

# Chapter 2

## Plasmodesmata: Channels for Viruses on the Move

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### Abstract

The symplastic communication network established by plasmodesmata (PD) and connected phloem provides an essential pathway for spatiotemporal intercellular signaling in plant development but is also exploited by viruses for moving their genomes between cells in order to infect plants systemically. Virus movement depends on virus-encoded movement proteins (MPs) that target PD and therefore represent important keys to the cellular mechanisms underlying the intercellular trafficking of viruses and other macromolecules. Viruses and their MPs have evolved different mechanisms for intracellular transport and interaction with PD. Some viruses move from cell to cell by interacting with cellular mechanisms that control the size exclusion limit of PD whereas other viruses alter the PD architecture through assembly of specialized transport structures within the channel. Some viruses move between cells in the form of assembled virus particles whereas other viruses may interact with nucleic acid transport mechanisms to move their genomes in a non-encapsidated form. Moreover, whereas several viruses rely on the secretory pathway to target PD, other viruses interact with the cortical endoplasmic reticulum and associated cytoskeleton to spread infection. This chapter provides an introduction into viruses and their role in studying the diverse cellular mechanisms involved in intercellular PD-mediated macromolecular trafficking.

**Key words** Plasmodesmata, Plant virus, Movement protein, Membrane, Cytoskeleton, RNA silencing

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### 1 Interaction of Viral Movement Proteins with PD

The trafficking of viruses and other macromolecules through PD depends on intracellular transport mechanisms and is restricted by the size of the PD aperture. The size exclusion limit (SEL) of PD, thus the upper limit of the size of molecules transported by PD, is under tight control and changes during plant growth and development. Younger leaves that act as physiological sinks for photo-assimilates have PD with an overall larger SEL than the PD in mature source tissues. Leaf maturation and the corresponding overall decrease in the SEL of PD have been correlated with a change in PD structure from “simple” to “branched” [1]. Nevertheless, despite the restricted SEL of PD in mature leaves, cells in both mature and immature leaves interact with neighboring

cells and can have PD in various states of aperture (closed, open, dilated), thus indicating that the PD aperture of cells is individually regulated and able to respond to specific signals [2].

A hallmark feature of viral MPs is their ability to manipulate the mechanism that determines the SEL and increase the aperture of PD even in mature leaves. This ability of MPs to “gate” PD was first discovered for the MP of *Tobacco mosaic virus* (TMV). Microinjection of fluorescent, membrane-impermeable size-specific dextran probes into the cytoplasm revealed that transgenic tobacco plants constitutively expressing the MP allowed the cell-to-cell diffusion of probes with an approximate size of 10 kDa whereas diffusion was restricted to a molecular size of 0.8 kDa in control plants [3]. The protein was then shown to spread itself between cells upon injection [4] or upon transient expression from a plasmid (introduced into tissue by microprojectile bombardment [2] or agroinfiltration [5]). Experiments using microprojectile bombardment have shown that only 2 % of the cells in mature leaves allowed the cell-to-cell diffusion of 2 × GFP (two fused copies of green fluorescent protein, 54 kDa) whereas 52 % of the cells allowed the trafficking of GFP fused to MP (MP:GFP; 58 kDa) [2]. The ability of MP to alter PD aperture is usually correlated with the accumulation of the protein at PD. Several studies indicated that MP accumulates preferentially to the complex, branched PD in mature tissues [6–9]. However, more recent studies have shown that MP also accumulates in simple PD of immature cells [2, 10]. Moreover, the presence of accumulated MP in PD does not necessarily indicate that the PD are gated. Microinjection of cytoplasmic probes into cells within different radial zones of spreading TMV infection sites has demonstrated that the gating of PD by MP is restricted to cells at the infection front although the MP resides in PD throughout the infection site [11]. Accumulation of MP in PD and gating may even represent independent functions of MP, since TMV was shown to spread through PD without MP being trapped in PD [10] and certain transiently expressed mutants of MP moved between cells in the absence of any accumulation in the channel [5]. Plants that express the MP and are able to complement the movement function of movement-deficient TMV mutants do not show obvious growth defects, suggesting the existence of mechanisms that tightly control the ability of MP to modify PD aperture and thus prevent the continuous trafficking of signaling macromolecules between cells. The nature of these mechanisms remains to be elucidated. However, the MP has several amino acids that are phosphorylated *in vivo* [12–16] and that may play a role in regulating MP functions. The C-terminus of MP is dispensable for function in virus movement but carries phosphorylation sites that if phosphorylated downregulate the ability of MP to move from cell to cell and to gate PD in a host-dependent manner [17, 18]. Consistent with this type of posttranslational control, PD are associated with several kinases [19–23]

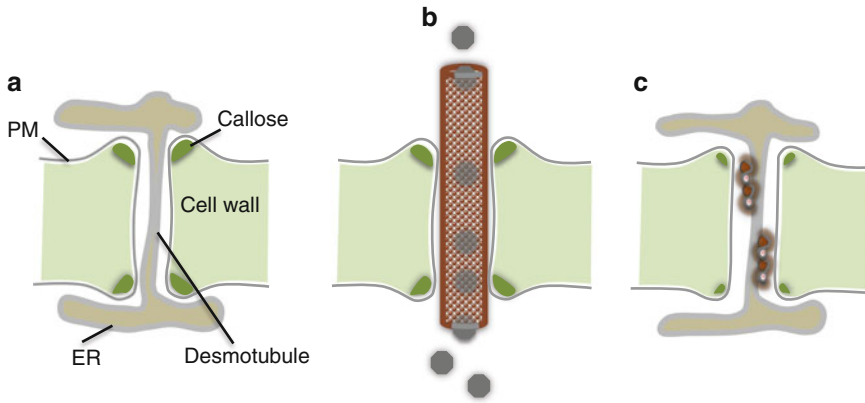
and a specific kinase, plasmodesmal associated kinase (PAPK), was shown to specifically phosphorylate the C-terminal phosphorylation sites of MP in vitro and also to be active on a subset of other MPs and non-cell-autonomous plant transcription factors [22]. Another hallmark of MP function is the ability of the protein to bind single-stranded nucleic acids in a sequence-independent manner in vitro [24]. Since TMV does not require its coat protein (CP) for intercellular movement [25], the MP is believed to form an MP:RNA complex with viral RNA (vRNA) and to support viral movement in the form of a non-encapsidated viral ribonucleoprotein complex (vRNP). This non-virion mode of movement by TMV has strong potential for providing insights into the cellular mechanisms by which cells support the intercellular trafficking of protein and RNA molecules.

Although TMV and its MP continue to play a major role in pioneering work addressing virus movement, many more viruses and their MPs have also been studied in the meantime. These studies revealed that viruses may encode more than one protein required for movement and that viral MPs may use different mechanisms for targeting PD and for facilitating virus movement through the channel. Thus, dependent on the virus species, intercellular virus movement occurs in virion or non-virion form and often depends on viral coat protein (CP) in addition to MP. The following paragraphs describe different MP-mediated mechanisms by which viruses target and move through PD. Since the movement of different viruses has been recently reviewed [26, 27], only selected virus models are described here.

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## 2 Virion Movement of Tubule-Forming Viruses

Viruses that move from cell to cell in the form of encapsidated virions encounter the problem that the size of the virion particles (>10 nm) exceeds the cytoplasmic channel diameter in PD (the cytoplasmic sleeve is at most 10 nm in diameter). As a result, the viruses must have adopted drastic strategies to allow the particles to pass through the pore. Several viruses known to move between cells in the form of assembled virions encode MPs able to assemble a large tubular transport structure inside PD [28–30] (Fig. 1). The desmotubule is absent from such modified PD and the overall diameter of the PD can be dilated [31]. The molecular mechanism of tubule-guided transport of viral particles through the pore is not known. However, some MPs of tubule-forming viruses interact with the CP of the respective virus, usually at the C-terminus of the MP. The C-terminus of the MP of *Compea mosaic virus* (CPMV) is located on the inside of the tubule [30], thus in close proximity to the virus particle passing through the tubule. Consistent with a requirement of MP:CP interactions between the inner tubule wall



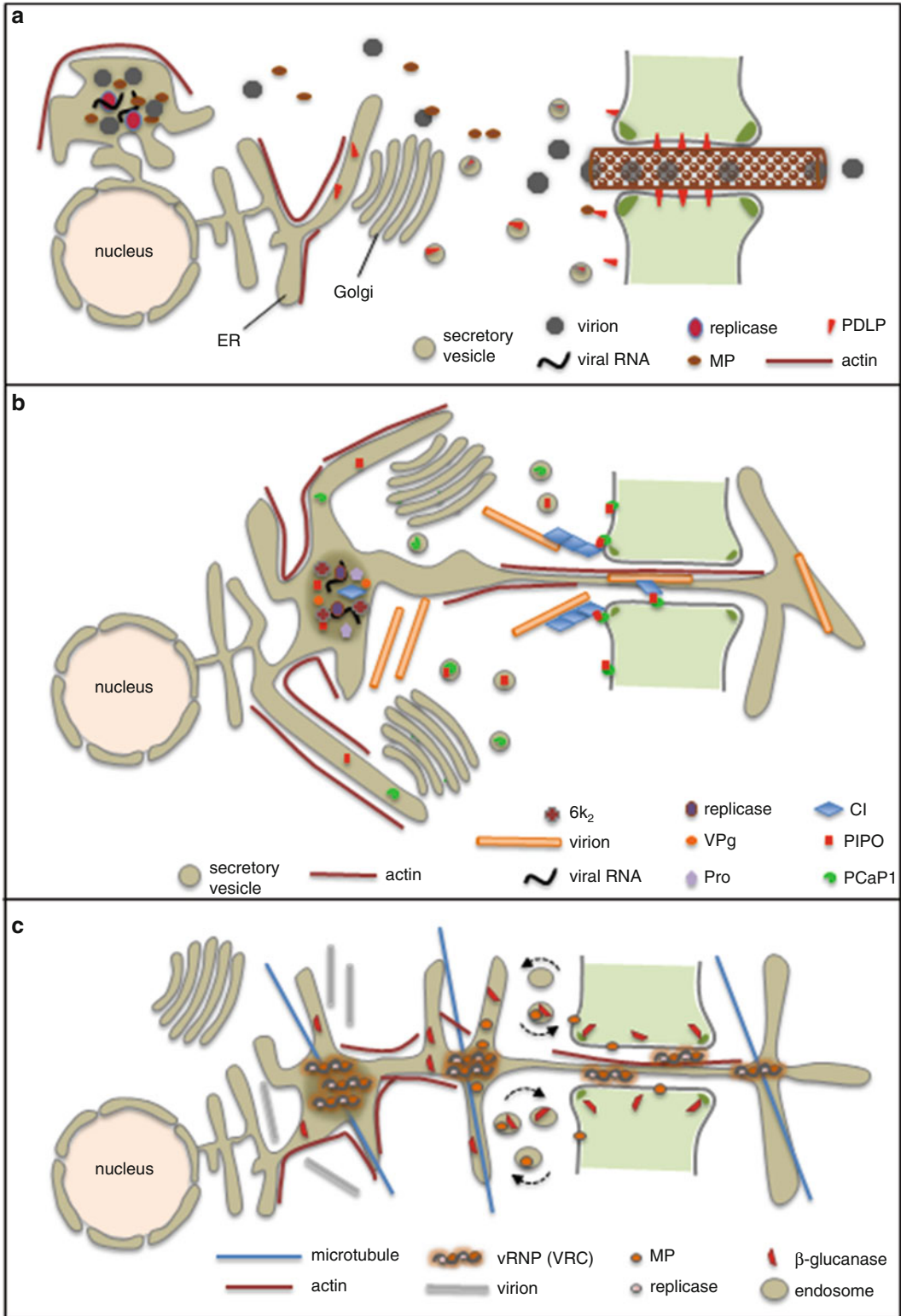
**Fig. 1** PD structure and modification by viruses. **(a)** General structure of primary PD. The PD pore maintains plasma membrane (PM) and endoplasmic reticulum (ER) continuity between cells. The ER traverses the pore as a thin tube known as the desmotubule. Callose deposits in the cell wall around the PD neck regions play an important role in controlling macromolecular transport through the channel. **(b)** Modification of PD by tubule-forming viruses. The tubule is assembled from viral MP and permits the movement of whole virion particles between cells. Since the tubule replaces the desmotubule, this type of movement disrupts ER continuity between cells. **(c)** Modification of PD by viruses that move from cell to cell in a non-encapsidated form. The MPs of these viruses cause an increase in PD SEL. An increased SEL is often linked to the degradation of PD-associated callose but may also involve a role of PD-associated actin. The viral ribonucleoprotein complexes traverse the PD channel likely in association with the fluid ER/desmotubule membrane

and CPMV particles for transport, only “empty” tubules were observed, when the C-terminus of the MP was deleted [32]. Specific interactions between tubules and virions mediated by the C-terminus of MP have also been observed for the *Grapevine fan-leaf nepovirus* (GFLV) [33]. The interaction between the tubule-forming MPs and the CPs of the respective tubule-forming viruses represents an important determinant for movement specificity. For example, the MP of CPMV does not interact with particles of other tested virus species [34] and the MP of the GFLV-related *Arabidopsis mosaic virus* failed to support GFLV movement unless the nine C-terminal amino acids of the MP were replaced by the nine C-terminal amino acids of the GFLV MP [33]. The interactions of tubule-forming MPs with the CP of the respective virus may suggest that virus transport may occur through polar assembly/disassembly of MP tubules that may propel MP-bound viral particles in a mechanism analogous to microtubule treadmilling [26]. Such a mechanism would depend on continuous delivery of MP to the base of the tubule within the infected cell.

As shown for GFLV and *Cauliflower mosaic virus* (CaMV), efficient tubule assembly and virus infection depend on interaction of the tubule-forming MP with PD-localized proteins (PDL), a multigene protein family that localizes to PD via the ER-Golgi

secretory pathway [35] (Fig. 2a). The MPs interact with PDLP at PD and not earlier within the secretory pathway, since the treatment with the secretory pathway inhibitor Brefeldin A (BFA) resulted in the accumulation of the MPs in the cytosol [36–38] and not, as would be expected, in BFA bodies, as seen for PDLP [35, 39]. The secretory pathway consists of interacting and highly dynamic organelles that move intracellularly with support of the actin cytoskeleton ([40] and citations herein). Thus, consistent with the role of the secretory pathway in the targeting of PDLP to PD, intracellular transport of PDLP as well as of Golgi bodies is strongly affected by the treatment with Latrunculin B (LatB: inhibitor of actin polymerization) and 2,3-butanedione monoxime (BDM, myosin ATPase inhibitor), both of which inhibit the actomyosin system in Golgi trafficking along the ER required for secretory cargo uptake [41, 42]. In agreement with these findings the PD targeting of PDLP as well as of GFLV MP, tubule formation, and virus movement depend on the activity of specific myosin XI classes (particularly myosin XI-2 and XI-K) [43] that are known to play important roles in cell dynamics, including F-actin organization, ER motility, and organelle trafficking [44–48]. The inhibition of myosin XI-K did not alter the subcellular distribution of cellular markers of the plasma membrane (PM), of lipid raft subdomains within the PM and PD, of the PD neck, or the vacuolar membrane, thus indicating that the XI-K-dependent PD targeting by PDLP follows a specific route [43].

Given these insights into the PD targeting of PDLP it now remains to determine how the MP and the viral particles of GFLV (and of other PDLP-dependent viruses) are targeted to PD. Previous studies indicated that the application of inhibitors that interfere with microtubule polymerization causes GFLV tubules to form at ectopic cortical sites rather than at PD [37]. This may be consistent with the recent proposal that cortical, ER-intersecting microtubules form specific cortical ER-associated landmarks for the proper positioning of organelles and membrane transport pathways in the cell cortex [49, 50]. Therefore, it is conceivable that the MP interacts with the ER and/or microtubules and that the lack of microtubules causes the disappearance of important positional information required for PD targeting. Since GFLV and CPMV replicate in perinuclear aggregates of recruited ER membranes [51, 52], the route by which the MP reaches PD may be via trafficking along the ER membrane, thus similar to the route taken by several other RNA viruses, like TMV (see below). The CP of GFLV has been localized to viroplasms and to the tips of MP tubules in the cell periphery [51]. However, how the CP or the assembled virions are targeted from viroplasms to PD remains to be studied.



**Fig. 2** Cellular mechanisms that target viruses to PD. **(a)** PD targeting by tubule-forming viruses, e.g., GFLV. This virus replicates in aggregates of recruited ER membrane near the nucleus and requires the secretory pathway



### 3 Tubule-Independent Virion Movement

In contrast to the tubule-forming viruses, most plant viruses move through PD without inducing major structural changes in channel architecture. This mode of virus movement must be associated with infectious particles and movement mechanisms that are adapted to PD structure and function and likely relies on existing cellular mechanisms for the transport of macromolecules. As a consequence of such adaptation the majority of the viruses not relying on a tubule-forming MP evolved mechanisms to move from cell to cell in a non-encapsidated form. These are described further below (*see* Subheading 4). Here, I first mention examples of viruses proposed to move through PD in the form of encapsidated virions despite that they have not been reported to form MP tubules (*see* Subheadings 3.1 and 3.2).

#### 3.1 Movement of Closteroviruses

Closteroviruses represent a first example for viruses apparently moving from cell to cell as virions through PD without tubule formation. These viruses have particularly large RNA genomes and form very long and flexible virions. Their movement involves four structural proteins and one ER-localized MP, which is required for virus movement but is not an integral virion component [53]. Three of the four structural components form a narrow tail that functions in virion movement [54]. One of these components, the Hsp70 homolog (HSP70h), localizes to PD in a manner dependent on myosin VIII and thus might be involved in targeting the virion to PD or in transporting the virion through the pore [55]. It has been proposed that the latter may be facilitated by the ATPase function of Hsp70h, which may generate mechanical force required for translocation [56].

**Fig. 2** (continued) as well as the actin cytoskeleton and associated myosin motors for intercellular movement. The mechanism that targets the MP and virions to PD is not known. However, efficient tubule formation and virus movement depend on the interaction of MP with PD-localized PDLP, which requires the secretory pathway to reach PD. **(b)** PD targeting of potyviruses, e.g., TuMV. Movement is thought to occur in the form of virions and involves the virus-encoded proteins CI and P3N-PIPO, as well as host-encoded PCaP1. Virions produced in membrane-associated replication complexes associate with CI, which targets PD through interaction with P3N-PIPO and the PM-associated protein PCaP1. The CI protein accumulates at PD where it forms characteristic inclusions that may direct the virions into the PD pore. Given that P3N-PIPO moves between cells, the PCaP1-bound P3N-PIPO may facilitate the movement of the virion through the PD into the neighboring cell. **(c)** PD targeting of non-encapsidated virus, e.g., TMV. This virus replicates in association with the ER and uses the ER-actin network for PD targeting and the transport of replication complexes (VRCs) into the neighboring cell. The MP interacts with microtubules to assist in the assembly and controlled release of VRCs from cortical microtubule-associated ER sites (c-MERs). VRCs that remain anchored at these sites continue replication and form viral factories that produce virions. The MP interacts with, or induces the recruitment of,  $\beta$ -glucanase to facilitate intercellular movement of the VRC by degradation of PD-associated callose. Both proteins may reach the PD neck region with support of an endosomal cycling pathway

### 3.2 Movement of Potyviruses

Another virus family potentially moving through PD in the form of virions without tubule formation may be represented by potyviruses, the largest group of plant viruses (Fig. 2b). Evidence for movement in the form of encapsidated virions comes from mutations in the conserved core region of the *Tobacco etch virus* (TEV) CP that abolished both virion assembly and cell-to-cell movement [57, 58]. Virus movement is facilitated by the cylindrical inclusion (CI) protein, which forms cone-shaped cylindrical structures at PD. The CI protein of *Potato virus A* (PVA) binds and copurifies with virions [59] and the *Turnip mosaic virus* (TuMV) CP, which is required for TuMV movement, co-localizes to PD-associated CI cones [60]. The localization of the TuMV CI protein to PD depends on a more recently identified potyviral protein termed P3N-PIPO, which targets PD via the ER-Golgi secretory pathway [60] and interacts with host factors that facilitate its own movement [61]. P3N-PIPO may function as the core MP of the virus by facilitating the transport of the CI with bound virus particles from ER-associated viral replication complexes (VRCs) to PD. The PIPO domain of P3N-PIPO interacts with a plasma membrane-associated cation-binding protein, PCaPI. Virus accumulation, movement, and disease symptoms were reduced in an Arabidopsis PCaPI knockout and it has been suggested that this protein may provide a membrane-binding function that may be required for potyviral movement through PD [61]. A recent study using specific inhibitors, dominant negative mutants, and virus-induced gene silencing to target different host cell transport systems led to the conclusion that TuMV movement depends on intact pre- and post-Golgi transport as well as on myosin XI-2 and XI-K but is independent of endosomes [62]. However, it may still be unclear whether all potyviruses indeed move between cells in the form of encapsidated virions. Although potyviruses have a flexuous filamentous virion morphology, the longitudinal and lateral dimensions (680–900 nm long and 11–15 nm wide) of the particles may be incompatible with the native structure of PD. Moreover, the CI protein of *Plum pox virus* (PPV) is an RNA helicase [63] and microinjection studies with proteins encoded by *Lettuce mosaic potyvirus* (LMV) and *Bean common mosaic necrotic potyvirus* (BCMNV) indicated that the CP and HC-Pro (helper component-protease) of these potyviruses are able to modify the SEL of PD, move from cell to cell, and facilitate the movement of viral RNA [64], which are hallmark features of the widespread non-virion mode of virus movement exemplified by TMV. Indeed, recent in vivo studies provide evidence supporting the conclusion that TuMV infection spreads between cells in the form of membrane-bound VRCs [65].



## 4 Virion-Independent Virus Movement (as vRNP)

Most non-tubule-forming viruses encode MPs that form vRNPs with vRNA and facilitate the intercellular spread of the vRNPs by interacting with the cellular machinery that transports macromolecules and regulates the SEL of PD. The prototype virus exemplifying this type of movement is TMV [3, 24, 66]. This type of movement can be independent of CP, as in the case of TMV [25], or may require CP as, for example, in the case of the bromoviruses *Brome mosaic virus* (BMV) and *Cucumber mosaic virus* (CMV) [67–69] or the potexviruses [70]. However, although the CP of TMV is dispensable for local movement of the virus, it is required for long-distance movement through the phloem [25]. As for many other viruses, this requirement of CP for long-distance movement may not necessarily reflect a requirement of virion formation but may indicate the requirement of additional stabilization of the vRNP for either entry or movement through the vasculature [71].

### 4.1 Movement of TMV

TMV may still be the best studied virus with regard to movement (Fig. 2c). Cell biological observations and mutational studies suggest that the vRNPs of TMV are associated with viral replication complexes (VRCs) that in addition to vRNA and MP also contain the viral 126 and 186 kDa replicase proteins and produce CP [72–75]. According to microscopical *in vivo* observations the VRCs assemble at sites in the cortical ER-actin network that coincide with cortical microtubules [50, 76–79]. These microtubule-associated ER sites (c-MERs) may represent important “cortical landmarks” at which various endomembrane and motor-driven organelle trafficking pathways are proposed to convene to catalyze the encounter and molecular exchange between organelles and macromolecular trafficking and signaling pathways [49, 50, 78]. The MP exhibits strong binding affinity to tubulin and microtubules [72, 80, 81], and therefore may assist in the anchorage, formation, and maturation of the ER-associated VRCs by an aggresomal mechanism that recruits host factors and membranes with support of the cytoskeleton and associated motor proteins [50, 78]. In addition to binding to microtubules, the MP also interacts with important regulators of the microtubule cytoskeleton, such as  $\gamma$ -tubulin [77] and microtubule END-BINDING 1 (EB1) [82], which may explain the observation of microtubule nucleation events at VRCs during early maturation stages and the occurrence of multiple microtubules joined together in the center of larger, more mature VRCs at later stages [50, 78]. Expression of MP in mammalian cells causes the displacement microtubule nucleation activity from the centrosome to ectopic sites in the cytoplasm [83], thus suggesting that the MP may subvert the plant

microtubule nucleation machinery to support VRC formation and growth. The MP may interact with a common mechanism recently reported for microtubule reorientation in plants [84]. This mechanism consists in the recruitment of  $\gamma$ -tubulin and the nucleation of new microtubules at existing microtubules and thus to the formation of microtubule crossovers, which act as templates for the production of additional microtubules with support of the microtubule-severing protein katanin. It seems possible that the MP interacts with  $\gamma$ -tubulin to recruit this mechanism to the site of the VRC and thus to support VRC maturation and growth with the help of nucleated microtubules.

In time-lapse movies monitoring the behavior of fluorescent protein-tagged MP, the MP-associated VRCs/MP particles are first visualized as very small cortical particles that either remain attached to c-MERs or get detached to move in a directional stop-and-go fashion along the ER-actin network between c-MERs, in a manner depending on a dynamic actin and microtubule cytoskeleton [50, 77]. Consistent with their VRC nature, the ability of MP to interact with microtubules and the formation of the mobile MP particles is correlated with MP function in virus movement [76, 77, 85]. Moreover, in agreement with the association of the spread of infection with VRC movement along the ER-actin network between c-MERs, the efficiency by which infection spreads between cells is independent of the secretory pathway [86] but depends on dynamic microtubules [87], on the integrity of actin filaments, and on the expression or activity of specific myosin motors [74, 88–90].

VRCs that do not move but remain anchored at c-MERs in the infected cell may grow into virus factories [50, 78]. Thus, as infection spreads forward into yet uninfected cells, the cells behind the infection front develop factories (or “X-bodies”) that accumulate high levels of replicase, coat protein, and viral RNA in addition to MP [72, 73, 91]. At this stage, highly expressed MP can show profound accumulation of MP in viral factories and along microtubules to which the virus factories are aligned [72, 81]. Subsequently, thus soon after accumulation along microtubules, the MP is degraded except for MP localized to PD. The process of degradation may be triggered by ER stress caused by the accumulation of unfolded or aggregated MP, which induces the AAA ATPase CDC48. This protein extracts the MP from the ER, and thus allows the ER to recover, and the delivery of MP to the cytoplasm where it first decorates the microtubule cytoskeleton and is then degraded by the 26S proteasome [92].

It is curious why TMV infection is associated with such high overaccumulating levels of MP. Indeed, only a small fraction (2 %) of the amount of MP produced during TMV infection is required for the spread of infection [93]. Consistently, virus variants that produce lower amounts of MP spread normally and MP

localization is restricted to small cortical MP particles/VRCs and to PD in these cases [72, 94]. It appears possible that the accumulating levels of MP during late stages of infection play a regulatory role. Accumulation of MP along microtubules, as seen during late stages of TMV infection, was shown to interfere with kinesin-mediated motility [80], with the movement of MP particles [76], and with virus movement [95, 96]. Thus, by producing high levels of MP the virus may be able to prevent the further PD targeting and spread of infection between cells that are already behind the infection front. Moreover, in agreement with the inhibition of MP targeting to PD during late infection stages, the ability of MP to alter the SEL is limited to cells at the infection front [11]. The inhibition of virus movement and MP trafficking between cells behind the infection front may facilitate a phase change from mechanisms that support virus movement towards mechanisms that rather support VRC growth and virion progeny production.

Further studies might reveal how the various functions of MP are regulated. Apart from the already mentioned C-terminal phosphorylation, the MP is regulated by ubiquitinylation [80, 97]. Moreover, the MP may assume different folds. The MP is a hydrophobic protein with two hydrophobic regions involved in ER association [98]. A structural model predicted that the hydrophobic regions act as transmembrane domains [99, 100]. In this model the MP domains required for RNA binding [101] and interaction with microtubules [85], chaperones [102], and cell wall-associated proteins [103] are buried in the membrane suggesting that either this model is not correct or additional protein folds must exist [104]. Assuming that the model is correct and supported by the observation that MP oligomerizes in vivo [105], we proposed that MP may form higher order complexes with monomers carrying different folds and thus combine different MP functions within the complex [50, 78]. However, according to a recent report the protein does not form transmembrane helices but rather localizes to the cytoplasmic face of the membrane [104]. In contrast to the transmembrane insertion model, the latter model allows cytoplasmic accessibility of the MP domains required for function.

## **4.2 Movement of “Triple-Gene-Block” Viruses**

Membrane-associated replication and transport of VRCs are documented for a diverse range of viruses [106–108] and, unlike for TMV, often involve more than one MP. The rod-shaped hordei-like (hordei-, pomo-, peclu-, and beny-) viruses and potexviruses encode three MPs in overlapping ORFs, the triple gene block (TGB). The mode of action of these proteins has been intensely studied [70, 109–111] and led to the proposal of somewhat varied movement strategies of the TGB-encoding viruses [110]. The general model for movement of these viruses involves the binding of viral RNA by TGB1 and the targeting of the TGB1:RNA complex to PD with the help of TGB2 and TGB3, transmembrane

proteins that are localized to the ER. Unlike for hordeiviruses, the movement of potexviruses and, presumably, of other viruses with potex-like TGB proteins depends on the CP in addition to the TGB proteins. However, whether potexviruses move in the form of virions or rather in a non-encapsidated form is unclear. Electron micrographs showing fibrillar material that appears to resemble PVX virions located within PD of infected leaves and the reaction of this material with antibody able to bind to PVX virus particles but not to isolated CP subunits led to the proposal that PVX moves between cells in the form of encapsidated virions [112]. In contrast, the results of microinjection experiments and the mutational analysis of CP indicating that virion formation is not sufficient for virus movement rather favored the proposal that potexvirus transport occurs in the form of a TGB1-CP-RNA complex [113]. The latter model was supported by the observation that potexvirus CP mutants capable of virion formation but not capable to move between cells could be functionally complemented by the CP of unrelated viruses such as the potyviruses *Potato virus A* or *Potato virus Y* or the sobemovirus *Cocksfoot mottle virus*, and even by the MP of TMV [114, 115]. The currently prevailing model proposes that PVX movement occurs in the form of a partially encapsidated viral RNA with a 5' associated TGB1 [110]. The requirement for CP in TGB virus movement may depend on the size and domain structure of TGB1. Whereas potexviruses have small TGB1 proteins and require CP for movement the viruses of the hordei-like group have TGB1 proteins with extended N-terminal domains and do not require CP for movement. The extended N-terminal domain may thus act as a chaperone able to sufficiently protect the viral RNA during movement and thus neutralize a need of CP for such function [116]. Recent studies on movement of the potexvirus *Bamboo mosaic virus* (BaMV) confirmed the role of the TGB2/TGB3 complex in the targeting of TGB1 to PD. Interestingly, the same complex was shown to be stably associated with virions, thus suggesting that BaMV targets PD in the form of virions [117]. Future research may show whether this virus moves through PD in virion form.

Many experiments have provided important insights into the host components involved in the targeting of TGB-containing viruses to PD. Several observations indicated that the TGB2 and TGB3 proteins associate with the ER and with ER-derived, TGB2-induced motile granules that are visualized along actin filaments, whereas the TGB1 protein localizes to the cytoplasm and requires TGB2 and TGB3 for PD targeting [117–135]. The TGB-RNA complexes (TGB-virion complexes in the case of BaMV, [117]) thus reach the cell wall by trafficking along the ER-actin network and once at PD facilitate transport of viral RNA (or virions) through the pore, presumably by increasing the PD SEL by mechanisms that involve TGB1 [136–138] and TGB2 [118, 139].

Studies with the pomovirus *Potato mop-top virus* (PMTV) indicated that the TGB2 and TGB3 proteins remain in the infected cell and are recycled by endosomal membrane trafficking for further rounds of transport [118].

The TGB complex that targets PD may be associated with viral replication or may itself represent a VRC. Indeed, the virion-associated TGB3-containing ER membrane complex of BaMV also contains viral replicase [117] and interactions between the CP and the helicase domain of the replicase are essential for virus movement [140]. PVX replicates in association with the ER [141] and the TGB2-induced motile ER-derived and TGB3-containing granules are associated with replicase, as well as with ribosomes and virions [119, 124], thus suggesting that these granules contain replication complexes (VRCs). At later stages of infection, the TGB proteins also colocalize with non-encapsidated viral RNA to replication factories (X-bodies) that are formed by TGB1-mediated ER membrane recruitment and produce virions [142, 143]. The vicinity of the TGB proteins and CP to viral RNA or replicase in the motile granules and factories suggests that the proteins are locally translated near VRCs and remain associated with them through their network interactions in motile granules and X-bodies during early and later stages of infection. Taken together, these observations suggest the model that during the course of infection TGB2/3-associated VRCs form along the ER. Initially motile along the dynamic ER-actin network, they soon encounter PD, where the TGB2 and TGB3 proteins interact with TGB1 to insert CP into the channel, probably in the form of a movement complex with viral RNA. This insertion process may be supported by continuous replication within the PD-associated VRC. At later stages of infection, the PD-associated VRCs and also the VRCs that remained along the ER network grow into ER-associated granules that finally accumulate in a perinuclear TGB1 aggregate (X-body) that acts as a viral factory and produces viral progeny [144].

The process by which PVX targets PD may be facilitated by the affinity of the potexvirus TGB3 protein to highly curved ER membrane domains enriched with reticulon-like proteins [145, 146]. It has been suggested that this affinity may target TGB3-containing complexes to the desmotubule, which may require reticulons for structural stabilization [147]. The PD targeting of PVX or its anchorage near the channel may also involve an interaction with specific PM domains since PVX movement was inhibited by expression of Remorin, a protein proposed to localize PD via plasma membrane (PM) rafts. Since Remorin was shown to interact with TGB1 it may inhibit virus movement by titrating TGB1 away from PD [148].

PD targeting and movement of TGB viruses may also involve a function of microtubules. The CP of PVX was shown to interact with microtubules and to interfere with MAP2 binding in vitro

[149]. Moreover, PVX movement was inhibited by overexpression of the microtubule-binding protein MPB2C [150]. The TGB1 protein of PMTV was shown to interact with microtubules *in vivo* and to form cortical particles along them [151, 152]. Deletion mutation analysis demonstrated a correlation between the microtubule association of TGB1 and its accumulation in PD. Microtubule disruption by colchicine treatment abolished the accumulation of TGB1 at PD as well as the formation of TGB1 particles, leading to accumulation of the protein along the ER network. These findings suggest a role of microtubules in the localized interaction of TGB1 with the membrane proteins TGB2 and TGB3. Since TGB1 interacts with viral RNA, these microtubule-associated processes may reflect the localized formation and transport of VRCs. Further studies may reveal whether or not the formation and transport of TGB virus VRCs along the ER occur in analogy to the formation and transport pathway proposed for TMV VRCs, thus involving c-MERs [50] (Fig. 2c).

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## 5 The Use of Biochemical Inhibitors May Be of Limited Value in the Analysis of the Cellular Components Involved in Virus Movement

Although there is accumulating evidence for a role of microtubules in macromolecular and viral trafficking and particularly in the formation and guided intracellular trafficking of VRCs and other macromolecules to PD [50, 78, 153], the spread of viruses is usually not affected by the presence of microtubule inhibitors [74, 80, 154]. This interesting feature has led to disagreements in the earlier literature but may be explained simply by inhibitors not being fully effective, either because they do not reach all cellular targets or because microtubules are stabilized. The treatment of plant cells with microtubule inhibitors can indeed fail to disrupt parts of the microtubule cytoskeleton as was demonstrated, for example, by antibody labeling and by showing that the MP of TMV still labels microtubules during infection in inhibitor-treated tissues [155]. In the case of TMV, resistance against microtubule inhibitors may be partly explained also by the ability of MP to manipulate the microtubule nucleation machinery and to confer superstability to microtubules [80, 83, 85]. Moreover, microtubule-associated cortical landmarks (c-MERs) are stable structures that require microtubules for their formation but not for their functional maintenance, at least over certain periods of time [49]. Thus, upon inhibition of microtubules, TMV may still be able to interact with c-MERs and the connecting ER-actin network for spread.

The lack of an effect on TMV spread is not limited to inhibitors of microtubules but may also be observed for inhibitors of the actin cytoskeleton. Although the virus moves along the ER-actin network, infection continued to spread during 24 h of treatment



with actin inhibitor [90]. While the latter finding could argue against a role of actin filaments in TMV movement, the same study showed that TMV movement was dominantly inhibited upon expression of an actin-binding protein that also inhibited the motor-dependent trafficking of Golgi complexes along the ER, thus clearly indicating a role of myosin-mediated trafficking along the ER membrane in TMV movement [90]. Moreover, inhibition of TMV movement by actin inhibitors was observed upon prolonged treatments with actin inhibitor and in plants silenced for actin or myosin motors [74, 89, 156]. Actin inhibition was also shown not to affect the accumulation of MP to PD [157], although FRAP experiments clearly indicated that the efficient targeting of PD by MP requires an intact ER-actin network [158]. The careful application of biochemical inhibitors may not be able to inhibit virus movement because inhibition is rarely complete and virus spread depends on the successful cell-to-cell movement of only few virus genomes [159]. Thus, even if the inhibitor is effective to some degree and induces a strong bottleneck for virus movement, infection may continue to spread normally. Similarly, MP can accumulate in PD over time although actin inhibitors interfere with the efficient functioning of the PD targeting pathway to some extent. Given these considerations, it is important that experiments applying biochemical inhibitors to the analysis of virus movement are carefully designed and cautiously interpreted.

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## 6 Viral Mechanisms to Control PD Aperture

Upon arrival at PD, the VRCs could, in principle, continue to diffuse along the ER membrane to pass along the desmotubule and into the adjacent cell. ER membrane-intrinsic and luminal probes readily diffuse between cells [160, 161], thus supporting the fluidity of the desmotubular membrane [162, 163] and the ability of the desmotubule to transport macromolecules. However, due to the large size of the viral complex, additional mechanisms that expand the PD aperture are necessary.

### 6.1 Interference with PD-Associated Actin

Several studies indicate that the aperture of PD is directly or indirectly controlled by actin filaments [164, 165] and structural models depict the PD channel with actin filaments wrapped around the desmotubule [166, 167]. Consistent with a role of actin in controlling PD aperture, the MPs of TMV and of *Cucumber mosaic virus* were shown to exhibit actin-severing activity in vitro. Moreover, the stabilization of actin filaments by treatment with phalloidin prevented the ability of MP to increase PD SEL in vivo [164]. Nevertheless, although these findings suggest that MPs manipulate actin to control the PD aperture, it remains to be

shown whether the actin-severing activity of these MPs occurs at PD or elsewhere in the cell. It is also yet unclear whether these MPs indeed interact with actin *in vivo*.

## 6.2 Interference with Callose Deposition

Another important mechanism that restricts PD aperture and that is indeed likely to be modified by the infecting virus is the deposition of callose in the cell walls surrounding the PD neck regions (Fig. 1). Callose is a  $\beta$ -glucan polysaccharide that is deposited at the PD neck region during different stresses, including wounding and pathogen attack [168]. The deposition of callose at PD has been linked to the salicylic acid (SA) defense signaling pathway that induces callose synthase activity [169] and leads to callose deposition at PD by a pathway involving EDS1, NPR1, and the PD-associated protein PDL5 [170, 171]. Experimental evidence indicates that the MP of TMV allows VRC movement through the PD by preventing the deposition of callose induced by infection. To account for this effect it has been proposed that the MP may recruit  $\beta$ -1,3 glucanases to degrade stress-induced callose at PD [160, 172] (Fig. 2c). This hypothesis is based on previous reports indicating a positive correlation between  $\beta$ -1,3-glucanase expression and viral spread [173–175]. A glucanase isoform that may be targeted to PD during TMV infection is AtBG<sub>pap</sub>. Similar to several recently isolated PD proteins, this protein is predicted to be a glycosylphosphatidylinositol (GPI)-anchored protein. This protein was localized to the ER, the cell periphery, and PD, and mutation of the protein-encoding gene by a T-DNA insertion led to a reduction in GFP cell-to-cell movement and stress-induced callose deposition at PD [176]. Moreover, the cell-to-cell movement of GFP-tagged *Turnip vein clearing virus* (TVCV), a TMV-related tobamovirus, was reduced in *Arabidopsis atbg<sub>pap</sub>* mutants. The same mutants showed highly increased PD-associated callose levels, thus confirming the role of AtBG<sub>pap</sub>-mediated callose degradation during virus movement [177]. While a direct role of this or another glucanase in the degradation of callose deposits during virus movement awaits to be demonstrated, the ability of MP to inhibit callose deposition at PD was shown to involve an ankyrin-repeat-containing protein (ANK), which facilitates TMV spread and interacts with MP [178]. The regulation of callose deposition at PD during infection may also involve the viral replicase. Evidence comes from the analysis of MP-transgenic *Nicotiana benthamiana* plants in which MP facilitated the non-cell-autonomous diffusion of ER-localized, GFP-tagged probes in the presence but not in the absence of infection and/or replicase [160]. This finding supports virus movement in the form of a VRC and is also consistent with a role of the replicase-encoding region of TMV in virus movement [75].

A role of callose deposition in restricting virus movement was also established for PVX [175] and a two-hybrid screen led to the

isolation of TGB2-interacting proteins (TIP1, TIP2, TIP3) that interact with  $\beta$ -1,3, glucanase and may play a role in regulating PD SEL [179].

A number of observations indicate that the callose deposition at PD and the regulation of PD SEL are regulated by redox homeostasis [180–182] and calcium [183–185]. Thus, it is conceivable that MPs may alter the PD SEL and facilitate virus movement also through interaction with pathways that influence the redox and calcium states of the infected cell.

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## 7 Requirement of Structural Unfolding for Movement Through PD

In addition to the modification of PD SEL, viral movement through PD may also require the structural modification of the transported complex. The observation that in vitro-assembled MP:RNA complexes have a thin and elongated appearance raised the proposal that the MP chaperones the vRNA through the dilated PD [101]. The hordeivirus TGB1 proteins have three RNA-binding domains and the interaction of these domains may play a role in VRC remodeling during movement [116]. Consistent with a requirement for structural unfolding for movement, the cell-to-cell movement of the non-cell-autonomous protein (NCAP) KN1 (see below) was inhibited by chemical cross-linking [186]. Moreover several chaperone proteins have been associated with macromolecular trafficking through PD [102, 187–190].

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## 8 Plant Viruses Use Existing Mechanisms for Macromolecular Transport

The MP of TMV was the first protein shown to alter the SEL of PD and to support its own spread between cells [3]. Later, it was demonstrated that this is a hallmark property of many viral MPs as well as of a special class of endogenous proteins commonly referred to as non-cell-autonomous proteins (NCAPs). Many NCAPs act as transcription factors playing important non-cell-autonomous roles in cell-type specification and differentiation [191–193]. NCAPs may also be involved in the cell-to-cell and long-distance transport of various RNA molecules, including mRNAs [193–197] and small RNAs [198–202]. It is likely that the ability of viruses to target and spread through PD evolved as an adaptation to essential mechanisms that also contribute to the transport of endogenous macromolecules. This hypothesis is supported by several observations. For example, expression of a dominant-negative form of the NON-CELL AUTONOMOUS PATHWAY PROTEIN 1 (NCAPP1) blocked the cell-to-cell trafficking of the MP of TMV as well as of the *Cucurbita maxima* PHLOEM PROTEIN 16 (CmPP16) [187]. Moreover, expression of the microtubule-associated protein

MPBP2C interfered with the cell-to-cell movement of TMV [96], PVX [150], as well as the *Zea mays* homeobox protein KNOTTED 1 (KN1) [203]. In addition, the intercellular trafficking of both KN1 and TMV was shown to be sensitive to mutations in CCT8, a chaperonin complex believed to act in the post-translocational refolding of transported proteins [188]. Also, the MP of TMV facilitated the spread of silencing signal [5] which may suggest that small RNAs and viral RNAs share a common pathway or mechanism for spread.

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## 9 Virus Movement and Defense Responses

The efficiency of virus movement through PD is affected by plant defense responses that cause a reduction of the PD SEL, reduce viral replication, or lead to degradation of the viral proteins or genome. As already mentioned above, virus infection triggers the salicylic acid (SA) signaling pathway that involves the deterrence of pathogens through increasing callose deposition at PD and viruses may have evolved a recruitment of glucanase enzymes to degrade callose and thus to reverse this defense-induced constriction of PD.

The efficiency of virus infection is also determined by the interaction of viruses with posttranscriptional RNA silencing that targets viral and host RNAs for cleavage or translational repression. As a counter strategy against this important defense response, plant viruses have evolved proteins able to suppress RNA silencing by interfering with different components of the RNA silencing pathway [204, 205]. The silencing suppressor of TMV resides in the 126 kDa small replicase subunit and likely acts through sequestration of virus-derived small RNAs [206, 207]. The ability of the viral MP to facilitate the spread of silencing [5] suggests that TMV and potentially also other viruses may facilitate their movement not only by suppression but also by exploitation of the host RNA silencing machinery. It is conceivable that MP enhances host cell susceptibility for the incoming virus by facilitating the PD-mediated intercellular spreading of virus- and host-derived small RNAs that may act as RNA-based effectors to downregulate defense-related genes in cells at the virus front, whereas the silencing suppressor may act only after virus movement, i.e., in cells containing replication factories and producing virions [208]. Pathogen-encoded small RNA effectors that target host defense genes are well known for several mammalian viruses [209] and have been recently reported also for the plant pathogenic fungus *Botrytis cinerea* [210]. However, whether motile, small RNA-based effectors spreading through PD indeed play a role in facilitating the cell-to-cell propagation of virus infection remains to be investigated. Recent observations indicate that Arabidopsis plants are able to sense virus infection by pathogen-recognition receptors (PRR) and to mount pathogen-associated

molecular pattern (PAMP)-triggered immunity (PTI) through the PAMP co-receptor BAK1 (for BRASSINOSTEROID INSENSITIVE1 (BRI1)-ASSOCIATED RECEPTOR KINASE1) [211]. It will be interesting to see whether this response, or its potential signaling propagation between cells, involves PD.

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## 10 Conclusions

Viruses transport their genomes between cells to spread infection and thus are convenient systems to study the cellular pathways by which macromolecules are targeted and transported through PD. As pathogens, viruses are also excellent systems to determine the manifold mechanisms by which cells defend themselves at PD against invaders. Although numerous viral systems suitable to address these questions are available, only few could be mentioned here. Plant viruses illuminate different pathways by which viruses and other macromolecules can be transported to and through PD. TMV exemplifies a mechanism that involves the viral manipulation of the PD SEL and depends on the ER-actin network that in coordination with specific microtubule system activities supports both the replication and the movement of the virus in a non-encapsidated form. Tubule-forming viruses are systems that depend on the secretory ER-Golgi-plasma membrane pathway for movement. The tubules formed by these viruses replace the desmotubule in PD and thus disrupt the ER connectivity between cells used by other viruses like TMV. Cytoplasmic plant viruses provide important insights into the structural and functional organization of the cortical cytoplasm. Studies with TMV suggest a role of specialized microtubule-associated ER sites (c-MERs) in the assembly of VRCs for either movement (early infection) or growth into viral factories (late infection). It will be interesting to see whether the same sites play a role also in the assembly of complexes of other viruses or in the intercellular movement of cellular proteins and RNAs [50]. Viruses also continue to provide important information with regard to the role of callose in the regulation of the PD SEL. However, since viruses are pathogens, many events occurring in the infected cells may be related to replication, defense, and degradation processes rather than to macromolecular movement through PD. Thus, it is important to dissect the *in vivo* observations with respect to their functional significance in movement. Future research can make use of an excellent panel of novel helpful *in vivo* techniques, such as superresolution microscopy [212]; fluorescent *in vivo* detection of RNA and RNA spread, e.g., [62, 77, 91, 213, 214]; *in vivo* analysis of complexes by FLIM-FRET and BiFC, e.g., [35, 61, 82, 105]; dominant-negative inhibition of cellular processes, e.g., [43, 62]; and novel dye

loading methods to measure PD conductivity, e.g., [171], to just name a few. In combination with genetic and novel next-generation sequencing-based approaches these will lead to a new era of understanding about the mechanisms of PD-mediated intercellular communication in plant development and disease.

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## References

1. Oparka KJ, Roberts AG, Boevink P et al (1999) Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell* 97: 743–754
2. Crawford KM, Zambryski PC (2001) Non-targeted and targeted protein movement through plasmodesmata in leaves in different developmental and physiological states. *Plant Physiol* 125:1802–1812
3. Wolf S, Deom CM, Beachy RN et al (1989) Movement protein of *Tobacco mosaic virus* modifies plasmodesmal size exclusion limit. *Science* 246:377–379
4. Waigmann E, Lucas W, Citovsky V et al (1994) Direct functional assay for *Tobacco mosaic virus* cell-to-cell movement protein and identification of a domain involved in increasing plasmodesmal permeability. *Proc Natl Acad Sci U S A* 91:1433–1437
5. Vogler H, Kwon MO, Dang V et al (2008) *Tobacco mosaic virus* movement protein enhances the spread of RNA silencing. *PLoS Pathog* 4:e1000038
6. Ding B, Haudenschild JS, Hull RJ et al (1992) Secondary plasmodesmata are specific sites of localization of the *Tobacco mosaic virus* movement protein in transgenic tobacco plants. *Plant Cell* 4:915–928
7. Epel B (1994) Plasmodesmata: composition, structure and trafficking. *Plant Mol Biol* 26:1343–1356
8. Hofius D, Herbers K, Melzer M et al (2001) Evidence for expression level-dependent modulation of carbohydrate status and viral resistance by a *Potato leafroll virus* movement protein in transgenic tobacco plants. *Plant J* 28:529–543
9. Roberts IM, Boevink P, Roberts AG et al (2001) Dynamic changes in the frequency and architecture of plasmodesmata during the sink-source transition in tobacco leaves. *Protoplasma* 218:31–44
10. Kim I, Kobayashi K, Cho E et al (2005) Subdomains for transport via plasmodesmata corresponding to the apical-basal axis are established during *Arabidopsis* embryogenesis. *Proc Natl Acad Sci U S A* 102:11945–11950
11. Oparka KJ, Prior DAM, Santa Cruz S et al (1997) Gating of epidermal plasmodesmata is restricted to the leading edge of expanding infection sites of *Tobacco mosaic virus*. *Plant J* 12:781–789
12. Haley A, Hunter T, Kiberstis P et al (1995) Multiple serine phosphorylation sites on the 30 kDa TMV cell-to-cell movement protein synthesized in tobacco protoplasts. *Plant J* 8:715–724
13. Tyulkina LG, Karger EM, Sheveleva AA et al (2010) Binding of monoclonal antibodies to the movement protein (MP) of *Tobacco mosaic virus*: influence of subcellular MP localization and phosphorylation. *J Gen Virol* 91: 1621–1628
14. Kawakami S, Padgett HS, Hosokawa D et al (1999) Phosphorylation and/or presence of serine 37 in the movement protein of *Tomato mosaic tobamovirus* is essential for intracellular localization and stability in vivo. *J Virol* 73:6831–6840
15. Watanabe Y, Meshi T, Okada Y (1992) *In vivo* phosphorylation of the 30-kDa protein of *Tobacco mosaic virus*. *FEBS Lett* 313: 181–184
16. Citovsky V, McLean BG, Zupan JR et al (1993) Phosphorylation of *Tobacco mosaic*



- virus* cell-to-cell movement protein by a developmentally regulated plant cell wall-associated protein kinase. *Genes Dev* 7:904–910
17. Trutnyeva K, Bachmaier R, Waigmann E (2005) Mimicking carboxyterminal phosphorylation differentially effects subcellular distribution and cell-to-cell movement of *Tobacco mosaic virus* movement protein. *Virology* 332:563–577
  18. Waigmann E, Chen M-H, Bachmeier R et al (2000) Regulation of plasmodesmal transport by phosphorylation of *Tobacco mosaic virus* cell-to-cell movement protein. *EMBO J* 19: 4875–4884
  19. Fernandez-Calvino L, Faulkner C, Walshaw J et al (2011) Arabidopsis plasmodesmal proteome. *PLoS One* 6:e18880
  20. Salmon MS, Bayer EM (2012) Dissecting plasmodesmata molecular composition by mass spectrometry-based proteomics. *Front Plant Sci* 3:307
  21. Jo Y, Cho WK, Rim Y et al (2011) Plasmodesmal receptor-like kinases identified through analysis of rice cell wall extracted proteins. *Protoplasma* 248:191–203
  22. Lee JY, Taoka K, Yoo BC et al (2005) Plasmodesmal-associated protein kinase in tobacco and Arabidopsis recognizes a subset of non-cell-autonomous proteins. *Plant Cell* 17:2817–2831
  23. Yaholom A, Lando R, Katz A et al (1998) A calcium-dependent protein kinase is associated with maize mesocotyl plasmodesmata. *J Plant Physiol* 153:354–362
  24. Citovsky V, Knorr D, Schuster G et al (1990) The P30 movement protein of *Tobacco mosaic virus* is a single-strand nucleic acid binding protein. *Cell* 60:637–647
  25. Holt CA, Beachy RN (1991) In vivo complementation of infectious transcripts from mutant *Tobacco mosaic virus* cDNAs in transgenic plants. *Virology* 181:109–117
  26. Niehl A, Heinlein M (2011) Cellular pathways for viral transport through plasmodesmata. *Protoplasma* 248:75–99
  27. Schoelz JE, Harries PA, Nelson RS (2011) Intracellular transport of plant viruses: finding the door out of the cell. *Mol Plant* 4:813–831
  28. Kasteel DTJ, Perbal M-C, Boyer J-C et al (1996) The movement proteins of *Cowpea mosaic virus* and *Cauliflower mosaic virus* induce tubular structures in plant and insect cells. *J Gen Virol* 77:2857–2864
  29. van Lent JWM, Schmitt-Keichinger C (2006) Viral movement proteins induce tubule formation in plant and insect cells. In: Baluska F, Volkmann D, Barlow PW (eds) *Cell-cell channels*. Springer, New York, NY
  30. van Lent J, Storms M, van der Meer F et al (1991) Tubular structures involved in movement of *Cowpea mosaic virus* are also formed in infected cowpea protoplasts. *J Gen Virol* 72:2615–2623
  31. van der Wel NN, Goldbach R, van Lent J (1998) The movement protein and coat protein of *Alfalfa mosaic virus* accumulate in structurally modified plasmodesmata. *Virology* 244:322–329
  32. Lekkerkerker A, Wellink J, Yuan P et al (1996) Distinct functional domains in the *Cowpea mosaic virus* movement protein. *J Virol* 70: 5658–5661
  33. Belin C, Schmitt C, Gaire F et al (1999) The nine C-terminal residues of the *Grapevine fanleaf nepovirus* movement protein are critical for systemic virus spread. *J Gen Virol* 80:1347–1356
  34. Carvalho CM, Wellink J, Ribeiro SG et al (2003) The C-terminal region of the movement protein of *Cowpea mosaic virus* is involved in binding to the large but not to the small coat protein. *J Gen Virol* 84:2271–2277
  35. Amari K, Boutant E, Hofmann C et al (2010) A family of plasmodesmal proteins with receptor-like properties for plant viral movement proteins. *PLoS Pathog* 6:e1001119
  36. Pouwels J, Van Der Krogt GN, Van Lent J et al (2002) The cytoskeleton and the secretory pathway are not involved in targeting the *Cowpea mosaic virus* movement protein to the cell periphery. *Virology* 297:48–56
  37. Laporte C, Vetter G, Loudes AM et al (2003) Involvement of the secretory pathway and the cytoskeleton in intracellular targeting and tubule assembly of *Grapevine fanleaf virus* movement protein in tobacco BY-2 cells. *Plant Cell* 15:2058–2075
  38. Huang Z, Han Y, Howell SH (2000) Formation of surface tubules and fluorescent foci in *Arabidopsis thaliana* protoplasts expressing a fusion between the green fluorescent protein and the *Cauliflower mosaic virus* movement protein. *Virology* 271:58–64
  39. Thomas CL, Bayer EM, Ritzenthaler C et al (2008) Specific targeting of a plasmodesmal protein affecting cell-to-cell communication. *PLoS Biol* 6:e7
  40. Brandizzi F, Wasteney GO (2013) Cytoskeleton-dependent endomembrane organization in plant cells: an emerging role for microtubules. *Plant J* 75:339–349
  41. Nebenführ A, Gallagher LA, Dunahay TG et al (1999) Stop-and-go movements of plant

- Golgi stacks are mediated by the acto-myosin system. *Plant Physiol* 121:1127–1142
42. Tominaga M, Yokota E, Sonobe S et al (2000) Mechanism of inhibition of cytoplasmic streaming by a myosin inhibitor, 2,3-butanedione monoxime. *Protoplasma* 213:46–54
  43. Amari K, Lerich A, Schmitt-Keichinger C et al (2011) Tubule-guided cell-to-cell movement of a plant virus requires class XI myosin motors. *PLoS Pathog* 7:e1002327
  44. Avisar D, Prokhnevsky AI, Makarova KS et al (2008) Myosin XI-K Is required for rapid trafficking of Golgi stacks, peroxisomes, and mitochondria in leaf cells of *Nicotiana benthamiana*. *Plant Physiol* 146:1098–1108
  45. Ueda H, Yokota E, Kutsuna N et al (2010) Myosin-dependent endoplasmic reticulum motility and F-actin organization in plant cells. *Proc Natl Acad Sci U S A* 107:6894–6899
  46. Prokhnevsky AI, Peremyslov VV, Dolja VV (2008) Overlapping functions of the four class XI myosins in Arabidopsis growth, root hair elongation, and organelle motility. *Proc Natl Acad Sci U S A* 105:19744–19749
  47. Peremyslov VV, Prokhnevsky AI, Avisar D et al (2008) Two class XI myosins function in organelle trafficking and root hair development in Arabidopsis. *Plant Physiol* 146:1109–1116
  48. Peremyslov VV, Prokhnevsky AI, Dolja VV (2010) Class XI myosins are required for development, cell expansion, and F-Actin organization in Arabidopsis. *Plant Cell* 22:1883–1897
  49. Hamada T, Tominaga M, Fukaya T et al (2012) RNA processing bodies, peroxisomes, Golgi bodies, mitochondria, and endoplasmic reticulum tubule junctions frequently pause at cortical microtubules. *Plant Cell Physiol* 53:699–708
  50. Peña EJ, Heinlein M (2013) Cortical microtubule-associated ER sites: organization centers of cell polarity and communication. *Curr Opin Plant Biol* 16:764–773
  51. Ritzenthaler C, Laporte C, Gaire F et al (2002) *Grapevine fanleaf virus* replication occurs on endoplasmic reticulum-derived membranes. *J Virol* 76:8808–8819
  52. Carette JE, van Lent J, MacFarlane SA et al (2002) *Cowpea mosaic virus* 32- and 60-kilodalton replication proteins target and change the morphology of endoplasmic reticulum membranes. *J Virol* 76:6293–6301
  53. Peremyslov VV, Pan YW, Dolja VV (2004) Movement protein of a closterovirus is a type III integral transmembrane protein localized to the endoplasmic reticulum. *J Virol* 78:3704–3709
  54. Dolja VV, Kreuze JF, Valkonen JPT (2006) Comparative and functional genomics of closteroviruses. *Virus Res* 117:38–51
  55. Avisar D, Prokhnevsky AI, Dolja VV (2008) Class VIII myosins are required for plasmodesmata localization of a closterovirus Hsp70 homolog. *J Virol* 82:2836–2843
  56. Peremyslov VV, Hagiwara Y, Dolja VV (1999) HSP70 homolog functions in cell-to-cell movement of a plant virus. *Proc Natl Acad Sci U S A* 96:14771–14776
  57. Dolja VV, Haldeman R, Robertson NL et al (1994) Distinct functions of capsid protein in assembly and movement of *Tobacco etch virus*. *EMBO J* 13:1482–1491
  58. Dolja VV, Haldeman-Cahill R, Montgomery AE et al (1995) Capsid protein determinants involved in cell-to-cell and long distance movement of *Tobacco etch potyvirus*. *Virology* 206:1007–1016
  59. Gabrenaite-Verkhovskaya R, Andreev IA, Kalinina NO et al (2008) Cylindrical inclusion protein of *Potato virus A* is associated with a subpopulation of particles isolated from infected plants. *J Gen Virol* 89:829–838
  60. Wei T, Zhang C, Hong J et al (2010) Formation of complexes at plasmodesmata for potyvirus intercellular movement is mediated by the viral protein P3N-PIPO. *PLoS Pathog* 6:e1000962
  61. Vijayapalani P, Maeshima M, Nagasaki-Takekuchi N et al (2012) Interaction of the trans-frame potyvirus protein P3N-PIPO with host protein PCaP1 facilitates potyvirus movement. *PLoS Pathog* 8:e1002639
  62. Agbeci M, Grangeon R, Nelson RS et al (2013) Contribution of host intracellular transport machineries to intercellular movement of *Turnip mosaic virus*. *PLoS Pathog* 9:e1003683
  63. Lain S, Riechmann JL, Garcia JA (1990) RNA helicase: a novel activity associated with a protein encoded by a positive strand RNA virus. *Nucl Acids Res* 18:7003–7006
  64. Rojas MR, Zerbini M, Allison RF et al (1997) Capsid protein and helper component-proteinase function as potyvirus cell-to-cell movement proteins. *Virology* 237:283–295
  65. Grangeon R, Jiang J, Wan J et al (2013) 6 K2-induced vesicles can move cell to cell during *Turnip mosaic virus* infection. *Front Microbiol* 4:351
  66. Deom CM, Oliver MJ, Beachy RN (1987) The 30-kilodalton gene product of *Tobacco mosaic virus* potentiates virus movement. *Science* 237:384–389

67. Kim SH, Kalinina NO, Andreev I et al (2004) The C-terminal 33 amino acids of the *Cucumber mosaic virus* 3a protein affect virus movement, RNA binding and inhibition of infection and translation. *J Gen Virol* 85: 221–230
68. Nagano H, Mise K, Furusawa I et al (2001) Conversion in the requirement of coat protein in cell-to-cell movement mediated by the *Cucumber mosaic virus* movement protein. *J Virol* 75:8045–8053
69. Sasaki N, Kaido M, Okuno T et al (2005) Coat protein-independent cell-to-cell movement of bromoviruses expressing *Brome mosaic virus* movement protein with an adaptation-related amino acid change in the central region. *Arch Virol* 150:1231–1240
70. Verchot-Lubicz J, Ye CM, Bamunusinghe D (2007) Molecular biology of potexviruses: recent advances. *J Gen Virol* 88:1643–1655
71. Hipper C, Brault V, Ziegler-Graff V et al (2013) Viral and cellular factors involved in phloem transport of plant viruses. *Front Plant Sci* 4:154
72. Heinlein M, Padgett HS, Gens JS et al (1998) Changing patterns of localization of the *Tobacco mosaic virus* movement protein and replicase to the endoplasmic reticulum and microtubules during infection. *Plant Cell* 10:1107–1120
73. Asurmendi S, Berg RH, Koo JC et al (2004) Coat protein regulates formation of replication complexes during *Tobacco mosaic virus* infection. *Proc Natl Acad Sci U S A* 101: 1415–1420
74. Kawakami S, Watanabe Y, Beachy RN (2004) *Tobacco mosaic virus* infection spreads cell to cell as intact replication complexes. *Proc Natl Acad Sci U S A* 101:6291–6296
75. Hirashima K, Watanabe Y (2001) Tobamovirus replicase coding region is involved in cell-to-cell movement. *J Virol* 75: 8831–8836
76. Boyko V, Hu Q, Seemanpillai M et al (2007) Validation of microtubule-associated *Tobacco mosaic virus* RNA movement and involvement of microtubule-aligned particle trafficking. *Plant J* 51:589–603
77. Sambade A, Brandner K, Hofmann C et al (2008) Transport of TMV movement protein particles associated with the targeting of RNA to plasmodesmata. *Traffic* 9:2073–2088
78. Niehl A, Peña EJ, Amari K et al (2013) Microtubules in viral replication and transport. *Plant J* 75:290–308
79. Peña EJ, Heinlein M (2012) RNA transport during TMV cell-to-cell movement. *Front Plant Sci* 3:193
80. Ashby J, Boutant E, Seemanpillai M et al (2006) *Tobacco mosaic virus* movement protein functions as a structural microtubule-associated protein. *J Virol* 80:8329–8344
81. Heinlein M, Epel BL, Padgett HS et al (1995) Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* 270:1983–1985
82. Brandner K, Sambade A, Boutant E et al (2008) *Tobacco mosaic virus* movement protein interacts with green fluorescent protein-tagged microtubule end-binding protein 1. *Plant Physiol* 147:611–623
83. Ferralli J, Ashby J, Fasler M et al (2006) Disruption of microtubule organization and centrosome function by expression of *Tobacco mosaic virus* movement protein. *J Virol* 80: 5807–5821
84. Lindeboom JJ, Nakamura M, Hibbel A et al (2013) A mechanism for reorientation of cortical microtubule arrays driven by microtubule severing. *Science* 342:1245533
85. Boyko V, Ferralli J, Ashby J et al (2000) Function of microtubules in intercellular transport of plant virus RNA. *Nat Cell Biol* 2:826–832
86. Tagami Y, Watanabe Y (2007) Effects of brefeldin A on the localization of tobamovirus movement protein and cell-to-cell movement of the virus. *Virology* 361:133–140
87. Ouko MO, Sambade A, Brandner K et al (2010) Tobacco mutants with reduced microtubule dynamics are less susceptible to TMV. *Plant J* 62:829–839
88. Harries PA, Schoelz JE, Nelson RS (2009) Covering common ground: F-actin-dependent transport of plant viral protein inclusions reveals a novel mechanism for movement utilized by unrelated viral proteins. *Plant Signal Behav* 4:454–456
89. Liu J-Z, Blancaflor EB, Nelson RS (2005) The *Tobacco mosaic virus* 126-kilodalton protein, a constituent of the virus replication complex, alone or within the complex aligns with and traffics along microfilaments. *Plant Physiol* 138:1877–1895
90. Hofmann C, Niehl A, Sambade A et al (2009) Inhibition of *Tobacco mosaic virus* movement by expression of an actin-binding protein. *Plant Physiol* 149:1810–1823
91. Tilsner J, Linnik O, Christensen NM et al (2009) Live-cell imaging of viral RNA genomes using a Pumilio-based reporter. *Plant J* 57:758–770
92. Niehl A, Amari K, Gereige D et al (2012) Control of *Tobacco mosaic virus* movement protein fate by CELL-DIVISION-CYCLE

- protein 48 (CDC48). *Plant Physiol* 160: 2093–2108
93. Arce-Johnson P, Kahn TW, Reimann-Philipp U et al (1995) The amount of movement protein produced in transgenic plants influences the establishment, local movement, and systemic spread of infection by movement protein-deficient *Tobacco mosaic virus*. *Mol Plant Microbe Interact* 3:415–423
  94. Szécsi J, Ding XS, Lim CO et al (1999) Development of *Tobacco mosaic virus* infection sites in *Nicotiana benthamiana*. *Mol Plant Microbe Interact* 2:143–152
  95. Chen MH, Tian GW, Gafni Y et al (2005) Effects of calreticulin on viral cell-to-cell movement. *Plant Physiol* 138:1866–1876
  96. Kragler F, Curin M, Trutnyeva K et al (2003) MPB2C, a microtubule-associated plant protein binds to and interferes with cell-to-cell transport of *Tobacco mosaic virus* movement protein. *Plant Physiol* 132:1870–1883
  97. Reichel C, Beachy RN (2000) Degradation of the *Tobacco mosaic virus* movement protein by the 26S proteasome. *J Virol* 74:3330–3337
  98. Fujiki M, Kawakami S, Kim RW et al (2006) Domains of *Tobacco mosaic virus* movement protein essential for its membrane association. *J Gen Virol* 87:2699–2707
  99. Brill LM, Dechongkit S, DeLaBarre B et al (2004) Dimerization of recombinant *Tobacco mosaic virus* movement protein. *J Virol* 78:3372–3377
  100. Brill LM, Nunn RS, Kahn TW et al (2000) Recombinant *Tobacco mosaic virus* movement protein is an RNA-binding,  $\alpha$ -helical membrane protein. *Proc Natl Acad Sci U S A* 97:7112–7117
  101. Citovsky V, Wong ML, Shaw AL et al (1992) Visualization and characterization of *Tobacco mosaic virus* movement protein binding to single-stranded nucleic acids. *Plant Cell* 4:397–411
  102. Shimizu T, Yoshii A, Sakurai K et al (2009) Identification of a novel tobacco DnaJ-like protein that interacts with the movement protein of *Tobacco mosaic virus*. *Arch Virol* 154:959–967
  103. Chen M-H, Citovsky V (2003) Systemic movement of a tobamovirus requires host cells pectin methylesterase. *Plant J* 35:386–392
  104. Peiroa A, Martinez-Gil L, Tamborero S et al (2014) The *Tobacco mosaic virus* movement protein associates with but does not integrate into biological membranes. *J Virol* 88: 3016–3026
  105. Boutant E, Didier P, Niehl A et al (2010) Fluorescent protein recruitment assay for demonstration and analysis of in vivo protein interactions in plant cells and its application to *Tobacco mosaic virus* movement protein. *Plant J* 62:171–177
  106. Verchot J (2011) Wrapping membranes around plant virus infection. *Curr Opin Virol* 1:388–395
  107. Laliberté JF, Sanfaçon H (2010) Cellular remodeling during plant virus infection. *Annu Rev Phytopathol* 48:69–91
  108. Grangeon R, Jiang J, Laliberté JF (2012) Host endomembrane recruitment for plant RNA virus replication. *Curr Opin Virol* 2: 683–690
  109. Morozov SY, Solovyev AG (2003) Triple gene block: modular design of a multifunctional machine for plant virus movement. *J Gen Virol* 84:1351–1366
  110. Verchot-Lubicz J, Torrance L, Solovyev AG et al (2010) Varied movement strategies employed by triple gene block-encoding viruses. *Mol Plant Microbe Interact* 23:1231–1247
  111. Solovyev AG, Kalinina NO, Morozov SY (2012) Recent advances in research of plant virus movement mediated by triple gene block. *Front Plant Sci* 3:276
  112. Cruz SS, Roberts AG, Prior DAM et al (1998) Cell-to-cell and phloem-mediated transport of *Potato virus X*: the role of virions. *Plant Cell* 10:495–510
  113. Lough TJ, Netzler NE, Emerson SJ et al (2000) Cell-to-cell movement of potexviruses: evidence for a ribonucleoprotein complex involving the coat protein and first triple gene block protein. *Mol Plant Microbe Interact* 13:962–974
  114. Fedorkin ON, Merits A, Lucchesi J et al (2000) Complementation of the movement-deficient mutations in *Potato virus X*: potyvirus coat protein mediates cell-to-cell trafficking of C-terminal truncation but not deletion mutant of potexvirus coat protein. *Virology* 270:31–42
  115. Fedorkin O, Solovyev A, Yelina N et al (2001) Cell-to-cell movement of *Potato virus X* involves distinct functions of the coat protein. *J Gen Virol* 82:449–458
  116. Makarov VV, Rybakova EN, Efimov AV et al (2009) Domain organization of the N-terminal portion of hordeivirus movement protein TGBp1. *J Gen Virol* 90:3022–3032
  117. Chou YL, Hung YJ, Tseng YH et al (2013) The stable association of virion with the triple-gene-block protein 3-based complex of *Bamboo mosaic virus*. *PLoS Pathog* 9:e1003405
  118. Haupt S, Cowan GH, Ziegler A et al (2005) Two plant-viral movement proteins traffic in the endocytic recycling pathway. *Plant Cell* 17:164–181

119. Ju HJ, Samuels TD, Wang YS et al (2005) The *Potato virus X* TGBp2 movement protein associates with endoplasmic reticulum-derived vesicles during virus infection. *Plant Physiol* 138:1877–1895
120. Lauber E, Janssens L, Weyens G et al (2001) Rapid screening for dominant negative mutations in the *Beet necrotic yellow vein virus* triple gene block proteins P13 and P15 using a viral replicon. *Transgenic Res* 10:293–302
121. Mitra R, Krishnamurthy K, Blancaflor E et al (2003) The *Potato virus X* TGBp2 protein association with the endoplasmic reticulum plays a role in but is not sufficient for viral cell-to-cell movement. *Virology* 312:35–48
122. Samuels TD, Ju HJ, Ye CM et al (2007) Subcellular targeting and interactions among the *Potato virus X* TGB proteins. *Virology* 367:375–389
123. Solovyev AG, Stroganova TA, Zamyatin AA Jr (2000) Subcellular sorting of small membrane-associated triple gene block proteins: TGBp3-assisted targeting of TGBp2. *Virology* 269:113–127
124. Bamunusinghe D, Hemenway CL, Nelson RS et al (2009) Analysis of *Potato virus X* replicase and TGBp3 subcellular locations. *Virology* 393:272–285
125. Chang BY, Lin NS, Liou DY et al (1997) Subcellular localization of the 28 kDa protein of the triple-gene-block of *Bamboo mosaic potexvirus*. *J Gen Virol* 78:1175–1179
126. Davies C, Hills G, Baulcombe DC (1993) Sub-cellular localization of the 25-kDa protein encoded in the triple gene block of *Potato virus X*. *Virology* 197:166–175
127. Zamyatin AA Jr, Solovyev AG, Savenkov EI et al (2004) Transient coexpression of individual genes encoded by the triple gene block of *Potato mop-top virus* reveals requirements for TGBp1 trafficking. *Mol Plant Microbe Interact* 17:921–930
128. Erhardt M, Vetter G, Gilmer D et al (2005) Subcellular localization of the Triple Gene Block movement proteins of *Beet necrotic yellow vein virus* by electron microscopy. *Virology* 340:155–166
129. Lim HS, Bragg JN, Ganesan U et al (2008) Triple gene block protein interactions involved in movement of *Barley stripe mosaic virus*. *J Virol* 82:4991–5006
130. Lim HS, Bragg JN, Ganesan U et al (2009) Subcellular localization of the *Barley stripe mosaic virus* triple gene block proteins. *J Virol* 83:9432–9448
131. Schepetilnikov MV, Solovyev AG, Gorshkova EN et al (2008) Intracellular targeting of a hordeiviral membrane-spanning movement protein: sequence requirements and involvement of an unconventional mechanism. *J Virol* 82:1284–1293
132. Tilsner J, Cowan GH, Roberts AG et al (2010) Plasmodesmal targeting and intercellular movement of *Potato mop-top pomovirus* is mediated by a membrane anchored tyrosine-based motif on the luminal side of the endoplasmic reticulum and the C-terminal transmembrane domain in the TGB3 movement protein. *Virology* 402:41–51
133. Cowan GH, Lioliopoulou F, Ziegler A et al (2002) Subcellular localization, protein interactions, and RNA binding activity of *Potato mop-top virus* triple gene block proteins. *Virology* 298:106–115
134. Krishnamurthy K, Mitra R, Payton ME et al (2002) Cell-to-cell movement of the PVX 12 K, 8 K, or coat proteins may depend on the host, leaf developmental stage, and the PVX 25 K protein. *Virology* 300:269–281
135. Krishnamurthy K, Heppler M, Mitra R et al (2003) The *Potato virus X* TGBp3 protein associates with the ER network for virus cell-to-cell movement. *Virology* 309:135–151
136. Angell SM, Davies C, Baulcombe DC (1996) Cell-to-cell movement of *Potato virus X* is associated with a change in the size-exclusion limit of plasmodesmata in trichome cells of *Nicotiana clevelandii*. *Virology* 216:197–201
137. Lough TJ, Shash K, Xoconostle-Cazares B et al (1998) Molecular dissection of the mechanism by which potexvirus triple gene block proteins mediate cell-to-cell transport of infectious RNA. *Mol Plant Microbe Interact* 11:801–814
138. Howard AR, Heppler ML, Ju HJ et al (2004) *Potato virus X* TGBp1 induces plasmodesmata gating and moves between cells in several host species whereas CP moves only in *N. benthamiana* leaves. *Virology* 328:185–197
139. Tamai A, Meshi T (2001) Cell-to-cell movement of *Potato virus X*: the role of p12 and p8 encoded by the second and third open reading frames of the triple gene block. *Mol Plant Microbe Interact* 10:1158–1167
140. Lee CC, Ho YN, Hu RH et al (2011) The interaction between *Bamboo mosaic virus* replication protein and coat protein is critical for virus movement in plant hosts. *J Virol* 85:12022–12031
141. Doronin SV, Hemenway C (1996) Synthesis of *Potato virus X* RNAs by membrane-containing extracts. *J Virol* 70:4795–4799
142. Linnik O, Liesche J, Tilsner J et al (2013) Unraveling the structure of viral replication complexes at super-resolution. *Front Plant Sci* 4:6

143. Tilsner J, Linnik O, Wright KM et al (2012) The TGB1 movement protein of *Potato virus X* reorganizes actin and endomembranes into the X-body, a viral replication factory. *Plant Physiol* 158:1359–1370
144. Tilsner J, Linnik O, Louveau M et al (2013) Replication and trafficking of a plant virus are coupled at the entrances of plasmodesmata. *J Cell Biol* 201:981–995
145. Lee SC, Wu CH, Wang CW (2010) Traffic of a viral movement protein complex to the highly curved tubules of the cortical endoplasmic reticulum. *Traffic* 11:912–930
146. Wu CH, Lee SC, Wang CW (2011) Viral protein targeting to the cortical endoplasmic reticulum is required for cell-cell spreading in plants. *J Cell Biol* 193:521–535
147. Tilsner J, Amari K, Torrance L (2011) Plasmodesmata viewed as specialised membrane adhesion sites. *Protoplasma* 248:39–60
148. Raffaele S, Bayer E, Lafarge D et al (2009) Remorin, a solanaceae protein resident in membrane rafts and plasmodesmata, impairs *Potato virus X* movement. *Plant Cell* 21:1541–1555
149. Serazev TV, Nadezhkina ES, Shanina NA et al (2003) Virions and membrane proteins of the potato X virus interact with microtubules and enables tubulin polymerization in vitro. *Mol Biol* 37:919–925
150. Cho SY, Cho WK, Choi HS et al (2012) Cis-acting element (SL1) of *Potato virus X* controls viral movement by interacting with the NbMPB2Cb and viral proteins. *Virology* 427:166–176
151. Wright KM, Cowan GH, Lukhovitskaya NI et al (2010) The N-terminal domain of PMTV TGB1 movement protein is required for nucleolar localization, microtubule association, and long-distance movement. *Mol Plant Microbe Interact* 23:1486–1497
152. Shemyakina EA, Solovyev AG, Leonova OG et al (2011) The role of microtubule association in plasmodesmal targeting of *Potato mop-top virus* movement protein TGBp1. *Open Virol J* 5:1–11
153. Heinlein M (2008) Microtubules and viral movement. In: Nick P (ed) *Plant microtubules*. Springer, Berlin, pp 141–173
154. Gillespie T, Boevink P, Haupt S et al (2002) Functional analysis of a DNA shuffled movement protein reveals that microtubules are dispensable for the cell-to-cell movement of *Tobacco mosaic virus*. *Plant Cell* 14:1207–1222
155. Seemanpillai M, Elamawi R, Ritzenthaler C et al (2006) Challenging the role of microtubules in *Tobacco mosaic virus* movement by drug treatments is disputable. *J Virol* 80: 6712–6715
156. Harries PA, Park JW, Sasaki N et al (2009) Differing requirements for actin and myosin by plant viruses for sustained intercellular movement. *Proc Natl Acad Sci U S A* 106: 17594–17599
157. Prokhnevsky AI, Peremyslov VV, Dolja VV (2005) Actin cytoskeleton is involved in targeting of a viral Hsp70 homolog to the cell periphery. *J Virol* 79:14421–14428
158. Wright KM, Wood NT, Roberts AG et al (2007) Targeting of TMV movement protein to plasmodesmata requires the actin/ER network: evidence from FRAP. *Traffic* 8:21–31
159. Gutierrez S, Yvon M, Thebaud G et al (2010) Dynamics of the multiplicity of cellular infection in a plant virus. *PLoS Pathog* 6:e1001113
160. Guenoun-Gelbart D, Elbaum M, Sagi G et al (2008) *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* 21:335–345
161. Barton DA, Cole L, Collings DA et al (2011) Cell-to-cell transport via the lumen of the endoplasmic reticulum. *Plant J* 66:806–817
162. Cantrill LC, Overall RL, Goodwin PB (1999) Cell-to-cell communication via plant endomembranes. *Cell Biol Int* 23:653–661
163. Grabski S, de Feijter AW, Schindler M (1993) Endoplasmic reticulum forms a dynamic continuum for lipid diffusion between contiguous soybean root cells. *Plant Cell* 5:25–38
164. Su S, Liu Z, Chen C et al (2010) *Cucumber mosaic virus* movement protein severs actin filaments to increase the plasmodesmal size exclusion limit in tobacco. *Plant Cell* 22: 1373–1387
165. White RG, Barton DA (2011) The cytoskeleton in plasmodesmata: a role in intercellular transport? *J Exp Bot* 62:5249–5266
166. Overall RL, Blackman LM (1996) A model of the macromolecular structure of plasmodesmata. *Trends Plant Sci* 1:307–311
167. Maule AJ (2008) Plasmodesmata: structure, function and biogenesis. *Curr Opin Plant Biol* 11:680–686
168. Zavaliev R, Ueki S, Epel BL et al (2011) Biology of callose (beta-1,3-glucan) turnover at plasmodesmata. *Protoplasma* 248:117–130
169. Dong X, Hong Z, Chatterjee J et al (2008) Expression of callose synthase genes and its connection with *Npr1* signaling pathway during pathogen infection. *Planta* 229:87–98



170. Lee JY, Wang X, Cui W et al (2011) A plasmodesmata-localized protein mediates crosstalk between cell-to-cell communication and innate immunity in Arabidopsis. *Plant Cell* 23:3353–3373
171. Wang X, Sager R, Cui W et al (2013) Salicylic acid regulates plasmodesmata closure during innate immune responses in Arabidopsis. *Plant Cell* 25:2315–2329
172. Epel BL (2009) 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* 20:1074–1081
173. Beffa RS, Hofer RM, Thomas M et al (1996) Decreased susceptibility to viral disease of  $\beta$ -1,3-glucanase-deficient plants generated by antisense transformation. *Plant Cell* 8:1001–1011
174. Bucher GL, Tarina C, Heinlein M et al (2001) Local expression of enzymatically active class I  $\beta$ -1,3-glucanase enhances symptoms of TMV infection in tobacco. *Plant J* 28:361–369
175. Iglesias VA, Meins F Jr (2000) Movement of plant viruses is delayed in a  $\beta$ -1,3-glucanase-deficient mutant showing a reduced plasmodesmatal size exclusion limit and enhanced callose deposition. *Plant J* 21:157–166
176. Levy A, Erlanger M, Rosenthal M et al (2007) A plasmodesmata-associated  $\beta$ -1,3-glucanase in Arabidopsis. *Plant J* 49:669–682
177. Zavaliev R, Levy A, Gera A et al (2013) Subcellular dynamics and role of Arabidopsis  $\beta$ -1,3-glucanases in cell-to-cell movement of tobamoviruses. *Mol Plant Microbe Interact* 26:1016–1030
178. Ueki S, Spektor R, Natale DM et al (2010) ANK, a host cytoplasmic receptor for the *Tobacco mosaic virus* cell-to-cell movement protein, facilitates intercellular transport through plasmodesmata. *PLoS Pathog* 6:e1001201
179. Fridborg I, Grainger J, Page A et al (2003) TIP, a novel host factor linking callose degradation with the cell-to-cell movement of *Potato virus X*. *Mol Plant Microbe Interact* 16:132–140
180. Benitez-Alfonso Y, Jackson D (2009) Redox homeostasis regulates plasmodesmal communication in Arabidopsis meristems. *Plant Signal Behav* 4:655–659
181. Benitez-Alfonso Y, Jackson D, Maule A (2011) Redox regulation of intercellular transport. *Protoplasma* 248:131–140
182. Stonebloom S, Brunkard JO, Cheung AC et al (2012) Redox states of plastids and mitochondria differentially regulate intercellular transport via plasmodesmata. *Plant Physiol* 158:190–199
183. Tucker EB (1990) Calcium-loaded 1,2-bis(2-aminophenoxy)ethane- $N,N,N',N'$ -tetraacetic acid blocks cell-to-cell diffusion of carboxyfluorescein in staminal hairs of *Setcreasea purpurea*. *Planta* 182:34–38
184. Tucker EB, Boss WF (1996) Mastoparan induced intracellular  $Ca^{2+}$  fluxes may regulate cell-to-cell communication in plants. *Plant Physiol* 111:459–467
185. Holdaway-Clarke TL, Walker NA, Hepler PK et al (2000) Physiological elevations in cytoplasmic free calcium by cold or ion injection result in transient closure of higher plant plasmodesmata. *Planta* 210:329–335
186. Kragler F, Monzer J, Shash K et al (1998) Cell-to-cell transport of proteins: requirement for unfolding and characterization of binding to a putative plasmodesmal receptor. *Plant J* 15:367–381
187. Lee J-Y, Yoo B-C, Rojas MR et al (2003) Selective trafficking of non-cell-autonomous proteins mediated by NtNCAPP1. *Science* 299:392–396
188. Fichtenbauer D, Xu XM, Jackson D et al (2012) The chaperonin CCT8 facilitates spread of tobamovirus infection. *Plant Signal Behav* 7:318–321
189. Aoki K, Kragler F, Xoconostle-Cazares B et al (2002) A subclass of plant heat shock cognate 70 chaperones carries a motif that facilitates trafficking through plasmodesmata. *Proc Natl Acad Sci U S A* 99:16342–16347
190. Xu XM, Wang J, Xuan Z et al (2011) Chaperonins facilitate KNOTTED1 cell-to-cell trafficking and stem cell function. *Science* 333:1141–1144
191. Wu S, Gallagher KL (2012) Transcription factors on the move. *Curr Opin Plant Biol* 15:645–651
192. Haywood V, Kragler F, Lucas WJ (2002) Plasmodesmata: pathways for protein and ribonucleoprotein signaling. *Plant Cell* 14(Suppl):S303–S325
193. Lucas WJ, Lee JY (2004) Plasmodesmata as a supracellular control network in plants. *Nat Rev Mol Cell Biol* 5:712–726
194. Lucas WJ, Yoo B-C, Kragler F (2001) RNA as a long-distance information macromolecule in plants. *Nat Rev Mol Cell Biol* 2:849–857
195. Hannapel DJ, Sharma P, Lin T (2013) Phloem-mobile messenger RNAs and root development. *Front Plant Sci* 4:257
196. Kim M, Canio W, Kessler S et al (2001) Developmental changes due to long-distance

- movement of a homeobox fusion transcript in tomato. *Science* 293:287–289
197. Kehr J, Buhtz A (2008) Long distance transport and movement of RNA through the phloem. *J Exp Bot* 59:85–92
  198. Yoo BC, Kragler F, Varkonyi-Gasic E et al (2004) A systemic small RNA signaling system in plants. *Plant Cell* 16:1979–2000
  199. Furuta K, Lichtenberger R, Helariutta Y (2012) The role of mobile small RNA species during root growth and development. *Curr Opin Cell Biol* 24:211–216
  200. Brosnan CA, Voinnet O (2011) Cell-to-cell and long-distance siRNA movement in plants: mechanisms and biological implications. *Curr Opin Plant Biol* 14:580–587
  201. Skopelitis DS, Husbands AY, Timmermans MC (2012) Plant small RNAs as morphogens. *Curr Opin Cell Biol* 24:217–224
  202. Melnyk CW, Molnar A, Baulcombe DC (2011) Intercellular and systemic movement of RNA silencing signals. *EMBO J* 30:3553–3563
  203. Winter N, Kollwig G, Zhang S et al (2007) MPB2C, a microtubule-associated protein, regulates non-cell-autonomy of the homeodomain protein KNOTTED1. *Plant Cell* 19:3001–3018
  204. Wu Q, Wang X, Ding SW (2010) Viral suppressors of RNA-based viral immunity: host targets. *Cell Host Microbe* 8:12–15
  205. Burgyan J, Havelda Z (2011) Viral suppressors of RNA silencing. *Trends Plant Sci* 16: 265–272
  206. Csorba T, Bovi A, Dalmay T et al (2007) The p122 subunit of *Tobacco Mosaic Virus* replicase is a potent silencing suppressor and compromises both small interfering RNA- and microRNA-mediated pathways. *J Virol* 81:11768–11780
  207. Vogler H, Akbergenov R, Shivaprasad PV et al (2007) Modification of small RNAs associated with suppression of RNA silencing by tobamovirus replicase protein. *J Virol* 81: 10379–10388
  208. Amari K, Vazquez F, Heinlein M (2012) Manipulation of plant host susceptibility: an emerging role for viral movement proteins? *Front Plant Sci* 3:10
  209. Umbach JL, Cullen BR (2009) The role of RNAi and microRNAs in animal virus replication and antiviral immunity. *Genes Dev* 23: 1151–1164
  210. Weiberg A, Wang M, Lin FM et al (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* 342:118–123
  211. Kørner CJ, Klauser D, Niehl A et al (2013) The immunity regulator BAK1 contributes to resistance against diverse RNA viruses. *Mol Plant Microbe Interact* 26:1271–1280
  212. Fitzgibbon J, Bell K, King E et al (2010) Super-resolution imaging of plasmodesmata using three-dimensional structured illumination microscopy. *Plant Physiol* 153: 1453–1463
  213. Tilsner J, Oparka KJ (2010) Tracking the green invaders: advances in imaging virus infection in plants. *Biochem J* 430:21–37
  214. Dunoyer P, Schott G, Himber C et al (2010) Small RNA duplexes function as mobile silencing signals between plant cells. *Science* 328:912–916

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