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Plant viral movement proteins: Agents for cell-to-cell trafficking of viral genomes

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Abstract

Plants viruses spread throughout their hosts using a number of pathways, the most common being movement cell to cell through plasmodesmata (PD), unique intercellular organelles of the plant kingdom, and between organs by means of the vascular system. Pioneering studies on plant viruses revealed that PD allow the cell-to-cell trafficking of virally encoded proteins, termed the movement proteins (MPs). This non-cell-autonomous protein (NCAP) pathway is similarly employed by the host to traffic macromolecules. Viral MPs bind RNA/DNA in a sequence nonspecific manner to form nucleoprotein complexes (NPC). Host proteins are then involved in the delivery of MPs and NPC to the PD orifice, and a role for the cytoskeleton has been implicated. Trafficking of NCAPs through the PD structure involves three steps in which the MP: (a) interacts with a putative PD docking complex, (b) induces dilation in the PD microchannels, and (c) binds to an internal translocation system for delivery into the neighboring cytoplasm. Viral genera that use this NCAP pathway have evolved a combination of a MP and ancillary proteins that work in concert to enable the formation of a stable NPC that can compete with endogenous NCAPs for the PD trafficking machinery. Incompatible MP–host protein interactions may underlie observed tissue tropisms and restricted infection domains. These pivotal discoveries are discussed in terms of the need to develop a more comprehensive understanding of the (a) three-dimensional structure of MPs, (b) PD supramolecular complex, and (c) host proteins involved in this cell-to-cell trafficking process.

Keywords: Cell-to-cell movement; Chaperone; Host factors; Local viral infection; Non-cell-autonomous proteins; Plasmodesmata; Viral movement proteins; Viral genome

Contents

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Introduction

Plant viruses spread throughout their hosts using a number of pathways, the most common being movement cell to cell through plasmodesmata, unique intercellular organelles of the plant kingdom (Ding, 1998; Lucas, 1995; Oparka, 2004), and between organs (i.e., systemic infection) by means of the vascular system and, more specifically, the sieve tube system of the phloem (Gilbertson and Lucas, 1996; Lucas et al., 2001; Oparka and Santa Cruz, 2000; van Bel, 2003). Pioneering ultrastructural studies, performed by Esau and others (Esau, 1968 and references therein), detected virus particles located within both modified plasmodesmata and mature, functional, sieve elements. Based on these collective studies, the concept emerged that plant viruses move their genomic material between cells in an encapsidated form, protected by the capsid protein (CP).

A limitation to these early works was the fact that molecular tools for identification of viral proteins were not yet available. Thus, detection of infected cells had to be based on the presence of virus particles (or inclusion bodies representing aggregates of particles), which are often produced rather late in the viral life cycle. This was particularly important as the presence of virus particles, lodged within highly modified plasmodesmata, may have resulted from cytological damage caused by the infection process, per se, rather than reflecting the actual form in which the virus had moved into neighboring uninfected cells. Research over the past two decades has led to the identification of the basic molecular steps underlying this process of viral cell-to-cell movement. The emerging picture is that, during the course of plant viral evolution, a number of mechanisms developed to allow the viral genome to cross the barrier of the host cell wall. In essence, two basic strategies are now well documented: one in which the viral genome moves as particles and another where the viral nucleic acids are trafficked through plasmodesmata in the form of ribonucleoprotein (RNP) complexes. In this review, we will discuss the identification and characterization of the viral and host proteins involved in the cell-to-cell trafficking of RNP complexes. Further, our attention will be focused on the cell biology of viral movement proteins in vRNP complex trafficking; extensive reviews on movement protein evolution have been dealt with elsewhere (Melcher, 2000; Morozov and Solovyev, 2003; Rojas et al., 2005; Waigmann et al., 2004).

Movement capacity encoded by the viral genome

An elegant series of experiments, performed on temperature-sensitive (*ts*) mutants of *Tobacco mosaic virus* (TMV), established that the capacity for viral replication, in protoplasts, was unaffected at restrictive temperatures but, under these same conditions, TMV was confined to sites of inoculation in plant tissues (Nishiguchi et al., 1978). Subsequently, peptide analysis indicated that a mutation in the TMV genome, in an ORF encoding a 30-kDa protein, was likely responsible for the observed *ts* capacity for virus spread (Leonard and Zaitlin, 1982). Support for this hypothesis was provided by inoculation of the TMV *ts* mutant onto transgenic tobacco plants producing the wild-type form of the 30-kDa protein (Deom et al., 1987). Here, under restrictive temperatures, the *ts* mutant was able to spread within the inoculated tissues and develop a systemic infection. Reciprocal experiments, in which either the ORF for the wild-type 30-kDa protein was replaced by the *ts* mutant form, or mutations were engineered into the wild-type 30-kDa ORF, blocked the capacity for TMV cell-to-cell movement (Meshi et al., 1987). These studies indicated that the TMV genome encodes a non-structural 30-kDa protein that is necessary and sufficient for local spread of this virus. Although no direct evidence was available as to whether or not the TMV 30-kDa protein was actually transported from the infected cell into neighboring cells, it became known as the transport or "movement" protein (MP) (Atabekov and Dorokhov, 1984).

TMV MP interacts with plasmodesmata and RNA

The general consensus of the day was that TMV, like other viruses, would move through plasmodesmata as virions (Esau, 1968; Gibbs, 1976; Weintraub et al., 1976). Under this scenario, its 30-kDa MP would be expected to interact, in some manner, with individual plasmodesmata to facilitate the transport of the virus particle. Evidence in support of this notion was provided by immunolocalization studies in which the TMV MP was detected in mesophyll plasmodesmata located within infected tissues (Tomenius et al., 1987). Furthermore, expression of the TMV MP in transgenic tobacco lines resulted in a modification to the normal properties of PD (Fig. 1). Microinjection experiments revealed that, in the presence of TMV MP, the molecular size exclusion limit (SEL) of PD was increased from approximately 1 kDa in control plants to greater than 10 kDa in TMV MP transgenic lines (Wolf et al., 1989). Even though this was a significant increase in PD SEL and these studies provided direct proof that a viral MP could interact with the host PD, the value of 10 kDa was considerably lower than that which would have been expected if the MP modified PD to allow the passage of virus particles (rigid rod-shaped virion: diameter 18 nm, length 300 nm).

A partial resolution to this conundrum was apparent from the finding that TMV mutants, lacking the CP, could still infect the inoculated leaves (Dawson et al., 1988; Holt and Beachy,

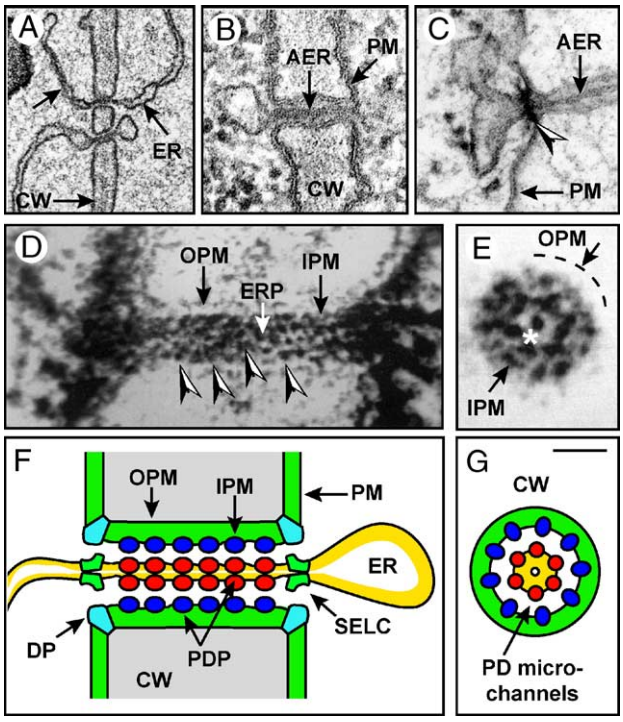

Fig. 1. Plasmodesmata represent special intercellular organelles that establish cytoplasmic and endomembrane (endoplasmic reticulum [ER] and nuclear envelope) continuity between neighboring cells. (A–E) Electron micrographs illustrating the general ultrastructural features of primary plasmodesmata developed across newly formed cell walls; (A–D, median longitudinal sections; E, transverse section). (A and B) Each plasmodesma exists as a discrete plasma membrane (PM)-lined intercellular cytoplasmic channel, in which the central region is occupied by an appressed form of the ER (also referred to as the desmotubule). Note the continuity of the ER between the neighboring cells. The space between the plasma membrane (PM) and the ER is termed the cytoplasmic annulus (CA). (C) Neck region, or orifice, of a PD illustrating the presence of electron dense material (dart) at the entrance to the cytoplasmic annulus. (D and E) Computer-enhanced high-resolution freeze-substituted images of primary PD. (D) PD in longitudinal section illustrating the presence of proteins (electron dense material; darts) located in the neck region and along the cytoplasmic annulus. (E) PD in transverse section; note the presence of proteins embedded in the inner plasma membrane (IPM) and outer ER leaflets (*). Broken line represents the location of the surface of the outer plasma membrane (OPM) leaflet. (F and G) Schematic representation of a PD in median longitudinal (F) and transverse section (G) showing the arrangement of the plasma membrane, ER, and cytoplasmic annulus formed by the continuity of these two membrane systems across the cell wall. Note that the PD proteins divide the cytoplasmic annulus into discrete microchannels that act as molecular sieves, allowing small metabolites and ions to diffuse between neighboring cells. This unique form of cytoplasmic continuity is termed the symplasm (Robards and Lucas, 1990). Plasmodesmata having equivalent features can be inserted across existing cell walls; these are termed secondary PD (Lucas et al., 1993a). Micrographs are from Lucas et al. (1993a, 1993b). Abbreviations are as follows: AER, appressed endoplasmic reticulum; CW, cell wall; DP, docking protein; ER, endoplasmic reticulum; ERP, ER proteins; IPM, inner plasma membrane leaflet; OPM, outer plasma membrane leaflet; PDP, plasmodesmal proteins; PM, plasma membrane; SELC, size exclusion limit complex. Scale bar in panel G, 25 μm.

1991): hence, cell-to-cell movement was unlikely to occur through the trafficking of TMV particles. However, the naked folded TMV RNA molecule is presumed to have a diameter on the order of 10 nm (Gibbs, 1976). Hence, it appeared unlikely that this viral RNA would be able to "diffuse" through PD microchannels, unless of course the PD structure was significantly modified during the early stages of infection. The discovery that the TMV MP was able to bind, in a cooperative but sequence nonspecific manner, to single-stranded nucleic acids (both RNA and DNA) provided the next clue as to the mechanism underlying movement of the infectious agent (Citovsky et al., 1990, 1992).

Insight into the physical structure of the RNP complex, formed between the TMV MP and the infectious RNA, was provided by transmission electron microscopy studies. In the presence of TMV MP, RNA molecules become coated and extended into long thread-like structures having diameters of approximately 2 nm (Citovsky et al., 1992). Atomic force microscopy has also been used to confirm the physical properties of TMV MP and other viral MP RNP complexes as having diameters in the range of 1.5–3.5 nm (Andreev et al., 2004; Kiselyova et al., 2001). The 2–3 nm diameter of the TMV MPvRNA complex is close to the dimensions of the dilated PD microchannels, those being on the order of 3–4 nm (Lucas et al., 1995; Oparka et al., 1999; Wolf et al., 1989). Thus, these findings were consistent with the TMV MP mediating the binding and trafficking of the infectious vRNP complex to and through PD.

Direct support for this hypothesis was provided by parallel experiments being performed on other viral MPs, in which fluorescently labeled recombinant MP was shown to move through PD along with fluorescently labeled RNA/DNA when co-injected into mesophyll cells (Fujiwara et al., 1993; Noueiry et al., 1994; Ding et al., 1995; Rojas et al., 1997; Lough et al., 1998). The capacity of the TMV MP to interact both with single-stranded RNA and PD/host proteins was established through the engineering of specific mutations in the MP ORF. A high correlation was observed between the capacity of these MP mutants to: (a) associate with PD, (b) increase PD SEL, and (c) traffic progeny viral RNA cell to cell (Berna et al., 1991; Lapidot et al., 1993; Waigmann et al., 1994): amino acid residues 3–5 and 195–213 were shown to be essential for MP localization to secondary PD (Ding et al., 1992), and amino acid residues 126–224 were responsible for increasing PD SEL. Two RNA-binding domains, located at residues 112–185 and 186–268, were also identified in the TMV MP (Citovsky et al., 1992).

In summary, the established functional characteristics of the TMV 30-kDa MP are as follows: it (a) binds single-stranded RNA in a sequence nonspecific manner to form a stable RNP complex; (b) interacts with the host PD machinery to induce an increase in the SEL; (c) gates PD at the leading edge of TMV infection (Oparka et al., 1997); (d) is present within the PD structure; and (e) mediates the cell-to-cell trafficking of itself and viral progeny RNA, leading to the infection of neighboring cells. These features provide an operational definition of a viral MP and are illustrated in Fig. 2.

Movement proteins are a general feature of many plant viruses

The pioneering studies conducted on TMV gave rise to similar investigations on other plant viruses, and it soon became apparent that MPs are a general feature of plant viral

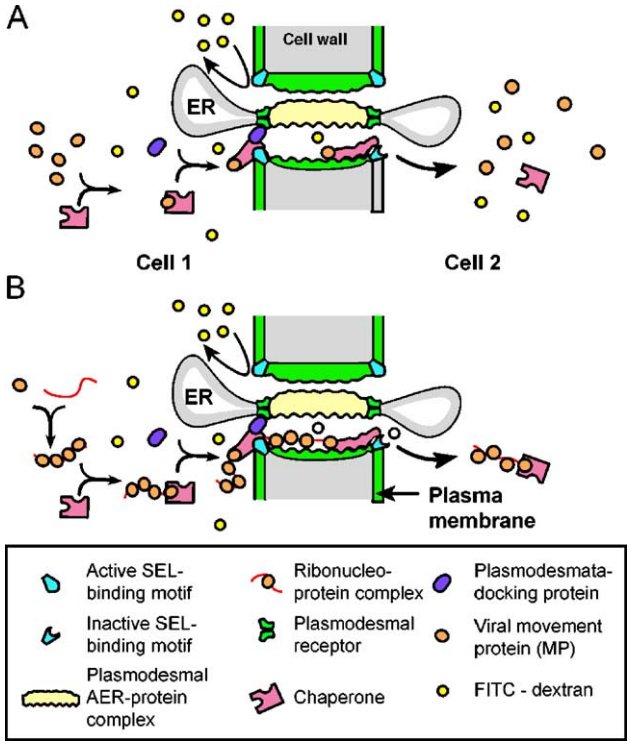

Fig. 2. Viral group 1 MPs interacts with PD to mediate the spread of MP–vRNA complexes. Model illustrating the steps thought to be involved in cell-to-cell trafficking of MPs and vRNP complexes. (A) MP binds to a host chaperone, and this complex then interacts with a PD docking protein which activates the SEL machinery. This step utilizes a structural motif in the MP rather than a conserved sequence for PD targeting. The increase in PD SEL allows the MP to engage the PD translocation machinery for movement into the neighboring cells; in this dilated state, the PD microchannels also allow the co-diffusion of 10–20-kDa fluorescein-isothiocyanate-labeled dextrans (FITC-dextrans). (B) MP–vRNA complexes interact with the same cellular constituents to traffic through PD.

genomes (Carrington, 1996; Lucas and Gilbertson, 1994; Maule, 1991). However, the range of complexity, in terms of the number and type of ancillary viral proteins required for RNP complex formation and movement, surely reflects the divergent evolutionary pathways taken by the wide range of plant-infecting viruses. Based on these extensive studies, the movement machinery required for cell-to-cell spread of vRNA/DNA, in the form of nucleoprotein complexes, can be divided into at least five basic operational groups. Representative viruses for each group and their MP and ancillary proteins are shown in Table 1. This arrangement is not meant to reflect evolutionary lineages (Koonin and Dolja, 1993; Melcher, 2000; Mushegian and Koonin, 1993), but, rather, it was developed to illustrate the increasing complexity displayed by plant viruses with respect to the identity and number of viral proteins essential for cell-to-cell movement.

Viral trafficking systems that require only the MP

Members of the *Tobamovirus*, *Dianthovirus*, and *Ubravirus* genera (group 1) encode a single MP gene with characteristic properties associated with MP function; however, each MP displays its own unique properties. For example, whereas the TMV MP and RCNMV MP bind RNA cooperatively, the GRV ORF4 binds non-cooperatively (Nurkiyanova et al., 2001). In contrast to the TMV MP, the RCNMV MP does not form an extended thread-like RNP complex; rather, its RNP complex is compacted (Fujiwara et al., 1993). Such significant differences may well reflect aspects of the host cellular machinery that each particular virus has hijacked for its delivery to and movement through PD.

Viral systems that can require two proteins for local movement

The *Bromovirus* and *Cucumovirus* genera represent an interesting situation in which cell-to-cell spread of infection always requires a MP; however, in some, but not all cases, the CP is also necessary for trafficking through PD. In such cases, the CP serves some form of ancillary role to the MP (Table 1, group 2). An excellent illustration of this situation is provided by studies performed on docot- (CCMV) and monocot-adapted (BMV) bromoviruses. Cell-to-cell movement of CCMV only requires the 3a MP as CP mutants move as wild-type virus (Rao, 1997). However, for BMV, both the MP and CP are required (Mise and Ahlquist, 1995; Schmitz and Rao, 1996). Recombinant CCMV, in which the 3a MP was replaced by that of BMV (CCMV [B3a]), was still able to undergo limited cell-to-cell movement (Mise and Ahlquist, 1995), but deletion of the CP in this recombinant background blocked infection (Sasaki et al., 2003). Interestingly, specific mutations, engineered within the central region of the BMV 3a MP, restored wild-type infectivity to both a BMV-ΔCP deletion mutant and CCMV(B3a) (Sasaki et al., 2005). These experiments suggest that the requirement for the BMV CP may be involved in some form of host response rather than being directly involved in the actual mechanism of cell-to-cell trafficking of the bromovirus vRNP complex.

The situation with respect to CMV is rather interesting as genetic studies indicated that both the 3a MP and the CP are

Table 1
Viral encoded proteins required for cell-to-cell movement of infectious RNA/DNA

Group	Genus	Virus	MP(s)	Ancillary proteins
1	*Tobamovirus*	TMV	30 kDa	–
	Dianthovirus	RCNMV	35 kDa	–
	Umbravirus	GRV	ORF4	–
2	*Bromovirus*	CCMV	3a	–
		BMV	3a	CP
	Cucumovirus	CMV	3a	CP
3	*Begomovirus*	BDMV/SLCV	BC1	BV1
4	*Potyvirus*	TEV/BCMNV/LMV	HC-Pro + CP	CI
	Hordei-like viruses	BSMV	TGBp1	TGBp2 + TGBp3
5	*Potex-like* viruses	PVX/WClMV	TGBp1	TGBp2 + TGBp3 + CP

Abbreviations are as follows: BCMNV, *Bean common mosaic necrosis virus*; BDMV, *Bean dwarf mosaic virus*; BMV, *Brome mosaic virus*; BSMV, *Barley stripe mosaic virus*; CCMV, *Cowpea chlorotic mottle virus*; CMV, *Cucumber mosaic virus*; GRV, *Groundnut rosette virus*; LMV, *Lettuce mosaic virus*; PVX, *Potato virus X*; RCNMV, *Red clover necrotic mosaic virus*; SLCV, *Squash leaf curl virus*; TEV, *Tobacco etch virus*; TMV, *Tobacco mosaic virus*; WClMV, *White clover mosaic virus*; CP, capsid protein; CI, cylindrical inclusion protein.

necessary for local infection (Suzuki et al., 1991; Canto et al., 1997; Kaplan et al., 1998). In contrast, microinjection of recombinant CMV 3a MP into mesophyll cells resulted in both an increase in PD SEL and cell-to-cell movement of fluorescently labeled MP, along with the co-injected CMV RNA (Ding et al., 1995). In agreement with microinjection studies, transient expression of a CMV 3a:GFP or 3a:GUS fusion protein resulted in movement into neighboring epidermal cells; a 3a MP mutant failed to move with both approaches (Ding et al., 1995; Itaya et al., 1997). Furthermore, when expressed in transgenic plants, the 3a MP could mediate the immediate cell-to-cell trafficking of injected fluorescently labeled CMV RNA; no such movement was detected in wild-type plants. These findings are consistent with viral movement being in the form of a 3a MP–vRNA complex, a conclusion supported by mutations in the CP demonstrating that virion formation was not a prerequisite for cell-to-cell movement (Kaplan et al., 1998). Thus, it is possible that the 3a MP:vRNA ratio used in the microinjection experiments allowed for the introduction of stable RNP complexes, whereas, during viral infection, the CP may be required to work in concert with the 3a MP to form such stable vRNP complexes for delivery to PD.

Evidence in support of this hypothesis was gained by a combination of RNA-binding and CMV 3a MP mutation experiments. First, the 3a MP does not form as stable an RNP complex as the TMV MP: additionally, not all regions of the 3a MP–RNA complex are RNase protected (Li and Palukaitis, 1996). Second, deletion of 33 amino acids from the C-terminus of the 3a MP (3a MPΔC) allowed for CP-independent CMV infection (Nagano et al., 2001). Third, an elegant series of experiments performed using atomic force microscopy revealed that the CMV 3a MPΔC bound more strongly to RNA than did wild-type MP; the unbinding forces for the 3a MPΔC were double those for wild-type MP (Kim et al., 2004a; Andreev et al., 2004). This increase in binding affinity would likely result in the formation of stabilized and hence movement-competent 3a MPΔC–vRNP complexes without involvement of the CP. Fourth, replacement of the CMV 3a MP by the GRV ORF4 enabled cell-to-cell movement of this engineered CMV, regardless of the presence or absence of its CP (Nurkiyanova et al., 2001); that is, the CP is dispensable in the presence of an MP having high RNA-binding affinity.

These studies provide insights both into the dynamic aspects of MP–RNA interaction that must take place in the infected cell and the selection forces that drove viral evolution towards the formation of stable, and movement-competent, infectious complexes. Clearly, in the case of CMV, the RNA-binding capacity of the CP was used to complement the 3a MP in forming an RNase protected vRNP complex.

Begomoviruses utilize an MP plus a nuclear shuttle protein

Plant DNA viruses can be separated based on their genomes and the mechanism employed for cell-to-cell movement; those with monopartite, double-stranded genomes are passaged as virus particles through tubular structures that cross the cell wall (Hull, 2002; Rojas et al., 2005). The DNA viruses having single-stranded, and often multipartite, genomes encode a protein that displays all the hallmarks of the RNA virus MP (Table 1, group 3). Here, the BC1 MP of the bipartite begomoviruses (family Geminiviridae) has been extensively studied (Rojas et al., 2005). This MP is rather unique as it binds cooperatively to DNA, but not RNA, in a form- and size-specific manner, reflecting the replicative state of the virus (i.e., ds-DNA) and the genome size (2.5–3.0 kb) (Noueiry et al., 1994; Rojas et al., 1998). DNA viruses replicate in the nucleus, and the export of progeny DNA (both ss- and ds-DNA) into the cytoplasm is mediated by BV1, a nuclear shuttle protein (NSP) (Noueiry et al., 1994; Sanderfoot and Lazarowitz, 1995). An interaction between BV1 and BC1 is thought to be required for the transfer of the DNA into a BC1-associated complex that can then be trafficked through the PD (Sanderfoot and Lazarowitz, 1996). This combination of MP (BC1) and the ancillary NSP (BV1) appears to be essential for the begomoviruses to exploit the endogenous RNA trafficking system of their hosts (Gilbertson et al., 2003).

Multiple genes are necessary for movement in other viral genera

A number of RNA viruses require an MP and two additional proteins for effective cell-to-cell spread of their vRNA (Table 1, group 4). In contrast to other plant viruses, members of the *Potyvirus* genus do not appear to have evolved a dedicated ORF to encode an MP. This is interesting as the *Potyvirus* is the largest genus of plant viruses (Revers et al., 1999). Rather, they co-opted existing proteins to fulfil this essential function. Genetic studies conducted on TEV established that the CP plays a role in cell-to-cell and long-distance movement (Dolja et al., 1994, 1995) and that the helper component-proteinase (HC-Pro; required for processing the polyprotein produced from the potyvirus genome) is involved in some way with systemic infection (Cronin et al., 1995; Kasschau et al., 1997). Microinjection studies performed with proteins encoded by two other potyviruses, BCMNV and LMV, revealed that both the CP and HC-Pro can (a) induce an increase in PD SEL, (b) traffic through PD, and (c) facilitate the cell-to-cell movement of vRNA (Rojas et al., 1997). Hence, these two multifunctional proteins must have also acquired the characteristics associated with MPs. Interestingly, the BCMNV HC-Pro was more effective than the CP at inducing an increase in PD SEL and trafficking cell to cell. Finally, these studies indicated that HC-Pro and the CP appear to act cooperatively in mediating the trafficking of vRNA, a finding also supported by protein–protein interaction experiments (Roudet-Tavert et al., 2002).

All other BCMNV proteins, including P1, CI, NIa and NIb, were unable to interact with and change the properties of PD (Rojas et al., 1997). This is of interest as the CI protein, an RNA helicase, is required for effective cell-to-cell spread of infection (Carrington et al., 1998). In this regard, it is noteworthy that the CI protein can be detected over the PD orifice only during the early stages of infection (Roberts et al.,

1998). Thus, this potyviral CI protein likely functions as an ancillary agent in the movement process, perhaps by facilitating the delivery and alignment at the PD pore of an HC-Pro-CP–vRNA complex (Rojas et al., 1997; Carrington et al., 1998).

A very different system is used by viruses that carry a specialized, evolutionarily conserved triple gene block (TGB) encoding for the TGBp1, TGBp2, and TGBp3, all of which are essential for viral cell-to-cell movement (Herzog et al., 1998; Lawrence and Jackson, 2001; Morozov and Solovyev, 2003). The TGBp1 of the Hordei-like virus movement system contains multiple RNA-binding sites (Donald et al., 1997) and exhibits strong, salt-resistant, cooperative binding activity (Cowan et al., 2002). These properties may account, in part, for the fact that viruses in this group do not require the CP for cell-to-cell movement (Quillet et al., 1989; Petty et al., 1990; Lawrence and Jackson, 2001). Consistent with this notion, TGBp1–vRNA complexes could be isolated from infected tissues (Brakke et al., 1988). The Hordei-like virus TGBp1 also displays ATP-dependent helicase activity (Kalinina et al., 2002), and mutations in the helicase domain established that this function is essential for cell-to-cell movement (Lawrence and Jackson, 2001; Zamyatnin et al., 2004). An interesting question that is yet to be resolved is whether this helicase activity, associated with TGBp1, performs a similar function to that of the potyviral CI protein.

An important operational characteristic of the Hordei-like virus TGBp1 is that, by itself, it does not appear to have the ability to modify PD SEL nor to mediate its own cell-to-cell movement (Morozov and Solovyev, 2003). It would therefore appear that the two ancillary proteins, namely, TGBp2 and TGBp3, are absolutely essential for intracellular delivery of the TGBp1–vRNA complex to PD. In support of this hypothesis, immunogold labeling identified TGBp1 in the PD of infected cells; however, in transgenic plants expressing TGBp1, alone, no immunolabeling of PD could be detected until leaves were infected with virus (Erhardt et al., 1999, 2000). Similar results were obtained in TGB:GFP experiments; here, both the TGBp2 and TGBp3 had to be expressed in the same cell in order for TGBp1-GFP to be delivered to and move through PD (Erhardt et al., 2000; Zamyatnin et al., 2004).

Further insight into the operation of a highly complex interrelationship between the Hordeivirus-like TGB proteins is provided by the finding that they cannot be substituted, individually, by other viral TGB proteins (Lauber et al., 1998). Thus, cell-to-cell movement by members of the Hordei-like viruses must be achieved through a highly specific interaction among the cognate TGB proteins. In support of this concept, the TGBp2 and TGBp3 both appear to be located in close proximity to PD (Gorshkova et al., 2003; Haupt et al., 2005).

The Potex-like viruses (Table 1, group 5) also encode this conserved TGB which is essential for their cell-to-cell movement (Beck et al., 1991). But, in addition, viruses within this group have an absolute requirement for the cognate CP (Forster et al., 1992; Oparka et al., 1996; Lough et al., 2000); thus the Potex-like viruses require four viral-encoded proteins for the cell-to-cell movement of the progeny RNA. In contrast to the situation with the Hordei-like viruses, numerous studies have documented the ability of the Potex-like TGBp1 to induce an increase in PD SEL as well as to mediate its own cell-to-cell movement (Angell et al., 1996; Lough et al., 1998; Yang et al., 2000; Howard et al., 2004).

These MP properties of TGBp1 are exhibited when the protein is introduced into wild-type plants, either by microinjection or biolistic bombardment techniques. Thus, in the absence of the ancillary proteins, TGBp2, TGBp3, and CP, the TGBp1 can move to and through PD. In general, the TGBp2 and TGBp3 appear to function as cell-autonomous agents that do not directly affect PD SEL (Lough et al., 1998). However, under some conditions and in certain hosts, these proteins may also influence PD SEL (Tamai and Meshi, 2001) or act non-cell-autonomously (Krishnamurthy et al., 2002; Verchot-Lubicz, 2005). In any event, most studies support the notion that the TGBp2 and TGBp3, as membrane-associated proteins (Morozov and Solovyev, 2003), function in the delivery of the infectious agent to PD for entry into uninfected cells.

Two models have been proposed to explain the requirement for the Potex-like viral CP in local infection (Forster et al., 1992; Baulcombe et al., 1995): genome movement through PD as (a) filamentous virions (Santa Cruz et al., 1998) or (b) a TGBp1–vRNA-CP complex (Lough et al., 1998, 2000). The fact that the PVX CP is targeted to PD and that it cannot induce an increase in SEL nor move cell to cell, when expressed alone (Oparka et al., 1996; Santa Cruz et al., 1998), is consistent with either model. The strongest evidence in support of the virion model is derived from the detection of fibrillar material, in PD of PVX-infected tissues, that is immunoreactive with virion-specific antisera (Santa Cruz et al., 1998). Also consistent with virion movement is the finding that the PVX CP can be co-translocated with the infectious material.

The case for vRNP trafficking is based on a number of lines of evidence. First, specific WClMV CP mutants could still form virions but were unable to move out of the inoculated cells (Forster et al., 1992). Next, an extensive series of microinjection experiments was performed with transgenic plants expressing all possible combinations of WClMV TGB proteins and the CP. The capacity of fluorescently labeled TGBp1 to traffic through PD was equivalent in transgenic plant cells expressing the CP, TGBp2, TGBp3, TGBp2 + TGBp3, and TGBp2 + TGBp3 + CP (Lough et al., 1998). However, this movement capacity was significantly impaired when labeled infectious WClMV RNA was co-injected, along with TGBp1, into all transgenic tissues except for those expressing the combination of TGBp2 + TGBp3 + CP. In addition, mutations engineered into the recombinant WClMV TGBp1 could inhibit the trafficking of both the protein and the fluorescently labeled vRNA.

A combination of microinjection and dual-channel confocal laser scanning microscopy (CLSM) provided direct evidence that labeled vRNA and TGBp1 can move, together, into neighboring cells (Lough et al., 1998). In addition, mutant analysis of the 5′ region of the WClMV genome identified an approximately 100 bp region that, when engineered onto a

heterologous gene such as GFP, mediated cell-to-cell transport of the chimeric transcript (T.L. Lough and W.J. Lucas, unpublished results). Lastly, an equivalent series of experiments to those performed with WClMV, conducted with PVX by using a transient expression approach, established that the PVX TGBp1 and CP, but not TGBp2 or TGBp3, moved between cells with viral RNA (Lough et al., 2000). In addition, encapsidation and cell-to-cell movement functions of the CP were demonstrated to be separable events.

The preponderance of evidence is fully consistent with the Potex-like TGBp1 functioning as the MP in mediating the cell-to-cell trafficking of an infectious TGBp1–vRNA-CP complex. This mode of action would be in line with the MPs that have been characterized for viruses in groups 1–4 (Table 1). Now that we have established the nature and properties of the viral encoded proteins required for local infection, it is time to turn our attention to the plant host to discover the cellular mechanisms underlying this cell-to-cell trafficking of macromolecular complexes.

Trafficking of endogenous non-cell-autonomous proteins and RNP complexes

The seminal discovery that viral MPs have the capacity to mediate the trafficking of proteins and vRNP complexes through the host PD raises the obvious question as to whether or not plants similarly use this pathway for the trafficking of macromolecules (Lucas et al., 1993a). A number of genetic lines of evidence suggested that certain aspects of plant development were indeed under non-cell-autonomous control (Carpenter and Coen, 1995; Lucas, 1995; Szymkowiak and Sussex, 1992; Huala and Sussex, 1993), but the active agents were generally considered to be phytohormones.

The first experimental evidence that plant proteins could traffic through PD was gained from studies conducted on *KNOTTED1* (*KN1*), a homeodomain transcription factor known to be involved in controlling cell fate in the plant meristem (Jackson and Hake, 1997). Microinjection experiments performed with recombinant KN1 revealed that this plant protein has functional properties almost identical to those of viral MPs (Lucas et al., 1995). Introduction of KN1 into cells resulted in an increase in PD SEL and the rapid cell-to-cell movement of the fluorescently labeled protein. Interestingly, KN1 was also able to mediate the cell-to-cell trafficking of mRNA, however, in contrast to viral MPs, KN1 could traffic only its own sense mRNA. Although these KN1 microinjection results were initially viewed with some level of skepticism, these findings have now been confirmed by a range of studies demonstrating cell-to-cell movement of KN1:GFP and the ability to traffic its RNA (Jackson, 2002; Kim et al., 2003, 2005).

The fundament difference in sequence specificity observed between KN1 and the viral MPs is probably indicative of the requirements associated with meristem development versus viral infection. The general lack of sequence specificity displayed by viral MPs presumable reflects the situation, in the infected cell, where the virus is able to produce many infectious transcripts. Under such conditions, there would be little selection pressure on the MP to evolve the capacity for interaction with the cognate viral transcripts in a sequence-specific manner. In contrast, in the plant, there is probably a strong selective advantage in being able to mediate the transport of a unique protein–mRNA complex across a given cellular boundary (Jackson and Hake, 1997; Lucas, 1995; Haywood et al., 2002). In any event, a number of plant non-cell-autonomous proteins (NCAPs) that can mediate the cell-to-cell transport of RNA have now been characterized, and they range in properties from being highly substrate specific, such as PSRP1 (Yoo et al., 2004), to CmPP16 (Xoconostle-Cázares et al., 1999), whose function is thought to involve the formation of RNP complexes with the many transcripts that move long distance through the phloem (Ruiz-Medrano et al., 1999; Lough and Lucas, in press); like viral MPs, CmPP16 binds sequence nonspecifically to RNA.

Competition between NCAPs and MPs: problems with mimicry

An ever widening array of plant NCAPs is currently under investigation (Cilia and Jackson, 2004; Haywood et al., 2002; Lucas and Lee, 2004). The emerging principle, based on these studies, is that PD have evolved a sophisticated mechanism for the regulated trafficking of NCAPs. With the exception of LEAFY (LFY), another non-cell-autonomous plant transcription factor (Sessions et al., 2000; Wu et al., 2003), mutations made in host NCAPs and viral MPs greatly impair or completely abolish movement function (Haywood et al., 2002). These results support the hypothesis that trafficking requires specificity, in terms of protein–protein interaction, at some stage in the delivery to or passage through PD. Control is considered to be conferred at several points along the trafficking pathway. One site of regulation is thought to involve the formation of an NCAP–chaperone complex that can (a) interact with cellular constituents for delivery to the PD orifice (Kragler et al., 1998) and (b) be recognized by the PD machinery (Lucas and Lee, 2004) (Fig. 2). Although the identity of these NCAP/MP-interacting chaperones remains to be elucidated, NON-CELL-AUTONOMOUS PATHWAY PROTEIN 1 (NCAPP1) appears to have the necessary prerequisites as (a) it can bind CmPP16 and a wide range of phloem NCAPs, (b) a mutant form blocks the trafficking of a subset of NCAPs and MPs, and (c) it is located on the ER in close proximity to the PD orifice (Lee et al., 2003).

A further requirement for NCAP/MP trafficking involves the capacity of the chaperone complex to interact with putative PD docking proteins. By some as yet unknown mechanism, this docking step activates a process leading to a spatial rearrangement of the proteins that control the SEL of the PD microchannels (Figs. 1F and G, 2). This property of the NCAP/MP–chaperone complex, to increase the basal SEL from 1 kDa to as high as 40 kDa (Lucas et al., 1995; Rojas et al., 1997), is a near ubiquitous feature; however, a few exceptions do exist to this rule (Waigmann and Zambryski, 1995). Of relevance to viral infection, modula-

tion of PD SEL appears to be under developmental control. Lastly, protein cross-linking experiments indicate that at least partial unfolding is necessary for NCAP/MP translocation through the PD microchannel (Kragler et al., 1998).

In addition to protein unfolding, an absolute requirement for microchannel dilation appears to exist for cell-to-cell trafficking of NCAP/MP–RNA complexes (Kragler et al., 1998, 2000). This operational feature may well explain why many viral MPs have an ATP-dependent helicase motif; vRNP trafficking would involve both an SEL increase and vRNA unfolding. In this regard, it is intriguing to note that the *INCREASED SIZE EXCLUSION LIMIT OF PLASMODESMATA (ISE)* gene of *Arabidopsis* (Kim et al., 2002) encodes a DEAD-box RNA helicase (Kim et al., 2004b). As indicated by the name, PD in the *ise* mutant background display a constitutive increase in SEL; this finding provides the first link between an endogenous helicase and PD function. As such helicases would require ATP for activity, the discovery of *ise* may provide an explanation as to why a reduction in the level of cytoplasmic ATP elicits an increase in PD SEL (Cleland et al., 1994). Collectively, these interactions likely represent pivotal control points in the cell-to-cell trafficking of macromolecules (Haywood et al., 2002; Oparka, 2004; Ruiz-Medrano et al., 2004).

A range of experimental approaches has been employed to establish that plant viruses compete for access to the NCAP trafficking pathway. Microinjection experiments, involving KN1 and CMV MP, established that dysfunctional KN1 blocks the cell-to-cell trafficking of fluorescently labeled CMV MP and vice versa (Kragler et al., 1998). In addition, in transgenic plants expressing a dysfunctional form of NCAPP1, the cell-to-cell transport of TMV MP and CmPP16 was fully inhibited, whereas that of CMV and KN1 remained unaffected (Lee et al., 2003). Based on results of this nature, it is thought that multiple pathways exist for NCAP/MP and RNP complex trafficking through PD. The existence of these different pathways clearly would have provided plant viruses with a range of mechanisms by which to "hitch" a ride into the neighboring cells. However, the caveat to this situation is at least twofold. First, efficient competition by a host NCAP could block the capacity of the viral MP to move the infectious agent to/through PD; this may well reflect specific cases of host incompatibility. Second, expression of the host proteins involved in a certain NCAP pathway will be under developmental and/or physiological control. Such regulation could result in the virus being confined to specific tissues/cell types; the plant virus literature is replete with examples of this type of restricted infection domains.

Regulation of MP/NCAP movement by phosphorylation

Viral MPs can be phosphorylated by plant protein kinases (PKs), suggesting that this form of posttranslational modification may play a role in regulating viral movement (Lucas and Lee, 2004; Waigmann et al., 2004). Support for this hypothesis is provided by experiments performed in vitro, with protoplasts and intact cells. No translation products were produced when a TMV MP–vRNA complex was added to an in vitro translation system, or introduced into protoplasts, which lack PD (Karpova et al., 1997). However, inoculation of this vRNP complex into cells, located within leaf tissues and thus connected by PD, resulted in viral infection. Phosphorylation of the TMV MP contained within the vRNP complex resulted in a conversion of the non-translatable form of the complex into one that was translatable in vitro and infectious in protoplasts and plant tissues (Karpova et al., 1999). These findings are consistent with TMV MP undergoing phosphorylation, during its movement through PD, a modification that may destabilize the RNP complex thereby allowing the ribosomal machinery access to the vRNA (Fig. 3A).

A direct role for phosphorylation, in terms of regulating MP trafficking through PD, has also been demonstrated. Building on the finding that residues Ser_{258}, Thr_{261}, and Ser_{265}, located in the TMV MP C-terminus, represent potential phosphorylation sites (Citovsky et al., 1993), Waigmann et al. (2000) developed mutant MPs in which Asp replaced all three target sites; that is, this mimics the phosphorylated MP condition. When microinjected into *Nicotiana tabacum* cells, this mutant could neither induce an increase in PD SEL nor traffic through PD; a TMV carrying this mutant MP was unable to infect this host. Interestingly, in *Nicotiana benthamiana* tissues, this mutant protein behaved as wild-type TMV MP in that it could move cell to cell and was infectious.

Based on these findings, phosphorylation on all three C-terminal target sites may negatively regulate an essential,

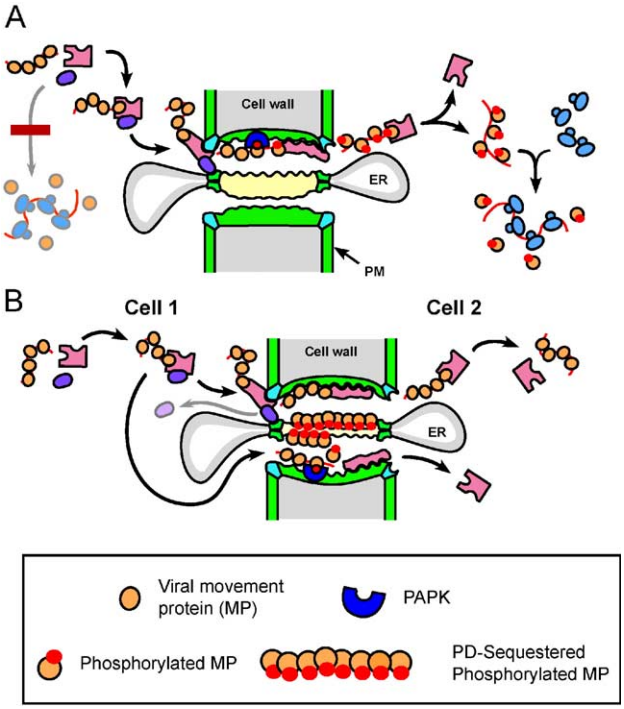

Fig. 3. Schematic illustrating the role of a PD-associated protein kinase (PAPK) in regulating the function of TMV MP in cell-to-cell trafficking and replication of vRNA. (A) Phosphorylation of TMV MP, during its passage through the PD microchannels, reduces the affinity for vRNA. (B) Super-phosphorylation of the TMV MP by the host leads to sequestration of this dysfunctional form in PD, where it accumulates in the ER lumen.

host-dependent, interaction between PD and the TMV MP (Waigmann et al., 2000) (Fig. 3B). A weakness of this hypothesis is that the C-terminal region of the TMV MP is dispensable for infection (Berna et al., 1991; Boyko et al., 2000a). In addition, if this were the case, one might anticipate that TMV would have mutated these potential regulatory sites. Retention of these target residues may impart positive attributes to this region of the MP (Lee and Lucas, 2001), a notion supported by TMV infection studies in which lesion size was shown to be altered, in a complex manner, for a number of MP C-terminal deletion mutants (Berna et al., 1991; Gafny et al., 1992). Another possibility is that the host may benefit from inactivation of the TMV MP; loss of its capacity to increase PD SEL may restore the ability of the plant to control this important aspect of PD function (Robards and Lucas, 1990). In this regard, it is interesting to note that, in transgenic plants expressing the TMV MP, physiological process associated with photosynthate partitioning were perturbed, causing a shift in resource allocation between shoots and roots (Lucas et al., 1993b). Lastly, in support of this notion, TMV only appears to upregulate PD SEL at the infection front (Oparka et al., 1997).

A range of PKs are able to phosphorylate the tobamovirus MPs, including calcium-dependent PK (Yahalom et al., 1998), casein-kinase-II-like (Matsushita et al., 2000, 2003), and PK C-like (Karpova et al., 1997) enzymes. A casein-kinase-II-like enzyme has been shown to recognize and phosphorylate the potyvirus CP, which functions as an MP, and this posttranslational modification reduced its RNA-binding affinity and caused a defect in cell-to-cell movement (Ivanov et al., 2003). Clearly, the identification of the specific location for these various PKs may shed light on their roles in modulating MP function. Molecular identification and characterization of one such PD-associated PK (PAPK1) have now been accomplished (Lee et al., 2005). This 34-kDa kinase was isolated from a plasmodesmal-enriched cell wall protein preparation (Lee et al., 2003) and is a member of the casein kinase I (CKI) family. PAPK1 displays substrate specificity in that it recognizes only a subset of viral MPs and endogenous NCAPs. An important property of PAPK1 is that it specifically phosphorylates the three C-terminal target residues, Ser_{258}, Thr_{261}, and Ser_{265} of the TMV MP, previously shown to affect its movement capacity (Waigmann et al., 2000). Subcellular localization studies established that PAPK1 and TMV MP colocalize within cross-walls in a pattern consistent with their localization within PD (Fig. 3).

Members of the CKI family are known to exhibit broad substrate specificities, thereby allowing them to participate in a spectrum of regulatory events, including signaling processes (Jia et al., 2004). In addition, CKI family members can phosphorylate substrates already modified by other PKs (Xu et al., 1995). This requirement for multiple levels of substrate phosphorylation is emerging as a paradigm in the control over NCAP/MP trafficking in plants (Waigmann et al., 2000; Ivanov et al., 2003; Lee and Lucas, 2004; Trutnyeva et al., 2005). Future studies will be required in order to provide insights into the relationship between PAPK1 phosphorylation and infection by plant viruses, including TMV. As one of the few identified molecular components of PD, PAPK1 should also act as a valuable tool for the elucidation of the cellular events involved in the trafficking of constituents to PD.

Delivery of NCAPs, MPs, and RNP complexes to PD orifice

Is simple diffusion adequate for cell-to-cell movement of macromolecules? In some tissues, GFP and other fluorescent probes up to 50-kDa can move through PD (Oparka et al., 1999; Fisher and Cash-Clark, 2000). As the heterologous proteins used in these experiments would not likely contain specific PD-targeting motifs, these findings suggest that random diffusion may be able to drive the movement of some macromolecules, provided their physical dimensions are commensurate with those of the dilated PD microchannels (Lucas and Lee, 2004). Consistent with this notion, fluorescence recovery after photobleaching studies established that molecules as large as 500 kDa can diffuse relatively freely within the cytoplasm (Seksek et al., 1997). Thus, in the presence of large diffusion gradients, some PD-targeted proteins may well access their docking sites by this delivery method. However, as MPs still appear to be essential for viral infection to occur in tissues in which the PD SEL is approximately 50 kDa (Oparka et al., 1999), we can conclude that vRNA/vRNP complexes cannot diffuse through dilated PD microchannels.

Importantly, studies on animal viruses have revealed a role for the cytoskeleton in transport of vRNP complexes within the cytoplasm (Ploubidou and Way, 2001; Sodeik, 2000). It is also generally accepted that the targeting of proteins and RNP complexes, within the cytoplasm, involves an interaction with the cytoskeleton (Bassell and Singer, 1997; Okita and Choi, 2002; de Heredia and Jansen, 2004). Based on this paradigm, one could expect that plants and also some viruses would similarly have recruited the cytoskeleton to assist in NCAP/MP and RNP complex delivery to PD. Indeed, numerous studies performed largely with TMV have demonstrated an association of viral MPs with both microfilaments and microtubules (Boyko et al., 2000a, 2000b; Heinlein et al., 1995, 1998; Más and Beachy, 1999; McLean et al., 1995).

The precise role that the plant cytoskeleton plays in the viral infection process still remains to be elucidated. A direct involvement of microtubules in MP/vRNP complex delivery to PD seems unlikely. Results obtained with a TMV vector, expressing a DNA-shuffled MP gene, failed to detect an association with microtubules, yet the vector displayed enhanced cell-to-cell movement capacity (Gillespie et al., 2002). Next, very early in the infection process, TMV MP is found in association with the cortical ER, whereas labeling of the microtubules is only detected during the latter stages of infection (Heinlein et al., 1998; Más and Beachy, 1999; Gillespie et al., 2002). Furthermore, pharmacological agents that disrupt microtubules do not appear to interfere with viral spread (Gillespie et al., 2002; Kawakami et al., 2004). In view of these findings, microtubules are unlikely to be directly involved in vRNP complex delivery to PD, but, rather, they

may assist in the sequestration of TMV MP for entry into the 26S proteosome for protein turnover. Whether this role of the microtubules is a general feature for other MP systems remains to be established.

The case for the microfilaments, as the motility system involved in viral spread, relies heavily on correlative events rather than direct linkages. A possible involvement of actin in PD function is indicated by microinjection experiments in which actin-filament-disrupting agents cause an increase in PD SEL (Ding et al., 1996). Naturally, this effect could be an indirect consequence of a perturbation to the cytoplasmic architecture rather than reflecting a specific actin-based process occurring in association with PD. Interestingly, both actin (Overall and Blackman, 1996) and myosin (Reichelt et al., 1999) have been detected within PD by immunogold labeling, and, furthermore, disruption of actin filaments blocks cell-to-cell spread of TMV (Kawakami et al., 2004). Taken together, these findings provide some credence to the notion that a microfilament-based system may be involved in MP delivery to and passage through PD (Oparka, 2004).

A preponderance of the studies investigating the relationship between the cytoskeleton and viral infection/cell-to-cell spread has relied on a combination of MP:GFP fusions and CLSM to observe events within cells. The limitations to this approach are three-fold: first, after GFP synthesis, a period of hours is required before it emits fluorescence (Heim et al., 1995); second, only very low levels of TMV MP are needed for cell-to-cell movement (Arce-Johnson et al., 1995; Szécsi et al., 1999); third, the complete infection cycle takes between 2 and 4 h (Derrick et al., 1992). Hence, signal strength, and thus detection capacity, is limiting when the important early events of viral replication and movement are probably occurring within the infected tissue. Solutions to these experimental limitations would likely result in a resolution to the question as to whether microfilaments are generally necessary for vRNP delivery to PD.

The ER has also been implicated in the infection process, a notion fully consistent with its known role as sites for viral replication and protein synthesis (Noueiry and Ahlquist, 2003). Viruses may indeed usurp the ER to form special structures, termed virus-replication complexes (VRCs; Asurmendi et al., 2004). Studies on TMV revealed that such VRCs contain vRNA, replicase, and MP (Más and Beachy, 1999). VRCs are not only closely associated with the ER, but their intracellular movement can be blocked by inhibitors of microfilaments (Kawakami et al., 2004). As large plant organelles, such as the Golgi apparatus, are known to traffic on the ER/actin network (Boevink et al., 1998), it may be that movement of these putative VRCs is driven by this same system. The reported positioning of VRCs at or near the PD orifice (Kawakami et al., 2004) may well allow the MP to overpower the capacity of the host defense system, perhaps involving several PKs (Fig. 3), and other recognition factors, thereby ensuring the rapid transfer of vRNP complexes, through PD, and efficient replication in the invaded cell. This scenario may be more feasible than the proposed cell-to-cell trafficking of entire VRCs (Kawakami et al., 2004).

TGB proteins: riding on the secretory actin-ER-driven and endocytic pathways

Extensive studies on the roles played by the three TGB proteins, in virus trafficking, have yielded an amazing picture as to how the Hordei-like and Potex-like viruses have adapted to hitch a ride on the cell's secretory and endocytic membrane pathways. Sequence analysis indicates that the Hordei-like and Potex-like TGBp2 have two conserved membrane spanning domains. The situation for TGBp3 is slightly different in that the Hordei-like protein has two membrane domains, whereas that of the Potex-like protein has only a single domain. In any event, these predicted properties suggested that both the TGBp2 and TGBp3 would function as integral membrane proteins. Consistent with this hypothesis, these proteins co-purify with the endomembranes (Morozov and Solovyev, 2003).

Subcellular localization studies, employing GFP fusion proteins, indicate that both TGBp2 and TGBp3 reside in the immediate proximity to the endomembrane system and predominantly over the ER (Cowan et al., 2002; Solovyev et al., 2000). Mutations in the predicted transmembrane domains of TGBp2 and TGBp3 both prevented association with the ER and blocked virus movement (Krishnamurthy et al., 2003; Mitra et al., 2003; Morozov and Solovyev, 2003). Interestingly, small fluorescent spots, or motile granules, have been observed to traffic along the ER–microfilament network, with the fluorescent signal eventually becoming stationary at the cell periphery, in a pattern suggestive of localization to PD (Haupt et al., 2005).

Expression of TGBp3, alone, results in its accumulation in the vicinity of PD, whereas TGBp2 does not seem to be able to self-target to PD. However, co-expression of TGBp2 and TGBp3 leads to their colocalization within the cytoplasm; here, TGBp3 appears to redirect TGBp2 from the ER network to peripheral bodies (Haupt et al., 2005; Solovyev et al., 2000). As expected based on microinjection studies, expression of TGBp1-GFP, in the presence of TGBp2 and TGBp3, gives a similar localization pattern to that observed for TGBp2 plus TGBp3 (Zamyatnin et al., 2004).

The potential involvement of the endocytic pathway in TGBp2 and TGBp3 recycling is supported by high-resolution imaging studies performed later in the time course of transient expression experiments. At this time, both proteins appear to be incorporated into membrane vesicles; here, incorporation of TGBp3 was dependent on TGBp2 (Haupt et al., 2005). In addition, the TGBp2 C-terminal hydrophobic region seems to act as a retrieval signal in Golgi-to-ER recycling. A model illustrating the pathway by which the Hordei-like and Potex-like TGB proteins may mediate the delivery of vRNA to PD is illustrated in Fig. 4. Insights into the operation of the endogenous NCAP pathway were afforded through the discoveries made with viral MPs. It may well be that the studies performed with the TGB proteins could again open the door to a better understanding of the role played by the secretory and endocytic pathways in host NCAP signaling.

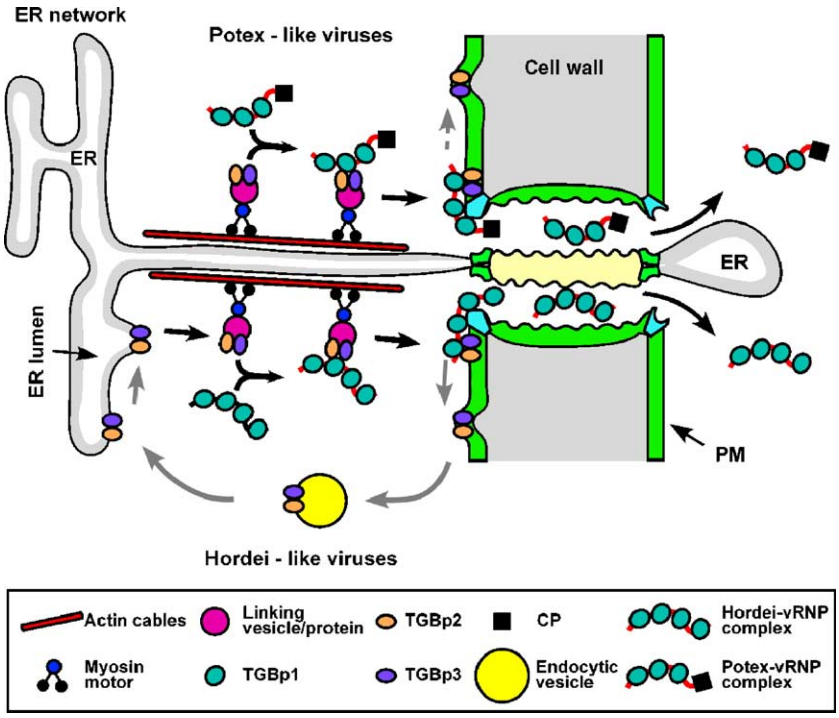

Fig. 4. Model illustrating the role of the actin-ER secretory and endocytic pathways in the cell-to-cell spread of Hordei-like and Potex-like viruses. Redrawn after Lough et al. (1998) and Haupt et al. (2005).

Host proteins interacting with MPs: a challenging jigsaw puzzle

It is now well established that viral MPs are multifunctional agents involved in many facets of viral infection, including the formation of vRNP complexes, mediating the cell-to-cell and long-distance trafficking of the viral genome, vRNA translation, and suppression of gene silencing (Harrison and Robinson, 2005; Kasschau and Carrington, 1998). To execute these functions, MPs must be able to interact with an equally diverse group of endogenous proteins, including PKs; this feature seems to have greatly complicated the identification of bona fide host interacting proteins.

The manner in which the cellular machinery of the host has been co-opted by the TGB-containing viruses is perfectly illustrated in Fig. 4. With all that is currently known regarding this trafficking system, it is interesting that details of the host proteins remain sparse. One TGBp2-interacting host protein has been identified using a yeast 2-hybrid screen (Fridborg et al., 2003): three TGBp2 interacting proteins (TIPs) displayed specificity in that they did not interact with either TGBp1 or TGBp3. TIP1 is thought to be homologous to HBP1, a tobacco ankyrin-repeat containing protein that likely is involved in protein–protein interaction. Interestingly, these TIPs also interact with β-1,3-glucanase, an enzyme that participates in regulating the level of callose which plays a role in both adjusting PD SEL (Wolf et al., 1991) and modulating virus infection (Iglesias and Meins, 2000). Hence, TIP1 may be involved in some way with PD SEL modulation (Fridborg et al., 2003). Once delivered to the plasma membrane, TGBp2 could bind to the β-1,3-glucanase, thereby stimulating its enzyme activity. However, many other potential scenarios exist, and it will be of great interest to watch as this puzzle unfolds.

A number of different experimental approaches have been employed to identify host proteins that interact with the TMV MP and related proteins. Although direct binding to actin and tubulin has been reported, the relevance of this interaction is yet to be established. Identification of MPB2C, an uncharacterized microtubule-associated tobacco protein that binds to TMV MP and colocalizes with microtubules, provides support for the notion that microtubules are involved in protein turnover. In vivo studies established that MPB2C mediates the accumulation of TMV MP within the cytoplasm but is not essential for cell-to-cell trafficking of the vRNP complex (Kragler et al., 2003). Hence, MPB2C appears to act as a negative regulator of the level of TMV MP available for vRNP complex formation and movement.

TMV MP has also been reported to bind to pectin methylesterase (PME), a cell-wall-located enzyme, and this protein was proposed to be a host receptor involved in cell-to-cell movement of vRNP complexes (Dorokhov et al., 1999). Mutant analysis of the TMV MP identified a domain that was necessary and sufficient for binding to PME (Chen et al., 2000); absence of PME binding capacity was correlated with impairment in movement function. The link between this wall enzyme and TMV MP trafficking through PD is somewhat enigmatic. As an extracellular protein, PME would be delivered to the cell wall via the secretory pathway. Hence, in a system similar to that described for the Hordei-like and Potex-like viruses, a TMV MP–vRNA complex may hitch a ride on the secretory vesicles contain-

ing PME. A number of simple tests can be performed to test this hypothesis.

A TMV MP affinity column has also been used to isolate host interacting proteins. A calreticulin was isolated and cloned and shown to localize to PD (Chen et al., 2005). Interestingly, overexpression of this calreticulin caused (a) an alteration in TMV MP targeting as it appeared to be redirected away from PD to microtubules and (b) a block to virus spread. Calreticulin is an ER chaperone that recognizes improperly folded proteins. This might well explain why it was detected over PD as dysfunctional TMV MP accumulates in the PD median cavity, a region representing the ER lumen (Ding et al., 1992), during infection. However, why overexpression of calreticulin would limit virus infection presents yet another interesting conundrum.

Future directions

Research into the mechanisms used by both viruses and plants to achieve cell-to-cell trafficking of macromolecules has contributed greatly to our understanding of plant and viral evolution. It is now imperative that progress be made with the isolation and characterization of host proteins underlying the NCAP pathway. The focus of these studies should be on the characterization of PD structural proteins, and especially those involved in NCAP/MP-induced increase in SEL, recognition/docking, and the actual translocation of macromolecules along the PD microchannels. Isolation of the putative chaperones will provide invaluable information on the number of different NCAP pathways and the degree to which host and viral MPs compete for these systems.

One of the most perplexing aspects of MP/NCAP trafficking relates to the apparent absence of simple motifs required for protein targeting to the PD. In order to resolve this problem, greater efforts are needed in the area of MP structure, especially in terms of elucidating, at high resolution, the three-dimensional structure of proteins from representative viral movement pathways (Table 1). Proof that generic structural motifs are necessary and sufficient for movement on particular chaperone-based pathways (Aoki et al., 2002) would open the door for a more extensive analysis of plant databases. Such studies could establish the extent to which plants utilize the symplasm for the exchange of informational macromolecules. Results of this kind would impact greatly on studies of long-distance transport of both viral and plant RNP complexes.

Interactions between plant viruses and their hosts are complex and continued studies on the role(s) played by MPs, in determining host, (in)compatibility will contribute towards unraveling the specific pathways involved. The many unanswered questions surrounding the manner in which host proteins, such as PME, function in the process of virus infection ensures that this area is ripe for the picking. Another outcome from this work will be knowledge on the evolution of host factors that limit viral passage across specific cellular and tissue boundaries. This could provide an explanation as to why certain viruses are confined to a particular tissue type, such as the phloem (Gilbertson and Lucas, 1996). Finally, the role of the viral MP in the battle between the host's RNA interference machinery and viral counter-surveillance is well established; however, molecular details for many MPs remain to be resolved.

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