It is time to move: heat-induced translocation events

Tingting Zhu[1,2], Shao-Li Yang[1,2], Ive De Smet[1,2,*]

[1]Department of Plant Biotechnology and Bioinformatics, Ghent University, B-9052 Ghent, Belgium

[2]VIB Center for Plant Systems Biology, B-9052 Ghent, Belgium

[*]Corresponding author: I. De Smet (ive.desmet@psb.vib-ugent.be)

ABSTRACT

Climate change-induced temperature fluctuations impact agricultural productivity through short-term intense heat waves or long-term heat stress. Plants have evolved sophisticated strategies to deal with heat stress. Understanding perception and transduction of heat signals from outside to inside cells is essential to improve plant thermotolerance. In this review, we will focus on translocation of molecules and proteins associated with signal transduction to understand how plant cells decode signals from the environment to trigger a suitable response.

KEYWORDS

Heat stress, translocation, signaling, thermotolerance

INTRODUCTION

An increasingly warmer planet impacts agricultural productivity in the short term (e.g. frequent crop damages caused by intense heat waves) and in the long term (e.g. loss of arable land and shifts of crop areas) [1]. Understanding how plants react to a high-temperature environment is therefore an essential first step to develop climate change-adapted crops. Plants use several strategies to deal with (moderate) heat stress. For example, to avoid heat stress, plants complete the entire reproductive period before summer or flower early in the morning to avoid heat at noon. Additionally, when exposed to high temperature plants coordinate their growth (elongation and upward orientation of organs, referred to as thermomorphogenesis) to enhance cooling [2]. To tolerate heat stress, plants also have heat-resistant buds or trigger leaf abscission by sealing off the vascular system and preventing nutrient or water loss [3–6]. However, temperatures above physiologically endured levels can result in deleterious effects and trigger distinct signaling pathways [7].

Two main strategies can be considered with respect to heat stress response and tolerance. Basal heat tolerance (also called intrinsic heat tolerance) is the natural capacity of plants to tolerate heat by inherent factors. Acquired heat tolerance (also called adaptive heat tolerance) occurs after priming or acclimation triggered by a short pre-exposure to non-lethal heat [8]. The perception mechanisms mediating heat stress responses are still largely unknown and likely to be different from those associated with thermomorphogenesis [2,9]. Different cellular compartments (e.g. plasma membrane, nucleus, chloroplast, endoplasmic reticulum) have the ability to sense temperature changes [10–13]. To efficiently transduce and decode the information from different organelles, one of the molecular mechanisms relies on protein translocation to trigger signaling modules [13,14]. Intracellular distribution of proteins changes dynamically depending on the organelle import and export. The translocation can be controlled by signal sequences (e.g. signal peptide, nuclear localization signal or targeting signal) that target proteins to correct organelles **(Figure 1a)** [15]. For example, all plant HSFs have a nuclear localization signal (NLS); but, a nuclear export signal (NES) only exists in class A HSFs (HSFAs) [16]. In addition, heat-induced protein translocation is also regulated through alternative splicing, protein-protein interactions, writing or erasing post-translational modifications (PTMs), and/or inducing protein cleavage **(Figure 1b)** [17,18]. Besides protein translocation, translocation of small molecules is also crucial to regulate heat responses. Calcium ion (Ca^{2+}), lipid derivates and reactive oxygen species (ROS)

translocate to play roles as second messengers during signal transduction **(Figure 1c)** [10,19–21]. This review focuses on how heat affects localization of molecules and proteins associated with signal transduction (mainly knowledge from *Arabidopsis thaliana*, unless otherwise indicated) to understand how plant cells decode signals from the high temperature environment to trigger heat tolerance responses.

TRANSLOCATION-MEDIATED HEAT STRESS SIGNALING AND THERMOTOLERANCE - FROM PLASMA MEMBRANE TO NUCLEUS

Phospholipids do not only play important structural roles (e.g. membrane fluidity), but also act as essential second messengers in response to stimuli to activate or inactivate downstream targets [22]. Among the lipid second messengers, phosphatidic acid (PA) is generated through two main pathways, namely PHOSPHATIDYLINOSITOL-PHOSPHOLIPASE C-DIACYLGLYCEROL (DAG) KINASE (PI-PLC-DGK) and PHOSPHATIDYLCHOLINE-PHOSPHOLIPASE D (PLD)-dependent pathways (PC-PLD) [23,24] (**Figure 2**). Heat stress elevates the levels of PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE [PI(4,5)P2] at the plasma membrane [24], followed by an enhanced level of a PLC-DGK-dependent or PLD-dependent product PA [10,24–26]. Intriguingly, PLD-produced PA binds to cytosolic GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPC) and helps mediating the nuclear translocation of GAPC via vesicle trafficking upon heat exposure [10,20] (**Figure 2**). PA-bound GAPC accumulates in the nucleus and activates the NUCLEAR FACTOR Y SUBUNIT C10 (NF-YC10) [27,28]. Activated NF-YC10 then promotes gene expression (e.g. *HSFAs* and *HSPs*) that confer basal thermotolerance [10,20,29] (**Figure 2**). In addition, heat stress also induces the nuclear translocation of PHOSPHATIDYLINOSITOL 3-PHOSPHATE (PI3P) produced by PHOSPHATIDYLINOSITOL 3-KINASE (PI3K)-phosphorylated PHOSPHATIDYLINOSITOL. In contrast, heat had no impact on the localization of DAG or PI(4,5)P2 [24,30]. However, the targets of PLC-DGK-produced PA or PI3P remain to be identified (**Figure 2**).

Heat stress induces the transient opening of CYCLIC NUCLEOTIDE GATED CHANNELS (CNGCs), resulting in the increase of Ca^{2+} signals from the plasma membrane, and the activation of kinases (e.g. CALMODULIN-BINDING PROTEIN KINASE 3, CBK3), which in turn activates the chaperone-liberated HSFA1 to promote expression of *HSP* genes [31–33]. In addition, Ca^{2+} influx controlled by heat-activated CNGCs (e.g. CNGC6) is required for the

activation of RESPIRATORY BURST OXIDASE HOMOLOGs (RBOHDs) which possess Ca^{2+}-binding EF-hand motifs [34–36]. ROS activates CNGCs to induce a Ca^{2+} signature, resulting in the activation of HSF-dependent pathways during heat stress [37,38], suggesting that ROS and Ca^{2+} signals generate regulatory circuits with a feedback function. In addition, under normal conditions, the transcription factor ETHYLENE RESPONSE FACTOR 74 (ERF74) is mostly bound to plasma membrane-localized ACYL-COA-BINDING PROTEIN (ACBP) to control ROS homeostasis [21]. Under heat stress, ERF74 is released from the plasma membrane and translocates to the nucleus to directly regulate *RBOHD* expression and improve heat tolerance [21]. However, how heat promotes ERF74 nuclear translocation remains unknown (**Figure 2**).

A NAC transcription factor, rice NAC WITH TRANSMEMBRANE MOTIF 1 (NTM1)-LIKE 3 (OsNTL3), localizes on the plasma membrane with a C-terminal transmembrane domain. Heat stress and endoplasmic reticulum (ER) stress promote the cleavage of OsNTL3, and OsNTL3 then relocates from the plasma membrane to the nucleus. The nuclear-localized truncated form of OsNTL3 without the transmembrane domain directly mediates the expression of *OsbZIP74* under heat stress [12] (**Figure 2**). However, the mechanism by which heat and heat-induced ER stresses promote the cleavage of OsNTL3 remains unknown.

HEAT STRESS SIGNALING TRANDUCED FROM ENDOPLASMIC RETICULUM TO NUCLEUS

Heat induces the unfolded protein response (UPR), the denaturation of proteins when temperature rises, to control plant thermotolerance. This process is associated with the endoplasmic reticulum (ER), which has the capacity to readjust protein folding, and the accumulation of misfolded proteins in the ER is referred to as ER stress. INOSITOL-REQUIRING ENZYME-1 (IRE1) localizes on the ER membrane and functions as an mRNA splicing enzyme. Unfolded proteins and chaperones in the ER lumen interact with IRE1 to activate its enzyme activity upon heat [11,12,39,40]. One of the IRE1 targets, *bZIP60* mRNA, is spliced to an isoform whose protein product lacks a transmembrane domain, allowing bZIP60 to migrate into the nucleus to regulate downstream genes to protect plants from heat stress [11,39,40] (**Figure 2**). Similarly, OsIRE1 induces the splicing of *OsbZIP74* mRNA. The spliced *OsbZIP74* mRNA encodes a nucleus-localized OsbZIP74 to upregulate *OsNTL3* expression, leading to heat tolerance in rice seedlings [12,41]. In contrast, when misfolded proteins accumulate in the ER, the transmembrane domain of

bZIP28 is cleaved via endomembrane-regulated intramembrane proteolysis (RIP) by the Golgi-resident SITE-2 PROTEASE (S2P), and the activated bZIP28 relocates to the nucleus to up-regulate downstream genes [42] (**Figure 2**). However, the mechanism underlying the cleavage of bZIP28 through heat stress or heat-induced ER stress is not clear. The RIP pathway-mediated nuclear-localized bZIP28 not only activates ER chaperone genes but also activates brassinosteroid signaling, which is required for stress acclimation and growth. A heat shock quickly induces dephosphorylation of BES1 to activate brassinosteroid signaling through S2P-cleaved and activated bZIP28 [42].

ER membrane-localized chaperone proteins also contribute to heat stress response. B-CELL LYMPHOMA2 (BCL-2)-ASSOCIATED ATHANOGENE 7 (BAG7) localizes on the ER membrane and interacts with bZIP28. Encountering heat stress, BAG7 undergoes sumoylation, enters into the nucleus, interacts with the transcription factor WRKY DNA-BINDING PROTEIN 29 (WRKY29) and regulates the expression of downstream chaperon genes to mitigate heat-induced ER stress [43] (**Figure 2**).

HEAT-REGULATED PROTEIN TRANSLOCATION FROM CYTOPLASM TO NUCLEUS

In animals, the TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL SUBFAMILY V MEMBER 1 (TRPV1) transiently opens under heat shock, allowing periplasmic Ca^{2+} to enter and bind to CaMs, which associate with the N- and C-terminal cytosolic domains of TRPV1 [44,45]. This induces HEAT SHOCK TRANSCRIPTION FACTOR 1 (HSF1) hyperphosphorylation and activation in the cytoplasm [46]. In plants, CYCLIC NUCLEOTIDE GATED CHANNELS (CNGCs) can initiate a cellular signal like the animal TRPV1 upon heat exposure [47,48]. Additionally, under normal conditions, HSFA1s remain inactive due to the inhibition by HSP70 and HSP90 in the cytoplasm [18,49]. Under heat stress, HSP70/90 bind to unfolded proteins and release HSFA1s [18,49]. Subsequently, heat-activated CALMODULIN-BINDING PROTEIN KINASE 3 (CBK3) phosphorylates HSFA1a [31,32] (**Figure 2**). Consequently, phosphorylated and activated HSFA1a translocates from the cytoplasm to the nucleus and binds heat shock elements in the promoters of *HSP* genes, leading to improved basal thermotolerance [48,50,51]. In parallel, BRASSINOSTEROID-INSENSITIVE 2 (BIN2), a key negative regulator in BR signaling, phosphorylates threonine 263 (Thr263) located in the NLS of HSFA1d, which prevents

nuclear localization and suppresses plant thermotolerance [52]. However, in response to heat, HSFA1d is activated and quickly accumulates in the nucleus through releasing BIN2-mediated phosphorylation and suppression on HSFA1d [52]. The different behavior of phosphorylated HSFA1a and HSFA1d can possibly be attributed to different phosphosites that play unique roles in controlling nuclear localization. In addition, BRI1-EMS-SUPPRESSOR 1 (BES1) is activated, even in the absence of brassinosteroids, through heat-induced BES1 de-phosphorylation and nuclear accumulation, and directly binds to heat shock elements in the promoter of *HSFs* [53]. Plants often encounter repeated and variable heat stress, thus moderate heat stress primes plants to establish a molecular 'thermomemory' that enables plants to withstand a more extreme-heat stress exposure. Interestingly, heat priming promotes high levels of dephosphorylated BES1 in the nucleus during the memory phase, which is essential for sustained activation of heat stress memory-associated genes, such as *HSFA3* [54] (**Figure 2**).

CHLOROPLAST AND VACUOLE LOCALIZATION-REGULATED THERMOTOLERANCE

Among several SMALL UBIQUITIN-LIKE MODIFIER (SUMO) isoforms, SUMO1 and SUMO2 are quickly conjugated to target proteins during short-term heat exposure, dependent on the SUMO E3 ligase SAP AND MIZ1 DOMAIN-CONTAINING LIGASE 1 (SIZ1) [55]. In addition, several proteins are conjugated with SUMO3 in the cytoplasm upon long-term heat stress exposure [55,56]. For example, the heat stress-induced SUMOylation on PHOTOSYSTEM II SUBUNIT R (PSBR) contributes to the maintenance of its chloroplast localization, which is dependent on its chloroplast import efficiency correlated with phosphorylation [56]. The chloroplast localization of these SUMOylation targets under long-term heat stress is partially maintained by the SUMO ligase SIZ1 [56]. SUMOylation is thus required for the import of nucleus-encoded chloroplast proteins under heat stress conditions [56] (**Figure 2**).

The rice genetic locus *THERMO-TOLERANCE 3* (*TT3*), containing two genes (*TT3.1* and *TT3.2*), contributes to transducing heat signals from the plasma membrane to chloroplasts [57]. Upon heat stress, the plasma membrane-localized E3 ligase OsTT3.1 translocates to the endosomes, where OsTT3.1 ubiquitinates the chloroplast precursor protein OsTT3.2. The ubiquitinated OsTT3.2 proteins localize to vacuole for vacuolar degradation. Low levels of OsTT3.2 in chloroplasts are crucial for protecting thylakoids from heat stress, which is essential

for thermotolerance in rice [57]. While ubiquitination is involved in vacuole localization of OsTT3.2, the mechanism for OsTT3.1 moving from the plasma membrane to endosomes under heat stress is still unknown (**Figure 2**).

STRESS GRANULES-RELOCALIZED PROTEINS PROTECT PLANTS FROM HEAT

Upon heat, some proteins relocalize into heat-induced stress granules and processing bodies to maintain balance between storage, translation, and degradation of mRNA. The granules are usually disassembled during the temperature recovery phase, the period during which stalled translation is being restored and plants restart growth processes [58–64]. ACETYLATION LOWERS BINDING AFFINITY 4/5/6 (ALBA4/5/6) contribute to the basal thermotolerance by relocalizing from the cytoplasm into stress granules and processing bodies, and directly binding and recruiting HSF mRNAs (e.g. *DREB2A*, *HSFA2*, *HSFA7b*) into these stress granules and processing bodies to protect them from degradation. Additionally, these ALBA proteins are associated with ribonucleoproteins (RNPs) in the granules [60,65] (**Figure 2**). However, heat-induced stress granules disassemble upon recovery, releasing the proteins and RNA components that are protected by HSPs upon recovery (e.g. HSP101), indicating that some HSPs are responsible for the acquired thermotolerance [66]. Similarly, wheat MULTIPROTEIN BRIDGING FACTOR 1 (MBF1) localizes into heat-induced cytoplasmic stress granules to regulate the translation efficiency of HSPs, which plays a crucial role in wheat heat tolerance [67]. In addition to heat-induced cytoplasmic stress granules, heat stress also induces the formation of plastidial (e.g. chloroplastic) stress granules in plants [59]. One of the heat-induced chloroplastic stress granule proteins, SNOWY COTYLEDON 1 (SCO1), is – together with other proteins, mRNAs and metabolites – sequestered to protect enzymes involved in chlorophyll biosynthesis and RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE (RuBisCo) activity (e.g. RUBISCO ACTIVASE) from heat-caused damage [59]. Interestingly, two RNA binding proteins, RNA-BINDING GLYCINE-RICE D2 and D4 (RBGD2 and RBGD4), undergo prion-like low complexity domain-mediated stress granule formation, and thus promote plant survival in response to heat stress [62]. However, the mechanism through which RGBD2/4 bind to mRNAs and proteins in stress granules and mediate heat tolerance in plants is still unknown.

CONCLUSIONS AND PERSPECTIVES

Because heat becomes more intense and long-lasting during the summer [68], a comprehensive understanding of acquired and basal heat tolerance is crucial. Especially, we should pay more attention to the mechanisms associated with basal heat tolerance to develop climate change-adapted crops. It is apparent that translocation of molecules and proteins plays a key role during heat stress signaling and response, and several mechanisms remain to be uncovered or require more attention. Importantly, coordination between and integration of different signals affecting the translocation of molecules or proteins requires further in depth analyses. For example, how ROS regulatory systems are integrated with Ca^{2+} signaling networks to control the activation and nuclear translocation of plant HSFs. HSFs are central regulators in plant heat stress tolerance in plants. Many details of the downstream events of HSFs have been uncovered, yet there is still limited knowledge on how plants sense heat signals and transduce the signals to intercellular organelles, contributing to thermotolerance. In mammalian cells, trimerization is required for the transcriptional activity of HSF1 [46]. However, in plants it is still unclear whether HSF monomers interact with each other to form a trimer that is hyperphosphorylated and translocated to nucleus, and then bind to heat shock elements of the downstream genes [14]. There are also several possible analogies with cold stress; but, if the same mechanisms occur during heat tolerance remains to be answered. For example, cold induces CALCIUM-DEPENDENT KINASE 28 (CPK28)-regulated phosphorylation of plasma membrane-localized protein NIN-LIKE PROTEIN 7 (NLP7), causing NLP7 to shuttle to the nucleus. This phosphorylation is essential for freezing tolerance in plants [69]. Additionally, the detailed mechanism on heat stimuli-triggered translocation of phospholipids from the plasma membrane to the intracellular compartments associated with heat sensing and thermotolerance, needs to be further explored. Finally, in contrast to other organisms [70], the regulatory mechanisms underlying stress granule disassembly during temperature stress recovery are still largely unexplored in plants.

Altogether, our increasing knowledge on the molecular mechanisms associated with heat stress-mediated translocation signaling will pave a way for developing thermoresilient crops.

ACKNOWLEDGEMENTS

T.Z. is supported by a postdoctoral research mandate from the UGent BOF (BOF22/PDO/113).

REFERENCES

1. Santos FD, Ferreira PL, Pedersen JST: **The Climate Change Challenge: A Review of the Barriers and Solutions to Deliver a Paris Solution**. *Climate* 2022, **10**:75.
2. Ludwig W, Hayes S, Trenner J, Delker C, Quint M: **On the evolution of plant thermomorphogenesis**. *J Exp Bot* 2021, doi:10.1093/jxb/erab310.
3. Delker C, Quint M, Wigge PA: **Recent advances in understanding thermomorphogenesis signaling**. *Curr Opin Plant Biol* 2022, **68**:102231.
4. Shekhawat K, Almeida-Trapp M, García-Ramírez GX, Hirt H: **Beat the heat: plant- and microbe-mediated strategies for crop thermotolerance**. *Trends Plant Sci* 2022, **27**:802–813.
5. Preston JC, Fjellheim S: **Flowering time runs hot and cold**. *Plant Physiol* 2022, **190**:5–18.
6. Zhu T, Fonseca De Lima CF, De Smet I: **The heat is on: how crop growth, development, and yield respond to high temperature**. *J Exp Bot* 2021, doi:10.1093/jxb/erab308.
7. Chen Z, Galli M, Gallavotti A: **Mechanisms of temperature-regulated growth and thermotolerance in crop species**. *Curr Opin Plant Biol* 2022, **65**:102134.
8. Halter G, Simonetti N, Suguitan C, Helm K, Soroksky J, Waters ER: **Patterns of thermotolerance, chlorophyll fluorescence, and heat shock gene expression vary among four Boechera species and Arabidopsis thaliana**. *Botany* 2017, **95**:9–27.
9. Kerbler SM, Philip A. Wigge: **Temperature Sensing in Plants**. *Annu Rev Plant Biol* 2023, **74**.
10. Kim S, Yao S, Zhang Q, Wang X: **Phospholipase Dδ and phosphatidic acid mediate heat-induced nuclear localization of glyceraldehyde-3-phosphate dehydrogenase in Arabidopsis**. *Plant J* 2022, **112**:786–799.
11. Li Z, Tang J, Srivastava R, Bassham DC, Howell SH: **The Transcription Factor bZIP60 Links the Unfolded Protein Response to the Heat Stress Response in Maize**. *Plant Cell* 2020, **32**:3559–3575.
12. Liu X, Lyu Y, Yang W, Yang Z, Lu S, Liu J: **A membrane-associated NAC transcription factor OsNTL3 is involved in thermotolerance in rice**. *Plant Biotechnol J* 2020, **18**:1317–1329.

** This is an elegant example of how a PM-localized transcription factor can be released upon heat stress and relocate to the nucleus to regulate heat responses. Heat stress leads to a truncated form of OsNTL3 without the transmembrance domain to positivly regulate the expression of downstream genes to increase heat tolerance.

13. Hayes S, Schachtschabel J, Mishkind M, Munnik T, Arisz SA: **Hot topic: Thermosensing in plants**. *Plant Cell Environ* 2021, **44**:2018–2033.
14. Ding Y, Yang S: **Surviving and thriving: How plants perceive and respond to temperature stress**. *Dev Cell* 2022, **57**:947–958.
15. Sun J-L, Li J-Y, Wang M-J, Song Z-T, Liu J-X: **Protein Quality Control in Plant Organelles: Current Progress and Future Perspectives**. *Mol Plant* 2021, **14**:95–114.
16. Scharf K-D, Berberich T, Ebersberger I, Nover L: **The plant heat stress transcription factor (Hsf) family: Structure, function and evolution**. *Biochim Biophys Acta - Gene Regul Mech* 2012, **1819**:104–119.
17. Hu Y, Mesihovic A, Jiménez-Gómez JM, Röth S, Gebhardt P, Bublak D, Bovy A, Scharf K, Schleiff E, Fragkostefanakis S: **Natural variation in HsfA2 pre-mRNA splicing is associated with changes in thermotolerance during tomato domestication**. *New Phytol* 2020, **225**:1297–1310.
18. Mesihovic A, Ullrich S, Rosenkranz RRE, Gebhardt P, Bublak D, Eich H, Weber D, Berberich T, Scharf K-D, Schleiff E, et al.: **HsfA7 coordinates the transition from mild to strong heat stress response by controlling the activity of the master regulator HsfA1a in tomato**. *Cell Rep* 2022, **38**:110224.
19. Cano-Ramirez DL, Carmona-Salazar L, Morales-Cedillo F, Ramírez-Salcedo J, Cahoon EB, Gavilanes-Ruíz M: **Plasma Membrane Fluidity: An Environment Thermal Detector in Plants**. *Cells* 2021, **10**:2778.
20. Kim S-C, Guo L, Wang X: **Nuclear moonlighting of cytosolic glyceraldehyde-3-phosphate dehydrogenase regulates Arabidopsis response to heat stress**. *Nat Commun* 2020, **11**:3439.
21. Yao Y, He RJ, Xie QL, Zhao X hai, Deng X mei, He J bo, Song L, He J, Marchant A, Chen X, et al.: **ETHYLENE RESPONSE FACTOR 74 (ERF74) plays an essential role in controlling a respiratory burst oxidase homolog D (RbohD)-dependent mechanism in response to different stresses in Arabidopsis**. *New Phytol* 2017,

213:1667–1681.

22. Shiva S, Samarakoon T, Lowe KA, Roach C, Vu HS, Colter M, Porras H, Hwang C, Roth MR, Tamura P, et al.: **Leaf Lipid Alterations in Response to Heat Stress of Arabidopsis thaliana**. *Plants* 2020, **9**:845.
23. Ren H, Gao K, Liu Y, Sun D, Zheng S: **The role of AtPLC3 and AtPLC9 in thermotolerance in Arabidopsis**. *Plant Signal Behav* 2017, **12**:e1162368.
24. Li T, Xiao X, Liu Q, Li W, Li L, Zhang W, Munnik T, Wang X, Zhang Q: **Dynamic responses of PA to environmental stimuli imaged by a genetically encoded mobilizable fluorescent sensor**. *Plant Commun* 2022, doi:10.1016/j.xplc.2022.100500.

* This study provides a fluorescent indicator, GREEN FLUORESCENT PROTEIN (GFP)-N160RbohD, to monitor PA dynamics in living cells under different conditions. Heat stress induces nuclear translocation of PA through a process that requires diacylglycerol kinase activity, as well as secretory and endocytic trafficking.

25. Qin F, Lin L, Jia Y, Li W, Yu B: **Quantitative Profiling of Arabidopsis Polar Glycerolipids under Two Types of Heat Stress**. *Plants* 2020, **9**:693.
26. Higashi Y, Saito K: **Lipidomic studies of membrane glycerolipids in plant leaves under heat stress**. *Prog Lipid Res* 2019, **75**:100990.
27. Zhao H, Wu D, Kong F, Lin K, Zhang H, Li G: **The Arabidopsis thaliana Nuclear Factor Y Transcription Factors**. *Front Plant Sci* 2017, **07**.
28. Petroni K, Kumimoto RW, Gnesutta N, Calvenzani V, Fornari M, Tonelli C, Holt BF, Mantovani R: **The Promiscuous Life of Plant NUCLEAR FACTOR Y Transcription Factors**. *Plant Cell* 2013, **24**:4777–4792.
29. Sato H, Mizoi J, Tanaka H, Maruyama K, Qin F, Osakabe Y, Morimoto K, Ohori T, Kusakabe K, Nagata M, et al.: **Arabidopsis DPB3-1, a DREB2A Interactor, Specifically Enhances Heat Stress-Induced Gene Expression by Forming a Heat Stress-Specific Transcriptional Complex with NF-Y Subunits**. *Plant Cell* 2014, **26**:4954–4973.
30. Vermeer JEM, van Wijk R, Goedhart J, Geldner N, Chory J, Gadella TWJ, Munnik T: **In Vivo Imaging of Diacylglycerol at the Cytoplasmic Leaflet of Plant Membranes**. *Plant Cell Physiol* 2017, **58**:1196–1207.
31. Yip Delormel T, Boudsocq M: **Properties and functions of calcium-dependent protein**

kinases and their relatives in Arabidopsis thaliana. *New Phytol* 2019, **224**:585–604.

32. Liu H-T, Gao F, Li G-L, Han J-L, Liu D-L, Sun D-Y, Zhou R-G: **The calmodulin-binding protein kinase 3 is part of heat-shock signal transduction in Arabidopsis thaliana**. *Plant J* 2008, **55**:760–773.
33. Cui Y, Lu S, Li Z, Cheng J, Hu P, Zhu T, Wang X, Jin M, Wang X, Li L, et al.: **CYCLIC NUCLEOTIDE-GATED ION CHANNELs 14 and 16 Promote Tolerance to Heat and Chilling in Rice**. *Plant Physiol* 2020, **183**:1794–1808.
34. Mhamdi A, Van Breusegem F: **Reactive oxygen species in plant development**. *Development* 2018, **145**.
35. Dietz K-J, Mittler R, Noctor G: **Recent Progress in Understanding the Role of Reactive Oxygen Species in Plant Cell Signaling**. *Plant Physiol* 2016, **171**:1535–1539.
36. Wang W, Zhang J, Ai L, Wu D, Li B, Zhang L, Zhao L: **Cyclic Nucleotide-Gated Ion Channel 6 Mediates Thermotolerance in Arabidopsis Seedlings by Regulating Hydrogen Peroxide Production via Cytosolic Calcium Ions**. *Front Plant Sci* 2021, **12**.
37. Katano K, Honda K, Suzuki N: **Integration between ROS Regulatory Systems and Other Signals in the Regulation of Various Types of Heat Responses in Plants**. *Int J Mol Sci* 2018, **19**:3370.
38. Katano K, Kataoka R, Fujii M, Suzuki N: **Differences between seedlings and flowers in anti-ROS based heat responses of Arabidopsis plants deficient in cyclic nucleotide gated channel 2**. *Plant Physiol Biochem* 2018, **123**:288–296.
39. Xie C, Yang L, Jia G, Yan K, Zhang S, Yang G, Wu C, Gai Y, Zheng C, Huang J: **Maize HEAT UP-REGULATED GENE 1 plays vital roles in heat stress tolerance**. *J Exp Bot* 2022, **73**:6417–6433.
40. Deng Y, Humbert S, Liu J-X, Srivastava R, Rothstein SJ, Howell SH: **Heat induces the splicing by IRE1 of a mRNA encoding a transcription factor involved in the unfolded protein response in Arabidopsis**. *Proc Natl Acad Sci* 2011, **108**:7247–7252.
41. Lu S-J, Yang Z-T, Sun L, Sun L, Song Z-T, Liu J-X: **Conservation of IRE1-Regulated bZIP74 mRNA Unconventional Splicing in Rice (Oryza sativa L.) Involved in ER Stress Responses**. *Mol Plant* 2012, **5**:504–514.
42. Che P, Bussell JD, Zhou W, Estavillo GM, Pogson BJ, Smith SM: **Signaling from the Endoplasmic Reticulum Activates Brassinosteroid Signaling and Promotes**

Acclimation to Stress in Arabidopsis. *Sci Signal* 2010, **3**.

43. Li Y, Williams B, Dickman M: **Arabidopsis B-cell lymphoma2 (Bcl-2)-associated athanogene 7 (BAG7)-mediated heat tolerance requires translocation, sumoylation and binding to WRKY29**. *New Phytol* 2017, **214**:695–705.
44. Lau S-Y, Procko E, Gaudet R: **Distinct properties of Ca2+–calmodulin binding to N- and C-terminal regulatory regions of the TRPV1 channel**. *J Gen Physiol* 2012, **140**:541–555.
45. Guihur A, Rebeaud ME, Bourgine B, Goloubinoff P: **How do humans and plants feel the heat?** *Trends Plant Sci* 2022, **27**:630–632.
46. Kmiecik SW, Mayer MP: **Molecular mechanisms of heat shock factor 1 regulation**. *Trends Biochem Sci* 2022, **47**:218–234.
47. Fischer C, Kugler A, Hoth S, Dietrich P: **An IQ Domain Mediates the Interaction with Calmodulin in a Plant Cyclic Nucleotide-Gated Channel**. *Plant Cell Physiol* 2013, **54**:573–584.
48. Bourgine B, Guihur A: **Heat Shock Signaling in Land Plants: From Plasma Membrane Sensing to the Transcription of Small Heat Shock Proteins**. *Front Plant Sci* 2021, **12**.
49. Tiwari LD, Khungar L, Grover A: **AtHsc70-1 negatively regulates the basal heat tolerance in Arabidopsis thaliana through affecting the activity of HsfAs and Hsp101**. *Plant J* 2020, **103**:2069–2083.
50. Zhang X, Guo H: **mRNA decay in plants: both quantity and quality matter**. *Curr Opin Plant Biol* 2017, **35**:138–144.
51. Andrási N, Rigó G, Zsigmond L, Pérez-Salamó I, Papdi C, Klement E, Pettkó-Szandtner A, Baba AI, Ayaydin F, Dasari R, et al.: **The mitogen-activated protein kinase 4-phosphorylated heat shock factor A4A regulates responses to combined salt and heat stresses**. *J Exp Bot* 2019, **70**:4903–4918.
52. Luo J, Jiang J, Sun S, Wang X: **Brassinosteroids promote thermotolerance through releasing BIN2-mediated phosphorylation and suppression of HsfA1 transcription factors in Arabidopsis**. *Plant Commun* 2022, doi:10.1016/j.xplc.2022.100419.

* In this study, authors reveal that BIN2, a component in the BR signaling pathway, interacts and phosphorylates HSFA1d to suppress the nuclear localization of HSFA1d. Heat-induced

BR signaling promotes plant thermotolerance through releasing the BIN2 suppression of HSFA1d to facilitate its nuclear localization and DNA binding activity.

53. Albertos P, Dündar G, Schenk P, Carrera S, Cavelius P, Sieberer T, Poppenberger B: **Transcription factor BES1 interacts with HSFA1 to promote heat stress resistance of plants**. *EMBO J* 2022, **41**.

* This study shows that heat mediates dephosphorylation and activation of BES1, that activated BES1 can interact with HSFA1a, which directly binds to heat shock elements in the promoters of heat shock proteins of the HSP70 and HSP90 type to increase thermotolerance.

54. Yao X, Li Y, Chen J, Zhou Z, Wen Y, Fang K, Yang F, Li T, Zhang D, Lin H: **Brassinosteroids enhance BES1-required thermomemory in Arabidopsis thaliana**. *Plant Cell Environ* 2022, doi:10.1111/pce.14444.

55. Rytz TC, Miller MJ, McLoughlin F, Augustine RC, Marshall RS, Juan Y, Charng Y, Scalf M, Smith LM, Vierstra RD: **SUMOylome Profiling Reveals a Diverse Array of Nuclear Targets Modified by the SUMO Ligase SIZ1 during Heat Stress**. *Plant Cell* 2018, **30**:1077–1099.

56. Zheng X, Wang C, Lin W, Lin C, Han D, Xie Q, Lai J, Yang C: **Importation of chloroplast proteins under heat stress is facilitated by their SUMO conjugations**. *New Phytol* 2022, **235**:173–187.

* Data in this paper indicate that heat stress-induced SUMOylation on PSBR, a subunit of photosystem II, contributes to the maintenance of its chloroplast localization. This provides hints for further investigation on protein modifications associated with plant organelles under heat stress conditions.

57. Zhang H, Zhou J-F, Kan Y, Shan J-X, Ye W-W, Dong N-Q, Guo T, Xiang Y-H, Yang Y-B, Li Y-C, et al.: **A genetic module at one locus in rice protects chloroplasts to enhance thermotolerance**. *Science (80-)* 2022, **376**:1293–1300.

** This study provides evidence how the plasma membrane senses heat sitimuli to communicate with chloroplasts. Here, the authors identied a quantitative trait locus, THERMO-TOLERANCE 3 (TT3), including two genes, TT3.1 and TT3.2, which are important for rice thermotolerance. Heat stress induces the plasma membrane-localized E3 ligase TT3.1 translocation to the endosomes, on which TT3.1 ubiquitinates the chloroplast precursor

protein TT3.2 for vacuolar degradation.

58. Yan C, Yan Z, Wang Y, Yan X, Han Y: **Tudor-SN, a component of stress granules, regulates growth under salt stress by modulating GA20ox3 mRNA levels in Arabidopsis**. *J Exp Bot* 2014, **65**:5933–5944.

59. Chodasiewicz M, Sokolowska EM, Nelson-Dittrich AC, Masiuk A, Beltran JCM, Nelson ADL, Skirycz A: **Identification and Characterization of the Heat-Induced Plastidial Stress Granules Reveal New Insight Into Arabidopsis Stress Response**. *Front Plant Sci* 2020, **11**.

60. Tong J, Ren Z, Sun L, Zhou S, Yuan W, Hui Y, Ci D, Wang W, Fan L-M, Wu Z, et al.: **ALBA proteins confer thermotolerance through stabilizing HSF messenger RNAs in cytoplasmic granules**. *Nat Plants* 2022, **8**:778–791.

** This study provides a mechanism on how heat stress transcription factors (HSFs) are regulated upon heat stress. They show that ACETYLATION LOWERS BINDING AFFINITY (ALBA) proteins phase separate into stress granules (SGs) and processing bodies (PBs) under heat stress, to directly bind selected messenger RNAs, including HSF mRNAs, and recruit these into SGs and PBs to protect them from degradation under heat stress in Arabidopsis.

61. Pomeranz MC, Hah C, Lin P-C, Kang SG, Finer JJ, Blackshear PJ, Jang J-C: **The Arabidopsis Tandem Zinc Finger Protein AtTZF1 Traffics between the Nucleus and Cytoplasmic Foci and Binds Both DNA and RNA**. *Plant Physiol* 2009, **152**:151–165.

62. Zhu S, Gu J, Yao J, Li Y, Zhang Z, Xia W, Wang Z, Gui X, Li L, Li D, et al.: **Liquid-liquid phase separation of RBGD2/4 is required for heat stress resistance in Arabidopsis**. *Dev Cell* 2022, **57**:583–597.e6.

63. Londoño Vélez V, Alquraish F, Tarbiyyah I, Rafique F, Mao D, Chodasiewicz M: **Landscape of biomolecular condensates in heat stress responses**. *Front Plant Sci* 2022, **13**.

64. Kearly A, Nelson ADL, Skirycz A, Chodasiewicz M: **Composition and function of stress granules and P-bodies in plants**. *Semin Cell Dev Biol* 2022, doi:10.1016/j.semcdb.2022.11.008.

65. Maruri-López I, Figueroa NE, Hernández-Sánchez IE, Chodasiewicz M: **Plant Stress Granules: Trends and Beyond**. *Front Plant Sci* 2021, **12**.

66. McLoughlin F, Kim M, Marshall RS, Vierstra RD, Vierling E: **HSP101 Interacts with the Proteasome and Promotes the Clearance of Ubiquitylated Protein Aggregates**. *Plant Physiol* 2019, **180**:1829–1847.
67. Tian X, Qin Z, Zhao Y, Wen J, Lan T, Zhang L, Wang F, Qin D, Yu K, Zhao A, et al.: **Stress granule-associated TaMBF1c confers thermotolerance through regulating specific mRNA translation in wheat (Triticum aestivum)**. *New Phytol* 2022, **233**:1719–1731.
68. Lo J: **China hit by longest and strongest heatwave on record**. *Clim HOME NEWS* 2022,
69. Ding Y, Yang H, Wu S, Fu D, Li M, Gong Z, Yang S: **CPK28-NLP7 module integrates cold-induced Ca 2+ signal and transcriptional reprogramming in Arabidopsis**. *Sci Adv* 2022, **8**.
70. Gwon Y, Maxwell BA, Kolaitis R-M, Zhang P, Kim HJ, Taylor JP: **Ubiquitination of G3BP1 mediates stress granule disassembly in a context-specific manner**. *Science (80-)* 2021, **372**.

FIGURES

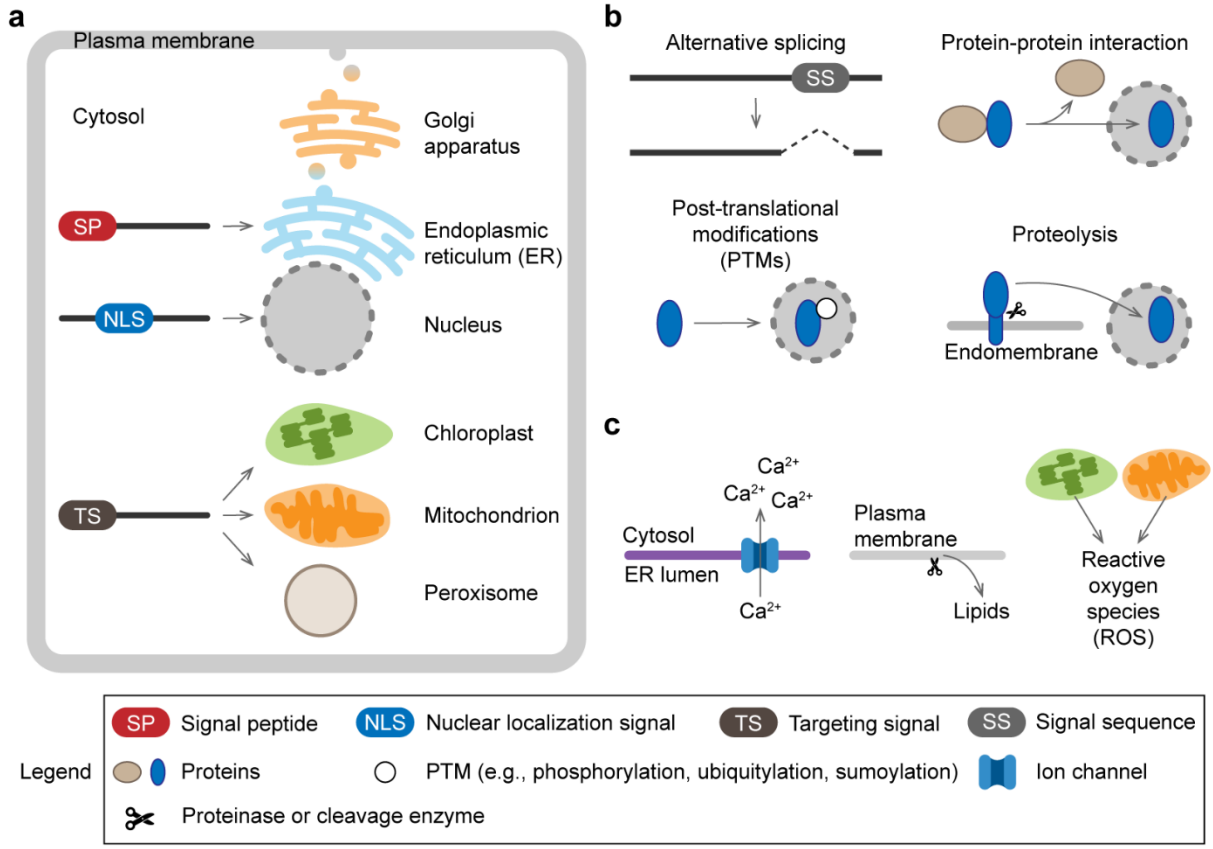

Figure 1. **A schematic overview of possible translocation events. (a)** Translocation of proteins by signal sequences. Most proteins are synthesized in the cytosol and translocate into organelles based on their signal amino sequences. Signal peptides (SP) are usually found at the N-terminus of proteins and target proteins to the endomembrane system or further on the plasma membrane. A nuclear localization signal (NLS) targets a protein to the nucleus. A targeting signal (TS), specifically named transit peptide for chloroplast proteins, can be recognized by a complex on the organelle membrane to translocate proteins into specific organelles. **(b)** Translocation of proteins by signaling or other regulatory mechanisms. Translocation occurs due to alternative splicing to retain or remove signal sequences (SS), protein-protein interaction to release protein from trapping, post-translational modifications (PTMs), or proteolysis to release a functional from transmembrane domains. **(c)** Translocation of (second) chemical messengers from their place of storage or production. The translocation of calcium ions (Ca^{2+}) from e.g. the endoplasm reticulum, digested lipid derivatives from the plasma membrane, or reactive oxygen species (ROS) production from chloroplasts and mitochondria are all crucial to transduce signals.

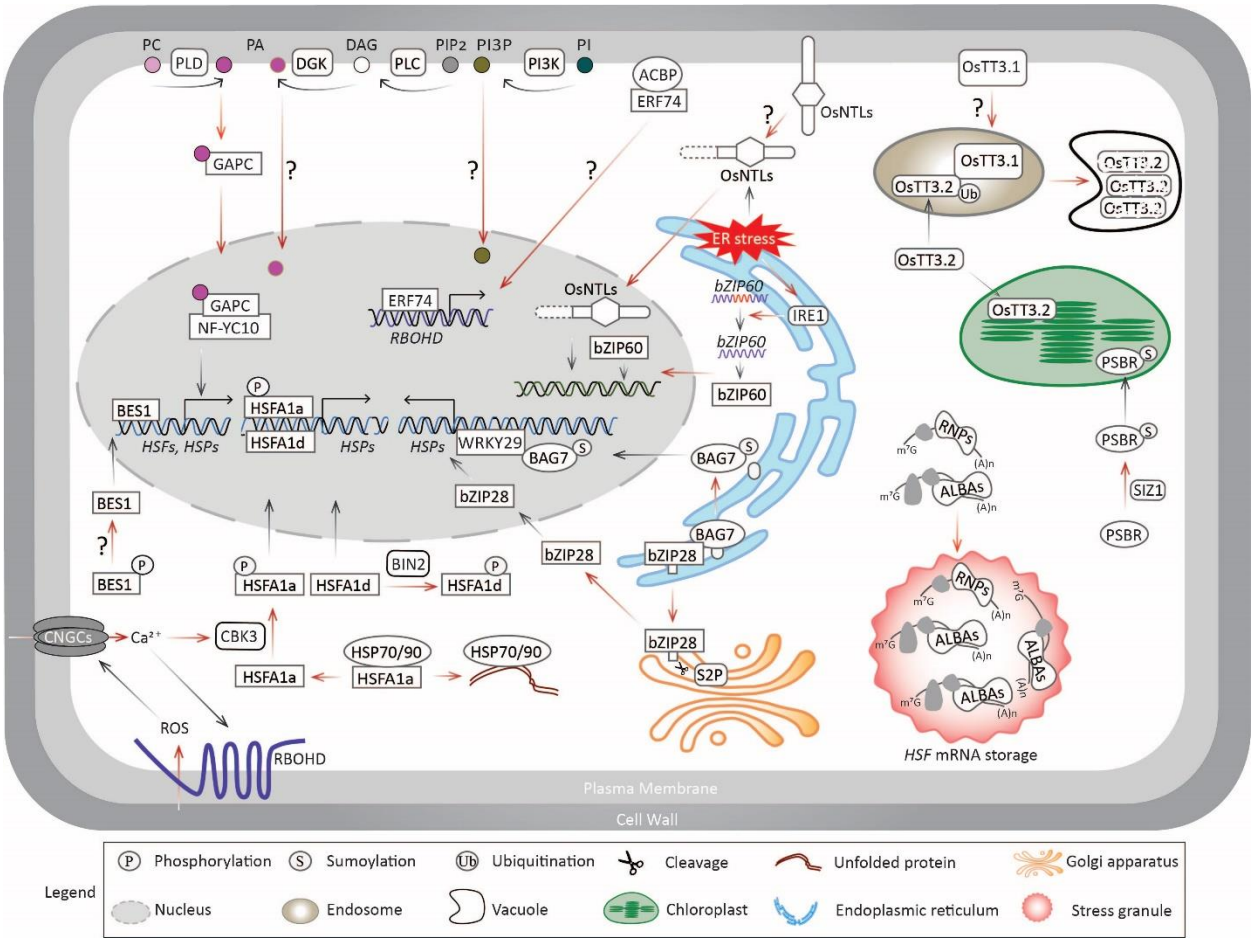

Figure 2. **Translocation events drive thermotolerance.** Simplified summary of the key signaling and translocation processes upon heat stress (details can be found in the main text). Red arrows mark a direct heat effect. Question marks indicate unknown mechanisms associated with heat-induced translocation. **Abbreviations:** ACBP: ACYL-COA-BINDING PROTEIN; ALBA4/5/6: ACETYLATION LOWERS BINDING AFFINITY 4/5/6; BAG7: B-CELL LYMPHOMA2-ASSOCIATED ATHANOGENE 7; BES1: BRI1-EMS-SUPPRESSOR 1; BIN2: BRASSINOSTEROID-INSENSITIVE 2; bZIP: BASIC-LEUCINE ZIPPER DOMAIN TRANSCRIPTION FACTOR; CBK3: CALMODULIN-BINDING PROTEIN KINASE 3; CNGC: CYCLIC NUCLEOTIDE GATED CHANNEL; DAG: DIACYLGLYCEROL; DGK: DAG KINASE; ERF74: ETHYLENE RESPONSE FACTOR 74; GAPC: GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE; IRE1: INOSITOL-REQUIRING ENZYME-1; HSF: HEAT SHOCK FACTOR; HSP: HEAT SHOCK PROTEIN; IRE1: INOSITOL-REQUIRING ENZYME-1; NF-YC10: NUCLEAR FACTOR Y SUBUNIT C10; NTL: NAC MTF (NTM1)-LIKE PROTEIN; PA: PHOSPHATIDIC ACID; PC: PHOSPHATIDYLCHOLINES; PI:

PHOSPHATIDYLINOSITOL; PIP2: PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE; PI3P: PHOSPHATIDYLINOSITOL 3-PHOSPHATE; PLC: PHOSPHOLIPASE C; PLD: PHOSPHOLIPASE D; PSBR: PHOTOSYSTEM II SUBUNIT R; RBOHD: RESPIRATORY BURST OXIDASE HOMOLOG; RNP: RIBONUCLEOPROTEIN; S2P: SITE-2 PROTEASE; SIZ1: SAP AND MIZ1 DOMAIN-CONTAINING LIGASE 1; TT3: THERMO-TOLERANCE 3; WRKY29: WRKY DNA-BINDING PROTEIN 29.