Arch. Phytopath. Pflanz., 2000, Vol. 33, pp. 99–110
Reprints available directly from the publisher
Photocopying permitted by license only

CELL-TO-CELL MOVEMENT OF PLANT VIRUSES THROUGH PLASMODESMATA: A REVIEW

STEVE KOLL[a,*] and CARMEN BÜTTNER[b,†]

[a]*University of Ghent, Faculty of Agriculture and Applied Biological Sciences, Lab. Biochemistry and Molecular Cytology, Coupure Links 653, 9000 Gent, Belgium;* [b]*Humboldt-Universität zu Berlin, Institut fuer Gartenbauwissenschaften, Fachgebiet Phytomedizin, Lentzeallee 55/57, D-14195 Berlin*

(Received 25 January 2000)

Plasmodesmata are cytoplasmic bridges in plants through which intercellular communication occurs. This involves the transport of ions, photoassimilates, growth hormones as well as protein-nucleic acid complexes. Although these molecules are rather small (< 1 kDa) plant viruses succeed in using these intercellular highways to transport their genome. These viruses alter the plasmodesmata in some way to allow the transport of such large molecules. This review deals with how plant viruses manage this with the help of movement proteins and the cytoskeleton.

Keywords: Plasmodesmata; movement protein; size exclusion limit; dextrans; trichome

INTRODUCTION

Intercellular communication in plants occurs through cytoplasmic bridges called plasmodesmata (PD) [1]. Historically, PD have been assigned a passive role: creating cytoplasmic continuity between plant cells to allow free transport of small metabolites and growth hormones less than 1 kilodalton (kDa). When it was discovered that plant viruses

*Corresponding author. Tel.: ++32 9 264 5969, Fax: ++32 9 264 6219, e-mail: steve.koll@rug.ac.be
†Tel.: +49 30 314 71 175/139/297, Fax: +49 30 314 71 178, e-mail: carmen.buettner@agrar.hu-berlin.de

pirate PD for movement of their genomes during infection, it was proposed that viruses altered the PD to allow transport of very large molecules. This review deals with this and other associated capabilities of plant viruses.

PLASMODESMATA: PLANT CHANNELS FOR MOLECULES ON THE MOVE

In recent years, plasmodesmata have been implicated not only in transport of small molecules such as water, ions and photoassimilates, but in intercellular traffic of proteins, nucleic acids, and protein-nucleic acid complexes. Interestingly, this ability to transport large molecules and complexes has been described for only one other biological channel, the nuclear pore [2].

The current structural model of a simple plasmodesma derives from electron microscopy experiments that used high-pressure freezing to preserve fine details of tobacco mesophyll and vascular cells [4, 5]. In contrast to their animal counterparts – gap junctions between closely appressed cells – PD are elongated structures that traverse the thick cell walls that surround plant cells [1]. The endoplasmic reticulum passes through the plasmodesma and is both surrounded by and filled with regularly-spaced globular particles approximately 3 nm in diameter; this membrane-protein complex is referred to as the desmotubule or appressed endoplasmic reticulum (Fig.1). The

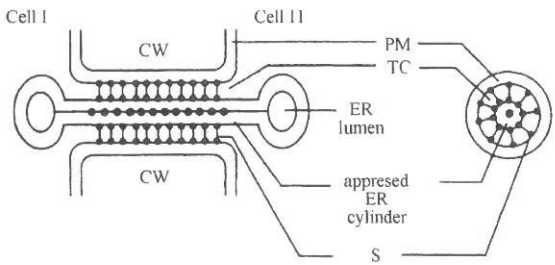

FIGURE 1 A schematic model of PD substructure based on studies on tobacco mesophyll and vascular cells. A median-longitudinal view is shown on the left and a transverse view on the right. Whether the spoke-like extensions (S) connect appressed ER and plasma membrane (PM) along the entire length, or partial length, of the PD needs further investigation. CW, cell wall; TC, putative transport channels (after [4]).

particles associated with the outer leaflet of the appressed endoplasmic reticulum appear to be connected to those on the inner leaflet by most likely proteinaceous filaments. Further, globular particles are embedded in the inner leaflet of the plasmalemma, greatly restricting the open space [5]. In addition to the endoplasmic reticulum, plasmodesmata may associate with cytoskeletal elements such as myosin and actin [2].

Plasmodesmata follow a complex developmental pathway. There are two types of plasmodesmata, which may be morphologically defined as simple and branched, or developmentally as primary and secondary. Primary or simple plasmodesmata are found predominantly in young tissue and consist of a simple, single channel, as pictured in Figure 1. Secondary or branched plasmodesmata are found in older tissues and show a higher degree of variability, often with many single channels leading into a larger central cavity [6]. Functionally, the secondary plasmodesmata differ in several ways from simple ones, most notably in response to viral infection. Viruses move through simple plasmodesmata [9].

CELL-TO-CELL TRAFFICKING OF VIRAL PROTEINS

Transport through plasmodesmata appears to be very complex. While micro-channels of PD have a diameter of ca. 2.5 nm [4], the unassisted, passive transport through PD, studied using microinjected dyes, appears to be limited to molecules up to 1.5–2.0 nm in diameter, equivalent to a molecular mass of 0.75–1.0 kDa [2]. The smallest diameter of plant viruses measured in any given angle is at least 10 nm. Even free, folded viral nucleic acids are estimated to be larger than 10 nm [4]. Thus, a virus cannot simply diffuse through PD. Whereas several factors act to decrease plasmodesmal permeability (*e.g.*, divalent cation, aromatic amino acids), only one endogenous plant protein, KN1, encoded by the maize knotted-1 homeobox gene, is known to increase plasmodesmal permeability. Furthermore, the KN1 protein also facilitates the movement of dextrans and proteins larger than 20 kDa [7]. Plant viruses have also developped strategies to overcome the limited plasmodesmal gate size. Many viruses have evolved to increase the plasmodesmal size exclusion limit (SEL) during

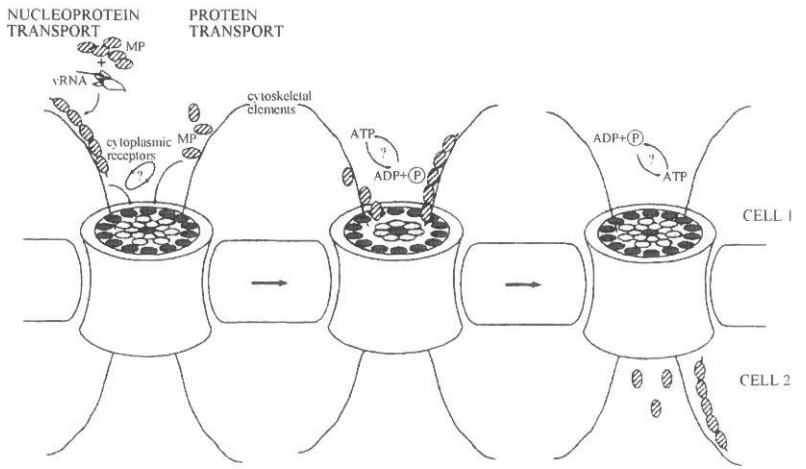

FIGURE 2 A model for plasmodesmal transport of viral movement proteins and movement protein-nucleic acid complexes. Left, targeting to PD; middle, interaction with PD and increase in permeability; right, translocation and decrease in permeability (after [2]).

local and systemic spread of infection. The mechanism by which this increase in plasmodesmal permeability occurs is unknow, but we do know that many known viruses encode one or more movement proteins (MPs) that interact with PD to facilitate spreading of viral genomes or virions from cell to cell. For example, it is possible that a conformational change in the aforementioned filaments enlarges the permeable space of plasmodesmal channels by pulling the globular particles into the appressed ER (Fig. 2) [2]. Specific mechanisms of MP interactions with PD vary from virus to virus. Two broad mechanisms of viral cell-to-cell movement are best characterized to date: one that involves viral movement as viral particles (virions) and the other that involves viral movement presumably as viral ribonucleoprotein complexes [4].

MOVEMENT PROTEIN-NUCLEIC ACID COMPLEXES

The best studied cell-to-cell movement protein is the 30 kDa protein (P30) of tobacco mosaic virus (TMV). P30 has been suggested to possess three biological activities [2]. It is thought to bind TMV RNA,

forming an extended P30-RNA complex that can penetrate the plasmodesmal channel. It also may interact with the cytoskeletal elements to facilitate transport of the P30-TMV RNA complexes from the cell cytoplasm to the PD. Finally, P30 functions to increase the SEL of PD. P30 binds both RNA and ssDNA, but not dsDNA, without sequence specificy. This sequence non-specific binding explains the observations that coinfection with TMV can complement cell-to-cell movement of plant viruses which normally do not spread through plasmodesmata.

At least for some viruses that are thought to traffic from cell to cell in the form of ribonucleoprotein complexes, viral proteins in addition to MPs appear to be required for cell-to-cell trafficking of viral genomes. For instance, cell-to-cell movement of cucumber mosaic virus (CMV) requires coat protein (CP); for potyviruses, CP and the cylindrical inclusion protein (CI) are all involved in viral cell-to-cell movement [4] for potexviruses, the triple gene block (TGB) proteins induce plasmodesmal gating [12].

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 (Fig. 2). Because the free folded TMV RNA has been estimated to be 10 nm in diameter, association with P30 likely shapes it in a thinner transferable form capable of transport through plasmodesmal channels [2]. However, it was shown that, for the red clover necrotic mosaic virus (RCNMV), unfolding of a vRNA during trafficking through the PD is likely necessary, but that it may be accomplished by viral and/or cellular proteins other than MP, or by a combination of MP and such putative viral and/or cellular proteins [4].

Numerous viruses appear to form tubular structures across the cell walls for viral particles to move intercellularly. These include for instance the tobacco ringspot virus (TRSV), the carnation etched ring virus (CERV) and the cauliflower mosaic virus (CaMV). Experiments in which a viral MP is expressed in plant protoplasts and/or insect cells indicate that, without the participation of other viral proteins or specific plant components, the MPs of some viruses (like CaMV) are sufficient to induce tubular structure formation by themselves [4].

Many other viruses, however, do not form such tubular structures during infection and have to rely on the biological activity of their

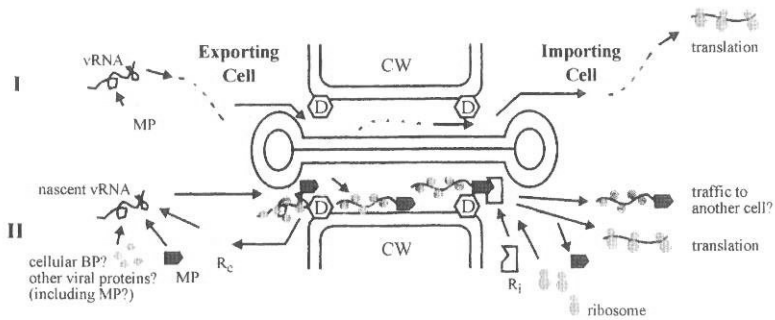

FIGURE 3 Models for viral ribonucleoprotein complex travelling from an exporting cell into an importing cell. In model I, MP binds to a nascent vRNA cooperatively and in a non-sequence specific manner. The MP-vRNA complex then travels from cell to cell. In model II, the protein components of a ribonucleoprotein complex may consist of more than MP. Cellular binding protein (BP), and/or other viral proteins are also involved, perhaps depending on viruses. The MP may bind to part of the RNA and serve as a pilot to be recognized by a cytosolic receptor (Re) in the exporting cell for targeting to the PD. At the orifice of PD, the complex interacts with a docking protein (D) presumably located in the plasma membrane, the receptor is recycled, and the ribonucleoprotein complex is translocated through the PD. On the importing cell side, a different cytosolic receptor (Ri) recognizes and imports the complex, presumably involving interactions between the receptor, MP, and the docking protein (D). CW, cell wall (after [4]).

MPs for their cell-to-cell movement. In general, trafficking of fluorescently labelled viral proteins form one cell to another can be detected within seconds to minutes, implying that MPs operate an endogenous PD transport pathway [1, 4].

Two models for a viral ribonucleoprotein trafficking through PD, based on studies on TMV and RCNMV MPs, respectively, are presented in Figure 3 for comparison [4]. It is important to keep in mind that direct experimental evidence for any model is still lacking.

THE MOVEMENT OF DNA VIRUSES

The movement of DNA viruses such as geminiviruses requires coordinated nuclear and PD trafficking of viral DNAs. Geminiviruses are the only plant virus group with a single stranded DNA genome. These viruses encode a BV1 protein that is responsible for nuclear trafficking of the viral DNA, and a BC1 protein that is involved in cell-to-cell trafficking of the viral DNA. The BC1 MP appears to function

similarly to the MPs of TMV and RCNMV in that it was able to traffic the viral genome in the absence of other viral proteins. It is been proposed, that BV1 binds the viral ssDNA, shuttles it between the cytoplasm and nucleus, and in the cytoplasm the BC1 traps the BV1-ssDNA complexes and trafficks them from cell-to-cell [4].

MOVEMENT TOWARDS THE PD: INVOLVMENT OF THE CYTOSKELETON

Because TMV RNA translation and therefore the production of P30 occurs in the host cell cytoplasm, P30-TMV RNA cell-to-cell transport complexes most likely are also formed in the cytoplasmic compartment [2]. How, then, do these complexes arive at plasmodesmata prior to the cell-to-cell movement? It has been suggested that P30 interacts with microtubuli and, to a lesser extent, with actin microfilaments. P30 association with tubulin was also demonstrated [8]. These observations suggest that P30 may interact with the cytoskeletal elements in the host cell cytoplasm and use them as tracks to migrate to the cell periphery and, ultimately, to plasmodesmata. In hindsight, these results are obvious because simple viscosity would hinder diffusion of an elongated viral genome coated with MP. That the MP proteins themselves track along the cytoskeleton suggests that association of a viral nucleoprotein complex with the cytoskeleton is not passive, but may be determined by specific signal sequences (see below) [1].

Because P30 is most likely associated with the viral RNA during infection, it is the P30-TMV RNA complex that may interact with the host cell microtubuli and microfilaments during transport to plasmodesmata (Fig. 2) [2].

Alternatively, interaction with the cytoskeleton may anchor P30 or P30-TMV RNA complexes in the cytoplasm, gradually releasing them in response to an, as yet unidentified, signal for plasmodesmal transport to occur. In this case, association with microtubuli and microfilaments would function as a regulatory mechanism for plasmodesmal transport rather than the targeting apparatus [2].

The cytoskeleton may also participate in the gating of PD. Actin filaments traverse PD channels [1], and actin may act as a sphincter at the neck region of PD. Additional cellular factors could interact with

actin to generate an open or closed PD conformation and so regulate transport.

INCREASE IN PLASMODESMAL PERMEABILITY

Once the P30-TMV RNA cell-to-cell transport complex reaches the plasmodesmal channel, it must transverse it to enter the neighbouring host cell. Although the estimated 2.0–2.5 nm diameter of this nucleoprotein complex is relatively small, it is still incompatible with the 1.5 nm diameter of the intact plasmodesmal channel [2]. To allow movement therefore, P30 induces an increase in plasmodesmal permeability (Fig. 2). Unlike the wild-type tobacco mesophyll plasmodesmata which can traffic only dextrans of up to 0.75–1.0 kDa, the P30 transgenic plants exhibited a plasmodesmal SEL of almost 10 kDa. Importantly, the increased SEL corresponds to a 5–9 nm diameter of the dilated channel, potentially allowing unrestricted traffic of the P30-TMV RNA complexes.

It was also noted that large fluorescent dextrans moved not only into the cells adjacent to the microinjected cell, but traveled as far as 20 to 50 cells away from the site of injection. This observation 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 these plasmodesmata can traffic protein molecules [9].

Additionally, gating of epidermal PD is restricted to the leading edge of expanding infection sites of TMV. This provides the demonstration that within an expanding infection site plasmodesmatal gating is under temporal control [10].

THE EXCEPTION TO THE RULE: TOBACCO MOSAIC VIRUS MOVEMENT PROTEIN-MEDIATED PROTEIN TRANSPORT BETWEEN TRICHOME CELLS [9]

Most studies have used mesophyll cells for analyzing MP function by observing an MP-mediated change in movement patterns of various fluorescently labeled molecules. However, there is a major drawback:

mesophyll cells are highly interconnected and form a complex three-dimensional cellular network such that only the surface cells can be clearly monitored under the light microscope. It is therefore not possible to observe or predict the exact pathway of a fluorescently labeled molecule. Studies of plasmodesmal transport would be greatly facilitated if the injected cell were part of a linear array of cells. Leaf trichomes consisting of four to eight cells arranged in a single file provide such a system. Moreover, trichomes are known to support movement of plant viruses.

It was demonstrated that the basal SEL of trichoma PD for dextrans lies between 7 kDa and 9 kDa [9] and that TMV MP does not gate trichome PD to increase their SEL for dextrans.

That viruses are capable of movement through trichomes strongly suggests an interactions between MP and trichome plasmodesmata. A direct way of testing the interaction between TMV MP and trichome PD is to determine whether TMV MP itself can move between trichome cells. And indeed, this was observed.

These results suggest that trichome PD are selective in supporting molecular transport because proteins (TMV MP) can move but dextrans larger than 7 kDa cannot. One possible basis for this selection is that a plasmodesmal transport signal is required for efficient translocation. To address this question, it was tested whether GUS, a bacterial enzyme, was capable of moving between cells. GUS (60 kDa) turned out to be unable to move between cells, but a purified GUS::TMV MP fusion protein (90 kDa) does move between trichome cells. Additionally, TMV MP cannot facilitate GUS movement *in trans*. These results indicate that TMV MP most likely contains functional information to promote its own movement through plasmodesmata.

Thus, the basal functional properties of the mesophyll and thrichome PD as well as their interaction with TMV MP are different. In trichomes, dextran movement might not reflect an MP-mediated increase in channel size, even though this increase takes place. Possibly, MP moves fast and only transiently increases plasmodesmal channel size, for example, only while MP moves through PD. Plasmodesmal channels could be opened rapidly and as soon as TMV MP has entered the passageway, reduced to normal size again, thus effectively preventing dextrans exceeding the basal SEL from entering the transport channels.

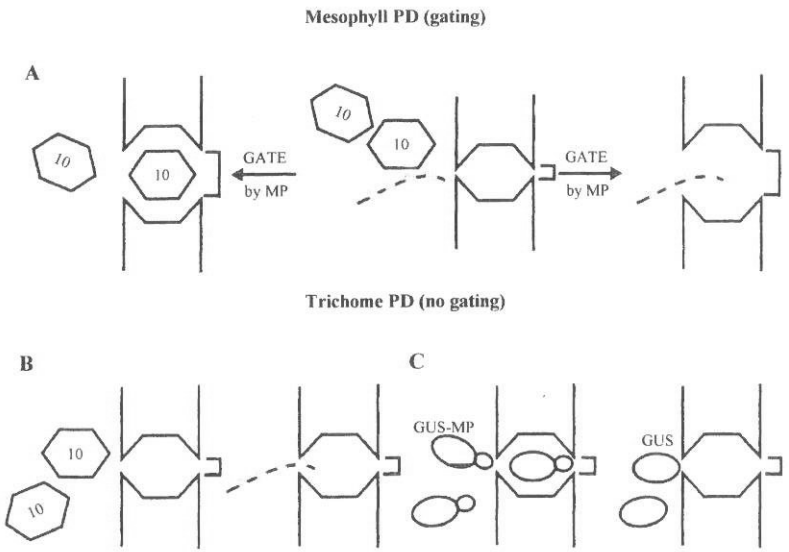

FIGURE 4 Model for mesophyll *versus* trichome PD transport. (A) Transport through mesophyll PD. PD size is small (center) before gating by TMV MP (at left and right). Movement of both 10-kDa dextrans and TMV MP-single-stranded nucleic acid complex occurs after MP gating. (B) and (C) Transport through trichome PD. The size of the PD channel is the same in all panels and is larger than the ungated mesophyll channel but smaller than the gated mesophyll channel. Neither 10-kDa dextrans nor GUS can move on their own, but a GUS::TMV MP fusion protein can (see text) (after [9]).

Clearly, in trichomes, protein movement is not a simple diffusion process, with size as a the major selection criterion for movement as assumed for dextrans. Instead the data suggest that a plasmodesmal transport signal resides within MP and is essential for an active transport mechanism. As summarized in Figure 4, the present results imply a complex situation with at least three general criteria for transport through PD: (1) size of PD channels (Fig. 4A), (2) size and shape of transported molecules (Fig. 4B), and (3) the presence or absence of a plasmodesmal targeting/transport signal sequence (Fig. 4C).

THE MECHANISM FOR PLASMODESMAL TRANSPORT

The mechanism by which macromolecules are transported through PD is completely unknown. However, it may be possible to predict the key features of this process by analogy to nuclear transport which is the

only other known example of traffic of large proteins and protein-nucleic acid complexes through a membrane pore [2].

Similar to nuclear import, and as indicated above, transport through PD most likely consists of two major steps: (1) recognition of the transported molecule in the cell cytoplasm and its targeting to the plasmodesmal channel, and (2) translocation (Fig. 2). Proteins and protein-nucleic acids complexes destined for cell-to-cell transport may be recognized by their putative targeting sequence, the plasmodesmata localization signal (PLS). The carboxyl terminal part of the 100 amino acid-long P30 domain required for interaction with PD may carry such a singal. PLSs may also mediate transport of nucleic acids associated with the PLS-containing protein, such as P30-TMV RNA complexes [2].

By analogy to nuclear import, another possibility for plasmodesmal regulation is cytoplasmic anchoring. P30 interaction 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 [2].

A CRITICAL ENDNOTE

As mentionned above, the SEL mostly is established by microinjection experiments in which the plasmodesmal passage of fluorescent probes (mostly dextrans) of increasing molecular mass is recorded. Recent data now shows that the effect on the SEL depends on the used microinjection technique [11]. Using two different microinjection methods the effect of transgenic expression of the MPs of tomato spotted wilt virus (TSWV) and TMV on plasmodesmal SEL was assessed. By pressure mediated injection of fluorescent probes of different molecular mass, in both TSWV and TMV MP-transgenic tissues a similar enlargement of the plasmodesmal SEL was observed, in line with the general conception that alteration of plasmodesmal gating is an intrinsic activity of viral MPs. Strikingly, when iontophoretic (diffusion mediated influx) injection of the same probe was performed, the plasmodesmal SEL was found to be decreased in transgenic tissues expressing either MP. As iontophoresis is a less invasive technique than pressure injection, these different results may indicate that increase of plasmodesmal SEL in the presence of viral

MPs, as measured by the broadly applied pressure injection technique, may not reflect a genuine biochemical activity of these viral proteins. Rather this increase may be the consequence of disturbance of the plasmodesmatal structure due to the application of pneumatic stress as additional stress on plasmodesmata which have become more prone to disruption by the presence of these viral proteins.

This finding may have major implications for our understanding of viral MP functioning and demonstrates that results obtained with the commonly applied pressure injection systems should be interpreted with more reservations.

References

[1] Zambryski, P. (1995). Plasmodesmata: plant channels for molecules on the move. *Science*, **270**, 1943–1944.

[2] Lartey, R., Ghoshroy, S., Sheng, J. and Citovsky, V. (1997). *Transport through plasmodesmata and nuclear pores: cell-to-cell movement of plant viruses and nuclear import of Agrobacterium T-DNA*. In: McCrae, M. A. *et al.*, pp. 253–280.

[3] McCrae, M. A., Saunders, J. R., Smyth, C. J. and Stow, N. D. (Eds.) (1997). *Molecular aspects of host-pathogen interactions*. Cambridge University Press, 361 pp.

[4] Ding, B. (1998). Intercellular protein trafficking through plasmodesmata. *Plant Molecular Biology*, **38**, 279–310.

[5] Ding, B., Turgeon, R. and Parthasarathy, M. V. (1992). Substructure of freeze-substituted plasmodesmata. *Protoplasma*, **169**, 28–41.

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

[7] Lucas, W. L., Bouche-Pillon, S., Jackson, D. P., Nguyen, L., Baker, L., Ding, B. and Hake, S. (1995). Selective trafficking of knotted1 homeodomain protein and its mRNA through plasmodesmata. *Science*, **270**, 1980–1983.

[8] McLean, B. G., Zupan, J. and Zambryski, P. (1995). Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. *The Plant Cell*, **7**, 2101–2114.

[9] Waigmann, E. and Zambryski, P. (1995). Tobacco mosaic virus movement protein-mediated protein transport between trichome cells. *The Plant Cell*, **7**, 2069–2079.

[10] Oparka, K. J., Prior, D. A. M., Santa Cruz, S., Padgett, H. S. and Beachy, R. N. (1997). Gating of epidermal plasmodesmata is restricted to the leading edge of expanding infection sites of tobacco mosaic virus (TMV). *The Plant Journal*, **12**, 781–789.

[11] Storms, M. M. H., van der Schoot, C., Prins, M., Kormelink, R., van Lent, J. W. M. and Goldbach, R. W. (1998). A comparison of two methods of microinjection for assessing altered plasmodesmal gating in tissues expressing viral movement proteins. *The Plant Journal*, **13**, 131–140.

[12] Santa Cruz, S., Roberts, A. G., Prior, D. A. M., Chapman, S. and Oparka, K. J. (1998). Cell-to-cell and phloem-mediated transport of potato virus x: the role of virions. *The Plant Cell*, **10**, 495–510.