XBP1 (XBP1s) and histone methyl transferase complex together shape the 12h epigenome and drive 12h nascent transcription of genes at dawn and dusk, which are enriched for basal transcription regulation, mRNA processing and export, ribosome biogenesis, translation initiation, and protein processing/sorting in the Endoplasmic Reticulum (ER)-Golgi in a temporal order consistent with the progressive molecular processing sequence described by the central dogma. In addition to tight control at the transcription initiation step, 12h rhythm of nuclear speckle liquid-liquid phase separation dynamics further regulates the 12h oscillator at the mRNA processing and transcription elongation step. Physiologically, liver-specific deletion of the 12h oscillator resulted in accelerated liver aging and steatosis, manifested with mitochondrial dysfunction, and dysregulated proteostasis, lipid and mRNA metabolism. Evolutionarily, the mammalian 12h oscillator likely evolves from the ancient circatidal clock of marine animals, whose behaviors are synchronized to the ~12h ebb and flow of the tides. 12h rhythms of orthologous gene expression are also found in species as divergent as crustacean, sea anemone, *C.elegans*, zebrafish, mouse and primates. The recent identification of 12h transcriptome in the prefrontal cortex region of humans, and altered 12h rhythms in genes associated with proteostasis and mitochondrial function in schizophrenia subjects further indicate the importance of 12h oscillator. Due to the absence of tidal cues for terrestrial animals, we conjecture that the 12h oscillator is co-opted to adapt to the 12h cycle of metabolic stress that peaks at transition times at dawn and dusk in in-land animals.

B580/P2959

A central helical fulcrum controls eIF2B conformation and metabolic signaling

R. E. Lawrence[1], S. Shoemaker[2], A. Deal[3], L. Wang[3], S. Marqusee[2], P. Walter[3]; [1]University of California, San Francisco, San Francisco, CA, [2]University of California at Berkeley, Berkeley, CA, [3]University of California at San Francisco, San Francisco, CA

The Integrated Stress Response (ISR) is an ancient cellular pathway that allows cells to remodel metabolism and translation in response to a wide variety of stresses. ISR signaling is mediated by dynamic interactions between members of a core circuit that regulates the rate of nucleotide loading of the GTPase eIF2. eIF2's heterodecameric guanine nucleotide exchange factor, eIF2B, contains core regulatory subunits eIF2Bβ and eIF2Bδ that have homology to archaeal ribulose 1-5-bisphoshate isomerases. eIF2B is dynamically controlled by phosphorylation of its eIF2 substrate, which ultimately inactivates eIF2B GEF activity, converting eIF2B from an active "A-state" conformation to an inactive "I-state conformation." A key open question in the field was how exactly eIF2-P binding at a site distal from eIF2B's catalytic domain allosterically induced the transition into the "I-state". Using hydrogen-deuterium exchange, biochemistry, cryo-EM, and cellular assays we uncovered a highly conserved helical switch in the eIF2Bδ core regulatory subunit ("δhelix13") that acts as a fulcrum for eIF2B's transition between the A-state to the I-state. In particular we found i) that δhelix13 can adopt two distinct switch conformations, and ii) that mutations that lock the switch into a particular state are sufficient to drive the entire eIF2B complex into the A-state or I-state. Furthermore, δhelix13 mutations clamp eIF2B activity and ISR signaling into defined states in cells, presenting attractive tools for the development of ISR-targeting drugs for neurodegenerative disease, as well as exploring metabolic consequences of chronic ISR activation.

B581/P2960

Investigations into Hsc70 phosphorylation in response to DNA damage

T. Moss, S. Moss, A. Wooldredge, O. Johnson, K. Shokat, J. Gestwicki, S. Mukherjee; University of California, San Francisco, San Francisco, CA

Hsc70 is a constitutively expressed member of the Hsp70 family of chaperone proteins. Hsc70 has numerous and diverse roles in the cytoplasm and nucleus, which include maintaining basal cellular functions and response to stress. Due to its myriad functions, Hsc70 activity must be tightly regulated, and dysregulation can lead to neurodegenerative disease and cancer. Given that Hsc70 is a highly abundant protein, its activity is often regulated via post translational modifications (PTMs). Using a *Legionella pneumophila (L.p.)* kinase, LegK4, we identified threonine 495, a highly conserved amino acid, as a phosphosite in Hsc70 with strong regulatory potential. In the context of *L.p.* infection, LegK4 phosphorylates cytosolic Hsc70, resulting in a reduction in both its ATPase activity as well as its ability to fold proteins. As a result, protein synthesis is severely reduced. The mechanism by which this single phosphorylation affects Hsc70 function remains unknown. Given that pathogens often hijack and/or mimic host enzymes, we investigated whether a host kinase can phosphorylate T495 of Hsc70. Indeed, we identified that this phosphorylation also occurs in response to stress — prolonged treatment of cells with the DNA alkylating agent methyl methanesulfonate (MMS) leads to an accumulation of phospho-Hsc70(T495) (pHsc70) in the nucleus. This phosphorylation is dependent on the DNA-damage response kinases DNA-PKcs, Chk2, and CK-1, and correlates with changes in nuclear architecture previously linked

to apoptosis. Thus, using *L.p.* as a tool, we have discovered a previously unknown role of Hsc70 phosphorylation during DNA damage.

B582/P2961

Cryo-EM reveals the dynamics interplay between mitochondrial Hsp90 and SdhB folding intermediates

Y. Liu; Chemistry and Biochemistry, University of Maryland, College Park, College Par, MD.

TRAP1 is a mitochondrion specific Hsp90, a ubiquitous chaperone family that mediates the folding and maturation of hundreds of "client" proteins. Through the interaction with client proteins, TRAP1 regulates mitochondrial protein homeostasis, oxidative phosphorylation/glycolysis balance, and plays a critical role in mitochondrial dynamics and disease. However, the molecular mechanism of client protein recognition and remodeling by TRAP1 remains elusive. Here we established the succinate dehydrogenase B subunit (SdhB) from mitochondrial complex II as a client protein for TRAP1 amenable to detailed biochemical and structural investigation. Cryo-EM structures of the TRAP1:SdhB complex show TRAP1 stabilizes SdhB folding intermediates by trapping an SdhB segment in the TRAP1 lumen. Unexpectedly, client protein binding induces an asymmetric to symmetric transition in the TRAP1 closed state. Our results highlight a client binding mechanism conserved throughout Hsp90s that transcends the need for cochaperones and provide molecular insights into how TRAP1 modulates protein folding within mitochondria. Our structures also suggest a potential role for TRAP1 in Fe-S cluster biogenesis and mitochondrial protein import. In addition, we used a combination of solution, biochemical and cryo-EM approaches to show that, independent of nucleotide state, a subpopulation of TRAP1 exists as tetramers. The biological function of TRAP1 tetrameric states remain to be explored.

B583/P2962

Characterization of a putative *C. elegans* ortholog of the ERAD protein, Valosin-containing Protein-interacting Membrane Protein (VIMP)

L. Dahlberg[1], L. Ellgaard[2], A. Dhalla[3], T. Marks[1]; [1]Western Washington University, Bellingham, WA, [2]University of Copenhagen, Copenhagen, Denmark, [3]Antelope Valley College, Palmdale, CA

The ER-associated Degradation machinery consists of large protein complexes whose makeup can change depending on the stress level of the ER. The Valosin-containing Protein-interacting Membrane Protein (VIMP, SelenoS) is a member of the ERAD machinery that can interact directly with the adaptor protein, p97, which shuttles misfolded proteins to the proteasome for degradation. Despite VIMP's central position in ERAD protein complexes, its precise roles in ERAD and cellular stress responses are still unclear. VIMP levels increase during ER stress, and some studies suggest that cells that lack VIMP have reduced response to ER stress, as measured by the induction of Unfolded Protein Response (UPR) genes including *xbp1 and hspa5* (BiP). In *C. elegans,* a putative ortholog to VIMP is the gene F26F4.9. F26F4.9 has high sequence homology with mammalian VIMP except for at its C-terminus, where it contains a glutaredoxin domain instead of the unstructured selenocysteine-containing domain. We used the ER stress reporter transgene, P*hsp-4*::GFP to determine if F26F4.9 is required for regulating *hsp-4* during an ER stress response. We find that a genetic deletion in F26F4.9 (*tm2433*) reduces the activation of P*hsp-4*::GFP that usually occurs when animals are treated with the ER stress inducing drug, Tunicamycin, or when animals are lacking other ERAD machinery. In particular, RNAi against *hsp-4* dramatically increases the activity of GFP expressed under the *hsp-4* promoter. However, in RNAi-fed

animals, the loss of F26F4.9 reduces the high P*hsp-4*::GFP expression by 50%. Our current work is addressing the mechanisms by which F26F4.9 may work within the ERAD machinery to regulate gene transcription as part of the unfolded protein response.

B584/P2963

Impairment of Endoplasmic Reticulum-Associated Degradation by Perturbed Lipid Biosynthesis

E. M. Rubenstein, S. M. Turk, C. J. Indovina, D. L. Overton, A. M. Runnebohm, C. J. Orchard, E. M. Doss, K. A. Richards, C. B. Irelan, M. M. Daraghmi, C. G. Bailey, J. M. Miller, J. M. Niekamp, S. K. Gosser, M. E. Tragesser-Tiña, K. P. Claypool, S. M. Engle, B. W. Buchanan, J. B. Olesen, P. J. Smaldino; Ball State University, Muncie, IN

The relationship between membrane lipid composition and endoplasmic reticulum (ER) protein homeostasis is complex and incompletely understood. We performed a screen to identify genes required for efficient ER-associated degradation (ERAD) of the Hrd1 ubiquitin ligase substrate *Deg1*-Sec62 in *Saccharomyces cerevisiae*. We identified and validated *INO4*, which encodes one component of the heterodimeric Ino2/Ino4 transcription factor, as required for *Deg1*-Sec62 degradation. Ino2/Ino4 regulates expression of genes encoding several enzymes contributing to lipid biosynthesis. *Deg1*-Sec62 degradation in *ino4* yeast was restored by supplementation with inositol, choline, and ethanolamine, metabolites whose synthesis and/or uptake are mediated by Ino2/Ino4-regulated genes. Mutation of several genes encoding enzymes contributing to phospholipid or sterol biosynthesis delayed *Deg1*-Sec62 degradation. Further, *INO4* deletion stabilized a panel of ER protein quality control substrates of the Hrd1 and Doa10 ubiquitin ligases and sensitized yeast to hygromycin B, which is associated with an elevated burden of aberrant and misfolded proteins. These results suggest ERAD is broadly sensitive to membrane lipid composition. Several human diseases are characterized by disrupted lipid synthesis. Impaired lipid homeostasis may delay ER protein degradation in individuals with these conditions.

B585/P2964

The RIDD activity of *C. elegans* IRE1 in sensory neurons supports survival in stressful environments.

M. Ying, Male[1], Y. Argon[1], T. Gidalevitz[2]; [1]Children's Hospital of Philadelphia, Philadelphia, PA, [2]Drexel University, Philadelphia, PA

Regulated IRE1-dependent RNA decay (RIDD) and *xbp-1* splicing are two RNase activities of ER stress sensor IRE1. Unlike *xbp-1* splicing, RIDD is only identified in few species, and its substrate repertoire is not well defined. Similarly, while it serves to reduce biosynthetic burden on ER under stress, the roles of RIDD in normal physiology are only beginning to emerge. We asked whether *C. elegans* IRE1 can cleave RIDD substrates and what are the physiological consequences to their degradation.To test RIDD capability of the worm IRE1, we fused its cytosolic kinase and RNase domains with the human transmembrane and luminal domains, to retain proper protein interactions within the ER, and expressed it in human *ire1(-/-)* cells. The chimeric protein efficiently spliced human *xbp-1*; this was increased by ER stress and inhibited by IRE1-selective inhibitor, 4μ8c, confirming chimera's activity. Importantly, chimeric IRE1 downregulated the canonical RIDD target *blos-1* mRNA, which was also inhibited by 4μ8c. Thus, the worm IRE1 possesses RIDD activity.To understand the physiological role(s) of RIDD in *C. elegans*, we looked for endogenous substrates. Using existing transcriptomic data, we identified messages whose downregulation during tunicamycin (Tm)-induced ER stress depended selectively on IRE1, including that of a TGFβ homologue, *daf-7*. Importantly, *daf-7* mRNA was efficiently degraded

under ER stress by human IRE1, along with its authentic RIDD substrate *blos-1*, establishing *daf-7* as a strong candidate for RIDD substrate.DAF-7 protein is secreted from sensory neurons in early larval stages, and if its secretion is decreased, it signals stressful environments and triggers several adaptive responses. We found that upon Tm exposure of young larvae, *daf-7* mRNA is selectively downregulated in IRE1-dependent manner. Remarkably, Tm concentrations too low to activate *xbp-1* splicing efficiently induce RIDD of *daf-7*, suggesting a sensing functionality. Because Tm is a natural metabolite of some *Streptomyces spp.*, which prey on the worm food bacteria, and because DAF-7 secretion signals food, we asked whether low Tm exposure may trigger an adaptive response. We found that low Tm improved survival by worm population of combined starvation and temperature stress. Thus, by utilizing RIDD to sense a danger signal, *C. elegans* prepares for upcoming environmental challenges.

B586/P2965

***In-Vivo* Detection of Stress-Dependent Sumoylation in the Budding Yeast Cytosol**

B. Humphries, O. Kerscher; College William & Mary, Williamsburg, VA

SUMO, a small ubiquitin-like modifier protein, modulates protein function to regulate essential cellular processes, including the response to stress. In yeast and mammalian cells, exposure to stressors such as elevated temperatures or oxidizing agents, initiates a wave of sumoylation that propagates throughout the nucleus and cytosol. This SUMO Stress Response (SSR) has been reported to protect protein complex integrity, prevent protein aggregation, and enhance stress-specific transcriptional responses. However, the choreography and functional targets of this stress-induced sumoylation in the cytosol are not well understood. Therefore, the objective of our research is to establish a novel SUMO stress reporter assay to visualize and quantify cytosolic sumoylation targets that respond to heat shock and peroxide stress. Our SUMO stress reporter assay is based on a bimolecular fluorescence complementation (BiFC) or Split-GFP approach. Two complementing fragments (GFP 1-10 and GFP 11) of the green fluorescent protein are fused to the SUMO ligase Siz1 and a *bona fide* cytoplasmic SUMO substrate of this E3 ligase, respectively. Our results indicate that Siz1 interacts specifically with a GFP 11-fusion protein tethered to the cytoplasmic side of the mitochondrial outer membrane. In contrast, a soluble fusion protein, GFP11-HXK1 was enriched with GFP1-10-Siz1 in the yeast cell nucleus. In conclusion, refining this assay may hold great utility for the study of SUMO-dependent stress-induced protein interactions in the cytosol and the study of genetic pathways that control them.

B587/P2966

Transcriptional control of proteome remodeling with cell size

D. You[1], M. Lanz[1,2], J. Elias[1,2], J. Skotheim[1,2]; [1]Stanford University, Stanford, CA, [2]Chan Zuckerberg Biohub, Stanford, CA.

Generally, growing cells pair increase in size with proportional increase in their biological components to maintain cell function. However, recent studies demonstrated that both cell function and proteome composition are not necessarily constant with cell size. Indeed, many proteins change their relative concentrations with cell size, and large cells are more likely to enter senescence, implicating cell enlargement as a potential source of stress. Whether these proteomic changes reflect a stress response and how these changes occur are unknown. To understand the mechanisms of proteome remodeling with cell size, we examined two pathways in the biosynthetic process - transcription and protein turnover. We employed pulsed-SILAC mass spectrometry and RNAseq in inducible cyclin-D knockdown

cells that yielded near steady-state cells of varying volumes. We find that changes at the transcriptomic level explains most of the proteome remodeling with cell size, and that protein turnover also partially contributes to this phenomenon. Our study points to a size-dependent transcriptional response program that reshapes the cell proteome

B588/P2967
Structure of the PAPP-A/IGFBP5 complex reveals mechanism of substrate recognition
Q. Hao; Calico Life Science (AlphaBet), South San Francisco, CA

Insulin-like growth factor (IGF) signaling is highly conserved and tightly regulated by proteases including Pregnancy-Associated Plasma Protein A (PAPP-A). PAPP-A and its paralog PAPP-A2 are metalloproteases that mediate IGF bioavailability through cleavage of IGF binding proteins (IGFBPs). Here, we present single-particle cryo-EM structures of the catalytically inactive mutant PAPP-A (E483A) in complex with a peptide from its substrate IGFBP5 (PAPP-A_{BP5}) and also in its substrate-unbound form, by leveraging the power of AlphaFold to generate a high quality predicted model as a starting template. We show that PAPP-A is a flexible *trans*-dimer that binds IGFBP5 via a 25-amino acid anchor peptide which extends into the metalloprotease active site. This unique IGFBP5 anchor peptide that mediates the specific PAPP-A-IGFBP5 interaction is not found in other PAPP-A substrates. Additionally, we illustrate the critical role of the PAPP-A central domain as it mediates both IGFBP5 recognition and trans-dimerization. We further demonstrate that PAPP-A trans-dimer formation and distal inter-domain interactions are both required for efficient proteolysis of IGFBP4, but dispensable for IGFBP5 cleavage. Together the structural and biochemical studies presented here reveal the mechanism of PAPP-A substrate binding and selectivity.

Ubiquitin and Proteasome Function

B589/P2968
Degradation of ubiquitinated post-ER membrane proteins via Ddi1, Rbd2 and the proteasome
A. Y. Minard, R. Piper; University of Iowa, Iowa City, IA.

Misfolded or surplus membrane proteins in the endosomal system are hazardous to cells and thus, in healthy cells, are typically subject to degradation by ER-Associated Degradation (ERAD) or, if the protein resides outside of the ER, by the multivesicular body (MVB) pathway. Proteins are marked for degradation with a ubiquitin (Ub) tag which, can vary in its topology. In the case of ERAD, membrane proteins are marked with K48-linked polyubiquitin (polyUb^{K48}), which directs retrotranslocation and Cdc48-mediated delivery to the proteasome. In contrast, substrates for the MVB pathway are modified with monoUb or polyUb^{K63}, which engages the ESCRT machinery to deliver membrane protein substrates into intralumenal vesicles. It is unclear, however, whether the topology of the ubiquitin chain dictates entry into either pathway, or if cells are able to process post-ER membrane proteins that are modified by polyUb^{K48}. Here we show that post-ER membrane proteins that are modified by polyUb^{K48} chains are in fact engaged by a degradative pathway distinct from ERAD and MVB sorting. By targeting a set of synthetic ubiquitin ligases to TGN/Endosomal membrane proteins, we find that polyUb^{K48} modified proteins are delivered to the proteasome, but without retrotranslocation from the membrane. Rather, they are subjected to two cleavage events: one by the ubiquilin Ddi1, which cleaves the cytosolic portion concomitant with degradation by the proteasome; and a second by the rhomboid protease Rbd2 in the lumenal portion of the protein, which is degraded in the lysosomal lumen. Importantly, polyUb^{K48}-

modification of post-ER membrane proteins does not sort them into the MVB pathway, despite the capacity of some of the ESCRT machinery to recognize a wide variety of polyUb linkages. These data reveal a new post-ER degradation pathway 'pERdeg' that we hypothesize operates in tandem with the MVB pathway and may be a major degradative route for a subset of membrane proteins as well as those artificially targeted for degradation by PROTAC drugs.

B590/P2969

Molecular Mechanisms for the Activation of 26S Proteasomes by ZFAND5

D. Lee[1], Y. Zhu[2], **L. Colson**[3], X. Wang[4], E. Tkacik[3], L. Huang[4], Q. Ouyang[5], A. L. Goldberg[1], Y. Lu[3]; [1]Department of Cell Biology, Harvard Medical School, Boston, MA, [2]Division of Structural Biology, University of Oxford, Oxford, UNITED KINGDOM, [3]Department of Systems Biology, Harvard Medical School, Boston, MA, [4]University of California Irvine, Irvine, CA, [5]Peking University, Beijing, CHINA.

Various factors increase intracellular protein degradation by stimulating the 26S proteasome's activities. We present the mechanisms of the proteasome activator ZFAND5, a 213 amino acid protein that contains a ubiquitin-binding, A20 zinc finger domain near its N-terminus and an AN1 zinc finger domain near its C-terminus (1, 2). Skeletal muscles upregulate ZFAND5 during atrophy, when proteolysis by the ubiquitin proteasome pathway increases leading to muscle wasting (1). Addition of ZFAND5to purified 26S proteasomes stimulates the hydrolysis of ubiquitinated proteins up to fourfold, short peptides up to six fold, and ATP up to twofold (2). This stimulation in the degradation of ubiquitinated proteins requires the A20domain, and the enhancement of the peptidase activity requires the AN1 domain.
To uncover the mechanism by which ZFAND5 stimulates the activities of the 26S proteasome, we determine the structures of ZFAND5-bound 26S proteasomes using cryo-electron microscopy. ~68% of all particles contain ZFAND5 (Z+) and show ZFAND5's AN1domain interacting with the 26S proteasome's ATPase subunit Rpt5 and core particle (CP) subunit α;;;;7.~20% of the Z+ particles exhibit a novel proteasome conformation where the diameter of the entrance to the ATPase's substrate translocation channel is threefold larger than in the basal state. In this novel conformation, ZFAND5'sC-terminus interacts with the ATPase subunit Rpt1 and the ubiquitin-binding subunit Rpn1. ZFAND5's stimulation of peptidase and ubiquitinated protein degradation activity requires this C-terminal region. The other ~32% of all particles lack ZFAND5 and exhibit an open gate on the CP, a fourfold larger population than 26S proteasomes alone (3). Using single-molecule fluorescence microscopy invitro, we find 26S proteasomes only degrade ~20% of ubiquitinated proteins that bind without ZFAND5 but degrade ~70% of the ubiquitinated substrates that bind with ZFAND5. ZFAND5 dissociates before deubiquitylation of the substrate. These findings suggest ZFAND5's C-terminal interaction with proteasomes triggers a substrate capturing state that directly precedes a translocation-competent state to promote the proteolysis of substrates.1. Hishiya,A., et al. (2006). The EMBO journal, 25(3), 554-564.2. Lee,D., et al. (2018). PNAS, 115(41), E9550-E9559.3. Chen,S., et al. (2016). PNAS, 113(46), 12991-12996.

B591/P2970

Rescue of proteotoxic stress and neurodegeneration by an endoplasmic reticulum Zn2+ transporter

X. Guo[1], **M. C. Mutch**[1], A. Y. Torres Espinosa[1], W. Dai[2], D. J. Montell[1]; [1]Molecular, Cellular & Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA, [2]Fudan University, Shanghai, CHINA.

Proteotoxic stress drives the progression of numerous degenerative diseases including Type I diabetes, Huntingtin's Disease, Parkinson's Disease, and retinitis pigmentosa. In response to misfolded proteins, cells adapt by activating the endoplasmic reticulum (ER) stress response including ER-associated protein degradation (ERAD). However, persistent stress overwhelms the response and triggers apoptosis. Identifying mechanisms that enhance ERAD and mitigate ER stress are expected to lead to new therapeutic approaches. Here, we show that overexpression of the conserved, ER membrane-localized, Zn^{2+} transporter ZIP7, potently enhances ERAD and rescues retinal degeneration caused by misfolded Rhodopsin (Rh) in Drosophila. We found that overexpressing Zip7 dramatically reduces the abundance of ubiquitinated proteins, likely through enhancing proteasome function. In contrast to prior work suggesting that ZIP7 promotes trafficking of membrane proteins such as Notch, we show that ZIP7 is necessary for ERAD, which is essential for cell functions such as motility and survival. We propose that Zn^{2+} transport is limiting for ERAD and that ZIP7 gene therapy could ameliorate retinitis pigmentosa due to folding-defective mutations in rhodopsin. We will present progress in elucidating the mechanisms of Zip7 function in maintaining proteostasis, mitigating ER stress, and preventing neurodegeneration *in vivo*.

B592/P2971

Altering PA28gamma expression in 4T1 cancer cells with butyrate

N. Bowman[1], I. Ma[1], N. Saran[1], L. F. Barton[2]; [1]Austin College, Sherman, TX, [2]Biology, Austin College, Sherman, TX.

PA28γ is an ATP and ubiquitin independent proteasome activator overexpressed in many cancers. In normal cells, PA28γ is regulated through AP-1 by p38, a transcriptional activator, and microRNA-7, a translational inhibitor. Both pathways may be deregulated in cancers to impact the expression of PA28γ. One drug treatment that has been used previously to reduce PA28γ expression is sodium butyrate, but the mechanism for reducing PA28γ expression is not understood. Immunoblots and qPCR were used to understand how sodium butyrate impacts PA28γ levels in 4T1 mouse mammary carcinoma cells. Sodium butyrate does reduce PA28γ protein concentration in a dose and time dependent manner. In contrast, *psme3* mRNA levels remained relatively constant, indicating that butyrate treatment is not affecting mRNA synthesis or degradation. These data suggest butyrate is impacting the expression of PA28γ post transcriptionally, but further testing is needed to elucidate the specific mechanism.

B593/P2972

Characterizing the role of the E3 ligase Rsp5 in stress granule clearance

M. K. Rivera, J. R. Buchan; University of Arizona, Tucson, AZ

Stress granules (SGs) are conserved cytoplasmic condensates of non-translating mRNA and associated RNA-binding proteins, whose assembly is induced by the accumulation of non-translating mRNAs during adaptation of cells to stressful environments. While SG assembly is relatively well understood, many

mechanisms including chaperone function, post-translational modifications, proteasomal and autophagic (granulophagy) clearance have been reported. However, the mechanisms, utilization and integration of these clearance pathways is poorly understood, likely impacting post-transcriptional gene expression. Studying SG clearance may aid in furthering our understanding of diseases such as ALS and Cancer, where aberrant persistence of SGs is theorized to contribute to disease pathology. In this work, we performed an unbiased genome wide screen Saccharomyces cerevisiae (budding yeast) to identify regulators of SG clearance via granulophagy. This screen identified several genes involved in Ubiquitin-related factors, including co-factors of the essential E3 ligase Rsp5, known to add K63-linked ubiquitin chains to substrates and facilitates vacuolar/lysosomal trafficking. Using conditional genetics, we find that Rsp5 strongly promotes SG clearance after transient heat shock (HS), but not sodium azide. Currently, we are characterizing if/when Rsp5 colocalizes to SGs during stress or stress recovery. Furthermore, we will purify HS-induced SGs, +/- Rsp5 activity, in efforts to identify Rsp5 interactors and modification substrates that facilitate SG clearance in an unbiased manner. Understanding the role of Rsp5-mediated SG clearance may aid in the development of novel therapeutic, finding alternative solutions to treat related diseases.

B594/P2973

Quantitative ubiquitination assays to rapidly design PROTACs and molecular glues

N. A. Warren, K. Wallace, D. Padykula, A. Russell, **J. Pedroza**; LifeSensors, Inc., Malvern, PA

PROTACs have emerged as a new class of drugs that can target the “undruggable” proteome by hijacking the ubiquitin proteasome system. Despite PROTACs’ success, most current PROTACs interface with a limited number of E3 ligases, hindering their expansion into applicable therapeutic uses. Currently, PROTAC drug discovery relies heavily on traditional Western blotting and reporter gene assays which are insensitive and prone to artifacts, respectively. New reliable methods to monitor true PROTAC function (i.e., ubiquitination and subsequent degradation of targets at physiological expression levels) without external tags are essential to accelerate the PROTAC discovery process and to address many unmet therapeutic areas. In this study, we developed a new high-throughput screening technology using “TUBEs” as ubiquitin-binding entities to monitor PROTAC-mediated poly-ubiquitination of native target proteins with exceptional sensitivity. As a proof of concept, targets including BRD3, Aurora A Kinase, and KRAS were used to demonstrate that ubiquitination kinetics can reliably establish the rank order potencies of PROTACs with variable ligands and linkers. PROTAC-treated cell lysates with the highest levels of endogenous target protein ubiquitination - termed “Ub_{Max}” - display excellent correlations with DC_{50} values obtained from traditional Western blots with the added benefits of being high throughput, providing improved sensitivity, and reducing technical errors.

B595/P2974

Expanding Chemical Libraries of E3 Ubiquitin Ligases to Unlock Targeted Protein Degradation

G. Jalagadugula, A. Russell, K. Wallace, J. Pedroza, **A. Haseeb**; LifeSensors, Inc., Malvern, PA

PROteolytic TArgeting Chimeras (PROTACs) have emerged as a new class of drug that can target the “undruggable” proteome to develop novel therapeutics, with specific focus on oncological therapeutics. PROTACs hijack the ubiquitin proteasome system (UPS) to ubiquitinate target proteins and subsequently degrade them in the proteasome. PROTACS are heterobifunctional small molecules with two active domains one binds to target protein and other binds to E3 ligase linked with a linker. The human

genome encodes ~ 600 E3 ligases that differentially expressed across various tissues. Only a few E3 ligases have been utilized to date for PROTAC applications. Expanding the chemical space to recruit largely unexplored E3 ligases can unlock the true potential degraders. This can be designed selectively to target proteins in selective tissues by regulating them under certain conditions, or by inducing specific type of ubiquitination and degradation through proteasome or lysosome. Therefore, identifying novel ligands for E3 ligases that perform non-degradative function can broaden the applicability in drug discovery. LifeSensors has developed technologies to identify novel ligands for E3 ligases that can recruit ligases to degrade undruggable proteome. In the current study, LifeSensors will highlight multiple approaches to identify novel ligands, and validate them to demonstrate their selectivity and applicability for target protein degradation (TPD).

Systems and Synthetic Biology

B597/P2975

Novel Exosome-based Platform for Rapid Production of Nano-vaccines with Enhanced Safety, Efficacy and Precision

J. Ku, R. A. Flojo, G. Chen, C. Olson, B. Lu; Santa Clara University, Santa Clara, CA.

Background: Technical advancements in bioinformatics, synthetic biology and surface display technology have opened new avenues for a rapid production of exosome-based nanovaccine with enhanced safety, efficacy and precision [1-3]. **Methods**: Using a combination of computational analysis, synthetic biology and exosome surface display technology [2-3], we designed and produced engineered exosome-based vaccines that display highly selective epitopes derived from the full-length antigens. **Results**: We initially developed a computational software and successfully used it to identify, extract and assemble highly immunogenic epitopes from both virus and cancer antigens. We then demonstrated that this software tool was able to condense highly immunogenic epitopes by removing low immunogenic segments from inclusion. We subsequently used DNA synthesis and gene cloning methods to incorporate these epitopes into an exosome-targeting scaffold, and successfully constructed a series of mammalian expression vectors. Finally, by transfecting these vectors into cultured human 293T cells, we were able to produce engineered exosomes that displayed the highly selective epitopes on their outer surface of secreted exosomes, therefore validating the vaccine production system.**Conclusion:** We have developed and validated a novel exosome-based platform that enables a rapid production of surface-displayed nanovaccine with improved safety, efficacy and precision.

Reference1.Dokholyan NV and Vishweshwaraisah YL. (2022) Toward rational vaccine engineering. *Advanced Drug Delivery Reviews*. 183:1141422.Meyer C, Losacco J, Stickney Z, Li L, Marriott G, Lu B. (2017) Pseudotyping exosomes for enhanced protein delivery in mammalian cells. *International Journal of Nanomedicine*. 12:3153-3170 3.Stickney Z, Losacco J, McDevitt S, Zhang Z, Lu B (2016) Development of exosome surface display technology in living human cells. *Biochem Biophys Res Commun*. 472(1):53-59

B598/P2976

Bacterial cell size and growth regulation in fluctuating nutrient environments

J. C. Kratz[1], S. Banerjee[2]; [1]Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, [2]Physics, Carnegie Mellon University, Pittsburgh, PA.

Bacteria dynamically regulate cell size and growth rate to thrive in changing environments. Previous work has found that bacterial cell size is coupled to nutrient-specific growth rate at steady-state, and that bacteria remodel their proteome in different environments to increase fitness. However, a mechanistic understanding of how bacteria dynamically regulate cell size and growth physiology in time-varying nutrient environments is lacking. We have developed a mathematical model based on cellular proteome allocation that accurately predicts *E. coli* cell size and growth rate control in response to nutrient shifts in both exponential and stationary phase. The model utilizes a threshold accumulation framework for cell division control, in which cells divide after accumulating a set amount of division proteins, and connects growth rate and division protein production rate to proteome allocation in time-varying environments. In response to pulsatile nutrient concentration, our model predicts that during upshift, *E. coli* transiently prioritize biomass accumulation and ribosome production over production of cell division machinery. This results in a rapid increase in single-cell growth rate, and a temporary overshoot in intergenerational added volume, leading to an increase in average cell size. Surprisingly, our model predicts the opposite behavior during downshift, namely that bacteria prioritize division over growth, despite needing to upregulate costly division machinery and increasing population size when nutrients are scarce. The transient upregulation of division protein production results in rapid cell division without biomass accumulation, leading to a sharp decrease in cell size, but a rapid increase in population growth rate. Importantly, we also find that both growth rate and cell size stabilization time after nutrient pulse is dependent on the pulse duration. Specifically, stabilization time increases with increasing nutrient pulse length until saturating at a constant stabilization time. Our model suggests that this transient memory of previous environments is a result of slow dynamics of proteome reallocation, and can confer increased fitness in fluctuating environments.

B599/P2977

Emergent dynamics and force generation in motor-free active contractile networks (Experiment)

X. Lei[1], T. Chakrabortty[1], J. Honts[2], S. Bhamla[1]; [1]Georgia Institute of technology, Atlanta, GA, [2]Drake Univeristy, Des moines, IA.

Living cells exploit contractile structures in the cytoskeleton to achieve various functions such as cell division and motility. Here we show in experimental and computational studies on a Ca^{2+}-triggered contractile cytoskeletal network composed of cortical proteins from ciliates (Tetrahymena). Our results demonstrate that this Ca^{2+}-triggered network exhibits viscoelastic behaviors that, under certain conditions, can be reversed. We propose a unifying phase diagram for these calcium-driven contractile dynamics. From simulations, we find that the difference in the molecular dynamics between the phases, such as diffusion coefficients, controls the network's morphology and contractility. Based on the simulation result, we created a new phase, experimentally combing the contractility and maintaining the micro-structure by pulsating energy input. We demonstrate how this viscoelastic system can be useful in generating controlled active forces and could have potential in an artificial cytoskeletal network in synthetic cells.

B600/P2978

Determining the neural circuits underlying female aggression in Drosophila

K. M. Martinez, I. J. Santiago, S. Sengupta, C. B. Palavicino-Maggio; Basic Neuroscience Divison, McLean Hospital, Belmont, MA.

Aggression is an intrinsic behavior that aids in defense, resources, and survival. The fruit fly, *Drosophila melanogaster*, is an excellent model for studying neural circuits. While both males and females elicit aggression, female aggression research has received less attention. Previously, our work in *Drosophila* identified a female-specific subgroup of cells in the pC1 brain region, known as pC1α neurons. Activation of pC1α neurons triggered female flies to fight at high-intensity levels. With this observance, our aim was to identify the neuronal clusters that relay sensory information to the pC1α neurons and ultimately lead to the activation of the female high aggression circuitry. To build on the circuitry of the pC1α neurons, our lab used an available electron microscopy database of the adult female fruit fly brain (FAFB). Our tracings revealed that pC1α neurons are morphologically distinct and present in both hemispheres of the female brain and are comprised of five neurons (pC1a-e). It also revealed interconnectivity between five subtypes. In general, pC1α neurons exhibited interconnectivity within the hemisphere (ipsilateral) and between hemispheres (contralateral). Interestingly, we uncovered several asymmetries in connectivity between and within the right and left hemispheres. We determined that pC1α neurons in the right hemisphere exhibit interconnectivity, while those in the left hemisphere do not. Additionally, we found more contralateral connections from left to right (207) than right to left (50). We also searched for non- pC1α neurons that send presynaptic inputs into the pC1α population. One major input contributor and presynaptic partner, the SMP093 L/R neurons, is in the superior medial protocerebrum (SMP) area, which is involved in circadian rhythm, feeding, and courting behavior. SMP093 showed a great deal of projections onto the left and right pC1α neurons indicating inputs not only bilaterally (L>R:263; R>L:157) but having interconnectivity (L>L: 163; R>R:387). Although it was examined that the left had limited interconnectivity, SMP093 L/R showed strong projections to those left pC1α neurons (320). We thus hypothesize that neurons found in the SMP region play a vital role in the circuitry of female aggression. Since pC1α neurons are only found in females, understanding how the circuitry behind fruit fly aggression works can be used to find new principles of how a sexually dimorphic brain works. In addition, this model might serve as a foundation for the development of therapeutic targets that can ameliorate aggression and behavioral abnormalities observed in psychiatric and neurodegenerative illnesses.

B601/P2979

Uncovering principles of cell organization using soft X-ray tomography and whole-cell integrated modeling of inter-organelle spatial relationships and physical interactions in *S. cerevisiae*

M. Mirvis, W. F. Marshall; Biochemistry & Biophysics, University of California, San Francisco, San Francisco, CA.

Cell organization - the cumulative morphology and relative arrangement of subcellular structures - forms distinct spatio-geometric patterns. Establishing causative links between cell organization and function is a major goal of cell biology. Robust methods are needed to define cell organization and distinguish organization of healthy cells from disorganization in stress and disease. Mesoscale cell organization arises from interactions between organelles. While the biochemical basis of organelle interactions has been well-studied, the role of physical interactions, such as packing, is poorly understood. To what

extent is cell organization a reflection of organelle confinement within the limited space of the crowded cytoplasm? We are developing a whole-cell integrated modeling approach to quantify the key constraints and variations in cell organization based on organelle physical and spatial relationships in *S. cerevisiae.* Adapting an approach developed by the Allen Institute (Viana, *et al*, doi: 10.1101/2020.12.08.415562), we perform single-cell and -organelle 3D morphometrics and spherical harmonic shape modeling from volumetric imaging. This generates a shape space for each structure, and integration of cell, nucleus, and vacuole shapes produces an "average cell" and a range of variant configurations. Sampling variation along various axes in the dimensionally-reduced shape space identifies principal components. A preliminary model based on a small confocal microscopy dataset suggests that cell volume, organelle-cell and inter-organelle scaling, and position are candidate top contributors to organizational variation, with a larger contribution by the vacuole than the nucleus. For higher spatial resolution and statistical power, we collected a dataset of 200-300 wild-type cells imaged with isotropic nanometer resolution by soft X-ray tomography in collaboration with the National Center for X-ray Tomography. Morphometric analysis of organelle volume and shape correlations indicates differential relative scaling patterns among organelles, and suggests asymmetric deformations due to vacuole-nucleus packing. Integrated shape modeling and analyses of co-varying geometric features in interacting organelle pairs are currently being developed. Future work will include comparative analysis between WT and large-vacuole mutants, characterizing physical packing relationships based on correlated morphological changes between the enlarged vacuole and neighboring organelles. This work will ultimately aim to quantify the magnitude of disorganization in a highly packed cytoplasm and characterize the impacts of shifting morphological and physical interdependencies between interacting organelles on global cell organization.

B602/P2980

Synthesis, characterization, and cellular imaging of a novel cyan emitting fluorescent α-amino acid

A. Gupta, B. Yan, M. Guo; Department of Chemistry and Biochemistry, University of Massachusetts Dartmouth, Dartmouth, MA.

The development of microscopy and fluorescent-based approaches to tag proteins have been advancing the fields of cellular and molecular biology, biotechnology, and medicine. Among others, small molecule-based approaches like incorporation of fluorescent unnatural α-amino acids (FAA) into proteins have been of particular interest to study protein interactions with other biomolecules, protein dynamics, and in live-cell imaging. The wide range of applications have attracted much scientific research interests. Here, we report the synthesis of a novel fluorescent α-amino acid 4-dibenzothiophen-4-yl-L-phenylalanine (DBT-FAA) capable of emitting cyan light in the visible region using optimized Suzuki cross-coupling reaction methodology in a reasonably good yield. The synthesized FAA has emission signals at ~440 nm, ~480 nm and ~540 nm with a tail extended beyond 600 nm. Upon incubation in Hela cells, the DBT-FAA at 10 μM gives strong fluorescent signals (upon 405 nm laser excitation) in cytosol. The cells remain viable with normal morphology with up to 50 μM DBT-FAA, suggesting no or very minimal toxicity of the novel fluorescent amino acid. It has high quantum yield (0.74) and is photostable as studied through a series of 100 fluorescence spectra for 5 hours in aqueous condition. These data indicate that DBT-FAA is biocompatible for imaging in live-cell. Being an *L*-α-amino acid, DBT-FAA may be utilized to site-specifically incorporate into peptides and proteins to unravel the biological processes for broader applications in therapeutics and biochemical research. We thank the NIH for funding this project.

B603/P2981

Computational Strategies for the Identification of a Robust Transcriptional Biomarkers Panel to Sense Multi-Stress States in *Bacillus subtilis*

Y. Huang[1], W. Smith[1], C. Harwood[1], N. Sinha[2], A. Wipat[1], J. Bacardit[1]; [1]Newcastle University, Newcastle upon Tyne, UNITED KINGDOM, [2]University of Pennsylvania, Philadelphia, PA.

Objective: One challenge in the engineering of biological systems is to be able to recognise the cellular stress states of bacterial hosts, as these stress states can lead to suboptimal growth and lower yields of target products. Our current knowledge of how different environmental treatments modulate gene regulation and bring about physiology adaptations is limited, and hence it is difficult to determine the mechanisms that lead to their effects. To enable the design of genetic circuits for reporting or mitigating the stress states, our study aimed to identify a relatively reduced set of gene biomarkers that can reliably indicate relevant cellular growth states in bacteria. Methods: We analysed transcriptome data for *Bacillus subtilis* which reveals patterns of gene expression of 269 samples grown in 104 different conditions. We proposed a pipeline of computational methods for the selection of a robust multi-stress states sensing biomarker panel: a) an unsupervised machine learning approach to extract clusters of samples with differentiated gene expression profiles; b) a feature selection model to identify biomarker genes that can predict different cellular growth states; c) a recommendation system incorporating complementary information from machine learning model, gene regulatory network and co-expression network to prioritise a robust biomarker panel with best predicted multi-stress states sensing power.Results: We revealed 10 clusters of samples that can be characterised as different cellular growth states and 949 panels of biomarker genes can indicate the cellular state. We identified a biomarker panel consisting of 10 genes with various functions corresponding to DNA repair, endopeptidase activity, the synthesis of essential proteins, etc. This biomarker panel showed great predicative performance in sensing a variety of stress states from the dataset used for biomarker identification as well as extended stress states from 9 independent datasets (average f1-score achieved at 0.99 and 0.98 respectively).Conclusion: The computational strategies introduced in our study has the potential for recognising biomarkers that show great sensitivity and robustness to a wide range of cellular stress states, enabling the implementation of a stress sensing system that can monitor the growth and stress state for bacteria host in biotechnology applications.
References1. Huang, et al. *Sensors* **2021**, *21*, 2436. https://doi.org/10.3390/s210724362. Huang, et al. *BioRxiv* **2022**. https://doi.org/10.1101/2022.06.20.496894

B604/P2982

The novel PAICE Suite reveals circadian timing of non-coding RNAs and the spliceosome components, indicating multiple levels of clock control in the post-transcriptional regulation of macrophages

S. Buel; Rensselaer Polytechnic, Troy, NY.

Background/ObjectivesThe innate immune system is the body's first line of defense against foreign pathogens. One of the cell types of interest in this system is the macrophage, for its ability to phagocytize and clear the area of pathogens, activate the adaptive immune system, and release cytokines which activate other immune cells. Macrophages are tightly regulated on many levels to ensure appropriate responses to foreign pathogens, with one of these layers being circadian rhythms (Keller 2009). In a circadian time course in *M. musculus* macrophages, Collins et. al (2021) determined

that the clock controls expression of 15% of the transcriptome and 29% of the proteome. However, there is only a 250-gene overlap in the circadian transcripts which are translated into circadian proteins, predicting a high level of post-transcriptional regulation by the clock. Using these comprehensive RNAseq and proteomic data sets (Collins 2021), we aimed to uncover these clock-controlled post-transcriptional mechanisms, particularly in the non-coding transcriptome and the spliceosome pathway.
Methods/ResultsUtilizing the PAICE suite to analyze the RNAseq and proteomics circadian time series data of *M. musculus* macrophages published by Collins et. al (2021), we found that, lncRNAs were enriched in circadian oscillations relative to the detectable non-coding transcriptome. Importantly, LncRNAs can bind to and regulate classes of DNA, RNA, and protein, and control expression and binding of genes (Statello, 2021). Additionally, we observed oscillations in the coding mRNA (89%), non-coding snRNA (15.6%), and proteins (37.5%) of the spliceosome machinery, indicating robustness in the clock's ability to control splicing events, suggesting a further mechanism of circadian post-transcriptional regulation. ENCORE's ontological analysis resolved that the overall proteome displayed damped waveforms, including immune pathways. Joint modeling of the transcriptome and proteome using MOSAIC improved upon ECHO's analyses, identifying more circadian transcripts and proteins than previously reported by Collins et. al (2021).
Conclusions
Using the PAICE suite of programs, we discovered previously unrecognized clock-controlled post-transcriptional regulation mechanisms at the level of the spliceosome and non-coding snRNA and lncRNA in macrophages.

B605/P2983

MinDE bacterial reaction-diffusion system generates synthetic spatiotemporal protein patterns in metazoan cells

R. Rajasekaran, S. Coyle; University of Wisconsin–Madison, Madison, WI.

Cells are molecular systems that use spatiotemporal patterns of biochemical activities to perform complex biological tasks. Thus, artificial systems that generate self-organizing subcellular protein patterns could enable novel cell morphologies, signal processing, or biological activities to be engineered. However, few synthetic biology tools exist that can perform these functions. Here, we repurpose the bacterial Min system as an orthogonal general-purpose spatiotemporal signaling node in metazoan cells. In bacteria, MinD and MinE proteins undergo pole-to-pole membrane oscillations to specify the midpoint of the cell; *in vitro*, these two proteins are sufficient to form a broad range of dynamic and stationary reaction-diffusion patterns on lipid bilayers. We demonstrate that the Min system can self-organize into synthetic oscillations, traveling waves, and stable sub-cellular patterns when expressed in metazoan cells. We have developed analytical workflows to quantitatively characterize reaction-diffusion phenomena across thousands of cells to identify trends that relate biochemical parameters to the frequency, power, and phase structure of patterning behavior. This allowed us to harness the orthogonality of MinDE circuits in metazoan cells to design artificial signaling networks that can interact with and regulate host-cell biology: MinDE circuits that react to host-cell biological processes enable dynamic encoding and amplification of real time cell-state information into MinDE reaction-diffusion behavior; MinDE circuits that regulate host-cell signaling can artificially drive subcellular protein activity to the synthetic patterns we genetically encode. By developing tools that can program dynamic protein distributions and biological activities within the cell, our work will establish a new class of strategies for cellular engineering in metazoans.

B606/P2984

Coordination of p53 and MAPK dynamics controls heterogeneous responses to genotoxic agents in single cells

R. Hanson, **E. Batchelor**; Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis, MN.

Heterogeneous cellular responses to chemotherapy are a significant obstacle in cancer treatment. One source of such heterogeneity is variability in the temporal expression and activity of key signal transduction pathways that detect cell stresses and coordinate appropriate responses in individual cells. We have shown that variable p53 expression dynamics can generate distinct cellular responses to genotoxic agents. However, in some cases distinct stresses can generate the same p53 dynamics but different cell fate outcomes, suggesting integration of dynamic information from other pathways is important for cell fate regulation. We focused on pancreatic cells and quantified the dynamics of p53 and the MAPKs, signaling systems frequently mutated in pancreatic cancer. To determine how MAPK activities affect p53-mediated responses to DNA double strand breaks and oxidative stress, we used time-lapse microscopy to simultaneously track p53 and ERK, JNK, or p38 MAPK activities in single cells. While p53 dynamics were comparable between the stresses, cell fate outcomes were distinct. Combining MAPK dynamics with p53 dynamics was important for distinguishing between the stresses and for generating temporal ordering of downstream cell fate pathways. Cross-talk between MAPKs and p53 controlled the balance between proliferation and cell death. These findings provide insight into how individual cells integrate signaling information from separate pathways with distinct temporal patterns of activity to encode stress specificity and drive heterogeneous cell fate decisions. Furthermore, our results identify timing windows during which combination drug treatments can effectively alter cell fate responses to genotoxic agents.

B607/P2985

Early and late IFNγ-responsive genes decode dynamic IFNγ stimulus in opposing ways in macrophages

B. Naigles[1], A. Narla[2], N. Hao[1]; [1]School of Biological Sciences, University of California, San Diego, La Jolla, CA, [2]Department of Physics, University of California, San Diego, La Jolla, CA.

It is essential for innate immune cells to respond appropriately to pathogens and other pro-inflammatory stimuli so that they exhibit the appropriate functional outcome to a given stimulus and neither under-, over-, or mis-react. One way in which inflammatory stimuli can vary is in their dynamics - that is, the amplitude and duration of stimulus experienced by the cell. In this study, we investigate how macrophages respond to dynamic interferon-gamma (IFNγ) stimulus. *In vivo*, macrophages experience IFNγ stimulus during both the innate and adaptive immune responses, and IFNγ induces pro-inflammatory gene expression and diverse functional outcomes, including the secretion of cytokines and chemokines. We specifically use long-term live-cell imaging in a microfluidic device to investigate how the genes IRF1, CXCL10, and CXCL9 respond to dynamic patterns of IFNγ stimulus. To do this, we use a mouse macrophage cell line with endogenous fluorescent reporters for IRF1, CXCL10, and CXCL9. IRF1 is an early-responding gene encoding a transcription factor for large portions of the downstream pro-inflammatory gene expression response. CXCL10 and CXCL9 are later-responding genes and encode chemokines that recruit T cells to further amplify the immune response. We find that IRF1 responds to low amplitude or short duration stimulus, while CXCL10 and CXCL9 require longer or higher-amplitude

stimulus to be expressed. We also investigate the heterogeneity in each gene, finding that CXCL10 has very high cell-to-cell variability and that this heterogeneity appears to be largely stochastic across all dynamic conditions and does not correlate with response amplitude. CXCL9 and IRF1 are more homogenous. We additionally perturb the response with inhibitors of chromatin regulators and find that inhibiting p300 delays CXCL10 gene expression but does not affect the cell-to-cell variability. We develop an ordinary differential equation model to capture the response of each gene to the dynamic IFNγ stimulus. We conclude that these three genes in the same signaling pathway respond to dynamic IFNγ stimulus with very different response features, including different levels of heterogeneity. This implies that each gene is employing a different strategy to decode dynamic IFNγ stimulus so that they each exhibit appropriate expression for the cell's functional outcomes downstream of each stimulus pattern.

B608/P2986

Local and global control of protein synthesis in a syncytium

A. P. Jalihal[1], C. M. Chai[2], S. Quinodoz[3], A. S. Gladfelter[1]; [1]University of North Carolina, Chapel Hill, NC, [2]HHMI Janelia Research Campus, Ashburn, VA, [3]Princeton University, Princeton, NJ.

Multinucleate "syncytial" cells are found widely across all eukaryotic kingdoms, notably in filamentous fungi and mammalian tissues like placenta and muscle. It is unknown what mechanisms allow nuclei in syncytia to collaboratively build these large and spatially complex cells. Previous work in the Gladfelter lab has demonstrated that neighboring nuclei in the multinucleate fungus *Ashbya gossypii* show high degrees of heterogeneity in gene expression and timing of nuclear division despite sharing a common cytoplasm. This suggests that the cytosol in these cells is composed of compositionally and functionally heterogeneous nuclear "territories" that regulate local cytoplasmic function. However, these locally autonomous nuclei must also coordinate across the cell to ensure integrated mycelial growth. The goal of this work is to dissect the basis of local and global coupling of the cell cycle to growth in a large syncytium.

It is known from prior work on large cells such as neurons that local compositional variability can be achieved via differences in local translation. We investigated the role of spatial variation in protein synthesis in shaping variable cytosolic territories. We first investigated spatial differences in mRNA translation using a fluorescence in situ hybridization (FISH) based approach that monitors translating mRNAs, which revealed heterogeneity in nuclear-proximal translation of cell-cycle transcripts. This indicates local translation is likely an element of asynchronous nuclear division. We hypothesized one basis for this difference in translation may be variable ribosome production between nuclei. Probing ribosome production via metabolic labeling of rRNAs in *Ashbya* revealed a high degree of inter-nuclear variability in rRNA output. Together, these lines of evidence suggest that local differences in protein synthesis may strongly influence local cytosolic composition over the micron scale and these may be accentuated by differences in ribosomal output. We next asked how overall mycelial growth is maintained despite variability in local translation. Mathematical modeling of ribosomal diffusion suggested that hyphal-scale (100s of microns) ribosomal concentrations may be buffered despite local inter-nuclear (5 microns) variation in rRNA production. Overall, this work provides insight into how length-scale dependent cytosolic function can emerge from differences ribosome production and activity in syncytial cells.

B609/P2987

Structure and mechanism of key enzymes involved in*M. tuberculosis*GDP-Heptose biosynthetic pathway: potential drug targets

S. Karan, **A. Saxena, Male**; School of Life Sciences, Jawaharlal Nehru University, New Delhi, INDIA.

D-α-D-Heptose is the fundamental constituent in the S layer or the capsular polysaccharide ofmycobacteria. Incorporation of D-α-D-heptose in arabinogalactan layer is crucial for themaintenance of mycobacterial cell wall. Hence, complete elucidation of the nucleotide activatedD-α-D heptose pathway will be critical to understand the biosynthetic mechanism and willcontribute in drug development against *M. tuberculosis.*The D-α-D heptose in *M. tuberculosis* is produced in four steps, (i) isomerization of S7P to M7P byGmhA isomerase (ii) phosphorylation at C1 position of D-glycero-D-manno- heptose-7 phosphate byHddA kinase (iii) Dephospho -rylation at C7 position of D-glycero-D-manno-heptose-1,7 bisphosphateby GmhB phosphatase and (iv) activation of D-glycero-D-manno-heptose-1 phosphate by HddCguanylyl-transferase to produce GDP-D-glycero-D-manno heptose.In current project, we have identified the substrate binding mechanism and low-resolution structures of allkey enzymes, *Mtb*GmhA, *Mtb*HddA, *Mtb*GmhB involved in this pathway using biochemical, SAXS anddynamics simulation techniques. Based on discovered mechanism, we are developing specific inhibitorsand will test *in vitro* and *in vivo* assays to determine clinical efficacy of these compounds. All theseresults will be presented in the meeting**Representative publications**1. Sumita Karan, Ankita Behl, Amin Sagar, Arkita Bandhopadhyay, **Ajay K. Saxena# (2021).** Structural studies on*Mycobacterium tuberculosis HddA* enzyme using small angle X-ray scattering and dynamics simulation techniques,*Int. J. Biol. Macromol.171:28-36.*2. S. Karan, B. Pratap, Shiv P. Yadav, FNU, Ashish & **Ajay K. Saxena# (2020).** Structural and functionalcharacterization of *M. tuberculosis* sedoheptulose- 7-phosphate isomerase, a critical enzyme involved inlipopolysaccharide biosynthetic pathway.*Nature Sci. Rep. 10(1):20813.*3. Sumita Karan, Bhanu pratap, Ashish, **Ajay K. Saxena# (2019).** Low-resolution SAXS and structural dynamicsanalysis on *M. tuberculosis* GmhB enzyme involved in GDP-heptose biosynthetic pathway.*Int. J. Biol. Macromol. 136: 676-685.*

B610/P2988

Gene expression noise accelerates the evolution of biological oscillators

Y. Lin[1], **N. Buchler**[2]; [1]Information Sciences Group (CCS-3), Los Alamos National Laboratory, Los Alamos, NM, [2]Molecular Biomedical Sciences, North Carolina State University, Raleigh, NC.

Gene expression is a biochemical process, where stochastic binding and un-binding events naturally generate fluctuations and cell-to-cell variability in gene dynamics. These fluctuations typically have destructive consequences for proper biological dynamics and function (e.g., loss of timing and synchrony in biological oscillators). Here, we show that gene expression noise counter-intuitively accelerates the evolution of a biological oscillator and, thus, can impart a benefit to living organisms. We used computer simulations to evolve two mechanistic models of a biological oscillator at different levels of gene expression noise. We first show that gene expression noise induces oscillatory-like dynamics in regions of parameter space that cannot oscillate in the absence of noise. We then demonstrate that these noise-induced oscillations generate a fitness landscape whose gradient robustly and quickly guides evolution by mutation towards robust and self-sustaining oscillation. These results suggest that noise helps dynamical systems evolve or learn new behavior by revealing cryptic dynamic phenotypes outside the bifurcation point.

B611/P2989

Exploring Forces During Hybrid Collective Cell Migration Using Synthetic Magnetic Cells (syMcells)

J. A. De Lora, 1, L. Motyckova, S. Pashapour, P. Fischer, I. Platzman, J. Spatz; Max Planck Institute for Medical Research, Heidelberg, Germany

The functioning of synthetic cells that are designed to execute predetermined biophysical tasks could be directly evaluated by interfacing with natural cells. This biophysical approach would validate built-in operations such as synthetic cell-natural cell adhesion interactions. It follows that hybrid cultures may, in the least confirm task achievement or, at the most trigger unpredicted emergent properties. In this work, a hybrid cell migration system that emulates E-cadherin-mediated cellular adhesions is built while implementing remote control of synthetic cells using magnetic fields. SyMcells are established using an emulsion to create a lipid-derived chassis that encapsulates magnetic cytoplasm comprising an oil-based ferrofluid. SyMcells are then functionalized with recombinant E-cadherin proteins by polyhistidine-Ni-NTA complexation. Finally, syMcells are cultured with natural cells genetically engineered to express E-cadherin-EGFP and within confinement chambers. Upon releasing the hybrid cultures from confinement, hybrid cell migration dynamics are observed by time-lapse, traction force, and monolayer stress microscopy. Parameters such as cell migration velocity, E-cadherin clustering, actin cytoskeleton organization, and traction fields are measured as a function of the presence/manipulation of syMcells. The morphologies of syMcells are actuated during the observation period by applying a homogeneous electromagnetic field from our custom-built microscope. We use this system to investigate the manipulation of hybrid cell migration characteristics including to gauge forces within migrating monolayer hybrid cultures and in the future to control leader *vs* follower phenotypes.

B612/P2990

Establishing measures for 'Innate Morphological Heterogeneity' in cell cultures

P. Pragya, A. Mittal[,1]; IIT Delhi, Delhi, India

Observations of asymmetries within cell populations, and within single cells, with respect to variables such as protein expression(s), cellular polarity (e.g., apical or basolateral) etc. continue to assist in better understanding of architecture-defined-functionalities in living cells. The concept of 'average phenotype' of a cell population, a supposed representative of a cell population based on assumption of the cells being similar (oblivious to deviations as noise/cell-culture-artifacts), is in contrast to an increasing appreciation for existence of heterogeneity amongst cells. This heterogeneity, resulting-from/in-form-of genetic/phenotypic/morphological variations, can be explored using various assays coupled with single-cell imaging techniques in cell cultures. In this work, we explicitly quantitate 'innate morphological heterogeneity' (IMH) in cell cultures by rigorous quantitative analyses of bright field image data, along with image data of fluorescently labelled subcellular organelles/features (e.g., nucleus, plasma membrane, tubulin, actin, lysosomes, endoplasmic reticulum and mitochondria) at a single-cell level for cell cultures of some common cell lines (RAW264.7, HeLa and Hek293T), acquired using a confocal microscope. We report cellular-density-based IMH in three-dimensional (3D) space for adherent cell cultures, i.e., in terms of X-Y, Y-Z and X-Z planes from bright field images. Similarly, we report fluorescence-based IMH at subcellular levels in 3D. We discuss the importance of our findings in the context of apical/basolateral asymmetries, and other aspects of cellular polarities, reported in literature pertaining to cell physiology. Representation of IMH for RAW264.7 cells (and their nuclei), in form of distribution of Average Pixel Values (APVs) per plane for each of the X-Y-Z axes along with Total Pixel

Number (TPN) for each of the octants in a 3D cartesian system, show a remarkable alteration in distributions of pixel intensities and inferred cellular polarities upon synchronization of cell cultures using thymidine and nocodazole treatment. Our results highlight possibly important "diagnostic" applications of IMH in general cell biology, which may not be limited to comparing non-synchronized (regular-passaged) and synchronized cells but also useful in investigations of other environmental/experimental cell culture variations.

B613/P2991

A modular, deep learning-based analysis framework for high resolution 3D timelapse imaging of cells and nuclei in hiPSC colonies

B. Morris, .. Allen Institute for Cell Science, S. M. Rafelski; Allen Institute for Cell Science, Seattle, WA.

The Allen Institute for Cell Science (allencell.org) aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain cellular organization, and how they transition between states during differentiation and disease. To this end, here we aim to characterize and understand how cell and nuclear size and shape behave over the course of a cell cycle. This requires high-resolution 3D movies and accurate determination of the cell and nuclear boundaries via segmentation within the same cell. However, high resolution imaging in 3D (e.g. 100x/1.25 NA) has many limitations such as high levels of phototoxicity and small fields of view that make imaging and tracking cells over long time periods difficult. To overcome these difficulties, we imaged colonies of hiPSCs expressing mTagRFP-t-CAAX (i.e. fluorescently labeled plasma membrane) in 3D for two days at five minute intervals at low magnification and resolution (20x/0.8NA). We developed deep-learning-based image processing modules to obtain accurate 3D cell and nuclear boundaries necessary for shape analysis and tracking. First, our transfer function module is used to generate predicted images of high magnification and resolution (100x/1.25 NA) from 20x images of cells expressing mTagRFP-t-CAAX. Next, a label-free module is employed to predict the nuclear boundary from bright-field images of cells expressing fluorescently tagged lamin B1 (demarcating the nuclear envelope). Then, semantic and instance segmentation modules are used to generate accurate 3D cell and nuclear segmentations, which a tracking module uses to track nuclei and cells over time. Finally, a time-point classifier module is used to identify cell division events. Each step in this pipeline is validated using metrics relevant to the downstream use of the data. For example, if the ultimate use of the segmented tracks is to examine nuclear and cellular shape over time, stepwise validation can consist of comparing the shape descriptors between segmentations of the real and model-predicted images. To make the data processing fully automated, scalable, and easily reproducible, these modules are linked using Snakemake, a Python-based workflow manager. The resulting dataset from a single colony movie consists of tracks of hundreds of cells and nuclei that can be temporally aligned for data analysis.

B614/P2992

Recent Deep Learning Studies for Microfluidics Assays of Yeast Lifespan

H. Qin[1], M. Ghafari[2], W. Dang[3]; [1]University of Tennessee At Chattanooga, Chattanooga, TN, [2]Sanford Burnham Prebys Medical Discovery, San Diego, CA, [3]Baylor College of Medicine, Houston, TX

We wish to highlight our deep learning methods to facilitate the microfluidic-based high throughput assay of the replicative life spans of the budding yeast [1-3]. The budding yeast is a proven model of cellular aging and has revealed many longevity-related genes with conserved roles in eukaryotic cells.

Microfluidics-based assays have become an effective way to monitor the aging process for a large number of cells, resulting in hundreds of time-lapse microscopic images. We are the first groups to demonstrate that the deep learning method can accurately classify cell division events. We developed a maximal likelihood approach to infer the cell family tree and cell lineages. We showed that YOLO and Mask-RCNN are complementary for cell object detections. We will compare our results with two other groups. Overall, it is promising that deep learning methods could potentially transform cellular aging research.

B615/P2993

Endogenous tagging for the intra-cellular cartography of the human proteome

S. Vaid[1], M. Vangipuram[1], K. C. Cheveralls[1], K. Kim[1], H. Kobayashi[1], L. A. Royer[1], M. Mann[2], M. D. Leonetti[1]; [1]Chan Zuckerberg Biohub, San Francisco, CA, [2]Max Planck Institute for Biochemistry, Martinsried, Germany

Elucidating the wiring diagram of the human cell is a central goal of the post-genomic era. Here, we combined fluorescent CRISPR tagging, confocal live-cell imaging, mass spectrometry, and data science to systematically map the localization and interactions of human proteins. Our approach provides a data-driven description of the molecular and spatial networks that organize the proteome. Unsupervised clustering of these networks delineates functional communities that facilitate biological discovery. We found that remarkably precise functional information can be derived from protein localization patterns, which often contain enough information to identify molecular interactions, and that RNA binding proteins form a specific subgroup defined by unique interaction and localization properties. Paired with a fully interactive website (opencell.czbiohub.org), our work constitutes a resource for the quantitative cartography of human cellular organization.

B616/P2994

Nuclear-cytoplasmic compartmentalization of cyclin B1-Cdk1 promotes robust timing ofmitotic events

G. Maryu, Q. Yang; University of Michigan, Ann Arbor, MI

The cyclin-dependent kinase (Cdk1) oscillator is widely characterized in homogenized cytosolic extracts, leaving unclear the impact of nucleocytoplasmic compartmentalization. Here, by developing a Förster (or fluorescence) resonance energy transfer (FRET) biosensor, we track Cdk1 spatiotemporal dynamics in reconstituted nucleus-present and nucleus-absent cells side-by-side and find compartmentalization significantly modulates clock properties previously reported in bulk studies. While nucleus-absent cells display highly tunable frequency, the nucleus-present cells maintain constant frequency against cyclin variations. Despite the high expression variability, all cyclin proteins degrade within the same mitotic duration, further ensuring robust timing. Moreover, Cdk1 and cyclin B1 cycle rigorously out-of-phase, producing wide phase-plane orbits, essential for oscillation robustness. Although homogenized Cdk1 is well-known for delayed spiky activation, we found steady nuclear accumulation of activated cyclin B1-Cdk1 until the nuclear envelope breakdown (NEB) before another abrupt activation to trigger anaphase. Cdk1 biphasic activation and spatial compartmentalization may together coordinate the accurate ordering of different downstream events.

B617/P2995

How to visualize weird multidimensional infection data.

D. Schneider; Stanford University, Stanford, CA

All our our science communications are metaphorical and we limit ourselves to a small set of these metaphors. This helps us communicate, but are we missing something? Would it be useful if we tried new metaphors? Are there some problems that are not solvable using one set of metaphors, but the solution becomes obvious when you look at the problem in a different way? I started using playful artistic ways of describing my data when I felt that my audiences seemed to be missing my point. I find these visualizations useful in a couple of ways: First, they help me think about things differently and can point to answers that aren't obvious from all points of view. Second, they help me talk about my science to people who aren't in my head. I'll be sharing both successful and unsuccessful attempts and creative data visualization and talk about what insight it can provide.

Engineering Tissue and Scaffold Models

B618/P2996

Generating a Perfusable Vascularized Cerebral Organoid Model

E. LaMontagne[1], L. J. Macdougall[2], F. Papes[3], E. L. Teng[1], J. Mayner[1], B. P. Sutherland[4], K. S. Anseth[2], A. M. Kloxin[4], A. R. Muotri[3], A. J. Engler[1]; [1]Bioengineering, University of California, San Diego, La Jolla, CA, [2]Chemical & Biological Engineering, University of Colorado, Boulder, Boulder, CO, [3]Pediatrics, University of California, San Diego, La Jolla, CA, [4]Chemical & Biomolecular Engineering, University of Delaware, Newark, DE.

Although brain organoids have advanced our understanding of the human brain, their growth, cellular complexity, and functionality are limited by the absence of vasculature. Current organoid vascularization efforts rely on either: (1) the *in situ* formation of neo-vessels in endothelial co-cultures, or (2) microfluidics channels that flow adjacent to organoids. Neither produces vasculature that resembles native cerebral vessels. We hypothesize that a system in which patent vessels form throughout the organoid as it matures will enable organoid growth beyond the diffusion limit of oxygen and promote the formation of more complex cerebral structures. To fabricate this system, we first developed a microfluidic device containing posts onto which a fiber scaffold is woven in a lattice resembling the cerebral vascular network. Photodegradable fibers are formed by extruding and crosslinking a solution of two multi-arm polyethylene glycol (PEG) components that contain allyl sulfide to facilitate degradation via radical addition. Brain organoids that we have generated from pluripotent stem cells (PSCs) are to be placed within the woven fiber network when at the neuroprogenitor cell (NPC) stage so that they grow around fibers as they enlarge. Upon radical addition, the polymer scaffold will solubilize and provide the framework for a continuous, hollow lumen that will be seeded with PSC-derived endothelial cells to generate vasculature. The microfluidic channels are perfusable at high flow rates and produce low wall shear stresses, confirmed using computational physics models. Photodegradable PEG fibers can be extruded, woven into a scaffold, and degraded via 365nm, 5mW/cm^2 light in under 5 minutes. All components of the sacrificial scaffold are biocompatible with brain organoids. Future experiments include the fabrication of multielectrode arrays to stimulate and read electrical activity of the organoids, long-term perfusion at varying shear stresses, and single-cell RNA sequencing analysis to evaluate maturation markers. To our knowledge, this will be the first vascularized brain organoid model

with highly integrated vasculature that can be perfused over long periods of time. This will generate opportunities to study neurovascular development, brain cancers, or other neurological conditions affecting vasculature using a completely human and *in vitro* system.

B619/P2997

DNA-programmed tissue patterning and controlled cell-cell interactions on hydrogel substrates

L. S. Prahl, C. M. Porter, A. J. Hughes; University of Pennsylvania, Philadelphia, PA.

Replicating the organizational principles that establish fine scale tissue structure - such as epithelial-mesenchymal interfaces and compartment boundaries - is critical to our understanding of organismal development and our capacity for building functional replacement tissues. Despite the wealth of technologies available to control *in vitro* tissue size, shape, and mechanical environment, fine-scale spatial control of cellular composition remains an engineering challenge. To overcome this challenge, we present a DNA-based cell micropatterning approach on photosensitive hydrogels that can support hierarchical patterning of multiple cell types onto the same substrate with high spatial precision. We photopattern single-stranded DNA (ssDNA) onto benzophenone-polyacrylamide (BP-PA) hydrogels and introduce lipid-ssDNA-labeled cells, which are specifically captured onto gel-bound ssDNA deposits. This means multiple cell types can be efficiently co-patterned onto the same hydrogel in a spatially controlled fashion using different ssDNA sequences. To promote cell adhesion for long-term 2D culture, we find that extracellular matrix proteins can be attached to the BP-PA gel surface using standard hydrogel chemistry without compromising cell capture fidelity. Finally, we apply this approach to study several biological processes: stiffness-dependent fibroblast spreading on ssDNA-patterned hydrogels, spatial and temporal quantification of Ret tyrosine kinase signaling dynamics in migrating epithelial colonies, and the interfacial behaviors of tissues containing multiple micropatterned cell populations over time. This technology therefore enables independent control of cell patterning, adhesion, and mechanical environment, constituting a powerful strategy for programming complex multicellular interactions and interfaces into engineered tissues.

B620/P2998

Production of biodegradable gelatin-elastin cryogels for excellent mechanical properties

E. Bahar[1], B. Reece[1], S. R. Russell[2], D. Oyetibo[3], U. Stevic[1,4], K. M. Myers[2], K. E. Kasza[2], K. E. Kasza[2], K. Kita[1]; [1]Department of Biology, St. Francis College, Brooklyn, NY, [2]Department of Bioengineering, Columbia University, New York, NY, [3]St. Francis College, Brooklyn, NY, [4]Keck School of Medicine, University of Southern California, Los Angeles, CA.

One of the important areas in regenerative medicine is to develop appropriate scaffolds to support the proper growth and differentiation of cells. There are reams of studies reporting the development of scaffolds to support cell growth. However, many of them require relatively complicated procedures for the synthesis. Ideal scaffolds should be easy to manufacture, biodegradable, and still possess excellent biophysical characteristics. Cryogels are porous scaffolds with interconnected pores that allow cell migration as well as an efficient exchange of nutrients inside scaffolds. Although gelatin-based cryogels were reported more than a decade ago, there is not much progress in that particular area since then. We also think that the improvement of biophysical parameters, such as elastic module and tensile strength, would be critical to developing clinically applicable scaffolds, because our body parts are flexible but also undergo mechanical forces. Among several extracellular matrices, we postulated that

the addition of elastin may give better flexibility as well as the stretchability of biodegradable scaffolds. We mixed and poured gelatin and the various concentration of elastin into casting plates after crosslinking with glutaraldehyde to create a smooth, sheet-like cryogel. After freezing at -20°C overnight followed by thawing, the remaining aldehyde was quenched with carbonate buffer containing sodium borohydride. Based on our preliminary measurement, the addition of elastin could increase the tensile strength of cryogels up to four-fold. In contrast, the linear elastic modulus appeared to be in the same range compared to gelatin-only (0% elastin) gel. The previously reported formula, the gelatin-fibrinogen (0.03%) cryogel, showed lower elastic module as well as tensile strength. Thus, it looks promising to utilize elastin as an additional component to develop biodegradable cryogels in regenerative medicine.

B621/P2999

Role of preconditioning with oxygen and glucose deprivation in promoting differentiation of dental pulp stem cells in 3D culture

I. A. Okkelman, W. Vandenberghe, **R. I. Dmitriev**; Ghent University, Ghent, BELGIUM.

Dental pulp stem cells (DPSC) are promising for bone tissue engineering and repair. To support angiogenesis and long-term viability within microfabricated and 3D-printed tissue constructs, they are often grown and differentiated together with endothelial cells in multicellular spheroids. However, this is often associated with the decline of endothelial cells and slow rate of DPSC differentiation towards osteoblasts. Interestingly, hypoxia affects cell viability and at the same time promotes angiogenesis with osteoblast differentiation. Here, we hypothesized that oxygen and glucose deprivation pre-conditioning can improve the process of DPSC differentiation in 3D culture with endothelial HUVEC cells. To do this, we performed oxygen and glucose deprivation experiments with 2D cultures of DPSC and HUVEC and tested effects on cell viability, redox potential and morphology. Interestingly, glucose deprivation did not significantly affect redox state of both HUVEC and DPSC. We therefore preconditioned these cell types with 0% O_2, followed by 24 h reperfusion, and produced heterocellular spheroids. To understand the effects of preconditioning in this 3D culture, we performed fluorescence microscopy analysis of cell death (Sytox Green) together with monitoring of spheroid oxygenation (ratiometric red/near-infrared O_2-sensing nanoparticles) during 7 days of differentiation. We found that cell death was not significantly different in the spheroids from non-differentiated and differentiated groups of cells. However, the periphery-to-core O_2 gradients were significantly lower ($p=0.05$) in non-differentiated hDPSC 21% / HUVEC 0% and hDPSC 0% / HUVEC 0% groups in comparison to hDPSC 21% / HUVEC 21% spheroids. During differentiation, this difference in spheroid oxygenation was slightly ameliorated, keeping the same trend as in non-differentiated spheroids. Thus under the deeper hypoxia, the heterocellular spheroids formed with 0% O_2-preconditioned endothelial cells demonstrated cell viability comparable to spheroids formed from non-preconditioned cells. This data correlates with the results on the alteration of redox potential in adherent (2D) DPSC and HUVEC cultures, where hypoxia-conditioned HUVEC cells demonstrated more profound response. We also found that preconditioned cells displayed stronger accumulation of hydroxyapatite and osteogenic differentiation. Collectively, our preliminary data indicates that hypoxia preconditioning, combined with imaging of oxygenation and cell death, can improve the quality of the spheroid-based ‘tissue building blocks’ in biofabrication.Supported by the Special Research Fund (BOF) grant of Ghent University (BOF/STA/202009/003).

B622/P3000

Automated Neural Network Solver for Cell Biology Applications

C. Lew; Palos Verdes Peninsula High School, Rancho Palos Verdes, CA.

Artificial intelligence (AI) has found increasing applications in cell biology. AI can analyze genetic sequences, predict chemical properties, and predict protein structure. However, AI also has indirect applications. One example is the use of AI to solve math models of biological processes. In cell biology, there are 2D and 3D models for simulating tumor growth, cell transport, cell chemistry, and cell signaling. In order for these models to work, they must be solved using a solver program. Traditional solvers use math methods such as the finite element method and the finite difference method. While traditional solvers have been a boon to cell biology, they have one major drawback: they can only solve a model by stepping forward in time one step at a time. This means that traditional solvers can be slow. For a 3D model at high resolution, a traditional solver can take hours to complete a simulation. The objective of this study is to reduce this time latency by creating an AI-based solver. We hypothesize that AI-solvers can solve 2D and 3D models hundreds of times faster than traditional solvers. Our method for building an AI solver followed multiple steps: we created a graphical user interface that allows users to enter a partial differential equation (PDE), initial conditions, parameters, and boundary conditions; we then created a Python code that automatically discretizes and solves the PDE within a grid; we set the Python code to run numerous random trials to generate a solution set; finally, we fed the dataset into a neural network to train it to understand the PDE. With sufficient training, the neural network will be able to solve the PDE for any time step within its training domain. As a trial run, we used a traditional solver and our AI-based solver to simulate the 2D reaction-diffusion Turing equation for 20 seconds of real time. The traditional solver required 30 seconds, while our AI-based solver required 0.078 second, a 385-fold improvement. We also performed trial runs on Fisher's equation for the spread of invasive cells, an mRNA-sRNA binding model, and the oxygenation of a red blood cell. In all cases, the AI-based solver was faster. We conclude that our AI-based solver will benefit those who use math models for their cell biology research. These researchers can train the AI-solver overnight and have a completed AI-based solver by morning.

B623/P3001

Evidence for intracellular glucose binding to proteins involved in secretion

T. Pramanik, F. Firdos, A. Mittal[,1]; IIT DELHI, Delhi, India

For glucose-stimulated-insulin-secretion (GSIS) by pancreatic β-cells in animals, it is believed that ATP generated from glucose metabolism is primarily responsible for GSIS. However, this ignores possible generation of intracellular ATP from other sources also. Thus, specificity of glucose in GSIS is not attributable to ATP from glucose metabolism alone; in fact, the reason behind this specificity remains undiscovered till date. In our group, transcriptomic analyses of hyperglycemic-exposed-Min6 cells identified upregulation of 3 secretion-specific genes corresponding to proteins KIF11, ATP6V0A4 and CACNB4. KIF11 is a motor protein which is involved in vesicular transport. ATP6V0A4 is required for acidification and maturation of secretory vesicles. CACNB4 is a voltage gated calcium channel subunit involved in β-cell insulin secretion. These had not been previously related, in any way, specifically to GSIS. Thus, we specifically asked whether these 3 proteins, obviously involved in insulin secretion, had any possible direct interaction with intracellular (but un-metabolized) glucose. Using AutoDock Tools 4.2.6, glucose was investigated as a possible ligand for the above 3 proteins. Glucose metabolites

involved in glycolysis (namely G6P, F6P, F-1,6-BP), and other epimers of glucose (Mannose and Galactose; aldohexoses - Altrose, Allose, Gulose, Idose, Talose; ketohexoses - Fructose, Psicose, Tagatose and Sorbose) as possible ligands were also investigated. Remarkably, glucose showed a statistically significant better score (in Kcal/mol) among all metabolites and hexoses ($C_6H_{12}0_6$'s) with all 3 proteins. Further, the glucose-affinity/binding scores with the 3 proteins were also compared with scores (in Kcal/mol) for glucose as a ligand for positive controls (proteins known to interact with glucose - GCK and GLUT2) along with negative controls (proteins known not to interact with glucose - RPA1, KU70-80, POLA1, ACAA1A, POLR1A). Again, remarkably, binding affinity scores of glucose molecules for KIF11, ATP6V0A4 and CACNB4 were found to be closer to positive controls with statistically significant higher scores compared to negative controls. Therefore, while reporting strong evidence for glucose binding ability of 3 secretion-related proteins in GSIS, we provide the first ever evidence of a possibly direct role of intracellular glucose molecules in GSIS.

B624/P3002

Imaging-ready model systems to study physical factors contributing to pre-metastatic niches

J. Flournoy, O. Perry, E. Chen, J. Michel, D. Yan, M. Brock, F. Housseau, Y. Chen; Johns Hopkins University, Baltimore, MD.

Metastases are the leading cause of cancer death and accounts for 90% of all cancer deaths. Metastasis occurs when cells disseminate from the primary tumor and colonize distant organs. Recent evidence showed prior to colonization, the tissue in the distant organs undergo compositional changes in cell population and structural changes in extracellular matrix (ECM) to form the pre-metastatic niche (PreMN). However, the cause that triggers such changes is unknown. We aim to dissect the process of PreMN formation and identify the key players for clinical intervention. Given significantly increased risk of death in patients with comorbidity of secondary lung cancer and muco-obstructive pulmonary diseases such as cystic fibrosis (CF), idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disorder (COPD), we hypothesize that high viscosity of the abnormal mucus contributes to the formation of PreMN in the lung tissue. Indeed, we have observed that mesenchymal cells that are activated by elevated viscosity, exhibit phenotypes similar to carcinoma-associated fibroblasts (CAFs). CAFs are known to remodel ECM and secrete tumor-promoting cytokines, thus enabling tumor progression. It is likely that the viscosity-activated fibroblasts in the distant organ behave similarly and form PreMN to accommodate the invasion of disseminated tumor cells (DTCs). It is ideal to observe the PreMN formation longitudinally by documenting cellular behaviors with detailed dynamic and spatial resolution. Therefore, we use a 3D biofabricated microphysiological model in mimicry of human tissues and an *ex vivo* model, both allowing multispectral live cell imaging. Our microphysiological model consists of melanoma-bearing skin mimetic tissue, blood vessels, and lung-mimetic tissue cultured in viscous mucus similar to that of IPF. During biofabrication, the mechanical properties representative of each tissue are recapitulated. For the *ex vivo* model, we use precision-cut lung slices (PCLS) from mice bearing Lewis Lung Carcinoma (LLC). Both the microphysiological system and PCLS are imaging-friendly. The features, such as ECM organization, cell morphological changes, cell migration and proliferation, are extracted from the 4D image data. We also examine the model systems using micro-CT, so that the changes in the tissue can be interrogated using techniques developed in radiomics. The result of micro-CT radiomics can provide valuable insight to screening PreMN formation thus granting the possibility of intervention before metastasis even occurs.

B625/P3003

Enhanced small green fluorescent proteins as a multisensing platform for biosensor development

G. Liang[1,2,3,4], **C. Lai**[1,2,3], Z. Yue[1,2,3,5], H. Zhang[1,2,3], D. Li[1,2,3], Z. Chen[6], X. Lu[6], L. Tao[1,2,3], F. Subach[7], K. Piatkevich[1,2,3]; [1]Westlake University, Hangzhou, China, [2]Westlake Laboratory of Life Sciences and Biomedicine, Hangzhou, China, [3]Institute of Basic Medical Sciences, Westlake Institute for Advanced Study, Hangzhou, China, [4]The Hong Kong Polytechnic University, Hong Kong, China, [5]Xi'an Jioa Tong University, Xi'an, China, [6]Key Laboratory of Precise Synthesis of Functional Molecules of Zhejiang Province, School of Science, Instrumentation and Service Center for Molecular Sciences, Westlake University, Hangzhou, China, [7]Complex of NBICS Technologies, National Research Center "Kurchatov Institute", Moscow, Russian Federation

Engineered light, oxygen, and voltage (LOV)-based proteins are able to fluoresce without oxygen requirement due to the autocatalytic incorporation of exogenous flavin as a chromophore thus allowing for live cell imaging under hypoxic and anaerobic conditions. They were also discovered to have high sensitivity to transition metal ions and physiological flavin derivatives. These properties make flavin-binding fluorescent proteins (FPs) a perspective platform for biosensor development. However, brightness of currently available flavin-binding FPs is limited compared to GFP-like FPs creating a need for their further enhancement and optimization. In this study, we applied a directed molecular evolution approach to develop a pair of flavin-binding FPs, named miniGFP1 and miniGFP2. The miniGFP proteins are characterized by cyan-green fluorescence with excitation/emission maxima at 450/499 nm and a molecular size of ~13 kDa. We carried out systematic benchmarking of miniGFPs in Escherichia coli and cultured mammalian cells against spectrally similar FPs including GFP-like FP, bilirubin-binding FP, and bright flavin-binding FPs. The miniGFPs proteins exhibited improved photochemical properties compared to other flavin-binding FPs enabling long-term live cell imaging. We demonstrated the utility of miniGFPs for live cell imaging in bacterial culture under anaerobic conditions and in CHO cells under hypoxia. The miniGFPs' fluorescence was highly sensitive to Cu(II) ions in solution with Kd values of 67 and 68 nM for miniGFP1 and miniGFP2, respectively. We also observed fluorescence quenching of miniGFPs by the reduced form of Cu(I) suggesting its potential application as an optical indicator for Cu(I) and Cu(II). In addition, miniGFPs showed the ability to selectively bind exogenous flavin mononucleotide demonstrating a potential for utilization as a selective fluorescent flavin indicator. Altogether, miniGFPs can serve as a multisensing platform for fluorescence biosensor development for in vitro and in-cell applications.

B626/P3004

Understanding the Molecular Mechanisms Regulating Cell Growth and Protein Production in CHO cells to Improve the Performance of CHO-Based Bioengineering

R. Singh, N. Kumar; Translational Health Science And Technology Institute (THSTI), Haryana, India

Chinese Hamster Ovary (CHO) cells are the most preferred host cellular machinery for industrial-scale production of modern-day protein bio-therapeutics. Currently, biphasic culture, where cells, once achieve requisite cell density in culture, are treated with sodium butyrate (NaB) to reduce cell growth and improve cell-specific productivity, is among the most commonly used methods to improve the yield of biotherapeutics from CHO-based cultures. However, the cost of such bio-therapeutics still remains high requiring improvement in the performance of the bioprocess to make them affordable. And, knowledge of the molecular mechanisms by which NaB improves cell-specific productivity is the only key

to achieving the objective. It is known that plasma cells are the natural and professional antibody-producing cells. During B-cell development, fast-grower but low-producer pre-B cells differentiate into non-growing but high-producer mature plasma cells. This B-cell development is associated with the expression of a cascade of molecules, each of which plays a key role in deciding the fate of the cell. The timing of the appearance of each of these molecules may be critical. Therefore, in this investigation, we mimicked different stages of the B-cell development process in CHO cells grown using gradually increasing concentrations (0, 0.125, 0.25, 0.5, 1, 2, 4 & 8mM) of NaB. The cell growth-arrest was observed at ≥0.5mM NaB ($p < 0.05$) and viability decreased gradually with increasing NaB concentration (2mM to 8mM) 24hr post-treatment. The increased mitochondria and ER content and decreased nucleus content were observed using flow cytometry following 0.5-1mM NaB treatment, a concentration known to cause growth arrest and improve productivity. The expression analysis of 10 genes that are well-known in the literature to regulate plasma cell differentiation and maturation enabled the identification of potential targets that may play a critical role in the regulation of cell growth and productivity. The untargeted intracellular metabolite analysis also enabled the identification of metabolites potentially involved in the regulation of cell growth (such as adenine, dihydroxyuracil, deoxycarnitine), cell-death (such as glutathione, spermine, spermidine) and productivity (such as acetate phenylalanine, glutamine, citrulline). The work is ongoing to validate the cell growth, viability- and/or productivity-associated targets in order to improve the CHO cell performances for recombinant protein production in bioprocess.

B627/P3005

Transfection of 3D bioprinted human skeletal muscle tissue to assess innate immune responses to mRNA vaccines

L. H. Brunner[1], H. Soliman[2], E. Miller[1], T. Gales[3], M. Maglaty[3], J.-L. Klein[3], K. M. Wisdom[3], A. S. Pitek[1], G. Maruggi[1], K. Luisi[1], A.-M. Steff[1], S. J. Wadsworth[2], J. McAuliffe[1]; [1]GSK- Rockville Center for Vaccines Research, Rockville, MD, [2]Aspect Biosystems, Vancouver, BC, Canada, [3]GSK- Upper Providence, Collegeville, PA

Human 3D *in vitro* models have been used for improving predictions of the safety and efficacy of small molecule medicines for years. These models have been shown to better mimic the physiological complexity and function of native human tissue compared to 2D or animal models. The use of appropriate 3D *in vitro* models for evaluation of vaccine candidates could similarly inform vaccine development and advancement into the clinic. To this end, a skeletal muscle model for studying intramuscularly administered vaccine candidates was developed. A unique microfluidic 3D bioprinting technology was used to manufacture skeletal muscle tissues consisting of primary human myoblasts printed within multi-layer fibers, developed using composite biomaterials, resulting in aligned, multinucleated and differentiated myotubes that resemble native human myobundle architecture, as confirmed by histological staining. Importantly for studying antigen expression upon administration of vaccine candidates, these printed microtissues were shown to be transfectable with self-amplifying mRNA (SAM) vaccines formulated in lipid nanoparticle (LNP) delivery vehicles. The bioprinted tissues not only expressed the antigens encoded by the SAM-LNP vaccines, but they also responded with the production of cytokines and chemokines, including IFN-beta, IFN-gamma, IL-6, TNF-alpha, G-CSF, and IP-10. These cytokines and chemokines are known to play a key role in human innate immune responses. Some of these cytokines have been detected in clinical trial participants receiving mRNA vaccines and may be indicators of reactogenicity to vaccines. To improve the translational relevance of these models,

experimental data will need to demonstrate a correlation between *in vitro* data from this model and clinical observations. Overall, our results indicate that these 3D bioprinted skeletal muscle microtissues may serve as a physiologically relevant *in vitro* model for studying the response to vaccine candidates administered through the intramuscular route.

B628/P3006

Towards a quantitative understanding of mitotic waves in *Xenopus laevis* extracts

O. Puls, Q. Yang; University of Michigan, Ann Arbor, MI

Over the years, various models were developed to capture the mechanism behind the mitotic clock. In short, various checkpoints controlled by the activity of cyclinB-Cdk1 direct the cell through a series of steps which define one mitotic cycle. The regulation of these processes facilitates the longevity of multicellular organisms by enforcing a regular, clock-like timing of mitotic events. When a collection of these oscillators couple, they synchronize. In particular, in various systems—e.g. *Drosophila* and *Xenopus*—early embryogenesis is marked by a series of synchronous cell divisions across the length of the embryo. The size of these embryos implies a faster coordinating effect than diffusion alone. Work in the field proposes a mechanism for such spatial coordination in the form of mitotic waves. However, this literature largely focuses, and in some sense is dependent on, the role of nuclei acting as pacemakers. Here, we explore these waves directly and more broadly. Using *Xenopus* extracts and a Cdk1 FRET sensor, we exploit a novel approach utilizing metaphase-arrested extracts, producing one-dimensional directed mitotic waves without reconstituted nuclei. With this setup, we can probe the possible differences between mitotic waves in systems with and without nuclei, finding the speed of the former to be significantly slower. We observe the frequency of the latter system to consistently lag behind the former, possibly explaining this speed disparity. Further, we show the slowing is primarily due to a lengthening of the mitotic phase. Finally, we also investigate undirected waves with and without nuclei, primarily focusing on the possible transition from sweep-like to trigger-like waves as period slows. In total, we display a unique method for exploring biochemical waves and offer insight into their properties.

B629/P3007

Probing cell-cell communication during neutrophil swarming

J. Strickland; University of California, San Francisco, San Francisco, CA

As the most abundant white blood cell in the body, neutrophils are able to rapidly enter tissues in large numbers when responding to tissue injury or infection. Instead of seeking out these sites of damage alone however, neutrophils enhance their own recruitment in a process known as neutrophil swarming. This mechanism provides for a strong positive feedback system in the early immune response that can help rapidly deploy cells to an area while infections and injuries might still be easy to contain. When neutrophils begin to overwhelm other immune regulatory systems however, the swarming mechanism can also contribute to a number of inflammatory diseases by creating excessive tissue damage and inflammation. While some of the key signals of this process have been identified such as the pro inflammatory lipid LTB4, the molecular logic of the process is still poorly understood. For instance, what signals initiate the signal amplification process? How exactly are these signals then amplified to reach a large radius of cells? To address these questions, I propose a set of in-vitro experiments to isolate and precisely perturb the signaling mechanisms of neutrophil swarming. This approach will have direct

relevance for key risk factors associated with atherosclerosis such as endotoxemia and subsequent neutrophil-mediated plaque destabilization events. By understanding the underlying mechanisms neutrophils use to amplify their own inflammation, this work will help build a foundation for new therapies aimed at preventing excess inflammation while still maintaining neutrophil's host defense abilities.

B630/P3008

Characterization of functional activity of forebrain organoids using longitudinal multiphoton calcium imaging

P. Harary[1], R. Kurzion[2], K. Mensah-Brown[3], D. Jgamadze[1], H. Song[2], G. Ming[2], H. Chen[1]; [1]Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [2]Neuroscience, University of Pennsylvania, Philadelphia, PA, [3]Neurosurgery, Mayo Clinic Rochester, Rochester, MN.

Understanding the baseline functional activity of brain organoids is highly relevant to their use as a tool to study neurological conditions as well as a substrate for transplantation-based therapeutic applications. Although a small number of previous studies have assessed synchronized activity in organoids, the significant heterogeneity inherent to brain organoids and diffusion limitations have impaired precise, long-term characterization of functional activity. Here, the genetically encoded calcium indicator GCaMP8m and a custom-designed culture plate were used to perform longitudinal multiphoton imaging of forebrain organoids. Organoids were grown from an induced pluripotent stem cellline derived from a healthy volunteer. At differentiation day (dd) 40, the organoids were sliced at a thickness of 500µm, with repeated slicing every 30 days. Viral transduction was performed on dd45 with AAV expressing GCaMP8m under a human synapsin promoter. Neural activity at two different depths within the organoids was assessed up to dd180. Analysis of the videos was performed using the Mesmerize platform for motion correction and region of interest (ROI) selection followed by Python scripts for analysis. Firing rates were found to differ significantly across both age and depth with older and deeper regions demonstrating increased firing rate ($p<0.05$). The level of Pearson correlation also varied across both age and depth, with shallower regions in more mature organoids showing the highest correlation. This platform enables investigation of neural activity throughout organoid maturation and uniquely allows for repeated recordings from the same organoid with high-resolution imaging. Additionally, multiphoton calcium imaging makes it possible to reliably image neural activity at depths up to 800µm beneath the surface from ROIs on the same imaging plane. Full characterization of the functional connectome of intrinsic organoid activity may allow for comparison with in vivo data to assess the strength of organoids as models of human neurodevelopment and repair substrates.

B631/P3009

SmartSpheroPlates - A Novel High-Throughput 3D *In Vitro* Assay for the Formation of Reproducible Anchored Spheroids

S. F. Robben[1,2], P. Davidson[1], R. S. R. Robeiro[1], M. Le Berre[1], C. Cerveau[1], P. Thiebaud[1]; [1]4Dcell, Paris, France, [2]KU Leuven, Leuven, Belgium

Spheroids are one of the most used and versatile *in vitro* models enabling research on cells organised in 3D. However, conventional methods for the formation of spheroids present shortcomings related to the variability in spheroid size and the lack of reproducibility. Additionally, it is difficult for researchers to keep track of individual spheroids over time as simple manipulations, such as medium exchange, can

cause spheroids to be aspirated together with the medium. To overcome these issues, a high throughput assay was developed using a PEG based microstructured hydrogel allowing for the formation of homogeneous spheroids with reproducible size and shape which are fixed to a specific location. The hydrogel structure, containing between 19 and 91 microwells per gel, provides a microenvironment that enables the cells to sediment and self-assemble into spheroids within hours. The presence of a small contact point allows the spheroids to attach to the glass bottom on which the gels are made, preventing spheroid loss during manipulations. Due to the design of the microwells and the optical transparency of the hydrogel, the assay is completely compatible with conventional microscopy techniques and spheroid growth can be tracked over time. Experiments using mesenchymal stem cells showed that the relation between the number of seeded cells and spheroid diameter follows a cubic trend. The standard deviation of the diameter of different spheroids, measured after 10 days is of 1.94%. It was also observed that due to the presence of anchoring points, less than 12% of the spheroids were lost during exaggerated medium exchange conditions compared to 86% of spheroids in similar conditions without a contact point. Additionally, the structural composition of the PEG-based hydrogel enables the diffusion of nutrients all around the spheroids, allowing them to be kept in culture for up to six weeks. These experiments showcase the potential of the assay to conduct research on spheroids with higher precision and control at a higher throughput. This overcomes most shortcomings of the conventional spheroid formation methods, and the simplicity of this tool makes it highly accessible to study fundamental research questions or to speed up drug screening and toxicology studies.

B632/P3010

Shallow-angle illumination with low-magnification imaging reveals micro-scale changes in scaffold surface morphology that can promote epithelial cell barrier formation

C. F. Lewallen[1], J. T. Montford[1], A. Maminishkis[2], K. Bharti[1]; [1]Ocular Stem Cell and Translational Research Section, Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD, [2]Translational Research CORE, Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD

Retinal pigment epithelium (RPE) is located in the back of the eye, which forms a polarized barrier and helps maintain the health and function of the distal retina. Many degenerative eye diseases can benefit from RPE transplantation to damaged eye regions to restore sight or halt disease progression. We focus on RPE monolayers grown on poly lactic-co-glycolic acid (PLGA) substrate to refine surgical delivery options. PLGA offers a strong substrate for surgical delivery and a biodegradable option to remove artificial substrates when RPE is introduced into the eye environment. Towards this goal, it is essential to quantify how the growth substrate (PLGA) architecture impacts key cell health indicators such as trans-epithelial resistance (TER); an indicator of RPE barrier integrity. Consequently, we leveraged common, low-angle lighting techniques to exaggerate the surface features of PLGA scaffolds under low-magnification microscopy and then assessed the TER of cultured RPE, on these same scaffolds, over the duration of 5 weeks. In this paper, we show that scaffolds with less than 1.6 skewness (Ssk) and less than 4 kurtosis (Sku) provided the RPE monolayer a better growth substrate, which allowed for a better quality RPE monolayer and higher resistance. These data provide a simple, yet novel method for identifying optimal PLGA scaffold morphology, prior to cell culture for future therapeutic use.

Germ Cells, Gametogenesis, and Fertilization

B634/P3011

Regulated Secretion Mediates Yeast Cell Fusion

U. Machi[1], E. Mazur[1], A. Sundlof[1], J. Smith[2], M. Rose[1]; [1]Biology, Georgetown University, Washington, DC, [2]Biology, Stetson University, DeLand, FL.

Cell-cell fusion is a critical event in many systems, including muscle development, fertilization, and immune response. In yeast mating, partner cells must first degrade the intervening cell wall material before plasma membrane fusion can be achieved. Cell wall removal is carried out by secreted remodeling enzymes such as glucanases, mannanases and chitinases. The action of these enzymes must be tightly controlled, as ectopic cell wall degradation causes cell lysis. Yeast cell fusion requires continuous secretion, as revealed by mutations in the standard secretory pathway. However, electron microscopy demonstrated the appearance of mating-specific vesicles in pheromone-responding cells and at the Zone of Cell Fusion (ZCF) in prezygotes. The vesicles dissipate after fusion is complete. The behavior of the vesicles appears to be regulated by the fusion proteins Fus1p, Fus2p, Rvs161p, and Cdc42p. Deletion of *FUS1* prevents vesicles from localizing to the ZCF; mutations of *FUS2, RVS161 or CDC42* appear to block fusion with the plasma membrane. All lead to defects in subsequent cell wall degradation, but do not show evidence of defects in general secretion. To study the possible role of a mating-specific regulated secretion pathway in yeast, we have developed a Fluorogen Activated Protein (FAP) assay to monitor secretion. The FAP assay works by fusing the FAP to a protein of interest and exposing the cell to a membrane-impermeable fluorogen. When the FAP binds the fluorogen, a fluorescent signal is emitted, allowing for visualization of protein secretion. We FAP-tagged Scw4p, Gas1p, and Scw10p, three pheromone-regulated glucanases, and monitored their secretion. We observed that Scw4p and Gas1p are secreted in a pulse to the ZCF in a Fus2p-dependent manner, whereas Scw10p secretion slowly accumulates and is Fus2p-independent. Interestingly, both *SCW4* and *GAS1* are downregulated in mating, in contrast to upregulation of *SCW10*. We conclude that Scw4p and Gas1p are preloaded into the mating-specific vesicles and specifically secreted during conjugation to help effect cell wall removal.

B635/P3012

Investigating changes in proteasome complex composition during budding yeast meiosis

M. C. Reyes[1], J. A. Cheng[1], E. Doron-Mandel[2], J. Kim Kim[2], M. Jovanovic[2], G. A. Brar[1]; [1]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, [2]Department of Biological Sciences, Columbia University, New York City, NY.

Protein complexes are essential for the proper execution of many cellular processes, including meiosis, the process in which diploid cells transform into haploid gametes. Our previous study leveraged parallel global measurements of translation and protein levels during meiosis and found that meiotic *Saccharomyces cerevisiae* synthesize the members of most protein complexes imprecisely. The protein levels of these complex members are post-translationally adjusted through degradation to achieve precise matching throughout meiosis. An interesting outlier to this high protein-level matching is the 26S proteasome, which is responsible for regulated degradation of ubiquitin-labeled proteins in the cell. The 26S proteasomes consists of two components: the 19S regulatory particle (RP), which is the best studied activator of proteasome and the 20S core particle (CP), in which proteolysis occurs. During late meiotic

stages, our data indicate that the RP is selectively degraded 2-3 hours before degradation of the CP, concurrent with an increase of the alternative CP activator Blm10. These findings suggest unanticipated stage-specific modulation of proteasome activity during meiosis. We performed co-immunoprecipitation on the CP over a meiotic time course and found that in late meiotic stages, there is a greater increase in Blm10-CP association compared to RP-CP association. The consequences of an increasing a Blm10-associated proteasome pool during specific meiotic stages remain mysterious. To further elucidate the role of Blm10 during late meiotic stages, we performed label free quantification (LFQ) mass spectrometry on wildtype and Blm10 deletion mutants. From these data, we have identified potential direct degradation targets of Blm10-proteasomes, and evidence that the absence of Blm10 delays timely completion of meiosis. In conclusion, we have defined a natural change in proteasome complex composition during meiosis and have begun to characterize functional roles for the late meiotic increase in Blm10-proteasomes.

B636/P3013

Role of LOTUS-domain proteins in *C. elegans* germline development

B. Radhakrishna Pillai, P. Czermak, J. Bindics, S. Saha; Institute of Molecular Biotechnology, Vienna BioCenter, Vienna, AUSTRIA.

Germ cells ensure the continuance of life by giving rise to totipotent zygotes. A conserved feature of germ cells is the presence of perinuclear non-membrane-bound condensates of proteins and RNA, called 'Nuage'. Mutations in nuage proteins result in teratomas and cause infertility. Among conserved components of nuage are the ATP-dependent RNA helicase 'Vasa' protein, LOTUS domain proteins, Tudor domain proteins, and argonauts. The molecular mechanisms underlying the functions of LOTUS-domain proteins in the germline remains poorly understood. In the nematode worm *C. elegans,* nuage is proposed to contain a collection of condensates – P granules, Z granules, SIMR foci and mutator foci. P granules contain two LOTUS-domain proteins MIP-1 and MIP-2, mutations of which reduce the fecundity of animals. MIP-1 and MIP-2 each contain two LOTUS domains (hereafter called L1 and L2) in addition to two intrinsically disordered regions. Recent studies have identified a role of these intrinsically disordered regions in mediating the association of P granules with nuclear pores. However, the role of the two LOTUS domains in MIP proteins remains unclear. Studies in Drosophila have shown that LOTUS domains can bind the Vasa protein and stimulate its RNA-dependent ATPase activity. Here, we show that in *C. elegans*, MIP-1 stimulates the RNA-dependent ATPase activity of the Vasa orthologue GLH-1. We also show that in vivo, L2 cannot complement for L1 function. We speculate that the LOTUS domains of MIP proteins have distinct roles in supporting the integrity and function of P granules.

B637/P3014

Cell Biological Analysis of Mouse Cryptorchidism Using *in vitro* Culture System

S. Tone[1], T. Nakatsuka[2], K. Hirata[2], S. Ohira[2], R. Hara[2], A. Kuriyama[1], A. Nagai[2]; [1]Graduate School of Science and Engineering, Tokyo Denki University, Hatoyama, Saitama, JAPAN, [2]Department of Urology, Kawasaki Medical School, Okayama, JAPAN.

Cryptorchidism is one of the most common abnormalities of male sexual development, and is characterized by the failure of the testis to descend into the scrotum. Despite extensive studies of cryptorchidism over the past century, the mechanisms for temperature-induced germ-cell loss are not well understood. All of the main cell types in the testis are believed to be affected by the elevated testis

temperature induced by cryptorchidism. The suitable temperature (about 34 °C) in the special environment of the scrotum is required for maintaining optional conditions for normal spermatogenesis. Many studies reported that experimentally induced cryptorchidism in mice caused germ cell apoptosis and suppressed spermatogenesis. However, other factors such as hormones, immunological circumstances or oxygen concentration must also be examined for cryptorchidism. To explore the mechanism for cryptorchidism, *in vitro* cultures of testes have been used, but complete spermatogenesis using *in vitro* methods has not been accomplished until 2011. In 2011, Sato *et al*. (Nature, 471, 504-507) reported the in vitro production of functional sperm in cultured neonatal mouse testes. Using this *in vitro* system, for the first time, we report that spermatogenesis was abrogated at 37 °C, in accordance with *in vivo* surgery-mediated cryptorchidism, while spermatogenesis proceeded at 34 °C in cultured testes. This result clearly showed that temperature is the sole determinant of cryptorchidism. Moreover, we found that spermatogenesis was arrested before early spermatocytes at 37 °C. While DNA synthesis could be detected in some germ cells at 34 °C in cultured testes, newly DNA synthesis could be hardly detectable at 37 °C. But after switching from 37 to 34 °C in culture, DNA synthesis was resumed within 24 hrs. In conclusion, using our *in vitro* system, we have demonstrated that (1) temperature is the determining factor for cryptorchidism, and (2) higher temperature (37 °C) suppresses DNA synthesis in spermatogenesis.

B638/P3015

Generation of functional primordial germ cells from embryonic stem cells in rats

M. Oikawa[1], M. Hirabayashi[2], T. Kobayashi[1,2]; [1]Institute of Medical Science, University of Tokyo, Tokyo, JAPAN, [2]National Institute for Physiological Sciences, Okazaki, JAPAN.

Primordial germ cells (PGCs), the precursors of sperm and eggs, are emerged from pre-gastrulating epiblast. It is important to understand PGC specification for the fundament of cell fate decisions, reproductive medicine and animal breeding. However, the fewness of PGCs (~40 in mice) and limited access to peri-implantation embryos had hindered to understand the process. To overcome these problems, *in vitro* system to induce PGC-like cells (PGCLCs) from pluripotent stem cells (PSCs) has been developed. This robust and scalable system allowed us to analyze PGC specification in several mammalian species. However, PGCLCs with function to produce fertile gametes had been only developed in mice. Here, we aim for the induction of functional PGCLCs from rat PSCs. We generated *Nanos3-T2A-tdTomato* (N3T) reporter rats to monitor the expression *Nanos3*, a marker specifically expressed in PGCs. We established embryonic stem cells (ESCs) from blastocysts of N3T reporter and used them to induce rat PGCLCs (rPGCLCs). Rat ESCs firstly differentiated into epiblast-like cells (EpiLCs) via floating aggregates, although adherent monolayer condition used in mouse ESCs did not work. Next, we transferred EpiLC-aggregates into the medium with BMP4, a critical cytokine for PGC fate. Within 2 days, N3T expression was started and almost maximized at day 2-3 in a part of cell aggregates. We also analyzed transcriptome of rPGCLCs and found their gene expression profiles was similar to migratory rPGCs. We next reconstructed gonads by rPGCLCs and gonadal somatic cells and observed maturation of rPGCLCs into gonadal stage PGCs with epigenetic reprogramming. Finally, we transplanted rPGCLCs into the seminiferous tubule of germline-less *Prdm14*-deficient rats to test their gametogenic capacity. After 10-12 weeks, spermatogenesis was found in rPGCLCs-transferred testis. Notably, rPGCLC-derived sperm/spermatids lead to birth of viable offspring via microinjection. These offspring appeared healthy and grow up to fertile adults for both sexes. In conclusion, we successfully generate functional PGCLCs from PSCs in rats, which is the first demonstration of fully functional non-mouse PGCLCs. We believe our

system will contribute to understanding fundamental mechanisms in pluripotency and germline development and help establish broad applicability for *in vitro* gametogenesis in various mammals.

B639/P3016

Precision reproductive genetics: pinpointing the effects of a maternally-inherited *Kif18a* variant on oocyte meiosis and aneuploidy

L. Biswas[1], M. Aboelenain[1,2], S. Sun[1], J. Xing[1], K. Schindler[1]; [1]Department of Genetics, Rutgers University, New Brunswick, Piscataway, NJ, [2]Theriogenology, Mansoura University, Mansoura, EGYPT.

A quarter of all pregnancies end in miscarriage; half of miscarriages are caused by chromosomal chromosome number imbalance, termed "aneuploidy." Aneuploidy most often arises in the egg and is almost always incompatible with life. Maternal age at ovulation is currently the only predictor of a given egg's risk of aneuploidy. However, for some individuals, egg aneuploidy occurs more often and earlier than would be predicted by maternal age. We hypothesized that genetic variants might directly cause aneuploidy in individuals for whom maternal age is an insufficient predictor. To test this hypothesis, we leveraged our novel biobank of whole-exome sequence data from infertility patients with extreme rates of aneuploid embryo conception relative to maternal age. From these sequence data, we identified a genetic variant enriched in patients with disproportionately high rates of embryonic aneuploidy, *KIF18A*^{MDM}. We found that overexpression of *KIF18A*^{MDM} causes dysmorphic meiotic spindles and increased aneuploidy in mouse oocytes. To validate our overexpression experiments, we generated a new knock-in mouse line bearing this variant, *Kif18a*^{MDM}. Eggs from *Kif18a*^{MDM} mice are more frequently aneuploid than those from wild-type counterparts and have unique morphological phenotypes, including micronuclei and fragmented cells. These studies lay the groundwork for further studies of *Kif18a*^{MDM}'s possible role as a maternal genetic variant leading to increased egg aneuploidy relative to maternal age.

B640/P3017

Expression of Piwi family and their interaction with genes regulating ovarian follicle development

Y. Kim[1], **S. Ku**[2]; [1]Seoul National University Hospital, Seoul, KOREA, REPUBLIC OF, [2]Department of Obstetrics and Gynecology, Seoul National University Hospital, Seoul, KOREA, REPUBLIC OF.

An ovarian follicle is a unit of interaction with hormones by surrounding somatic cells for the maturation of the oocyte. Among the follicles, only one follicle is selected as the dominant follicle and develops into a mature follicle and this ability decreases naturally with age and leads to infertility. For the preservation of fertility, uncovering molecular regulators of follicular development has been reported using advanced transcriptomics. The correlation of Piwil2 with miRs was reported by our group (Kim et al., Int J Mol Sci., 2021). Based on these results, we analyzed the expression of the Piwi family on the maturation of ovarian follicles. Ovaries of 2-week- and 16-week-old mice were surgically retrieved, and single follicles were isolated. Pre-antral follicles of 100~120 μm in diameter were seeded in each media droplet. After 4 days of *in vitro* culture, the mimics and inhibitors of the Piwi family were transfected, then the follicles were further cultured until they reached the maturation stage. The expressions of Piwi and target genes were analyzed and their effects on follicle maturation were evaluated via the assessment of follicular maturation and oocyte fertilization. The expression of the Piwi family varied in 2-week- and 16-week-old ovaries. The expression of target genes, CYP19A1, HDAC6, ZP3, Figla, Nobox, and FSH receptors was downregulated in 16-week-old mice. Taken together, these data suggest that the Piwi regulates the

critical genes involved in female fertility in a reproductive stage-specific manner (2022R1A2B5B01002541).

B641/P3018

The properties of moringa that promote sea urchin egg viability

M. R. Lee[1], C. Wu[2]; [1]Borough of Manhattan Community College, New York, NY, [2]Boston College, Boston, MA.

The Properties of Moringa that Promote Sea Urchin Egg Viability

Marshall Lee, Chenyang Wu and Dr. Lalitha Jayant Borough of Manhattan Community College, New York

ABSTRACT

Sea urchins, like humans, are deuterostomes, organisms whose anus forms first relative to their mouth during embryological development. Sea urchin embryos could provide valuable insight into embryological development of humans and other deuterostomes. However, when left by themselves, the eggs of sea urchins disintegrate and die within 24 hours. This study aims to expand upon a previous study, which found that aqueous extracts of Moringa oleifera, a tree found in the Indian subcontinent, was able to preserve the viability of eggs obtained from the sea urchin species Lytechinus variegatus for up to seven days. To identify the properties of moringa that allowed it to preserve sea urchin eggs, aqueous moringa leaf extracts were obtained by brewing commercial moringa leaf powder in water at different temperatures. The extracts were tested for their antibacterial, anti-protist, antiviral and anti-oxidant properties using slight modifications of standard methods. Results indicate that the aqueous extracts brewed at 90℃ and 100℃ showed increasing levels of antibacterial activity when used at 50% concentration. The extracts were also able to hinder the growth of protists that co-isolate as cysts with the urchin eggs. Furthermore, the extracts, especially the ones extracted at 90°C and 100°C, showed a Total Antioxidant Capacity (TAC) of 5 ± 0.5 mmol Trolox equivalent TR/g. Antiviral activity was not detected in these extracts. More studies are needed to further ascertain the role of aqueous moringa leaf extracts on the viability of sea urchin eggs especially, the effects of various flavonoids present in moringa that contribute to the TAC.

B642/P3019

Biomolecular condensates in *C. elegans* oocytes are physiological markers for age-related viability loss

P. A. Bhatia, R. Amin, J. Woodruff; UT Southwestern Medical Center, Dallas, TX

Female age-related infertility is a growing field of research due to the increase in delayed motherhood and rise in demand for fertility treatment amongst older women. While we have methods to assist in pregnancies past the most fertile years, we do not have a good understanding of the underlying cellular processes that might be contributing to oocyte-specific aging.Since there are major limitations to studying human or even mammalian oocytes (both *in vivo* and *ex vivo*), our lab makes use of a *C. elegans* model. *C. elegans* nematodes naturally produce males and hermaphrodites but can be induced to a female phenotype. Our lab uses a temperature sensitive *fem-1* model that produces arrested oocytes that show a natural decline in viability with age. These arrested oocytes contain unique membrane-less condensates that are thought to store proteins and RNA.We hypothesize these condensates play a protective function, either through transcriptional regulation or by maintaining the integrity of RNA and proteins, in anticipation of fertilization. We analyzed a few key components in the condensates (IFET-1, CGH-1, CAR-1, mRNA) and are investigating their necessity/sufficiency and how changes to condensate

formation and morphology coincides with changes to oocyte viability. I've found that both by *in vitro* reconstitution and by *in vivo* knockdowns, I can understand the effect each protein has on condensate morphology and oocyte viability, which helps me elucidate their ultimate function. I have observed, with proteomics, that there is a large decrease in certain proteins in knockdown populations, suggesting that transcriptional repression is not be the primary mode of protection. Further studies will be done to elucidate the specific protective mechanism of the condensates that lead to alterations in oocyte viability.

B643/P3020

Characterization of the SLC9C2 (NHE11) Protein in Mammalian Sperm

C. C. Gardner, J. A. Abele, P. F. James; Miami University, Oxford, OH

The Na^+/H^+ exchangers (NHEs) are a family of transporters that are found in the membranes of many cells. Na^+/H^+ Exchangers are encoded by the SLC9 gene family of solute carriers and mainly function to regulate pH. Twelve of the thirteen NHE proteins have been characterized to some degree, yet nothing has been published about SLC9C2 (encoding the putative NHE11 protein) beyond the existence of the gene in several mammalian species. We have thus sought to characterize the expression and localization of the final unstudied SLC9 gene family member, SLC9C2 (NHE11), to begin to identify its physiological role. We extracted RNA from a panel of rat tissues and RT-PCR was performed to discover that NHE11 expression is limited to the testis. We also cloned and sequenced NHE11 transcripts from both rat and human testis and found that the deduced full-length NHE11 proteins are expected to contain a NHE domain at the N-terminus, followed by a voltage-sensing domain (VSD), and an intracellular cyclic nucleotide binding domain (CNBD) at the C-terminus. The predicted protein structure NHE11 suggests that it is likely a NHE transporter that may be regulated by membrane potential and/or cyclic nucleotides. This predicted protein structure of NHE11 shares a similar predicted structure with its paralog NHE10, which also exhibits testis-specific expression and has been shown to be essential for male fertility in mice and humans. We developed a polyclonal anti-NHE11 antibody against the deduced rat NHE11 protein. Using this anti-NHE11 antibody we were able to confirm the expression of the full-length NHE11 protein in both rat testis and rat sperm via immunoblotting. When performing immunofluorescence experiments on rat testis sections, we found that rat NHE11 localizes to the developing sperm head in testis sections, specifically to the acrosomal granule. In mature rat sperm cells, NHE11 localizes specifically to the plasma membrane of the sperm head, overlaying the acrosome. Like rat, human NHE11 protein co-localizes with the acrosomal granule of developing spermatids. The localization of NHE11 to the sperm head is significant because there are no known NHEs shown to localize to the head of mature sperm. Our group's work to characterize the expression and localization of SLC9C2 (NHE11) suggests that this protein is likely important in mammalian sperm physiology.

B644/P3021

Identification of cadherin superfamily RNA and protein in the sea star mature egg

A. Whetzel, D. J. Carroll; Midwestern University, Glendale, AZ

Little is known about how sperm and egg interact at fertilization and initiate egg activation. In the sea star, the sperm protein bindin interacts with the egg bindin receptor EBR1, but it is unlikely that these interactions alone are sufficient for successful fertilization. To identify other potential egg cell surface proteins that could play a role at fertilization, the sea star mature egg transcriptome was examined for

the expression of RNAs encoding cadherins. The sea star is a useful model system with abundant, synchronized oocytes that can be easily collected and manipulated. Cadherins are a family of Ca^{2+}-dependent proteins involved in cell-cell adhesion and signal transduction. Outside of a few model systems, little is known about their expression in germ cells or their role in fertilization. There are thought to be over 100 different types of cadherins found in vertebrates. The shared features include at least two extracellular Ca^{2+}-binding domains called the *cadherin repeats*, a transmembrane domain, and a short cytoplasmic tail. Cadherin mRNAs in the sea star egg were discovered by examining orthologs of known cadherins in a mature sea star egg transcriptome (Accession PRJNA398668). Putative cadherin RNAs were identified using the National Center for Biotechnology Information (NCBI) tblastn software with known vertebrate cadherin proteins as the target sequences. The putative proteins were predicted using Open Reading Frame finder (ORFinder) followed by blastp against the NCBI nonredundant protein database. From 22 unique vertebrate cadherin sequences, six different cadherin RNAs were identified in the *Patiria miniata* egg transcriptome. The smallest is a protocadherin Fat 1-like isoform with an RNA open reading frame (ORF) of 5445 nucleotides and a predicted protein of 1814 amino acids and the largest a protocadherin Fat 4-like isoform with an RNA ORF of 15096 nucleotides and a prediction protein of 5031 amino acids. Expression of these transcripts was confirmed by RT-PCR with RNA freshly isolated from *P. miniata* eggs. Protein expression of one of the cadherins was subsequently verified via immunoblotting oocyte and egg protein with an antibody directed against vertebrate protocadherin-10. The discovery and detection of cadherins in the sea star egg provides beneficial information for future functional studies of the fertilization pathway.

B645/P3022

Identification of Receptor Tyrosine Kinase family RNAs expressed in the sea star mature egg

A. Holden, A. Whetzel, D. Carroll; Midwestern University, Glendale, AZ

Ca^{2+} release is regulated by Src kinases and PLCγ leading to IP3 production. The molecules upstream of this signaling pathway, including those at the cell surface that may interact with the fertilizing sperm, are unknown. Since Src family tyrosine kinases and PLCγ are known to be regulated by receptor tyrosine kinases (RTK), we identified the RTK mRNAs that are expressed in the sea star egg by searching for orthologs of known RTKs in the mature sea star egg transcriptome (Accession PRJNA398668) using the National Center for Biotechnology Information (NCBI) tblastn software. Putative proteins were predicted using ORFinder followed by blastp against the NCBI nonredundant protein database. Representative orthologs of each of the 20 known subfamilies of mammalian RTKs were found in the *Patiria miniata* transcriptome with varying degrees of similarity. Expression of these sea star RNAs was confirmed using RT-PCR on freshly isolated sea star egg mRNA. The percent identity between the putative sea star protein and the human orthologue can be very high, close to 50% in some cases, such as with the tyrosine-protein kinase transmembrane receptor Ror1, a protein that may function to inhibit WNT-mediated signaling. The largest RTK open-reading frame discovered shared 31% percent identity with the protooncogene Orphan receptor tyrosine kinase ROS1, with a length of 10,761 nucleotides and an *in silico* predicted protein of 2513 amino acids. Additional findings include an 81% identity match in the sea star transcriptome with the ErbB2 RTK which is a member of the epidermal growth factor family, known for its role in regulating cell proliferation. The fibroblast growth factor receptor also displayed a high percent match of 42% in the sea star transcriptome with a length of 4310 nucleosides. Domain analysis using the NCBI CDD (conserved domain database) shows that the predicted sea star proteins possess similar, or identical, domain architecture to their mammalian orthologs. Future experiments will focus

on determining which of these are translated into proteins in the egg using western blotting and commercially available anti-RTK antibodies, and which may play a role at fertilization by the production of recombinant dominant-negative constructs for injection into the mature eggs.

B646/P3023

Mapping CBD-1 domains responsible for vitelline layer protein recruitment during *C. elegans* eggshell assembly

A. Wang, J. Prieto, S. K. Olson; Pomona College, Claremont, CA

To defend the embryo from polyspermy and foreign substances, the extracellular matrix of *C. elegans* oocytes undergoes rapid remodeling to form an impermeable protective eggshell after fertilization. Chitin-binding domain protein 1 (CBD-1), responsible for independently anchoring the EGG complex (EGG1-5/CHS-1/MBK-2) and the PERM complex (PERM-2/PERM-4) to the outermost vitelline layer, is crucial for eggshell formation. While previous studies indicate the PERM complex is reliant on the N-terminus of CBD-1 for proper localization, little is known about the relative contributions of CBD-1's various chitin-binding domains, interdomain loops, and predicted mucin chain sites to PERM-2 and PERM-4 recruitment. Fluorescence microscopy of CRISPR-generated *cbd-1* mutants revealed that removing chitin-binding domains 3 and 4 resulted in reduced and irregular recruitment of PERM-2::mCherry along oocyte and embryo surfaces, while loss of individual loops between chitin-binding domains 3-4, 4-5, 5-6, and 8-9 did not affect localization along oocyte membranes. In embryos, however, loop 4-5 knockout mutants exhibited a similar phenotype as domain 3-4 knockout mutants, with PERM-2::mCherry failing to be recruited to, or retained on, the eggshell. Further experiments to determine effects on PERM-4 and EGG complex binding will help to elucidate how vitelline protein binding domains contribute to vitelline layer remodeling.

B647/P3024

Mitotic Regulation in *Drosophila* pole cells

C. Smits[1], H. Nunley[2], E. Gavis[1], S. Shvartsman[1,2]; [1]Princeton University, Princeton, NJ, [2]Flatiron Institute, New York, NY

The proper development of an embryo often requires careful regulation of the timing and number of mitotic divisions. Here, we focus on mitotic regulation in the precursor cells to the germline in *Drosophila*: the pole cells. Pole cells are the first cells to form during embryogenesis, when syncytial nuclei migrating outward from the center of the embryo enter a specialized cytoplasm, the germ plasm, at the posterior of the embryo. Approximately 6 nuclei then bud out and form complete cells, while the somatic nuclei remain in a syncytium and continue to divide synchronously. The pole cells divide approximately 2 times (with some variability), eventually forming a cluster of ~30 cells that arrests in G2 and becomes quiescent until later stages of development. While many of the components and processes that lead to this specialized behavior have been well characterized, much of the previous research has relied on fixed embryos, precluding our ability to characterize single cell dynamics and understand how divisions are regulated in real time. Here, we use light sheet microscopy to visualize the process of pole cell formation and subsequent divisions in living embryos. Through our analysis of endogenously tagged Cyclin E-sfCherry, along with the pole cell marker Vasa-GFP, for each embryo we construct a forest of lineage trees, each tree corresponding to the lineage of a single pole bud. We first test whether one pole bud's tree affects the trees of other buds; for example, over-proliferation of one bud's tree may cause

under-proliferation in others. Then, using Cyclin E and Vasa levels, we test whether we can relate cell cycle asynchrony between daughters in a tree to unequal sharing of germ plasm at division. Our study sheds light onto how the number of proliferative divisions in this small population of cells is controlled during development.

B648/P3025

Investigating the role of a novel secreted protein in sperm development and fertilization in *C. elegans*

M. Hwang, **S. K. Olson**; Pomona College, Claremont, CA

Fertilization is an essential process in sexual reproduction where sperm and egg fuse together to create the next generation. Successful fertilization requires the proper development of gametes and subsequent sperm-egg recognition, binding, and fusion. Despite the importance of fertilization, the molecular underpinnings of this process remain elusive. In the nematode *Caenorhabditis elegans*, the specific proteins that govern sperm-egg fusion have not yet been described. We recently identified an uncharacterized gene, *spe-61*, that results in a sperm-specific fertility defect when a null mutation was introduced by CRISPR-Cas9 gene editing. *spe-61* is predicted to encode a secreted extracellular protein expressed specifically in the spermatheca. We are currently characterizing the localization pattern of a CRISPR-generated SPE-61::mScarlet fusion protein to better understand its site of action. Brood count assays led to the classification of the SPE-61 mutant as a spermatogenesis defective (SPE) mutant due to an observed low progeny count and abundance of unfertilized oocytes on the plate. The fertilization defect was rescued by mating with wild-type males, demonstrating that the *spe-61(null)* phenotype is specifically due to a sperm defect. We also found that *spe-61(null)* male and hermaphrodite mutants make spermatids that are morphologically normal and can properly differentiate into spermatozoa. Future studies will characterize sperm motility and migratory behavior, sperm competition, and sperm-egg interactions. This work will contribute to our understanding of the genes that play a role in spermatogenesis, spermiogenesis, and/or fertilization. In addition, the determination of the specific role of SPE-61 in fertilization may offer important insights into fertilization mechanisms across evolution, particularly as few fully secreted sperm proteins have been implicated in sperm-egg interaction to date.

B649/P3026

Investigating the functional role of PERM-2 and PERM-4 amyloidogenic domains in *C. elegans* eggshell vitelline layer assembly

Y. Alcaraz, **E. Sisay**, J. Carranza, S. Olson; Pomona College, Claremont, CA

C. elegans embryos are protected during early development by a multilayered eggshell, the outermost layer of which is called the Vitelline Layer. We are interested in exploring vitelline layer structure due to its importance in promoting fertilization, polyspermy prevention, egg activation, and eggshell barrier assembly. Previous work from our lab showed that the vitelline layer is comprised of at least three proteins, with CBD-1, PERM-2, and PERM-4 assembling together to build a structural barrier that prevents diffusion of high molecular weight compounds. To better understand how CBD-1, PERM-2, and PERM-4 interact to build the vitelline layer, we used the protein prediction programs PASTA and AmylPred to identify domains in each protein that may promote homo- and hetero-polymerization. Both PERM-2 and PERM-4 contain putative aggregation-prone and amyloidogenic domains, reminiscent of similar domains found in the ZP1-3 proteins that polymerize to form the mammalian zona pellucida. We therefore deleted the putative PERM-2 and PERM-4 aggregation domains using CRISPR-Cas9 in order to

investigate how these domains impact protein localization to the vitelline layer, eggshell integrity, and embryonic viability. Deletion of amino acids 161-184 in PERM-2 (the putative aggregation domain) prevented both PERM-2::mScarlet$^{\Delta 161\text{-}184}$ and PERM-4::mScarlet from localizing to the vitelline layer. Likewise, deletion of amino acids 70-86 in PERM-4 prevented PERM-2::mScarlet and PERM-4::mScarlet$^{\Delta 70\text{-}86}$ localization. However, CBD-1::GFP localization was not affected by the PERM-2 $^{\Delta 161\text{-}184}$ or PERM-4 $^{\Delta 70\text{-}86}$ domain deletions. These data suggest that CBD-1 serves to recruit PERM-2 and PERM-4, while PERM-2 and PERM-4, via their aggregation-prone domains, recruit and/or maintain each other on the vitelline layer. The *perm-2* $^{\Delta 161\text{-}184}$ and *perm-4* $^{\Delta 70\text{-}86}$ mutants also exhibited reduced brood sizes, similar in magnitude to those of *perm-2(null)* and *perm-4(null)* mutants, further highlighting the importance of the aggregation domains in PERM-2 and PERM-4 function. To further explore the role of the aggregation/amyloidogenic domains and determine whether PERM-2/4 assembly mirrors that of the mammalian ZP1-3 proteins, we are currently using CRISPR to mutate Cysteines predicted to be involved in stabilization of the domain structure via disulfide bridges. This work is crucial to understanding nematode fertilization and early embryonic development, and to identifying reproductive mechanisms that have been conserved or have diverged across evolution.

B650/P3027

Identifying Genes Required for Nuclear Rejuvenation during Gametogenesis

B. Styler; University of California, Berkeley, Berkeley, CA

Lifespan is reset during gametogenesis. In budding yeast, senescence-associated factors are excluded from gametes through sequestration into a distinct nuclear envelope-bound compartment, known as the Gametogenesis Uninherited Nuclear Compartment (GUNC). This compartment is subsequently degraded through vacuolar lysis, contributing to cellular rejuvenation. However, the mechanism by which age-induced damage is selectively sequestered into the GUNC remains unknown. To approach this question, we are developing a genetic screen that aims to identify factors involved in GUNC compartmentalization and selective nuclear inheritance. The screen will be conducted by targeting a plasmid containing a selectable marker to the GUNC and using the yeast deletion collection to identify genes required for plasmid sequestration and subsequent elimination. To target the plasmid to the GUNC, we engineered a conditionally-excisable centromere, thereby mimicking an extrachromosomal circular DNA (ecDNA) from the rDNA locus that normally accumulates in aged cells but is excluded from gametes. However, this alteration alone is not sufficient to mimic ecDNA. To improve plasmid exclusion from gametes, we fused aggregate-prone nucleolar proteins to the plasmid. Excitingly, one such fusion enhanced plasmid exclusion by 2-fold. In parallel, we employed a candidate-based approach to determine whether genes involved asymmetric segregation during mitosis are also required for exclusion of senescence-associated factors during gametogenesis. Together, these approaches offer a mechanistic understanding of how gametes can become devoid of age-induced nuclear defects.

Tissue Development and Morphogenesis 2

B651/P3028

Mechanistic Understanding of Transepithelial Fluid Transport

M. Chugh, S. Megason; Harvard Medical School, Boston, MA.

Morphogenesis, the process by which tissues develop into functional shapes, requires coordinated mechanical forces. Most current literature ascribes contractile forces derived from actomyosin networks as the major driver of tissue morphogenesis. Recent works from diverse species have shown that pressure derived from fluids can generate deformations necessary for tissue morphogenesis. Here, using zebrafish inner ear morphogenesis and its fluid filled lumen as a model system, I aim to tackle one of the long standing questions in physiology: how does fluid/water transport occur across epithelia? Several models for water movement across epithelia have been proposed, but their accuracy in mature physiology and especially in development remain unclear. By employing high spatiotemporal live imaging and quantitative analysis, I aim to tease apart real time water transport across inner ear epithelia and its regulation. Water transport and induced hydrostatic pressure in interstitial spaces and epithelial lumens can help us uncover new principles of tissue organisation during development and disease.

B652/P3029

Engineering septin disease alleles in *C. elegans*

J. A. Perry, D. E. Sloan, A. Brown, A. S. Maddox; Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Cell function and tissue integrity rely on the maintenance of cell shape and organization. The cytoskeleton, consisting of polymers and motor proteins, serves to structure and remodel the cell and determines tissue architecture. A highly conserved family of GTP-binding proteins called septins forms filaments and other higher-order structures, including gauzes and rings, from the non-polar arrangement of core proteins (ex. ABCDDCBA). Many cellular functions, including cell division and cell motility, are thought to involve the septin higher-order structures, where they serve to scaffold and recruit or sequester other proteins at the plasma membrane. Septin dysregulation by mutation or abnormally high or low expression occurs in many human diseases, including cancer, fibrosis, and neuropathies. Yet, how septin perturbation influences disease is unknown. A significant obstacle to defining septins' role in human health and pathology is the number of septin genes encoded in the human genome (13, some of which also have splice isoforms). Additional complexity arises from the functional substitution of septins within each of the four subfamilies. By contrast, the simple model animal *Caenorhabditis elegans* has only two septins. *C. elegans* septins, like septins in other species, can form filaments and localize to the cytokinetic ring during cell division. This project aims to understand how septins contribute to cell and tissue behavior and organization through the creation of transgenic *C. elegans* "avatars" containing septin mutations in conserved residues and mutations derived from human disease septin alleles. The phenotype of the *C. elegans* avatars will be compared to that of septin-null worms and previously characterized septin loss-of-function alleles. Preliminarily, we found that septins are required for normal development and function of the *C. elegans* germline, which is a simple single-cell model for stem cell biology, cell migration, and the regulation of cell cycle progression and differentiation. *C. elegans* bearing septin mutations have reduced brood size and oocyte production.

Mutant septin germlines also develop more slowly than control, perhaps indicating a defect in cell proliferation. Using *C. elegans* to study septin mutation will elucidate how septin dysfunction perturbs cell and tissue organization, with the long-term goal of providing insights into human development and disease.

B653/P3030

Modeling epithelial tissue as a 3D, self-sculpting, viscoelastic slab with active surfaces

X. Du, M. Shelley; Flatiron Institute, New York, NY.

During morphogenesis, epithelial monolayers actively alter their shape to create future body parts of metazoans; this makes the epithelium one of the most active and critical structures in early animal development. Even though epithelial cells exist and move in three dimensions, mathematical models frequently describe them as merely two-dimensional. However, recent imaging technology has begun to reveal pertinent dynamics in the third dimension of the tissue. With the importance of the third dimension in mind, we have developed a self-sculpting, three-dimensional, computational model of epithelia whose dynamics are driven by active forces on its surface. We present an initial, fundamental study for a reduced version of an epithelia that investigates how surface forces affect its internal dynamics. Our model captures the 3D slab-like geometry of epithelia, viscoelasticity of tissue response, fluid surroundings, and driving from active surface forces. We represent epithelial tissue as a thick slab, a 3D continuum comprised of a viscous Newtonian fluid with an extra viscoelastic stress. Employing this model, we simulate and make quantitative predictions about 3D cell dynamics and cell shape deformations as they respond to surface forces in common morphological contexts. In particular, we elucidate the initiation of ventral furrow invagination and T1 transitions in Drosophila embryogenesis. In the former, we demonstrate the importance of fluid and geometric surroundings to drive invagination. In the latter, we show the limitations of surface forces alone to drive T1 transitions. Our methods allow for detailed, physics-based studies of animal morphogenesis in 3D that includes the full geometric, fluid context of live systems.

B654/P3031

Cell contacts undergo systematic planar angle rotation, continually replenishing the pool of contractile junctions and mechanistically contributing to tissue elongation in the early Drosophila embryo

T. Vanderleest[1], **L. J. Russell**[1], K. Bond[2], H. Miao[3], Y. Xie[3], J. T. Blankenship[3], D. Loerke[2]; [1]Biological Sciences, Physics and Astronomy, University of Denver, Denver, CO, [2]Physics and Astronomy, University of Denver, Denver, CO, [3]Biological Sciences, University of Denver, Denver, CO.

Tissue elongation through epithelial remodeling during morphogenesis is a fundamental process that occurs through dynamic cell behaviors. In the early *Drosophila* embryo, tissue elongation is achieved through organized cell intercalation through directional t1-transitions. Previous work has suggested that the initialization of planar polarity within the epithelial sheet creates an initial population of vertical cell-cell contacts/interfaces (aligned with the dorsal-ventral axis) that undergoes a single round of contraction into higher-order vertices, followed by the resolution of these vertices into new horizontal interfaces (aligned with the anterior-posterior axis) which promotes tissue elongation. Here, we show that the dynamics of specific interfaces - i.e. contraction/elongation behaviors - are not specified permanently by a single initialization event at the beginning of germband extension, but that the planar polarity of interface behaviors is instead continuously re-interpreted on the basis of planar orientation

angle. In addition, our data shows that interfaces systematically and continuously rotate from horizontal towards more vertical orientations. Intriguingly, as these individual interfaces change their orientation, their contraction or elongation behaviors also change, demonstrating an ongoing dynamic 'rectification' mechanism. These changes in interface behaviors are also observable at the level of molecular machinery that directs contractile behaviors, with rectifying interfaces acquiring Myosin II as they approach more vertical orientation angles. Disruptions to the contractile molecular machinery, namely in F-actin and Myosin II function, show reductions in rotation rates, consistent with rotation being driven, at least in part, by interface contraction. These data demonstrate that intercalation and planar polarity are continuous processes that are constantly updated within the tissue context. As a result of the angular rectification, cells in the germ band can have several interfaces that acquire a contractile identity over time, meaning cells are continually "refreshing" T1 interfaces that can then participate in multiple staggered rounds of oriented neighbor exchange to produce higher net tissue extension.

B655/P3032

Tissue Structure and Cell-cell Adhesion of the Fully Elongated Amnioserosa in the *Drosophila* Embryo

T. Ray, T. J. C. Harris; Cell and Systems Biology, University of Toronto, Toronto, ON, CANADA.

Epithelia are crucial for embryogenesis and exhibit diversity in the way they are shaped and arranged. Significant literature exists on the contribution of tall, or columnar, epithelial cells to embryogenesis through the cell shape changes, cell division, and neighbour exchanges that are often mediated through actomyosin activity. However, other types of epithelial cells are less understood. For example, the Drosophila embryo amnioserosa (AS) forms a squamous epithelium made up of elongating spindle-like cells that facilitate germ band extension, and later reshape themselves for germband retraction and dorsal closure. What are the tissue structure and cell adhesion properties of the fully elongated AS? We imaged multiple probes by confocal microscopy to characterize fully elongated AS tissue structure. Plasma membrane probes showed long, spindle-like cells that taper both laterally and in depth to points at either end of each cell. Probing the nuclei showed pseudostratification, with some nuclei at the tissue surface and others below tapered ends of neighbouring cells. Strong microtubule (MT) bundles were parallel to and concentrated at the level of adherens junctions (AJs), with both running the full length of the cells. Investigating these long AJs began with Canoe, a probe for tension at AJs, which was found accumulated only at the tips of elongated amnioserosa cells, whereas its bi-cellular junction levels were less than those of epidermal cells. Maternal knock down of α-catenin, a component of AJs that binds F-actin and Canoe, surprisingly revealed that E-cadherin was retained at AS AJs but strongly disrupted at epidermal AJs. Although AS cells still elongated in α-catenin RNAi embryos, they were no longer aligned in the same direction, possibly due to a non-autonomous effect of the germband disruption. With α-catenin depletion, AS cells also showed retention of MTs and the junctional organizer Bazooka at AJs. When depleted of Bazooka, AS cells showed a breakdown of AJs and disorganized MTs. Probing for Bazooka localization showed a unique distribution of large puncta along move evenly distributed AS AJs. Overall, my project highlights a unique organization of an elongated and pseudostratified epithelium with AJs that rely less on a tension-based α-catenin mechanism and more on a Baz-dependent mechanism.

B656/P3033

Rab11 direction of lumen formation and pancreatic morphogenesis is required for proper cell differentiation

H. R. Barlow, N. Ahuja, L. Fassetta, Y. Htike, T. Bierschenk, D. Marciano, O. Cleaver; UT Southwestern Medical Center, Dallas, TX.

The molecular links between epithelial lumenogenesis, cell shape determination and the resulting tissue-level morphogenesis remain murky despite decades of rigorous studies. Here we describe a double knockout of Rab11A and Rab11B (Rab11) in the developing murine pancreatic epithelium that disrupts all three of these processes. Rab11-null pancreata are unable to form or connect their lumens. This is due at least in part to failures in vesicle trafficking, as apical components remain trapped within Rab11-null cells. Furthermore, these cells have severe defects in coordinating singular apical membrane initiation sites between groups of cells. In addition to polarity and lumenal defects, Rab11-null cells are significantly rounded, and the structure of acinar rosettes is disrupted. All these deficiencies combine to cause significant morphogenetic defects that eventually lead to a severe skewing of cell fate determination in the developing pancreas. These observations suggest Rab11 is indirectly or directly regulating lumenogenesis, cell shape and cell fate specification. These findings begin to link a number of cellular processes in vivo, and present tantalizing possibilities for decoding pancreatic morphogenesis.

B657/P3034

Transcriptional profiles along cell programming into corneal epithelial differentiation

M. T. Ortiz Melo[1], M. J. Garcia Murillo[1,2], V. M. Salazar Rojas[3], J. E. Campos Contreras[3], F. Castro-Munozledo[2]; [1]FES Iztacala, UNAM Universidad Nacional Autonoma De Mexico, Mexico, MEXICO, [2]Department of Cell Biology, Centro de Investigacion y de Estudios Avanzados del IPN (CINVESTAV), Mexico, MEXICO, [3]UBIPRO, FES Iztacala, UNAM Universidad Nacional Autonoma De Mexico, Mexico, MEXICO.

To study the transitional stages along the differentiation of corneal epithelial cells and discern its differential regulation, we used cultured RCE1(5T5) cells, which reproduce in vitro the developmental stages of corneal epithelial cell differentiation, as a model. We analyzed three differentiation stages based on the growth state of the cells and after studying the expression of transcription factors such as Oct4, Pax6, ΔNp63α, and selected differentiation, signaling, and epigenetic markers. Namely, proliferative non-differentiated cells, committed cells, and cells that constitute a stratified epithelium with a limbal epithelial-like structure. RNAseq-based transcriptome analysis showed 4891 genes differentially expressed among these stages, displaying distinctive gene signatures: proliferative cells had 1278 genes signature and seemed to be early epithelial progenitors with Oct4+, KLF4+, Myc+, ΔNp63α+, ABCG2+, Vimentin+, Zeb1+, VANGL1+, Krt3-, Krt12- phenotype. Committed cells with 417 genes as signature, displayed markers indicative of the beginning of corneal differentiation and genes characteristic of proliferative cells; with possible participation of Six3 and Six4 transcription factors along this stage. The third stage matches with a stratified corneal epithelium (979 genes signature), showing an increase in the expression of WNT10A, NOTCH2, NOTCH3, and Cux1, besides Pax6, KLF4, or Sox9. The differentiated cells express about 50% of the Epidermal Differentiation Complex (EDC) genes. Analysis of differences between corneal epithelium and epidermis could be crucial to understanding the regulatory mechanisms of the differentiated phenotype.

B658/P3035

Radial astroglia form large spherical structures during development in the zebrafish optic tectum

H. M. Barber[1,2], S. Kucenas[3,2,1]; [1]Cell & Developmental Biology Graduate Program, University of Virginia, Charlottesville, VA, [2]Program in Fundamental Neuroscience, University of Virginia, Charlottesville, VA, [3]Department of Biology, University of Virginia, Charlottesville, VA.

Radial glia (RG) are bipolar cells with long radial processes which serve as precursors for both neurons and glia. In zebrafish, RG persist into adulthood and share many properties with mammalian astrocytes, including interactions with blood vessels and neurons. In the zebrafish optic tectum (OT), RG bodies line the ventricular surface and extend long processes towards the pial surface, where they elaborate extensively. Using *in vivo,* time-lapse imaging of *Tg(slc1a3b:myrGFP-P2A-H2AmCherry)*-expressing zebrafish larvae, we observed OT RG extending projections from their pial processes basally towards the ventricular zone. These small processes often formed large spherical structures, measuring approximately 3 to 7 micrometers in diameter and lasting up to 6 hours before dissipating. Currently, the identity and function of these projections are unclear. Using molecular characterization, we have begun assaying the nature of this developmental phenomenon. From our preliminary data, at least some of the spherical structures contain markers of neuronal nuclei and dying cells but not of phagosomes. Due to their shape, size, movement, and containment of other cell nuclei, we tentatively dub these structures “scyllate heads”, in reference to the multi-headed Odyssean figure Scylla. Here we present our preliminary investigations and descriptions of OT RG scyllate heads. Due to an observed association with microglia, we hypothesize that scyllate heads sequester developmental debris, including exuberant neurons, for later phagocytosis by professional phagocytes.

B659/P3036

TEX264 induces reticulophagy in human keratinocytes to drive degradation of the endoplasmic reticulum during epidermal differentiation

C. Johnson, A. Tiwaa, **C. L. Simpson**; University of Washington, Seattle, WA

Formation of the cutaneous barrier depends upon a unique program of cellular differentiation termed cornification. As maturing keratinocytes reach the outermost layers of the epidermis, they initiate wholesale organelle breakdown to generate compacted cellular sheets that form a protective shield for the body. The mechanisms regulating organelle degradation during epidermal development remain unclear, but could reveal novel strategies to augment skin barrier function and restore tissue integrity in disorders of cornification. Our prior work uncovered that human keratinocytes engage the autophagy machinery via the mitophagy receptor NIX and the GTPase DRP1 to degrade mitochondria during their final stage of differentiation. These findings led us to ask if keratinocytes utilize distinct autophagy receptors to orchestrate breakdown of other organelle compartments as they form the epidermal barrier. To examine organelle dynamics in a live stratified tissue model, we coupled spinning-disk confocal microscopy with three-dimensional organotypic cultures of human epidermis grown from primary keratinocytes. We found the endoplasmic reticulum (ER) undergoes fragmentation in the outermost cell layers and subsequent routing into lysosomes. Supporting our hypothesis that keratinocytes utilize selective autophagy to degrade the ER, we found the reticulophagy receptor TEX264 is upregulated in the outermost cell layers in RNA sequencing and protein expression data from both murine and human epidermis. Ectopic expression of TEX264 was sufficient to induce the formation of ER fragments, which colocalized with the autophagosome marker LC3. Moreover, mutation of its LC3-

interacting region abolished the ability of TEX264 to fragment the ER, confirming that interaction with Atg8 family proteins is essential to its function in keratinocyte reticulophagy. Finally, treatment with tunicamycin, which induces ER stress and reticulophagy, upregulated TEX264 expression in keratinocytes and resulted in reduced tissue thickness and premature cornification of organotypic cultures, suggesting precocious ER breakdown accelerates maturation of the epidermis. In sum, our findings establish that TEX264 functions as a reticulophagy receptor in keratinocytes and reveal a novel role for ER remodeling and degradation in epidermal morphogenesis.

B660/P3037

Fluid Pressure Promotes Morphogenesis of the Lung

C. M. Nelson; Princeton University, Princeton, NJ.

The embryonic lung is comprised of a hollow epithelium, which is filled with an aqueous fluid and surrounded by a loose mesenchyme. The initially simple epithelium ramifies into the more complex architectures of the mature lung to enable sufficient airflow and gas exchange after birth or hatching. Reciprocal interactions between the epithelium and its adjacent mesenchyme have long been known to be essential for instructing morphogenesis of the lung. However, mechanical forces from the non-cellular compartment of the embryonic lung - namely, the pressure and flow of the luminal fluid - also appear to play a critical role in regulating morphogenesis of both the epithelium as well as the mesenchyme. Here, we combined genetic, imaging, and microfluidic approaches to uncover the specific effects of luminal fluid pressure on lung morphogenesis in mammals and reptiles. Using transgenic and reporter mice, we found that luminal fluid pressure regulates the rate of airway branching morphogenesis in the embryonic mouse lung, in part by activating mechanical signaling through the Hippo pathway effector, Yap. Activation of Yap in the epithelium leads to an increase in retinoic acid signaling, which induces the mesenchyme to differentiate into airway smooth muscle, the stiffness of which sculpts the growing epithelium into branches. Surprisingly, Yap is also required within the mesenchyme itself, suggesting that forces from fluid pressure are transmitted between tissue compartments. We found a more direct role for luminal fluid pressure in morphogenesis of the embryonic lizard lung. Using the brown anole lizard and timelapse imaging analysis, we found that the epithelium dramatically inflates with fluid. The pressure of this luminal fluid transmits forces that direct the migration of the smooth muscle layer into a mesh. Fluid pressure then pushes the epithelium through the holes in the mesh to generate the gas exchange surface of the lung. These data suggest a conserved role for mechanical signaling downstream of fluid pressure in lung morphogenesis.

B661/P3038

Increased intracellular pH promotes cell death in the developing*Drosophila* eye

J. Reyna Pacheco, J. Mendez, D. Orozco, R. Soriano, B. Grillo-Hill; San José State University, San José, CA

Constitutively increased intracellular pH (pHi) is common to most cancers regardless of their tissue of origin or genetic background. Cancer research has traditionally focused on cancer-associated mutations and dysregulated signaling pathways. However much less is known about how changes in cellular chemistry, including pHi, regulate cancer cell behaviors. One objective of our current work is to understand how pHi dynamics influences growth control during development, as previous studies suggest that higher pHi inhibits cell death through apoptosis. Our lab developed tools to increase pHi in the absence of other transforming mutations by over-expressing the *Drosophila* sodium-proton

exchanger, *DNhe2*, in the *Drosophila* eye. We previously showed that flies overexpressing *DNhe2* have a "rough" or mispatterned adult eye. To determine the underlying cause of this rough eye, we examined fly eyes at two earlier stages of development (larval and pupal), and characterized cell morphology and quantified cell numbers. We previously showed that flies overexpressing *DNhe2* show increased proliferation in larval eye discs. Paradoxically, we found fewer cells at the end of patterning in pupal eyes (11.4 compared to 15 cells per counting area in control). We next tested whether this decrease in cell number was due to increased cell death. We found that the pH-dependent cell death is p53-dependent but caspase independent, which is inconsistent with apoptosis, but suggests autophagy. We are currently testing whether molecular markers of autophagy correlate with increased pHi. Second, we want to identify the genes that mediate this increased cell death at higher pHi. A dominant modifier screen identified overexpression of the oncogene *Myc* as a strong suppressor of the *DNhe2*-induced rough eye. We tested whether this suppression is due to Myc attenuating the hyperproliferative effects of over-expressing *DNhe2*, but we saw no effect. However, we found that co-expression of *Myc* inhibits the cell death seen with over-expression of *DNhe2*. Together, our findings elucidate mechanisms for pH-regulation of conserved, critical developmental processes and provide evidence for new paradigms in growth control.

B662/P3039

A zebrafish model of CREB3L1 loss and gain-of-function to elucidate the role of CREB3L1 in bone development and regeneration

P. VanWinkle[1], T. Nawara[1], H. Rosengrant[1], H. Thomas[2], E. Lee[1], J. Parant[2], E. Sztul[1]; [1]Cellular, Integrative, and Developmental Biology, University of Alabama- Birmingham, Birmingham, AL, [2]Pharmacology and Toxicology, University of Alabama- Birmingham, Birmingham, AL.

cAMP-responsive element binding protein 3-like-1 (CREB3L1) is a bZIP transcription factor highly expressed in osteoblasts during mammalian development. This is demonstrated in mice deleted of CREB3L1, as they exhibit a fragile skeleton, with poor fracture healing capacity. Further, human patients with homozygous CREB3L1 mutations exhibit severe osteogenesis imperfecta with multiple fractures and severe decreases in bone mineralization. Despite a clear role in mammalian bone physiology, our data demonstrate that zebrafish lacking CREB3L1 do not exhibit gross abnormalities in developmental osteogenesis typical of other zebrafish models of congenital bone disease. However, zebrafish have unique regenerative abilities in many tissues, including bone. To evaluate the role of CREB3L1 in bone regeneration, we utilized two strains of zebrafish: CREB3L1$^{-/-}$, which lacks CREB3L1 function, or CREB3L1$^{TA/TA}$, which express a constitutively active fragment of CREB3L1 under the endogenous CREB3L1 promoter. Via tail-fin transection, we evaluated the regenerative capacity of both CREB3L1$^{-/-}$ and CREB3L1$^{TA/TA}$ fish. We observed an overall decrease in regeneration in the CREB3L1$^{-/-}$ fish, although physiological morphology of the bones was maintained. In contrast, fish expressing the constitutively active (TA) CREB3L1 fragment showed gross abnormalities in the regeneration of the fin, including fin ray fusion and malformation. Bone regeneration is an important topic in overall bone health, with applications to both traumatic and degenerative bone pathologies. The role of CREB3L1 in regeneration of whole bone has yet to be evaluated and as such, our model will help to elucidate the specific physiological function of CREB3L1 in regenerating bone tissue.

B663/P3040

Characterization of Intermediate cells during epidermal development

A. Prado Mantilla[1], W. Ning[2], T. Lechler[1]; [1]Cell Biology and Dermatology Department, Duke University, Durham, NC, [2]School of Life Sciences, Yunnan University, Kunming, Yunnan, CHINA.

The main role of the skin is to function as a barrier keeping external pathogens/toxins out and moisture in. This barrier is formed in the late stages of epidermal stratification. In mice, this process starts at E14.5, when basal cells start to divide asymmetrically and form the first layer of suprabasal cells, a transient cell population called intermediate cells (ICs). Then, at E15.5, basal cells start to produce spinous cells which subsequently mature into granular cells and later into corneocytes that form part of the epidermal barrier by E17.5. The role of ICs in this process has gone largely unstudied. These cells have unique characteristics in the epidermis such as their ability to proliferate without being attached to the basement membrane, and while expressing differentiation markers like Keratin1/Keratin10. Understanding the mechanisms behind these unique epidermal features could be beneficial for clinical applications. The aims of this study are to characterize/find markers specific for intermediate cells and to understand their role in epidermal stratification. To explore the differences between proliferating ICs and postmitotic spinous cells at E16.5, we performed RNA-Seq analysis of FACS purified cells from both cell types. From these data sets, we got a list of candidate markers that were enriched in each cell population. By immunostaining, we validated that the transcription factor MafB is not expressed in ICs and is a good marker for spinous cells. Further, mis-expression of MafB in intermediate cells results in loss of proliferation. We also found markers of ICs by validating the mRNA expression of St8sia6 and Scara5 only in ICs using RNAscope. Furthermore, we detected that transglutaminase1, an important protein for epidermal barrier formation expressed in granular cells, was expressed in ICs but not in spinous cells at E16.5. In addition, we did a gene ontology enrichment analysis of biological processes upregulated in ICs. We found that some of their enriched pathways were reminiscent of granular cell function, like establishment of skin barrier and lipid metabolic processes. Currently, the theory in the field is that ICs become spinous cells before maturing into granular cells. However, when we traced IC cell fate through EdU-pulse labeling, we found that ICs directly transitioned to granular cells without first expressing markers of spinous cells. Altogether, this suggests that, unlike the sequential cell maturation that happens in adults, ICs did not mature into spinous cells before becoming granular cells. Thus, ICs are precursors of the first granular cells during epidermal development.

B664/P3041

Alcohol consumption in mice during puberty affects the normal development of the mammary gland

M. Dunbar, I. Shibley; Pennsylvania State Univ/Berks Campus, Reading, PA

Alcohol consumption by women is associated with an increased risk of breast cancer. While many studies have examined the effect of alcohol on the mature mammary gland, little is known about the effects of alcohol on the developing mammary gland. While it is becoming increasingly clear that early exposure to alcohol, especially during puberty, can affect a woman's lifetime risk of breast cancer, the mechanisms remain unknown. In the present study, we studied the effect of alcohol consumption on the pubertal development of the mouse mammary gland. Three-week-old female mice were fed a liquid diet containing ethanol at 0, 10 or 20% of calories throughout puberty. Structural development of the mammary glands was examined by whole mount analysis of the #4 inguinal gland. Alcohol treatment resulted in a significant increase in the overall length of epithelial ducts as well as the number of

terminal end buds and branch points of 8-week old mice. Additionally, there was a significant increase in the more mature alveolar bodies in mice that consumed 20% of calories of ethanol when compared to control mice. Proliferation of mammary epithelial cells in the TEB was increased in alcohol fed mice, as measured by BrdU incorporation. Previous studies have shown that overexpression of parathyroid hormone related protein (PTHrP) in the mammary gland inhibits estrogen induced growth of the mammary gland during puberty. Therefore, we were interested in determining what effect alcohol would have on the pubertal development in PTHrP-overexpressing mice. Interestingly, there was no difference in duct length, TEB number or epithelial proliferation in alcohol fed PTHrP-overexpressing mice when compared to pair fed controls. Together, these studies suggest that alcohol exposure during pubertal development alters the normal development of the mammary gland perhaps by interfering with estrogen signaling.

B665/P3042

Developmental reprogramming of adipose tissue by maternal obesity.

M. BISWAS[1,2], S. Bayar[1], I. Splichalova[1], J. Zurkovic[3], C. Thiele[3], F. Shakeri[4], A. Buness[4], A. Pfeifer[2], E. Mass[1]; [1]Developmental Biology of Innate Immune System, LIMES Institute, Bonn, GERMANY, [2]Institute of Pharmacology and Toxicology , University hospital of Bonn, Bonn, GERMANY, [3]Biochemistry and cell biology of lipids, LIMES Institute, Bonn, GERMANY, [4]Institute for Genomic Statistics and Bioinformatics, University hospital of Bonn, Bonn, GERMANY.

Background: Macrophages have different developmental origin such as yolk sac derived tissue resident macrophage and bone marrow derived, monocyte derived macrophage. Adipose tissue macrophages (ATMs) are the predominant immune component in adipose tissue and they govern the metabolic fate of white adipose tissue (WAT), depending on their ontogeny. We propose that maternal obesity can impart unfavorable effects on the metabolic profile of offspring by developmental reprogramming of these adipose tissue macrophages. Our objective is to show these macrophages undergo chronic immune activation, possible epigenetic changes and pathological activation of specific signaling pathways such as Hif1a signaling and affects adipose tissue development and function, thereby contributing to the pathogenesis of metabolic diseases in offspring's later life. **Material & methods:** We created different dietary conditions consisting of maternal and post-weaning diet in mice. We used conditional HIF1α knockout mice model and studied effects of macrophages on the adipose tissue microenvironment in homeostasis, metabolic stress and maternal metabolic challenge. We used high dimensional flow cytometry and morphological assessment by histology, quantified by a specific machine learning algorithm. **Results:** Using specific immune markers, we could identify distinct subsets of macrophages and monocytes. Specific macrophage subsets like Tim4- macrophages and monocytes were associated with obese and maternal obese animals. Interestingly, adipocytes in the corresponding animals showed statistically significant increase in size. This adipose tissue hypertrophy was rescued by specific knockouts of HIF1α signaling in macrophages. Lipidomic analysis identified specific lipid subclasses associated with the maternal obese group. **Conclusion**: Maternal obesity modulates the metabolic profile in offspring and adipose tissue macrophages play a key role in it. Identifying specific genes or proteins responsible for expression of specific macrophage phenotypes can pinpoint potential therapeutic targets for obesity.

B666/P3043

Characterization of irregular chondrocyte stacking during craniofacial development in a zebrafish model of *cblC* syndrome.

B. E. Pinales, D. Paz, B. S. Castellanos, N. G. Reyes-Nava, L. Jimenez Madrigal, I. Perez, V. L. Castro, A. M. Quintana; University of Texas at El Paso, El Paso, TX

Methylmalonic aciduria and homocystinuria, cblC type (*cblC*) is a multiple congenital anomaly syndrome caused by mutations in the *MMACHC* gene, which encodes an enzyme required for B12 metabolism that, when mutated, causes an accumulation of toxic metabolites, specifically, methylmalonic acid (MMA) and homocysteine (HC). Additional phenotypes of *cblC* include microcephaly, neurological abnormalities, hematological manifestations, ocular deficits, and craniofacial dysmorphia. Although craniofacial abnormalities are not typically regarded as a hallmark of *cblC*, a subset of individuals have been reported with these phenotypes. The deletion of *Mmachc* in mice is embryonic lethal, and therefore, craniofacial phenotypes have not been fully characterized. Specifically, it is unknown whether the accumulation of MMA/HC is a prerequisite for craniofacial deficits associated with the mutation of *MMACHC*. Here we used a zebrafish model of *cblC* syndrome to determine metabolic accumulation in relation to the onset of craniofacial phenotypes. MMA levels were measured at 1-, 3-, and 6-days post fertilization (DPF) and facial development was evaluated at 1-, 2-, and 5-DPF. Our findings revealed moderate craniofacial anomalies such as increased Meckel's cartilage protrusion from the ceratohyal, increased palatoquadrate width, and irregular chondrocyte organization between neighboring chondrocytes in the hyosymplectic cartilage region. These moderate phenotypic modifications did not cause gross morphological defects in craniofacial development but were highly penetrant and were restored by a ubiquitin driven *MMACHC* transgene. Most fascinating was the fact that site-directed mutagenesis of the MMACHC cobalamin binding domain did not affect the ability of MMACHC to restore chondrocyte development to normal levels. Collectively, these data suggest that *mmachc* regulates craniofacial development independent of cobalamin binding and in the absence of accumulation of MMA.

B667/P3044

Bisphenol A Exposure During Pregnancy until Lactation Affects Expression of Androgen Receptor in Adult Gerbil Prostate and Impairs Sperm Motility

S. J. Colleta, T. F. R. Ruiz, V. Reame, S. R. Taboga; Universidade Estadual Paulista "Júlio De Mesquita Filho" - UNESP, São José do Rio Preto, Brazil

Estrogens are crucial for sexual differentiation and modulation of sexual organs. Bisphenol A (BPA) has estrogens effects and act as an endocrine disruptor on reproductive system. This experiment was aimed to test whether gerbil (*Meriones unguiculatus*) exposure to bisphenol-A, on gestacional to lactation period, could cause impacts on ventral prostate in adulthood. For this, gerbil females were allocated with fertile males, randomly divided into 4 experimental groups: control (**C**) gavage free; oil control (**CO**) gavage vehicle; low dose (**LBPA**) (50 µg/kg BPA) and high dose (**HBPA**) (5000 µg/kg BPA) - the BPA was dilute in corn oil (0.1 ml), the pregnant gerbils were gavage daily, from the 8th day of gestation until lactation end. The male offspring were allocated in insulators until 90 days-age and then euthanized. Sperm motility was performed. Prostate was removed, weighted, fixed in 4% paraformaldehyde and processed in histology and immunohistochemistry (IHC) routines. Testosterone (T) and estradiol (E2) were analyses by ELISA in blood samples. Histological sections stained with Hematoxylin-Eosin were

used for the morphology, stereology and lesions analysis. IHC analysis for androgen receptor (AR) were performed. Quantitative analysis was based on parametric (ANOVA) and nonparametric (Kruskal-Wallis) tests for group differences. Exposure to BPA did not alter the body, ventral prostate and prostatic complex weight. BPA had a negative effect on sperm motility, **HBPA** group show a reduction in the rate of spermatozoa with progressive movement and an increase in the rate of immotile spermatozoa in relation to **C** groups. There were no drastic morphology alterations in the ventral prostatic lobe in the different groups and no differences in stereological analysis and on incidence and multiplicity of prostate lesions. **HPBA** group had a decreased in E2 levels when compared with **LBPA** group. There was a decrease in the number of AR-positive cells in the of animals in the **CO**, **LBPA** and **HBPA** groups relative to the **C** group. These data indicate that exposure to BPA from gestation to lactation, besides caused no alteration in morphology and lesion predisposition, can cause alterations on hormonal signaling in the prostate at adulthood, and these alterations are probably related to impairments in sperm motility. Furthermore, corn oil, a widely used diluent vehicle, also led to alterations in AR expression.

B668/P3045

Changes in the dynamics of gastric cell proliferation are induced by early weaning

I. Rattes; University of São Paulo, São Paulo, Brazil

Epithelial cells proliferate along the gastric gland during rat postnatal development, whereas after weaning, they are concentrated between the isthmus and neck areas. In adult animals, an additional inactive, a reserve niche, is also found at gland base. Breastfeeding period is critical for stomach maturation in terms of supply of nutrients and growth factors, and so the abrupt cessation of breastfeeding can disturb the organ development. Thus, considering the importance of breastfeeding period, our hypothesis is that an abrupt change in diet with early weaning (EW) will lead to disturbances in the proliferative niches that can be extended to adulthood. Our current aims were to evaluate the late effects of EW in cell proliferation (PI) and expression of genes related to stem cell niches. Wistar rats were submitted to EW (15d) and gastric samples were collected at 18, 30, 60 and 120 d for RT-qPCR, tissue and cell morphology (Ethics Committee CEUA ICB USP 18/2015; 115/2017; 4532180222). Our previous results indicated that at 18 d EW increased the PI and pushed proliferative cells into the isthmus area, but at 60d, their distribution increased at the gland base (doi.org/10.3389). We found that later on, at 120d, the PI was lower than younger ages, and such decrease was more evident in the S group. We also registered that growth dynamics of the gland varied with the advancing age in the EW rat. As for genes involved in signaling and stem cell niches, EW induced differential effects at 18d and 60d, whereas at 120d, expression was not influenced. Among the responsive genes, Notch1 and Notch2 were the most affected by the abrupt diet change during development. Regarding the stem cell marker Troy, we found it during postnatal development: from the 3rd to the 15th postnatal day the number of Troy+ cells did not show differences, and from the 18th day on the number of Troy+ cells increased in the gastric mucosa. RT-qPCR shows an increase of Tnfrsf19 (Troy) at 60d EW, and Troy protein levels are elevated in 18d EW, but reduced at 60d. We can suggest that the dynamic of gastric growth was altered by EW through the regulation of genes involved in the signaling and organization of stem cell niches. Grant FAPESP: #2018/05064-0, #2018/07782-8, #2020/05117-7. Key words: early weaning, stomach, stem cell

B669/P3046

Intracellular pH dynamics during zebrafish neural crest development

C. Chou-Freed, D. E. Wagner, D. L. Barber; University of California, San Francisco, San Francisco, CA

Many cell behaviors, including cell proliferation and migration, require dynamic changes in intracellular pH (pHi). Our group recently reported that pHi dynamics act as a critical regulator of cell fate determination, including the differentiation of mouse embryonic stem cells and lineage specification of adult intestinal stem cells. However, it remains unresolved how pHi dynamics impact embryonic development *in vivo*. To address this gap, we generated zebrafish lines expressing a ratiometric fluorescent pHi sensor and confirmed the ability to reproducibly quantify pHi using live cell imaging of 24-30 hpf embryos. Using this system, we are testing the hypothesis that pHi dynamics regulate the development of neural crest, a highly conserved vertebrate embryonic cell population that gives rise to diverse cell types, including neurons, cartilage, and bone. Given the many pHi-regulated cell behaviors necessary for neural crest development, including multilineage specification, epithelial-to-mesenchymal transition, and cell migration, neural crest is an ideal system to address the gaps in our understanding of pHi dynamics during embryonic development. We found a significantly higher pHi in migratory compared to premigratory neural crest cells, consistent with the established role of increased pHi in coordinating directed cell migration in many cell types. To resolve the functional significance of increased pHi in migratory neural crest cells, we are experimentally perturbing pHi in zebrafish neural crest by CRISPR-Cas9-mediated knockout of plasma membrane H+ transporters. The experimental models generated here will provide new insights into the cellular and molecular factors regulating neural crest development, with important implications for human congenital diseases and tissue repair.

B670/P3047

Investigating the role of cyclooxygenase signaling in ectodermal derivative development

T. Leathers, C. Rogers; Anatomy, Physiology, and Cell Biology, University of California, Davis, Davis, CA.

During neurulation, the ectoderm-derived neural plate undergoes cytoskeletal rearrangements and folds until its borders meet and fuse at the dorsal side of the embryo, forming the neural tube. Differential expression of morphogens in the ventral and dorsal neural tube then patterns this tissue into separate lineages, the central nervous system (CNS) and neural crest (NC) cells. Though neural tube defects are the second most common congenital malformations in humans, the molecular mechanisms regulating neural tube closure and patterning are poorly understood. Additionally, dietary and environmental perturbations increase the risk of neural tube closure defects. Use of non-steroidal anti-inflammatory drugs (NSAIDs) during embryonic development is linked to a greater risk of neural tube defects, but the mechanistic reasons are unclear. NSAIDs inhibit cyclooxygenase (COX) enzymes, which synthesize the hormone-like lipid prostaglandin E2 (PGE2). To uncover the mechanistic pathway by which COX signaling inhibition leads to neural tube defects, we have characterized the expression of *Cox* genes and the genes encoding tissue-specific PGE2 receptors, *Ep3* and *Ep4*, during neurulation. *Cox1* expression is ubiquitous in early embryos, but *Cox2* is expressed in the anterior and trunk non-neural ectoderm. *Ep3* is localized to the developing neural tube, while *Ep4* is expressed in developing intermediate mesoderm. Knockdown of EP3 caused morphological defects in neural tube closure and significantly reduced the number and migration distance of NC cells. This phenocopies the defects observed after embryonic NSAID exposure, while knockdown of COX2 alone did not cause significant

developmental defects. Our work has identified a previously uncharacterized role of an enzymatic and lipid-based pathway in ectodermal derivative patterning.

B671/P3048

Modeling Tissue Injury in a dish

Y. Woappi; Columbia University, New York, NY

Epithelial damage is the node for a variety of human conditions and diseases, including chronic wounding, inflammation, and over 80% of all human cancers. As the largest lining tissue, the skin epithelium is often subject to injury and has evolutionarily adapted by acquiring the cellular plasticity necessary to repair the damaged site. Over the years, several efforts were made to study epithelial plasticity using *in vitro* and *ex vivo* cell-based models. However, these efforts have been limited in their capacity to recapitulate the various phases of wound healing. To meet this challenge, we established the human skinoid culture system, developed by cultivating organoid-like 3D cultures of primary neonatal human keratinocytes in alternating adhesive and suspension culture environments. We found that this skinoid technology was capable of enriching for a subpopulation of epidermal stem cells with enhanced regenerative potential and enabled us to study a variety of processes, including oncogenic neoplasia, wound healing, and tissue biofabrication. Importantly, skionids patterned all four major phases of tissue reconstruction: a) homeostatic renewal, b) differentiation halt/reversal c) stress lineage activation, and d) tissue restoration, enabling us to conduct unprecedented spatiotemporal multi-omics analysis of the human wound healing procedure in a dish.

B672/P3049

Investigating seamless tubulogenesis and unicellular branching morphogenesis in a course-based undergraduate research experience

J. Schottenfeld-Roames, T. Simpson, L. Lorenz; Princeton University, Princeton, NJ

Objective: To identify proteins required for seamless tube formation and branching morphogenesis in *Drosophila* tracheal terminal cells. Transformation of epithelial cells into complex functional organs is a critical step in development. Organs such as the lungs and kidney are composed of multicellular tubes (two or more cells attached to one other by junctions), whereas a large percentage of capillaries in the vertebrate vascular system are composed of seamless tubes (single cells that lack junctions). Although the morphogenetic events that help generate a multicellular tube can vary, it is clear how cells joined together by epithelial junctions create a membrane-bounded lumen; what is not obvious is how a single cell hollows out to create an internal lumenal membrane *de novo*. Specialized tips cells in the *Drosophila* tracheal system called “terminal cells” go on to form branched seamless tubes. Using terminal cells as a model, we have uncovered a number of cytoskeletal and membrane trafficking regulators that help orchestrate this seamless cell-to-tube transformation in previous work. Candidate RNAi screens to identify additional factors required for terminal cells morphogenesis have served as the foundation for an upper-level undergraduate research course. One such protein identified by students in the course is the actin contractibility regulator, Mysoin II. Here, we show a novel role for Myosin II during the process of seamless tubulogenesis and unicellular branching morphogenesis the fly tracheal system. RNAi against the fly *myosin II regulatory light chain* (*spaghetti-squash)* and *myosin II heavy chain* (*zipper)* results in changes to terminal cell architecture and discoordination between branch and lumen formation. Sholl and Strahler analyses, which capture changes in overall branch complexity, reveal

limited differences between control and *myosin II* knockdown cells; however, these analyses are limited in their ability to describe global changes to cell shape. A student-developed method for analyzing branch density within defined quadrants of space around a designated plane of a terminal cell reveals that Myosin II depleted cells do have reduced spreading compared to wild type control cells. Efforts to determine the mechanism by which Myosin II regulates branching and tube formation in terminal cells is currently underway.

B673/P3050

How Do the MyoD Binding Regions Upstream and Downstream of the Acta1 Gene Influence Transcription of a-actin mRNA?

A. L. Littlefield[1], G. M. Beas[1], G. De Salvo[2], S. B. Sharp[1]; [1]California State University, Los Angeles, Los Angeles, CA, [2]California Institute of Technology, Pasadena, CA

The transcription factor MyoD contributes to myogenesis (muscle development) by binding to conserved regulatory sequences typically found upstream of target genes. Interestingly, binding sites for MyoD have been found downstream as well as upstream of Acta1, one of MyoD's target genes. The role of the downstream binding regions is unknown. I hypothesize that MyoD binding both upstream and downstream of Acta1 is necessary for developmentally regulated, muscle-specific Acta1 transcription. Testing this hypothesis will increase our knowledge of longer-distanced, downstream transcriptional regulation, and will provide regulatory mutations to test for in ACTA1-related myopathies that have not been ascribed to a coding-region mutation. I am performing transient plasmid transfections with normal and mutated Acta1 regulatory regions inserted upstream and downstream of a firefly luciferase reporter gene. The constructs are transfected into mouse pre-muscle and fibroblast cells, and a dual luciferase assay is performed at both myoblast (undifferentiated) and myocyte (differentiated) stages. I have determined the quantity of normalizer NanoLuc plasmid to use; have found that apparent NanoLuc activity is lower in growth versus differentiation conditions; and have observed an apparent effect of the specific plasmids present in the transfection mixture on NanoLuc activity. I address how these findings will inform in our data analyses. I have performed a single trial transient transfection and luciferase assay on constructs containing the 5', 5' and various 3', truncated 5', and mutated 5' binding sites. My results showed that normalized Acta1 promoter-driven firefly luciferase expression is higher in the presence of the upstream MyoD binding region than in its absence in myocytes, but not in myoblasts, consistent with previous reports. Additionally, constructs containing both the 5' and 3' binding sites, showed higher transcriptional activation when compared to constructs containing just the 5' binding sites. So far, these results show that the 3' binding site may play a role in increasing expression of Acta1 mRNA. More trials with these constructs, as well as experiments testing additional constructs are underway.(Funded by RISE NIH-R25 GM061331)

B674/P3051

The AP-1 clathrin adaptor protein complex coordinates human cortical tissue morphogenesis and neurogenesis

A. E. Carleton[1], S. Wang[1], D. L. Gumucio[2], M. C. Duncan[2], K. Taniguchi[1]; [1]Medical College Wisconsin, Milwaukee, WI, [2]University of Michigan, Ann Arbor, MI.

A handful of rare human neurodevelopmental disorders has been linked to several mutation variants in the subunits of the AP-1 clathrin adaptor protein complex, a critical regulator of clathrin-dependent

polarized membrane trafficking. Mechanistically, the role of AP-1 in clathrin-dependent trafficking at the Golgi and endosomal networks has been extensively investigated, and, based on these studies, the AP-1-associated disorders are thought to be due to impaired AP-1-dependent trafficking of copper transporters, sortilins and/or secretases. To gain additional insights into the AP-1 pathology in the context of developing human brain, we generated human cortical organoids (HCO) derived from control human embryonic stem cells (hESC) and hESC lacking AP-1-γ1. Strikingly, AP-1-γ1-KO HCO displayed defective morphogenesis of cortical epithelium in which apical lumen fails to properly expand, an apical morphogenesis defect similar to in vitro AP-1-γ1-KO human epiblast models (Wang et al. Science Advances 2021). In addition to this defective neuroepithelium morphogenesis, single cell transcriptomic analysis comparing control and KO HCOs showed several cell subpopulations that are depleted in the KO, revealing a role of AP-1 in neural specification. Together, these studies provide the first comprehensive analysis for the role of the AP-1 complex during human cortical development, and provides a crucial insight into mechanisms underlying the AP-1 associated neural disorders.

Cell Fate Determination 2

B675/P3052

Adipose Tissue Derived Mesenchymal Stem Cells Differentiation is Improved by Photobiomodulation Therapy

N. P. Bueno[1], R. L. Bighetti Trevisan[1], M. M. Beloti[2], P. Arany[3], M. M. Marques[4], **E. P. Ferraz**[1]; [1]School of Dentistry, University of Sao Paulo, São Paulo, BRAZIL, [2]School of Dentistry of Ribeirao Preto, University of Sao Paulo, São Paulo, BRAZIL, [3]University at Buffalo, Buffalo, NY, [4]School of Dentistry, Ibirapuera University, São Paulo, BRAZIL.

Mesenchymal stem cells (MSC) from different sources have been widely explored for bone tissue regeneration purposes. MSCs from adipose tissue (at-MSC) are an attractive alternative since a higher number of cells are easily harvested at lesser morbidity compared to cells obtained from other sources. In spite of it, their use for bone tissue regeneration is limited due to their lower osteogenic differentiation potential. To circumvent this problem, photobiomodulation (PBM) emerges as a non-invasive therapy, which is able to enhance cell differentiation accelerating the repair process. In this context, this study aimed to investigate the PBM as a therapeutic alternative to improve the osteoblastic differentiation of at-MSC. For this, rat at-MSC were cultured in an osteogenic medium to differentiate into osteoblasts (OB) and submitted to the PBM protocol (660 nm; 0.14 J; 20 mW; 0.714 W/cm2 and 5 J/cm2). Non-irradiated OB were used as Control. The effect of PBM on osteoblastic differentiation (OB-PBM) was evaluated by measuring gene and protein expression of alkaline phosphatase (*Alp*) and runt-related transcription factor-2 (*Runx-2*) on days 3 and 7 using quantitative PCR and Western Blot. The ALP activity in situ was evaluated at 7 and 10 days using Fast red protocol. Data were compared by ANOVA, and the p-value was set at 0.05. PBM therapy led to an increased gene and protein expressions of ALP and RUNX2 after 3 and 7 days compared to Control ($p<0.001$ for both gene/proteins at all experimental periods). ALP activity in situ was also higher on OB-PBM on days 7 and 10 compared to non-irradiated cells ($p<0.05$ for all periods). To superficially evaluate the PBM osteoinductive properties, at-MSCs were cultured in non-osteogenic conditions, and the same parameters were assessed. Under this condition it was observed a slight increased *Alp* gene expression and ALP activity were noticed on at-MSC-PBM at early days ($p<0.05$), without differences in later periods of culture. Our results indicate the positive effect of PBM in improving the osteoblastic differentiation of at-MSC cultured under osteogenic

conditions, which may contribute to strategies to improve the aesthetic-functional reconstruction of bone defects. Financial support Fapesp: #21/04874-1; 21/03665-0.

B676/P3053

Islet-1 is differentially regulated by Scratch2 in the embryonic DRG and spinal cord.

V. Samartin Botezelli, S. Mirna Anticona, C. Purcell Goes, **C. Yan**; Cell and Developmental Biology, University of São Paulo, Sao Paulo, BRAZIL.

Sensory information is coded by peripheral sensory neurons in the dorsal root ganglion (DRG) and conveyed to interneurons in the spinal cord. The embryonic source of sensory neurons and spinal cord interneurons are distinct - neural crest and neural tube, respectively. However, both populations express a similar set of neural transcription factors after mitotic arrest. This suggests that intermediate progenitors of different sensory components could share genetic pathways. Here, we compared the genetic relationship between the same set of transcription factors (Scratch2, Islet-1 and NeuroD4) in the context of embryonic DRG and progenitors of dorsal spinal cord interneurons. In both tissues, Scratch2 (Scrt2), Islet-1, and NeuroD4 are expressed in early progenitor cells in the intermediate zone. However, in the DRG, Scrt2, Islet-1, and NeuroD4 are co-expressed, while in the embryonic spinal cord Islet-1 is only found in Scrt2-negative progenitors. Accordingly, overexpression of Scrt2 in the DRG increases Islet-1 labeling. In contrast, an increase of Scrt2 in the spinal cord decreases the dorsal most Islet-1-positive population. We then identified Scrt2 binding sites on the genome of embryonic spinal cord cells. The CUT&RUN tracks that overlapped with evolutionarily conserved non-coding regions were further analyzed for additional neural transcription factors target sites. We are now performing comparative functional studies of a genomic region ~100kb upstream of Islet-1 that is recognized by Scrt2 and has potential binding sites for multiple neural transcription factors.

B677/P3054

The folic acid receptor (Far1) regulates early development in *Dictyostelium*

K. Swier; Biological Sciences, Chicago State University, Chicago State University, IL.

Dictyostelium discoideum is a social amoeba that proliferates as a single-celled organism when nutrients are plentiful, but aggregates and develops into a multicellular fruiting body upon starvation. Many of the genes upregulated by acute starvation, such as the discoidin genes, are also turned on during proliferation as cell density increases and nutrients are depleted. Discoidin expression is negatively regulated by folic acid during proliferation. Some time ago, this regulation was shown to be dependent upon signaling through a G protein coupled receptor (GPCR) as Gβ-null cells could not suppress discoidin expression. Recently, the folic acid receptor (Far1) was identified. Far1 is a GPCR that binds folate and LPS and mediates both chemotaxis toward folate or LPS and phagocytosis of bacteria. The availability of far1 knockouts (KO) allowed us to test whether Far1 is indeed the GPCR mediating the negative signal provided by folate. Parental Ax2 cells and far1 KO cells were transformed with an expression vector encoding green fluorescent protein under control of the discoidin promoter. In axenic cultures, folate repressed the discoidin promoter in wild-type cells but not in far1 KO cells. LPS had no effect on expression from the discoidin promoter. When cells were grown in bacteria suspension, the activity of the discoidin promoter was completely repressed in both wild type and far1 KO cells. Surprisingly, far1 KO cells were unable to differentiate upon starvation in submerged cultures, whether grown axenically

or on bacteria prior to starvation. However, development on non-nutrient agar appeared normal. Far1 may have a role in early development of *Dictyostelium*.

B678/P3055

Investigating the roles of Etv4 and Etv5 during the self-renewal and differentiation of nephron progenitor cells in kidney organogenesis

K. Day[1,2], K. Thorner[3], A. Hosawi[4], B. Hammond[5,2], M. Fraunfelter[6], M. Kofron[6], F. D. Costantini[7], C. Cebrian[6,8]; [1]Molecular and Developmental Biology Graduate Program, Cincinnati Children's Research Foundation, Cincinnati, OH, [2]University of Cincinnati, Cincinnati, OH, [3]Developmental Biology/Admin & Core Services, Cincinnati Children's Research Foundation, Cincinnati, OH, [4]Division of Clinical Pharmacology, Cincinnati Children's Research Foundation, Cincinnati, OH, [5]Biomedical Research Technologies MS Program, Cincinnati Children's Research Foundation, Cincinnati, OH, [6]Division of Developmental Biology, Cincinnati Children's Research Foundation, Cincinnati, OH, [7]Department of Genetics & Development, Columbia University, New York, NY, [8]Department of Pediatrics, University of Cincinnati, Cincinnati, OH.

Kidney organogenesis is a complex process that relies on the interaction of different cell populations at defined spatial and temporal locales. In mammals, the reciprocal signaling between the ureteric bud (UB) and the metanephric mesenchyme (MM) drive the branching of the UB and the differentiation of nephrons from the MM. During nephrogenesis, nephron progenitor cells (NPCs) condense and epithelialize. Subsequently, morphogenetic movements and differential gene expression drive the progression from the nascent nephron into the specialized cell types and segments of the filtering units, including podocytes, proximal tubule, loop of Henle, and distal and connecting tubule. Each of these segments are essential to kidney function. My thesis project focuses on two genes that play a key role in signaling during kidney development: *Etv4* and *Etv5*. *Etv4/5* are members of the polyoma enhancer activator 3 (PEA3) subfamily of E-twentysix (ETS) transcription factors and are expressed in multiple tissues during development including in the MM, UB tips and developing nephrons. ETV4/5 act redundantly in the kidney and their ablation results in kidney agenesis.To identify any specific role for *Etv4/5* in nephrogenesis, we have used a *Six2-GFP-cre; Etv4*$^{-/-}$*; Etv5*$^{F/F}$ mouse model to interrogate the consequences of removing *Etv4/5* from the SIX2^{+} NPCs and its derived cells. Our data show that knocking out *Etv4/5* in the *Six2*$^{+}$ cells leads to hypoplastic kidneys, NPC premature depletion and a cystic phenotype. Moreover, immunofluorescent analyses of mutant kidneys indicate a decrease in the number of segments that stain for distal markers such as TFAP2B and SLC12A1, while other non-distal markers such as HNF1B do not exhibit a significant decrease. These results indicate that, while removing ETV4/5 reduces the overall number of nephrons, there is a disproportionate decrease in segments that are distal in nature. Furthermore, a large proportion of the cysts stain positively for AQP1, a marker of the upper thin descending limb of LOH, which could indicate that a blockage is occurring in the distal segments of these nephrons. In summary, our data support a model in which *Etv4/5* are required for the development of the distal segments of the nephron, and we hypothesize that the cystic phenotype seen in these mutants is a consequence of disrupting the development of these segments.

B679/P3056

Single Cell Mapping of Cell Differentiation in a System Derived from a Single Cell Type, the Platelet

I. Pokrovskaya[1], S. Rhee[2], M. W. Sebb[1], J. Kamykowski[1], O. Zhao[3], D. Cruz[4], M. A. Aronova[5], R. D. Leapman[4], **B. Storrie**[1]; [1]Physiology and Cell Biology, University of Arkansas Medical Scis (UAMS), Little Rock, AR, [2]Pharmacology and Cell Biology, University of Arkansas Medical Scis (UAMS), Little Rock, AR, [3]NIH, NIH, Bethesda, MD, [4]NIBIB, NIH, Bethesda, MD, [5]PNIBIB, NIH, Little RockBethesda, MD.

One of the long-standing problems in cell biology is how a single cell type, here the platelet, can produce an organized structure comparable in complexity to an early-stage embryo or an organoid. In the case of a puncture wound clot in mice, be it in a vein or an artery, the structure formed is complex, consisting of several different stable platelet activation states that nucleate the formation of platelet-rich pedestals and columns that lead to the formation of an organized platelet aggregate, a thrombus, that produces bleeding cessation. Here, we have used computer automated electron microscopy approaches to map differentiation state, I.e., platelet activation state, across total thrombus volumes and at the individual platelet level across full thrombus cross sections. The goal was to morphologically define at 3-5 nm resolution, platelet activation state and overall thrombus organization in a structure of near millimeter size. In so doing, we also used a region of interest, ROI approach, to 3-dimensionally render individual platelets in situ to probe platelet-platelets interactions in 3D space. The net outcome of this analysis was to establish that the forming clot,i.e., thrombus, consisted of 5 morphologically distinct activation states based on platelet shape, adherence and granule content. Each of these states were derived progressively from a basal state circulating platelet and once achieved appeared to have varying temporal stability. Even at relatively late times in thrombus formation relative to bleeding cessation, multiple platelet activation states were present within the thrombus. Based on single cell neighbor analysis, 2-micron radius, these activation states were loosely zoned within the thrombus. i.e., a favored but not strict association of like with like. Efforts to automate platelet stratification across sections through machine learning to give full 3D outcomes are in progress.

B680/P3057

Effect of Normoglycemia on the Disrupted Osteoblast Differentiation of Mesenchymal Stem Cells Induced by Type 1 and Type 2 Diabetes Mellitus

D. Weffort[1], L. F. Adolpho[1], A. T. P. Souza[1], G. P. Freitas[2], H. B. Lopes[1], F. S. Oliveira[1], R. L. Bighetti-Trevisan[1], L. Pitol-Palin[3], D. H. Matsushita[3], R. Okamoto[3], **M. M. Beloti**[1], A. L. Rosa[1]; [1]School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, BRAZIL, [2]School of Dentistry, Federal University of Goiás, Goiânia, BRAZIL, [3]Araçatuba Dental School, São Paulo State University Júlio de Mesquita Filho, Araçatuba, BRAZIL.

Diabetes mellitus (DM) disrupts insulin secretion, action or both, which is characterized by hyperglycemia and most of the cases is categorized as type 1 (T1DM) or type 2 (T2DM), both causing damages to bone through several mechanisms, including effects on osteoblasts. Based on the different etiopathologies of T1DM and T2DM, we hypothesized that T1DM and T2DM induce distinct levels of inhibition of the osteoblast differentiation of mesenchymal stem cells (MSCs) that is recovered when the hyperglycemic stimulus is removed. Thus, the aims of this study were to evaluate the osteoblast differentiation of MSCs derived from rats with either T1DM or T2DM as well as the effect of a normoglycemic environment on the osteogenic potential of these MSCs. To induce T1DM, rats received a single injection of streptozotocin (60 mg/kg) and for T2DM induction, rats were fed with a high-fat diet

and received a single injection of streptozotocin (35 mg/kg). Rats that received the streptozotocin vehicle were used as control and animals with blood glucose levels above 300 mg/dL were considered with T1DM and above 198 mg/dL, with T2DM. MSCs from healthy rats were grown in normoglycemic medium (control) and MSCs from rats with either T1DM or T2DM were cultured under hyperglycemic or normoglycemic conditions. The data were submitted to appropriate statistical analyses ($p \leq 0.05$). Morphometric parameters of the rat femurs (n=5 for control and T1DM, and n=6 for T2DM) showed that T1DM and T2DM induced bone loss, with more deleterious effects of T2DM than T1DM. Both T1DM and T2DM reduced the osteoblast differentiation of MSCs grown in hyperglycemic media, with more pronounced effects of T1DM, as demonstrated by alkaline phosphatase activity (n=5), RUNX2 protein expression (n=3) and extracellular matrix mineralization (n=5), and modulated the gene expression of several components of the bone morphogenetic protein signaling pathway (n=3). The retrieve of the normoglycemic environment partially recovers the osteogenic potential of MSCs from rats with T1DM but not with T2DM. Our findings shed light on the need for specific therapeutic approaches to treat either T1DM- or T2DM-induced bone loss as both disrupt osteoblast differentiation at distinct levels and most likely by different mechanisms. **Financial support:** FAPESP (Grants # 2019/18711-7 and 2017/12622-7) and CNPq (Grant # 307698/2021-1).

B681/P3058

Characterizing the sexual dimorphism in lung microvascular angiogenesis and the role of sex hormones

B. Hayward-Piatkovskyi[1], C. R. Gonyea[1], C. N. Clark[1], K. Lingappan[2], J. P. Gleghorn[1]; [1]University of Delaware, Newark, DE, [2]Children's Hospital of Philadelphia, Philadelphia, PA.

Bronchopulmonary dysplasia (BPD) exhibits a striking sexual dimorphism wherein prematurely born male infants are almost twice as likely to develop BPD compared to their female counterparts. While this difference is well established, the mechanisms contributing to this sexual dimorphism remain unknown. Previous work has identified sex differences in lung microvascular angiogenesis, though the exact nature of the dimorphism remains poorly characterized. Sex hormones are known to play a key role in early organ development, including lung development. Characterizing the sexual dimorphism in lung angiogenesis and identifying the role sex hormones play in this dimorphism is necessary to build the foundation for understanding the sex differences in BPD. The objective of this study was to characterize the sexual dimorphism in pulmonary microvascular angiogenesis and identify the way sex hormones modify these characteristics. We used a bead sprouting assay to model angiogenesis of neonatal human pulmonary microvascular endothelial cells (HPMEC). Standard culture mediums contain sex hormones in the form of fetal bovine serum (FBS) and phenols. We used commercially available charcoal-stripped FBS to create a hormone-free medium for these experiments. Using this hormone-free medium, we characterized angiogenesis, focused on the number of sprouts per bead and the maximum length of those sprouts. We found that male HPMECs produced fewer but longer sprouts compared to females. To identify which sex hormones were responsible for these changes, we dosed the medium with specific concentrations of β-estradiol (E2) or dihydrotestosterone (DHT). Female HPMECs maintained the female phenotype in angiogenesis, regardless of the presence of E2 or DHT. This suggests the female HPMEC phenotype is sex hormone independent. Interestingly, male HPMECs produce more sprouts in the presence of low doses of E2 or high doses of DHT. This data defines a male and female phenotype in angiogenesis while demonstrating that sex hormones modify the male, but not

female, phenotype. These findings provide a springboard for further investigation into the sex-dependent response to sex hormones in angiogenesis and warrant further investigation.

B682/P3059

Regulation of muscle subtype-specific *Myomaker* expression in a chordate with facultative myoblast fusion

C. Johnson[1], H. Zhang[2], Z. Zhang[2], P. Bi[3], A. Stolfi[1]; [1]School of Biological Science, Georgia Institute of Technology, Atlanta, GA, [2]Center for Molecular Medicine, University of Georgia, Athens, GA, [3]Department of Genetics, University of Georgia, Athens, GA.

Vertebrate myoblast fusion allows for multinucleated muscle fibers to compound the size and strength of individual mononucleated cells, but we have only recently started to understand the evolution of this important process. The phylum Chordata hosts closely related groups that span distinct myoblast fusion states: no fusion in cephalochordates, facultative fusion and multinucleation in tunicates, and extensive, obligatory fusion in vertebrates, which comprise the sister group to the tunicates. We previously reported that the chordate-specific muscle fusogen Myomaker likely arose through gene duplication in the last common ancestor of tunicates and vertebrates, This revealed an unexpectedly complex evolutionary history of myoblast fusion in chordates. A pre-vertebrate phase of muscle multinucleation driven by Myomaker was followed by the later emergence of another factor, Myomixer, which enables the highly efficient fusion system of vertebrates. Here we show that facultative activation of *Myomaker* transcription in multinucleated muscles of tunicate juveniles (but not mononucleated larval tail muscles) is carried out by a post-metamorphic myogenesis program based on the cooperative activity of Myogenic Regulatory Factor (MRF) and Early B-Cell Factor (EBF) transcription factors. This regulatory logic stands in stark contrast to the transcriptional activation of *Myomaker* in mammalian myoblasts by the MRF ortholog MYOD1 alone. Thus, we reveal the *cis*-regulatory basis of facultative vs. obligate myoblast fusion in chordates.

B683/P3060

Roles for the Planar Cell Polarity Pathway in postnatal intestinal patterning and architecture

R. Lee, K. Sumigray; Yale University, New Haven, CT

The coordination and patterning of cells required for proper tissue architecture play a critical role in organ development and function. This process is especially important in the small intestine, which relies heavily on its unique architecture of “hills” (villi) and “valleys” (crypts) to maximize nutrient absorption. Crypts are the architectural unit of the intestinal stem cell niche and form postnatally in mice. We have identified a role for crypts in mesoscale intestinal patterning through the regulation of villus ribbon patterning. Stem cell clones from a single crypt generate daughter cells that migrate up the villus in a linear ribbon pattern, while progressively differentiating into zonated cell states. This differentiation is tightly correlated to distinct digestive functions through compartmentalized expression of nutrient transporters. Despite the potential functional insights of intestinal patterning on both gross and mesoscale levels, *how this mesoscale patterning is established and maintained is not well understood.* We found that planar cell polarity (PCP) pathway genes expressed in crypts are essential to maintain villus ribbons. Furthermore, loss of PCP genes caused gross architectural defects, including villus branching, crypt translocation, and loss of epithelial cell attachment to the extracellular matrix. Our data suggest that PCP regulates directional cell migration out of the crypt and into the villus. In addition, as

the crypt is the architectural unit of the intestinal stem cell niche, this may imply that positioning and orientation of a crypt may affect gross villus architecture but may not be necessary for stem cell function. Future studies will focus on identifying the cellular and molecular mechanisms by which PCP regulates intestinal architecture and patterning and the physiological consequences of disrupted villus ribbon patterning. These studies demonstrate that mesoscale patterning plays an essential role in the organization and maintenance of intestinal architecture that is important for tissue physiology.

B684/P3061

Guiding kidney organoid morphogenesis through size and compositional control

C. Porter, A. Hughes; Bioengineering, University of Pennsylvania, Philadelphia, PA.

Human kidney organoids offer an exciting future avenue for renal replacement therapy. However, current kidney organoids, derived from induced pluripotent stem cells, lack the reproducibility, physiologic structure, and functionality necessary to make organoid-based therapies a reality. Recently, production of kidney organoids from multiple progenitor cell types displayed more maturity than previous organoids derived from single precursors. However, in these studies, precursor cells were intermixed in uncontrolled ratios and numbers, resulting in heterogeneous organoids with unpredictable composition. This has motivated our objective to determine how controlling initial kidney precursor ratios and quantities modulates final organoid composition and structure. To this end, we developed a novel cell photopatterning technology to capture precise numbers of multiple cell types within microwell arrays. Using immortalized renal epithelial cells, we validated the ability of this culture system to: 1.) define initial cell numbers, ratios, and positions through 2D cell patterning, 2.) allow cells to seamlessly transition to 3D suspension culture, with a single organoid per microwell, and 3.) produce highly parallel and trackable cultures. We show that long-term, spheroid sizes and compositions directly depend on the initial number of cells and ratios. Applying our culture system to the production of human kidney organoids, we found that final nephron organoid size and composition (the proportion of proximal versus distal tissue structures) also depend on the initial number of nephron progenitors. We are currently examining the effect of nephron to ureteric epithelium precursor ratio on kidney organoid outcomes. We expect to identify ratios of renal cell types that will restrict and modulate emerging renal tissues toward more physiologic structures. Providing the appropriate cell ratios and timing to cultures could even foster the fabrication of designer organoids or lead to emergent self-organization not previously seen in cultures that lack engineering control.

B685/P3062

Terminal cell differentiation by suppression of CDK4/6 activation to terminate clonal expansion

S. Sharma, M. N. Teruel; Dept. of Biochemistry, Drukier Institute for Children's Health & Weill Center for Metabolic Health, Weill Cornell Medicine, New York, NY.

Terminal cell differentiation is essential for the development and maintenance of tissues in all multi-cellular organisms. It is typically composed of an early “clonal expansion” period in which progenitor cells undergo one or more rounds of cell division, followed by a differentiation period when cells commit to differentiate and permanently exit the cell cycle. Different models have been proposed how proliferation and differentiation cooperate with each other during terminal cell differentiation. Here we used adipogenesis (fat cell differentiation) as a model for terminal cell differentiation and carried out live single-cell imaging to monitor cell cycle events and differentiation progression simultaneously in

thousands of progenitor cells undergoing adipogenesis. Our preliminary results support that CDK4/6 activity determines the duration of clonal expansion and number of cell divisions before differentiation. The CDK4/6 activity is regulated by a competition between cyclin D and timed expression of the CDK inhibitors p21, p27, and p18 during adipogenesis. Timed regulation of the competition allows high or low numbers of differentiated cells to be produced from a fixed number of progenitor cells.

B686/P3063

Establishing Different Conditions to Generate Epithelial to Mesenchymal Transition (EMT) in Human Induced Pluripotent Stem Cells to Study Cell State Transitions

G. Dalgin[1], . Allen Institute for Cell Science[1], J. Theriot[2], C. Hookway[1], R. N. Gunawardane[1]; [1]Allen Institute for Cell Science, Seattle, WA, [2]University of Washington, Seattle, WA

The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how cells transition between states during differentiation and disease. We are building a standardized framework by integrating 3D live-cell imaging with scRNAseq-informed multiplexed immunolabeling and RNA-FISH to understand the relationship between cell behavior, organization and cell identity. EMT serves as a powerful model system to investigate cell state changes where non-motile epithelial cells differentiate to form motile mesenchymal cells. EMT happens in both normal and pathological conditions, and it is identified in multiple different contexts (embryogenesis, wound healing, fibrosis, etc.) based on features such as switching from apical-basal to front-rear polarity and the homology of the genes involved. However, the progression from one cell state to another is not well understood. We aim to address this using multi-modal analysis, where we induce EMT in hiPSCs under three different conditions and compare them. In the first condition, we treated cells with a GSK3b inhibitor to upregulate WNT signaling to generate the mesoderm. For the second and third conditions, we treated cells with Activin^{low} BMP4^{low} or Activin^{high} BMP4^{high}, to generate the mesoderm and endoderm respectively. These experiments showed that conditions that generate both mesoderm and endoderm had similar impact on cell migration behavior. We are currently comparing the differences in spatiotemporal expression of key markers in between conditions. This experimental set up lets us perform extensive quantitative comparisons of EMTs generated under different conditions. We propose that this multi-modal, multi-scale approach can serve as a framework for studying EMT in varied settings and understanding cell states and transitions in a broader context. These studies have been performed on gene-edited cell lines from the Allen Cell Collection. Detailed information about these lines can be found at allencell.org.

B687/P3064

Inhibition of BMP4-mediated cross-activation of signaling pathways enhances Totipotent-like stem cells with Blastoid forming potential

T. Meharwade[1,2], L. Joumier[1,2], M. Parisotto[1], V. Huynh[1,2], E. da Rocha[3], M. Malleshaiah[1,2,2,4,5]; [1]Montreal Clinical Research Institute, Montreal, QC, Canada, [2]University of Montreal, Montreal, QC, Canada, [3]Federal University of Santa Catarina SC, Brazil, Florianópolis - SC, Brazil, [4]McGill University, Montreal, QC, Canada, [5]McGill Regenerative Medicine Network,, Montreal, QC, Canada

Embryonic stem cells (ESC) are defined by their inherent ability to self-renew and give rise to all cell types within the organism. Recent evidence suggests that cultured mouse ESCs co-exist in multiple

phenotypic states such as unbiased pluripotent state (naïve), totipotent (2Cell-like), EpiSC (primed) and are driven by distinct transcriptional regulators. An important aspect of these specific cell states is that they have different developmental potency: while totipotent cells can give rise to both embryonic and extraembryonic tissues, pluripotent cells give rise to only embryonic tissues and primed only to certain embryonic tissues. However, the signaling mechanisms initiating this remarkable developmental plasticity (co-existence of diverse cell fates) in genetically identical cells are unclear. Moreover, it remains unresolved which signaling pathway (if any) among those commonly active in ESC culture conditions is important to induce the Totipotent state. In this study, by examining various ESC culture conditions, we identified BMP4 signalling to promote Totipotent state. In addition, we also observed alternative cell states to be co-expressed and found BMP4 mediated signaling to curtail the induction of totipotent state through cross-activation of FGF, TGF-β and WNT pathways associated in promoting pluripotent and primed cell states, respectively. Furthermore, perturbation of cross-activated pathways using putative small molecules enabled in identification of a reprogramming condition (namely LBPXRS) to enhance totipotent state in ESCs. Integrated single-cell RNA-seq analysis of pre- and post-implantation mouse embryo, various ESC culture conditions and LBPXRS confirm the molecular features of LBPXRS akin to totipotent cell stages of pre-implantation embryo. Lastly, generation of in-vitro blastocyst-like structures (Blastoid) and *in-vivo* analysis of phenotypically reprogrammed totipotent cells exhibited functional and molecular features akin to totipotent cell stages of preimplantation embryos. Therefore, our findings reveal a novel BMP4-mediated signaling mechanism in ESCs to regulate diverse cell fates, and close association between BMP4 signaling cross-activation and totipotent-like reprogramming.

Host-pathogen/Host-commensal Interactions 2

B689/P3065

Malaria parasites harness Rho GTPase signaling and host cell membrane ruffling for productive invasion of hepatocytes

A. Schepis, S. Kappe; Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, WA.

Plasmodium sporozoites are the motile forms of the malaria parasite that infect hepatocytes. The initial invasion of hepatocytes is thought to be actively driven by sporozoites with little contribution by the host cell. The sporozoite contacts the host plasma membrane and enters the cell using its own actin-dependent propulsion, concurrent with invagination by the hepatocyte plasma membrane. The sporozoite modifies this membrane generating the parasitophorous vacuole, which supports the replication and growth of the nascent liver stage. Using fast live confocal microscopy, we observed that the initial interactions with sporozoites induce hepatocyte plasma membrane ruffles and filopodia extensions. These changes were triggered by sporozoites of rodent malaria parasites as well as *P. falciparum* sporozoites that infect human hepatocytes. Importantly, we find that these host cell processes strongly facilitate invasion and increase infection. Furthermore, Rho GTPase signaling, which regulates membrane ruffling and filopodia extension, is critical for productive infection. Interestingly, sporozoite cell traversal stimulates these processes, suggesting that it might increase hepatocyte susceptibility to productive infection. Our study unveils host cell signaling involved in plasma membrane dynamics as a critical host component of malaria parasite infection of hepatocytes.

B690/P3066

***Plasmodium falciparum* PfMORC is associated with nuclear proteins and controls the blood stage development**

M. Singh[1], G. Tesserin-Almeida[2], B. Dias[3], V. Santiago[3], J. M. Przyborski[4], G. Palmisano[3], C. R. S. Garcia[1]; [1]Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, BRAZIL, [2]Department of Physiology, Institute of Bioscience, University of Sao Paulo, Sao Paulo, BRAZIL, [3]Department of Parasitology, Institute of Biomedical Science, University of Sao Paulo, Sao Paulo, BRAZIL, [4]Department of Biochemistry and Molecular Biology (BiMoBi), Interdisciplinary Research Center (iFZ), Justus-Liebig-University, GIESSEN, GERMANY.

Malaria is one of the leading lethal parasitic diseases in developing countries where eradication efforts lack proper infrastructure. In 2021 malaria report of World Health Organization estimates 241 million new cases and more than 600 thousand deaths in 2020 (World Malaria Report 2021). Malaria is caused by a parasite of genus *Plasmodium*, a unicellular protozoan of Apicomplexa and *Plasmodium falciparum* is most virulent with the highest fatalities. The clinical symptoms of malaria are associated with the periodic rupture and exponential increase of parasites in the host's circulation system. The endogenous hormone plays a significant role in modulating parasite progression in both vector and the host. We have previously reported the role of melatonin and its metabolic intermediates in modulating the cell cycle of human *P. falciparum* and murine *P. chabaudi* within red blood cells [1]. Moreover, melatonin up-regulates a subset of genes involved in the ubiquitin-proteasome system (UPS) thus showing a complex and multi-tasking signaling cascade for hormone action in *P. falciparum*. The leading factors regulating the parasite cell cycle are poorly understood. Recently, we have found a nuclear protein PfMORC and shown that melatonin treatment increases PfMORC expression at asexual stage. We also found that cell cycle modulatory effect of melatonin is hampered when PfMORC is knocked down using a glmS self-cleaving ribozyme system [2]. Our data reveal the potential role of PfMORC during intraerythrocytic parasite development and also indicates the complex signaling mechanism linked with the host hormone melatonin. It would be interesting to investigate how PfMORC affect the parasite developmental cycle. Studies from various groups suggest that PfMORC is associated to *Apetala2* (ApiAP2) family of transcription factors and likely to play important role in transcriptional and epigenetic regulation. Further validation of the protein complex interaction is required to understand the molecular insights of apetela2 transcription factors in *Plasmodium* and exemplify the efforts to develop a new alternative therapeutic target **Key Words**: *Plasmodium*, Melatonin, Cell-cycle, PfMORC, *Apetala2*
References 1. Hotta, C.T., et al., *Calcium-dependent modulation by melatonin of the circadian rhythm in malarial parasites.* Nat Cell Biol, 2000. **2**(7): p. 466-8. 2. Singh, M.K., et al., *A nuclear protein, PfMORC confers melatonin dependent synchrony of the human malaria parasite P. falciparum in the asexual stage.* Sci Rep, 2021. **11**(1): p. 2057.

B691/P3067

***Legionella pneumophila* inhibits type I Interferon signaling to avoid cell intrinsic host cell defense**

D. Banks[1], **C. N. S. Allen**[1], M. Shuster[1], T. J. O'Connor[2], V. Briken[1]; [1]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, College Park, MD, [2]Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD.

The type I Interferon (IFN) host response to *Legionella pneumophilia* infections provides some cellular protection. Other pathogens have been shown to inhibit type I IFN-mediated cell signaling, but the

interaction of *L. pneumophila* with this signaling pathway has not been described. We showed that *L. pneumophila* inhibited IFN-β cell signaling via increasing doses of IFN-β up to 1000 pg/ml. However, *L. pneumophila* failed to inhibit IFN-γ-mediated cell signaling. The addition of high doses of IFN-β to *L. pneumophila*-infected macrophages limited the bacterial growth. Infected host cells produced increased amounts of nitric oxide (NO) after the addition of IFN-β, and the inhibition of NO-production via a chemical inhibitor resulted in increased bacterial growth. Finally, we showed that the *L. pneumophila* type IV secretion system is required to inhibit IFN-β-mediated cell signaling. In conclusion, we identified a novel host cell signaling pathway inhibited by *L. pneumophila* to improve survival in infected macrophages.

B692/P3068

Exploring the cell death mechanism induced by *Vibrio parahaemolyticus* Thermostable Direct Hemolysin

P. Verma, K. Chattopadhyay, A. Mukhopadhaya; Department of Biological Sciences, Indian Institute of Science Education and Research (IISER) Mohali, Punjab, INDIA.

Thermostable Direct Hemolysin (TDH) is the key virulence factor secreted by the marine bacterial pathogen *Vibrio parahaemolyticus*, one of the major causal organisms for human gastro-enteric diseases. It is a potent pore-forming toxin and can damage cellular homeostasis, presumably due to its ability to form pores on the plasma membrane of the target cells. TDH exhibits potent cytotoxicity against nucleated mammalian cells. However, the mechanism of the TDH-mediated cell death pathway is yet to be elucidated. Here, we show that TDH evokes features of apoptosis-like programmed cell death, such as phosphatidylserine flipping and a laddering pattern of DNA fragmentation. However, we have observed that unlike apoptosis, TDH-induced cell death is independent of any caspase activation. Thus, a caspase-independent programmed cell death is induced by TDH in the nucleated mammalian cells. Further, our results have revealed that TDH causes mitochondrial membrane permeability transition (MMPT), resulting in the release of important mitochondrial factors that take part in the subsequent execution of the caspase-independent cell death pathway and apoptosis-like DNA laddering pattern induced by TDH. Interestingly, TDH alone fails to induce MMPT in isolated mitochondria suggesting the involvement of other cytoplasmic factors in the process. We have observed that TDH shows a remarkable ability to translocate to the mitochondria of the target cells. Furthermore, we have observed the interaction of TDH with the pro-apoptotic molecule Bax. Thus, we speculate that TDH and Bax together possibly constitute a functional mitochondrial permeability transition pore (MPTP)-like structure that may result into MMPT induction. In sum, our study elucidates a novel mechanism of cell death induction by TDH that, to the best of our knowledge, has not been documented earlier for any other PFT family member. Our study adds valuable new insights regarding the role of TDH in bacterial infection and host-pathogen interaction processes.

B693/P3069

***Cryptosporidium* Uses its Cspv1 Virus to Activate Host Type I IFN Signaling and Attenuate Epithelial Antiparasitic Defense**

S. Deng[1,2], W. He[2], A. Gong[1,2], M. Li[2], Y. Wang[2], Z. Xia[2], X. Zhang[2], A. S. H. Pacheco[3], A. Naqib[4], M. Jenkins[5], P. C. Swanson[2], K. M. Drescher[2], J. K. Strauss-Soukup[6], M. A. Belshan[2], X. Chen[1,2]; [1]Microbial Pathogens and Immunity, Rush University Medical Center, Chicago, IL, [2]Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, NE, [3]Pediatric Gastroenterology, University of Nebraska Medical Center, Omaha, NE, [4]Anatomy and Cell Biology, Rush University Medical Center, Chicago, IL, [5]Animal Parasitic Diseases Laboratory, the United States Department of Agriculture, Beltsville, MD, [6]Chemistry, Creighton University College of Arts and Sciences, Omaha, NE.

Cryptosporidium is an obligate intracellular apicomplexan parasite that infects mammalian gastrointestinal epithelium. It is a leading cause of infectious diarrhea and diarrheal-related death in children worldwide. There is currently no vaccines and no fully effective therapy available for the infection. Humans are infected by ingesting *Cryptosporidium* oocysts, mainly the *C. parvum* and *C. hominis* species. Intestinal epithelial cells provide the first line of defense and play a critical role in the initiation, regulation, and resolution of both innate and adaptive immune reactions in response to infection. It is well established that type I IFN (IFN-γ) and type III IFN (IFN-lambda) play a critical protective role in intestinal anti-cryptosporidial immunity. Nevertheless, the role for type I IFN signaling in intestinal anti-*Cryptosporidium* defense remains unclear. *Cryptosporidium parvum* virus 1 (CSpV1) is a double-stranded RNA (dsRNA) virus carried by many *Cryptosporidium* spp. We report here that intestinal epithelial conditional *Ifnar1*$^{-/-}$ mice (deficient in type I IFN receptor) show resistance to *C. parvum* infection. CSpV1-dsRNAs are delivered into host cells and trigger type I IFN responses in infected cells through activating the PKR/RIG-I/MAVS signal pathways. Whereas *C. parvum* infection attenuates epithelial response to type II IFN-γ stimulation, knockout of type I IFN signaling or inhibition of CSpV1-dsRNA delivery can restore IFN-γ-mediated protective response. Accordingly, CSpV1 could be a new therapeutic target for cryptosporidiosis as antiviral reagent can promote the inhibitory effects of antiparasitic drugs on *C. parvum* infection of intestinal epithelium using *in vitro*, *ex vivo*, and *in vivo* infection models. Our data indicate the detrimental effect of type I IFN signaling in intestinal epithelial cells on intestinal anti-*C. parvum* defense and reveal a new strategy of immune evasion by the parasite.

B694/P3070

Role of putative toxin in stress response and pathogenicity of mycobacterium tuberculosis

S. Zarin; National Institute of Pathology, ICMR, New Delhi, INDIA.

The extremely notorious *Mycobacterium tuberculosis* is a highly successful pathogen since it can sneakily proliferate for decades escaping the host immune system. *M.tb* encodes an exceptionally large number of toxin-antitoxin (TA) systems, suggesting their important role in pathogenesis. TA systems are linked to stress adaptation, persistence and drug tolerance. These genes are promising candidates for novel therapeutic development to target persistent bacteria. *M.tb* encodes 90 TA systems, of which 50 belong to VapBC family. Proteomics study revealed 9 TA genes are unique to MTBC of which Rvzzzz was found to be present only in *M.tb* and *M.bovis*. Rvzzzz codes for VapC toxin protein harbouring a PIN domain characteristic of Ribonucleases. So to explore the role of Rvzzzz toxin in stress adaptation and persistence, Recombinant *M. smegmatis* (Ms_zzzz) expressing VapC protein was subjected to different abiotic stresses (pH, oxidative, nutritional and antibiotics) mimicking the environment encountered by

the pathogen inside host phagosome and checked for survivability by CFU assay. Infection studies was carried out on RAW macrophages to check for enhanced survivability and modulation of cell death pathways. Rvzzzz protein interactome was studied using Phage display technique and was also characterised biophysically with an aim to find its inhibitors in future. **Result:** Rvzzzz-y genes are co-operonic proving to be a bonafide TA system. Rvzzzz is a surface localised protein present predominantly in cell wall. Interacting partners obtained are major players of stress adaptation pathways. . Ms_zzzz presented significant tolerance to different stresses. It induced biofilm formation and the cells isolated from biofilm were recalcitrant to antibiotics. Ms_zzzz survived better inside infected RAW macrophages, inducing pro-inflammatory cytokines using TLR4 as receptor. The expression of cell surface markers such antigen-presenting MHC II and co-stimulatory CD80 and CD86 molecules was increased post infection along with enhanced ROS and RNS production together indicative of M1 polarization. Ms_zzzz inhibited apoptosis of RAW cells using it as a protective niche avoiding exposure to host Immune system. Overall Rvzzzz is involved in adapting to environmental cues in the host and is a promising candidate for the development of novel therapies to target persistent bacteria.

B695/P3071

Oxoeicosanoid signaling promotes early antimicrobial defense in zebrafish

Y. Ma[1], K. Hui[1], Z. Gelashvili[1,2], P. Niethammer[1]; [1]Cell Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, [2]Gerstner Sloan Kettering Graduate School of Biomedical Sciences, New York, NY.

5-oxoETE is a bioactive lipid derived from arachidonic acid (AA) generated when phospholipase A_2 activation coincides with oxidative stress. Through its G-protein coupled receptor OXER1, pure 5-oxoETE is a potent leukocyte chemoattractant. Yet, its physiological function, if any, has remained elusive for decades owing to the unusual OXER1 conservation pattern. OXER1 is conserved from fish to primates but not in rodents, precluding genetic loss-of-function studies in mouse. To determine its physiological role, we combined transcriptomic, lipidomic and intravital imaging assays with genetic perturbations of the OXER1 ortholog *hcar1-4* in zebrafish. *Pseudomonas aeruginosa* (PA) infection induced the synthesis of 5-oxoETE and its receptor, along with known inflammatory pathways. *Hcar1-4* deletion attenuated neutrophil recruitment and decreased post-infection survival, which could be rescued by ectopic expression of *hcar1*-4 or human *OXER1*. Our work reveals 5-oxoETE as a dominant lipid mediator of the early antimicrobial response in a non-rodent vertebrate. This is the first genetic proof for a conserved immune function of this enigmatic pathway with important implications for innate immune defense and perhaps sterile inflammation in humans.

B696/P3072

Parvovirus infection alters the nucleolar organization

S. Mattola[1], S. Leclerc[1], S. Hakanen[1], V. Aho[1], C. R. Parrish[2], M. Vihinen-Ranta[1]; [1]University of Jyväskylä, Jyväskylä, FINLAND, [2]University of Cornell, Ithaca, NY.

The nucleolus and nucleolar proteins are essential for the progression of infection by several viruses. Many viruses target the nucleoli to manipulate cellular antiviral defense and to facilitate their replication by recruiting nucleolar proteins. Consequently, viral infection often induces alterations in nucleolar structure and composition. Autonomous parvoviruses with small genomes and only a small number of encoded proteins depend on the cellular nuclear machinery for their efficient replication. Capsids of dependoparvoviruses, such as AAV, are assembled in the nucleolus. However, much less is known about

how autonomous parvoviruses interact with the nucleolus. Previously, we utilized proximity-dependent biotin identification (BioID), a powerful mass spectrometry-based tool capable of recognizing dynamic and transient interactions to elucidate the role of the viral NS2 protein in infection. The interactome linked NS2 to multiple proteins with possible relevance to the progression of infection. Notably, some gene ontology functions that were represented among the identified proteins were chromatin remodeling and DNA damage response. Moreover, the resolved BioID analysis revealed NS2 association with nucleolar proteins. Here, we applied a deep learning algorithm segmentation and nucleolin labeling to analyze the nucleolar changes induced by autonomous parvovirus infection. Our results show that the size of the detected nucleolin foci decreases, and nucleolin is released into the nucleoplasm in late infection. Analyses of ki-67, the strongest hit protein with the highest spectral count identified by BioID analysis, showed that the interaction between ki-67 with DNA increases in infection. In addition, the infection initiated by a viral clone lacking an intact NS2 failed to decrease the size of the detected nucleolin foci. All in all, our results suggest that parvoviruses modify and exploit nucleoli and nucleolar proteins during infection, and viral NS2 protein might play a role in regulating these processes.
Mattola S, Salokas K, Aho V, Mäntylä E, Salminen S, et al. (2022) Parvovirus nonstructural protein 2 interacts with chromatin-regulating cellular proteins. PLOS Pathogens 18(4): e1010353. https://doi.org/10.1371/journal.ppat.1010353 Mattola S, Leclerc S, Hakanen S, Aho V, Parrish CR, Vihinen-Ranta M. Parvovirus infection alters the nucleolar structure. bioRxiv, doi: https://doi.org/10.1101/2022.06.07.495090

B697/P3073

***Bifidobacterium bifidum*prevents cytokine induced increase of intestinal epithelial tight junction permeability by a novel mechanism involving peroxisome proliferator activated receptor gamma (PPAR-g) inhibition of myosin light chain kinase**

R. Abdulqadir, R. Al-Sadi, T. Ma; Medicine, Penn State College of Medicine, Hershey, PA.

Background: Recent studies have shown that *Bifidobacterium bifidum* (BB) causes a marked enhancement of the intestinal epithelial tight junction (TJ) barrier in a strain-specific manner. TNF-α is a ubiquitous pro-inflammatory cytokine that contribute to the development of intestinal inflammation in part by causing an increase in intestinal permeability. The TNF-α induced increase in intestinal epithelial TJ permeability is mediated by an NF-κB-dependent activation of myosin light chain kinase (MLCK) gene. The peroxisome proliferator-activated receptor gamma (PPAR-γ) is a nuclear receptor which is known to have an anti-inflammatory activity by interfering with NF-κB activation. However, the role of BB (and the possible mechanism involved) in protecting against cytokine-induced increase in intestinal permeability remain unclear. **Aims:** The major purpose of this study was to delineate the protective effect of BB against the TNF-α induced increase in intestinal TJ permeability and the mechanism involved. **Methods:** Filter-grown Caco-2 monolayers (*in vitro*) and recycling intestinal perfusion of live mice (*in vivo*) were used to asses intestinal TJ permeability by a paracellular marker (dextran-10 kDa). **Results:** TNF-α caused a NF-κB and MLCK-dependent increase in intestinal TJ permeability in Caco-2 monolayers and in live mice, BB inhibited the TNF-α increase in intestinal TJ permeability in a strain-specific manner. BB enhancement of the TJ barrier was associated with an increase in PPAR-γ expression and activity and inhibition of NF-κB activation and MLCK activity in Caco-2 monolayers and in mouse enterocytes. The inhibitory effect of BB on TNF-α induced increase in intestinal permeability, NF-κB activation, MLCK expression, and activity was abolished by siRNA-induced knock-down of PPAR-γ in Caco-2 monolayers. We also generated Villin-cre intestinal epithelial-specific PPAR-γ knock-out mice to study the

involvement of enterocytes PPAR-γ in the BB effect. BB also inhibited the TNF-a induced activation of NF-κB, increase in MLCK expression and increase in mouse intestinal permeability in PPAR-γ intestinal deficient mice (Villin Cre). **Conclusion**: These studies provide a novel insight into BB the mechanism of protection against cytokine-induced increase in intestinal TJ permeability *in-vitro* and *in-vivo*. Our data show that BB protects against the TNF-α induced increase in intestinal TJ permeability by PPAR-γ mediated inhibition of NF-κB activation and MLCK gene activation.

B698/P3074

Identifying *Brachyspira* virulence factors in infection of human colonic cells

A. Rajan, P. Gallego Alonso, M. Simrén, T. Pelaseyed; University of Gothenburg, Gothenburg, SWEDEN.

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder affecting 10-15% of people in developed countries and 10-45% of the global population and having an impact on mental well-being, behaviour, attitude and overall quality of life. The definitive causes of IBS are still unknown, however a link between intestinal spirochetosis, caused by the anaerobic spirochete *Brachyspira*, and IBS has been suggested, where around 40% of patients with IBS are infected by the pathogenic bacteria *Brachyspira*. *Brachyspira* is an intestinal spirochete that colonises the colon. *Brachyspira aalborgi* and *Brachyspira pilosicoli* are two species that cause human intestinal spirochetosis. *Brachyspira* bind microvillus-studded apical surface of intestinal epithelial cells in a characteristic end-on manner giving an appearance of a "false brush border" as observed in human colorectal biopsies. The pathogenesis of *Brachyspira* infection in humans is not well understood. *B. pilosicoli* produce enzymes that can modify glycosaminoglycans to create attachment sites and to enhance colonisation, which along with the bacteria's twisting motility, aids in accessing underlying intestinal epithelial cells. However, bacterial outer membrane proteins responsible for attachment to intestinal epithelial cells remain unknown. **My project aims to identify *B. pilosicoli* outer membrane proteins that act as virulence factors and host cell surface proteins involved in the attachment step of *Brachyspira* to intestinal epithelial cells and subsequently determine molecular alterations in the brush border of IBS patients**, which cause susceptibility to *Brachyspira* infection. So far, **I have identified three *B. pilosicoli* outer membrane proteins that are potential adhesins. These proteins bind both cultured intestinal epithelial cells and human tissue sections. I have solved the structure for one of these proteins (OMP1) and preliminary data show that it binds to glycosylated mucin proteins.** The next steps are to validate the function of OMP1 as an attachment factor and to identify its ligand on the host cell.

B699/P3075

***Borrelia burgdorferi* induces changes in the physical forces and immunity signaling pathways of endothelial cells early but not late during *in vitro* exposure**

R. Aparicio Yuste[1,2], **M. Münkel**[1], K. Axarlis[1], M. Gómez Benito[2], A. Reuss[1], G. Blacker[3], M. Caspi Tal[3,4], P. Kraiczy[5], E. Bastounis[1]; [1]Interfaculty Institute of Microbiology and Infection Medicine, (CMFI, EXC 2124), University of Tuebingen, Tuebingen, GERMANY, [2]Department of Mechanical Engineering, University of Zaragoza, Zaragoza, SPAIN, [3]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, [4]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, [5]Institute of Medical Microbiology and Infection Control, University Hospital of Frankfurt, Goethe University Frankfurt, Frankfurt am Main, GERMANY.

Borrelia burgdorferi (*Bb)* is the causative bacterial agent of Lyme disease which is on the rise in the USA and Europe alike. Once transmitted to the human host via an arthropod vector, *Bb* spreads through tissues by traveling along and through the vasculature, whose inner lumen is lined by a monolayer of endothelial cells (ECs). What the precise mechanisms that *Bb* employs to achieve efficient dissemination through the vasculature are is not well understood. Could extracellular *Bb* induce alterations in the EC force generation machinery like several other intracellular bacterial pathogens, promoting its own spread by potentially weakening the EC barrier integrity? Using videomicroscopy coupled with traction force and monolayer stress microscopy, we monitored the response of ECs to prolonged exposure to *Bb* and found a sharp, transient increase in EC traction and intercellular stresses, followed by a prolonged decrease in EC traction and monolayer stresses 15 hours post exposure (hpe). At a later stage of exposure though (>24 h) all variables return to levels similar to those observed for never-exposed ECs. Using RNA-sequencing to better understand the underlying biochemical mechanisms involved, we discovered an upregulation of EC innate immune signaling pathways during early but not late exposure to *Bb*. In marked contrast to exposure of ECs to live *Bb*, when we generated heat-inactivated *Bb* and exposed them to ECs, we evidenced no sharp increase in traction forces at early exposure, neither reversal at late exposure, but found a sustained weakening of physical forces all throughout the exposure. Thus, we discovered a differential response of EC physical force generation and innate immune signaling to live versus heat-inactivated *Bb*. This indicates a tight interplay between the two and is suggestive of an active modulation of both processes that might be key in establishing infection.

B700/P3076

Malaria derived extracellular vesicles inhibit primary neutrophils reactive oxygen species production and neutrophil extracellular trap formation.

K. Babatunde[1], M. Walch[2], P.-Y. Mantel[2]; [1]University of Calgary, Calgary, AB, Canada, [2]University of Fribourg, Fribourg, Switzerland

Introduction A dysfunctional innate immune response is believed to provide immune evasion of the malaria parasites, but also to cause increased susceptibility to bacterial infections. Neutrophils are the most abundant cells found in the blood circulation in direct contact with parasite-infected red blood cells (iRBCs). However, the neutrophil populations with reduced oxidative burst activities is present during malaria infection. These observations suggest that neutrophil responses are fundamentally defective in malaria patients. In this present work, we investigated, how extracellular vesicles (EVs) derived from iRBCs and containing both parasite and host materials, including microRNAs, modulate neutrophil response by transferring regulatory micro-RNAs. **Methods and Results** We have previously reported that malaria EVs contain miR451a, a microRNA that is known to regulate neutrophil activity when EVs are phagocytized by neutrophils. Thus we monitored the uptake of EVs by neutrophils through fluorescence microscopy, confocal microscopy, and real-time polymerase chain reaction techniques as well as potential pathways involved in their uptakes by neutrophils. Furthermore, our data demonstrated the influence of malaria-induced EVs on human neutrophil functions in vitro by inhibiting their ability to produce ROS and suppressing cytokine secretion. Neutrophils are known to produce neutrophil extracellular traps (NETs) during an immune response to pathogens; therefore, we quantified the production of NETs with incubation of EVs. **Conclusion** Our data indicate that EVs are actively taken up by neutrophils to deliver miR451, thereby interfering with their capacity to kill bacteria by inhibiting ROS and NETs formation. We describe a new mechanism of cellular communication between parasites and the host immune system. While EVs might increase tolerance to the parasites, they dramatically

affect the resistance to a coinfection by bacteria. The elucidation of the immune regulatory role of EVs might lead to the development of new therapies.

B701/P3077

***Pseudomonas aeruginosa*Extracellular Vesicles Regulate Innate Immune Responses in Corneal Epithelial Cells and Neutrophils**

R. Ayilam Ramachandran, D. M. Robertson; UT Southwestern Medical Center, Dallas, TX

ABSTRACT

Pseudomonas aeruginosa Extracellular Vesicles Regulate Innate Immune Responses in Corneal Epithelial Cells and Neutrophils

Purpose: *Pseudomonas aeruginosa* (PA) keratitis is a devastating complication that can arise as a result of trauma or contact lens wear. Extracellular vesicles (EVs) are nanoscale membrane vesicles secreted by various cells including gram negative bacterial pathogens. These EVs are known as bacterial-derived vesicles or bacterial EVs (BEV). BEVs carry biomolecular cargo, including virulence factors, and can play a role in cellular communication. The objective of this study is to characterize the biomolecular cargo and host innate immune responses during PA-keratitis. **Methods:** A standard invasive test strain of PA (strain PA01) was used in these studies. BEVs were isolated from supernatant collected from an overnight suspension culture and isolated using size exclusion chromatography. Pooled BEV enriched fractions F1/F2 and secretory protein enriched fractions F4/F5 were subjected to mass spectrometry at the UTSW Proteomics Core Facility. EV size and concentration was determined by nanoparticle tracking analysis (NTA). Telomerase-immortalized human corneal epithelial cells and a dimethyl sulfoxide (DMSO) differentiated neutrophil cell line HL-60 were treated with BEVs and secretory proteins to determine their functional role during ocular PA infection. **Results:** BEVs from F1/F2 were < 150 nm in size. Protein concentration was much lower in BEVs and highest in secretory fractions. The BEV particle:protein purity was ratio was > 7×10^9. 954 and 359 unique proteins were identified in BEV and secretory fractions, respectively. Gene enrichment analysis indicated that most of the proteins from both groups belonged to

metabolic and cellular processes. BEVs and secretory proteins were not cytotoxic, but increased corneal epithelial intracellular PA survival. BEVs induced IL-6 and IL-8 secretion. Secretory proteins downregulated cytokine responses. Both BEVs and secretory proteins induced neutrophil activation, chemotaxis and respiratory burst. However, secretory proteins attenuated neutrophil bacterial killing. **Conclusions:** BEVs and secretory proteins exhibited unique proteomic profiles with virulence proteins enriched in BEVs. This led to differential functional effects on innate immune responses in corneal epithelial cells and neutrophils during PA keratitis.

B702/P3078

***C. burnetii*downregulates IL-17 signaling in macrophages to block neutrophil recruitment**

T. Mordente Clemente, L. Augusto, S. D. Gilk; University of Nebraska Medical Center, Omaha, NE

Coxiella burnetii is a highly infectious pathogen that causes human Q fever, a flu-like acute illness that can develop into a chronic life-threatening endocarditis. *C. burnetii* first targets alveolar macrophages and promotes formation of a phagolysosome-like vacuole called the *Coxiella* Containing Vacuole (CCV). Successful host cell infection requires the Type 4B Secretion System (T4BSS), which translocates bacterial effector proteins across the CCV membrane into the host cytoplasm, where they manipulate a

variety of cell processes. We recently demonstrated that the *C. burnetii* T4BSS downregulates expression of IL-17 target genes as well as IL-17-stimulated chemokine secretion, and confers protection against IL-17 mediated killing by the macrophage. Given that IL-17 is known to protect against pulmonary pathogens, we hypothesize that *C. burnetii* T4BSS effector proteins downregulate intracellular IL-17 signaling pathways in order to evade the host innate immune response and promote bacterial pathogenesis. To further determine whether *C. burnetii* blocks IL-17-induced gene transcription, we utilized a stable IL-17 promoter reporter cell line which expresses secreted embryonic alkaline phosphatase (SEAP) under the control of one NF-kB and five AP-1 promoter elements. While there was a robust induction of SEAP following IL-17 activation in both mock and cells infected with the *C. burnetii* T4BSS mutant *dotA,* infection with wild type *C. burnetii* decreased SEAP by 75%, indicating that *C. burnetii* blocks IL-17-induced transcriptional activation. In addition, by assessing the phosphorylation state of NF-kB, MAPK, and JNK in mock- or *C. burnetii*-infected alveolar macrophages treated with IL-17, we confirmed that *C. burnetii* downregulates activation of these proteins. To determine whether the IL17RA-ACT1-TRAF6 pathway is required for IL-17-mediated killing of *C. burnetii*, we generated IL-17-RA and TRAF6 knockouts in macrophages. IL-17 stimulation decreased *C. burnetii* viability in WT cells, but not in the knockout cells, indicating that the IL17RA-ACT1-TRAF6 pathway is essential for the IL-17 bactericidal effect. Because IL-17 plays a critical role in recruiting neutrophils, we tested whether *C. burnetii* blocks IL-17-induced neutrophil chemotaxis using migration assays of neutrophils in the presence of supernatants from untreated and IL-17 treated infected macrophages. We found that *C. burnetii* actively inhibits IL-17-mediated neutrophil recruitment, and we identified CXCL5/LIX as an IL-17 induced neutrophil-chemoattractant downregulated in *C. burnetii*-infected macrophages. Together, our findings indicate that *C. burnetii* blocks IL-17 signaling to avoid direct killing by the macrophages and to decrease neutrophil recruitment to the infection site.

B703/P3079

***Toxoplama* exploits the host autophagy to acquire amino acids to persist in the brain.**

E. Funke[1], **L. Augusto**[2]; [1]University of Nebraska Omaha, Omaha, NE, [2]University of Nebraska Medical Center, Omaha, NE

Toxoplasma gondii is one of the most prevalent parasites worldwide. It is estimated that nearly 2 million people in the world have been infected with this pathogen. Given that healthy individuals usually can control parasite replication, they typically remain asymptomatic or present mild flu-like symptoms during acute infection. However, parasites that escape from immune response encyst in the brain, becoming resistant to both immune response and available drugs. One of the reasons for this successful infection is *Toxoplasma*'s ability to infect virtually any nucleated cell in warm-blooded vertebrates. However, the parasite relies on host cells' nutrients, especially amino acids and fatty acids, to chronically persist in the brain, which is associated with neurological alterations. The currently recommended therapy for toxoplasmosis causes severe adverse effects and only targets acute forms of *Toxoplasma*. Therefore, one of the highest priorities in the field is elucidating how latent parasites persist in the brain in order to identify potential drug targets against chronic forms. Curiously, upon infection *Toxoplasma* recruits the host cell's endoplasmic reticulum (ER) into close proximity to the parasitophorous vacuole (PV), although the reasons for these high affinity interactions are not completely understood. Here, we show that *Toxoplasma* exploits ER-phagy in infected cells, which culminates in lysosomal amino acid accumulation. Remarkably, the viability of chronic forms is drastically decreased when this accumulation of amino acids is blocked, and the ER function and homeostasis are restored. Finally, by restricting the

amino acid intake in chronically infected mice, the number of chronic forms in the brain was significantly decreased and neurological alterations caused by infection were restored. Our findings identified the underlying mechanisms used by *Toxoplasma* to exploit host ER and lysosomal pathways to acquire nutrients during chronic infection in the brain. These findings provide new insights into strategies for the treatment of toxoplasmosis.

B704/P3080

Evidence that a Palladin-VASP interaction influences Enteropathogenic *E.coli* (EPEC) actin-rich pedestal height

K. M. Bruzzini, S. Mann, J. A. Guttman; Simon Fraser University, Burnaby, BC, Canada

Enteropathogenic *E.coli* (EPEC) generate actin-rich protrusions at sites where the bacteria bind to their host cells. These structures enable the bacteria to rise off of the membrane surface onto "actin pedestals" that provide motility to the bacteria when docked to their host cells. The actin and VASP-associated protein palladin has been previously found to be important for actin structure formation in other systems and can functionally replace the Arp2/3 complex at branched actin comet tails generated by *Listeria monocytogenes*. Here we examined the role of palladin during EPEC infections. We found that palladin colocalized with actin throughout the stalk of EPEC pedestals. When an actin-binding mutant of palladin was transfected into host cells, pedestals appeared normal. However, when a VASP binding mutant form of Palladin was examined, pedestal height was impaired suggesting that a Palladin-VASP interaction may be needed for efficient pedestal height.

B705/P3081

The role of Myristoylated, Alanine-rich C-kinase Substrate (MARCKS) in Macrophages upon stimulation of Toll-like receptor 4.

J. Issara-Amphorn[1,2], V. Sjoelund[2], A. Nita-lazar[2]; [1]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, [2]Functional Cellular Networks Section, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD

MARCKS (Myristoylated Alanine-rich C-kinase Substrate) is a membrane protein expressed in many cell types including macrophages and functionally related to cell adhesion, phagocytosis and inflammatory responses. LPS (lipopolysaccharide), one of the strongest PAMPs (Pathogen-associated molecular patterns), triggers inflammation via TLR4 (Toll like receptor 4). During TLR4 stimulation, MARCKS is phosphorylated by PKC (Protein kinase C) resulting in its release to the cytosol followed by activation of inflammatory signal transduction pathways. The phosphorylation site of MARCKS (phospho-MARCKS) on serine (S163) may have a regulatory role, since we found it changes upon TLR stimulation. Serine phosphorylation serves as a key regulator of many physiological processes including innate and adaptive immune responses. Although MARCKS and the formation of phospho-MARCKS in macrophages have been described, the cellular role(s) of MARCKS and phospho-MARCKS in regulating macrophage functions remain unclear. As a proof-of-concept study, we activated macrophages with LPS with or without addition of a PKC inhibitor. We found that PKC inhibition substantially decreased macrophage IL6 and TNF cytokine production. In addition, confocal microscopy showed that MARCKS and phospho-MARCKS increased localization to endosomes in response to LPS stimulation. Moreover, CRISPR-CAS9 mediated knockout of MARCKS in macrophages downregulated TNF and IL6 cytokine production following LPS stimulation, suggesting a role for MARCKS in inflammatory responses. Our comprehensive

proteomics analysis comparing LPS-stimulation of WT and CRISPR-CAS9 MARCKS knock-out macrophages provides insight into the involvement of MARCKS in specific biological processes including inflammatory responses and cytokine-mediate signaling pathways, uncovering the specific proteins involved in regulating MARCKS activity upon LPS stimulation. The discovery of the mechanism by which MARCKS contributes to the inflammatory response may provide new strategies to manipulate inflammation-related diseases.
This research was supported by the Intramural Research Program of NIAID, NIH.

B706/P3082

***Legionella pneumophila* effector Lpg2409 binds PI(3)P and targets host mitochondria**

M. Grossi, C. Pike, M. Neunuebel; University of Delaware, Newark, DE

Mitochondria are dynamic organelles in both their constant energy turnover and in regulation of their structure through elongation/fission and mitophagy. *L. pneumophila* effectors can exploit these functions by fragmenting mitochondria to promote its survival. Here we present evidence that Lpg2409 targets mitochondrial components. HEK293T lysate pulldowns followed by mass spectrometry revealed that mCherry-Lpg2409 associates with Mic60, a protein that regulates mitochondrial structure, and ubiquitin ligase scaffolding proteins. In HeLa cells, EmGFP-Lpg2409 colocalized with endogenous Mic60 and induced cytosolic cytochrome C, yet no cell death occurred. Lpg2409 bound PI(3)P *in vitro,* and mCherry-Lpg2409 colocalized with EGFP-2xFYVE, indicating that Lpg2409 localized to PI(3)P-enriched vesicles such as autophagosomes. Indeed, Lpg2409 colocalized with endogenous autophagy markers p62 and ubiquitin in transfected HeLa cells. Lastly, immunostaining of host cells infected with *L. pneumophila* expressing HaloTag-Lpg2409 demonstrate that Lpg2409 likely accumulates later during infection. These findings suggest Lpg2409 targets key components involved in mitochondrial architecture and autophagy.

B707/P3083

Human B-lymphoblastoid Cells Exhibit Vastly Different Susceptibility to Shigatoxin 2

G. J. Chaudry[1], A. K. Strickland[1], O. N. Tran[1], S. N. Asin[1], A. Melton-Celsa[2]; [1]Science & Technology, 59th Medical Wing, San Antonio, TX, [2]School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD

Diarrheal infections are typically food-borne and occur globally. Two bacteria that can cause hemorrhagic colitis (HC) are *Shigella dysenteriae* and Shigatoxigenic strains of *Escherichia coli* (STEC) such as serotype O157:H7. The pathologic and clinical severity of diarrheal disease due to STEC is quite variable, but in extreme forms it involves the potentially fatal hemolytic uremic syndrome (HUS). Shiga toxins produced by *S. dysenteriae* and STEC are crucially important in HUS pathogenesis. STEC strains produce Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2), or both. Stx1 is essentially identical to the Shiga toxin produced by *S. dysenteriae* type 1. Stx2 has the same mode of action as Stx1, but there is significant amino acid divergence from Stx1. In addition, Stx2 is more frequently associated with HC and HUS. Both Stx1 and Stx2 are AB5 toxins with potent cytotoxic activity. The toxins bind to the cellular receptor globotriaosylceramide (Gb3) via the B pentamer, then are trafficked in a retrograde manner to the endoplasmic reticulum (ER). From the ER the toxin enzymatic moiety (A subunit) enters the cytosol. The Stx1 and Stx2 A subunits are N-glycosidases that target the 28S rRNA. This action renders the 28S rRNA nonfunctional, arrests protein synthesis, and kills the cell. The work reported here aimed to assess

susceptibility of 90 individual-specific human B-lymphoblastoid cell lines to Stx2. Toxicity assays were done at 37°C for 72 hours. Of the cell lines evaluated, many showed complete resistance to Stx2 (IC_{50} > 500 ng/mL), while others showed very high sensitivity (IC_{50} ~ 0.007 ng/mL). To discern gene expression and other genetic differences between the highly sensitive and resistant cell lines, we performed RNA seq in triplicate on 15 of these cell lines. The RNA seq results revealed that in the Stx2-resistant group 37 genes had >5-fold increased expression compared to Stx2-sensitive group. Conversely, 34 genes showed >5-fold increased expression in Stx2-sensitive group. There were 25,506 transcripts with no expression difference between resistant and sensitive cells ($P > 0.05$). We hypothesize that some of the gene expression differences account for the differential Stx2 sensitivity and resistance phenotypes in these lymphoblastoid cells. Work is now in progress to elucidate the steps where there are blocks to Stx2 toxicity in the resistant cell lines.

Prokaryotic Cell Biology

B708/P3084

Identification of bacterial species present in a concrete Pennsylvania bridge

P. Tadley, K. Hansen, J. Colosi, L. K. Goudsouzian; Biology, DeSales University, Center Valley, PA.

Concrete is the most prevalent material used in the construction of roads and bridges. Concrete corrosion is a leading cause of structural damage that can lead to bridge failure. While concrete contains low biomass, some bacteria can survive and even grow in the low oxygen, highly alkaline environment. There is little understanding of the microbial communities present in concrete, and whether those microorganisms present contribute to its degradation. The Pennsylvania Department of Transportation supplied a concrete sample collected from Pier 8 of the Minsi Trail Bridge (PennDOT #48-3007-0022-0071) in Bethlehem, Pennsylvania during renovation. Under stringent aseptic conditions, DNA was extracted from the interior of the sample using a silica suspension. A control sample (washed glass beads) was extracted alongside the concrete sample, to identify and rule out contaminant taxa which were introduced by the laboratory environment. A surface sample of the concrete was collected to determine the effectiveness of sterilization before collection of the interior sample. The V3-V4 region of the 16S gene was amplified in all samples and sequenced using massive parallel sequencing (next generation sequencing) with modifications for low-biomass samples. *Firmicutes*, *Proteobacteria*, and *Actinobacteriota* were the three most abundant phyla found in the Minsi Trail Bridge sample. The three most abundant individual bacterial genera were *Streptococcus*, *Corynebacterium*, and *Pajaroellobacter*. Eleven of the bacteria sequenced contained the gene *narG*, a subunit of nitrate reductase alpha. This enzyme causes the degradation of nitrogen used in concrete, thus contributing to its corrosion. Remarkably, 18S sequencing of the concrete surface sample yielded multiple examples of a DNA sequence identified as the eukaryotic midge, *Belgica antarctica*. This insect's range is limited to Antarctica, which leads us to believe that the eukaryote detected during sequencing is, in fact, a novel species of midge with a genome most closely related to that of *Belgica antarctica,* or a species of midge never characterized by genetic means.

B709/P3085

***Sulfolobus acidocaldarius* adhesion pili power twitching motility in the absence of a dedicated retraction ATPase**

A. Charles-Orszag[1], M. van Wolferen[2], S. J. Lord[1], S. Albers[2], D. Mullins[1]; [1]University of California, San Francisco, San Francisco, CA, [2]University of Freiburg, Freiburg, GERMANY.

Type IV pili are ancient and widespread filamentous organelles found in most bacterial and archaeal phyla. They support a wide range of fundamental functions including adhesion to substrates, DNA uptake, self aggregation and motility. In particular, disassembly of type IV pili mediated by PilT-family ATPases allows pilus retraction, which is responsible for twitching motility that is important for surface colonization in bacteria. Archaea do not possess homologs of a PilT retraction ATPase, and it was therefore hypothesized that archaeal cells are not capable of twitching motility. Yet, recent microscopic observations of live Sulfolobus acidocaldarius showed that cells exhibit short-range surface-motility that resembles bacterial twitching motility. S. acidocaldarius is a thermoacidophilic crenarcheon that encodes three different type IV pili systems with non-redundant functions. While archaella are rotating type IV pili that allow swimming motility, UV-inducible pili promote cell aggregation and DNA exchange upon exposure to UV light, and adhesion pili are responsible for surface attachment and biofilm formation. Here, using a combination of automated single cell tracking at high temperature, high-temperature fluorescence imaging, and genetic manipulations, we demonstrate that S. acidocaldarius exhibits bona fide twitching motility, and that this behavior depends specifically on adhesion pili. Our results show that adhesion pili are capable of retraction in the absence of a PilT retraction ATPase. While PilT-independent pilus retraction was previously observed in bacteria such as C. crescentus, our study suggests that in archaea, which do not encode a dedicated retraction ATPase, this might be a common mechanism for pilus retraction. Our results also suggest that the ancestral type IV pilus machinery was capable of dynamic retraction and extension cycles in the absence of a dedicated retraction ATPase.

B710/P3086

Cellular dynamics of early stages of *P.aeruginosa* infection by giant bacteriophages

O. S. Sokolova[1], A. Moiseenko[2], Y. Danilova[2], D. Antonova[3], I. Vishnyakov[3], M. V. Yakunina[3]; [1]Biology, MSU-BIT University, Shenzhen, CHINA, [2]Biology, Moscow M.V.Lomonosov University, Moscow, RUSSIAN FEDERATION, [3]Peter the Great St. Petersburg Polytechnic University, St. Petersburg, RUSSIAN FEDERATION.

The large-scale use of antibiotics in medicine and agriculture has led to the emergence and rapid spread of antibiotic-resistant strains of pathogenic bacteria. The problem is complicated by the fact that 1) the spread of antibiotic-resistant strains is faster than the development of new antibiotics; 2) numerous strains resistant to several antibiotics at once (multidrug-resistant strains) are emerging; 3) antibiotics used to fight a number of bacteria are highly toxic to humans. The natural enemies of bacteria, lytic bacteriophages, have evolved many molecular mechanisms over the course of evolution that allow them to successfully and rapidly infect bacteria, evolve in them, suppressing or modifying important cellular processes, and ultimately destroying infected bacteria. PhiKZ-related phages infect a wide range of bacteria, including clinically important pathogens of the genera Pseudomonas, Yersinia and Salmonella, which are characterized by resistance to antibiotics. In this regard, phiKZ and related phages are considered components of phage cocktails for the treatment of infections caused by these bacteria. To develop more effective and safe antibacterial agents based on these phages and the proteins encoded

by them, it is necessary to study the development of phage infection within bacterial pathogens. Here, we used RT-PCR, fluorescent *in situ* hybridization (FISH), cryo-electron microscopy and electron tomography to study the morphology of early stages of bacteriophage infection of Pseudomonas *aeruginosa* cells. The maturation of the pseudo-nucleus was traced in short intervals for 40 min after infection and revealed the continuous spatial separation of the phage and host DNA. Immediately after ejection, phage DNA was located inside the new-identified round compartments (RC); we used cryo-EM to demonstrate how the RC are organized. At a later infection stage, phage DNA was replicated inside the pseudo-nucleus; in the mature pseudo-nucleus, a saturated internal network of filaments was observed. This network consisted of DNA bundles in complex with DNA-binding proteins. On the other hand, the bacterial nucleoid underwent significant rearrangements during phage infection, yet the host DNA did not completely degrade until at least 40 min after phage application. Energy dispersive x-ray spectroscopy (EDX) analysis revealed that, during the infection, the sulfur content in the bacterial cytoplasm increased, which suggests an increase of methionine-rich DNA-binding protein synthesis, whose role is to protect the bacterial DNA from stress caused by infection. Analytical EM was supported by the Innovation Committee of Science and Technology of Shenzhen, China (20200828172651001) and cryo-EM by the Russian Science Foundation (20-04-60258).

B711/P3087

***Legionella pneumophila* infection of human macrophages retains golgi structure but reduces o-glycans**

R. E. Heineman[1], Y. Fu[2], V. Macwan[2], M. R. Terebiznik[2], R. E. Harrison[2]; [1]Cells and Systems Biology, University of Toronto, Toronto, ON, CANADA, [2]University of Toronto, Toronto, ON, CANADA.

Legionella pneumophila is an accidental pathogen that replicates intracellularly within the *Legionella*-containing vacuole (LCV) in macrophages. Within an hour of infection, *L. pneumophila* secretes effectors to manipulate Rab1 and intercept ER-derived vesicles to the LCV. The downstream consequences of interrupted ER trafficking on the Golgi of macrophages are not clear. We examined the Golgi structure and function in *L. pneumophila*-infected human U937 macrophages. Intriguingly, the size of the Golgi in infected macrophages remained similar to uninfected macrophages. Furthermore, TEM analysis also did not reveal any significant changes in the ultrastructure of the Golgi in *L. pneumophila*-infected cells. Drug-induced Golgi disruption impacted bacterial replication in human macrophages, suggesting that an intact organelle is important for bacteria growth. To probe for Golgi functionality after *L. pneumophila* infection, we assayed glycosylation levels using fluorescent lectins. Golgi O-glycosylation levels, visualized by the fluorescent cis-Golgi lectin, *Helix pomatia* agglutinin (HPA), significantly decreased over time as infection progressed, compared to control cells. N-glycosylation levels in the Golgi, as measured by L-PHA lectin staining, were not impacted by *L. pneumophila* infection. To understand the mechanism of reduced O-glycans in the Golgi we monitored UDP-GalNAc transporter levels in infected macrophages. The solute carrier family 35 membrane A2 (SLC35A2) protein levels were significantly reduced in *L. pneumophila*-infected U937 and HeLa cells and *L. pneumophila*growth in human macrophages benefitted from GalNAc supplementation. The pronounced reduction in Golgi HPA levels was dependent on the translocation apparatus DotA expression in bacteria and occurred in a ubiquitin-independent manner. Thus, *L. pneumophila* infection of human macrophages maintains and requires an intact host Golgi ultrastructure despite known interference of ER-Golgi trafficking. Finally, *L. pneumophila* infection blocks the formation of O-linked glycans and reduces SLC35A2 protein levels in infected human macrophages.

B712/P3088

Characterization of a putative trehalose biosynthetic pathway in *Salmonella typhimurium*

C. J. Larmore, A. R. Storm; Western Carolina University, Cullowhee, NC.

Trehalose is a non-reducing disaccharide composed of two glucose monomers. In living organisms, this sugar is predominantly connected by an α-1,1-glycosidic linkage. Trehalose has been shown to benefit many bacterial species by protecting biomolecules from denaturation, increasing cell wall stability, and serving as a carbon source. Genes believed to encode a trehalose biosynthetic pathway, including the enzymes maltooligosyl trehalose synthase (StMTSase) and maltooligosyl trehalose trehalohydrolase (StMTHase), have been identified and shown by knock-out studies to be essential in *Salmonella typhimurium*. However, the protein products have yet to be experimentally characterized. Because *S. typhimurium* is of medical concern and becoming increasingly antibiotic resistant, better understanding these essential genes will improve our understanding of potential targets for treatment. The objective of this study was to characterize the putative StMTSase and StMTHase enzymes to determine if this pathway is functional. Comparative sequence analysis revealed that both StMTSase and StMTHase are highly conserved with homologous proteins that have been previously characterized and retain amino acid residues known to be both catalytic and involved in substrate binding. The enzymatic activity of StMTSase was experimentally tested using the Nelson-Somogyi (NS) method for detection of reducing sugars, as MTSase enzymes convert reducing maltooligosaccharides into the non-reducing sugar maltooligosyl trehalose. StMTSase was shown to utilize maltohexaose as a substrate and function optimally at 40°C and a pH of 7.0. Overall, our results suggest that the putative trehalose biosynthetic pathway identified in *S. typhimurium* is functional and that StMTSase behaves optimally under similar conditions to MTSase enzymes in other bacterial species. Moving forward, enzyme activity assays will be performed across increasing substrate concentrations to determine the maximum rate at which StMTSase catalyzes its reaction (V_{max}) and its affinity for maltohexaose (K_m). Once these key parameters have been determined, the activity of two mutant StMTSase enzymes will be evaluated. One mutant lacks a key catalytic residue (E279), while the other contains substitutions of tyrosine at two positions (M460 and V464) to match binding site residues identified in known functional MTSases.

B713/P3089

Multiple ParA/MinD ATPases Coordinate the Positioning of Disparate Cargos in a Bacterial Cell

L. T. Pulianmackal, J. I. Limcaoco, K. Ravi, S. Yang, J. Zhang, M. K. Tran, M. J. O'Meara, A. G. Vecchiarelli; University of Michigan, Ann Arbor, MI.

In eukaryotes, linear motor proteins govern intracellular transport and organization. In bacteria, where linear motors are absent, the ParA/MinD (A/D) family of ATPases spatially organize an array of genetic- and protein-based cellular cargos. ParA is well known to segregate plasmids and chromosomes, as is MinD for its role in divisome positioning. Less studied is the growing list of ParA/MinD-like ATPases found across prokaryotes and involved in the spatial organization of diverse protein-based organelles, such as Bacterial Microcompartments (BMCs), flagella, chemotaxis clusters, and conjugation machinery. Given the fundamental nature of these processes in both cell survival and pathogenesis, it is unsurprising that the positioning of these cargos has been independently investigated to varying degrees in several organisms. However, it remains unknown whether multiple A/D ATPases can coexist and coordinate the positioning of such a diverse set of fundamental cargos in the same cell. If so, what are the mechanistic commonalities, variation, and specificity determinants that govern the positioning

reaction for each cargo? Here, we find that over a third of sequenced bacteria encode multiple A/D ATPases. Among these bacteria, we identified several human pathogens as well as the experimentally tractable organism, *Halothiobacillus neapolitanus*, which encodes seven A/D ATPases. We directly demonstrate that five of these A/D ATPases are each dedicated to the spatial regulation of a single cellular cargo: the chromosome, the divisome, the carboxysome BMC, the flagellum, and the chemotaxis cluster. We identify putative specificity determinants that allow each A/D ATPase to position its respective cargo. Finally, we show how the deletion of one A/D ATPase can have indirect effects on the inheritance of a cargo actively positioned by another A/D ATPase, stressing the importance of understanding how organelle trafficking, chromosome segregation, and cell division are coordinated in bacterial cells. Together, our data show how multiple A/D ATPases coexist and function to position a diverse set of fundamental cargos in the same bacterial cell. With this knowledge, we anticipate the design of minimal autonomous positioning systems for natural- and synthetic-cargos in bacteria for synthetic biology and biomedical applications.

B714/P3090

Dynamic heterogeneity inside the bacterial cytoplasm

A. Papagiannakis, C. Jacobs-Wagner; Stanford University, Stanford, CA.

When thinking of the bacterial cytoplasm, the nucleoid (DNA with its associated proteins) and the polysomes (mRNAs with their associated ribosomes) come to mind. In *Escherichia coli*, the nucleoid has been shown to spread through more than half of the cell volume[1], whereas the actively translating ribosomes account for about one fifth of the total proteome in fast growing cells[2]. Yet, despite their size and abundance, little is known about the biophysical interactions between these two macromolecules during the cell division cycle of *E. coli*.

Using quantitative epi-fluorescence microscopy in combination with microfluidics for long-term observation of single *E. coli* cells, we found that in this seemingly symmetric rod-shaped organism, the ribosomes and the DNA exhibit remarkable spatial and temporal heterogeneity which cannot be explained by free cytoplasmic diffusion and equilibrium processes. Our work proposes a self-organizing biophysical mechanism for chromosome segregation and positioning at the expense of ribosomal homeostasis in bacterial cells, which lack cytoskeleton mediated systems for these essential processes.

References

[1] Gray WT, Govers SK, *et al.* (2019) *Cell* 177(6):1632-1648.e20

[2] Dai X, Zhu M, *et al.* (2016) *Nat Microbiol* 2:16231

B715/P3091

Regulation of sporulation by proteolysis in *Bacillus subtilis*: mechanisms of the adaptor protein YjbA

J. A. Lyda, E. P. Riley, J. D. Tibocha-Bonilla, K. Zengler, K. Pogliano; UCSD/SDSC, NIST, La Jolla, CA

Previous studies in the Pogliano lab have demonstrated that the adaptor protein YjbA is responsible for the metabolic reprogramming of the forespore during sporulation in *Bacillus subtilis* however, the targeting mechanisms of this adaptor protein and the benefits of the metabolic reprograming are still unknown. Here we examine the function of an N-terminal sequence of one such substrate CitZ, which is conserved in CitZ homologues from endospore forming bacteria. *B. subtilis* responds to severe nutrient limitation using a process called sporulation wherein the vegetative cell differentiates into 2 cell types, eventually producing a spore resistant to oxidative stress and antibiotics. This highly durable spore

makes it difficult to treat infections created by other similar bacterial pathogens that also produce spores. During the sporulation process, the vegetative cell divides at one pole, giving rise to the smaller forespore and the larger mother cell. The mother cell then engulfs the forespore in the process called engulfment, nurturing it in its cytoplasm until maturation. Once the spore is mature, the mother cell lyses, releasing the spore. Research from the Pogliano lab has shown that the forespore and mother cell become metabolically differentiated, with metabolic enzymes including the TCA cycle and enzymes required to produce metabolic building blocks being lost from the forespore, which thereby depends on the mother cell for macromolecular synthesis. We have shown that these enzymes are degraded from the forespore during development by one of the cell's core proteases, ClpCP, and identified a forespore expressed adaptor protein, YjbA. To identify the targeting mechanisms of YjbA we made use of chimeras of CitZ, a degraded metabolic enzyme from the TCA cycle, and the bacterial-two-hybrid assay. We also employed a metabolic model of a sporulating cell to simulate the consequences of the metabolic reprogramming of the forespore. Our results suggest that YjbA does not recognize a specific pattern on its substrates but rather likely activates or enhances ClpCP activity in the developing forespore, thereby initiating metabolic differentiation.

B716/P3092

Green Syntheses and Characterizations of Silver Nanoparticles for Food Safety Applications

J. Gonzales[1], A. Geremew[2], L. Carson[2]; [1]Prairie View A&M University, Prairie View, TX, [2]Cooperative Agriculture Research Center, Prairie View A&M University, Prairie View, TX.

There is a growing demand and application of nanoparticles in various industries such as electronics, cosmetics, food safety, optics, and medicine. Green synthesis of silver nanoparticles (AgNPs) using medicinal plants has been investigated due to the presence of phytochemicals that play a role in the reduction process. Thus, this study aimed to synthesize and characterize AgNPs using aqueous leaf extracts of *Salvia blepharophylla* and *Salvia greggii*, and to assess their antimicrobial activity on the multidrug-resistant species of bacteria, *Escherichia coli, Salmonella typhimurium, Listeria*, and *Staphylococcus aureus*. The formation of AgNPs was confirmed using UV-vis spectroscopy as the reaction solution changed from a light brown to a darker brown. The particle stability and sizes were determined using the Anton Paar Litesizer. Agar well diffusion method was used to measure zones of inhibition (ZOI) to determine the antimicrobial properties of the AgNPs. Preliminary data indicated that AgNPs sizes were between 80-92 nm, and 80-84 nm, using extract of *S. blepharophylla* and *S. greggii*, respectively. Additionally, zeta potentials of AgNPs synthesized using *S. blepharophylla* ranged from -23 to -26 mV, and -20 to -25 mV using *S. greggii.* Overall results showed that *E. coli, S. typhimurium, Listeria,* and *S. aureus* were inhibited by AgNPs synthesized using the extract of *S. blepharophylla* with ZOIs were 0.9-1.2 mm, 0.9-1.1 mm, 0.3-1.3 mm, and 0.95-1.25 mm, respectively. Additionally, *E. coli, S. typhimurium, Listeria,* and *S. aureus* were inhibited by AgNPs synthesized using the extract of *S. greggii* ZOIs were 1-1.15 mm, 0.9-1.3 mm, 1-1.4 mm, and 1-1.3 mm, correspondingly. *S. aureus* seemed to be the most sensitive bacteria overall, against both plant-based AgNPs.

B717/P3093

Characterization of Multidrug Resistant Bacteria from the Lane College Soil

J. Wright, L. Allen, M. Van Stry; Lane College, Jackson, TN

Antibiotics are substances that eliminate or slow down the growth of bacteria. Beta-lactam antibiotics, such as penicillin, are antibiotics that contain a beta-lactam ring that inhibits bacterial cell wall biosynthesis. Bacteria are becoming resistant to antibiotics being that antibiotics are highly prescribed when people seek health care. My goal for this project is to isolate and characterize soil bacteria that are resistant to antibiotics. I hypothesize that the bacteria present in the soil on Lane College campus is resistant to beta-lactams. Furthermore, I hypothesize that these bacteria have a beta-lactamase gene, which is an enzyme that inactivates the bacteria by cleaving the beta-lactam ring. I collected soil samples from Lane College campus and cultured the bacteria on two nutrient agar plates with ampicillin and cultured over night at room temperature. For further characterization, I then chose 3 different colonies from the plates (S1A, S1B, and S2A) and streaked them on to new nutrient agar plates with ampicillin. The strains were tested for drug resistance using the Kirby-Bauer method. They were incubated overnight at room temperature. I then streaked separate nutrient agar plates from all 3 colonies and added antibiotic disks to each plate (ampicillin, penicillin, amoxicillin, and streptomycin) with E. coli as a control group. Strains S1A, S1B, and S2A were resistant to ampicillin, penicillin, and amoxicillin, but were sensitive to streptomycin. We then further characterized by Gram staining and morphological observation. All samples were Gram negative and the cells were a coccus shape. I am planning to use the 16S rDNA gene to identify the bacteria using Nanopore next generation sequencing, and to look for the presence of beta-lactamase genes by whole genome sequencing.

Muscle Structure, Function, and Disease

B719/P3094

A small internal deletion in Titin yields transient cardiogenic defects followed by ion channel-dependent cardiomyopathy and arrhythmia

X. Jiang[1,2], O. T. Ly[3,4], H. Chen[3], Z. Zhang[5], B. A. Ibarra[1,2], M. A. Pavel[3], A. Sridhar[3,6], D. Tofovic[3], A. Swick[1,2], R. Marszalek[6], F. Navales[1,2], Y. Gao[5], D. Darbar[3,6], A. Saxena[1,2]; [1]Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, [2]University of Illinois Cancer Center, Chicago, IL, [3]Division of Cardiology, University of Illinois at Chicago, Chicago, IL, [4]Department of Biomedical Engineering, University of Illinois at Chicago, Chicago, IL, [5]Department of Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL, [6]Department of Physiology, University of Illinois at Chicago, Chicago, IL.

Atrial fibrillation, the most common arrhythmia, has been occasionally shown to correlate with congenital heart defects, but causal relationships are not well-defined. Additionally, it is unclear how adult cardiac function might compensate for developmental defects and, in doing so, mask the potential for adult atrial fibrillation. Here, we deleted just 9 amino acids (Δ9) within the structural protein Titin in both zebrafish and human induced pluripotent stem cell-derived atrial cardiomyocytes (hiPSC-aCMs), revealing new connections between cardiac development and disease. We found that *ttna*$^{\Delta 9/\Delta 9}$ homozygous zebrafish embryos' atrial morphology is perturbed and accompanied by reduced cardiac function. Surprisingly, this function recovers within a few days of embryonic development, and most *ttna*$^{\Delta 9/\Delta 9}$ embryos reach adulthood and are externally indistinguishable from wild-type clutchmates. However, *ttna*$^{\Delta 9/\Delta 9}$ adults exhibit persistent atrial enlargement and weak atrial contraction, and a subset

of adults demonstrate atrial fibrillation with a near-absence of fibrosis. Patch-clamp assays and shotgun proteomics revealed that *ttna*$^{\Delta 9/\Delta 9}$ adult hearts have substantial ion channel remodeling in response to the Δ9 deletion, and inhibiting the function of potassium channel Kv7.1 in *ttna*$^{\Delta 9/\Delta 9}$ embryos accelerates developmental recovery of atrial contraction. Upon recapitulating the Δ9 deletion in hiPSC-aCMs, we found increased expression of Kv7.1 and impaired interactions between Kv7.1 and its regulatory subunit KCNE1, resulting in increased I_{Ks} current. Suppression of I_{Ks} rescues arrhythmia and partially restores contractility. Taken together, these findings reveal how a small deletion in Titin unexpectedly contributes to ion channel dysfunction, demonstrating how subtle developmental abnormalities can recover functionally yet increase the risk of subsequent adult cardiac disease.

B720/P3095

Visualizing sarcomere and cellular dynamics to improve cell transplantation and fusion in striated muscle

M. Gotthardt[1], J. Hüttemeister[1], M. Radke[1], F. Rudolph[1], C. Fink[1], S. Lehnart[2], E. Wagner[2]; [1]Max-Delbrueck Center, Berlin, GERMANY, [2]University Medical Center Göttingen, Goettingen, GERMANY.

The giant striated muscle protein titin integrates into the developing sarcomere to form a stable myofilament system that is extended as cells fuse. The logistics underlying myofilament assembly and disassembly have started to emerge with the possibility to follow labeled sarcomeric components. Here, we generated the mCherry knock-in at titin's Z-disk to study skeletal muscle development and remodeling. We find that titin integration into the sarcomere is tightly regulated and its unexpected mobility facilitates a homogenous distribution of titin after cell fusion - an integral part of syncytium formation and maturation of skeletal muscle. Implantation of titin-eGFP myoblasts into the muscle of mCherry-titin mice reveals how cells fuse and contribute to the continuous myofilament system across cell boundaries. Unlike in immature primary cultures, titin proteins are retained at the proximal nucleus and do not diffuse across the whole syncytium. These findings have implications for future cell-based therapies of skeletal muscle disease.

B721/P3096

Identification of PAKAP as a Novel Regulator of Myoblast Fusion in Vertebrates

A. Hazak, S. Sinha, G. Zarfati, K. Huk, E. Ozer-Partuk, O. Avinoam; Department of Biomolecular Sciences, Weizmann Institute Of Science, Rehovot, ISRAEL.

Myoblast cell fusion is an essential process in skeletal muscle development and regeneration. However, the molecular machinery that drives membrane fusion in myoblasts remains incompletely understood. One of the challenges in studying cell fusion is that it is a rare and transient event, making it difficult to identify the proteins involved. We have recently found that ERK1/2 inhibition induces synchronous myoblast differentiation and fusion in culture, making it possible to capture large amounts of fusing myoblasts. Additionally, we found that simultaneous inhibition of ERK1/2, which is required for primary myoblast proliferation, and P38, which is required for myoblast differentiation, uncouples differentiation and fusion, leading to the accumulation of differentiated unfused cells. I used mass spectrometry proteomics mapping to identify proteins that are specifically enriched during cell-to-cell fusion, comparing the differentiated unfused and fused cells. This analysis pointed to Paralemmin A-kinase anchor protein (PAKAP), a protein of unknown function, as a potential regulator of myoblast fusion. PAKAP is expressed as a natural fusion protein between Paralemmin-2 (PALM2) and the

downstream gene A-kinase anchor protein 2 (AKAP2). Interestingly, paralemmins are involved in membrane dynamics and filopodia formation, while AKAPs can bind to the regulatory subunit of protein kinase A (PKA) and direct it to a specific cellular location. Filopodia formation and PKA signaling are known to be involved during myoblast fusion. However, the exact mechanism remains unknown. To test whether PAKAP is essential for myoblast fusion, we generated a PAKAP knockout (KO) in C2C12 myoblast cell line and found that KO cells have a severe fusion failure phenotype. Immunofluorescence and membrane proteomics experiments showed that PALM2 and PAKAP localize to membrane protrusions on the surface of fusing cells. Taken together, these experiments suggest that PAKAP is specifically involved in myoblast fusion, possibly by directing PKA signaling to the plasma membrane, where it might activate PALM2 to generate fusogenic filopodia. We are currently testing this exciting hypothesis by live-cell imaging. Hence, our work will provide new insight into the mechanism of myoblast fusion as a paradigm for other cell fusion processes.

B722/P3097

Klhl41 plays a critical role in er homeostasis in skeletal muscle

T. Moretti, J. Essner, J. Kim; Iowa State University, Ames, IA.

Nemaline myopathy (NM) is a congenital skeletal muscle disorder characterized by hypotonia, muscle weakness, and atrophy. Thirteen genes have been linked to NM, the majority encoding the components of the sarcomeric thin filament. Within the NM genes, KLHL40, KLHL41, and KBTBD13 belong to the Kelch-like gene family whose gene products affect protein turnover, bringing a different mechanistic perspective to the disease. The Kelch-like proteins are involved in ubiquitination of target proteins as a substrate-recognizing component of a cullin3 RING ubiquitin ligase. Here we report a novel role of KLHL41 in ER homeostasis. When we knocked down the expression of KLHL41 in C2C12 myotubes, it led to an increase in several ER proteins. In particular, levels of Sarco/endoplasmic reticulum Ca2+-ATPase 1 (SERCA1) and ryanodine receptor (RYR) increased several folds. Overexpression of a recombinant KLHL41 reduced SERCA1 levels considerably, suggesting that KLHL41 is critical for maintaining SERCA1 levels. We generated zebrafish klhl41 knockout animals using CRISPR-Cas9 technology. There are two klhl41 orthologs (klhl41a and klhl41b) in zebrafish. klhl41a knockout (KO) alone caused no evident phenotype, while klhl41b or klhl41a/b KO caused strong phenotypes. The klhl41b and klhl41a/b mutants presented with disorganized and thinner myofibers, actin aggregation, reduced muscle integrity, and smaller somite size. In addition, levels of SERCA1 increased over three folds in klhl41 KO embryos. Furthermore, the cisternae of the sarcoplasmic reticulum (SR) were enlarged in the mutants. We conclude that KLHL41 is crucial for the organization of the sarcomeric thin filament as well as the homeostasis of the SR. Our experiments now focus on understanding the molecular mechanism by which KLHL41 affects SERCA1 and other SR proteins in skeletal muscle.

B723/P3098

Defining the differential function, localization, and protein interaction partners of wild type and expanded PABPN1, an RNA binding protein associated with a late onset muscle disease

A. T. Mezzell, K. E. Vest; Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, Cincinnati, OH.

The polyadenylate binding protein nuclear 1 (PABPN1) is an RNA binding protein that facilitates multiple steps in RNA processing. PABPN1 protein contains an amino-terminal alanine tract of unknown function.

Small expansions of the polyalanine tract cause the late-onset disease oculopharyngeal muscular dystrophy (OPMD), which affects craniofacial and proximal limb muscles and causes drooping eyelids, trouble swallowing, and loss of mobility. OPMD pathology remains poorly understood and no pharmacologic treatments are available. Therefore, a deeper understanding of the function, localization, and protein binding partners of both wild type and expanded PABPN1 is needed. Expanded PABPN1 forms nuclear aggregates, which have been implicated in OPMD pathology. However, soluble alanine-expanded PABPN1 interacts with a different subset of proteins than wild type PABPN1, suggesting that alanine expansion impacts PABPN1 function. We aim to define the protein binding partners of wild type and expanded PABPN1. Most previous models used to study PABPN1 and expanded PABPN1 over expressed the proteins, which can impair RNA binding protein function. We have generated stable muscle cell lines containing PABPN1 fused with the promiscuous biotin ligase TurboID under the control of an inducible promoter to allow for native levels of expression. PABPN1 is predominantly nuclear, with a small fraction located in the cytoplasm. We found that overexpression of TurboID fusion constructs results in mislocalization of both wild type and expanded PABPN1 to the cytoplasm. When we expressed the wild type PABPN1-TurboID fusion at endogenous levels, the protein localized normally and labeled known PABPN1 binding partners. When we expressed expanded PABPN1 at endogenous levels, we found that it is not detected in the cytoplasm suggesting that it is mislocalized to another compartment. Our future studies will focus on comparing wild type PABPN1 to alanine expanded PABPN1. We will extend our assays to explore aberrant localization of expanded PABPN1 and determine how mislocalization affects interaction with PABPN1 protein binding partners. Our findings will expand the knowledge of PABPN1 function and provide additional insight into the mechanisms that cause OPMD.

Defining Therapeutic Targets and New Therapeutics-for Diverse Diseases

B724/P3099

Laser Delivery of Optogenetic Therapy in Murine Pain Models

A. Fijany[1,2], D. Narcisse[3], R. Benkowski[2], A. Tripathy[3], N. Al-Adli[1], S. Mohanty[3]; [1]TCU School of Medicine, Fort Worth, TX, [2]Designplex Biomedical, LLC, Fort Worth, TX, [3]Nanoscope Technologies, LLC, Bedford, TX.

Introduction: Pain is an essential sensation that occurs due to tissue damage. However, it can occur chronically and become debilitating for individuals. Therapies for pain conditions are costly and complicated by adverse events (often related to off-target effects). Targeted treatments to areas involved in pain perception with a specific and easily modulated therapy can avoid these pitfalls. Optogenetics allows site-specific and temporally precise modulation of genetically targeted cells expressing an opsin protein. Traditionally, gene therapies have been delivered by viral vectors. However, previous studies have demonstrated that viral vectors can induce a maladaptive immunologic response that may be responsible for tissue degeneration in a dose-dependent manner.[1] In our own experience, we have experienced greater control with laser gene delivery in a manner that is superior to that seen in the viral vector studies.

Methods: Nanoscope Technologies developed an opsin with high sensitivity to red light and a significant depolarizing effect when activated named Multi-Characteristic Opsin (MCO). Using an injection of functionalized gold nanorods (fGNRs) and MCO-encoding DNA, we were able to induce the expression of MCO in target areas of the nervous system by illuminating them with concentrated bursts of attuned light. We then used the wireless Optogenetic Pain Modulator (OPM) device developed by Designplex Biomedical to stimulate the newly expressed MCO with precise control of the frequency, intensity, and

repetition rate of optogenetic stimulation in experimental models of pain. We used a model of acute inflammatory pain in the form of a formalin test in which optogenetic stimulation was used to mitigate pain in stereotyped pain behaviors. Sciatic nerve ligation served as a model of chronic neuropathic pain; we stimulated neurons related to pain information processing and measured the changes in the reflex response of a mice's hind paws to mechanical allodynia.
Results: We demonstrated that optogenetic stimulation of MCO-sensitized inhibitory neurons in the CNS leads to a significant reduction in acute pain reactions and a significant increase in the applied force threshold for paw withdrawal in previously hypersensitive limbs secondary to nerve ligation.
Conclusion: Our results support modulation of the inhibitory pathways within the CNS as a viable alternative for inhibiting chronic neuropathic pain. Given the need for reliability, portability, and convenience for pain treatment to be therapeutically effective and advantageous, the ease of application and easily customized optogenetic stimulation inherent to our approach make it ideal for future application in the field of neuromodulation.

B725/P3100

Peroxisome Proliferator Activated Receptor Alpha Regulates Interplay between Senescence, Mitochondrial Functions and Autophagy in Retinal Cells

J. Blasiak[1], M. Hytti[2], A. Koskela[2], S. Felszeghy[2], K. Kaarniranta[2]; [1]University of Lodz, Lodz, POLAND, [2]University of Eastern Finland, Kuopio, FINLAND.

Age-related macular degeneration (AMD), an eye disease is a world-wide emerging problem, but exact mechanisms of its pathogenesis is poorly known. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) encoded by the *PPARG1* gene is a master regulator of mitochondrial biogenesis and an important factor in antioxidant defense. We have created a model to study mechanisms of AMD pathogenesis including mice with knockout in the *PPARG1* gene and a series of lines of retinal pigment epithelium (RPE) cells derived from induced pluripotent stem cells (iPSCs) obtained from AMD patients. The aim of our study was to determine the expression of genes involved in mitochondrial functions, autophagy and senescence in *PPARG1*-KO mice and RPE-iPSCs as compared with respective controls. We also assessed the influence of age on the observed effects. Gene expression was evaluated by RT-PCR, immunochemistry and immunoblotting. Mitochondrial metabolism was evaluated by the Seahorse FX test. Bafilomycin a1 (Baf1) and MG-132 were used as autophagy inhibitor and inducer, respectively. The 3-months- or 1-year-old *PPARG1*-KO mice were derived from the C57BL/6J strain. We observed difference between the expression of *HMGB1* (*High Mobility Group Box 1*) and *CDKN2A (Cyclin Dependent Kinase Inhibitor 2A*) (senescence), *SDH* (*Succinate Dehydrogenase Complex Subunit D), MT-CO1* (*Mitochondrially Encoded Cytochrome C Oxidase I*) (mitochondria)*, MTOR* (*Mechanistic Target of Rapamycin Kinase*)*, MAP1LC3B* (*Microtubule Associated Protein 1 Light Chain 3 Beta*)*, SQSTM1* (*Sequestosome 1*) and *PRKN (Parkin RBR E3 Ubiquitin Protein Ligase*) (macroautophagy and mitophagy) genes in the RPE cells from KO animals and AMD patients. Observed effects were more pronounced in 1-year- than in 3-months-old animals. The PGC-1α deficiency results in altered mitochondrial metabolism, accelerated decline of the expression of autophagy-related genes with age and alterations in mitochondria- and senescence-related genes in RPE cells. The mice with knockout in the PGC-1α-encoding gene and RPE-iPSCs obtained from AMD patients can be a useful complex model to investigate the role of impaired mitochondrial and autophagic functions associated with cellular senescence in AMD pathogenesis.
This work was supported by National Science Centre, Poland grant number 2017/27/B/NZ3/00872.

B726/P3101

***Maackia amurensis* Seed Lectin (MASL) Increases Movement Velocity of Mice with TNFα Induced Rheumatoid Arthritis**

K. L. Hamilton[1], A. A. Greenspan[2], C. M. Laugier[2], A. J. Shienbaum[3], B. Fischer[4], A. Bottaro[4], G. S. Goldberg[2]; [1]Georgetown University, Washington, DC, [2]Rowan University, Stratford, NJ, [3]Keystone Medical Laboratories, Throop, PA, [4]Rowan University, Camden, NJ.

Up to 70 million people around the world suffer from rheumatoid arthritis. Current treatment options have varied efficacy and can cause unwanted side effects. New approaches are needed to treat this condition. Sialic acid modifications on chondrocyte receptors have been associated with arthritic inflammation and joint destruction. The transmembrane mucin receptor protein podoplanin (PDPN) has been identified as a functionally relevant receptor that presents extracellular sialic acid motifs. PDPN signaling promotes inflammation and invasion associated with arthritis and, therefore, has emerged as a target that can be used to inhibit arthritic inflammation. *Maackia amurensis* seed lectin (MASL) can target PDPN on chondrocytes to decrease inflammatory signaling cascades and reduce cartilage destruction in a lipopolysaccharide induced osteoarthritis mouse model. Here, we investigated the effects of MASL on rheumatoid arthritis progression in a TNFα transgenic (TNF-Tg) mouse model. Results from this study indicate that MASL can be administered orally to ameliorate joint malformation and increase velocity of movement exhibited by these TNF-Tg mice. These data support the consideration of MASL as a potential treatment for rheumatoid arthritis.

B727/P3102

Potential antioxidant, cytotoxic, antiviral, and antibacterial activities of extracts from three species of *Piper*, an African pepper

A. Meyer[1], S. Akbar[2], D. Sheridan[1], H. Cogburn[1], J. A. Fernandez Romero[1], B. Rafferty[1], A. Koroch[1], C. Priano[1]; [1]Borough of Manhattan Community College, New York, NY, [2]Brooklyn College, Brooklyn, NY.

Peppers of the genus *Piper* are commonly used as spices and traditional medicines in Africa. The aim of this study was to identify total phenolic content and antioxidant, cytotoxic, antiviral, and antibacterial activities of extracts from seven commercial sources of *P. nigrum, P. guineense,* and *P. borbonense.* Crude extracts were prepared in different solvents from dried crushed seeds for each sample. Total phenolic compounds and antioxidant capacities were determined using the Folin-Ciocalteu assay and the 2,2'-azino-bis -ABTS assay. The cytotoxicity in different cell lines and viral entry inhibition by aqueous extracts were explored using the XTT colorimetric assay and the SARS-CoV-2 Delta variant pseudoviral model. Antibacterial activity was determined by growing *Escherichia coli* and *Bacillis subtilis* in microplate cultures in the presence or absence of each aqueous extract and monitoring growth by spectrophotometry. Variations in total phenolic content and antioxidant capacity between samples and extraction solvents were observed. Samples with high total phenolics exhibited the highest antioxidant capacity. The SARS-CoV-2 Delta variant pseudoviral model in HeLa ACE-2 cells showed half-maximal effective concentrations (EC_{50} values) between 0.7 and 3.7 mg/mL. The half-maximal cytotoxic concentration and EC_{50} ratio (selective index) showed promising viral entry inhibition in four of seven extracts with selective indexes between 8.2 and 14.9. Aqueous extracts from *P. borbonense* showed the best antiviral selectivity. The cytotoxicity in Caco-2 cells showed that most of the aqueous extracts did not decrease cell viability, with no dose-response observed. Whereas *P. guineense* and *P. borbonense*

extracts inhibited growth of *B. subtilis*, there was no activity observed against *E. coli*. Differential results are possibly due to differences in bacterial cell wall structure. Samples of *P. borbonense* that exhibited the highest suppression of *B. subtilis* growth had relatively high phenolic content and antiviral activity. The data collectively support a scientific basis for traditional health benefits of *Piper* extracts and warrant further investigation into the actions of specific phenolic compounds present in these extracts and their potential biological activities.

B728/P3103

Reduction of senescence of chondrogenic progenitor cells by Fisetin in knee osteoarthritis

A. Aggarwal[1], J. Jacob[1], A. Aggarwal[2]; [1]Postgraduate Institute of Medical Education & Research Chandigarh India, Chandigarh, INDIA, [2]Postgraduate Institute of Medical Education & Research Chandigarh India, CHANDIGARH, INDIA.

Introduction: Knee osteoarthritis is a debilitating disorder that results in the degradation of articular cartilage. Not only chondrocytes are affected, but the stem cells are also prone to oxidative stress and cellular senescence. Not only the senescence is restricted to the cells but is propagated via Senescence associated secretory phenotype (SASP) that contributes to overall degradation of the cellular matrix. The Objective was to study the effect of Fisetin, the natural flavonoid on the cellular senescence in the chondrogenic progenitor cells (CPCs) and the impact on the overall regeneration of articular cartilage. **Methods**: Chondrogenic progenitor cells were isolated and cultured from the medial tibial plateaus of knee osteoarthritis patients that were clinically and radiologically diagnosed according to the American College of Rheumatology criteria. The cells were evaluated for the cellular senescence using SA-β galactosidase assay. IC_{50} value was calculated using MTT assay and cells were treated with fisetin for 24 hours. Senotherapeutic activity of fisetin was assessed post treatment by staining the cells with SA-β galactosidase and evaluating the cellular senescence genes using real time PCR (p16, p21, p53, and p38MAPK) and proteins using Western Blot. In addition, the matrix degradation enzymes MMP-9 and MMP-13 as well as IL-1 β was evaluated at both gene and protein levels. **Results**: Determination of cell viability upon 24 hours treatment with natural drug fisetin, using a concentration ranging from 5µM-100µM revealed that fisetin was not cytotoxic and safe in this range of concentration (99.5%-86.03% viability; p=0.61). Treatment of CPCs with fisetin at a concentration of 100µM significantly decreased the senescence index compared to the untreated control (42.93%, p=0.01). With higher doses of fisetin treatment i.e., 50µM and 100µM, gene expression of p53 (50µM: p=0.005, 100µM: p<0.0001) and p38 (50µM: p=0.04, 100µM: p<0.007) was significantly downregulated. Protein expression of p53 and p38 was further verified by western immunoblots supported the decline in the cellular senescence proteins post treatment. Similarly, SASP related gene expression of MMP-9, MMP-13 and IL-1β was significantly downregulated at high dose of fisetin. However, significant downregulation of protein expression of MMP-9 (p=0.04) and IL-1β (p=0.03) was observed at 100 µM. **Conclusion**: Overall, fisetin was found to be effective in reducing the burden of senescence in CPCs and SASP including proteases MMP9 and MMP13 associated with cartilage matrix degradation and inflammatory cytokine IL-1β.

B729/P3104

Arctiin attenuates high cholesterol-induced bone loss by decreasing oxidative stress in osteoclasts

G. Li, **H. Choi**; Biological Sciences, University of Ulsan, Ulsan, KOREA, REPUBLIC OF.

Arctiin, arctigenin glycoside is a bioactive ingredients of *Arctium lappa L* that has been used as a medicininal herb in Asia. Arctiin-containing plants has been considered to be effective to treat rheumatic arthritis, inflammatory disease, and infection in traditional oriental medicine. Our previous studies demonstrated that high cholesterol diet induced bone loss in mice along with oxidative stress. We hypothesized arctiin may protect high cholesterol diet-induce bone loss via decreasing oxidative stress. To investigate action mechanism of arctiin to protect from cholesterol-induced bone loss, arctiin was orally treated at a dose of 10 mg/kg in atherogenic diet-fed C57BL/6 male mice for 6 weeks. In vitro, the anti-oxidant effects of arctiin was evaluated in osteoclasts (OCs) that was stimulated by 7-ketocholesterol (7-KC). Arctiin decreased the number and activity of 7-KC stimulated OCs and inhibited autophagy via disruption transcription factor EB (TFEB) nuclear localization by reducing oxidized TFEB signaling pathway. Arctiin decreased cytosolic ROS to decrease its effects on OCs. These findings provide a therapeutic potential of arctiin to prevent from cholesterol-induced bone loss by decreasing oxidative stress. Funding sources: Basic Science Research Programs (2021R1A2C1003423; 2018R1A2B6001276; 2014R1A6A1030318) of the NRF grants funded by the Korea government

B730/P3105

Insulin like growth factor -binding protein 2 ameliorates glucose imblanced state by regulating Amp-activated kinase signal pathway in Thioacetamide injured rat ovary model

D. Lee[1,2], H. Park[1,2], K. Na[1], J. Seok[3], Y. Kim[4], G. Kim[1,2]; [1]CHA University, Seongnam-si, KOREA, REPUBLIC OF, [2]PLABiologics.Co.Ltd, Seongnam-si, KOREA, REPUBLIC OF, [3]University of Chicago, Chicago, MD, [4]CHA Bundang Medical Center, Seongnam-si, KOREA, REPUBLIC OF.

Glucose is an important energy substrate for the generation of ATP for the metabolic and physiological functions of the ovary. However, impaired glucose metabolism, insulin resistance is an important cause of ovarian dysfunction and mitochondrial dysfunction due to the central importance of glucose as a source of energy. Glucose dysregulation can interfere with reproductive hormones and cause reproductive diseases including polycystic ovary syndrome and ovarian insufficiency. PD-MSCs could have therapeutic effects on metabolic dysfunction and ovarian function via activating AMPK signaling pathway. The objectives of this study are to analyze the therapeutic effects of PD-MSCs on glucose metabolism in TAA-injured rat model and to investigate the correlation between glucose metabolism and ovarian function through AMPK signaling.The expression of glucose metabolism factors (e.g.,AMPK,SIRT1,GLUT4,IR,IRS1,PI3K,AKT) were significantly increased in PD-MSCs compared to TAA group (*$p<0.05$). The follicular development hormones, AMH, FSH and E2 levels were also higher than TAA group. In addition, the expressions of mitochondrial dynamics factors and ATP uptake were significantly increased in PD-MSCs transplanted group than TAA group (*$p<0.05$). Ex vivo cocultivation of TAA treated ovaries and PD-MSCs or treatment with recombinant IGFBP2(100ng/ml) and IGF-1 inhibitor(100ng/ml) increased glucose metabolism marker and ovarian function and insulin signal and Autophagy marker. Notably, the mRNA expression of AMPK,SIRT1,GLUT4,IGFBP2,IGF-1,IRS1,IR was significantly increased in cocultivation with PD-MSCs group and recombinant treat group (*$p<0.05$). In addition, the protein level of AMPK,GLUT4 was significantly increased in PD-MSCs group and IGFBP2 treated group(*$p<0.05$). Also, the mRNA expression of Autophagy marker(BECN1,LC3b) was significantly

increased in PD-MSCs group and IGFBP2 treaed group but mTOR expression was opposite. These findings suggest that secreting IGFBP2/IGF-1 by PD-MSCs plays an important role in glucose metabolism, mitochondrial dysfunction,ovarian function,Autophagy and offer new insight into the effects of stem cell therapy for reproductive systems.

B731/P3106

Comparative analysis of co-cultured amniotic cell conditioned media with cell-free amniotic fluid reveals differential effects on epithelial-mesenchymal transition and myofibroblast activation

N. Liu[1], C. M. Bowen[1], M. M. Shoja[1], K. L. Castro de Pereira[1], L. P. Dongur[1], S. Antonio[1], W. K. Russell[1], T. C. Broderick[2], J. H. Fair[1], **W. S. Fagg**[1]; [1]University of Texas Medical Branch, Galveston, TX, [2]Merakris Therapeutics, Research Triangle Park, NC

Myofibroblast activation is a cellular response elicited by a variety of physiological or pathological insults whereby cells initiate a coordinated response intended to eradicate the insult and then revert back to a basal state. However, an underlying theme in various disease states is persistent myofibroblast activation that fails to resolve. Based on multiple observations, we hypothesized that the secreted factors harvested from co-culturing amniotic stem cells might mimic the anti-inflammatory state that cell-free amniotic fluid (AF) elicits. We optimized an amnion epithelial and amniotic fluid cell co-culture system, and tested this hypothesis in the context of myofibroblast activation. However, we discovered that co-cultured amniotic cell conditioned media (coACCM) and AF have opposing effects on myofibroblast activation: coACCM activates the epithelial-mesenchymal transition (EMT) and stimulates gene expression patterns associated with myofibroblast activation, while AF does the opposite. Intriguingly, extracellular vesicles (EVs) purified from AF are necessary and sufficient to activate EMT and inflammatory gene expression patterns, while the EV-depleted AF potently represses these responses. In summary, these data indicate that coACCM stimulates myofibroblast activation, while AF represses it. We interpret these findings to suggest that coACCM, AF, and fractionated AF represent unique biologics that elicit different cellular responses that are correlated with a wide variety of pathological states, and therefore could have broad utility in the clinic and the lab.

B732/P3107

Purinosome assembly in various biological models of DNPS disorders

V. Barešová, O. Součková, V. Škopová, S. Kmoch, M. Zikánová; Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic

Purinosome assembly in various biological models of DNPS disordersObjectives: Purine metabolism is an integral part of human life, as purines are essential compounds that provide energy to the body and make up our genetic code. Purines are synthesized in de novo purine synthesis (DNPS) by coordinated action of six enzymes to catalyze ten consecutive reactions. These enzymes are organized in a multi-enzyme structure, the purinosome, which is transiently and reversibly assembled in the cell cytoplasm as the demand for purines changes. Disorders of purine metabolism include rheumatological, neurodevelopmental, immunological or renal diseases. Their pathogenesis is not fully understood.Methods: We investigated purinosome formation in a purine-depleted media by immunofluorescence labeling of endogenous proteins involved in the DNPS pathway in skin fibroblasts from patients with DNPS disorders. We then performed knockout of genes encoding DNPS enzymes in HeLa cells using GeneArt® CRISPR Nuclease Vector with OFP Reporter Kit and analyzed the ability of

knockout cells to form the purinosome in the same manner. To observe dynamic purinosome formation, we prepared a transgenic C. elegans model carrying the fluorescently tagged DNPS proteins PPAT-1:GFP and F38B6.4 (GART):mCherry.Results: We demonstrated that mutations in skin fibroblasts derived from patients with DNPS, AICAribosiduria and ADSL, PAICS and PFAS deficiencies disrupt purinosome assembly. Purinosome formation was also impaired in HeLa cells where DNPS enzymes were knocked out. Fibroblasts from patients with Lesch-Nyhan syndrome form the purinosome not only in a purine-depleted but also in a purine-rich medium. A similar observation was seen in the HGPRT enzyme knockout HeLa cell line. The purinosome formation was normalized by transfection of wt protein. In the C. elegans model purinosomes colocalized with mitochondria and were strongly formed in the dauer state. We did not observe purinosome formation in L1 and adult states.Conclusion: The mechanism of purinosome assembly and the contribution of various biological, genetic and metabolic factors have not been fully investigated. The aim of our study was to describe the purinosome in different pathological conditions. This characterization will improve our understanding of purine synthesis and regulation and provide better insight into the pathophysiology and possible treatment of purine disorders.

Digestive and Excretory Organs

B733/P3108

A Macromolecular Complex of CFTR, NHERF2, and LPA_2 in Intestinal Epithelium and Its Role in the Pathological Process of Secretory Diarrhea

W. Zhang[1,2,3], S. Kong[2], Y. Zhang[4], A. P. Naren[5]; [1]USDA-ARS, Stoneville, MS, [2]Departments of Pediatrics, University of Tennessee Health Science Center, Memphis, TN, [3]Departments of Physiology, University of Tennessee Health Science Center, Memphis, TN, [4]Department of Bioscience Research, University of Tennessee Health Science Center, Memphis, TN, [5]Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Diarrhea is the second leading cause of death in children under five years old and the leading cause of malnutrition in this age group. The hyperactivation of the cystic fibrosis transmembrane conductance regulator (CFTR) channels in the gastrointestinal tract plays a central role in the pathogenic process of some secretory diarrheas. CFTR forms a macromolecular complex with Na^+/H^+ exchanger regulatory factor 2 (NHERF2) and lysophosphatidic acid receptor 2 (LPA_2) at the apical plasma membrane of intestinal epithelial cells. This study aimed to investigate the roles of CFTR-NHERF2-LPA_2 complex in fluid homeostasis and inflammatory responses in the gut. **Methods:** (1) Study models: Intestinal epithelial cells (human HT29-CL19A cells, mouse m-ICc12 cells), mouse intestinal epithelial tissues, mouse intestine fluid secretion models. (2) Techniques: immunofluorescence imaging, Western blotting, PCR, proximity ligation assay, ELISA, Ussing chamber, etc. **Results:** (1) LPA_2 is a major LPA receptor in human and mouse intestinal epithelial cells. (2) CFTR complexes with NHERF2 and LPA_2 at the plasma membrane of HT29-CL19A cells and m-ICc12 cells. (3) LPA inhibits CFTR channel function through an LPA_2-mediated G_i pathway. LPA substantially reduced the cholera toxin (CTX)-induced and CFTR-mediated intestinal fluid secretion in mice. (4) GRI977143 (a specific LPA_2 agonist) inhibited the forskolin-induced CFTR channel activity in polarized HT29-CL19A cells and mouse intestinal epithelial tissues. GRI977143 significantly decreased the CTX-induced closed-loop intestinal fluid secretion in mice. (5) Knock-down of LPA_2 in HT29-CL19A cells and m-ICc12 cells significantly reduced the gene and protein level of IL-8. GRI977143 also inhibited IL-8 secretion. (6) IL-8 level is decreased in the intestines of LPA_2 knockout mice compared to wild type mice. **Conclusion:** CFTR-NHERF2-LPA_2 complex plays a critical role

in the pathological process of certain secretory diarrheas. In addition to modulating the CFTR-mediated fluid secretion, it also regulates IL-8 secretion from intestinal epithelial cells which could contribute to excessive inflammatory responses in the gut. Specific and potent anti-secretory agents can be useful additions to oral rehydration therapy for secretory diarrheas. **Acknowledgment:** Supported in part by NIH grant R01HL147351.

B734/P3109

Bile Inhibits Bacterial Toxin Activity by Instigating Structural Destabilization and Oxidation

J. E. S. Taylor[1], D. Heisler[1,2], E. Kudryashova[1], D. Kudryashov[1]; [1]Department of Chemistry and Biochemistry, Ohio State University, Columbus, OH, [2]University of Texas Southwestern Medical Center, Dallas, TX.

While bile primarily solubilizes fat in the gut, it also regulates the colonization of the gut microbiome. To counteract this, some bacteria have evolved to modulate the production of virulence factors in response to bile. We speculate that due to their typically high conformational plasticity and low stability, virulence factors are easy targets for the denaturing activities of bile salts. If confirmed, toxin denaturation by bile salts may influence the pathogenic potential of bacteria and justify bile-related adjustments in the expression of virulence factors. In this work, we explored the effects of reconstituted bovine bile, sodium deoxycholate (DOCh), and sodium taurocholate (TCh) on the stability and activity of effector domains of multifunctional auto-processing repeats-in-toxin (MARTX) from *V. cholerae* and *A. hydrophila*. We assessed changes in protein structure and stability using collisional quenching of intrinsic Trp residues and pelleting. Most toxins responded to bile and DOCh treatments by enhanced quenching of Trp fluorescence by acrylamide. We observed increased precipitation of toxins in the presence of DOCh. The effects of bile on solubility were milder and more discriminative, slightly promoting precipitation of some toxins, showing no effect on others, and even improving the solubility of yet others, e.g., actin crosslinking domain (ACD) toxin from psychrophilic *A. hydrophila*, when added above its melting temperature. Upon entry of the host cell cytoplasm, MARTX domains are separated by activation of the cysteine protease domain (CPD). Cleavage of the CPD-containing four- and two-domain constructs was unaffected by DOCh and TCh but was inhibited by whole bile. This inhibition was accompanied by the formation of a high molecular weight protein species that could be reversed by the addition of a reducing agent, as revealed by non-reducing SDS-PAGE. Overall, these observations suggest that bile can compromise the structural integrity of the tested bacterial toxins, instigate their precipitation, and induce oligomerization via reversible cysteine oxidation, justifying the need for bacteria to tune toxin expression levels.

B735/P3110

Basigin is expressed in mouse intestines and feces

J. D. Ochrietor[1], A. Preciado[1], V. J. Ochrietor[1], J. Gelsleichter[1], A. Arikawa[2]; [1]Biology, University of North Florida, Jacksonville, FL, [2]Nutrition and Dietetics, University of North Florida, Jacksonville, FL.

Basigin is a cell-adhesion protein belonging to the immunoglobulin superfamily (IgSF). This ubiquitously expressed protein plays roles in metabolism and immunity, with its most notable functions related to vision, cancer, and COVID-19. Recent studies by this research group showed that Basigin expression in blood vessel endothelial cells of the mouse brain is altered in response to ex vivo exposure to lipopolysaccharide (LPS), which is a component of the Gram negative bacterial outer membrane. Blood

vessel endothelial cells of the brain contribute to the blood-brain barrier (BBB), which may be functionally altered in response to an inflammatory stimulus. Because the intestinal epithelium also forms a barrier, the expression of Basigin in that tissue may also be altered in response to LPS. Therefore, expression of Basigin in mouse intestine, specifically the colon and rectum, was analyzed as a first step in understanding the potential role of the IgSF molecule in acute and chronic inflammation of the tissue. Mouse feces was also assessed because it may serve as a non-invasive method for assessing colon health. Colorectal samples from male and female mice at various post-natal ages were obtained and the contents were removed and served as the fecal sample analyses. Protein lysates were generated from the intestine and feces samples for subsequent quantitative ELISA analyses. In addition, intestines containing feces were fixed in 4% paraformaldehyde and subjected to immunohistochemical analyses. It was determined that male and female intestine samples were similar in expression of Basigin, with the highest concentration expressed at post-natal day 14. Basigin was observed on intestinal epithelial cells, with less prominent expression near the caecum and more prominent expression near the rectum. Basigin was detected in the fecal samples as well, with the highest concentration at post-natal day 21. No correlation between Basigin concentrations in the fecal and intestinal samples was observed. Because of the differential expression of Basigin through the length of the colon, future studies will examine specific regions of the intestine to better understand the gradient of expression within the tissue. Additionally, it would be useful to analyze external fecal pellets, rather than samples isolated from the intestines themselves, as samples isolated from regions near the caecum had less exposure to Basigin-expressing epithelial cells. It can be concluded that Basigin is expressed on both male and female mouse epithelial cells. The usefulness of fecal samples to assess Basigin expression in the colon, and hence the health of the tissue, has yet to be determined.