Contents lists available at ScienceDirect

Plant Science

Review article

Review: Membrane tethers control plasmodesmal function and formation

Chaofan Chen [a,b], Steffen Vanneste [c,d,e], Xu Chen [b,*]

[a] College of Life Science and Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, Fujian Agriculture and Forestry University, Fuzhou, China
[b] FAFU-UCR Joint Center for Horticultural Biology and Metabolomics, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, China
[c] Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium
[d] Department of Plants and Crops, Ghent University, Coupure links 653, 9000 Ghent, Belgium
[e] Lab of Plant Growth Analysis, Ghent University Global Campus, Songdomunhwa-Ro, 119, Yeonsu-gu, Incheon 21985, Republic of Korea

A B S T R A C T

Cell-to-cell communication is crucial in coordinating diverse biological processes in multicellular organisms. In plants, communication between adjacent cells occurs via nanotubular passages called plasmodesmata (PD). The PD passage is composed of an appressed endoplasmic reticulum (ER) internally, and plasma membrane (PM) externally, that traverses the cell wall, and associates with the actin-cytoskeleton. The coordination of the ER, PM and cytoskeleton plays a potential role in maintaining the architecture and conductivity of PD. Many data suggest that PD-associated proteins can serve as tethers that connect these structures in a functional PD, to regulate cell-to-cell communication. In this review, we summarize the organization and regulation of PD activity via tethering proteins, and discuss the importance of PD-mediated cell-to-cell communication in plant development and defense against environmental stress.

1. Introduction

Cell-to-cell communication is the interactive exchange of information between neighboring cells, which is fundamental in maintaining cellular homeostasis in multicellular organisms and contributes to diverse aspects of developmental processes and responses to environmental stresses [1,2]. Plants have evolved plant-specific cell-to-cell communication via nanosized channels between two adjacent cells, called plasmodesmata (PD). PD are embedded in the cell wall and connect the cytoplasm and endoplasmic reticulum (ER) of adjacent cells [3]. PD-mediated cell-to-cell communication and dynamics of PD pores facilitate the transport of mobile molecules, such as small RNAs, transcriptional factors, and viruses, among others [4–7].

Structurally, PD are cylindrical, membrane-lined tunnels with a diameter of 30–50 nm, which are classified into two types, primary and secondary PD [8]. Primary PD are established during cytokinesis in the newly forming cell wall or cell plate, and secondary PD form in cell walls of non-dividing cells during or after cytokinesis [9]. The PD formation is a highly orchestrated process, which requires the participation of various intracellular organelles [10,11]. During cytokinesis, the ER is positioned across the developing cell plate, and the initial matrix of cell wall and plasma membrane (PM) fuse into the vicinity of the ER for PD

initiation. Then, the ER is appressed, forming the central axial structure of primary PD, called a desmotubule [12]. The membrane portion of PD is a continuous extension of the PM, and is enriched in sterols and very long chain saturated fatty acids sphingolipids [13]. During cell plate expansion, the ER membrane and PM separate, leaving an intermembrane gap filled with cytoplasm, the so-called cytosolic sleeve [14,15]. Actin filaments, possibly located within the cytosolic sleeve, participate in maintaining the structural integrity of PD [16,17](Fig. 1A).

Eukaryotic cells contain subcellular compartments with unique functions. Non-vesicular inter-organellar communication is possible at specific contact-sites where close apposition of the membranes of two organelles is established via specialized tethering proteins [18,19]. Such contact sites are currently emerging as important for lipid transfer, Ca^{2+} homeostasis, signal transduction, and molecule exchange [7,18,20,21]. For example, the ER has an expansive membrane network that works closely with multiple organelles, such as the Golgi apparatus, ribosomes, endosomes, mitochondria, and particularly with the PM for protein secretion [22–26]. The close apposition of specialized domains of ER and PM are tethered by spoke-like filamentous structures in the PD, which allows defining them as a plant-specific subtype of ER–PM contact sites [15,27]. Based on the current observation, ER–PM contact sites probably participate in PD initiation, differentiation, and conductivity

---

* Corresponding author.
E-mail address: chenxu@fafu.edu.cn (X. Chen).

regulation [27,28]. In this review, the recent findings on inter-organellar communication during PD development and regulation are summarized.

2. ER-PM contact sites determine PD morphology

The ER is the major source of biosynthesis of PM-associated proteins and a variety of lipids. Additionally, it is as an important intracellular store of Ca^{2+} that is used in a wide variety of signaling events. Therefore, the direct association between the ER and other membrane compartments, such as the contacts between ER and PM, is of great importance for cell signaling, organelle morphology and ER function [28–30]. Such contact sites require tethering structures to keep the two membranes closely together, facilitating the location of specialized proteins and signal exchange [31]. PD comprise two types of membrane systems: the plasma membrane, which lines the PD pore; and the ER membrane, which is tightly constricted into a rod-like structure [32–34]. These two membranes work closely together, with distances varying from very tight contacts to 10 nm intermembrane gaps [15]. Due to the presence of tethers that keep ER and PM specialized domains in close apposition, PD are accordingly grouped into two morphotypes: type I PD that display a

very tight connection between ER and PM; and type II PD that display a typical PD structure with obvious intermembrane gaps, and appear usually in the thicker, presumably older cell walls [15]. PD structural plasticity was recently re-established by electron tomography and the researchers proposed that ER-PM contacts occur already at the onset of PD biogenesis, which might be correlated with type I PD formation [15]. In type II PD, tether-like structures connecting the compressed ER and the PM are visible. The tether length might be correlated with the opening of the cytosolic sleeve and the aperture of PD [15]. Thus, ER-PM contacts probably promote the transition between these two PD types and are of great importance in information exchange and intercellular communication between adjacent cells.

The PD structure can be modulated to adjust symplastic transport efficiency, which may be caused by the regulation of ER–PM contact spacing [35–37]. For example, the increase in turgor pressure that occurs during cell growth leads to a reduction in PD permeability [38,39]. A novel mathematical model of pressure-controlled PD permeability showed that PD conductivity is strongly influenced by pressure-induced movement of the ER–desmotubule complex and the filamentous proteins that tether the ER and PM [40].

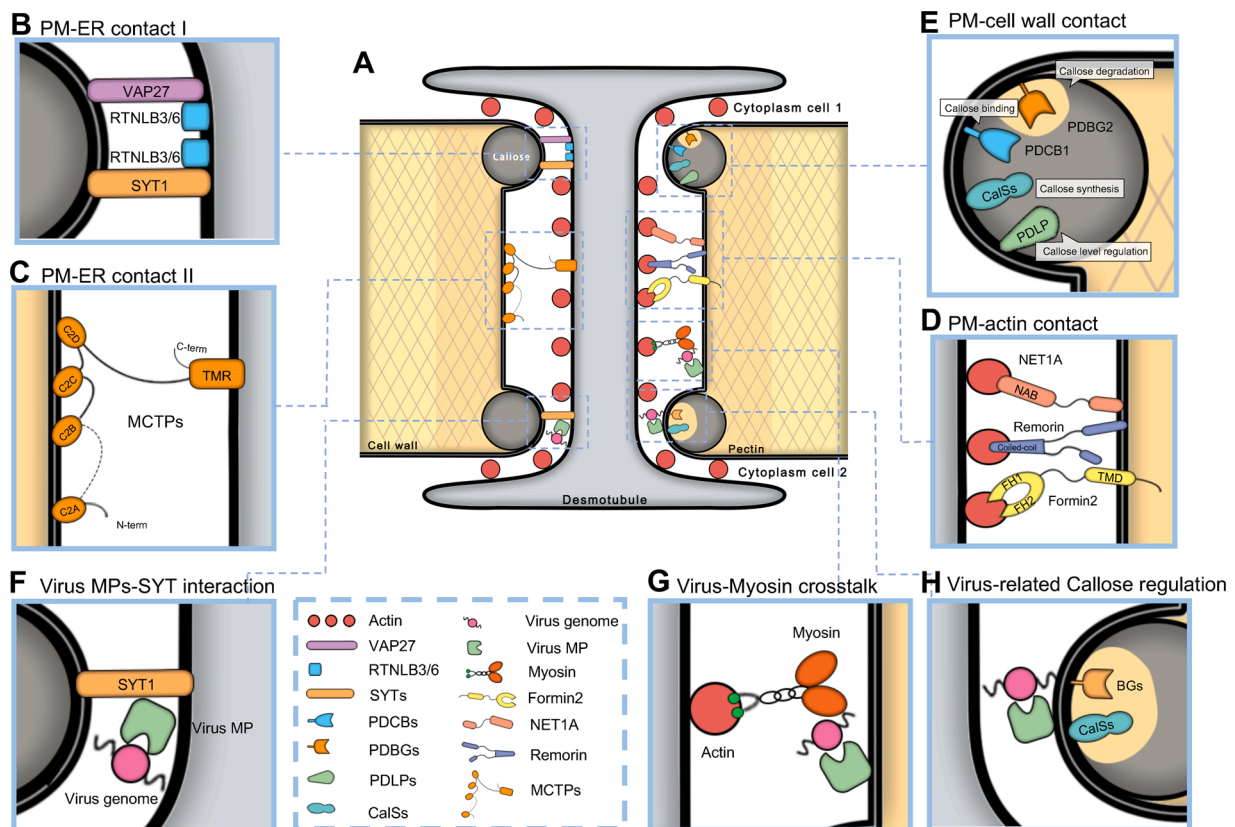The participation of ER–PM contact sites in Ca^{2+} dynamics, lipid

Fig. 1. Plasmodesmata-associated proteins might serve as tethers to participate in the communications between subcellular compartments and organelles at PD. (A) Schematic model of PD structure. PD originate from specialized endoplasmic reticulum (ER)–plasma membrane (PM) contacts that are encased by the PM, traverse the cell wall, and are associated with actin filaments. (B) VAP27 and SYT1 are two tethering proteins involved in ER–PM contact. Both interact with RTNLB3/6 and are involved in remodeling the cortical ER into desmotubules. (C) MCTPs may serve as filamentous proteins to tether ER and PM. MCTPs insert into the ER membrane via transmembrane regions (TMR) and into the PM via four C2 domains (C2A, C2B, C2C and C2D). (D) Three adaptors of PM–actin contact at PD. NET1A targets to the PD membrane and associates with actin through a novel actin-binding (NAB) domain. Rice remorin protein GSD1 locates at the PD membrane and directly interacts with actin via its coiled-coil domain. Formin2 is distributed at the PD membrane through its transmembrane domains (TMD), and it also catalyzes the nucleation of actin monomers via C-terminal formin homology 1 and 2 domains (FH1 and FH2 domains). (E) Callose synthases (CalSs), PD-located protein (PDLP), callose binding protein 1 (PDCB1), and β-1,3 glucanase 2 (PDBG2) probably connect PM and cell wall. Callose is synthesized by CalSs and degraded by PDBGs. CalSs are transmembrane proteins, and PDBG2 is a membrane-anchored apoplastic enzyme. PDCB1 embeds in the extracellular leaflet of the PM via the GPI motif and regulate callose accumulation. PDLPs, membrane-associated proteins, modulate the callose level at PD. (F) In early stage of virus infection, virus movement protein (MP) recruits SYT1 to PD, facilitating virus movement. (G) Virus movement requires myosins as a motor to coordinate actin bundles for virus trafficking through PD. (H) PD localized BG is crucial for *Turnip vein clearing virus* infection by degrading callose. CalS genes can be silenced during *Potato spindle tuber viroid* replication.

trafficking, membrane exchange, and inter-organellar communication has been well characterized in animals and yeast [23], and is also emerging in plants [28]. Notably, many tethering proteins that localize to ER–PM contact sites regulate PD architecture and function [23,27,41, 42]. In most PD, the desmotubule is a tightly furled tube in which the ER membranes are constricted [34]. Thus, it is reasonable to predict that desmotubule formation requires an induction of extreme membrane curvature. Reticulons are important for tubulation of the ER [43–47]. During cell plate formation, the reticulons, RTNLB3 and RTNLB6 associate with the desmotubule of primary PD where they are involved in remodeling of the cortical ER into desmotubules [34].

Using these two recticulons as baits, many PD-located proteins have been identified [48], including the well-known PD-regulatory proteins synaptotagmin A (SYTA/SYT1) [49], Remorin1.2 and 1.3 [50,51]. SYT1 is recognized as a tethering factor in ER–PM contacts that is essential in the formation of ER–PM junctions [49,52,53]. SYT1 is particularly targeted to the edges of ER sheets that are transformed into immobile ER tubules, but not to those of mobile tubules [54]. Deficiency in SYT1 causes a reduction in the immobile tubules and an enlargement of the ER meshes, while producing large intracellular vesicles attached to the PM [54–56]. Recently, two additional tethering proteins, SYT5 and SYT7, were identified at ER–PM contact sites, that can interact with SYT1 [42, 53]. The *syt1,5,7* triple mutant displays ER detachment from the PM, weak constriction of the ER at the entrance of PD, and disruption of MP location [42], supporting the importance of ER–PM contact sites in PD formation (Fig. 1B).

Other tethering factors, such as NETWORKED 3C (NET3C) and vesicle-associated membrane protein-associated protein 27 (VAP27−1) are associated with the cytoskeleton and coordinate ER–PM contact site formation with the cytoskeleton [57]. In the dual presence of VAP27 and NET3C, the association between the ER and PM increases substantially, whereas the expression of VAP27−1 alone does not induce ER–PM contacts [58]. However, the exact contributions of VAP27−1 and NET3C in PD formation have not been directly investigated. In a search for additional ER–PM contact tethers, a PD proteome approach was utilized to collect the abundant proteins during the transition phase from type I to type II PD. Multiple C2 domains and transmembrane region proteins (MCTPs) were more abundant in type I PD with tight ER-PM contacts compared to type II PD with wider cytosolic sleeve, suggesting that MCTPs are most likely the filamentous proteins that connect ER and PM in the PD [41](Fig. 1C). PD-mediated molecular trafficking and the level of PD-associated proteins is substantially altered in *Atmctp3/Atmctp4* double mutants [41].

Although tethering proteins are possibly responsible for the junctions between ER and PM, the ER–PM crosstalk at PD remains hypothetical.

3. Actin–PM contacts modify PD permeability

As a basic cytoskeleton component, actin is involved in cellular migration of organelles and dynamic movement of proteins [59–61]. Data based on pharmacological treatment show that destabilization or stabilization of actin filaments, respectively increases or decreases PD permeability [62,63]. Immunolocalization revealed the presence of actin along the entire length of PD [64,65], probably localizing in the cytosolic sleeve [17]. Several actin-associated proteins were shown to participate in the regulation of PD permeability [16,66,67]. However, it is questionable whether any cytoskeletal elements incorporate into cytosolic sleeve [33,68]. The PD pore is approximately 50 nm in diameter, and the desmotubule diameter is between 15–20 nm. Single actin fibers have a diameter of ~7 nm [69], and they usually constitute a double helix conformation of two strings of monomers, which is much larger than the supposed globular proteins (2.5–4.5 nm) which surrounding the desmotubule [32,70,71]. It has been proposed that if actin filaments are arranged in a spiral manner around the desmotubule, they have to be arranged in an unusual conformation surrounding desmotubule [33]. Thus, while the actin-directed cytoskeleton network is

clearly important in the regulation of PD conductivity, it is unclear how actin filaments are organized within the cytosolic sleeve and how they behave as regulatory elements to contact the membranes.

Animal cells employ a wide variety of adaptor proteins to connect actin and membranes, whereas plants do not have adaptors of this type. NETWORKED (NET) are likely the first adaptor proteins identified in actin–PM contact in plants [72]. NET proteins are plant-specific actin-binding proteins which anchor the PM and associate with PD [72]. Although NET1A likely tethers actin and PM, no following study has been conducted on its genetic function in PD regulation (Fig. 1D). Notably, the NET family is only present in tracheophytes and is absent in mosses and more ancient plant species [72]. Thus, it is intriguing to consider whether actin–PM tethering proteins is an innovation during the evolution of vascular plants.

FORMINs are another type of actin-binding protein [73–75], of which the FORMIN HOMOLOGY2 (FH2) domain is sufficient to catalyze the nucleation of actin monomers [76]. FORMINs with multiple domains can interact with various partners in remodeling of cytoskeletons, in determining organelle mobility, and in shaping cells [77]. In *Arabidopsis*, FORMINs are divided into two clades (I and II), containing respectively 11 and 10 members [78]. Typical class I formins are transmembrane proteins that anchor cortical actin to the cell wall [79–81]. FORMIN2 is a PD-localized class I FORMIN, that restricts the PD permeability [66]. Therefore, FORMIN2 tethers actin filaments to the PM at PD and where they are stabilized by its barbed-end capping activity [66]. These results, indicate that actin filaments at PD form a physical barrier that blocks PD [82], which is consistent with the conclusion obtained from CMV-MP [62].

The membrane system is a mixture of liquid-ordered and liquid-disordered sub-resolution nanodomains [83]. These nanodomains are enriched with lipids to restrict the lateral segregation of proteins and lipids, which serve as platforms to modulate signal perception, specificity, and transduction [84]. Small changes in nanodomain organization or lipid phase can induce rapid and large-scale alteration in signaling transduction on the PM [50,51]. The PM that lines PD contains similar lipid species as the neighboring PM, but in different proportions [13], implying a specific contribution of these lipid components to PD regulation.

The REMORIN family is a type of well-characterized membrane nanodomain-resident proteins, which have been widely used as a paradigm to study nanodomain organization pattern [50,51,85]. Owing to the abundant membrane components at PD, REMORINs are also detectable at the PD membrane [50,51,86]. Upon virus infection or during plant defense against a virus, REMORINs aggregate, leading to the assembly of membrane nanodomains and an increase in the ordered lipid phase [50,51,87,88]. Overexpression of REMORINs significantly blocks virus movement through PD [50,51,88]. Notably, the rice REMORIN, GRAIN SETTING DEFECT1, interacts with actin through its coil-coil domain [67], indicating that REMORINs connect the PM and actin at PD (Fig. 1D).

Actin filaments are closely associated with cortical ER tubules [89–91]. Removal of actin filaments destroys the ER network and its dynamic remodeling [92], suggesting a role of actin in the formation of ER network [89,90]. Additionally, ionic stress increases ER-PM connectivity by promoting the expansion of SYT1-enriched ER-PM contact sites in an actin-cytoskeleton dependent manner [53]. As mentioned above, reticulons probably induce the tubulation of the ER and participate in shaping the cortical ER into desmotubules [34]. Interestingly, in yeast cells the emergence of ER membrane curvature starts with an invagination that subsequently grows into a tubule [93]. Actin polymerization is associated with the emergence of membrane curvature and is necessary for tubule elongation [93]. Thus, the formation and maintenance of desmotubules are possibly actin-dependent. Additionally, the actin cytoskeleton is one of the most important factors that influence membrane organization [94] via stabilizing the liquid-ordered phase and preventing large-scale nanodomain separation [95]. Several studies

have proven that the actin cytoskeleton serves as a scaffold controlling lateral diffusion and dynamic movement of proteins at the PM [95–99]. The assembly of PM-associated proteins possibly depends on the interaction between actin and membrane adaptor proteins [100]. REMOR-INs, crucial regulators of PD permeability, determine the organization of PM nanodomains and associate with actin filaments [50,51,67]. Therefore, it is possible that actin-REMORIN complexes tether the desmotubule and PD-PM to establish an appropriate PD aperture and prevent the collapse of the cytosolic sleeve. Once actin filaments are destroyed at PD, the stability of the ER-desmotubule network is possibly affected, and membrane nanodomains at PD are assembled into a less tightly packed liquid-disordered (Ld) phase, resulting in an increased PD size exclusion limit (Fig. 1D).

Although the presence of actin at PD and its involvement in the regulation of PD conductivity has been demonstrated [62,63], the underlying molecular mechanisms are largely unknown. The available evidences suggest that actin filaments may contribute to establishing the adhesion of PM and cell wall, thereby, influencing the surrounding environments of PD. The limited microscopic technologies do not yet allow visualizing actin dynamics at the PD because the filaments are small and buried deep within the cell wall. Therefore, the unraveling of how actin at PD regulates cell-to-cell communication depends greatly on the identification and characterization of native components of the actin cytoskeleton that specifically localize to PD.

4. Cell wall–PM nexus maintain PD architecture

Plant cells are encased by rigid cell walls that give shape to the protoplast within and protect the plant against environmental stress. The two types of PD have distinct origins, associated with cell wall actions. The primary PD, that occur as discrete and linear entities, originate during cytokinesis on the new cell plates where new cell wall materials are deposited, and the secondary PD, with branched and multiple channels, that entirely *de novo* established in post-cytokinetic cell walls [101–103]. Since the stiffness generated by the cell wall is much higher than the PM, we speculate that the rigidity of the cell wall determines the architecture of PD, from simple, to branched, to highly branched [101]. Moreover, many cell wall-related proteins found in the PD proteome are potentially involved in specifying PD–cell wall connections [104]. Callose (β-1,3-glucan) deposited around the neck of the PD pore, physically constricts the PD aperture [105]. Callose is a cell wall component that is under control of callose synthases and degrading enzymes [103,106], and is often observed at PD in post-cytokinetic cell walls. Callose synthases are transmembrane proteins, and callose-degrading β-1,3-glucanases are membrane-anchored apoplastic enzymes [107–109]. Both types of enzymes associate with the PM, indicating the importance of PM–cell wall contacts in the formation of PD architecture. Visualization of the PD ultrastructure indicates possible spoke-like connections between the PD-PM and the cell wall at both the neck region of the PD and deeper within the wall [110]. Specific enzymatic digestion of cell wall components indicates that cellulose or pectin possibly stabilizes the PM–cell wall tethering spokes [110]. Notably, a pectin methylesterase, which catalyzes the demethylesterification of cell wall polygalacturonans and controls wall porosity and cell-cell adhesion, is localized preferentially around PD [111]. During virus infection, pectin methylesterase specifically binds the viral MP and is required for virus movement via PD [112].

As described above, the PD aperture is fine-tuned by the level of deposited callose. β-1,3 glucanase 2 (PDBG2), which degrades callose, and callose binding protein 1 (PDCB1), which directly binds callose and promotes callose accumulation at the PD neck, both regulate PD-mediated symplastic transport [13,113]. PDCB1 localizes to the neck region of PD, potentially provide a structural anchor between PD-PM and the cell wall [113]. PDBG2 and PDCB1 are glyco-sylphosphatidylinositol (GPI)-anchored proteins (GAPs), which attach to the extracellular leaflet of the PM via the GPI motif [107,113].

Cleavage of the GPI motif allows the diffusion of GAPs into the extracellular matrix, and where they participate in the cross-linking of wall polysaccharides [114]. To date, an increasing number of GAPs are found to contribute to cell wall morphogenesis [115–118]. Notably, the GPI-anchor motif also specifically recognizes PD components and serves as a primary sorting signal of GAPs, such as PDCB1 and PDBG2, to PD [119]. Therefore, GAPs are likely tethering proteins that connect PD and cell wall (Fig. 1E).

Sterols and sphingolipids with very long-chain saturated fatty acids are enriched in membranes around PD, and this unique membrane system can recruit cell wall remodeling enzymes, generating a PM–cell wall connection at PD [13]. For example, PD-located proteins (PDLPs) are membrane-associated proteins, which target to PD [120]. During eukaryotic filamentous pathogen *Hyaloperonospora arabidopsidis* infection, *pdlp1,2,3* mutants fail to accumulate callose at the haustoria, which are specialized feeding structures enabling exchange of nutrients and effectors between the host and pathogen. In contrast, overexpression of PDLP1 elevates callose deposition around the haustoria and enhance plant resistance to pathogen [121]. These observations suggest that PDLPs induce callose deposition at PD near the infection site via a similar mechanism. PDLP5 directly binds to the phytosphinganine (t18:0)-type of membrane-sphingolipid species with high affinity and modulates the callose level at PD, although the exact reason is unknown [122,123]. The ability of PDLP5 to directly bind PM components and alter callose deposition at PD opens the possibility that PDLP5 is a tethering factor in the cell wall–PM connection (Fig. 1E). Therefore, an understanding of how these adaptors mediate cell wall–PD interaction will allow further insight into the regulation and architecture formation of PD.

5. Plasmodesmal tethering elements are crucial for plant defense to virus attack

As channels that connect cells, PD are crucial for the local and systemic spread of signals in a cell-to-cell manner, as well as transportation of virus particles among plant cells [124]. Most RNA virus exhibit cytoplasmic infection cycles and replicate on the surface of various cell membranes, in which membrane contact sites provide an ideal location for the formation of viral replication complex and PD targeting [124]. During virus infection, the movement proteins (MPs) of the virus associate with PD where they interact with PD-associated proteins (such as remorin), and manipulate the size exclusion limit of PD [86,125]. The dilation of PD facilitates the symplastic spreading of RNA virus genomes. Even though the structure of MPs of different viruses is variable, their functions remain similar [126]. MPs associate with the cytosolic face of the ER membrane [127], target to microtubule–ER junctions [128], and recruit ER membranes to microtubules [129]. In addition to association with the ER, the MPs of *Cucumber mosaic virus* (CMV) target to PD where they sever F-actin and inhibit actin polymerization [62]. Thus, virus movement between adjacent cells requires participation of the ER–actin network. In addition to CMV MPs, the MPs of many other viruses target to PD through an ER–actin cytoskeleton network, such as *Tobacco mosaic virus* MP [92,130], *Potato leafroll virus* 17-kD MP [131], and *Oilseed rape mosaic virus* MP [132]. Interestingly, plasmodesmal tethering elements play a potential role in maintenance of PD architecture, facilitating virus trafficking. For example, SYT1 is recruited by MP to PD early in virus infection where SYT1 is dramatically accumulated and the cortical ER is subsequently remodeled to form viral replication site at PD [49]. Thus, SYT1 is necessary for the accumulation of MP at PD, and SYT1 and MP form a feedback regulatory loop for virus movement through PD [49, 133]. SYT1 also collaborates with SYT5 and SYT7 for the cell-to-cell movement of *Youcai mosaic virus* (YoMV) MPs [42]. Besides, truncated forms of ER-PM localized SYT1 and SYT5 or truncations of the Golgi-associated SYTs can influence *Turnip mosaic virus* (TuMV) movement [134] (Fig. 1F). Another ER-PM tethering protein, VAP27−3, collaborates with plant oxysterol-binding protein related proteins

(ORPs) to induce membrane proliferation at the foci site of viral RNA synthesis [135].

Myosin represents a diverse category of cytoskeleton motors, which possess a motor domain associated with actin filaments and a tail domain involved in cargo binding [136,137]. Myosins, having close association with ER and actin, are considered as potential candidates to tether ER, PM and actin [19,138]. There are at least 35 myosin classes in nature, of which only classes VIII and XI are found in higher plants [139, 140]. One member of the class VIII myosins (containing four members: ATM1, ATM2, myosin VIIIA, and myosin VIIIB), ATM1 is localized to PD, ER, and PM [141], while myosin XI is localized on the ER of tobacco BY-2 cells [142]. Deficiency in myosin XI-K induces an aberrant ER configuration and mobility, which causes a random orientation of actin filament bundles [143]. Therefore, myosin is responsible for organizing actin filament bundles and controls the dynamics of ER strands (Fig. 1G). Upon *Beet yellows virus* (BYV) infection, the Hsp70 homolog that functions in virus assembly and cell-to-cell movement, is autonomously recruited to PD [144]. Ectopic expression of the tail domain of class VIII myosin inhibits the localization of the Hsp70 homolog at PD [145]. A putative MP of *Rice stripe virus* (RSV), NSvc4 protein, also uses myosin VIII-1 in virus trafficking to PD [146]. Deficiency in class VIII and XI myosins affects the dynamic behavior of the ER and thus leads to a delay in MP accumulation at PD [147]. A PD-localized receptor-like protein, PLASMODESMATA LOCATED PROTEIN1 (PDLP1) promotes the movement of viruses by interacting with MPs within PD [148]. Notably, the distribution of PDLP1 to PD specifically requires class XI myosins, and inactivation of these myosins results in mislocalization of PDLP1 and MPs, and suppression of virus movement [149].

One of the cell wall components, callose is accumulated at the neck region of PD to limit PD opening upon abiotic and biotic stress [150–155]. Interestingly, to enhance virus trafficking, virus MPs may promote callose degradation and prevent callose synthesis to increase PD dilation. In the absence of *Tobacco mosaic virus* (TMV) MPs, TMV replication results in callose accumulation at the PD neck. By contrast, in the presence of TMV MPs, plasmodesmal callose level is reduced [154]. The fine-tuning in callose amounts by virus MPs suggests a regulatory function of callose synthase (CalS) and degrading enzyme (BG) during virus attack. Not surprisingly, AtBG2 is able to enhance virus spread by degrading callose at the PD [156]. In uninfected cells, AtBG2 is retained within ER; upon TMV infection, AtBG2 associates with the viral MPs in the ER-derived bodies; as the infection progressed, these ER bodies enlarge and connect with PD [156]. Apparently, the PD-associated BG is directly involved in regulation of callose at PD and cell-to-cell movement of virus. Beside of BG enzymes, virus may utilize another approach to decrease callose level by suppression of callose synthases' activity (review in [105]) (Fig. 1H).

Collectively, emerging evidences illustrate the great importance of PD tethering elements for virus spread through PD. Single virus may apply multiple approaches to modify PD architecture or permeability by utilization of different PD tethering elements. Questions remaining to be addressed are related to the mechanism by which MPs regulate the trafficking capacity of PD for virus movement and how these PD tethering elements are involved in this process.

6. PD structuring requires the coordination of member tethers as well as the signaling network away from PD

PD permeability and architecture formation are regulated by a variety of cellular compartments and the tethering proteins that associate with PD membrane. The membrane lipid environment of the PD pores which contains an enrichment of sphingolipids and a higher ratio of sterols to glycerolipids, is distinct from that of the bulk PM [13]. Several recent studies have highlighted an importance of membrane lipid compositions for the regulation of PD permeability [122,157–159]. Mutation of a sphingolipids biosynthesis component, a putative enzyme phloem unloading modulator (PLM), results in defective ER-PM tethers

and a consequently enhanced PD conductivity [158]. Recent technological advances have developed a computational pipeline to detect PD distribution and clustering pattern in a quantitative manner [160]. Interestingly, the *plm* mutant interferes with the clustering of PD, and causes a thicker cell wall around PD [160]. As PLM-regulated sphingolipids are enriched at the PD membrane and are also cross-linked with pectins in the cell walls, PLM is involved in both processes of ER-PM tethers formation within PD and cell wall regulation surrounding PD. Other membrane tethering proteins also manipulate PD structure and its surrounding components. Remorin proteins tightly engage with local lipid nanodomains [161], essential for ordered lipid structure at the PD. On one hand, over-accumulated remorin proteins promote higher ordered lipid structure, which decreases PD plasticity [159]. On the other hand, remorins restrict virus movement through PD by competitively binding actin filaments with a virus-interacting protein [162]. Therefore, PD membrane serves as a platform, incorporated with different kinds of lipid molecules and membrane-associated proteins to facilitate signaling transduction in a collaborative manner, resulting in the current structures of PD.

We have summarized the importance of PD tethering elements for PD structuring and functionality. Whereas, accumulating evidence shows that non-related signals, such as hydrogen peroxide and oxidative stress, also influence PD architecture. At elevated ROS levels, *ise1* (increased size exclusion limit1), *ise2*, and *gat1* (GFP-arrested trafficking1) mutants display severe defects in PD structure, with more branched PD [163–165]. This observation suggests that ISE1, ISE2, and GAT1 proteins are functionally crucial in the formation of PD structure. However, ISE1 is localized in the mitochondria while ISE2 and GAT1 are in the chloroplasts [163,165,166]. None of them are associated with PD, indicating that these proteins are not locally nor directly involved in PD formation. The pleiotropic effects of ROS stress in the cell, affecting actin polymerization, cytoskeleton organization, protein movement, and membrane assembly, make it difficult to judge whether the changes in behavior at PD are directly or indirectly caused by ROS. A recent study additionally illustrates that rapamycin (TOR)-glucose metabolic signaling network coordinates redistribution of carbohydrate transport from sink to source via a PD-dependent pathway [167]. TOR is a protein kinase that is activated by nutrients, which is a housekeeper gene in cytoplasm and nucleus to coordinate plant development and metabolism [168]. The activation of glucose-TOR signaling restricts PD transport, whereas the direct regulatory mechanism of TOR kinase on PD permeability is unknown [167].

Overall, PD mediated cell-to-cell communication in plant is a complicated system, which may require well-orchestrated crosstalk between proteins, lipid compositions and signal molecules located within or away from PD-associated components.

7. Conclusions and future perspectives

Plasmodesmata-mediated symplastic transport is a unique cell-to-cell communication pathway in plants and is essential in plant development and for coping with environmental stress. Although the PD structure was described decades ago, many open questions remain regarding the architecture formation of different PD morphotypes, and the regulatory mechanisms of PD conductivity. In recent years, rapidly developing microscopy technologies, such as novel electron tomography approaches, have helped obtain insights in this mysterious nano-sized area. We are just starting to identify and characterize the PD-localized proteins that tether the ER and the PM, which may help uncovering the origin of PD. Cytoskeleton networks, especially actin dynamics and the actin–myosin network at PD, have been widely investigated during virus trafficking. The actin–PM contacts at PD are most likely required in the adjustment of the size exclusion limit of cytosolic sleeves and therefore in the control of PD conductivity, via a process that remains poorly understood. The cell wall is unique in plant cells and might be one of the major reasons that a specific plant cell-to-cell communication

system evolved. To date, many tethering proteins that are localized in the interactive contact sites of subcellular compartments at PD have been characterized. In addition, as a result of great efforts to understand the relationship between PD conductivity and plant development, a series of mobile proteins have been described that move via PD.

Although these advances increase knowledge of the plasticity of PD regulation and the complicated formation of PD structure, much remains unknown about how these processes occur. For example, very little is known about the unique signaling and metabolism that occur at the PD. In addition, several PD-associated proteins involved in PD regulation are directly or indirectly related to callose homeostasis, and therefore, it is necessary to clarify their specific contributions to the different compositions of PD. New questions are also generated: How does the cell wall participate in the differentiation of secondary PD? What is the functional difference, if any, between primary and secondary PD?

The investigation of adaptor proteins that tether PD components has only begun but can help to answer such questions. In the future, their functions at different stages of PD development need to be specified and their contributions in specific cell types need to be clarified. The PD membrane contains a variety of associated proteins for downstream signaling events. On the basis of speculation that membrane nano-domains serve as signaling hubs, the nanodomain-associated remorin or tetraspanin webs [169] may act as new adaptors, facilitating further understanding of specific signaling transduction that occurs at PD.

Author contributions

Xu Chen and Chaofan Chen discussed and wrote the manuscript, Steffen Vanneste helped revising the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interests.

Acknowledgements

References

[1] J.M. Van Norman, N.W. Breakfield, P.N. Benfey, Intercellular communication during plant development, Plant Cell 23 (2011) 855–864.

[2] J. Ariazi, A. Benowitz, V. De Biasi, M.L. Den Boer, S. Cherqui, H. Cui, N. Douillet, E.A. Eugenin, D. Favre, S. Goodman, K. Gousset, D. Hanein, D.I. Israel, S. Kimura, R.B. Kirkpatrick, N. Kuhn, C. Jeong, E. Lou, R. Mailliard, S. Maio, G. Okafo, M. Osswald, J. Pasquier, R. Polak, G. Pradel, B. de Rooij, P. Schaeffer, V. A. Skeberdis, I.F. Smith, A. Tanveer, N. Volkmann, Z. Wu, C. Zurzolo, Tunneling nanotubes and gap junctions-their role in long-range intercellular communication during development, health, and disease conditions, Front. Mol. Neurosci. 10 (2017) 333.

[3] J.O. Brunkard, A.M. Runkel, P.C. Zambryski, Plasmodesmata dynamics are coordinated by intracellular signaling pathways, Curr. Opin. Plant Biol. 16 (2013) 614–620.

[4] C. Cheval, C. Faulkner, Plasmodesmal regulation during plant-pathogen interactions, New Phytol. 217 (2018) 62–67.

[5] R. Sager, J.Y. Lee, Plasmodesmata in integrated cell signalling: insights from development and environmental signals and stresses, J. Exp. Bot. 65 (2014) 6337–6358.

[6] Y. Long, B. Scheres, I. Blilou, The logic of communication: roles for mobile transcription factors in plants, J. Exp. Bot. 66 (2015) 1133–1144.

[7] Z.P. Li, A. Paterlini, M. Glavier, E.M. Bayer, Intercellular trafficking via plasmodesmata: molecular layers of complexity, Cell. Mol. Life Sci. (2020).

[8] K. Ehlers, R. Kollmann, Primary and secondary plasmodesmata: structure, origin, and functioning, Protoplasma 216 (2001) 1–30.

[9] V. Haywood, F. Kragler, W.J. Lucas, Plasmodesmata: pathways for protein and ribonucleoprotein signaling, Plant Cell 14 (Suppl) (2002) S303–325.

[10] P.K. Hepler, Endoplasmic reticulum in the formation of the cell plate and plasmodesmata, Protoplasma 111 (1982) 121–133.

[11] F. Kragler, L. William, M. Jan, Plasmodesmata: dynamics, domains and patterning, Ann. Bot. 81 (1998) 1–10.

[12] A.J. Maule, Plasmodesmata: structure, function and biogenesis, Curr. Opin. Plant Biol. 11 (2008) 680–686.

[13] M.S. Grison, L. Brocard, L. Fouillen, W. Nicolas, V. Wewer, P. Dormann, H. Nacir, Y. Benitez-Alfonso, S. Claverol, V. Germain, Y. Boutte, S. Mongrand, E.M. Bayer, Specific membrane lipid composition is important for plasmodesmata function in Arabidopsis, Plant Cell 27 (2015) 1228–1250.

[14] D. Yan, S.R. Yadav, A. Paterlini, W.J. Nicolas, J.D. Petit, L. Brocard, I. Belevich, M.S. Grison, A. Vaten, L. Karami, S. El-Showk, J.Y. Lee, G.M. Murawska, J. Mortimer, M. Knoblauch, E. Jokitalo, J.E. Markham, E.M. Bayer, Y. Helariutta, Sphingolipid biosynthesis modulates plasmodesmal ultrastructure and phloem unloading, Nat. Plants 5 (2019) 604–615.

[15] W.J. Nicolas, M.S. Grison, S. Trepout, A. Gaston, M. Fouche, F.P. Cordelieres, K. Oparka, J. Tilsner, L. Brocard, E.M. Bayer, Architecture and permeability of post-cytokinesis plasmodesmata lacking cytoplasmic sleeves, Nat. Plants 3 (2017) 17082.

[16] R.G. White, D.A. Barton, The cytoskeleton in plasmodesmata: a role in intercellular transport? J. Exp. Bot. 62 (2011) 5249–5266.

[17] R.L. Overall, R.G. White, L.M. Blackman, J.E. Radford, Actin and myosin in plasmodesmata. Actin: A Dynamic Framework for Multiple Plant Cell Functions, Springer, 2000, pp. 497–515.

[18] J.D. Petit, Z.P. Li, W.J. Nicolas, M.S. Grison, E.M. Bayer, Dare to change, the dynamics behind plasmodesmata-mediated cell-to-cell communication, Curr. Opin. Plant Biol. 53 (2020) 80–89.

[19] A.V. Pankratenko, A.K. Atabekova, S.Y. Morozov, A.G. Solovyev, Membrane contacts in plasmodesmata: structural components and their functions, Biochemistry Mosc. 85 (2020) 531–544.

[20] C.M. Schauder, X. Wu, Y. Saheki, P. Narayanaswamy, F. Torta, M.R. Wenk, P. De Camilli, K.M. Reinisch, Structure of a lipid-bound extended synaptotagmin indicates a role in lipid transfer, Nature 510 (2014) 552–555.

[21] S. Carrasco, T. Meyer, STIM proteins and the endoplasmic reticulum-plasma membrane junctions, Annu. Rev. Biochem. 80 (2011) 973–1000.

[22] S. Cohen, A.M. Valm, J. Lippincott-Schwartz, Interacting organelles, Curr. Opin. Cell Biol. 53 (2018) 84–91.

[23] A. Gallo, C. Vannier, T. Galli, Endoplasmic reticulum-plasma membrane associations:structures and functions, Annu. Rev. Cell Dev. Biol. 32 (2016) 279–301.

[24] J.R. Friedman, L.L. Lackner, M. West, J.R. DiBenedetto, J. Nunnari, G.K. Voeltz, ER tubules mark sites of mitochondrial division, Science 334 (2011) 358–362.

[25] B. Mesmin, J. Bigay, J. Moser von Filseck, S. Lacas-Gervais, G. Drin, B. Antonny, A four-step cycle driven by PI(4)P hydrolysis directs sterol/PI(4)P exchange by the ER-Golgi tether OSBP, Cell 155 (2013) 830–843.

[26] C. Raiborg, E.M. Wenzel, N.M. Pedersen, H. Olsvik, K.O. Schink, S.W. Schultz, M. Vietri, V. Nisi, C. Bucci, A. Brech, T. Johansen, H. Stenmark, Repeated ER-endosome contacts promote endosome translocation and neurite outgrowth, Nature 520 (2015) 234–238.

[27] J. Tilsner, W. Nicolas, A. Rosado, E.M. Bayer, Staying Tight: Plasmodesmal Membrane Contact Sites and the Control of Cell-to-Cell Connectivity in Plants, Annu. Rev. Plant Biol. 67 (2016) 337–364.

[28] E.M. Bayer, I. Sparkes, S. Vanneste, A. Rosado, From shaping organelles to signalling platforms: the emerging functions of plant ER-PM contact sites, Curr. Opin. Plant Biol. 40 (2017) 89–96.

[29] A.G. Manford, C.J. Stefan, H.L. Yuan, J.A. Macgurn, S.D. Emr, ER-to-plasma membrane tethering proteins regulate cell signaling and ER morphology, Dev. Cell 23 (2012) 1129–1140.

[30] G. Stefano, C. Hawes, F. Brandizzi, ER - the key to the highway, Curr. Opin. Plant Biol. 22 (2014) 30–38.

[31] J. Perez-Sancho, J. Tilsner, A.L. Samuels, M.A. Botella, E.M. Bayer, A. Rosado, Stitching Organelles: Organization and Function of Specialized Membrane Contact Sites in Plants, Trends Cell Biol. 26 (2016) 705–717.

[32] B. Ding, R. Turgeon, M.V. Parthasarathy, Substructure of freeze-substituted plasmodesmata, Protoplasma 169 (1992) 28–41.

[33] J. Tilsner, K. Amari, L. Torrance, Plasmodesmata viewed as specialised membrane adhesion sites, Protoplasma 248 (2011) 39–60.

[34] K. Knox, P. Wang, V. Kriechbaumer, J. Tilsner, L. Frigerio, I. Sparkes, C. Hawes, K. Oparka, Putting the squeeze on plasmodesmata: a role for reticulons in primary plasmodesmata formation, Plant Physiol. 168 (2015) 1563–1572.

[35] J.O. Brunkard, A.M. Runkel, P.C. Zambryski, The cytosol must flow: intercellular transport through plasmodesmata, Curr. Opin. Cell Biol. 35 (2015) 13–20.

[36] S. Otero, Y. Helariutta, Y. Benitez-Alfonso, Symplastic communication in organ formation and tissue patterning, Curr. Opin. Plant Biol. 29 (2016) 21–28.

[37] A. Schulz, Plasmodesmal widening accompanies the short-term increase in symplasmic phloem unloading in pea root tips under osmotic stress, Protoplasma 188 (1995) 22–37.

[38] K.J. Oparka, D.A.M. Prior, Direct evidence for pressure-generated closure of plasmodesmata, Plant J. 2 (1992) 741–750.

[39] Y.L. Ruan, D.J. Llewellyn, R.T. Furbank, The control of single-celled cotton fiber elongation by developmentally reversible gating of plasmodesmata and coordinated expression of sucrose and K+ transporters and expansin, Plant Cell 13 (2001) 47–60.

[40] K. Park, J. Knoblauch, K. Oparka, K.H. Jensen, Controlling intercellular flow through mechanosensitive plasmodesmata nanopores, Nat. Commun. 10 (2019) 3564.

[41] M.L. Brault, J.D. Petit, F. Immel, W.J. Nicolas, M. Glavier, L. Brocard, A. Gaston, M. Fouche, T.J. Hawkins, J.M. Crowet, M.S. Grison, V. Germain, M. Rocher, M. Kraner, V. Alva, S. Claverol, A. Paterlini, Y. Helariutta, M. Deleu, L. Lins, J. Tilsner, E.M. Bayer, Multiple C2 domains and transmembrane region proteins (MCTPs) tether membranes at plasmodesmata, EMBO Rep. 20 (2019), e47182.

[42] K. Ishikawa, K. Tamura, Y. Fukao, T. Shimada, Structural and functional relationships between plasmodesmata and plant endoplasmic reticulum-plasma membrane contact sites consisting of three synaptotagmins, New Phytol. 226 (2020) 798–808.

[43] J. Hu, Y. Shibata, C. Voss, T. Shemesh, Z. Li, M. Coughlin, M.M. Kozlov, T. A. Rapoport, W.A. Prinz, Membrane proteins of the endoplasmic reticulum induce high-curvature tubules, Science 319 (2008) 1247–1250.

[44] I. Sparkes, N. Tolley, I. Aller, J. Svozil, A. Osterrieder, S. Botchway, C. Mueller, L. Frigerio, C. Hawes, Five Arabidopsis reticulon isoforms share endoplasmic reticulum location, topology, and membrane-shaping properties, Plant Cell 22 (2010) 1333–1343.

[45] N. Tolley, I. Sparkes, C.P. Craddock, P.J. Eastmond, J. Runions, C. Hawes, L. Frigerio, Transmembrane domain length is responsible for the ability of a plant reticulon to shape endoplasmic reticulum tubules in vivo, Plant J. 64 (2010) 411–418.

[46] N. Tolley, I.A. Sparkes, P.R. Hunter, C.P. Craddock, J. Nuttall, L.M. Roberts, C. Hawes, E. Pedrazzini, L. Frigerio, Overexpression of a plant reticulon remodels the lumen of the cortical endoplasmic reticulum but does not perturb protein transport, Traffic 9 (2008) 94–102.

[47] G.K. Voeltz, W.A. Prinz, Y. Shibata, J.M. Rist, T.A. Rapoport, A class of membrane proteins shaping the tubular endoplasmic reticulum, Cell 124 (2006) 573–586.

[48] V. Kriechbaumer, S.W. Botchway, S.E. Slade, K. Knox, L. Frigerio, K. Oparka, C. Hawes, Reticulomics: Protein-Protein Interaction Studies with Two Plasmodesmata-Localized Reticulon Family Proteins Identify Binding Partners Enriched at Plasmodesmata, Endoplasmic Reticulum, and the Plasma Membrane, Plant Physiol. 169 (2015) 1933–1945.

[49] A. Levy, J.Y. Zheng, S.G. Lazarowitz, Synaptotagmin SYTA forms ER-plasma membrane junctions that are recruited to plasmodesmata for plant virus movement, Curr. Biol. 25 (2015) 2018–2025.

[50] D. Huang, Y. Sun, Z. Ma, M. Ke, Y. Cui, Z. Chen, C. Chen, C. Ji, T.M. Tran, L. Yang, S.M. Lam, Y. Han, G. Shu, J. Friml, Y. Miao, L. Jiang, X. Chen, Salicylic acid-mediated plasmodesmal closure via Remorin-dependent lipid organization, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 21274–21284.

[51] D. Huang, Y. Sun, Z. Ma, M. Ke, Y. Cui, Z. Chen, C. Chen, C. Ji, T.M. Tran, L. Yang, S.M. Lam, Y. Han, G. Shui, Z. Wei, S. Tan, K. Liao, J. Friml, Y. Miao, L. Jiang, X. Chen, Correction for Huang et al., Salicylic acid-mediated plasmodesmal closure via Remorin-dependent lipid organization, Proc. Natl. Acad. Sci. 117 (2020), 8659-8659.

[52] J. Pérez-Sancho, S. Vanneste, E. Lee, H.E. McFarlane, A. Esteban del Valle, V. Valpuesta, J. Friml, M.A. Botella, A. Rosado, The Arabidopsis synaptotagmin1 is enriched in endoplasmic reticulum-plasma membrane contact sites and confers cellular resistance to mechanical stresses, Plant Physiol. 168 (2015) 132–143.

[53] E. Lee, S. Vanneste, J. Perez-Sancho, F. Benitez-Fuente, M. Strelau, A.P. Macho, M.A. Botella, J. Friml, A. Rosado, Ionic stress enhances ER-PM connectivity via phosphoinositide-associated SYT1 contact site expansion in Arabidopsis, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 1420–1429.

[54] K. Ishikawa, K. Tamura, H. Ueda, Y. Ito, A. Nakano, I. Hara-Nishimura, T. Shimada, Synaptotagmin-associated endoplasmic reticulum-plasma membrane contact sites are localized to immobile ER tubules, Plant Physiol. 178 (2018) 641–653.

[55] J.D. Lewis, S.G. Lazarowitz, Arabidopsis synaptotagmin SYTA regulates endocytosis and virus movement protein cell-to-cell transport, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 2491–2496.

[56] A. Uchiyama, H. Shimada-Beltran, A. Levy, J.Y. Zheng, P.A. Javia, S. G. Lazarowitz, The Arabidopsis synaptotagmin SYTA regulates the cell-to-cell movement of diverse plant viruses, Front. Plant Sci. 5 (2014) 584.

[57] P. Wang, T.J. Hawkins, C. Richardson, I. Cummins, M.J. Deeks, I. Sparkes, C. Hawes, P.J. Hussey, The plant cytoskeleton, NET3C, and VAP27 mediate the link between the plasma membrane and endoplasmic reticulum, Curr. Biol. 24 (2014) 1397–1405.

[58] P. Wang, C. Richardson, T.J. Hawkins, I. Sparkes, C. Hawes, P.J. Hussey, Plant VAP27 proteins: domain characterization, intracellular localization and role in plant development, New Phytol. 210 (2016) 1311–1326.

[59] P.J. Hussey, T. Ketelaar, M.J. Deeks, Control of the actin cytoskeleton in plant cell growth, Annu. Rev. Plant Biol. 57 (2006) 109–125.

[60] M.K. Kandasamy, R.B. Meagher, Actin-organelle interaction: association with chloroplast in arabidopsis leaf mesophyll cells, Cell Motil. Cytoskeleton 44 (1999) 110–118.

[61] K. Van Gestel, R.H. Köhler, J.P. Verbelen, Plant mitochondria move on F-actin, but their positioning in the cortical cytoplasm depends on both F-actin and microtubules, J. Exp. Bot. 53 (2002) 659–667.

[62] S. Su, Z. Liu, C. Chen, Y. Zhang, X. Wang, L. Zhu, L. Miao, X.C. Wang, M. Yuan, Cucumber mosaic virus movement protein severs actin filaments to increase the plasmodesmal size exclusion limit in tobacco, Plant Cell 22 (2010) 1373–1387.

[63] B. Ding, M.-O. Kwon, L. Warnberg, Evidence that actin filaments are involved in controlling the permeability of plasmodesmata in tobacco mesophyll, Plant J. 10 (1996) 157–164.

[64] R.G. White, K. Badelt, R.L. Overall, M. Vesk, Actin associated with plasmodesmata, Protoplasma 180 (1994) 169–184.

[65] L.M. Blackman, R.L. Overall, Immunolocalisation of the cytoskeleton to plasmodesmata of Chara corallina, Plant J. 14 (1998) 733–741.

[66] M. Diao, S. Ren, Q. Wang, L. Qian, J. Shen, Y. Liu, S. Huang, Arabidopsis formin 2 regulates cell-to-cell trafficking by capping and stabilizing actin filaments at plasmodesmata, Elife 7 (2018), e36316.

[67] J. Gui, S. Zheng, J. Shen, L. Li, Grain setting defect1 (GSD1) function in rice depends on S-acylation and interacts with actin 1 (OsACT1) at its C-terminal, Front. Plant Sci. 6 (2015) 804.

[68] K. Bell, K. Oparka, Imaging plasmodesmata, Protoplasma 248 (2011) 9–25.

[69] K.C. Holmes, D. Popp, W. Gebhard, W. Kabsch, Atomic model of the actin filament, Nature 347 (1990) 44–49.

[70] C. Botha, B. Hartiey, R.H.M. Cross, The ultrastructure and computer-enhanced digital image analysis of plasmodesmata at the kranz mesophyll-bundle sheath interface of Themeda triandra var. Imberbis (Retz) A. Camus in conventionally-fixed blades, Ann. Bot. 72 (1993) 255–261.

[71] R.L. Overall, J. Wolfe, B.E.S. Gunning, Intercellular communication inAzolla roots: I. Ultrastructure of plasmodesmata, Protoplasma 111 (1982) 134–150.

[72] M.J. Deeks, J.R. Calcutt, E.K. Ingle, T.J. Hawkins, S. Chapman, A.C. Richardson, D.A. Mentlak, M.R. Dixon, F. Cartwright, A.P. Smertenko, K. Oparka, P.J. Hussey, A superfamily of actin-binding proteins at the actin-membrane nexus of higher plants, Curr. Biol. 22 (2012) 1595–1600.

[73] A.Y. Cheung, H.M. Wu, Overexpression of an Arabidopsis formin stimulates supernumerary actin cable formation from pollen tube cell membrane, Plant Cell 16 (2004) 257–269.

[74] H. Ingouff, J.N. Fitz Gerald, C. Guerin, H. Robert, M.B. Sorensen, D. Van Damme, D. Geelen, L. Blanchoin, F. Berger, Plant formin AtFH5 is an evolutionarily conserved actin nucleator involved in cytokinesis, Nat. Cell Biol. 7 (2005) 374–380.

[75] M.J. Deeks, M. Fendrych, A. Smertenko, K.S. Bell, K. Oparka, F. Cvrckova, V. Zarsky, P.J. Hussey, The plant formin AtFH4 interacts with both actin and microtubules, and contains a newly identified microtubule-binding domain, J. Cell. Sci. 123 (2010) 1209–1215.

[76] D. Breitsprecher, B.L. Goode, Formins at a glance, J. Cell. Sci. 126 (2013) 1–7.

[77] M.A. Chesarone, A.G. DuPage, B.L. Goode, Unleashing formins to remodel the actin and microtubule cytoskeletons, Nat. Rev. Mol. Cell Biol. 11 (2010) 62–74.

[78] M. Grunt, V. Zarsky, F. Cvrckova, Roots of angiosperm formins: the evolutionary history of plant FH2 domain-containing proteins, BMC Evol. Biol. 8 (2008) 115.

[79] F. Cvrckova, Are plant formins integral membrane proteins? Genome Biol. 1 (2000). RESEARCH001.

[80] A. Martiniere, P. Gayral, C. Hawes, J. Runions, Building bridges: formin1 of Arabidopsis forms a connection between the cell wall and the actin cytoskeleton, Plant J. 66 (2011) 354–365.

[81] P.A. van Gisbergen, M. Bezanilla, Plant formins: membrane anchors for actin polymerization, Trends Cell Biol. 23 (2013) 227–233.

[82] C. Chen, Y. Zhang, L. Zhu, M. Yuan, The actin cytoskeleton is involved in the regulation of the plasmodesmal size exclusion limit, Plant Signal. Behav. 5 (2010) 1663–1665.

[83] K. Simons, W.L. Vaz, Model systems, lipid rafts, and cell membranes, Annu. Rev. Biophys. Biomol. Struct. 33 (2004) 269–295.

[84] Y. Jaillais, T. Ott, The nanoscale organization of the plasma membrane and its importance in signaling: a proteolipid perspective, Plant Physiol. 182 (2020) 1682–1696.

[85] A. Legrand, D. Martinez, A. Grelard, M. Berbon, E. Morvan, A. Tawani, A. Loquet, S. Mongrand, B. Habenstein, Nanodomain clustering of the plant protein remorin by solid-state NMR, Front. Mol. Biosci. 6 (2019) 107.

[86] S. Raffaele, E. Bayer, D. Lafarge, S. Cluzet, S. German Retana, T. Boubekeur, N. Leborgne-Castel, J.P. Carde, J. Lherminier, E. Noirot, B. Satiat-Jeunemaitre, J. Laroche-Traineau, P. Moreau, T. Ott, A.J. Maule, P. Reymond, F. Simon-Plas, E. E. Farmer, J.J. Bessoule, S. Mongrand, Remorin, a solanaceae protein resident in membrane rafts and plasmodesmata, impairs potato virus X movement, Plant Cell 21 (2009) 1541–1555.

[87] N. Sasaki, E. Takashima, H. Nyunoya, Altered subcellular localization of a tobacco membrane raft-associated remorin protein by tobamovirus infection and transient expression of viral replication and movement proteins, Front. Plant Sci. 9 (2018) 619.

[88] A. Perraki, J. Gronnier, P. Gouguet, M. Boudsocq, A.F. Deroubaix, V. Simon, S. German-Retana, A. Legrand, B. Habenstein, C. Zipfel, E. Bayer, S. Mongrand, V. Germain, REM1.3's phospho-status defines its plasma membrane nanodomain organization and activity in restricting PVX cell-to-cell movement, PLoS Pathog. 14 (2018), e1007378.

[89] I.K. Lichtscheidl, S.A. Lancelle, P.K. Hepler, Actin–endoplasmic reticulum complexes in Drosera: their structural relationship with the plasmalemma, nucleus, and organelles in cells prepared by high pressure freezing, Protoplasma 155 (1990) 116–126.

[90] P. Boevink, K. Oparka, S. Santa Cruz, B. Martin, A. Betteridge, C. Hawes, Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network, Plant J. 15 (1998) 441–447.

[91] J. Runions, T. Brach, S. Kuhner, C. Hawes, Photoactivation of GFP reveals protein dynamics within the endoplasmic reticulum membrane, J. Exp. Bot. 57 (2006) 43–50.

[92] K.M. Wright, N.T. Wood, A.G. Roberts, S. Chapman, P. Boevink, K.M. Mackenzie, K.J. Oparka, Targeting of TMV movement protein to plasmodesmata requires the actin/ER network: evidence from FRAP, Traffic 8 (2007) 21–31.

[93] J. Encinar Del Dedo, F.Z. Idrissi, I.M. Fernandez-Golbano, P. Garcia, E. Rebollo, M.K. Krzyzanowski, H. Grotsch, M.I. Geli, ORP-mediated ER contact with endocytic sites facilitates actin polymerization, Dev. Cell 43 (2017) 588–602, e586.

[94] D.V. Koster, S. Mayor, Cortical actin and the plasma membrane: inextricably intertwined, Curr. Opin. Cell Biol. 38 (2016) 81–89.

[95] A. Honigmann, S. Sadeghi, J. Keller, S.W. Hell, C. Eggeling, R. Vink, A lipid bound actin meshwork organizes liquid phase separation in model membranes, Elife 3 (2014), e01671.

[96] D. Goswami, K. Gowrishankar, S. Bilgrami, S. Ghosh, R. Raghupathy, R. Chadda, R. Vishwakarma, M. Rao, S. Mayor, Nanoclusters of GPI-anchored proteins are formed by cortical actin-driven activity, Cell 135 (2008) 1085–1097.

[97] T.K. Fujiwara, K. Iwasawa, Z. Kalay, T.A. Tsunoyama, Y. Watanabe, Y. M. Umemura, H. Murakoshi, K.G. Suzuki, Y.L. Nemoto, N. Morone, A. Kusumi, Confined diffusion of transmembrane proteins and lipids induced by the same actin meshwork lining the plasma membrane, Mol. Biol. Cell 27 (2016) 1101–1119.

[98] S. Saha, I.H. Lee, A. Polley, J.T. Groves, M. Rao, S. Mayor, Diffusion of GPI-anchored proteins is influenced by the activity of dynamic cortical actin, Mol. Biol. Cell 26 (2015) 4033–4045.

[99] V. Mueller, C. Ringemann, A. Honigmann, G. Schwarzmann, R. Medda, M. Leutenegger, S. Polyakova, V.N. Belov, S.W. Hell, C. Eggeling, STED nanoscopy reveals molecular details of cholesterol- and cytoskeleton-modulated lipid interactions in living cells, Biophys. J. 101 (2011) 1651–1660.

[100] E. Sezgin, I. Levental, S. Mayor, C. Eggeling, The mystery of membrane organization: composition, regulation and roles of lipid rafts, Nat. Rev. Mol. Cell Biol. 18 (2017) 361–374.

[101] T.M. Burch-Smith, S. Stonebloom, M. Xu, P.C. Zambryski, Plasmodesmata during development: re-examination of the importance of primary, secondary, and branched plasmodesmata structure versus function, Protoplasma 248 (2011) 61–74.

[102] C. Faulkner, O.E. Akman, K. Bell, C. Jeffree, K. Oparka, Peeking into pit fields: a multiple twinning model of secondary plasmodesmata formation in tobacco, Plant Cell 20 (2008) 1504–1518.

[103] N. De Storme, D. Geelen, Callose homeostasis at plasmodesmata: molecular regulators and developmental relevance, Front. Plant Sci. 5 (2014) 138.

[104] L. Fernandez-Calvino, C. Faulkner, J. Walshaw, G. Saalbach, E. Bayer, Y. Benitez-Alfonso, A. Maule, Arabidopsis plasmodesmal proteome, PLoS One 6 (2011), e18880.

[105] R. Zavaliev, S. Ueki, B.L. Epel, V. Citovsky, Biology of callose (beta-1,3-glucan) turnover at plasmodesmata, Protoplasma 248 (2011) 117–130.

[106] S.W. Wu, R. Kumar, A.B.B. Iswanto, J.Y. Kim, Callose balancing at plasmodesmata, J. Exp. Bot. 69 (2018) 5325–5339.

[107] A. Levy, M. Erlanger, M. Rosenthal, B.L. Epel, A plasmodesmata-associated beta-1,3-glucanase in Arabidopsis, Plant J. 49 (2007) 669–682.

[108] Z. Hong, A.J. Delauney, D.P. Verma, A cell plate-specific callose synthase and its interaction with phragmoplastin, Plant Cell 13 (2001) 755–768.

[109] X. Dong, Z. Hong, M. Sivaramakrishnan, M. Mahfouz, D.P.S. Verma, Callose synthase (CalS5) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis, Plant J. 42 (2005) 315–328.

[110] S. Brecknock, T.P. Dibbayawan, M. Vesk, P.A. Vesk, C. Faulkner, D.A. Barton, R. L. Overall, High resolution scanning electron microscopy of plasmodesmata, Planta 234 (2011) 749–758.

[111] O. Morvan, M. Quentin, A. Jauneau, A. Mareck, C. Morvan, Immunogold localization of pectin methylesterases in the cortical tissues of flax hypocotyl, Protoplasma 202 (1998) 175–184.

[112] M.H. Chen, J. Sheng, G. Hind, A.K. Handa, V. Citovsky, Interaction between the tobacco mosaic virus movement protein and host cell pectin methylesterases is required for viral cell-to-cell movement, EMBO J. 19 (2000) 913–920.

[113] C. Simpson, C. Thomas, K. Findlay, E. Bayer, A.J. Maule, An Arabidopsis GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking, Plant Cell 21 (2009) 581–594.

[114] T.H. Yeats, A. Bacic, K.L. Johnson, Plant glycosylphosphatidylinositol anchored proteins at the plasma membrane-cell wall nexus, J. Integr. Plant Biol. 60 (2018) 649–669.

[115] C.S. Gillmor, W. Lukowitz, G. Brininstool, J.C. Sedbrook, T. Hamann, P. Poindexter, C. Somerville, Glycosylphosphatidylinositol-anchored proteins are required for cell wall synthesis and morphogenesis in Arabidopsis, Plant Cell 17 (2005) 1128–1140.

[116] S. Hayashi, T. Ishii, T. Matsunaga, R. Tominaga, T. Kuromori, T. Wada, K. Shinozaki, T. Hirayama, The glycerophosphoryl diester phosphodiesterase-like proteins SHV3 and its homologs play important roles in cell wall organization, Plant Cell Physiol. 49 (2008) 1522–1535.

[117] G. Schindelman, A. Morikami, J. Jung, T.I. Baskin, N.C. Carpita, P. Derbyshire, M. C. McCann, P.N. Benfey, COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in Arabidopsis, Genes Dev. 15 (2001) 1115–1127.

[118] P. Vaddepalli, L. Fulton, J. Wieland, K. Wassmer, M. Schaeffer, S. Ranf, K. Schneitz, The cell wall-localized atypical beta-1,3 glucanase ZERZAUST controls tissue morphogenesis in Arabidopsis thaliana, Development 144 (2017) 2259–2269.

[119] R. Zavaliev, X. Dong, B.L. Epel, Glycosylphosphatidylinositol (GPI) modification serves as a primary plasmodesmal sorting signal, Plant Physiol. 172 (2016) 1061–1073.

[120] C.L. Thomas, E.M. Bayer, C. Ritzenthaler, L. Fernandez-Calvino, A.J. Maule, Specific targeting of a plasmodesmal protein affecting cell-to-cell communication, PLoS Biol. 6 (2008) e7.

[121] M.C. Caillaud, L. Wirthmueller, J. Sklenar, K. Findlay, S.J. Piquerez, A.M. Jones, S. Robatzek, J.D. Jones, C. Faulkner, The plasmodesmal protein PDLP1 localises

[122] to haustoria-associated membranes during downy mildew infection and regulates callose deposition, PLoS Pathog. 10 (2014), e1004496.

[122] N.J. Liu, T. Zhang, Z.H. Liu, X. Chen, H.S. Guo, B.H. Ju, Y.Y. Zhang, G.Z. Li, Q. H. Zhou, Y.M. Qin, Y.X. Zhu, Phytosphinganine affects plasmodesmata permeability via facilitating PDLP5-Stimulated callose accumulation in Arabidopsis, Mol. Plant 13 (2020) 128–143.

[123] J.Y. Lee, X. Wang, W. Cui, R. Sager, S. Modla, K. Czymmek, B. Zybaliov, K. van Wijk, C. Zhang, H. Lu, V. Lakshmanan, A plasmodesmata-localized protein mediates crosstalk between cell-to-cell communication and innate immunity in Arabidopsis, Plant Cell 23 (2011) 3353–3373.

[124] A. Levy, J. Tilsner, Creating Contacts Between Replication and Movement at Plasmodesmata - A Role for Membrane Contact Sites in Plant Virus Infections? Front. Plant Sci. 11 (2020) 862.

[125] A. Perraki, M. Binaghi, M.A. Mecchia, J. Gronnier, S. German-Retana, S. Mongrand, E. Bayer, A.M. Zelada, V. Germain, StRemorin1.3 hampers Potato virus X TGBp1 ability to increase plasmodesmata permeability, but does not interfere with its silencing suppressor activity, FEBS Lett. 588 (2014) 1699–1705.

[126] B.C. Reagan, T.M. Burch-Smith, Viruses reveal the secrets of plasmodesmal cell biology, Mol. Plant Microbe Interact. 33 (2020) 26–39.

[127] A. Peiro, L. Martinez-Gil, S. Tamborero, V. Pallas, J.A. Sanchez-Navarro, I. Mingarro, A. Simon, The tobacco mosaic virus movement protein associates with but does not integrate into biological membranes, J. Virol. 88 (2013) 3016–3026.

[128] A. Sambade, K. Brandner, C. Hofmann, M. Seemanpillai, J. Mutterer, M. Heinlein, Transport of TMV movement protein particles associated with the targeting of RNA to plasmodesmata, Traffic 9 (2008) 2073–2088.

[129] J. Ferralli, J. Ashby, M. Fasler, V. Boyko, M. Heinlein, Disruption of microtubule organization and centrosome function by expression of tobacco mosaic virus movement protein, J. Virol. 80 (2006) 5807–5821.

[130] K.M. Wright, S. Chapman, A.G. Roberts, Plasmodesmal targeting and accumulation of TMV movement protein, Plant Signal. Behav. 2 (2007) 180–181.

[131] F. Vogel, D. Hofius, U. Sonnewald, Intracellular trafficking of Potato leafroll virus movement protein in transgenic Arabidopsis, Traffic 8 (2007) 1205–1214.

[132] A. Niehl, A. Pasquier, I. Ferriol, Y. Mely, M. Heinlein, Comparison of the Oilseed rape mosaic virus and Tobacco mosaic virus movement proteins (MP) reveals common and dissimilar MP functions for tobamovirus spread, Virology 456-457 (2014) 43–54.

[133] K. Ishikawa, M. Hashimoto, A. Yusa, H. Koinuma, Y. Kitazawa, O. Netsu, Y. Yamaji, S. Namba, Dual targeting of a virus movement protein to ER and plasma membrane subdomains is essential for plasmodesmata localization, PLoS Pathog. 13 (2017) e1006463.

[134] D.G. Cabanillas, J. Jiang, N. Movahed, H. Germain, Y. Yamaji, H. Zheng, J. F. Laliberte, Turnip mosaic virus uses the SNARE protein VTI11 in an unconventional route for replication vesicle trafficking, Plant Cell 30 (2018) 2594–2615.

[135] D. Barajas, K. Xu, I.F. de Castro Martin, Z. Sasvari, F. Brandizzi, C. Risco, P. D. Nagy, Co-opted oxysterol-binding ORP and VAP proteins channel sterols to RNA virus replication sites via membrane contact sites, PLoS Pathog. 10 (2014), e1004388.

[136] R. Aaziz, S. Dinant, B.L. Epel, Plasmodesmata and plant cytoskeleton, Trends Plant Sci. 6 (2001) 326–330.

[137] K.J. Oparka, Getting the message across: how do plant cells exchange macromolecular complexes? Trends Plant Sci. 9 (2004) 33–41.

[138] R.L. Overall, L.M. Blackman, A model of the macromolecular structure of plasmodesmata, Trends Plant Sci. 1 (1996) 307–311.

[139] F. Odronitz, M. Kollmar, Drawing the tree of eukaryotic life based on the analysis of 2,269 manually annotated myosins from 328 species, Genome Biol. 8 (2007) R196.

[140] A.S. Reddy, I.S. Day, Analysis of the myosins encoded in the recently completed Arabidopsis thaliana genome sequence, Genome Biol. 2 (2001). RESEARCH0024.

[141] L. Golomb, M. Abu-Abied, E. Belausov, E. Sadot, Different subcellular localizations and functions of Arabidopsis myosin VIII, BMC Plant Biol. 8 (2008) 3.

[142] E. Yokota, S. Ueda, K. Tamura, H. Orii, S. Uchi, S. Sonobe, I. Hara-Nishimura, T. Shimmen, An isoform of myosin XI is responsible for the translocation of endoplasmic reticulum in tobacco cultured BY-2 cells, J. Exp. Bot. 60 (2009) 197–212.

[143] H. Ueda, E. Yokota, N. Kutsuna, T. Shimada, K. Tamura, T. Shimmen, S. Hasezawa, V.V. Dolja, I. Hara-Nishimura, Myosin-dependent endoplasmic reticulum motility and F-actin organization in plant cells, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 6894–6899.

[144] V. Medina, V.V. Peremyslov, Y. Hagiwara, V.V. Dolja, Subcellular localization of the HSP70-homolog encoded by beet yellows closterovirus, Virology 260 (1999) 173–181.

[145] D. Avisar, A.I. Prokhnevsky, V.V. Dolja, Class VIII myosins are required for plasmodesmatal localization of a closterovirus Hsp70 homolog, J. Virol. 82 (2008) 2836–2843.

[146] Z. Yuan, H. Chen, Q. Chen, T. Omura, L. Xie, Z. Wu, T. Wei, The early secretory pathway and an actin-myosin VIII motility system are required for plasmodesmatal localization of the NSvc4 protein of Rice stripe virus, Virus Res. 159 (2011) 62–68.

[147] K. Amari, M. Di Donato, V.V. Dolja, M. Heinlein, Myosins VIII and XI play distinct roles in reproduction and transport of tobacco mosaic virus, PLoS Pathog. 10 (2014), e1004448.

[148] K. Amari, E. Boutant, C. Hofmann, C. Schmitt-Keichinger, L. Fernandez-Calvino, P. Didier, A. Lerich, J. Mutterer, C.L. Thomas, M. Heinlein, Y. Mely, A.J. Maule,

C. Ritzenthaler, A family of plasmodesmal proteins with receptor-like properties for plant viral movement proteins, PLoS Pathog. 6 (2010), e1001119.

[149] K. Amari, A. Lerich, C. Schmitt-Keichinger, V.V. Dolja, C. Ritzenthaler, Tubule-guided cell-to-cell movement of a plant virus requires class XI myosin motors, PLoS Pathog. 7 (2011), e1002327.

[150] W. Cui, J.Y. Lee, Arabidopsis callose synthases CalS1/8 regulate plasmodesmal permeability during stress, Nat. Plants 2 (2016) 16034.

[151] X. Wang, R. Sager, W. Cui, C. Zhang, H. Lu, J.Y. Lee, Salicylic acid regulates Plasmodesmata closure during innate immune responses in Arabidopsis, Plant Cell 25 (2013) 2315–2329.

[152] B.L. Epel, Plant viruses spread by diffusion on ER-associated movement-protein-rafts through plasmodesmata gated by viral induced host beta-1,3-glucanases, Semin. Cell Dev. Biol. 20 (2009) 1074–1081.

[153] J.Y. Lee, H. Lu, Plasmodesmata: the battleground against intruders, Trends Plant Sci. 16 (2011) 201–210.

[154] D. Guenoune-Gelbart, M. Elbaum, G. Sagi, A. Levy, B.L. Epel, Tobacco mosaic virus (TMV) replicase and movement protein function synergistically in facilitating TMV spread by lateral diffusion in the plasmodesmal desmotubule of Nicotiana benthamiana, Mol. Plant Microbe Interact. 21 (2008) 335–345.

[155] A.G. Roberts, K.J. Oparka, Plasmodesmata and the control of symplastic transport, Plant Cell Environ. 26 (2003) 103–124.

[156] R. Zavaliev, A. Levy, A. Gera, B.L. Epel, Subcellular dynamics and role of Arabidopsis beta-1,3-glucanases in cell-to-cell movement of tobamoviruses, Mol. Plant Microbe Interact. 26 (2013) 1016–1030.

[157] Z. Zhang, Y.L. Ruan, N. Zhou, F. Wang, X. Guan, L. Fang, X. Shang, W. Guo, S. Zhu, T. Zhang, Suppressing a putative sterol carrier gene reduces plasmodesmal permeability and activates sucrose transporter genes during cotton Fiber elongation, Plant Cell 29 (2017) 2027–2046.

[158] D. Yan, S.R. Yadav, A. Paterlini, W.J. Nicolas, J.D. Petit, L. Brocard, I. Belevich, M.S. Grison, A. Vaten, L. Karami, S. El-Showk, J.Y. Lee, G.M. Murawska, J. Mortimer, M. Knoblauch, E. Jokitalo, J.E. Markham, E.M. Bayer, Y. Helariutta, Publisher Correction: Sphingolipid biosynthesis modulates plasmodesmal ultrastructure and phloem unloading, Nat. Plants 5 (2019) 1023.

[159] D. Huang, Y. Sun, Z. Ma, M. Ke, Y. Cui, Z. Chen, C. Chen, C. Ji, T.M. Tran, L. Yang, S.M. Lam, Y. Han, G. Shui, Z. Wei, S. Tan, K. Liao, J. Friml, Y. Miao, L. Jiang,

X. Chen, Correction for Huang et al., Salicylic acid-mediated plasmodesmal closure via Remorin-dependent lipid organization, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 8659.

[160] A. Paterlini, I. Belevich, E. Jokitalo, Y. Helariutta, Computational tools for serial block Electron microscopy reveal plasmodesmata distributions and wall environments, Plant Physiol. 184 (2020) 53–64.

[161] I.K. Jarsch, T. Ott, Perspectives on remorin proteins, membrane rafts, and their role during plant-microbe interactions, Mol. Plant Microbe Interact. 24 (2011) 7–12.

[162] G. Cheng, Z. Yang, H. Zhang, J. Zhang, J. Xu, Remorin interacting with PCaP1 impairs Turnip mosaic virus intercellular movement but is antagonised by VPg, New Phytol. 225 (2020) 2122–2139.

[163] Y. Benitez-Alfonso, M. Cilia, A. San Roman, C. Thomas, A. Maule, S. Hearn, D. Jackson, Control of Arabidopsis meristem development by thioredoxin-dependent regulation of intercellular transport, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 3615–3620.

[164] T.M. Burch-Smith, P.C. Zambryski, Loss of INCREASED SIZE EXCLUSION LIMIT (ISE)1 or ISE2 increases the formation of secondary plasmodesmata, Curr. Biol. 20 (2010) 989–993.

[165] S. Stonebloom, T. Burch-Smith, I. Kim, D. Meinke, M. Mindrinos, P. Zambryski, Loss of the plant DEAD-box protein ISE1 leads to defective mitochondria and increased cell-to-cell transport via plasmodesmata, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 17229–17234.

[166] K. Kobayashi, M.S. Otegui, S. Krishnakumar, M. Mindrinos, P. Zambryski, INCREASED SIZE EXCLUSION LIMIT 2 encodes a putative DEVH box RNA helicase involved in plasmodesmata function during Arabidopsis embryogenesis, Plant Cell 19 (2007) 1885–1897.

[167] J.O. Brunkard, M. Xu, M.R. Scarpin, S. Chatterjee, E.A. Shemyakina, H. M. Goodman, P. Zambryski, TOR dynamically regulates plant cell-cell transport, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 5049–5058.

[168] E. Baena-Gonzalez, J. Hanson, Shaping plant development through the SnRK1-TOR metabolic regulators, Curr. Opin. Plant Biol. 35 (2017) 152–157.

[169] S.J. van Deventer, V.E. Dunlock, A.B. van Spriel, Molecular interactions shaping the tetraspanin web, Biochem. Soc. Trans. 45 (2017) 741–750.