PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Tobacco mosaic virus: a pioneer to cell–to–cell movement

Vitaly Citovsky

Phil. Trans. R. Soc. Lond. B 1999 **354**, 637-643
doi: 10.1098/rstb.1999.0415

THE ROYAL SOCIETY

Tobacco mosaic virus: a pioneer of cell-to-cell movement

Vitaly Citovsky

Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794-5215, USA (vitaly.citovsky@sunysb.edu)

Cell-to-cell movement of tobacco mosaic virus (TMV) is used to illustrate macromolecular traffic through plant intercellular connections, the plasmodesmata. This transport process is mediated by a specialized viral movement protein, P30. In the initially infected cell, P30 is produced by transcription of a subgenomic RNA derived from the invading virus. Presumably, P30 then associates with a certain proportion of the viral RNA molecules, sequestering them from replication and mediating their transport into neighbouring uninfected host cells. This nucleoprotein complex is targeted to plasmodesmata, possibly via interaction with the host cell's cytoskeleton. Prior to passage through a plasmodesma, the plasmodesmatal channel is dilated by the movement protein. It is proposed that targeting of P30-TMV RNA complexes to plasmodesmatal involves binding to a specific cell-wall-associated receptor molecule. This protein, designated p38, also functions as a protein kinase, phosphorylating P30 at its carboxy-terminus and minimizing P30-induced interference with plasmodesmatal permeability during viral infection.

Keywords: plasmodesmata; movement protein; protein–nucleic acid complexes; plasmodesma targeting signals; phosphorylation; regulation of plasmodesmatal permeability

1. INTRODUCTION

Katherine Esau was the first to postulate that viruses moved throughout the plant via plant intercellular connections, the plasmodesmata (Esau 1948). Viral spread through plant intercellular connections occurs in two distinct steps: local and systemic. Following initial infection, usually by mechanical or insect-mediated inoculation, many plant viruses spread from cell to cell through plasmodesmata until they reach the vascular system; the viruses are then transported systemically through the vasculature. Presumably, viral spread through the vascular tissue is a passive process, occurring with the flow of photoassimilates (reviewed by Leisner & Howell 1993); in contrast, cell-to-cell movement is an active function, requiring specific interaction between the virus and the plasmodesmata. With tobacco mosaic virus (TMV) and several other viruses, this interaction is mediated by virus-encoded non-structural movement proteins, which act to increase plasmodesmatal permeability and transport viral nucleic acids through the enlarged plasmodesmatal channels (reviewed by Lucas & Gilbertson 1994; Carrington *et al*. 1996; Ghoshroy *et al*. 1997).

The best-studied cell-to-cell movement protein is the 30 kDa protein (P30) of TMV (Deom *et al*. 1987). To date, four biological activities have been postulated for P30: (i) binding to TMV RNA, forming an extended P30-RNA complex that can penetrate the plasmodesmatal channel (Citovsky *et al*. 1990, 1992*a*); (ii) interacting with cytoskeletal elements to facilitate transport of the P30-TMV RNA complexes from the cell cytoplasm to plasmodesmata (Heinlein *et al*. 1995; McLean *et al*. 1995); (iii) increasing the size exclusion limit of plasmodesmata (Wolf *et al*. 1989); and (iv) interacting with a cell-wall-associated receptor, which then phosphorylates the bound movement protein, inactivating its ability to dilate plasmodesmata. Here, we examine these P30 activities and integrate them into a model for P30-mediated intercellular transport of TMV RNA.

2. MOVEMENT PROTEIN–NUCLEIC ACID COMPLEXES

In vitro studies showed that P30 binds both RNA and single-stranded (ss)DNA, but not double-stranded (ds)DNA, cooperatively and without sequence specificity (Citovsky *et al*. 1990, 1992*a*). This sequence non-specific binding explains the observation that coinfection with TMV can complement cell-to-cell movement of plant viruses in hosts in which they normally do not spread through plasmodesmata (reviewed by Carr & Kim 1983; Atabekov & Taliansky 1990). Electron microscopic observations revealed that P30 binding unfolds the nucleic acid molecule, creating an extended protein–RNA complex of 2.0–2.5 nm in diameter (Citovsky *et al*. 1992*a*). Because free-folded TMV RNA has been estimated to be 10 nm in diameter (Gibbs 1976), association with P30 probably converts it to a thinner form capable of transport through plasmodesmatal channels.

Following the demonstration that TMV P30 binds single-stranded nucleic acids (Citovsky *et al*. 1990, 1992*a*), cell-to-cell movement proteins of many other plant viruses were found to exhibit similar activity. For example, P1 of cauliflower mosaic virus (CaMV) was

shown to associate with both ssDNA and RNA to form long, thin complexes that closely resembled P30–ssDNA and P30–RNA complexes (Citovsky *et al*. 1991; Thomas & Maule 1995). Furthermore, the affinity of P1 for RNA was greater than that toward ssDNA, suggesting that P1–CaMV RNA complexes may be involved in the cell-to-cell spread of this virus (Citovsky, *et al*. 1991). This TMV-like mechanism for cell-to-cell movement may complement the previously characterized spread of CaMV in the form of whole viral particles through virus-modified plasmodesmata (Kitajima & Lauritis 1969). In addition to TMV and CaMV, the movement proteins of alfalfa mosaic virus (AMV), red clover necrotic mosaic dianthovirus (RCNMV) and several other viruses have been shown to bind single-stranded nucleic acids (Osman *et al*. 1992; Schoumacher *et al*. 1992; Pascal *et al*. 1994). As with TMV P30, these movement proteins bound nucleic acids cooperatively (Citovsky *et al*. 1991; Osman *et al*. 1992; Schoumacher *et al*. 1992); however, the movement protein of RCNMV did not appear to lengthen bound RNA molecules significantly (Fujiwara *et al*. 1993). Thus, transport through plasmodesmata via movement protein–nucleic acid intermediates may represent a common mechanism for cell-to-cell spread of many plant viruses.

Although many movement proteins function to transport viral nucleic acids, the degree of transport selectivity varies. For example, the P30 protein of TMV can bind and, by implication, transport any single-stranded nucleic acid (Citovsky *et al*. 1990, 1992*a*). In contrast, the RCNMV movement protein is capable of trafficking ssRNA but not ssDNA or dsDNA (Fujiwara *et al*. 1993), whereas the bean dwarf mosaic begomovirus (BDMV) BL1 movement protein facilitates transport of dsDNA but not ssDNA nor ssRNA molecules (Noueiry *et al*. 1994). However, transport of BDMV dsDNA seems incompatible with biochemical and genetic evidence obtained with another bipartite begomovirus, squash leaf curl virus (SqLCV), for which the transported form is thought to be genomic ssDNA (Pascal *et al*. 1994). Although BL1 binds nucleic acids only weakly (Pascal *et al*. 1994), it interacts with the second begomovirus movement protein, BR1 (Sanderfoot & Lazarowitz 1995), which directly associates with the transported nucleic acid molecule (Pascal *et al*. 1994). Thus, BR1 most likely binds the viral nucleic acid and transports it out of the host cell's nucleus, where begomoviruses replicate (Goodman 1981). BR1–ssDNA complexes (or BR1–dsDNA complexes, in the case of BDMV) associate with BL1, which then mediates plasmodesmatal transport (see also below).

3. MOVEMENT PROTEIN–CYTOSKELETON INTERACTION

Because TMV RNA translation, and therefore the production of P30, occurs in the host cell's cytoplasm (Palukaitis & Zaitlin 1986), cell-to-cell transport complexes of P30 and TMV RNA are most probably also formed in the cytoplasmic compartment. How, then, do these complexes arrive at plasmodesmata prior to cell-to-cell movement? Recent data suggest that P30 interacts with microtubules and, to a lesser extent, with actin microfilaments (Heinlein *et al*. 1995; McLean *et al*. 1995). This interaction was inferred from colocalization of P30,

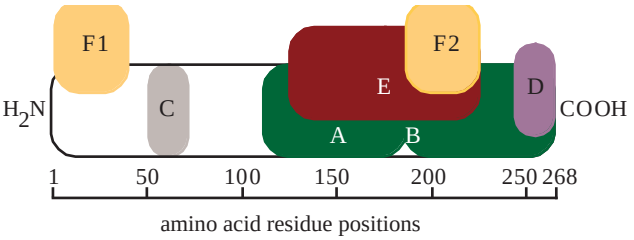

Figure 1. Functional domains of TMV P30. Domain A (aa 112–185), single-stranded nucleic acid binding; domain B (aa 186–268), single-stranded nucleic acid binding; domain C (aa 65–86), correct protein folding; domain D (aa 258, 261, 265), phosphorylation by a cell-wall-associated protein kinase; domain E (aa 126–224), increase in plasmodesmatal permeability; domains F1 and F2 (aa 165 and 185–224), binding to the cell-wall-associated p38 protein (P30 receptor).

transiently expressed in tobacco protoplasts, with tubulin as well as with actin filaments (McLean *et al*. 1995). The association of P30 with actin and tubulin was also demonstrated using *in vitro* binding assays (McLean *et al*. 1995). Furthermore, P30 tagged by translational fusion to the jellyfish green fluorescent protein (GFP) formed a filamentous network following transient expression in plant protoplasts. Interestingly, these filamentous arrays of GFP–P30 were best detected 18–20 h after expression. After 48–72 h, most GFP–P30 appeared as aggregates along the periphery of the protoplasts (McLean *et al*. 1995). Protoplasts from transgenic tobacco plants that express P30, which represents a steady-state expression system, contained P30 predominantly near the plasma membrane and the residual cell walls, where, in intact tissue, the plasmodesmata would be found (McLean *et al*. 1995). Finally, GFP–P30 was introduced into the TMV genome and the resulting modified virus retained infectivity (Heinlein *et al*. 1995). Fluorescent GFP–P30 expressed in tobacco protoplasts and leaf tissue following infection formed an intracellular network that co-aligned with cellular microtubules; however, no association of P30 with F-actin was detected in these experiments (Heinlein *et al*. 1995). Although the movement proteins of other plant viruses have not yet been tested for interaction with the cytoskeleton, it is likely that such an interaction will be found for many viral species.

4. INCREASE IN PLASMODESMATAL PERMEABILITY

The P30–TMV RNA cell-to-cell transport complex must transverse plasmodesmata to enter the neighbouring host cell. Although the estimated diameter of this nucleoprotein complex (Citovsky *et al*. 1992*a*) is relatively small (2.0–2.5 nm), it is still too large for the 1.5 nm-diameter channel through normal intact plasmodesmata (Wolf *et al*. 1989). To allow movement, therefore, P30 induces an increase in plasmodesmatal permeability. The ability of P30 to increase the plasmodesmatal size exclusion limit was first detected by injection of fluorescently labelled dextrans into leaf mesophyll cells of transgenic tobacco plants expressing P30 (Wolf *et al*. 1989). Unlike wild-type tobacco mesophyll plasmodesmata, which can traffic only dextrans up to 0.75–1.0 kDa, the P30-transgenic plants exhibited a plasmodesmatal size exclusion limit of almost 10 kDa (Wolf *et al*. 1989). Because the transgenic plant

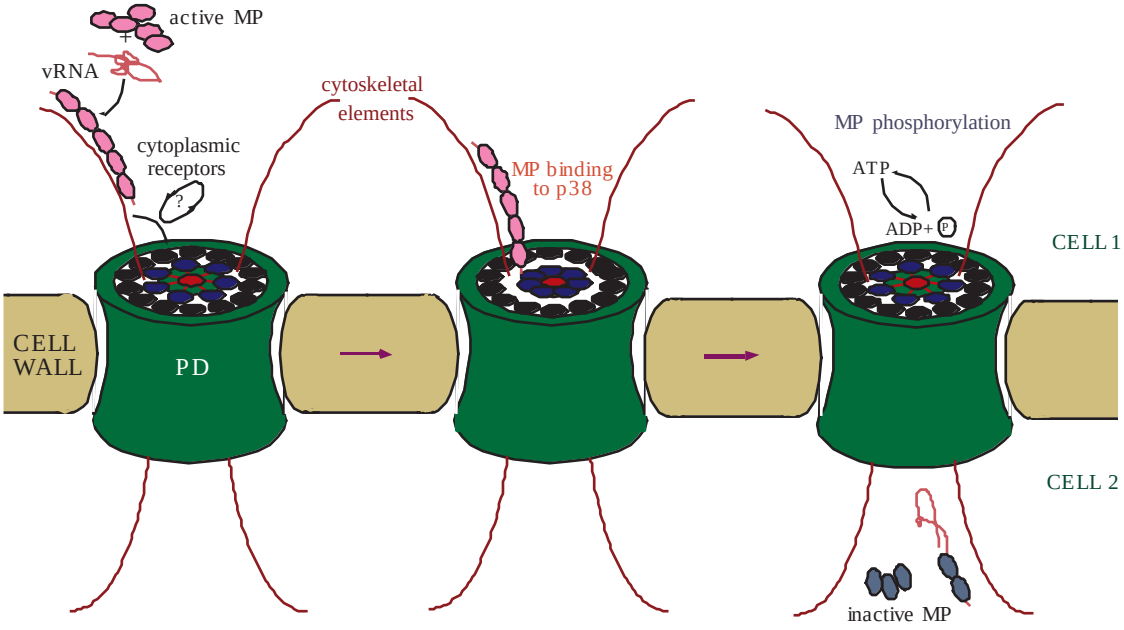

Figure 2. A model for production and plasmodesmatal transport of P30–TMV RNA complexes. MP, viral cell-to-cell movement protein, e.g. P30; vRNA, viral genomic RNA. See text for further details. (*a*) Targeting to plasmodesmata; (*b*) increase in plasmodesmatal permeability; (*c*) translocation, MP inactivation, and decrease in permeability.

tissue represents a steady state of P30 accumulation and activity, it remained unclear whether the increase in plasmodesmatal size exclusion limit was due to activation of an endogenous plasmodesmatal transport pathway or induction of a specific host response to this viral protein. Direct microinjection of purified P30 into wild-type tobacco mesophyll cells resulted in a relatively fast (3–5 min) increase in the size exclusion limit up to 20 kDa, indicating that P30 functions via the existing plasmodesmatal transport machinery (Waigmann *et al*. 1994). The increased size exclusion limit of 10–20 kDa (Wolf *et al*. 1989; Waigmann *et al*. 1994) corresponds to a dilated channel diameter of 5–9 nm, potentially allowing passage of 2.0–2.5 nm-wide P30–TMV RNA complexes (Citovsky *et al*. 1992*a*).

Microinjection experiments provided another important clue to P30 function. It was noted that large fluorescent dextrans moved not only into the cells adjacent to the microinjected cell, but also that they travelled as far as 20–50 cells away from the site of injection (Waigmann *et al*. 1994). These observations indicated that P30 itself must have moved through plasmodesmata to induce the increase in size exclusion limit in the distant mesophyll cells, providing the first evidence that plasmodesmata can allow the passage of protein molecules. An alternative and unlikely possibility, i.e. that P30 induced an intracellular signalling pathway rather than its own cell-to-cell movement, was ruled out later by immunolocalization experiments which showed that microinjected P30 indeed moves between plant cells (Waigmann & Zambryski 1995).

As for single-stranded nucleic acid binding, the ability to increase plasmodesmatal size exclusion limit is probably a property of many viral movement proteins. Presently, the cell-to-cell movement proteins of RCNMV, AMV, cucumber mosaic virus (CMV), tobacco rattle virus (TRV), potato virus X and the BL1 movement protein of BDMV have been shown to mediate transport of large fluorescent dextrans between plant cells (Derrick *et al*. 1992; Fujiwara *et al*. 1993; Poirson *et al*. 1993; Noueiry *et al*. 1994; Vaquero *et al*. 1994; Angell *et al*. 1996). RCNMV, CMV and BDMV movement proteins were also shown to move from cell to cell themselves (Fujiwara *et al*. 1993; Noueiry *et al*. 1994; Ding *et al*. 1995; Waigmann & Zambryski 1995). Collectively, these experiments have established the use of large fluorescent dextrans as an assay for interaction between plasmodesmata and viral movement proteins. Recent observations, however, questioned the biological relevance of this approach. P30 was coinjected with fluorescent dextrans into tobacco trichomes, which consist of a linear file of cells and, consequently, allow better visualization of intercellular transport (Waigmann & Zambryski 1995). Surprisingly, no increase in size exclusion limit could be detected, although trichome cells support viral infection and cell-to-cell movement. However, when P30 was fused translationally to the reporter enzyme, β-glucuronidase (GUS), the microinjected GUSP30 protein, moved efficiently between trichome cells (Waigmann & Zambryski 1995). This result strongly suggests that P30 is a *cis*-acting mediator of plasmodesmatal transport. In other words, P30 has to be physically associated with any transported molecule, such as viral RNA or GUS. This idea is consistent with most known examples of protein transport and targeting. For example, the increase in the nuclear pore size exclusion limit during nuclear import does not allow passage of protein molecules that are not directly associated with the nuclear localization signal (NLS) sequence (Goldfarb *et al*. 1986). Cell-to-cell transport of large fluorescent dextrans coinjected with P30 into the leaf mesophyll, then, may be only an 'afterglow' of the P30 biological activity when mesophyll (but not trichome) cell plasmodesmata remain open following microinjection or overexpression of the movement protein. True plasmodesmatal transport,

however, probably requires direct interaction between the movement protein and the transported molecule.

5. INTERACTION WITH A CELL-WALL-ASSOCIATED RECEPTOR

Presently, almost all our current knowledge about the process of TMV cell-to-cell movement derives from studies of its viral component, i.e. P30. Other than the cytoskeleton (Heinlein *et al*. 1995; McLean *et al*. 1995), no plant cell component that interacts with P30 has been identified. Recently, we have isolated a plant cell receptor for P30. Because P30 has been shown to localize to plasmodesmata within plant cell walls (Tomenius *et al*. 1987; Ding *et al*. 1992), it may specifically interact directly with a cell-wall-associated receptor. Thus, we developed a renatured blot overlay-binding assay for interaction between P30 and cell-wall proteins. In this technique, a protein mixture containing a putative receptor is separated by SDS-polyacrylamide gel electrophoresis (Laemmli 1970) and electrotransferred on to a PVDF membrane, followed by guanidine hydrochloride extraction of SDS from the blotted proteins. The membrane is then renatured, incubated with purified P30, washed and any P30 binding is detected using anti-P30 antibodies. These experiments identified a single protein band with a relative electrophoretic mobility of 38 kDa. This protein, designated p38, was not detected in the absence of P30, or upon probing an empty PVDF membrane with P30. Furthermore, no p38 band or any other P30-interacting proteins were detected in the soluble fractions of tobacco cells, suggesting that p38 may represent a specific cell-wall receptor for TMV P30.

To correlate binding of P30 to p38 with its biological activity, the P30 domains required for interaction with p38 were determined and compared to the known functional regions of P30. Figure 1 shows that two non-contiguous regions of P30 are involved in its association with p38. The first p38-binding domain, designated F1, is located at the amino terminus of the protein; this protein region has been shown to be essential for the movement function *in vivo* (Gafny *et al*. 1992; Lapidot *et al*. 1993). The second p38 binding domain, designated F2 and positioned in the carboxy-terminal part of the protein, overlaps with the previously identified P30 domain E required for the increase in plasmodesmatal permeability (Waigmann *et al*. 1994). Interestingly, it had been suggested that the E region interacts with a putative P30 receptor (Waigmann *et al*. 1994). Thus, P30 binding to p38 involves protein regions known to be required for the biological activity of this cell-to-cell transport protein. Because both F1 and F2 domains are necessary for binding, these protein regions probably form a single p38 recognition site within the native conformation of P30. However, it is possible that these major binding domains are augmented by the context of the native protein.

Previously, we have shown that P30 is phosphorylated by a cell-wall-associated protein kinase, at its carboxy-terminal serine and threonine residues (Citovsky *et al*. 1993). The ability of this protein kinase to phosphorylate P30 bound on the PVDF membrane was tested. A similar approach has been used to identify a protein kinase activity of photosystem II (Race & Hind 1996). To detect phosphorylation, P30 binding was followed by addition of radioactively labelled ATP and autoradiography. In these tests, protein kinase activity was found consistently in a single band with electrophoretic mobility similar to that of p38, following incubation of the electrophoresed cell-wall proteins with P30. No phosphorylation occurred when a P30 mutant, in which phosphorylation site residues Ser-258, Thr-261 and Ser-265 were replaced with non-phosphorylatable alanines (Citovsky *et al*. 1993), was used as substrate. However, this P30 mutant retained its ability to bind p38, suggesting that the receptor and protein kinase activities are independent and may represent two distinct protein functions. We also found that this protein kinase activity required the presence of Mg^{2+} but not Ca^{2+} cations, indicating that it is not responsible for the general Ca^{2+}-dependent protein kinase activity of plant cell walls (Epel 1994).

The possible biological function of P30 carboxy-terminal phosphorylation was examined using negatively charged amino-acid substitutions within the phosphorylation site. Substitution with aspartate or glutamate is known to mimic the electrostatic effects of phosphorylation (Dean & Koshland 1990). For example, replacement of serine by aspartate in the HPr protein has been shown to cause shifts in two-dimensional NMR spectra similar to those elicited by phosphorylation (Wittekind *et al*. 1989). Also, inactivation by phosphorylation of Ser-113 in isocitrate dehydrogenase is mimicked when aspartate is substituted at this site (Thorsness & Koshland 1987). Similarly, microinjection experiments showed that substituting Ser-258, Thr-261 and Ser-265 in P30 with aspartate residues abolished its ability to increase plasmodesmatal permeability, suggesting that phosphorylation has a role in down-regulation of the biological activity of P30 (J. S. Sheng, L. Waigmann, S. Ghoshroy, J. Hind, P. Zambryski and V. Citovsky, unpublished data).

The biological function of p38 is probably not limited to its role in virus movement. Because viruses often adapt existing cellular machinery for their own needs, TMV probably makes use of an endogenous pathway for cell-to-cell transport of proteins and nucleic acids. Indeed, it was suggested that plasmodesmatal transport of RNA is involved in signalling post-translational gene silencing (Palauqui *et al*. 1997). Thus, it is tempting to speculate that p38 has a natural cellular ligand that may mediate plasmodesmatal traffic of RNA and/or protein in uninfected tobacco plants.

6. POSSIBLE MECHANISMS FOR PLASMODESMATAL TRANSPORT

Transport through plasmodesmata most likely consists of two major steps: (i) recognition of the transported molecule in the cell cytoplasm and its targeting to the plasmodesmatal channel; and (ii) translocation (figure 2). In the initially infected cell, P30 is produced by translation of a subgenomic RNA produced during replication of the invading virus. Presumably, this protein then associates with a certain proportion of positive-strand genomic viral RNA molecules, sequestering them from replication and mediating their transport into the neighbouring uninfected host cells.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

P30–TMV RNA complexes destined for cell-to-cell transport may be recognized by their putative targeting sequence, the plasmodesma localization signal (PLS). P30 domains required for interaction with plasmodesmata (domain E) and/or binding to p38 (domains F1 and F2, figure 1) may carry such a signal. Similar transport of nucleic acids via a protein import pathway has been proposed for the nuclear import of *Agrobacterium* T-DNA associated with the bacterial VirD2 and VirE2 proteins (Citovsky *et al*. 1992*b*, 1994; Howard *et al*. 1992; Citovsky & Zambryski 1993; Guralnick *et al*. 1996; Zupan *et al*. 1996) and for that of the influenza virus genomic RNANP nucleoprotein complexes (O'Neill *et al*. 1995).

The putative PLS potentially interacts with specific cytoplasmic receptors (figure 2). These, as yet unidentified, receptor proteins may function to transport the PLS-containing protein to the plasmodesmatal annulus. Alternatively, the transported protein may be guided to a plasmodesma simply by association with cytoskeletal fibres (see above). In the latter case, the question of specific targeting remains to be resolved, i.e. whether or not there are microfilaments and microtubules that lead only to plasmodesmata.

Once at the plasmodesmatal channel, the transported protein or protein–PLS receptor complex must increase plasmodesmatal permeability to allow translocation. By analogy to nuclear import, GTPase and/or ATPase activities may be involved. Indeed, an ATPase activity has been localized to plasmodesmata (Zheng *et al*. 1985; Didehvar & Baker 1986), but further studies are necessary to ascertain the molecular nature of this enzyme. It is also possible that interaction of the transported protein with the cytoskeleton directly 'relaxes' the plasmodesmatal annulus, increasing its size exclusion limit for translocation. Elucidation of the mechanism of plasmodesmatal gating will depend on successful purification and characterization of the protein components of this channel.

Interaction of viral movement proteins with plasmodesmata may interfere with normal intercellular communication, and thus be detrimental to the physiology of the host plant. It is likely, therefore, that a mechanism exists to regulate the activity of P30 and, possibly, cellular proteins capable of plasmodesmatal transport (Lucas *et al*. 1995). Potentially, P30 phosphorylation is involved in such regulation, minimizing P30 interference with plasmodesmatal permeability during viral infection. Thus, two distinct biological functions may be required for the TMV cell-to-cell transport process. First, a specific receptor mediates P30 targeting to the host cell wall and, probably, to plasmodesmata. This function may be essential for plasmodesmatal transport; therefore, P30 domains involved in p38 binding are required for its biological activity. Second, a cell-wall-associated protein kinase phosphorylates P30 that has already performed its function within plasmodesmata (figure 2). This activity may play a role in down-regulation of P30, but is not directly required for its function. Indeed, the phosphorylation domain D of P30 (figure 1) is dispensable for the activity of this protein since its removal results in slightly, but consistently, enhanced viral movement (Gafny *et al*. 1992).

By analogy to nuclear import, another possibility for plasmodesmatal regulation is cytoplasmic anchoring. Interaction of P30 with the cytoskeleton may serve such a function by immobilizing P30 in the cell cytoplasm. This interaction may also mask the putative PLS sequence on the transported protein. Similar regulation by cytoplasmic anchoring has been described for nuclear import of the NF-κB transcription factor (reviewed by Dingwall 1991).

7. FUTURE DIRECTIONS

In recent years, the previously dormant field of plasmodesmatal macromolecular traffic has gained much impetus. Characterization of plasmodesmatal transport mechanisms will have a profound effect on our understanding of intercellular signalling and plant–pathogen interactions. Undoubtedly, biochemical and structural characterization of plasmodesmata and microinjection studies will continue to enhance our knowledge of intercellular communication and its role in plant development and morphogenesis. The greatest progress, however, may come from applying a genetic approach to dissect the molecular pathway for plasmodesmatal transport. For example, *Arabidopsis* mutants may be isolated that are resistant to systemic and/or cell-to-cell virus spread, potentially due to specific blockage in transport through plasmodesmata. One such mutant resistant to systemic spread of tobamoviruses has already been identified (Lartey *et al*. 1998). In addition, *Arabidopsis* mutants (Mahajan *et al*. 1998) and ecotypes with altered systemic movement of other plant viruses have been reported (Leisner *et al*. 1993; Lee *et al*. 1994; Simon 1994). Ultimately, isolation of additional mutants will identify most genes involved in plasmodesmatal transport and reveal the functional components of this pathway.

I thank the editors for critical reading and editing of the manuscript. Research in my laboratory is supported by grants from National Institutes of Health (grant no. R01-GM50224), the US Department of Agriculture (grant no. 94-02564), and the US–Israel Binational Research and Development Fund (BARD) (grant no. US-2247-93) to V.C.

REFERENCES

Angell, S. M., Davies, C. & Baulcombe, D. C. 1996 Cell-to-cell movement of potato virus X is associated with a change in the size exclusion limit of plasmodesmata in trichome cells of *Nicotiana clevelandii*. *Virology* **216**, 197–201.

Atabekov, J. G. & Taliansky, M. E. 1990 Expression of a plant virus-coded transport function by different viral genomes. *Adv. Virus Res.* **38**, 201–248.

Carr, R. J. & Kim, K. S. 1983 Evidence that bean golden mosaic virus invaded non-phloem tissue in double infections with tobacco mosaic virus. *J. Gen. Virol.* **64**, 2489–2492.

Carrington, J. C., Kassachau, K. D., Mahajan, S. K. & Schaad, M. C. 1996 Cell-to-cell and long-distance transport of viruses in plants. *Plant Cell* **8**, 1669–1681.

Citovsky, V. & Zambryski, P. 1993 Transport of nucleic acids through membrane channels: snaking through small holes. *A. Rev. Microbiol.* **47**, 167–197.

Citovsky, V., Knorr, D., Schuster, G. & Zambryski, P. 1990 The P30 movement protein of tobacco mosaic virus is a single-strand nucleic acid binding protein. *Cell* **60**, 637–647.

Citovsky, V., Knorr, D. & Zambryski, P. 1991 Gene I, a potential movement locus of CaMV, encodes an RNA binding protein. *Proc. Natl Acad. Sci. USA* **88**, 2476–2480.

Citovsky, V., Wong, M. L., Shaw, A., Prasad, B. V. V. & Zambryski, P. 1992*a* Visualization and characterization of tobacco mosaic virus movement protein binding to single-stranded nucleic acids. *Plant Cell* **4**, 397–411.

Citovsky, V., Zupan, J., Warnick, D. & Zambryski, P. 1992*b* Nuclear localization of *Agrobacterium* VirE2 protein in plant cells. *Science* **256**, 1803–1805.

Citovsky, V., McLean, B. G., Zupan, J. & Zambryski, P. 1993 Phosphorylation of tobacco mosaic virus cell-to-cell movement protein by a developmentally-regulated plant cell wall-associated protein kinase. *Genes Dev.* **7**, 904–910.

Citovsky, V., Warnick, D. & Zambryski, P. 1994 Nuclear import of *Agrobacterium* VirD2 and VirE2 proteins in maize and tobacco. *Proc. Natl Acad. Sci. USA* **91**, 3210–3214.

Dean, A. M. & Koshland, D. E. 1990 Electrostatic and steric contributions to regulation at the active site of isocitrate dehydrogenase. *Science* **249**, 1044–1046.

Deom, C. M., Shaw, M. J. & Beachy, R. N. 1987 The 30-kilo-Dalton gene product of tobacco mosaic virus potentiates virus movement. *Science* **327**, 389–394.

Derrick, P. M., Barker, H. & Oparka, K. J. 1992 Increase in plasmodesmatal permeability during cell-to-cell spread of tobacco rattle tobravirus from individually inoculated cells. *Plant Cell* **4**, 1405–1412.

Didehvar, F. & Baker, D. A. 1986 Localization of ATPase in sink tissue of *Ricinus*. *Ann. Bot.* **40**, 823–828.

Ding, B., Haudenshield, J. S., Hull, R. J., Wolf, S., Beachy, R. N. & Lucas, W. J. 1992 Secondary plasmodesmata are specific sites of localization of the tobacco mosaic virus movement protein in transgenic tobacco plants. *Plant Cell* **4**, 915–928.

Ding, B., Li, Q., Nguyen, L., Palukaitis, P. & Lucas, W. J. 1995 Cucumber mosaic virus 3a protein potentiates cell-to-cell trafficking of CMV RNA in tobacco plants. *Virology* **207**, 345–353.

Dingwall, C. 1991 Transport across the nuclear envelope: enigmas and explanations. *BioEssays* **13**, 213–218.

Epel, B. L. 1994 Plasmodesmata: composition, structure and trafficking. *Plant. Mol. Biol.* **26**, 1343–1356.

Esau, K. 1948 Some anatomical aspects of plant virus disease problems. II. *Bot. Rev.* **14**, 413–449.

Fujiwara, T., Giesman-Cookmeyer, D., Ding, B., Lommel, S. A. & Lucas, W. J. 1993 Cell-to-cell trafficking of macromolecules through plasmodesmata potentiated by the red clover necrotic mosaic virus movement protein. *Plant Cell* **5**, 1783–1794.

Gafny, R. M. L., Berna, A., Holt, C. A., Deom, C. M. & Beachy, R. N. 1992 Effects of terminal deletion mutations on function of the movement protein of tobacco mosaic virus. *Virology* **187**, 499–507.

Ghoshroy, S., Lartey, R., Sheng, J. & Citovsky, V. 1997 Transport of proteins and nucleic acids through plasmodesmata. *A. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 27–49.

Gibbs, A. J. 1976 Viruses and plasmodesmata. In *Intercellular communication in plants: studies on plasmodesmata* (ed. B. E. S. Gunning & A. W. Robards), pp. 149–164. Berlin: Springer-Verlag.

Goldfarb, D. S., Gariepy, J., Schoolnik, G. & Kornberg, R. D. 1986 Synthetic peptides as nuclear localization signals. *Nature* **322**, 641–644.

Goodman, R. M. 1981 Geminiviruses. In *Handbook of plant virus infection and comparative diagnosis* (ed. E. Kurstak), pp. 879–910. New York: Elsevier/North Holland Biomedical Press.

Guralnick, B., Thomsen, G. & Citovsky, V. 1996 Transport of DNA into the nuclei of *Xenopus* oocytes by a modified VirE2 protein of *Agrobacterium*. *Plant Cell* **8**, 363–373.

Heinlein, M., Epel, B. L. & Beachy, R. N. 1995 Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* **270**, 1983–1985.

Howard, E., Zupan, J., Citovsky, V. & Zambryski, P. 1992 The VirD2 protein of *A. tumefaciens* contains a C-terminal bipartite nuclear localization signal: implications for nuclear uptake of DNA in plant cells. *Cell* **68**, 109–118.

Kitajima, E. W. & Lauritis, J. A. 1969 Plant virions in plasmodesmata. *Virology* **37**, 681–685.

Laemmli, U. K. 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **277**, 680–685.

Lapidot, M., Gafny, R., Ding, B., Wolf, S., Lucas, W. J. & Beachy, R. N. 1993 A dysfunctional movement protein of tobacco mosaic virus that partially modifies the plasmodesmata and limits virus spread in transgenic plants. *Plant J.* **4**, 959–970.

Lartey, R. T., Ghoshroy, S. & Citovsky, V. 1998 Identification of an *Arabidopsis thaliana* mutation (*vsm1*) that restricts systemic movement of tobamoviruses. *Molec. Pl. Microbe Interact.* **11**, 706–709.

Lee, S., Stenger, D. C., Bisaro, D. M. & Davis, K. R. 1994 Identification of loci in *Arabidopsis* that confer resistance to geminivirus infection. *Plant J.* **6**, 525–535.

Leisner, S. M. & Howell, S. H. 1993 Long-distance movement of viruses in plants. *Trends Microbiol.* **1**, 314–317.

Leisner, S. M., Turgeon, R. & Howell, S. H. 1993 Effects of host plant development and genetic determinants on the long-distance movement of cauliflower mosaic virus in *Arabidopsis*. *Plant Cell* **5**, 191–202.

Lucas, W. J., Bouche-Pillon, S., Jackson, D. P., Nguyen, L., Baker, L., Ding, B. & Hake, S. 1995 Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science* **270**, 1980–1983.

Lucas, W. J. & Gilbertson, R. L. 1994 Plasmodesmata in relation to viral movement within leaf tissues. *A. Rev. Phytopathol.* **32**, 387–411.

McLean, B. G., Zupan, J. & Zambryski, P. 1995 Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. *Plant Cell* **7**, 2101–2114.

Mahajan, S. K., Chisholm, S. T., Whitham, S. A. & Carrington, J. C. 1998 Identification and characterization of a locus (RTM1) that restricts long-distance movement of tobacco etch virus in *Arabidopsis thaliana*. *Plant J.* **14**, 177–186.

Noueiry, A. O., Lucas, W. J. & Gilbertson, R. L. 1994 Two proteins of a plant DNA virus coordinate nuclear and plasmodesmatal transport. *Cell* **76**, 925–932.

O'Neill, R. E., Jaskunas, R., Blobel, G., Palese, P. & Moroianu, J. 1995 Nuclear import of influenza virus RNA can be mediated by viral nucleoprotein and transport factors required for protein import. *J. Biol. Chem.* **270**, 22 701–22 704.

Osman, T. A. M., Hayes, R. J. & Buck, K. W. 1992 Cooperative binding of the red clover necrotic mosaic virus movement protein to single-stranded nucleic acids. *J. Gen. Virol.* **73**, 223–227.

Palauqui, J. C., Elmayan, T., Pollien, J. M. & Vaucheret, H. 1997 Systemic acquired silencing: transgene-specific post-translational silencing is transmitted by grafting from silenced stocks to non-silenced scions. *EMBO J.* **16**, 4738–4745.

Palukaitis, P. & Zaitlin, M. 1986 Tobacco mosaic virus: infectivity and replication. In *Plant viruses*, vol. 2. *The rod-shaped plant viruses* (ed. M. H. V. Van Regenmortel & H. Fraenkel-Conrat), pp. 105–131. New York: Plenum Press.

Pascal, E., Sanderfoot, A. A., Ward, B. M., Medville, R., Turgeon, R. & Lazarowitz, S. G. 1994 The geminivirus BR1 movement protein binds single-stranded DNA and localizes to the cell nucleus. *Plant Cell* **6**, 995–1006.

Poirson, A., Turner, A. P., Giovane, C., Berna, A., Roberts, K. & Godefroy-Colburn, T. 1993 Effect of the alfalfa mosaic virus movement protein expressed in transgenic plants on the permeability of plasmodesmata. *J. Gen. Virol.* **74**, 2459–2461.

Race, H. L. & Hind, G. 1996 A protein kinase in the core of photosystem II. *Biochemistry* **35**, 13 006–13 010.

Sanderfoot, A. A. & Lazarowitz, S. G. 1995 Cooperation in viral movement: the geminivirus BL1 movement protein interacts with BR1 and redirects it from the nucleus to the cell periphery. *Plant Cell* **7**, 1185–1194.

Schoumacher, F., Erny, C., Berna, A., Godefroy-Colburn, T. & Stussi-Garaud, C. 1992 Nucleic acid binding properties of the alfalfa mosaic virus movement protein produced in yeast. *Virology* **188**, 896–899.

Simon, A. E. 1994 Interactions between *Arabidopsis thaliana* and viruses. In *Arabidopsis*. (ed. E. M. Meyerowitz & C. R. Somerville), Monograph 27, pp. 685–704. Cold Spring Harbor Laboratory Press.

Thomas, C. L. & Maule, A. J. 1995 Identification of the cauliflower mosaic virus movement protein RNA binding domain. *Virology* **206**, 1145–1149.

Thorsness, P. E. & Koshland, D. E. 1987 Inactivation of isocitrate dehydrogenase by phosphorylation is mediated by the negative charge of the phosphate. *J. Biol. Chem* **262**, 10 422–10 425.

Tomenius, K., Clapham, D. & Meshi, T. 1987 Localization by immunogold cytochemistry of the virus coded 30 K protein in plasmodesmata of leaves infected with tobacco mosaic virus. *Virology* **160**, 363–371.

Vaquero, C., Turner, A. P., Demangeat, G., Sanz, A., Serra, M. T., Roberts, K. & Garcia-Luque, I. 1994 The 3a protein from cucumber mosaic virus increases the gating capacity of plasmodesmata in transgenic tobacco plants. *J. Gen. Virol.* **75**, 3193–3197.

Waigmann, E., Lucas, W., Citovsky, V. & Zambryski, P. 1994 Direct functional assay for tobacco mosaic virus cell-to-cell movement protein and identification of a domain involved in increasing plasmodesmatal permeability. *Proc. Natl Acad. Sci. USA* **91**, 1433–1437.

Waigmann, E. & Zambryski, P. 1995 Tobacco mosaic virus movement protein-mediated transport between trichome cells. *Plant Cell* **7**, 2069–2079.

Wittekind, M., Reizer, J., Deutscher, J., Saier, M. H. & Klevit, R. E. 1989 Common structural changes accompany the functional inactivation of HPr by seryl phosphorylation or by serine to aspartate substitution. *Biochemistry* **28**, 9908–9912.

Wolf, S., Deom, C. M., Beachy, R. N. & Lucas, W. J. 1989 Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. *Science* **246**, 377–379.

Zheng, G.-C., Nie, X.-V., Wang, Y.-X., Jian, L.-C., Sun, L.-H. & Sun, D.-L. 1985 Cytochemical localization of ATPase activity during cytomixis in pollen mother cells of David lily-*Lilium davidii* var. Willmottiae and its relation to the intercellular migrating chromatin substance. *Acta Acad. Sin.* **27**, 26–32.

Zupan, J., Citovsky, V. & Zambryski, P. 1996 *Agrobacterium* VirE2 protein mediates nuclear uptake of ssDNA in plant cells. *Proc. Natl Acad. Sci. USA* **93**, 2392–2397.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES