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Dare to change, the dynamics behind plasmodesmata-mediated cell-to-cell communication

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Plasmodesmata pores control the entry and exit of molecules at cell-to-cell boundaries. Hundreds of pores perforate the plant cell wall, connecting cells together and establishing direct cytosolic and membrane continuity. This ability to connect cells in such a way is a hallmark of plant physiology and is thought to have allowed sessile multicellularity in *Plantae* kingdom. Indeed, plasmodesmata-mediated cell-to-cell signalling is fundamental to many plant-related processes. In fact, there are so many facets of plant biology under the control of plasmodesmata that it is hard to conceive how such tiny structures can do so much. While they provide 'open doors' between cells, they also need to guarantee cellular identities and territories by selectively transporting molecules. Although plasmodesmata operating mode remains difficult to grasp, little by little plant scientists are divulging their secrets. In this review, we highlight novel functions of cell-to-cell signalling and share recent insights into how plasmodesmata structural and molecular signatures confer functional specificity and plasticity to these unique cellular machines.

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Introduction

All forms of multicellularity depend on two fundamental pillars: cell-to-cell contact and cell-to-cell communication. Both functions have emerged independently and multiple times throughout evolution, resulting in the development of different forms of connections with diversified molecular strategies [1]. In plants, cell-to-cell communication is largely assisted by plasmodesmata intercellular pores, which ensure concerted cellular actions during tissue growth, development and response to environmental cues [2]. In concert with the vascular system, plasmodesmata support long-range signalling to integrate local responses at the organism level [3•,4–6]. These unique cellular machines can be viewed as gates through the plant cell wall, providing cytosolic and membrane continuity from cell-to-cell and eventually throughout the whole plant body. They are involved in multiple tasks like conveying organic nutrients [7•,8•], regulating crucial steps during organ initiation and growth [9,10], assisting tissue patterning by conveying positional information [11••], acting as signalling hubs and contributing to defence response [12•,13•,14–17]. Despite their central role in plant physiology, their operating mode remains elusive. Yet, they keep on fascinating and intriguing scientists. In this review, we recapitulate recent and significant advances in our understanding of plasmodesmata-mediated cell-to-cell communication and their central function for plant biology.

Reaching out further: new insights into plasmodesmata-mediated short-distance and long-distance signalling

A wide range of developmental and physiological processes depends on symplastic communication. Examples include shoot meristem maintenance [18–21], tissue patterning and organ growth [9,10], bud dormancy [22], defence signalling [5,6,16], adaptation to environmental stresses [12•,13•,14,15] and exchange of nutrients between cells and organs [7•,8•,23]. In the last two years, the realm of symplastic communication has grown even bigger and it now embraces symbiotic interaction [24•], calcium-based long-distance signalling [6] and unfolded protein response (UPR) [3•].

Transcription factors (TFs) were amongst the first endogenous factors to be shown to act non-cell autonomously through plasmodesmata, a decisive condition for both tissue patterning and meristem maintenance [10,11••,18,21]. Since then, a growing number of signalling molecules, from RNAs [4,25,26] to hormones [27] and even lipids [5,16], were reported to move through plasmodesmata. In all cases, and regardless of the trafficking mechanisms (selective or passive), these signalling gradients are tightly controlled both spatially and temporally. A recent study, by Helariutta and De Rybel's teams [11••], illustrates how, through a complex feed-back loop between TFs, miRNAs and

hormones, spatial information can be generated to create sharp boundaries. During radial growth initiation in root procambial tissues, cytokinin promotes the expression of the mobile PHLOEM EARLY DOF (PEAR) TFs, which then form a short-range gradient and activate genes promoting radial growth at protophloem sieve elements. PEAR action is in turn antagonised by class III HOMEODOMAIN LEUCINE ZIPPER (HD-ZIP III) transcription factors, their expression being controlled by auxin, miRNA165/166 and PEAR TFs [11••]. Not only movement but also the transcription of PEARs must be regulated to achieve proper growth pattern. This work perfectly illustrates the complexity of intercellular communication networks where both intra-cellular and inter-cellular processes are integrated at a multiscale level, and where symplastic trafficking is only one of the many key components.

Information exchange between distant organs is crucial to prime integrated responses at the body level. This is often achieved by combining cell-to-cell transport through plasmodesmata and long-distance trafficking via the phloem [4–6,16,26,28,29]. A well-established example is the florigen, Flowering Locus T, which moves from the leaves into the phloem to reach out the shoot apex and reprogram leaf production into flowers [28–32]. Failure in moving through plasmodesmata results in late flowering [29–32]. Plant stress responses also rely on long-distance communication. Herbivore feeding triggers glutamate-dependent calcium signalling at the wounded site, which rapidly propagates to distant leaves to presumably activate defence responses in non-damaged regions [6]. Calcium itself is unlikely to move long distances. Instead, calcium waves may require a relay-based system, potentially coupled with reactive oxygen species (ROS) [33]. Likewise, the ER-embedded UPR response, which until recently has been regarded as a cell-autonomous process, acts systemically through non-cell autonomous signalling and long-range movement of bZIP60 TF [3•]. By combining short-range and long-distance movement, plants can perceive and prime stress responses in regions far away from initiation sites. Plasmodesmata crucial functions also rely on their capacity to integrate a wide range of environmental and developmental signals and accordingly regulate the movement of many different classes of molecules at specific interfaces. The molecular mechanisms regulating transport across plasmodesmata rely, for a large part, on their structural and functional plasticity.

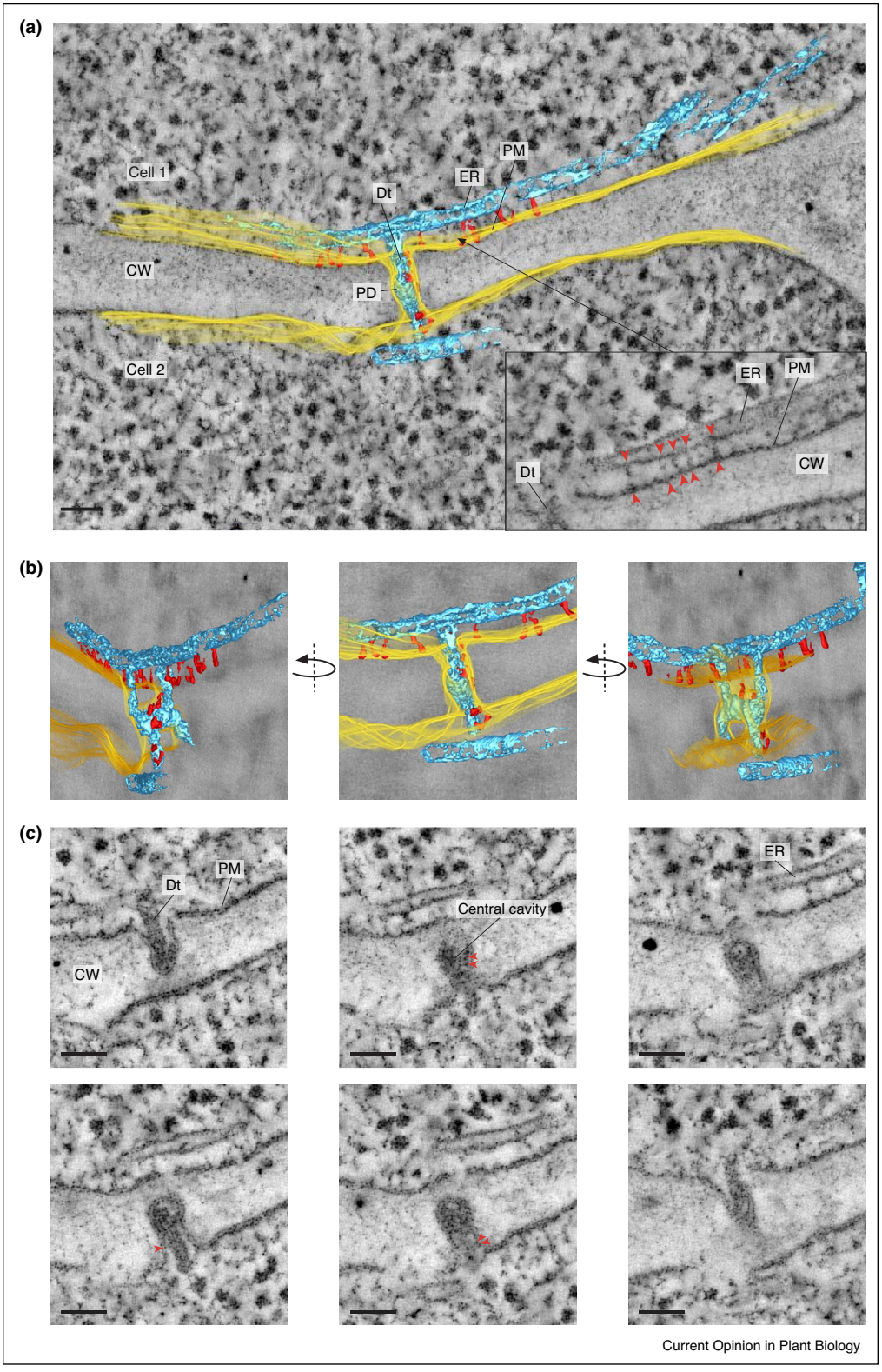
Plasmodesmal structure defines plasmodesmal function

Plasmodesmata bridge cells across the wall creating physical continuity between three compartments: the plasma membrane (PM), the cytoplasm and the endoplasmic reticulum (ER) (Figure 1) [2,34]. Distinguished by its lipid profile, membrane curvature, and protein composition, the PM lining plasmodesmata is considered as a specialized microdomain, which dictates plasmodesmata-specific function. For example, enrichment of distinctive sterol and sphingolipid species participate in the recruitment of cell-wall remodelling enzymes which create a unique cell wall environment and impacts on plasmodesmata permeability [35]. In apposition to the PM, and as it enters the pores, the ER becomes tightly constricted into a highly differentiated ER subdomain called the desmotubule. Constriction of the ER inside plasmodesmata presumably restricts cell-to-cell diffusion of ER-associated molecules. Inside and at the entry of the pores, the desmotubule/ER establishes contacts with the PM through tethering elements, which function and molecular identity have remained unknown until recently (see next section) [35,37]. Altogether, membranes and the immediate wall environment present a unique molecular signature, supporting plasmodesmata function [12•,13•,14,16,31,35,36••,37–39].

So far, no consensus plasmodesmata targeting motif has been identified and the emerging picture is that plants rely on a diversity of strategies to regulate symplastic trafficking. Both passive and selective transports occur, with the latter implying direct interaction between mobile factors and plasmodesmata 'receptors' to facilitate movement [16,30,31,39–41]. Dynamic cell-to-cell communication is also achieved through the controlled opening or closure of plasmodesmata. In canonical models, molecules traffic through the cytoplasmic sleeve and the size exclusion limit (SEL) of the pores is defined by the ER-PM gap [42]. In other words, the wider is the gap, the more transport there is. This model has however been recently challenged by two independents studies [7•,34]. Using electron tomography Nicolas *et al.* showed that post-cytokinesis plasmodesmata (called Type I), previously shown to offer high transport capacity, present a very narrow cytoplasmic sleeve not exceeding 2–3 nm. Later during cell growth/differentiation, the ER-PM gap extends to 8–10 nm leading to open-sleeved Type II plasmodesmata. *Arabidopsis* plants missing the *Phloem Unloading Modulator* (*PLM*) gene, present a defect in Type I to Type II transition at the phloem-pole-pericycle/endodermis interface, which results in higher symplastic unloading capacity. These data indicate that very narrow-sleeved plasmodesmata are actually more conductive than wide-sleeved ones, questioning the current trafficking model.

Over the years, callose has emerged as a chief regulator of plasmodesmata SEL and dynamically modulates the pore conductivity in response to environmental and developmental cues [13•,14,15,22,24•,37,42]. Although its mode of action remains poorly understood, the current model proposes that local callose synthesis at the plasmodesmata neck region forms an extracellular ring, which squeezes the PM against the ER, contracting the cytoplasmic sleeve [37]. This model, however, implies that the PM can accommodate rapid local deformation through stretching, an unlikely event for lipid bilayers, which present limited elastic properties [43]. Such local deformation would imply membrane remodelling

Figure 1



Plasmodesmata viewed by electron tomography. 3D segmentation **(a, b)** and reconstructed sections **(c)** of a branched plasmodesma at the phloem pole pericycle-endodermis interface in *Arabidopsis* root. (a) Plasmodesmata are embedded within the cell wall (CW) and create PM (yellow) and ER (blue) continuity from cell-to-cell. Tethering elements (red) are visible between the ER and the PM within and outside the pores. Inset represents a reconstructed micrograph section showing tether elements (red arrowheads) at the entrance of the pore. (b) Different views of

through lipid redistribution and possibly the action of membrane-shaping proteins. Callose accumulation could also lead to the re-organisation of PM-located callose-binding proteins and their interacting partners, thus changing not only immediate PM environment but also the ER-PM interface.

Using callose-cellulose biopolymer mixture, Abou-Saleh *et al.* recently suggested that, at certain concentrations, callose could in fact increase the elasticity rather than rigidify the wall matrix, leaving open the question of how this polymer could influence the properties of the cell wall at plasmodesmata and the conductive properties of the pores [44•]. Aside from callose, the 'I' shape ER that passes through the pore has recently been proposed to control rapid plasmodesmata closure upon osmotic pressure through mechanosensing [45•]. According to this model, the tether elements bridging the ER to the PM offer physical elasticity which in turn determines the sensibility to the pressure-induced movement of the desmotubule. This interesting piece of work revealed an alternative option for plasmodesmata regulation that directly take into account the mechanics associated with the desmotubule positioning in the context of cell–cell junction.

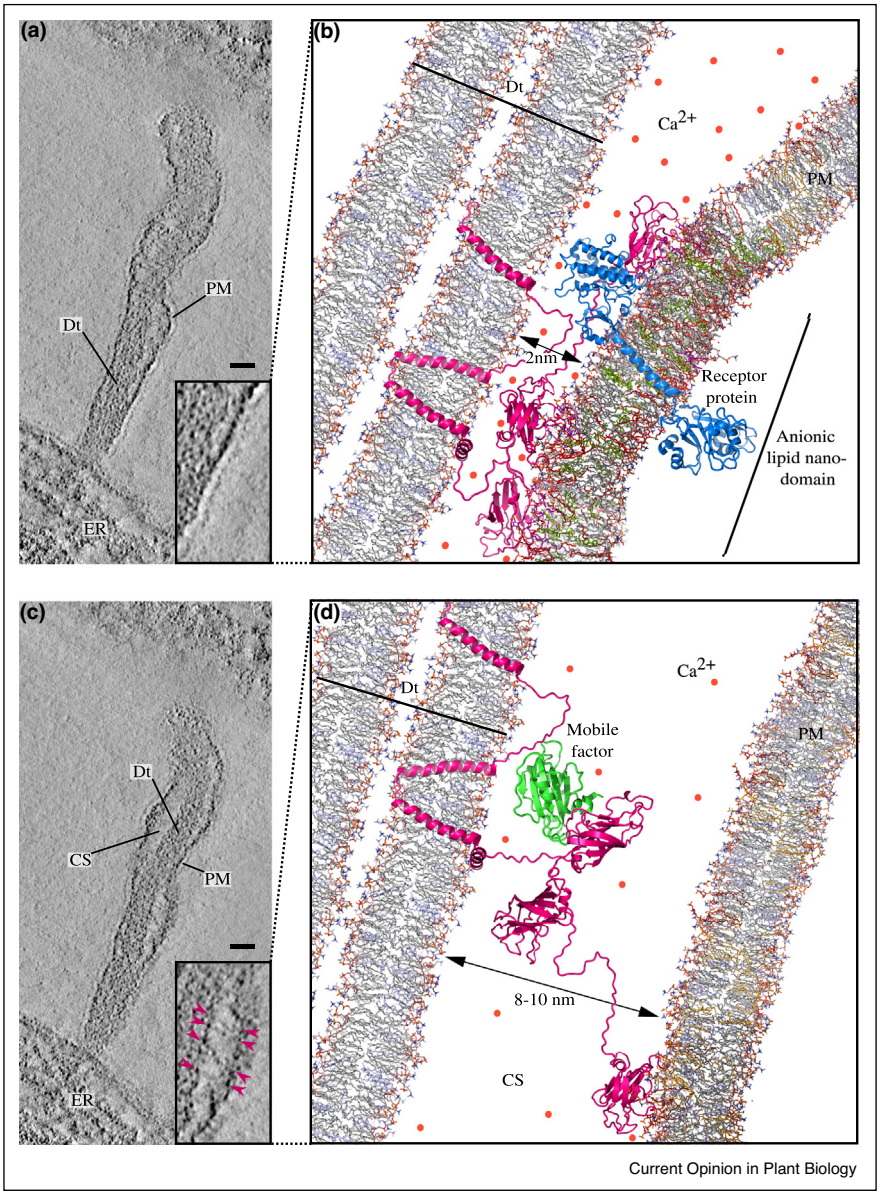
Besides plasmodesmata SEL, many additional elements influence symplastic trafficking. These include, plasmodesmata density at cellular interface [8•,46], wall thickness [47], expression level of mobile factors [11••], ability for a given molecule to 'enter' the symplastic pathway, which can be influenced by complex formation [19,20,48] or binding to a membrane-compartment [49,50•]. Ultimately, these diversified strategies, which rely on both the structural and functional properties of plasmodesmata, need to operate synergistically to precisely regulate symplastic trafficking and integrate a complex network of signalling pathways.

Acting at the ER-PM interface: the multiple C2 domains transmembrane region protein family

An additional way of considering plasmodesmata is to view them as specialised ER-PM membrane contact sites [2,51]. The gap between the two membranes inside the pore is remarkably flexible and presumably impacts intercellular trafficking (Figure 2) [7•,34]. Now, to the questions as to what elements regulate this structural plasticity, we still have no answer. Historically the molecular identity of the elements bridging the ER to the PM was in favour of cytoskeletal proteins such as actin or myosin, but their function in membrane tethering still remains hypothetical [34,51]. Recently, the Multiple C2 domains and Transmembrane region Proteins (MCTPs) have emerged as plasmodesmata-specific ER-PM tethers [36••]. MCTPs are a conserved family in higher eukaryotes, yet, while *Homo sapiens* and *Drosophila* spp. only have two members, the *Arabidopsis* genome contains 16 members, suggesting a larger functional diversity. At least six members of the *Arabidopsis* family cluster at plasmodesmata, where they seem to serve different functions detailed below [8•,31,36••,38,39,52]. MCTPs present the structural organisation of a typical tether, with a C-terminal transmembrane region, which inserts into the ER and three to four C2 domains, which act as PM docking sites through anionic lipid-binding [36••] (Figure 2). Unlike other tethers, MCTPs are not only involved in bridging membranes, they also regulate intercellular trafficking of non-cell autonomous signals. AtMCTP1/FT-interacting protein 1 (FTIP1) interacts with Flowering locus T to promote its transfer at the companion cell-sieve element interface [31]. AtMCTP3 and AtMCTP4 antagonise the movement of the TF SHOOT MERISTEM LESS, although here it is not clear whether they act from endosomes or directly at plasmodesmata [36••,50•]. Nevertheless, loss-of-function *mctp3/4 Arabidopsis* mutants display pleiotropic developmental defects [36••,50•], reduced SEL and altered plasmodesmata protein composition [36••]. In *Arabidopsis*, MCTP15/QUIRKY regulates CAPRICE movement and root epidermis patterning by directly modulating the activity and stability of the receptor-like kinase STRUBBELIG/ SCRAMBLED and downstream cell-to-cell signalling [39]. In maize, the AtMCTP15 homologue, Carbohydrate Partitioning Defective 33, promotes symplastic transport of carbohydrates into sieve elements, possibly by regulating plasmodesmata formation at the companion cell–sieve element interface [8•]. From their optimal position at the ER-PM interface, MCTPs appear to control multiple aspects of plasmodesmata-mediated cell-to-cell communication, including 1) selective transport of mobile factors, 2) activation of receptor-mediated cell-to-cell signalling, 3) plasmodesmata SEL, hence passive transport and 4) formation/ stabilisation of the pores. This multifaceted function of MCTPs may partially be attributable to the diversity of actions of their multiple C2 domains. Similar to other ER-PM tethers [53•,54], the C2 domains of AtMCTP4 and 15/QUIRKY most likely interact with anionic lipids, potentially in a calcium-dependant manner. This implies that the surface charges of the plasmodesmal PM and/or calcium could influence membrane docking inside the pores in a conditional and reversible manner, which in turn could change the cytoplasmic sleeve conducting properties (Figure 2). Furthermore, the same C2 cytosolic regions of AtMCTP15/QUIRKY, AtMCTP3/4

(Figure 1 Legend Continued) the 3D segmentation depicted in (a) showing the branched-structure with a central cavity where the two desmotubules are connected. (c) Reconstructed sections through the volume of the tomogram shown in panels (a, b). Tether elements are visible along the pore and in the central cavity (red arrowheads). PD: plasmodesmata, CW: cell wall, Dt: desmotubule. Scale bar is 50 nm.

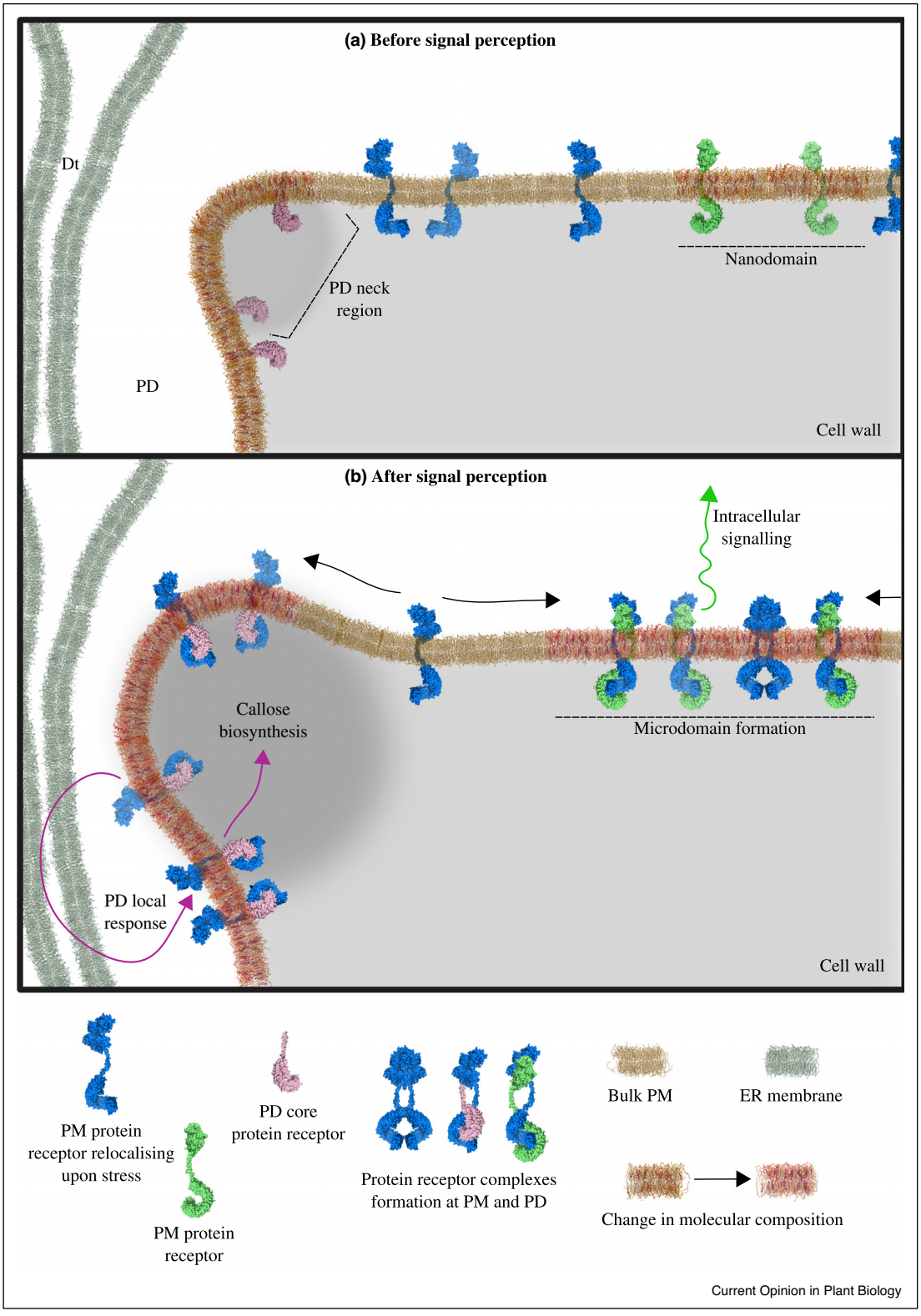
Figure 2



Hypothetical model of MCTP operating mode at the plasmodesmal ER-PM interface. **(a, c)** Two reconstructed sections at a ~2.5 nm interval of an electron tomogram of a plasmodesma in *Arabidopsis* root tip. The spacing between the desmotubule (Dt) and the PM varies from very close contacts with no observable electron-lucent cytoplasmic sleeve (CS) (inset in panel (a)), to larger gap with electron-lucent readily identifiable CS and spoke-like tethering elements connecting the two membranes (inset in panel (c), pink arrowheads indicate tethers). Scale bar is 20 nm.
(b, d) Molecular representation of MCTP with three C2 domains (pink) connecting the Dt to the PM inside the pores in tight ~2 nm (b) and 'open' ~8–10 nm (d) CS configurations. MCTPs insert into the ER/Dt membrane through their transmembrane region and interact with the PM in the presence of anionic lipids. The molecular re-arrangement of MCTP cytoplasmic tail in response to calcium (orange beads) and/or changes in membrane lipid composition influences the ER-PM gap inside the pore and the conductive properties of the CS. (b) Upon elevated local calcium concentration and the presence of anionic lipids, all C2 domains dock to the PM, restricting the ER-PM gap. C2 domains interaction with the PM could then stabilise/re-enforce anionic lipid nanodomains, changing the PM surface charge inside plasmodesmata and recruiting/activating/stabilising receptor proteins (blue). Note that calcium could also compete with C2 domains by shielding the polar heads of anionic lipids (not represented). (d) Upon low calcium concentration, PM lipid modification or binding to mobile factors (green), some C2 domains dissociate from the PM leading to the opening of the ER-PM gap.

and AtMCTP1/FTIP are known to be involved in protein–protein interactions [30,31,38,39,50•]. By analogy with Extended-Synaptotagmins [53•], it is tempting to speculate that MCTPs protein-binding and lipid docking functions work together to regulate transport (Figure 2). By uniting intercellular and inter-organellar functions, MCTPs are one-of-a-kind tethers, playing a master regulator function at plasmodesmata.

Figure 3



Putative model illustrating signal-triggered dynamic re-organisation of receptor-complexes induces local and distinct responses at plasmodesmata versus the PM. **(a)** The PM lining the plasmodesmata pores and the PM nanodomains provide a membrane environment distinct from the bulk PM, with a unique set of lipids and proteins, including protein receptors, and function as signalling platforms. **(b)** Biotic and abiotic-derived signals induce a re-organisation of PM protein receptors, which includes changes in localisation, protein-protein interactions, and clustering in

Plasmodesmata define membrane nanoterritories, which serve as dynamic signalling platforms

Plasmodesmata act at the interface between intra-cellular and extra-cellular compartments. As such, they are ideally located to integrate apoplastic, symplastic and endomembrane signalling to coordinate cellular responses. The PM lining plasmodesmata hosts receptor-like activities, which sense developmental and environmental apoplastic signals and regulate symplastic exchanges [12•,13•,14,15,17,55]. Some receptors are shared components between the PM and plasmodesmata, but they orchestrate distinct signalling pathways by assembling into different complexes depending on their localisation. For example, the receptor-kinases CLAVATA1 (CLV1) and CRINKLY4 (ACR4), which function in stem cell maintenance, have variable complex composition depending on if they locate at the bulk PM or plasmodesmata [55]. Likewise, STRUBBELIG/ SCRAMBLED associates with the PM but only forms heteromeric complex with AtMCTP15/QUIRKY at plasmodesmata, from where it initiates non-cell autonomous signalling [38].

Conventionally, plasmodesmata-signalling was believed to be exerted exclusively by plasmodesmata-located receptors. However, recent studies show that PM receptors can conditionally relocate to plasmodesmata to trigger local response (Figure 3). The Cysteine-Rich Receptor-like kinase 2 (CRK2), strictly located at the PM, associates to plasmodesmata within 30 min after salt treatment and promotes callose deposition [13•]. CRK2 re-organisation depends on Phospholipase D α1, indicating that changes in membrane lipid composition is instrumental to recruit receptors to plasmodesmata [13•]. Upon osmotic stress, the PM-associated Leucine-Rich repeat RLKs Qian Shou Kinase 1 (QSK1) and Inflorescence Meristem Kinase 2 (IMK2) rapidly relocalise, within less than 2 min, to plasmodesmata and into PM-nanodomains [12•]. Similar to CRK2, the recruitment of QSK1 to plasmodesmata is correlated with callose-accumulation and also partially depends on its phosphorylation [12•]. Stimuli-dependant re-organisations of receptor-like activities at plasmodesmata are therefore frequent events and may in fact be a common strategy to modulate symplastic trafficking. In a similar fashion, the immune fungal elicitor chitin induces redistribution of the chitin-receptor complex at both PM and plasmodesmata [14]. Upon chitin sensing at the PM, the CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) interacts with LysM receptor-like kinase LYK5, which initiates intracellular defence responses [14,17]. Simultaneously, LYSIN MOTIF DOMAIN CONTAINING GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN (LYM2) accumulates to plasmodesmata, where it associates with LYK4 to promote localised callose synthesis [14]. Taken together, these data indicate that the molecular composition of plasmodesmata is rapidly modified through either biotic or abiotic stresses to induce cellular responses.

Inherent to its role as a barrier, the PM constitutes an optimal interface for signal perception and intracellular signal transduction. The nanoscale composition and segregation of the PM contribute to the emergence of distinct membrane territories, which not only directly impact on receptor activation/deactivation, but also differentiate signalling pathways sharing common components[56–58]. Likewise, plasmodesmata create PM subdomains with a unique protein/lipid signature and facilitate localised and specific responses. In addition, signalling events triggered at the PM also need to be coordinated with local responses at plasmodesmata to specifically and independently regulate cell-to-cell communication. Until now, callose deposition-mediated plasmodesmata closure has been the main signalling output but other local responses could also be triggered in parallel. These could include changes in plasmodesmata membrane electrostatic signature, re-arrangement of plasmodesmata tethers which could then change the cytoplasmic sleeve conducting properties. Furthermore, we can wonder what are the molecular mechanisms underlying the rapid mobility of receptors between PM and plasmodesmata, but also how the system deactivates such processes.

Concluding remarks

Recent years have seen remarkable progress in our structural and functional understanding of plasmodesmata-mediated cell-to-cell communication and how these structures can create dynamic areas of cell-to-cell connectivity in response to a wide range of developmental and environmental signals. They have also highlighted the complexity of plant intercellular communication and the intricacies of short and long-range communication networks, where hormone-signalling, receptor-signalling and symplastic-signalling pathways intersect in a very dynamic manner to create coherent responses at the organism level. Challenges in studying plasmodesmata also lie in their nanoscale dimensions and their high plasticity, making it hard to pin down particular morphological states and link them to functional/physiological states. A comprehensive understanding of symplastic transport will benefit from multidisciplinary approaches that combine emerging fields and technologies such as *in silico*

(Figure 3 Legend Continued) microdomains. Alongside, a specific set of PM-associated receptors are rapidly and actively recruited to plasmodesmata where they accumulate and interact with plasmodesmal receptor proteins to induce local responses, such as callose deposition. With the receptor protein moving between the PM and plasmodesmata, perception of one signal can translate into both intracellular signalling cascades at the PM (green arrow) and local plasmodesmata responses (pink arrow), facilitated by the local protein interactors. PD: Plasmodesmata, Dt: Desmotubule, ER: Endoplasmic Reticulum, PM: Plasma Membrane.

molecular dynamics, electron microscopy, super resolution light microscopy and *in vitro* biophysical analyses with more classical genetics and cell biology approaches. Without doubt, future research will continue to uncover the fascinating and multifaceted mechanisms that govern plasmodesmata intercellular communication.

Conflict of interest statement

Nothing declared.

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This paper elegantly illustrates how signal communication between mother and daughter cells through plasmodesmata helps to establish cell identities and boundaries during ontogenesis in procambium cells. PEAR TFs mark the active dividing protophloem-sieve-elements and move to neighbour cells to promote procambial cell division. In the daughter cells, target of PEAR proteins, HD ZIP III antagonizes PEAR function and mobility to specify the un-dividing cell population.

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