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Cell-to-Cell and Long-Distance Transport of Viruses in Plants

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INTRODUCTION

The idea that viruses move through plants in two distinct modes was accurately concluded by G. Samuel in a 1934 paper describing the transport of tobacco mosaic virus (TMV) through solanaceous hosts: "It is considered that these facts favour the theory of a slow cell to cell movement of the virus via the plasmodesmen, combined with a rapid distribution through the plant via the phloem" (Samuel, 1934). It is now firmly established that plant viruses move from cell to cell and over long distances by exploiting and modifying preexisting pathways for macromolecular movement within cells, between cells, and between organs. In this review, we focus on the roles of viral and host components in the movement of viruses through these pathways. Exhaustive coverage of all aspects of movement is not possible, but the reader is referred to several excellent reviews that emphasize various facets of short- and long-range virus transport (Atabekov and Taliansky, 1990; Maule, 1991; Deom et al., 1992; Citovsky, 1993; Leisner and Turgeon, 1993; Lucas and Gilbertson, 1994; Lucas, 1995).

VIRUS MOVEMENT INVOLVES SPECIFIC VIRAL AND HOST FACTORS

Plant viruses encode functions specifically required for movement. This fact was demonstrated elegantly by Nishiguchi et al. (1978, 1980), using the temperature-sensitive Ls1 mutant of TMV. At the restrictive temperature, Ls1 replicates efficiently at the single-cell level and forms virus particles but is incapable of movement out of initially infected cells. The Ls1 defect maps to the gene encoding the 30-kD protein (Deom et al., 1987; Meshi et al., 1987), now known as the movement protein (MP). MPs have since been identified in most families of plant viruses and in most cases perform dedicated functions in intercellular movement. Besides MP, many viruses encode structural proteins or genome replication proteins with additional functions in cell-to-cell and long-distance transport.

MPs encoded by diverse virus families are genetically interchangeable in many cases (DeJong and Ahlquist, 1992; Giesman-Cookmeyer et al., 1995). Additionally, movement defects of a virus or virus strain in a particular host can often be complemented by coinfection with an unrelated virus that is movement competent (Atabekov and Taliansky, 1990). For example, brome mosaic bromovirus (BMV) gains the ability to move through tomato if it is coinoculated with TMV (Taliansky et al., 1982). Cell-type restrictions can also be overcome by the movement functions of heterologous viruses. The block to movement of potato leafroll luteovirus into mesophyll cells from phloem cells, in which it is normally restricted, is overcome by coinfection with potato virus Y (Barker, 1987). The exchangeability and complementation of movement functions from unrelated virus families suggest that disparate viruses can be routed through common intercellular movement pathways.

The host dependence of virus invasion suggests that specific host factors play key roles in movement. In fact, the hypothesis that virus movement requires compatibility between virus-encoded movement factors and host components is well supported. Movement-restricted interactions can result in limiting of the virus to initially infected cells or primary infection foci. Sulzinski and Zaitlin (1982) showed that certain plants, such as cowpea and cotton, support only subliminal infections by TMV in which virus fails to move from initially infected cells. Strain-specific restriction in movement can often be conditioned by mutations at one or a few loci within a host species. Such strain-specific restrictions have been described for several virus–host systems, including TMV in tomato (Motoyoshi and Oshima, 1977), tobacco etch potyvirus (TEV) in tobacco (Schaad and Carrington, 1996), and cauliflower mosaic caulimovirus (CaMV), turnip crinkle carmovirus, and beet curly top geminivirus in Arabidopsis (Simon et al., 1992; Leisner et al., 1993; Lee et al., 1994). In the case of TMV in tomato, the movement restrictions conditioned by either the *Tm-2* or the allelic *Tm-2*^{2} gene may involve a direct or indirect interaction between MP and the Tm-2 protein, as compensatory mutations within the TMV MP gene overcome the restrictions (Meshi et al., 1989; Weber et al., 1993).

THE VIRUS MOVEMENT PATHWAY IN PLANTS

At the organismal level, the movement pathway for a systemically infecting virus involves traversal of several cell types and

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tissues. If infection starts in an epidermal cell, the virus must move from cell to cell sequentially into mesophyll, bundle sheath, and phloem parenchyma and companion cells (Figure 1). Long-distance transport to other leaves is facilitated by movement from vascular parenchyma or companion cells into sieve elements through which the virus moves rapidly (centimeters/hour) by bulk flow to tissues that are sinks for photoassimilate (Leisner and Turgeon, 1993). Invasion of cells in systemic tissues at a distance away from the initial site of infection likely requires entry into companion cells from sieve elements, then cell-to-cell movement into bundle sheath, mesophyll, and epidermal cells (Figure 1). This entire pathway is part of an elaborate symplastic network (Lucas, 1995), so at no point after inoculation does a plant virus need to cross the plasma membrane. Rather, each step requires transport through an intercellular channel, the plasmodesma (Lucas et al., 1993). The utilization of intercellular channels for spread, instead of release into the extracellular space and attachment to cell surface receptors, is perhaps the most significant evolutionary adaptation that distinguishes plant viruses from animal viruses.

At the one- and two-cell level, the cell-to-cell movement pathway involves the transport of newly synthesized genomes to and through plasmodesmata. In fact, short-distance movement

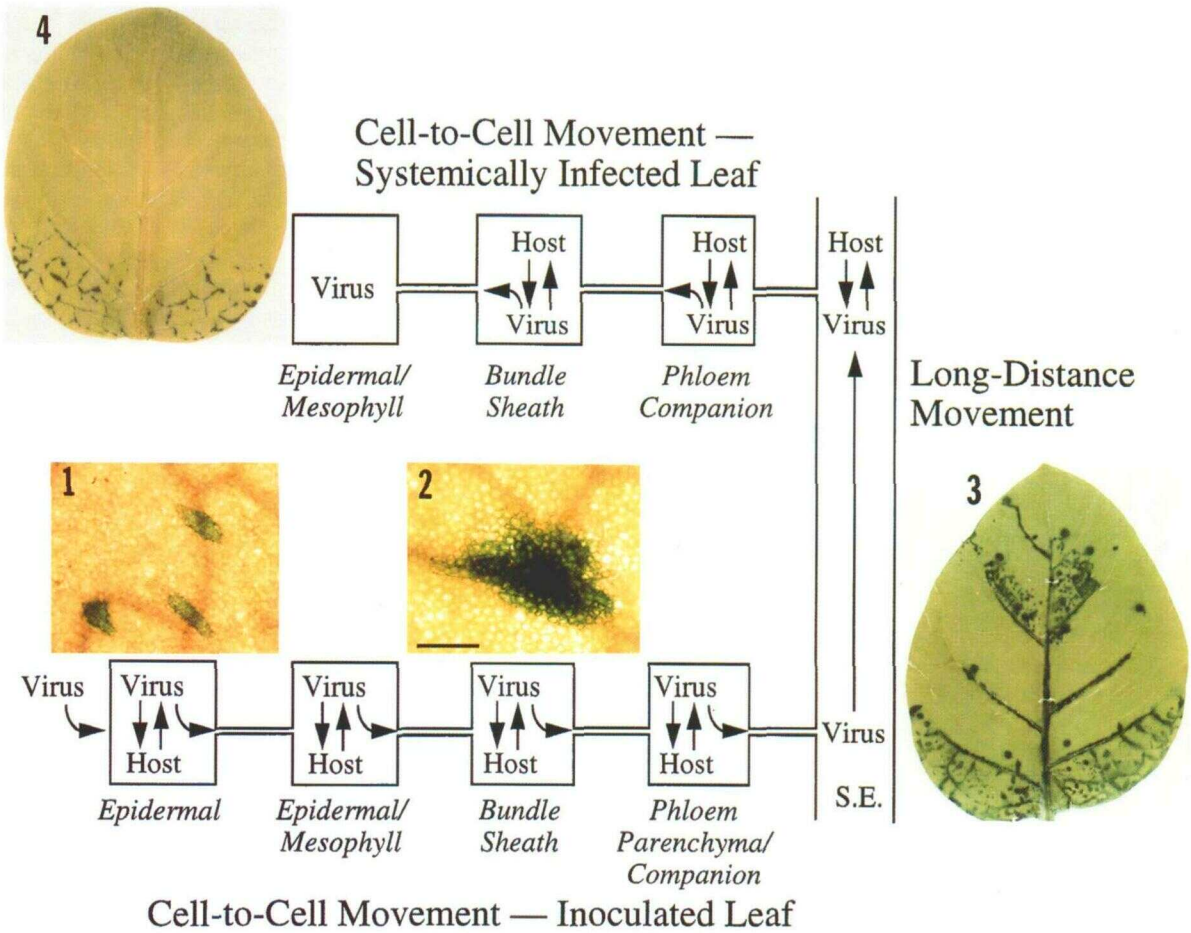

Figure 1. Cell-to-Cell and Long-Distance Movement Pathway in Plants.

Several points along the pathway are illustrated schematically, using TEV-GUS infection of Arabidopsis (1 and 2) or tobacco (3 and 4). Photographs 1 to 3 show the extent of TEV-GUS movement at 24-hr postinoculation (p.i.), 48-hr p.i., and 96-hr p.i., respectively, in inoculated leaves. Virus is evident only in initially inoculated epidermal cells at 24-hr p.i. (1) and in foci resulting from cell-to-cell movement at 48-hr p.i. (2). Primary infection foci and secondary spread through the vasculature are detected in the inoculated leaf at 96-hr p.i. (3). Photograph 4 shows long-distance movement of TEV-GUS to an upper, noninoculated leaf, where virus is moving cell to cell after exiting the vasculature. Infected cells were visualized by infiltration with the colorimetric GUS substrate 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid (X-gluc), as described by Dolja et al. (1992). Photographs 3 and 4 are from Verchot and Carrington (1995; reprinted with permission of the American Society for Microbiology) and Dolja et al. (1992), respectively. The scale for photographs 1 and 2 is indicated by the bar in photograph 2 (200 μm). The interactions between virus and host and the direction of virus movement are represented by arrows in the schematic diagram. S.E., sieve element.

can best be thought of as a process of genome movement, because many (but not all) viruses do not require virion assembly or a capsid protein to move to adjacent cells. We will consider the cell-to-cell movement pathway as a process divided into three major steps: (1) transfer of newly synthesized genomes from sites of replication to intracellular transport systems; (2) directed, facilitated transport of genomes to plasmodesmata; and (3) transit through plasmodesmata. These steps are discussed in the following sections.

CELL-TO-CELL MOVEMENT

Transfer of Genomes from Sites of Replication to Intracellular Transport Systems

Genome replication for positive-strand RNA viruses, which represent the overwhelming majority of plant viruses, occurs in the cytoplasm in close association with membrane surfaces. Genome replication for several other types of viruses, such as single-stranded (ss) DNA-containing geminiviruses and some negative-strand RNA viruses, occurs in the nucleus. Replication proteins are obviously involved in the amplification of viral genomes that are destined to move to adjacent cells. They can also influence the quantity and timing of synthesis of MP and therefore indirectly affect cell-to-cell movement functions (Watanabe et al., 1987).

Considering the compartmentalization of viral genome replication and the intracellular distances that genomes must traverse to reach plasmodesmata, it is possible that cell-to-cell or long-distance movement involves a critical interface between the replication apparatus and the transport machinery, including the MP. Interactions between MPs and replication proteins or nascent genomes may initiate the transport process and provide some degree of specificity for trafficking viral RNA. Furthermore, genetic evidence implicates replication proteins of several viruses, including BMV (Traynor et al., 1991), cucumber mosaic virus (CMV; Gal-On et al., 1994), and TMV (Holt et al., 1990; Nelson et al., 1993), as modulators of cell-to-cell or long-distance movement. For example, BMV mutants lacking parts of the 2a protein (RNA-dependent RNA polymerase) coding sequence have systemic spread deficiencies despite being able to replicate efficiently in protoplasts (Traynor et al., 1991). Whether replication proteins of these viruses participate in the movement process by communicating with the MP, by performing transport functions not associated with their role in genome replication, or by affecting a cellular factor or process remains to be determined.

Facilitated Intracellular Transport of Viral Genomes

Events required for intracellular transport of a genome from the site of replication to the plasmodesma are likely to involve interactions between the genome and viral MP and, in some cases, other viral proteins, as well as interactions between the nucleoprotein transport complex and an intracellular trafficking system. Recent findings discussed below shed light on these processes.

Geminivirus DNA is synthesized in the nucleus, requiring that genomes destined for intercellular transport must first traverse the nuclear–cytoplasmic boundary. Bipartite geminiviruses encode two MPs, BR1 and BL1, which act in a cooperative fashion to facilitate cell-to-cell movement (Brough et al., 1988; Etassami et al., 1988; von Arnim and Stanley, 1992; Pascal et al., 1993). The squash leaf curl geminivirus (SqLCV) BR1 protein contains localization signals that direct it to the nucleus of infected and transfected cells (Pascal et al., 1994; Sanderfoot et al., 1996), and purified SqLCV BR1 binds tightly to ssDNA (Pascal et al., 1994). In plant and insect cells coexpressing SqLCV BR1 and BL1, however, BR1 is directed away from the nucleus and toward the cell periphery (Sanderfoot and Lazarowitz, 1995). Furthermore, microinjection studies revealed that the bean dwarf mosaic geminivirus (BDMV) BR1 protein redirects double-stranded (ds) DNA and ssDNA from the nucleus to the cytoplasm (Noueiry et al., 1994). The BL1 protein is associated with the cell wall fraction and facilitates transport of macromolecules through plasmodesmata (see below).

These data support a model in which BR1 provides a nuclear shuttle activity to deliver viral DNA to the cytoplasm, after which BL1 mediates trafficking of DNA to and through plasmodesmata (Figure 2). The nuclear shuttle function of BR1 undoubtedly occurs by directed transport through nuclear pores (Sanderfoot et al., 1996) and thus may involve the nuclear export pathway used by cellular proteins, RNAs, and ribonucleoproteins (Görlich and Mattaj, 1996). It is not clear whether the nucleocytoplasmic and intercellular transit form of the viral genome is ssDNA or dsDNA, possibly because different analytical methods have been used to investigate distinct geminiviruses in various laboratories (Noueiry et al., 1994; Pascal et al., 1994). The epistatic effects of mutations affecting the SqLCV capsid protein, which encapsidates ssDNA, on the activity of BR1 favors the hypothesis that BR1 interacts functionally with ssDNA (Ingram et al., 1995). However, it is formally possible that SqLCV and BDMV differ fundamentally in their movement forms.

Facilitated intracellular transport of virus genomes that replicate in the cytoplasm or in association with cytoplasmic membranes is understood best for TMV and other viruses that use TMV-like movement mechanisms. Biochemical data support the notion that MPs form complexes with viral genomes. The TMV MP possesses cooperative, nonspecific ssRNA and ssDNA binding properties, with a minimal binding site of four to seven nucleotides per MP monomer (Citovsky et al., 1990, 1992). Similar nucleic acid binding properties have been characterized for several TMV-like MPs, including those of red clover necrotic mosaic dianthovirus (RCNMV), CMV, and alfalfa mosaic virus (AMV; Osman et al., 1992; Schoumacher et al., 1992; Giesman-Cookmeyer and Lommel, 1993; Li and Palukaitis, 1996). The TMV MP/nucleic acid complex is a thin,

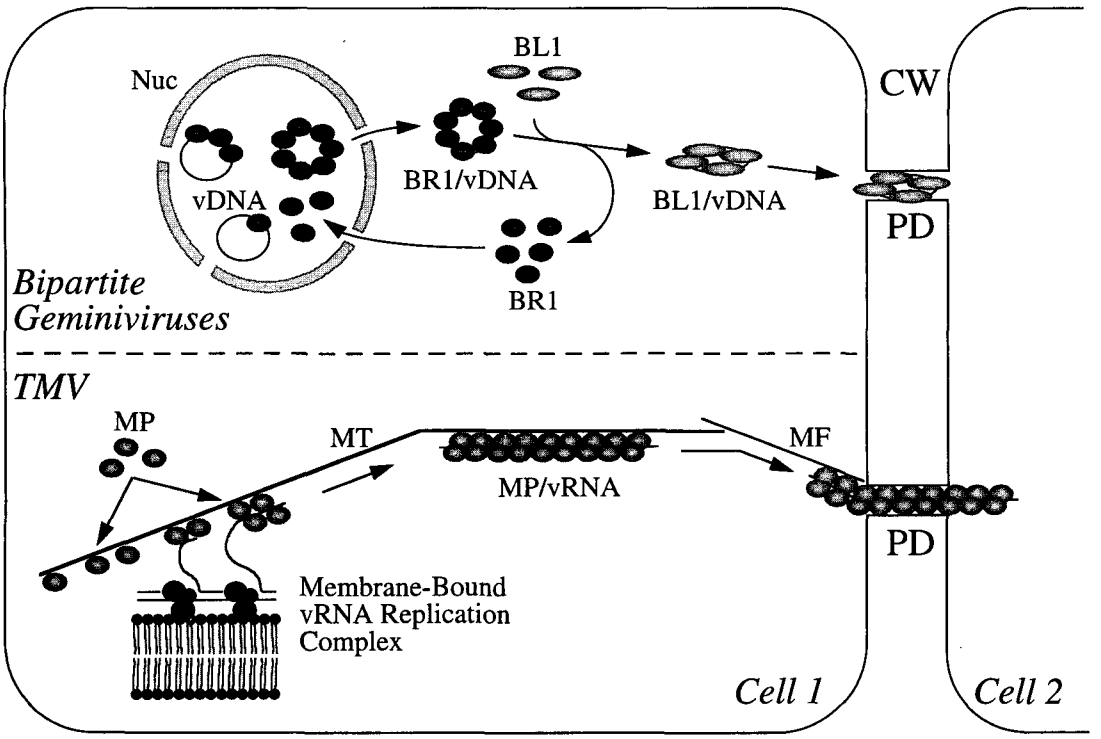

Figure 2. Models for Intracellular Transport of Bipartite Geminivirus and TMV Genomes.

For the geminiviruses, BR1 functions as a nuclear shuttle to escort newly synthesized genomes to the cytoplasm, where BL1 functions to traffic genomes to and through plasmodesmata. For TMV, complexes of MP with genomic RNA are taken from membranous sites of replication to plasmodesmata through microtubule- and microfilament-based facilitated transport. CW, cell wall; MF, microfilament; MP, movement protein; MT, microtubule; Nuc, nucleus; PD, plasmodesmata; vDNA, viral DNA; vRNA, viral RNA.

unfolded structure 1.5 to 2.0 nm in diameter (Citovsky et al., 1992), whereas the RCNMV MP/RNA complex retains considerable secondary structure with MP bound to single-stranded regions of the folded RNA (Fujiwara et al., 1993). Despite the functional similarity of these different MPs, they possess distinct domains required for nucleic acid binding and protein–protein (cooperativity) interactions (Citovsky et al., 1992; Osman et al., 1992; Schoumacher et al., 1992; Giesman-Cookmeyer and Lommel, 1993).

Several groups of cytoplasmically replicating viruses encode MPs with structural or functional differences from those encoded by the TMV-like viruses. In each case, however, nucleic acid binding activity constitutes a highly conserved feature. At least four groups of viruses have a triple gene block encoding a set of three MPs, each of which participates in systemic spread through plants (Petty et al., 1990; Beck et al., 1991; Gilmer et al., 1992). The largest of the three proteins from foxtail mosaic potexvirus (open reading frame 2 protein) and barley stripe mosaic hordeivirus (βb protein) bind ssRNA cooperatively, have ATPase activity, and contain highly conserved helicase-like sequence motifs (Rouleau et al., 1994; Donald et al., 1995). Although the role for putative helicase activity and the nature of the interactions among the three MPs are not clear, the RNA binding activity may serve to form a ribonucleoprotein complex analogous to that formed by the TMV-like MPs. The MP (P1 protein) of CaMV, a dsDNA-containing pararetrovirus, also possesses ssRNA binding activity (Citovsky et al., 1991; Thomas and Maule, 1995b). Citovsky et al. (1991) hypothesized that the 35S RNA reverse transcription template is the entity that moves from cell to cell. Although the binding domain of P1 clearly overlaps with a larger region required for movement (Thomas and Maule, 1995a, 1995b), it remains an open question whether or not the RNA binding function is involved directly in transport because other evidence indicates that CaMV moves from cell to cell as an icosahedral virion in which dsDNA is packaged (Maule, 1991). Furthermore, the P1 protein induces, and is an integral component of, cell wall–spanning tubules through which virions are proposed to pass (Perbal et al., 1993). The disparate activities of the P1 protein have led to the provocative suggestion that CaMV may actually use two distinct movement strategies at different stages in the multiplication cycle or in different tissues (Citovsky and Zambryski, 1991; Thomas and Maule, 1995b).

How does a genome bound to MP traverse the cytoplasm

en route to a plasmodesma? Two groups have provided evidence that MPs may facilitate trafficking through their interactions with the cytoskeleton. Protoplasts infected by wild-type TMV or a modified TMV encoding an MP–green fluorescent protein (MP-GFP) fusion protein contain MP or MP-GFP associated with cortical microtubules (Heinlein et al., 1995; McLean et al., 1995). The MP-GFP fusion protein accumulates in a filamentous network as well as in punctate bodies at the cell periphery in cells of intact leaves infected by the modified virus (Heinlein et al., 1995). Colocalization with microtubules and a subset of actin microfilaments is observed in protoplasts transiently expressing an MP-GFP fusion protein (McLean et al., 1995). Additionally, TMV MP has the potential to interact directly with tubulin and actin in vitro (McLean et al., 1995).

Because microtubules and microfilaments facilitate directed movement of large macromolecular complexes, organelles, vesicles, and mRNA through the cytoplasm (Vale, 1987; Langford, 1995; St Johnston, 1995), it is appealing to consider that these structures provide tracks upon which viral movement complexes ride to plasmodesmata (Figure 2). Such a model predicts that viruses exploit a normal intracellular pathway for trafficking nucleoprotein complexes to plasmodesmata, which raises a number of important questions. Does transport of movement complexes to plasmodesmata involve motors associated with microtubules (such as kinesin or dynein) and microfilaments (myosin)? Does the cortical endoplasmic reticulum (ER), which is often aligned with microtubules (Lee et al., 1989) and which spans the plasmodesma (Lucas et al., 1993), have any role in the intracellular transport of movement complexes? The genomes of most or all positive-strand RNA viruses are synthesized on endomembrane surfaces; therefore, it is possible that nascent genomes are transferred directly to cytoskeleton-bound MPs as they emerge from replication complexes. It is even possible that active replication complexes with MP bound to nascent RNA are transported on ER/microtubule networks. Does the MP–cytoskeletal interaction determine host range or cell-type specificity in those virus–host combinations in which movement is restricted? Although it is often assumed that receptor-like proteins at plasmodesmata provide specificity for intercellular trafficking, specificity may be equally important at the point of facilitated intracellular transport.

Intercellular Transport of Genomes through Plasmodesmata—TMV-like Mechanisms

By serving as intercellular channels that maintain a plantwide symplastic domain, plasmodesmata offer a means to overcome the problem all plant viruses face, namely, how to enter and exit a cell that is encased within a cell wall matrix. A plasmodesma formed during cytokinesis (a primary plasmodesma) of mesophyll cells is ~50 nm in diameter and is lined with plasma membrane that is contiguous with both adjoining cells (Lucas et al., 1993; Lucas and Gilbertson, 1994; Figure 3A). Extending through the plasmodesma is a desmotubule, sometimes referred to as appressed ER, that links the endomembrane systems of the neighboring cells. Some plasmodesmata contain a central cavity between the plasma membrane and desmotubule. High resolution electron microscopy indicates that the plasma membrane and desmotubule are each associated with protein globules (Ding et al., 1992b). Bridging proteins form linkages between the plasma membrane and desmotubule globules across the central cavity. Secondary plasmodesmata form by the branching of primary plasmodesmata or by the formation of new channels through preexisting cell walls.

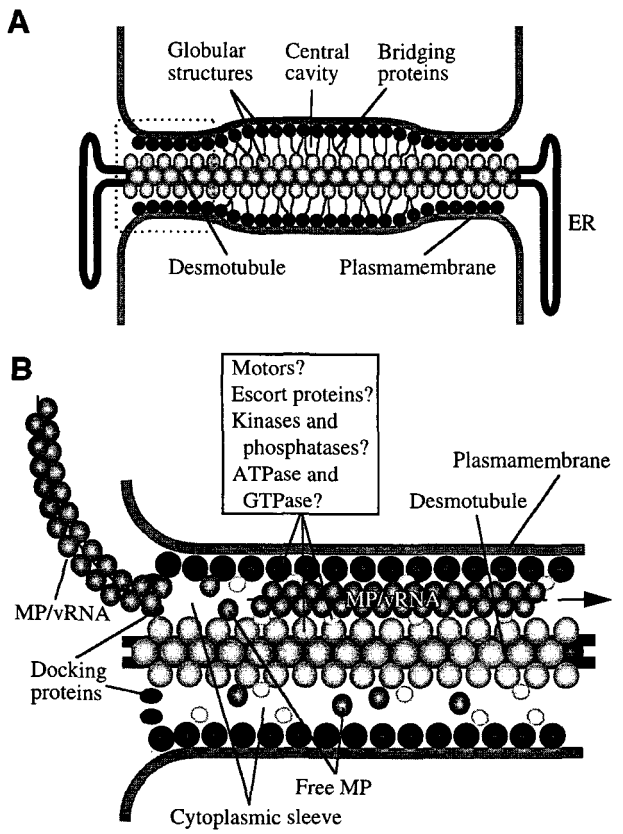

Figure 3. Models of Primary Plasmodesma and TMV-like Transport Complexes.

(A) Diagrammatic representation of one type of primary plasmodesma, based on high-resolution electron microscopic analyses (Ding et al., 1992b).

(B) Enlargement of boxed area in **(A)**. Movement complexes are shown interacting with putative docking proteins at the plasmodesmal opening and with globular structures and putative regulatory proteins in the cytoplasmic sleeve. The involvement of molecular motors, escort proteins and chaperones, kinases and phosphatases, and ATPase and GTPase in plasmodesmal transit is speculative.

ER, endoplasmic reticulum; MP/vRNA, movement protein/viral RNA complex.

It is the space between the plasma membrane and desmotubule, the cytoplasmic sleeve, through which viruses and other macromolecules are proposed to move. The limited effective diameter of the sleeve, however, results in a size exclusion limit (SEL) for passive diffusion of molecules with a mass of <1 kD (Goodwin, 1983; Terry and Robards, 1987; Wolf et al., 1989). How, then, do virus genomes traverse such a restricted channel? It is now clear that TMV-like MPs interact with plasmodesmata, modify the gating properties of plasmodesmata, and directly facilitate the transfer of large macromolecules.

The association of TMV-like MPs with the interior of plasmodesmata is indicated by immunocytochemical electron microscopy (Tomenius et al., 1987; Atkins et al., 1991; Ding et al., 1992a). Plasmodesmal targeting signals have yet to be identified, although broad regions encompassing the central and C-terminal sequences of the TMV and RCNMV MPs are necessary for plasmodesmal localization or function (Berna et al., 1991; Fujiwara et al., 1993; Giesman-Cookmeyer and Lommel, 1993; Waigmann et al., 1994). McLean et al. (1995) proposed that MP is targeted by sequential transport on microtubules and then on microfilaments. This is consistent with the detection of actin in and around plasmodesmata (White et al., 1994).

A defined activity of TMV MP in plasmodesmata was first demonstrated by Wolf et al. (1989), using microinjection techniques to introduce fluorescent dextrans of varying size. Plasmodesmata between mesophyll cells of transgenic plants expressing MP possess an SEL that is ~10-fold higher than that of plasmodesmata of control plants. In transgenic plants expressing a temperature-sensitive allele of TMV MP, the plasmodesmal gating activity is suppressed at restrictive temperatures (Wolf et al., 1991). Truncated MPs lacking C-terminal sequences fail to accumulate in the cell wall fraction (presumably plasmodesmata) and are unable to alter plasmodesmal SEL (Berna et al., 1991). The generality of this gating activity among TMV-like MPs from several other viruses is now well documented (Derrick et al., 1992; Fujiwara et al., 1993; Poirson et al., 1993; Vaquero et al., 1994; B. Ding et al., 1995). In addition, the BL1 MP of bipartite geminiviruses (Noueiry et al., 1994) and the open reading frame 2 (25 kD) protein encoded by the potato virus X (Angell et al., 1996) also alter plasmodesmal gating.

Although the SEL effect is a consistent feature of TMV-like MP–plasmodesma interaction in microinjection experiments with mesophyll cells, the significance of the effect per se is debatable. Cell type–specific and developmental effects on gating activity have been detected. For example, in transgenic plants, the TMV MP accumulates mostly in secondary, branched plasmodesmata in older tissues and only increases the SEL in mature leaves (Deom et al., 1990; Ding et al., 1992a). Considering that TMV can move through young, expanding leaves, the former observations do not correlate with the known pattern of TMV infection. The TMV MP fails to increase the SEL of plasmodesmata between individual trichome cells and between bundle sheath and phloem parenchyma cells (Ding et al., 1992a; Waigmann and Zambryski, 1995), even though TMV normally traverses these plasmodesmata during infection. It is possible that the nonspecific gating effect in some tissues and cell types is an indirect consequence of other important MP–plasmodesmal interactions.

Rather than facilitating intercellular movement by merely increasing the SEL of plasmodesmata, MPs are likely to mediate the active transport of genomes from cell to cell. Coinjection experiments reveal that the MPs of RCNMV, CMV, or BDMV (BL1) can induce the transport of fluorescently labeled nucleic acids to adjacent cells (Fujiwara et al., 1993; Noueiry et al., 1994; B. Ding et al., 1995). The RCNMV MP is capable of trafficking ssRNA but not ssDNA or dsDNA, whereas the BDMV BL1 MP facilitates transport of dsDNA but not ssDNA or ssRNA (however, see Pascal et al. [1994] for an opposing viewpoint). The molecular masses of the nucleic acids transported are several orders of magnitude larger than the dextrans that define the SEL in the presence of MP. In general, the coinjection experiments suggest that MPs facilitate transport of nucleic acids in a sequence-nonspecific manner. The ability of MP from one virus to complement movement defects of unrelated viruses also argues for considerable nonspecific genome function under physiological conditions. A basis for any sequence specificity of MP-mediated cell-to-cell transport of viral genomes has yet to be identified.

Do MPs only escort genomes to plasmodesmata, or do they also escort genomes through plasmodesmata and into the adjacent cell? Several studies indicate that MPs by themselves can traffic through plasmodesmata. The MPs of TMV, RCNMV, CMV, and BDMV rapidly move to adjacent cells and beyond after microinjection (Fujiwara et al., 1993; Noueiry et al., 1994; B. Ding et al., 1995; Waigmann and Zambryski, 1995). Certain mutant forms of MP that debilitate cell-to-cell transport of virus are unable to traffic through plasmodesmata, although the point in the trafficking pathway at which the altered proteins are arrested is not known. Fusion proteins consisting of TMV MP and β-glucuronidase (GUS) also traffic between cells, implying the presence of a plasmodesmal transport signal in MP (Waigmann and Zambryski, 1995).

The passage of MP–genome complexes through plasmodesmata is likely to require three general steps—binding at the plasmodesmal surface, transit through the channel, and release into the adjacent cell. Binding of MP–genome complexes at, and internalization into, plasmodesmata may occur by a process mediated by receptors or docking proteins at the plasmodesmal surface. Alternatively, movement of complexes into plasmodesmata may occur by a default pathway defined by the particular cytoskeletal-associated components involved in intracellular trafficking. Transit of MP–genome complexes may be driven by active mechanisms in which both MP and genome components move via interactions with a plasmodesmal trafficking apparatus. This apparatus is likely comprised of resident escort proteins, chaperones, and/or molecular motors. Whether genomes are transported as stable nucleoprotein complexes or as dynamic complexes in which MP subunits cycle on and off the genome is not known. The mechanisms governing release or delivery of the transport complex into the

adjacent cell are also poorly understood. By analogy with other cellular transport processes, plasmodesmal transport likely involves an energy requirement. Both TMV and CMV MPs have been shown to bind GTP (Li and Palukaitis, 1996), which could conceivably be transferred to and hydrolyzed by a plasmodesmal-associated GTPase during transport.

The interaction between MP and the plasmodesmal trafficking apparatus may also involve cycles of phosphorylation and dephosphorylation of MP. The TMV MP is phosphorylated in vivo and in vitro at Ser and Thr residues near the highly variable C-terminal region (Watanabe et al., 1992; Citovsky et al., 1993). The significance of phosphorylation is unclear, however, because although the C-terminal region contains host-range information, it is dispensable for cell-to-cell movement of TMV in tobacco (Berna et al., 1991; Fenczik et al., 1995). Many features of this intercellular transport model, including the involvement of GTP hydrolysis, are reminiscent of import and export through nuclear pores (Görlich and Mattaj, 1996). In fact, the structural and functional similarities between plasmodesmata and nuclear pores have been discussed extensively (Lucas et al., 1993).

It would seem generous of plants to provide a plasmodesmal trafficking pathway simply to accommodate invasion by viruses. Lucas (1995) has argued that such a pathway must be important for cell-to-cell trafficking of macromolecules required for normal plant growth and development. Direct evidence is now available to support this hypothesis. The maize *knotted1* (*kn1*) homeobox gene encodes a nuclear-localized transcriptional regulator, KN1, that influences cell fate at the vegetative meristem (Hake, 1992). The *kn1* mRNA is expressed in only a subset of shoot apical meristem cells that contain KN1. Microinjection experiments in tobacco mesophyll cells reveal that KN1 behaves like a viral MP: it increases the SEL of plasmodesmata and moves between cells (Lucas et al., 1995). Interestingly, KN1 also facilitates transport of its own mRNA. Thus, viral MPs almost certainly tap into an endogenous pathway for macromolecular trafficking. Furthermore, because evidence clearly suggests that plant virus evolution has involved the acquisition of cellular genes (Koonin and Dolja, 1993), it is possible that cellular proteins with plasmodesmal trafficking activity were the progenitors to viral MPs.

Intercellular Transport of Genomes by Tubule-Based Mechanisms

Several diverse viruses, including como-, caulimo-, nepo-, and tospoviruses, employ a cell-to-cell transport system that involves formation of tubules through cell walls and/or plasmodesmata (van Lent et al., 1990; Perbal et al., 1993; Wieczorek and Sanfaçon, 1993; Storms et al., 1995). In cowpea mosaic comovirus (CPMV)–infected cells, the tubules are composed of MP and possibly cellular constituents and possess an internal diameter of roughly the width of virions (van Lent et al., 1990, 1991; Kasteel et al., 1993). Electron microscopy suggests that the tubules project unidirectionally into one cell and are derived from plasmodesmata that have lost their desmotubules (Maule, 1991).

A striking feature of the CPMV, CaMV, and tomato spotted wilt tospovirus MPs is their ability to induce tubules in protoplasts and cultured insect cells. In both cell systems, expression of MP results in tubular protrusions of up to 50 μm in length extending from the cell surface (van Lent et al., 1991; Kasteel et al., 1993, 1996; Perbal et al., 1993; Wellink et al., 1993; Storms et al., 1995). The plasma membrane is contiguous with the exterior of the tubules. These structures exhibit many of the features of tubules visualized in plants in that they contain MP and, in the case of infected protoplasts, are filled with virus particles. Mature virions, however, are not required for CPMV tubule formation (Kasteel et al., 1993; Wellink et al., 1993). The protoplast data clearly indicate that plasmodesmata are unnecessary for most aspects of tubule development, but it is certainly possible that a plasmodesmal channel provides an opening to initiate tubule extension in intact tissue. The demonstration that tubules can be induced in insect cells suggests that these MPs interact with fundamentally important cellular components that are conserved across the boundaries of the animal and plant kingdoms.

A speculative transport model that integrates the known features of these tubule-inducing viruses can be proposed. Viral MPs are localized to plasmodesmata, where they induce removal of the desmotubule. Assembly of MP into tubules, possibly in association with the plasma membrane and host proteins, results in unidirectional extension of the tubule into the adjacent plant cell. Extending tubules may be anchored by cytoskeletal structures in one or both cells. Virions assembled in the cytoplasm are escorted to tubular structures through interactions with MP. Virions are then transported through tubules via specific MP–capsid protein interactions (Wellink and van Kammen, 1989) and deposited in the adjacent cell. It should be emphasized that besides its role in tubule formation, the CaMV MP displays ssRNA binding activity and limited sequence similarity with the TMV MP (Citovsky et al., 1991; Koonin et al., 1991; Thomas and Maule, 1995b), findings that could reflect multiple modes of CaMV transport.

Besides the tubule-forming viruses described above, some other viruses induce substantial physical modification of plasmodesmata within infected tissue. Plasmodesmata between cells infected by any of several potyviruses contain laminate, needlelike projections of a virus-encoded RNA helicase (CI protein; Langenberg, 1986; Lesemann, 1988). Unlike the tubules discussed above, the CI projections occur on each side of the plasmodesma of adjoining cells. The potyvirus capsid protein, which forms the coat of the flexuous rod-shaped virion, is essential for both cell-to-cell and long-distance movement (Dolja et al., 1994, 1995). Although evidence to indicate a direct role for the CI projections in movement is lacking, it is conceivable that the CI structures guide virions, or nonvirion complexes containing genomes and capsid protein, to and/or through plasmodesmata.

LONG-DISTANCE MOVEMENT

Long-distance or phloem-dependent movement requires that the virus be able to enter and exit bundle sheath cells, phloem parenchyma and companion cells, and sieve elements (Figure 1). Plasmodesmata provide symplastic connectivity between the epidermal/mesophyll cells and cells within the vasculature, including sieve elements. There are good reasons to believe, however, that long-distance movement involves viral and host functions that are distinct from those involved in movement through mesophyll cells.

Two critical points along the long-distance movement pathway are the entry into sieve elements and the exit from sieve elements. Plasmodesmata connecting a sieve element with its supporting companion cell possess a unique morphology that includes extensive branching on the companion cell side (Leisner and Turgeon, 1993). On the sieve element side, the plasmodesma forms a pore that lacks ER, although ER may be present over the pore within the sieve element. Limited analysis of SEL suggests that the companion cell/sieve element plasmodesmata have gating capacities that differ from those of plasmodesmata between mesophyll cells (Kempers et al., 1993). Because the sieve element lacks protein synthesis and virus genome replication activities, the exit process may involve a novel set of viral and host factors.

Most viruses that move cell to cell by a TMV-like mechanism require the capsid protein for long-distance movement. Virion assembly–defective mutants of TMV and RCNMV, for example, move cell to cell efficiently but exhibit defects in phloem-dependent long-distance transport (Dawson et al., 1988; Saito et al., 1990; Xiong et al., 1993; Vaewhongs and Lommel, 1995). In the case of TMV and its close relative, Odontoglossom ringspot virus (ORSV), the capsid protein controls in part the ability (TMV) or inability (ORSV) to move long distance in tobacco (Hilf and Dawson, 1993). Similarly, the ability of CMV and the inability of tomato aspermy cucumovirus to move long distance in cucumber is conditioned by the capsid protein (Taliansky and Garcia-Arenal, 1995). In the case of bipartite geminiviruses, the capsid protein may or may not be required, depending on the host and the viral genetic background, leading to the suggestion that long-distance movement can occur by both capsid protein–dependent and capsid protein–independent pathways (Pooma et al., 1996). Viruses encoding triple gene block MPs have differing requirements for capsid protein: potexviruses require capsid protein for systemic infection, whereas hordeiviruses do not (Petty et al., 1990; Forster et al., 1992; Baulcombe et al., 1995). The potyviruses have a capsid protein with discrete domains required for cell-to-cell and long-distance movement; the core domain is necessary for virion assembly and cell-to-cell movement, whereas the surface-oriented N- and C-terminal domains are required for long-distance movement (Dolja et al., 1994, 1995). Specific interactions between capsid protein and other viral or cellular factors necessary for long-distance movement have yet to be identified, but it is hypothesized that they facilitate entry into, flow through, or exit from the phloem.

What is the role of MP in phloem-dependent transport? Information concerning the role of MP in long-distance transport is scarce, mainly because of the difficulty in analyzing long-distance movement independent of cell-to-cell movement. However, there is limited genetic evidence that MP performs specific long-distance movement functions. Hybrid TMV genomes containing the ORSV MP are cell-to-cell movement competent in tobacco but defective in phloem-dependent transport (Hilf and Dawson, 1993). Removal of the C-terminal 11 amino acid residues of the ORSV MP confers on the hybrid virus long-distance movement function in tobacco but has little effect on cell-to-cell movement, implying that MP provides specific cell-to-cell and long-distance movement functions (Fenczik et al., 1995). Furthermore, several alanine-scanning mutants of RCNMV with substitutions affecting MP exhibit host-specific defects in long-distance transport (D. Giesmann-Cookmeyer and S.A. Lommel, personal communication). These mutants encounter a block to transport at the bundle sheath–phloem companion cell boundary.

Many viruses also encode proteins that provide functions needed for phloem-dependent but not cell-to-cell transport. For example, tombusviruses encode two proteins, p19 and p22, both of which are required for systemic infection. Cell-to-cell movement functions are provided by p22, whereas p19 promotes long-distance transport in a host-specific manner (Scholthof et al., 1995). CMV also encodes a protein, 2b, that promotes host-specific long-distance movement (S.-W. Ding et al., 1995), and the potyvirus HC-Pro protein provides functions required for both long-distance movement and efficient genome replication (Klein et al., 1994; Cronin et al., 1995). Additionally, many viruses encode replication proteins that appear to have specific roles in long-distance transport (Traynor et al., 1991; Nelson et al., 1993; Gal-On et al., 1994; X.-S. Ding et al., 1995; Weiland and Edwards, 1996). It must be stressed, however, that the biochemical roles of these proteins in long-distance transport are not yet known. Some of these proteins may actually have no direct role in movement but instead have an indirect function, such as that of a suppressor of a long-distance movement–restricting host response.

At which points in the long-distance movement pathway do unique virus–host interactions occur? Because TMV MP fails to dilate plasmodesmata between bundle sheath and phloem parenchyma cells, Ding et al. (1992a) postulated that movement past the bundle sheath layer requires functions in addition to MP, such as those provided by capsid protein. However, a correlation between plasmodesmal dilation in response to functional MP and the ability to support virus movement does not always hold (Deom et al., 1990; Waigmann and Zambryski, 1995). Also, TMV mutants lacking the capsid protein gene are able to reach phloem parenchyma cells by cell-to-cell movement (Ding et al., 1996), indicating that the capsid protein function in long-distance movement is only necessary after the virus has traversed the bundle sheath–phloem cell plas-

modesmata. The "masked" strain of TMV exhibits a delay in long-distance movement in tobacco compared with the U1 strain, and this correlates with an ~50% reduction in the numbers of phloem companion and vascular parenchyma cells infected in inoculated leaves (X.-S. Ding et al., 1995).

Host functions specifically affecting long-distance movement have also been inferred by the effects of variation in one or a few host genes. Cowpea chlorotic mottle virus is blocked at the bundle sheath/phloem cell boundary in inoculated leaves of the long-distance movement–restricting soybean line, PI 346304 (Goodrick et al., 1991). In contrast, TEV invades phloem cells in inoculated leaves of a tobacco variety, V20, which suppresses long-distance movement (Schaad and Carrington, 1996), implying that the transport block is encountered at the point of entry into and/or exit from sieve elements. In both of these examples, the movement restriction results from recessive alleles at two nonlinked loci. These genes might encode factors that interact with the respective viruses at unique points in the long-distance movement pathway. Alternatively, these genes might actually be conditioning a host defense response that limits movement into or through phloem.

Deposition of viral long-distance transport complexes into sieve elements and their presumed passive flow remain poorly understood processes. Loading of transport complexes in source tissues likely occurs through the plasmodesma pore by mechanisms similar to those used to load phloem proteins into sieve elements. The companion cell–sieve element plasmodesma is well adapted for transport of soluble macromolecules, allowing proteins synthesized in companion cells of one organ or tissue to be transported long distances (Fisher et al., 1992). For those virus–host combinations in which virion formation is necessary, the roles of MP or long-distance movement factors within sieve elements are not known. If these factors are transported, they may move either as free protein or as part of a complex with virions.

CONCLUDING REMARKS

The long overdue union between molecular plant virology and cell biology is yielding exciting insights into viral movement processes. We now have a base of information concerning the participation of viral proteins in cell-to-cell and long-distance movement and some clues about sites and structures within the cell at which they function. However, before assuming that we have a detailed picture of intercellular transport, it is important to consider the following gaps in our current state of knowledge. Little is known about the coupling between genome replication and movement. The factors that facilitate cytoskeletal transport of movement complexes toward plasmodesmata have yet to be identified. It is possible that these factors, which might include molecular motors and other microtubule- and microfilament-associated proteins, may provide a high degree of specificity in directing vectorial transport and therefore might represent key host determinants that govern virus or strain specificity. Despite emerging models of the structural organization of plasmodesmata, almost nothing is known about the identities and functions of plasmodesmal proteins. These proteins should fall into a number of structural and regulatory classes. Of particular importance are those proteins that may serve as receptors for movement complexes, because they may also contribute to host range specificity. Plasmodesmal proteins that function as shuttles between cells, that phosphorylate/dephosphorylate MP, that provide energy through ATP or GTP hydrolysis, and that mediate release of movement complexes have yet to be identified. Other important issues to resolve concern the mechanisms involved in plasmodesmal modification by tubule-forming MPs and the unique molecular requirements for long-distance transport.

Future advances will depend on the application of both biochemical and genetic approaches, combined with the creative use of virological tools. For example, progress in elucidating the cellular components in virus movement will depend in part on isolation of host mutants with defects at various points along the transport pathway. Considering that viruses exploit intra- and intercellular pathways that are necessary for normal plant growth and development, the isolation of large numbers of such mutants may require development of novel conditional screens.

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