Plant cell division from the perspective of polarity

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Highlight

Cell division orientation lies at the interface of tissue and cell polarities. The combination of developmental and cell biological approaches has recently brought, and will continue to bring, most progress in this enigmatic area of plant biology.

Abstract

The orientation of cell division is a major determinant of plant morphogenesis. In spite of considerable efforts over the past decades, the precise mechanism of division plane selection remains elusive. The majority of studies on the topic have addressed division orientation from a predominantly developmental or a cell biological perspective, respectively. Thus, mechanistic insights into the links between developmental and cellular factors affecting division orientation are particularly lacking. Here, I review recent progress in the understanding of cell division orientation in the embryo and primary root meristem of *Arabidopsis* from both developmental and cell biological standpoints. I offer a view of multilevel polarity as a central aspect of cell division: On the one hand, the division plane is a readout of tissue- and organism-wide polarities; on the other hand, the cortical division zone can be seen as a transient polar subcellular plasma membrane domain. I argue that a polarity-focused conceptual framework and the integration of developmental and cell biological approaches hold a great promise to unravel the mechanistic basis of plant cell division orientation in the near future.

Introduction

Biological polarity refers to *"persistent asymmetrical and ordered distribution of structures along an axis"* (Cove, 2000). In this sense, multicellular plants are polarized at all levels of organization: Asymmetric subcellular distribution of proteins and other molecules defines cell polarity (Grebe *et al.*, 2001; Ramalho *et al.*, 2021); the arrangement of different cell types and gradients of hormones, peptides, proteins, RNAs or transcriptional signatures can be seen as constituents of tissue polarity (Dolan *et al.*, 1993; Friml *et al.*, 2003; Carlsbecker *et al.*, 2010; Roszak *et al.*, 2021); and ordered distribution of tissues and organs hallmark the polarity of the whole organism.

Except for relatively rare cell fusion events, such as during fertilization, all cells are formed by cell division (See Box 2). In plants, the orientation of cell division is a key determinant of morphogenesis, as plant cells, owing to their rigid cell wall, cannot move within tissues and are thus permanently restricted to their original position, defined at cytokinesis by the position of the division plane (Facette *et al.*, 2019; Müller, 2019).

Plant cytokinesis is orchestrated by the cell plate, a transient endomembrane compartment which forms in the center of the cell. The cell plate grows centrifugally, guided by a cytoskeletal array called the phragmoplast, until it fuses with the plasma membrane at the cortical division site and thus partitions the mother cell into two daughters (reviewed by Boruc and Van Damme, 2015; Smertenko *et al.*, 2017). The cortical division site forms from a broader cortical division zone (CDZ), which is selected prior to cell division and its position determines the division plane. As the CDZ is defined by the specific presence and absence of a set of marker proteins, it is a prime example of a polar domain (Müller, 2019), and the division plane can then be considered a transient axis of cell polarity.

To divide in a developmentally meaningful way, a plant cell thus needs to 1) perceive cues of tissue polarity; 2) interpret such cues by deciding on a particular division plane; and 3) execute this decision by forming the CDZ at the correct position with respect to its own cell polarity determinants (Figure 1). Despite substantial recent progress (see Box 1), the molecular mechanisms governing each of these steps and their relationships are only beginning to emerge.

Here, I review recent progress in the understanding of developmental and cellular factors governing division orientation. I advocate the concept of multilevel polarity as a central aspect of plant cell division and highlight recently discovered factors that might integrate

tissue and cell polarity cues to guide division plane selection. To complement the extensively covered relationships between cell polarity, division orientation and fate in the leaf stomatal lineage (i.a. Shao and Dong, 2016; Muroyama and Bergmann, 2019; Guo *et al.*, 2021), I focus on studies using the *Arabidopsis* embryo and primary root meristem as models here. For general overview of cell polarity and polar proteins not immediately linked to division orientation, I refer the reader to the excellent reviews by Muroyama and Bergmann (2019), Wallner (2020) and Ramalho *et al.* (2021).

Cell division orientation as a readout of global polarity cues

In the absence of signaling inputs, plant cells by default select the division plane resulting in the smallest possible cross wall (the *minimal surface area* rule), or one which aligns with the maximal local mechanical stress (Besson and Dumais, 2011; Louveaux *et al.*, 2016). Nonetheless, the formation of a functional multicellular body requires that cell division planes often deviate from these default rules, and are positioned according to tissue polarity cues instead (Figure 1a). How individual cells perceive larger scale polarity is not known. The decisive cues might in principle be biochemical (e.g. a gradient of a signaling molecule) or mechanical, the latter hypothesis receiving increasing attention (Gorelova *et al.*, 2021). In the following paragraphs, I review the factors that govern division orientation downstream of global polarity perception, yet upstream of CDZ establishment, in different developmental contexts (Figure 1b).

Early embryogenesis

Upon fertilization followed by initial shrinkage, the zygote elongates, its nucleus migrates to the apical region and finally the zygote undergoes a highly asymmetric cell division (see Box 2), producing a 1-cell embryo and a suspensor cell. The unidirectional zygote elongation coincides with, and is dependent on, the formation of a subapical transverse ring of microtubules (MTs), while the organization of F-actin to an apical cap and longitudinal cables appears necessary to sustain nuclear migration (Kimata *et al.*, 2016). The first three rounds of embryo divisions leading to the 2-, 4- and 8-cell stage are geometrically symmetric (but only the latter two follow the *minimal surface area* rule), followed by a round of exemplary asymmetric divisions to reach the 16-cell stage (Yoshida *et al.*, 2014; Vaddepalli *et al.*, 2021). Similarly to the first division of the zygote, the correct orientation of these divisions depends on auxin signaling, cell shape, and actin and MT cytoskeleton. Nonetheless, there is no correlation between nuclear position and division orientation of the 4- and 8-cell embryo cells

(Chakrabortty *et al.*, 2018; Vaddepalli *et al.*, 2021). Auxin signaling in the embryo controls the expression of multiple cytoskeleton-related genes, including the MT-associated IQD6; and *iqd678* mutants display similar defects in MT organization, cell shape and division orientation to auxin response mutants. This suggests a scenario in which auxin signaling exerts its role in determining division orientation by modulating MT dynamics to control cell geometry (Vaddepalli *et al.*, 2021). Such a regulatory module would enable auxin-based tissue polarity cues (Friml *et al.*, 2003) to feed directly feed into cell polarity systems via the control of MTs, and could thus be central to the control of cell division orientation. Interestingly, the establishment of the inner-outer polarity axis is not affected by the auxin response mutants that cause severe defects of division orientation in the embryo (Vaddepalli *et al.*, 2021), supporting the notion that a global, auxin response-independent polarity system is established prior to the 4-cell embryo stage, possibly already in the zygote. Such a universal polarity plan would then be interpreted by various downstream polarity effectors – including the auxin-cytoskeleton-cell shape module controlling the orientation of cell divisions – throughout development (Ramalho *et al.*, 2021; Vaddepalli *et al.*, 2021).

Ground and outer tissue development

During post-embryonic development, both ground tissue cell layers, endodermis and cortex, originate from a single layer of stem cells, the cortex/endodermis initials (CEI). CEIs divide anticlinally (see Box 2), producing a new CEI and a CEI daughter, which subsequently undergoes a periclinal division to produce a cortex and an endodermis cell. The CEI daughter periclinal divisions are regulated by the SHORTROOT (SHR)-SCARECROW (SCR) transcriptional module, a key regulator of inner-outer tissue polarity (Helariutta *et al.*, 2000). The SHR-SCR pathway activates the expression of *CYCD6;1*, encoding a cyclin that is required specifically for periclinal divisions (Sozzani *et al.*, 2010), and *INFLORESCENCE AND ROOT APICES RECEPTOR KINASE* (*IRK*), an LRR kinase with a putative role in suppressing periclinal divisions (Campos *et al.*, 2019). The IRK-GFP fusion protein is polarly localized to the inner-lateral domain in cortex cells, outer-lateral domain in endodermis, and centrally at the apical/basal domains in CEIs (Campos *et al.*, 2019). The SHR-SCR-IRK pathway might thus integrate both tissue-wide and cellular inner-outer polarity cues in the control of division orientation of the ground tissue.

Similarly to endodermis and cortex, the epidermis and lateral root cap (LRC) cell layers originate from common protoderm initials, which alternate between anticlinal and periclinal

divisions to produce daughters that differentiate into epidermis and LRC, respectively (Abrash and Bergmann, 2009). The protoderm initial periclinal divisions are controlled by the NAC transcription factors FEZ and SOMBRERO (Willemsen *et al.*, 2008), other downstream regulators remain so far elusive.

Vascular development

Unlike the ground and outer tissues that divide almost exclusively anticlinally once leaving the stem cell niche, the patterning of the vascular bundle relies on periclinal and radial divisions of the outer protoxylem, protophloem and procambium cells adjacent to the pericycle throughout the meristem (Smet and De Rybel, 2016; Qian *et al.*, 2018; Miyashima *et al.*, 2019; El Arbi *et al.*, 2021). The vascular periclinal divisions are regulated by a signaling pathway centered around two bHLH transcription factors, TARGET OF MONOPTEROS 5 (TMO5) and LONESOME HIGHWAY (LHW), which form a heterodimer that is both necessary and sufficient to trigger periclinal divisions. The activity of TMO5-LHW is controlled by auxin response, while downstream, the heterodimer activates cytokinin (CK) signaling by promoting CK biosynthesis and de-conjugation (De Rybel *et al.*, 2014; Yang *et al.*, 2021). The TMO5-LHW-induced CK is thought to act as a mobile signal between the xylem cells, where the heterodimer is active, and the procambium cells that respond to the TMO5-LHW pathway by switching the division plane from anticlinal to periclinal (De Rybel *et al.*, 2013, 2014, 2016; Ohashi-Ito *et al.*, 2014; Vera-Sirera *et al.*, 2015; Yang *et al.*, 2021). The transcription factors PHLOEM EARLY DOF (PEAR) and DOF2.1 promote vascular periclinal/radial divisions downstream of the CK response (Miyashima *et al.*, 2019; Smet *et al.*, 2019). Nonetheless, only *DOF2.1*, but not the *PEAR*s, are transcriptionally activated by TMO5-LHW. Furthermore, while CK signaling is necessary for the TMO5/LHW-mediated periclinal divisions (De Rybel *et al.*, 2014), and ectopic CK treatment promotes the expression of *PEARs* and *DOF2.1* (Miyashima *et al.*, 2019; Smet *et al.*, 2019), it is not sufficient to trigger ectopic periclinal divisions. Therefore, the relationships between the TMO5-LHW – DOF2.1 and PEAR pathways, as well as the precise role of CK response in vascular cell division orientation, remain to be clarified. A recent report established that PEARs promote periclinal formative phloem pole divisions by activating Rho-Of-Plants (ROP) GTPase signaling (Roszak *et al.*, 2021). This pathway might thus mechanistically link tissue and cell polarities in the regulation of cell division orientation: The *PEAR* genes, being expressed specifically in young phloem pole cells, can be seen as a readout of apical-basal tissue polarity; while a local reduction of active ROP-GTP

levels appears as an early causal step of CDZ establishment (Roszak *et al.*, 2021) and thus a prominent cell polarization event.

Wound healing

Regeneration of wounded plant tissues involves the proliferation of undifferentiated callus cells, followed by re-polarization and re-differentiation following global polarity cues (Sena *et al.*, 2009; Ikeuchi *et al.*, 2019). Recent studies involving laser ablation of single cells have established how wound-induced periclinal cell divisions contribute to root tissue regeneration. The ablation of any cell in the root meristem triggers a periclinal division of the inner neighbor; the outer daughter adopting the fate of the ablated cell to restore tissue integrity (Marhavá *et al.*, 2019). Upon ablation of a cortex cell, the wound-induced periclinal divisions of the endodermis are correlated with the induction of SHR, SCR and CYCD6;1 reporters, and defective in the respective mutants. Likewise, LRC ablation involves the re-activation of, and is dependent on, the SMB-FEZ module. These findings strongly suggest that the wound-induced periclinal divisions are triggered by the same signaling pathways as the formative divisions in the stem cell niche (Marhavá *et al.*, 2019). Additionally, restorative periclinal divisions depend on auxin signaling and involve nuclear migration and localized bulging of the mother cell (Hoermayer *et al.*, 2020), urging comparison with the requirement of the same mechanisms for division orientation in early embryogenesis (Kimata *et al.*, 2016; Vaddepalli *et al.*, 2021; reviewed above).

Overall, it appears that cell division orientation in early embryogenesis, postembryonic root development as well as wound healing relies on distinct, but in many cases overlapping developmental factors (Figure 1b). Nonetheless, the full molecular details of these pathways are still far from understood. In particular, at what points do these developmental factors and tissue polarity cues feed into the cellular mechanisms of CDZ establishment, discussed in the following chapter, remains mostly unknown.

The cortical division zone as a transient polar domain

To successfully execute cytokinesis, a plant cell must i) form the CDZ within its plasma membrane; ii) reroute virtually all vesicle traffic to the cell plate (this has consequences for the localization of polar proteins and thus affects the polarity of the cell itself, see Box 3); iii) guide the growing cell plate to the CDZ; and finally iv) ensure the fusion of the cell plate with the plasma membrane (Boruc and Van Damme, 2015; Smertenko *et al.*, 2017; Livanos and Müller, 2019). In polarity terms put forward by Ramalho *et al.* (2021), CDZ formation

requires distinct establishment, reinforcement and maintenance steps (Figure 1c), and the cell plate guidance can be seen as implementation of the division plane polarity axis. As discussed above, the importance of division orientation for plant development implies that the site of CDZ formation must be precisely regulated and coordinated with existing organ, tissue and cell polarity axes.

Pre-prophase band

One of the earliest indications of the site of CDZ formation is the preprophase band (PPB), a circular array of MTs, actin microfilaments (MFs) and associated proteins that forms before the onset of mitosis and disappears during prometaphase (Pickett-Heaps and Northcote, 1966; Palevitz, 1987; Traas *et al.*, 1995; Smertenko *et al.*, 2017). PPB localization reliably predicts the division plane orientation and genetic or pharmacological interference with MT dynamics causes severe division orientation defects. These observations have led to the long-standing paradigm of the PPB as a central regulator of CDZ establishment (Traas *et al.*, 1995; Rasmussen *et al.*, 2013). This view was further supported by the discovery of TANGLED (TAN), the first known protein thar persistently marks the CDZ throughout cytokinesis and is required for phragmoplast guidance, whose initial CDZ recruitment, but not subsequent residence is MT-dependent (Walker *et al.*, 2007). Nonetheless, not all dividing plant cells form a PPB (Kosetsu *et al.*, 2017). Moreover, the *trm678* mutant, where the lack of PPB was uncoupled from defects in other MT arrays for the first time in *Arabidopsis*, failed to display severe defects in division plane positioning and overall morphology expected to result from the lack of a key division orientation regulator (Schaefer *et al.*, 2017). As the division orientations in *trm678* show increased variability, but are on average correct, the prevalent current understanding is that PPB acts in the reinforcement, rather than establishment, of the CDZ polar domain (Schaefer *et al.*, 2017; Livanos and Müller, 2019). In strong support of this hypothesis, the recruitment of key CDZ maintenance factor Phragmoplast Orienting Kinesin 1 (POK1) (Müller *et al.*, 2006; see below) is delayed and less efficient, but not impaired, in the *trm678* mutant (Schaefer *et al.*, 2017). Importantly though, most studies including Schaefer *et al.*, 2017 judge the presence/absence/localization of the PPB by the localization of MTs only. Therefore, it cannot be formally excluded that in the *trm678* mutant, other PPB components such as MFs (Palevitz, 1987), could still form a PPB-like structure which is indeed required for CDZ establishment (Livanos and Müller, 2019).

Rho of plants (ROP) GTPases

Among the known CDZ-resident proteins (for an extensive overview, see Smertenko *et al.*, 2017 and Livanos and Müller, 2019), POK1 and POK2 stand out as key regulators of CDZ maintenance, as they are necessary for correct division plane positioning and the localization of other CDZ markers including TAN, RanGAP1, and PHGAP1/2 (Müller *et al.*, 2006; Xu *et al.*, 2008; Lipka *et al.*, 2014; Stöckle *et al.*, 2016).

PHGAP 1 and 2 are putative GTPase Activating Proteins of ROP GTPases, the closest homologues of the yeast master polarity regulator Cdc42 (reviewed in Chiou *et al.*, 2017) which have been implied in a plethora of polarity-related processes in plants (Molendijk *et al.*, 2001; Nagawa *et al.*, 2012; Stanislas *et al.*, 2015; Feiguelman *et al.*, 2018; Denninger *et al.*, 2019; Kulich *et al.*, 2020). The identification of ROP-GAPs as important CDZ components hinted at the role of ROPs also in division orientation (Stöckle *et al.*, 2016). In strong support of this hypothesis, ROP signaling has recently been identified as key division orientation regulatory module downstream of tissue polarity during phloem development (Roszak *et al.*, 2021; see above). ROP9 as well as ROP-GEF3 and 5 are transcriptionally upregulated in young phloem pole cells that undergo formative periclinal cell divisions; and multiple different ROP signaling manipulations lead to pronounced division orientation defects. On the subcellular level, both anticlinal and periclinal divisions were preceded and predicted by a specific depletion of a ROP-GEF5 reporter and a ROP activity biosensor from the CDZ (Roszak *et al.*, 2021).

Collectively, these findings suggest that a localized reduction of ROP activity, achieved by a combination of polarized GAP accumulation and GEF depletion, is a key event in CDZ formation. The ROP signaling module might thus be the missing link between the developmental and cellular control of division orientation, reading out both tissue and cell polarity cues and integrating them into the quintessential output of division plane positioning.

IQD proteins

Another major recent advance in the understanding of division orientation is the discovery of IQ67 domain proteins IQD8 and its homologues IQD6 and IQD7 (Kumari *et al.*, 2021). The MT associated, plant-specific IQD family proteins had been proposed to mediate the effects of auxin and calcium signaling on cell and tissue geometry via controlling MT dynamics (Bürstenbinder *et al.*, 2017; Wendrich *et al.*, 2018; Yang *et al.*, 2020). IQD6, 7 and 8 are transcriptionally enriched in dividing cells, where they localize to a broader polar domain

overlapping with the CDZ. IQD8 interacts with POK1, POK2, PHGAP1 and PHGAP2, and the *iqd678* triple mutant displays division orientation defects and occasional aberrant localization of POK1, PHGAP2, and PPB; collectively indicating that IQD6-8 function redundantly early in CDZ specification (Kumari *et al.*, 2021). The cellular defects and overall phenotypes of the *iqd678* and *trm678* mutants are very similar, suggesting that the IQDs might function in CDZ reinforcement via regulating PPB assembly together with TRMs. Importantly, the very same *IQD* genes were independently found to act in asymmetric divisions during early embryogenesis downstream of auxin signaling, a pivotal regulator of tissue polarity (Friml *et al.*, 2003; Vaddepalli *et al.*, 2021; see above). This hints that IQDs might act as universal division orientation regulators that would connect tissue polarity cues to the cellular CDZ positioning machinery, and demands further functional characterization of the IQD family, including the generation and analysis of a full *iqd678* triple knock-out (the *iqd678* line described by Kumari et al. contains by residual *IQD6* and *IQD8* transcripts) and eventually higher order *iqd* mutant combinations.

Other putative cortical division zone establishment factors

Other recently discovered promising candidates for key cellular division orientation regulators belong to the SOSEKI (SOK) family. SOKs are conserved plant-specific proteins that share structural and functional homology with known animal polarity determinants. SOK reporters display remarkable edge-enriched polar localization patterns that are cell type-dependent, but robust towards perturbations of known signaling modules affecting the localization of other polar proteins, indicating they might indeed be part of a core system of coordinates integrating cell-, tissue- and organism polarity axes (Yoshida *et al.*, 2019; van Dop *et al.*, 2020). Crucially, SOK mis-/over-expression is sufficient to trigger cell division orientation alterations (Yoshida *et al.*, 2019). Nonetheless, it is not yet known whether SOKs are also required for proper cell division orientation and/or other polarity-related processes, as single *sok* mutants did not reveal distinct phenotypes (Yoshida *et al.*, 2019), and higher-order mutants have not yet been reported.

The identities and micro- and nano-organization of cellular membranes are largely defined by minor signaling lipids including sphinoglipids, sterols and anionic phosphoinositides (reviewed in Mamode Cassim *et al.*, 2019; Boutté and Jaillais, 2020). The plasma membrane is hallmarked by phosphatidylinositol-4-phosphphate (PI(4)P) (Simon *et al.*, 2016), while phosphatidylinositol-4,5-bisphosphphate ($PI(4,5)P_2$) promotes the asymmetric PM association

of multiple polar proteins involved in auxin transport, root hair formation and protophloem development (Stanislas *et al.*, 2015; Barbosa *et al.*, 2016; Marhavá *et al.*, 2020). It is thus reasonable to hypothesize that the specific lipid signatures of different polar domains might be key cellular determinants of division orientation (Caillaud, 2019). The differential phosphoinositide composition of the cell plate compared to the plasma membrane (Simon *et al.*, 2016) supports the idea that asymmetric lipid distribution is indeed important for cytokinesis. Nonetheless, evidence of a specific lipid composition of the CDZ, or of polar plasma membrane domains defining axial cell polarity, is currently lacking (the enrichment of PI(4)P and PI(4,5)P_2 at apical/basal domains reported by Tejos *et al.* (2014) could not be confirmed by Simon *et al.* (2016)) and the hypothesis thus awaits experimental validation.

MFs are initially localized at the PPB and later excluded from the Actin depleted zone (ADZ), which overlaps with the CDZ; nonetheless, as MFs require MTs for PPB localization, but not vice versa, these dynamic actin rearrangements have been assigned secondary importance for division plane determination (Palevitz, 1987; Liu and Palevitz, 1992; Rasmussen *et al.*, 2013; Smertenko *et al.*, 2017). In another polarized actin rearrangement coinciding with, and potentially regulating CDZ and division plane establishment, MFs are enriched at the apical and basal poles of anticlinally dividing root meristem cells (Collings *et al.*, 2005; Lebecq *et al.*, 2022). Additionally, numerous observations of division orientation defects resulting from interference with MFs in different tissues (i.a. Glanc *et al.*, 2019; Vaddepalli *et al.*, 2021) support a key function of actin cytoskeleton in division plane determination, which should be addressed in more detail in the future.

Future perspectives and concluding remarks

Despite substantial recent progress outlined above, which molecular and cellular factors are involved in the symmetry breaking step of CDZ establishment, and how are tissue and cell polarity cues mechanistically linked in the context of cell division orientation, remains unknown. The auxin-MTs, SHR-SCR-IRK, and PEAR-ROP modules operating during early embryogenesis, ground tissue patterning and phloem development, respectively (Campos *et al.*, 2019; Vaddepalli *et al.*, 2021; Roszak *et al.*, 2021), might have the power to guide division orientation in response to both tissue and cell polarity cues. Further characterization of these pathways should thus be assigned high priority in future investigations.

In addition to these and other hypothesis-driven approaches outlined above, the key factor(s) of CDZ establishment and its developmental control might be identified via traditional gene

discovery techniques, such as forward genetic or transcriptomic identification of downstream targets of the known gene regulatory networks, and proteomic screening for interactors of the known CDZ constituents. In this respect, the recent adoption and optimization of single-cell transcriptomics (Seyfferth *et al.*, 2021; Minne *et al.*, 2022) and proximity labeling (Mair *et al.*, 2019; Arora *et al.*, 2020) in the plant field will likely prove particularly useful. Nonetheless, protein abundance and post-translational modification analysis of dividing cells at single-cell resolution would likely be the true game-changer. Despite the technology to perform such experiments is not yet available, the rapid advances in the single-cell proteomics field (Vistain and Tay, 2021; Ctortecka *et al.*, 2022) hold a great promise for the future.

E. P. Eleftheriou and B.A. Palevitz began the introduction to their 1992 paper in Journal of Cell Science as follows: *"Determination of the division plane is of critical importance in growth, differentiation and morphogenesis in plants. However, despite considerable progress in identifying cell structures that may participate in division plane control, our understanding of the process remains incomplete."* Thirty years later, any research paper in the field could start with the exact same words, as the determination of cell division plane remains one of the biggest open questions of plant cell and developmental biology. The core of this question, how the output of complex developmental signals is integrated with cell and tissue polarity cues and mechanistically translated into the asymmetrical localization of several molecules that define the cortical division zone, lies exactly in between its developmental and cellular aspects. Consequently, the successful strategy to solve this problem will require an integrative approach combining the knowledge and methodology of both fields, and to my best belief also a strongly polarity-oriented mindset. With the advanced methods available today, spanning from single cell transcriptomics to live super-resolution imaging, solving the long-standing mystery of how is plant cell division orientation determined, might be at hand's reach.

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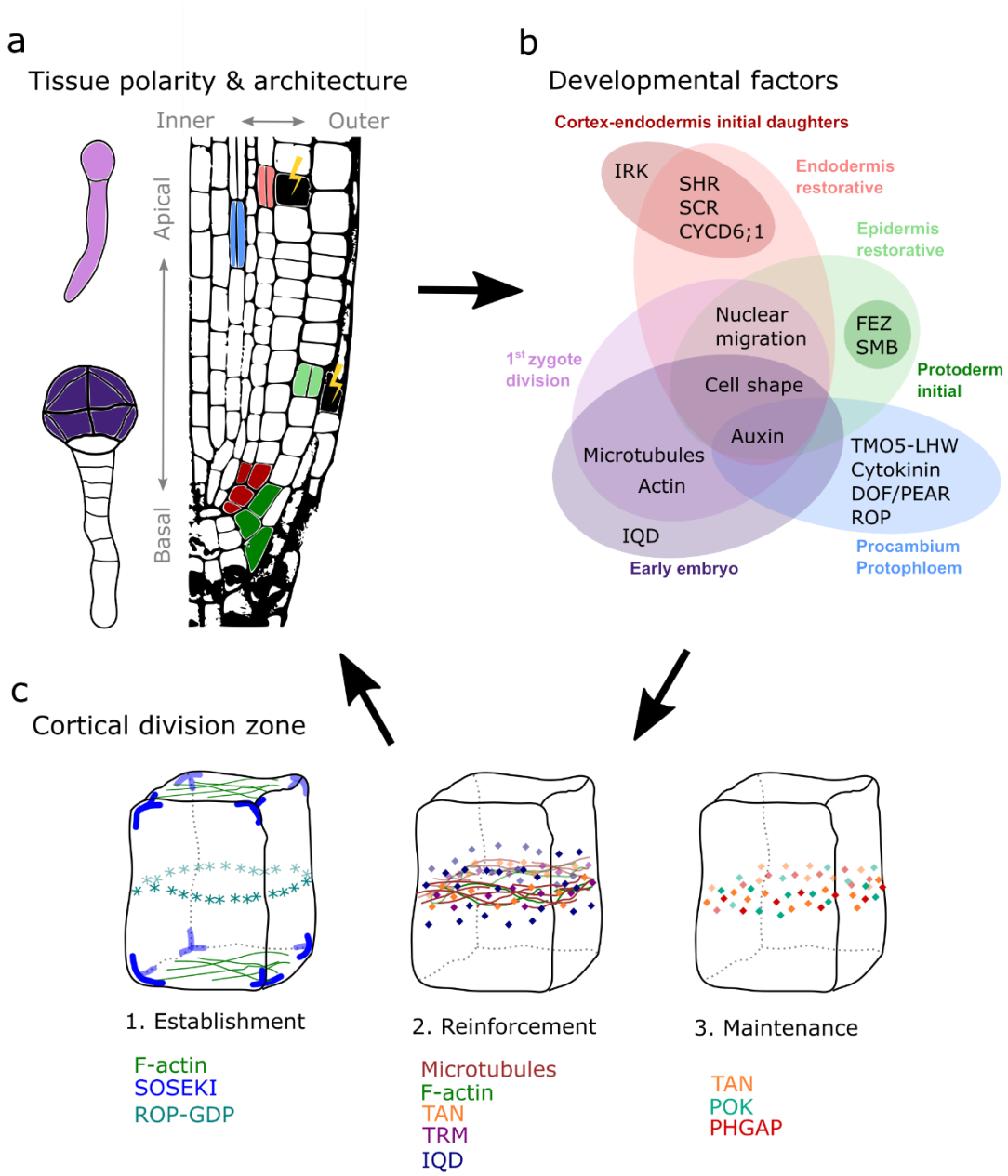

Figure 1: Multilevel polarity as a key determinant as well as result of oriented cell division

a: Schematic representation of the 1-cell and 16-cell embryo and the root apical meristem of *Arabidopsis*. Cells in which division orientation is of particular developmental importance are highlighted in the same color as the captions in **b**. Black cells with lightning icons represent laser-ablated cells.

b: Developmental factors that govern cell division orientation in the cells highlighted in **a,** downstream of tissue polarity cues and upstream of the cytokinesis machinery. Colors of the Venn diagram fields and captions correspond to the cell types highlighted in **a**. Assignment of factors to the Venn diagram fields reflects direct experimental evidence presented in a report(s) discussed in this review.

c: Schematic representation of CDZ in 3 steps characteristic of polar domain formation - establishment, reinforcement and maintenance, with selected (potential) key cellular regulators involved in each of the three steps.

BOX 1: Key developments in understanding the regulation of cell division orientation

Vaddepalli *et al.* (2021) Demonstrate the pivotal roles of auxin signaling, cell geometry, cytoskeletal dynamics and the IQD family proteins in the regulation of oriented divisions in early embryogenesis.

Kumari *et al.* (2021) Establish the role of the microtubule-associated IQD proteins in cortical division zone organization and division orientation in the root meristem.

Roszak *et al.* (2021) Find that cell division orientation during phloem development is controlled by ROP signaling downstream of the PEAR transcription factors, with a local reduction of ROP activity correlating with the site of CDZ establishment.

Marhavá *et al.* (2019) and **Hoermayer *et al.* (2020)** Reveal the induction of periclinal cell divisions as a major wound healing mechanism in the *Arabidopsis* root. They further show that the wound-induced periclinal divisions involve the reactivation of stem cell transcriptional pathways, as well as auxin signaling, nuclear migration and changes in cell shape.

Yoshida *et al.* (2019) and **van Dop *et al.* (2020)** Discover the novel plant-specific SOSEKI family of proteins polarly localized at the corners and edges of cells. They demonstrate that SOSEKI are evolutionarily conserved in the plant lineage, play a role in cell division orientation and are functionally homologous with the animal polarity regulator DISHEVELLED.

BOX 2: Cell division nomenclature

Karyokinesis & cytokinesis

A typical eukaryotic cell division event consists of karyokinesis, or the partitioning of chromatin into two daughter nuclei by mitosis or meiosis; and subsequent cytokinesis, a process in which the cytoplasm is partitioned by physical separation of the daughter cells. The regulation and implications of karyokinesis are out of scope of this review; and I generally use the term "cell division" as a synonym for cytokinesis.

Symmetric vs. asymmetric & proliferative vs. formative divisions

Some authors, i.a. Yoshida *et al.*, 2014, use the terms symmetric and asymmetric to refer solely to volume of the daughter cells, which is identical or very similar in symmetric divisions, and different in asymmetric ones. Other authors, i.a. Abrash and Bergmann, 2009, use these terms more broadly, and consider a cell division to be asymmetric not only when the volume, but also the shape, function and/or fate of the daughter cells are different.

Another related, yet more functional classification distinguishes between formative and proliferative cell divisions. Proliferative divisions produce daughters of identical cell type and fate needed for proliferation and growth, while formative divisions result in cells of different fates and are the driving force of pattern formation (Abrash and Bergmann, 2009). Proliferative divisions are often symmetric, while formative ones are typically asymmetric; these terms are consequently sometimes used as synonyms (i.a. by Müller, 2019; Rodriguez-Furlan *et al.*, 2022).

In the absence of cell migration, cell division and growth remain as the main determinants of patterning and morphogenesis. In this context, the position of each cell becomes as defining as its size, shape, fate or biochemical composition. As the position of each cell is determined exclusively at its birth during cell division by the positioning of the division plane of the mother cell, and two cells cannot have an identical position, one could argue that in the *sensu lato* understanding of the terms as used. i.a. by Abrash and Bergmann (2009), no cell division in a multicellular plant can ever be entirely symmetric or strictly proliferative.

Anticlinal vs. periclinal vs. radial

The relationship between the division plane and the main organ axes defines anticlinal, periclinal and radial divisions. The plane of division is perpendicular to the main organ axis in anticlinal divisions; parallel to both the main axis and organ surface in periclinal divisions,

and parallel to the axis, but perpendicular to the surface in radial divisions. In the primary root, anticlinal divisions thus add cells to existing cell files and contribute to longitudinal growth; radial and periclinal divisions promote radial growth by adding new cell files to existing tissue layers or generating additional cell layers, respectively (Smet and De Rybel, 2016).

Box 3: Reverse perspective: cell polarity in the context of cytokinesis

During cell division, most, if not all vesicle traffic is redirected to the cell plate (Dhonukshe *et al.*, 2006; Reichardt *et al.*, 2007; Richter *et al.*, 2014; Glanc *et al.*, 2018). This implies that most membrane cargos, including polarly localized proteins, localize to the cell plate during and immediately after cytokinesis. As the cell plate-derived new PM domains emerge at adjacent, and thus opposite poles of the two daughter cells, polar cargos will always end up transiently localized to the "wrong" domain in at least one of the daughter cells. The cells must thus possess mechanism(s) to assign the new PM domain the correct identity, and reroute the trafficking pathways to ensure the correct localization of polar proteins after cytokinesis (Geldner *et al.*, 2001; Men *et al.*, 2008; Glanc *et al.*, 2018). This does not however imply the need for symmetry breaking, as the identity of all other PM domains, and thus the general polarity of the cell and its alignment with tissue polarity, are inherited from the mother cell to its daughters (Glanc *et al.*, 2018; Ramalho *et al.*, 2021). Post-cytokinetic protein polarity re-establishment has, like many other aspects of protein and cell polarity, been so far studied mainly on the example of the PIN auxin efflux carriers, and was shown to require Clathrin-mediated endocytosis, *de novo* protein secretion and the PID/WAG AGCVIII kinases, but not basal-to-apical transcytosis or intact cytoskeleton (Boutté *et al.*, 2006; Men *et al.*, 2008; Mravec *et al.*, 2011; Yoshinari *et al.*, 2016; Glanc *et al.*, 2018, 2019). Identifying the mechanisms responsible for post-cytokinetic polarity re-establishment of the ever-growing number of other polar proteins will likely lead to substantial progress in our understanding of plant cell polarity as such: since true symmetry breaking might only happen in the zygote (Ramalho *et al.*, 2021) and the cell and tissue polarity cues are passed on from mother to daughter during each cell division event in a cell-intrinsic manner (Glanc *et al.*, 2018), the post cytokinetic polarity *re-establishment* might be as close to general polarity *establishment* as any cell other than the zygote ever gets.

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