Virus-Host Interactions during Movement Processes[1]

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Plant viruses must invade and infect as much of their hosts as possible to maximize their chances of successful perpetuation. They move cell to cell via plasmodesmata (PD), which they modify to a greater or lesser extent, and to distant parts of the plant through the vascular system. Plant viruses encode one or more nonstructural proteins specifically required for movement within their hosts and many also require their capsid (coat) protein(s). Classically, a viral movement protein (MP) is defined by its ability to increase the plasmodesmal size exclusion limit (SEL) and to move cell to cell; however, other viral proteins that do not themselves move may be essential for the movement process. Viruses that infect plants have developed a variety of strategies to move from cell to cell and are heavily dependent on endogenous host transport systems during movement, as with all aspects of their life cycles. Rather than attempt to cover all reported virus-host interactions during movement, in this short review, we would like to focus on some common themes that appear in the literature regarding each of the steps involved in viral cell-to-cell movement. These are the use of the endoplasmic reticulum (ER)/actin network as an intracellular transport pathway, recognition of adhesion sites at the cell periphery, modification of PD by alteration of the cell wall structure, heat shock protein (Hsp) 70-class chaperones as potential translocation factors, and regulation of movement. We will discuss how the movement processes of different viruses may utilize these steps in different ways or may not involve all of these steps. Other reviewers have covered different aspects of short and long distance movement processes, such as the role of the cytoskeleton and the requirement for suppression of host defense responses (for example, Reichel et al., 1999; Oparka, 2004; Waigmann et al., 2004; Voinnet, 2005).

INTRACELLULAR MOVEMENT USING THE ER

With the constant streaming of the plant cell cytoplasm, one could imagine that viral MPs would only need to go with the flow and bind to PD or other peripheral target sites when they encounter proteins that they recognize. Evidence from numerous studies suggests that this is not generally the case, although one cannot discount that a proportion of any MP may arrive at its destination in this way. Cell-to-cell movement is generally an early event in the infection process, occurring in 4 h for tobacco rattle virus in *Nicotiana clevelandii* or 5 h for tobacco mosaic virus (TMV) in *N. tabacum* (Fannin and Shaw, 1987; Derrick et al., 1992), and the level of MP produced in the first few hours is likely to be quite low. Thus, random movement with the cytoplasmic flow may be too inefficient. Many viruses form replication centers enriched in ER (for examples, see Schaad et al., 1997; Heinlein et al., 1998; Mas and Beachy, 1999; Carette et al., 2000; Ritzenthaler et al., 2002), and many viral proteins required for movement appear to be membrane proteins, often shown to locate to the ER. For example, the triple gene block (TGB) proteins 2 and 3 of potato mop top virus (PMTV) and potato virus X (PVX) when fused to fluorescent proteins label the ER (Solovyev et al., 2000; Cowan et al., 2002; Krishnamurthy et al., 2003; Mitra et al., 2003; Haupt et al., 2005). Only TGB2 of poa semilatent virus locates to the ER (Solovyev et al., 2000; Zamyatnin et al., 2002). TMV MP fused to fluorescent proteins labels the ER early in infection (Heinlein et al., 1998; Gillespie et al., 2002) and the 126/183-kD protein(s), which are also required for movement (Hirashima and Watanabe, 2001, 2003) and associate with movement complexes (MCs; complexes of viral RNA, MPs, and other viral, and perhaps host, proteins; Kawakami et al., 2004), have been shown to locate to membranes (Hagiwara et al., 2003). The p6 MP of beet yellows closterovirus and the MP of alfalfa mosaic virus fused to green fluorescent protein (GFP) also label the ER (Huang and Zhang, 1999; Peremyslov et al., 2004).

The MCs of viruses with ER-located MPs are likely to be assembled on the ER (Figure 1a). The ER passes through PD in the form of the desmotubule and is intimately entwined with the actin cytoskeleton (Boevink et al., 1998); therefore, the simplest and most efficient route for MCs of these viruses to take to PD from replication centers would be along the ER membrane. ER-attached MCs may specifically interact with myosin motors either directly or indirectly for transportation along the ER. The TMV MP appeared to colocalize with actin filaments in protoplasts (Mclean et al.,
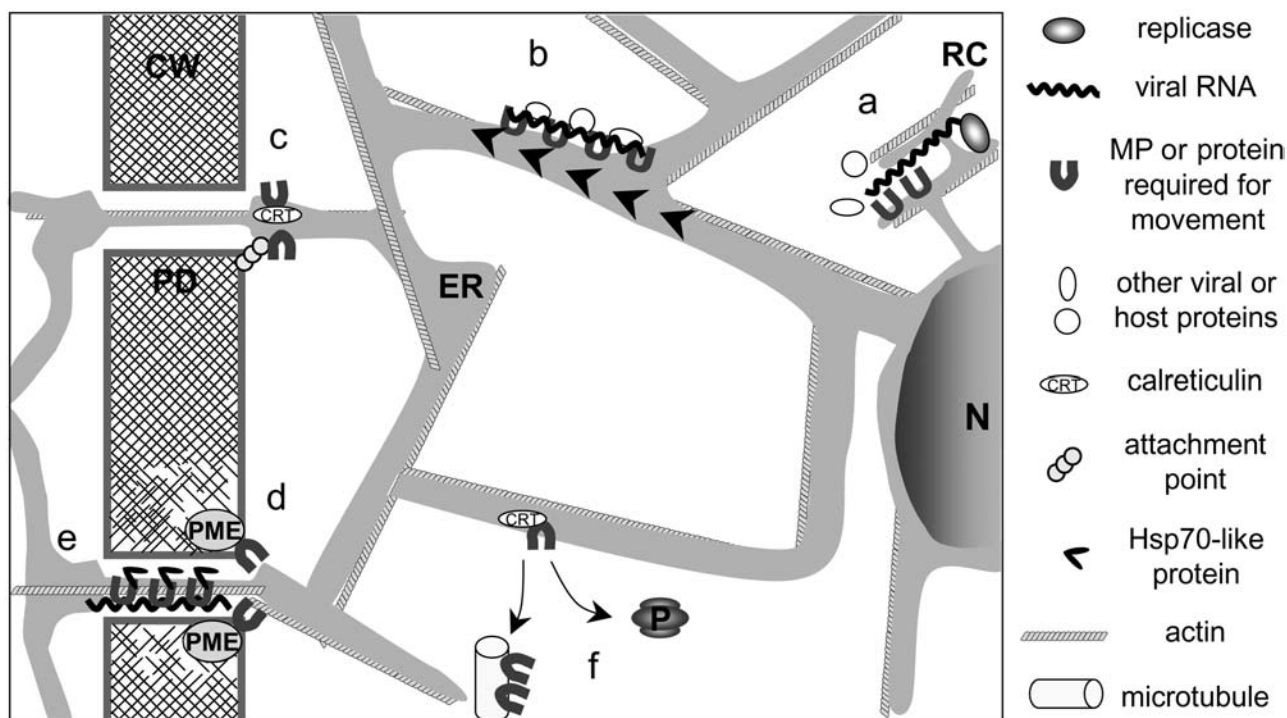
Figure 1. Diagram depicting possible explanations for some of the virus–host interactions with host proteins during movement. Many viruses replicate in replication centers (RC) rich in ER. The viral RNA extending from the replicase is bound by proteins involved in movement, and the MC is assembled with viral and host factors (a). The MC may then move with the rapid flow of the ER membranes (indicated by the chevrons; b). The viral proteins may bind to peripheral markers such as attachment points or PD-targeted proteins such as calreticulin (CRT) when encountered (c). Through associations with cell wall enzymes such as PME, viral-movement-associated proteins may loosen the wall structure (d), and Hsp70-like proteins may translocate the MP-RNA complex through the pore (e). Later in infection, movement-associated proteins are probably targeted for degradation. For TMV, calreticulin may play a role in removing the MP from the ER for degradation by the proteasome (P), with the excess binding to the microtubules (f). N and CW indicate the nucleus and cell wall, respectively.

1995), although this apparent localization could perhaps have been a result of the ER association of the MP. The tomato spotted wilt virus (TSWV) MP has been found to interact with a protein showing homology to myosin and kinesin motor proteins, At-4/1 (von Bargen et al., 2001). A Rab-like protein was found to be associated with the MC of groundnut rosette virus (N. Kalinina and M. Taliansky, personal communication), and the cauliflower mosaic virus MP interacts with a Rab acceptor homolog (Huang et al., 2001). Rab proteins, which are better known as regulators of vesicle fusion events, have been shown to interact with myosins (Pruyne et al., 1998; Schott et al., 1999), and a Drosophila Rab11 homolog was shown to be involved in RNA trafficking (Dollar et al., 2002).

Proteins in the ER membrane flow very rapidly, so it is extremely difficult to photobleach patches of labeled ER, and this flow is directional and dependent on the actin/myosin system (Runions et al., 2005). When the actin is depolymerized, membrane proteins move in a slower diffusive manner (Runions et al., 2005). Therefore, MPs may not require specific interactions with actin to move rapidly through the ER; they may simply be carried with the extremely rapid flow, relying on actin/myosin to maintain the speed of that flow (Figure 1b). In this scenario, they would have the ability to diffuse within the membrane if the actin was disrupted. This may explain why the recovery of fluorescence of TMV MP-GFP in PD after bleaching of labeled PD was not completely abolished by actin depolymerization or myosin motor inhibition (K.M. Wright, N.T. Wood, A.G. Roberts, S. Chapman, P. Boevink, K.M. MacKenzie, and K.J. Oparka, unpublished data).

The MPs of the tubule forming grapevine fanleaf virus (GFLV) and cowpea mosaic virus, which are representative of a large group of viruses that modify PD extensively for movement, were not noted to associate with the ER (Pouwels et al., 2002; Laporte et al., 2003). During tubule formation in PD, the desmotubule is lost; thus, the direct ER connection to PD would be severed. Therefore, despite the fact that GFLV replicates in ER-derived replication centers (Ritzenthaler et al., 2002), the ER would not serve as a direct route to PD once tubule construction had commenced.

An alternative route to PD would be to bind to a protein that was being targeted there by the host secretory pathway, such as the recently identified reversibly glycosylated polypeptide (Sagi et al.,

2005). This has been suggested for TMV MP binding to pectin methylesterase (PME; Jackson, 2000; Oparka, 2004) and could also be suggested for PVX TGB2 and the TGB12K-interacting proteins that interact with β-1,3-glucanase (Fridborg et al., 2003). However, the plasmodesmal targeting abilities of TMV MP and PMTV TGB3 were shown to be disrupted by actin depolymerization but not by inhibition of the secretory pathway; therefore, targeting of these viral proteins in secretory vesicles is unlikely (Haupt et al., 2005; K.M. Wright, N.T. Wood, A.G. Roberts, S. Chapman, P. Boevink, K.M. MacKenzie, and K.J. Oparka, unpublished data). Targeting of the cowpea mosaic virus MP to peripheral foci was not dependent on either the secretory pathway or cytoskeleton (Pouwels et al., 2002), whereas targeting of the GFLV MP was (Laporte et al., 2003), suggesting that there are fundamental mechanistic variations among tubule-forming viruses.

RECOGNITION OF PD OR PERIPHERY

Few plasmodesmal protein-virus MP interactions have been reliably demonstrated. TMV, turnip vein clearing virus, and cauliflower mosaic virus MPs bind PME (Dorokhov et al., 1999; Chen et al., 2000). PMEs were found to be present in the wall around PD and in other regions of the wall in flax and tobacco (Morvan et al., 1998; Chen et al., 2000). Therefore, if PME is a receptor for MPs, then it is likely to be a general peripheral target rather than a PD-specific one. Interactions with PMEs may help the MPs remain at the periphery. The TMV MP also interacts with a cell-wall-associated kinase (Citovsky et al., 1993). The proteins that interact with PVX TGB2 (Fridborg et al., 2003) and the At-4/1 myosin-kinesin-like protein that interacts with TSWV MP (von Bargen et al., 2001) are also potential candidates for PD-associated proteins, but they have not been localized.

Several virus MPs appear to bind to peripheral attachment sites (Heinlein et al., 1998; Huang et al., 2000; Satoh et al., 2000; Pouwels et al., 2002; Laporte et al., 2003). These are points at which the plasma membrane and the ER are anchored to the cell wall. Results from several virus movement studies suggest that the protein complexes associated with these attachment sites are also associated with PD (i.e. that there are attachment points at PD; Figure 1c). Hechtian strands emanate from PD and other attachment points. GFLV MP fused to GFP-labeled peripheral Hechtian attachment sites that also contained calreticulin. However, it is not clear what the GFLV MP binds to at these sites (Laporte et al., 2003). Calreticulin is a ubiquitous ER lumen-located chaperone that has a variety of functions, including the regulation of integrin-mediated cell adhesion in animals (Coppolino et al., 1997). Fluorescently tagged calreticulin has been reported to label the ER, PD, and/or peripheral sites in different studies (Denecke et al., 1995; Baluska et al., 1999, 2001; Torres et al., 2001; Laporte et al., 2003). Turnip crinkle virus

MP p8 interacts with an Arabidopsis (*Arabidopsis thaliana*) Arg-Gly-Asp (RGD)-containing protein Atp8 (Lin and Heaton, 2001). RGD motifs are recognized by plant integrin-like proteins (references in Lin and Heaton, 2001). It would be a convenient convergence of logic if GFLV MP also bound to an RGD-containing protein or calreticulin. An exciting finding is that the TMV MP has been found to bind calreticulin (Chen et al., 2005).

An apparently conserved Tyr-based sorting motif YXXΦ (where Y is Tyr, X is any amino acid, and Φ is an amino acid with a bulky hydrophobic side chain) has been noted in some very different viral MPs. For PMTV TGB3, the motif YQDLN, and in particular the Y residue, was shown to be essential for correct targeting of the protein (Haupt et al., 2005). Targeting of poa semilatent virus TGB3 was disrupted when the distance between the conserved Y and L residues was increased by insertion of four other amino acids (Solovyev et al., 2000). Laporte et al (2003) noted that the YXXΦ motif was conserved among nepovirus MPs, in KNOLLE and other syntaxins, and also in KORRIGAN. YXXΦ motifs are among those recognized by clathrin-coated vesicle adaptors at PM and Golgi in animals (Bonifacino and Lippincott-Schwartz, 2003). The presence of these motifs in viral MPs may therefore indicate involvement of secretory and/or endocytic vesicles rather than being peripheral targeting motifs per se. Recent studies indicate that both GFLV and PMTV may utilize vesicular trafficking (Laporte et al., 2003; Haupt et al., 2005).

MODIFICATION OF PD

Since the first demonstration of the ability of the TMV MP to increase plasmodesmal SELs, a process referred to as gating (Wolf et al., 1989), this has been regarded as a key property of classic MPs. We now know that many MCs are more complex than originally thought, and the various functions required for movement may be carried out by separate proteins. However, gating appears to be a fundamental requirement for cell-to-cell movement of nontubule-forming viruses.

There is not a great deal known about how the plasmodesmal aperture is regulated in the plant. Ding et al. (1996) demonstrated that actin is involved by depolymerizing the actin and measuring a subsequent increase in PD SEL. Callose deposition is known to close PD during defense and wound responses (for review, see Roberts and Oparka, 2003). Several studies indicate that deposition of callose acts as a barrier to virus movement (Wolf et al., 1991; Beffa et al., 1996; Iglesias and Meins, 2000; Bucher et al., 2001). The demonstration of an interaction between PVX TGB2 and proteins that interact with β-1,3-glucanase, a callose degrading enzyme (Fridborg et al., 2003), suggests that one strategy PVX may use to gate PD is to accelerate callose degradation.

It has been proposed that myosin spokes line the plasmodesmal channel, linking actin to the plasma membrane (Overall and Blackman, 1996), and it may be that structural alteration of these spokes or release of the spokes from one of their anchor points alters the SEL (Oparka, 2004). The myosin/kinesin-like protein At-4/1 found to interact with TSWV MP (von Bargen et al., 2001) may be required for trafficking to the PD, but another exciting possibility is that it could be a PD component involved in gating.

The interaction of TMV MP with PME may regulate the activity of PME and thus loosen the cell wall around PD, allowing the PD to open more easily (Figure 1d). With this in mind, an alternative view of the interaction between PME and TMV MP might be that the MP could be recruiting additional PME to the PD in order to assist gating rather than merely relying on PME for targeting. The association of TMV MP with calreticulin (Chen et al., 2005) also has potential regulatory possibilities by influencing local Ca^{2+} concentrations (for a discussion of the regulation of PD by calcium, see Roberts and Oparka, 2003) or by regulating the PD-associated adhesion site structure.

TRANSLOCATION THROUGH THE PORE

Hsp70 family chaperones are involved in many cellular processes (Mayer and Bukau, 2005). Viruses may well exploit Hsp70s in general protein folding (for reviews of Hsp70 chaperone functions, see Bukau and Horwich, 1998; Hartl and Hayer-Hartl, 2002), virion construction (Napuli et al., 2000; Satyanarayana et al., 2000; Alzhanova et al., 2001), and perhaps also regulation of host defenses (Kanzaki et al., 2003) either directly or indirectly through interactions with J-domain proteins.

The demonstration of PD trafficking of Hsp70-class proteins from the phloem (Aoki et al., 2002) and the fact that Hsp70s have motor activities that drive protein translocation (Pilon and Schekman, 1999; Voisine et al., 1999) suggest that Hsp70 proteins may have the ability to actively translocate viral MCs through PD pores (Figure 1e).

Closteroviruses are the only known group to encode their own Hsp70 homologs, and these proteins have been shown to be MPs (Peremyslov et al., 1999; Alzhanova et al., 2001). The beet yellows closterovirus Hsp70 homolog could also function as a MP for TGB viruses from both classes of TGB viruses (Agranovsky et al., 1998). The interaction of PMTV TGB2 with a J-domain protein may therefore play a role in translocation through PD in addition to the proposed role in protein recycling (Haupt et al., 2005). The MPs of viruses unrelated to closteroviruses have also been shown to interact with J-domain proteins (Soellick et al., 2000; von Bargen et al., 2001), suggesting a conserved role for Hsp70 in plant virus cell-to-cell movement.

REGULATION OF MOVEMENT

It is unlikely that the observed regulation of viral MPs is entirely due to host defense responses, as it is in the interests of a virus to minimize the damage to its host. Thus, viruses may collude in the down-regulation of their movement functions. The efficient down-regulation or removal of viral MP would ensure that the disruption of PD SEL and hence of signal and nutrient flow does not continue ad infinitum. The TMV MP is rapidly degraded about six cells away from the leading edge of a viral infection site, forming the classic halo pattern observed when the MP is fused to fluorescent proteins (Szecsi et al., 1999). The 26S proteasome has been demonstrated to be involved in MP degradation, and this was specifically suggested to be a damage limitation activity (Reichel and Beachy, 2000). The gating of PD in TMV infection was found to be limited to the leading edge even though the PD of cells in the centers of infection foci were labeled with the MP-GFP (Oparka et al., 1997). This indicated that the MP must be rendered nonfunctional in the PD of cells in the central region. Phosphorylation of the TMV MP has been demonstrated to down-regulate its gating ability (Waigmann et al., 2000; Trutnyeva et al., 2005), and it has been shown to be phosphorylated by a cell-wall-associated kinase (Citovsky et al., 1993). A putative PD kinase may therefore phosphorylate the MP to inactivate it. Alternatively, or in addition, insertion of the MP into the cavities of branched PD (Ding et al., 1992) may remove it from its active site in the PD.

Two proteins shown to interact with TMV MP, the microtubule-associated protein MPB2C (Kragler et al., 2003) and calreticulin (Chen et al., 2005), were found to inhibit cell-to-cell movement when overexpressed. Calreticulin overexpression also increased the amount of MP targeted to microtubules. Calreticulin is a chaperone that plays a role in removal of misfolded proteins from the ER for degradation by the proteasome (for review, see Michalak et al., 1999); therefore, it may help to remove excess MP from the ER membrane (Figure 1f), an activity that may have been exaggerated by its overexpression. The increased labeling of microtubules by the released TMV MP may simply be a result of higher concentrations of MP in the cytoplasm and the high affinity of the MP for microtubules (Boyko et al., 2000), or it may signify an involvement of microtubules in MP degradation as we have previously suggested (Gillespie et al., 2002).

PMTV TGB2 was found to associate with vesicle-like structures and interact with an RME8 homolog, a J-domain protein involved in endocytosis (Haupt et al., 2005). TGB3 was only found in vesicle structures when coexpressed with TGB2. Endocytic recycling of TGB2 and 3 may regulate the viral movement in a number of ways. TGB2 may remove TGB3 from PD, thus preventing permanent gating of the pore and reducing cytopathic effects of infection. At later stages of infection, the recycling of TGB2 and 3 may inhibit further movement by increasing the concentrations of these

proteins (Yang et al., 2000); conversely, early in infection, the recycling may assist movement by increasing the quantities of TGB2 and 3 available to form MCs.

CONCLUSION

Although viruses are capable of enormous variation, their cell-to-cell movement strategies are necessarily limited by the cellular equipment available for them to exploit and by the fact that all viruses studied to date must pass through PD. Some of the interactions between viral and host proteins may be explained in several ways, suggesting that these interactions may be multifunctional. The indications of common themes arising from viral movement studies imply that there has been convergent evolution of viral cell-to-cell movement mechanisms. The convergence of data suggests that we may be uncovering the fundamental cellular processes involved in macromolecular trafficking such as those that may be used by non-cell autonomous host proteins like KNOTTED1 (Lucas et al., 1995; Kim et al., 2002, 2005).

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