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# Tubule-Guided Movement of Plant Viruses

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**Abstract** Plant viruses move from cell to cell through plasmodesmata, which are complex gatable pores in the cell wall. While plasmodesmata normally allow the diffusion of only small molecules, they can be biochemically or structurally modified by virus-encoded movement proteins to enable the passage of either infectious ribonucleoprotein complexes or entire virus particles. In the latter case, the movement protein forms a transport tubule inside the plasmodesmal pore or at the surface of isolated cells. In this review, we describe the functional relevance of the tubules in the transport of viruses, speculative models for this movement mechanism, as well as the host components that seem to contribute to this type of transport.

## 1 Introduction

Successful propagation of viral infection in host plants comprises distinct and sequential stages: the initial penetration of the virus by mechanical wounding or vector transmission, the replication of the viral genome, and its transport from the initially infected cell into adjacent neighboring cells, a process referred to as local or cell-to-cell movement. Ultimately, propagation to the entire plant results from a chain of events altogether termed systemic movement that consists of viral entry into the vascular tissue, systemic invasion through the phloem stream, and unloading of the virus into non-infected tissues. Within this complex series of events, cell-to-cell movement is certainly one of the most important bottlenecks a virus has to overcome for successful invasion of its host. To do so, viruses exploit plasmodesmata (singular, plasmodesma), microscopic cell wall-embedded channels that provide symplastic continuity throughout most of the plant (for recent reviews Haywood et al. 2002; Heinlein 2002a; Lucas and Lee 2004; Roberts and Oparka 2003; Zambryski and Crawford 2000).

Plasmodesmata can be divided into two major groups (Ehlers and Kollmann 2001; Haywood et al. 2002). The primary plasmodesmata form during cytokinesis, whereas the secondary plasmodesmata develop between cells that are not necessarily clonally related (for recent reviews see Alfonso et al. 2006; Ehlers and Kollmann 2001; Heinlein and Epel 2004; van Bel and van

Kesteren 1999). Although plasmodesmata are subjected to large variations in size, structure, and composition depending on the tissue and the stage of development, they all appear to show a common basic structural architecture consisting of three main elements, the plasma membrane, the cytoplasmic sleeve and the desmotubule, all of which show continuity to the adjoining cells (Ding et al. 1992; Ehlers and Kollmann 2001; Heinlein and Epel 2004; Overall and Blackman 1996, Figs. 1A and 2A; see also Waigmann et al. 2007, in this volume. The plasma membrane inside plasmodesma is continuous with the cellular plasmalemma whereas the cytoplasmic sleeve is enclosed by the plasma membrane and is an extension of the cytosol. The desmotubule is a tightly woven phospholipid bilayer directly connected to the endoplasmic reticulum of each of the adjacent cells, thus forming an endomembrane

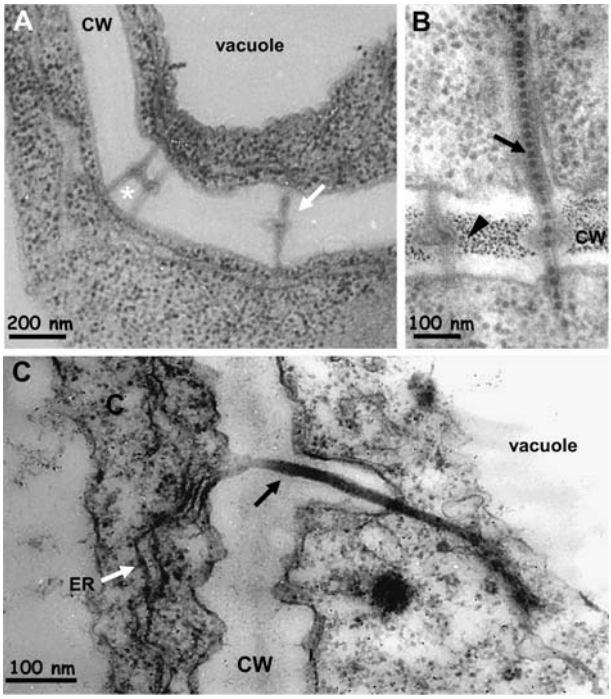


**Fig. 1** Transmission electron microscopy images of plasmodesmata in *Chenopodium quinoa* leaf tissue. **A** Transversal section through a leaf showing simple (*white arrow*) and branched plasmodesmata (*) spanning the cell wall. **B** Plasmodesma in GFLV-infected leaf tissue modified by a tubule filled with individually detectable icosahedrical GLFV-virions (*black arrow*). As indicated by the *black arrowhead*, infected tissue also contains non-modified plasmodesmata. **C** Longitudinal section of transgenic tobacco BY-2 cells expressing GFP:MP (GFLV), showing the presence of a tubule (*black arrow*). Thus, tubule formation does not require viral factors other than MP. Since the cells are not infected, the tubule is devoid of any virion particles

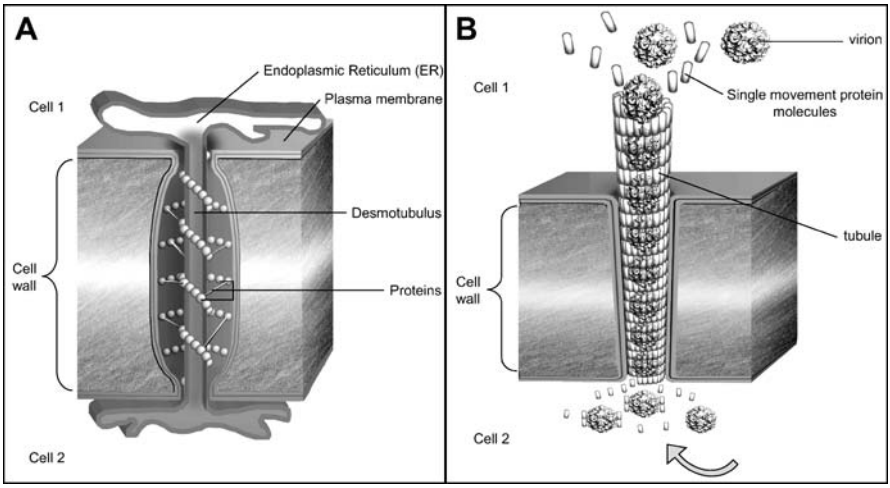


**Fig. 2** Schematic representation of a plasmodesma in healthy tissue (**A**) and in tissue infected with a tubule-forming virus (**B**). **A** Non-modified plasmodesma. The plasma membrane and the ER are continuous through the channel. The ER forms a central rod-like structure called the desmotubule. Proteins are embedded in the desmotubule and in the plasma membrane, and also form spoke-like structures connecting both membranes. The cytoplasmic annulus between the ER and the desmotubule likely functions as the major conduit for intercellular communication. **B** MP-modified plasmodesma. The desmotubule is replaced by a tubular structure composed of multiple MP-subunits. The tubulus serves to transport virions into non-infected cells. As indicated by the *grey arrow*, the tubule assembles in the infected cell (Cell 2) and disassembles for the release of the virions in the adjacent, non-infected cell (Cell 1)

continuum. Longitudinal views indicate that plasmodesmata are often narrowed at either end forming a so-called collar, or neck constriction (Olesen and Robards 1990). This constriction is thought to result from the deposition of callose between the plasma membrane and the wall in response to stresses such as plasmolysis or physical wounding (Radford et al. 1998) and may be one point of regulation of molecular flow from cell to cell.

The size exclusion limit of the different types of plasmodesmata can be measured using fluorescent tracer molecules. Initial studies of plasmodesmata gave rise to the idea that only small molecules of less than 1 kDa such as sugar and amino acid can easily pass through plasmodesmata by passive diffusion (Terry and Robards 1987; Tucker 1982). More recent investigations revealed that plants possess a macromolecular trafficking system, facilitating the plasmodesmal movement of endogenous RNA molecules such as silencing RNA molecules as well as proteins such as transcription factors (Heinlein 2002a; Heinlein and Epel 2004; Jorgensen et al. 1998; Lucas et al. 1995; Lucas and Lee 2004; Oparka 2004; Roberts and Oparka 2003). In general, proteins that can move between plant cells have been termed non-

cell autonomous proteins (NCAPs) (Lee et al. 2003). Some proteins, such as green fluorescent protein (GFP) or the transcription factors LEAFY, can pass through plasmodesmata by simple diffusion without requiring a specific interaction with components of the plasmodesmal pore (Oparka et al. 1999; Roberts and Oparka 2003; Wu et al. 2003). However, of the many NCAPs identified to date, most appear to show selective transport through plasmodesmata and also to increase the size exclusion limit of the plasmodesmal pore (Lucas and Lee 2004; Oparka 2004). Similar properties apply also to NCAPs of viral origin termed "movement proteins (MPs)". Originally discovered using temperature-sensitive mutants of *Tobacco mosaic virus* (TMV) (Deom et al. 1987; Meshi et al. 1987), they were given this name because they potentiate the transport of viruses from cell-to-cell (Atabekov and Dorokhov 1984).

The pioneering work that led to the discovery of the 30K MP of TMV gave rise to similar investigations on other plant viruses. It soon became evident that MPs are a general feature of plant viral genomes (Carrington et al. 1996; Lucas and Gilbertson 1994; Maule 1991). Based on their primary structure, viral MPs have been divided into at least four superfamilies, the largest of which being the "30K" superfamily, named after the 30K MP of TMV (Koonin and Dolja 1993; Koonin et al. 1991; Melcher 2000; Mushegian and Koonin 1993). Although structurally related, cell-to-cell movement of viruses belonging to the 30K superfamily can be divided into two main categories that are exemplified by TMV and *Cowpea mosaic virus* (CPMV, genus *Comovirus*). Thus, TMV MP alters the size exclusion limit of plasmodesmata without inducing obvious ultrastructural changes and mediates cell-to-cell transport of a complex of viral RNA and MP by a mechanism that does not involve the viral coat protein (CP) (see Waigmann et al. 2007, in this volume). This mechanism is probably closest to the one that regulates the trafficking of cellular NCAPs that has been extensively reviewed recently (Heinlein and Epel 2004; Lucas 2006; Waigmann et al. 2004). On the other hand, MPs from CPMV and other closely related virus such as *Grapevine fanleaf virus* (GFLV) target plasmodesmata to form tubular structures or nanotubules through which virions are transported from cell to cell (Fig. 1B; for recent review van Lent and Schmitt-Keichinger 2006). A third intermediate category is formed by viruses belonging to the family *Bromoviridae* whose MP assembles into tubules to promote the movement of CP/RNA ribonucleoprotein complexes rather than entire virus particles (Palukaitis and Garcia-Arenal 2003; Sánchez-Navarro and Bol 2001; Sánchez-Navarro et al. 2006). In this chapter, priority will be given to the tubule-guided movement of virions and how this process may relate to other types of mechanisms of cell-to-cell movement.

## 2
## Tubule Formation, an Intrinsic Property of Some Movement Proteins within the 30K Superfamily

First evidence of ultrastructural modifications of plasmodesmata during viral infection was provided by Esau, who detected virus-like particles located within both modified plasmodesmata and mature, functional, sieve elements (Esau 1968). Similar tubule-like structures were subsequently observed with numerous plant viruses belonging to different families and genera with either RNA or DNA genomes. Examples are found predominantly within the *Comoviridae*, *Bromoviridae* and *Caulimoviridae* (Table 1). Based on these collective studies, the concept emerged that some plant viruses move their genomic material between cells in an encapsidated form through specialized structures termed tubules or nanotubules (Fig. 2B).

The tubule-forming capacity is an intrinsic property of some MPs belonging to the 30K superfamily. Evidence was first provided using CPMV. Early electron microscopy studies of plant cells infected with CPMV revealed the presence of long tubular structures that often extended from the entry of plasmodesmata in one cell into the cytoplasm of a neighboring cell and that contained a single row of virus-like particles (van der Scheer and Groenewegen 1971). Genetic data later revealed that both the coat proteins and the RNA2-encoded 48K protein are essential for cell-to-cell movement of CPMV (Wellink and van Kammen 1989). Immunogold labeling with an antibody against the 48K protein showed that this protein is part of the tubules (van Lent et al. 1990), suggesting that it probably functions as the MP of CPMV by building tubules through plasmodesmata for the transport of virus particles. Further studies revealed that plasmodesmata are not required for tubule assembly, since their formation was also observed in protoplasts, isolated plant cells deprived of their cell wall and, consequently, lacking plasmodesmata. Thus, in CPMV-infected cowpea protoplasts, plasma-membrane lined virus-containing tubules protrude up to tens of micrometers from the cell surface into the medium (van Lent et al. 1991). Once it was established that tubules could not only form in plant tissues but also in protoplasts, it became much easier to identify the viral determinants required for tubule formation and virus movement. Thus, CPMV capsid proteins were shown to have no role in the morphogenesis of the tubules, since a mutant virus which failed to produce the capsid proteins was still capable of inducing tubules that appeared identical to those formed upon infection apart from the presence of virions (Kasteel et al. 1993). On the other hand, virus mutants that fail to produce the 48K protein or that produced a truncated 48K protein were no longer able to induce tubules, suggesting that at least the 48K protein is essential for tubule formation (Kasteel et al. 1993). Final demonstration that the 48K MP is the only viral protein needed for tubules formation was provided by using a 35S promoter-driven 48K expression system in protoplasts (Wellink et al. 1993).

**Table 1** Examples of plant viruses whose MPs belong to the 30K superfamily and assemble into tubule-like structures. Plant viruses are taxonomically grouped according to virus family and genus. References of publications demonstrating tubule-formation by their MPs are shown

| Family | Genus | Virus | Refs. |
|---|---|---|---|
| *Comoviridae* | *Comovirus* | *Cowpea mosaic virus* (CPMV) | van der Scheer and Groenewegen 1971; (van Lent et al. 1990) |
| | | *Bean pod mottle virus* (BPMV) | Kim and Fulton 1971 |
| | *Nepovirus* | *Grapevine fanleaf virus* (GFLV) | Kalasjan et al. 1979; Ritzenthaler et al. 1995; Stussi-Garaud et al. 1994 |
| | | *Arabis mosaic virus* (ArMV) | Stussi-Garaud et al. 1994 |
| | | *Tomato ringspot virus* (TomRSV) | Wieczorek and Sanfaçon 1993 |
| *Bromoviridae* | *Oleavirus* | *Olive latent virus 2* (OLV2) | Castellano 1987; Grieco et al. 1999 |
| | *Alfamovirus* | *Alfalfa mosaic virus* (AMV) | Godefroy-Colburn et al. 1991; van der Wel et al. 1998 |
| | *Ilarvirus* | *Tobacco streak virus* (TSV) | Martelli and Russo 1985 |
| | *Cucumovirus* | *Tomato aspermy virus* (TAV) | Francki et al. 1985 |
| *Caulimoviridae* | *Caulimovirus* | *Cauliflower mosaic virus* (CaMV) | Kitajima and Lauritis 1969; Linstead et al. 1988 |
| | | *Dahlia mosaic virus* (DMV) | Kitajima et al. 1969 |
| | *Badnavirus* | *Commelina yellow mottle virus* (ComYMV) | Cheng et al. 1998 |
| *Sequiviridae* | *Sequivirus* | *Parsnip yellow fleck virus* (PYFV) | Cheng et al. 1998 |
| *Geminiviridae* | *Begomovirus* | *Euphorbia mosaic virus* (EuMV) | Kim and Lee 1992 |
| *Bunyaviridae* | *Tospovirus* | *Tomato spotted wilt virus* (TSWV) | Kormelink et al. 1994 |

The above-mentioned pioneering work on CPMV performed in the groups of van Kammen and Goldbach paved the way to the discovery of numerous other MPs with tubule forming capacity. In agreement with the predicted function of tubules in virus movement, all the MP of the aforementioned tubule-forming viruses, when tested, assembled into tubules upon expression in protoplasts, including the MPs of GFLV (Ritzenthaler et al. 1995) (Fig. 3A,B), *Olive latent virus 2* (OLV2) (Grieco et al. 1999), *Cauliflower mo-*

*saic virus* (CaMV) (Perbal et al. 1993), *Tomato spotted wilt virus* (TSWV) (Storms et al. 1995) and *Alfalfa mosaic virus* (AMV) (Kasteel et al. 1997; Zheng et al. 1997). These studies revealed that the MP is the only viral requirement for tubule assembly. Amazingly, a number of these proteins, when tested, maintained their capacity to form tubules when expressed in insect cells (Kasteel et al. 1996; Storms et al. 1995). In all respects, tubules formed in insect and plant cells appear to be similar. Remarkably, the infectivity data of scanning deletion mutants that revealed the existence of a large C-terminal domain necessary for tubule formation (Thomas and Maule 1995a) largely mirrored those obtained with MP mutants expressed in insect cells (Thomas and Maule 1999) emphasizing the importance of tubule formation in aiding virus movement. Thus, Maule and colleagues nicely established that the majority of the CaMV MP (aa 1 to 282) is required for tubule formation, whereas the C-terminus could project into the lumen of the tubule to interact with the virions (Thomas and Maule 1995a, 1999).

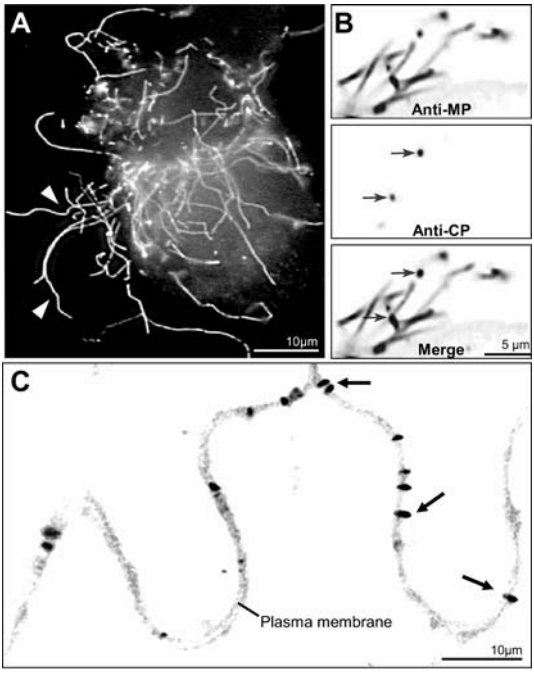


**Fig. 3** Localization of GFLV MP and coat protein **A** GFLV-infected BY-2 protoplast stained with anti-MP(GFLV)-antibody. The antibody stained tubules (*white arrowheads*) protruding from the cell surface. **B** Surface of GFLV-infected BY-2 protoplast stained with anti-MP-antibody (*top panel*: anti-MP) and anti-coat protein antibody (*middle panel*: anti-CP). The merged image (*bottom panel*: Merge) reveals detection of the viral coat protein at the tubule extremities. **C** *N. benthamiana* epidermal cells expressing an MP : RFP fusion protein. Red fluorescent tubules (*arrows*) are present in the epidermal cell wall

Rather unexpectedly, a number of other MPs within the 30K family were able to produce tubules at the surface of protoplasts, although tubules have never been found in infected tissues. This includes members of the *Bromoviridae* family such as *Cucumber mosaic virus* (CMV) and *Brome mosaic virus* (BMV) (Canto and Palukaitis 1999; Kasteel et al. 1997) and also the flexuous viruses *Apple chlorotic leafspot trichovirus* (ACLSV) (Satoh et al. 2000), *Grapevine berry inner necrosis virus* (GINV) (Isogai et al. 2003) and *Apple stem grooving capillovirus* (ASGV) (Isogai et al. 2003). The specific case of these viruses that require the CP for movement and show a discrepancy between plants (no tubules) and protoplasts (tubules) will be discussed more specifically below. More intriguing is the tubule-forming capacity of the MP of TMV in infected protoplasts (Heinlein et al. 1998a; Mas and Beachy 1998). Tubules were never found in plasmodesmata of TMV-infected plants. Moreover, TMV is the type member of the viruses that are thought to move as ribonucleoprotein complexes without the functional requirement of the CP (Dawson et al. 1988; Knapp et al. 2001). In transgenic plants, TMV MP is found as fibrillar material in secondary Pd cavities (Ding et al. 1992), while upon expression in multicellular cyanobacteria this MP forms tubular arrangements of fibrils that traverse the septum between cells (Heinlein 2006; Heinlein et al. 1998b). Fibrillar material has also been reported to be present in plasmodesmal pores modified by the MP of TSWV (van Lent and Schmitt-Keichinger 2006), suggesting that TSWV could move in a similar manner as TMV. In agreement with this view, Storms and co-workers established that the MP of TSWV (NSm) had similar effects on plasmodesmal gating as TMV (Storms et al. 1998). A similar mode of action may also be indicated by the finding that the NSm protein can complement movement-defective TMV (Lewandowski and Adkins 2005). While this also demonstrates that virion formation is not required for NSm to traffic TMV RNA (Lewandowski and Adkins 2005), previous experiments suggested that nucleoprotein complexes of TSWV viral RNA and N protein move through tubules, as deduced from the interaction between the N and NSm proteins and their co-localization within tubules (Soellick et al. 2000; Storms et al. 1995). More importantly, this finding questions the requirement of N protein for the movement of TSWV RNAs and, more generally, the relevance of tubules in virus movement. One possible explanation for these observed discrepancies could be that some viruses use two alternative movement strategies to establish systemic infection: movement as a viral RNA-MP complex, as exemplified by TMV (see Waigmann et al. 2007, in this volume), or alternatively, movement as complete virions by a tubule-guided mechanism as exemplified by CaMV and BMV (Jansen et al. 1998; Thomas and Maule 1995b). Such explanation could also account for the fact that the MPs of a number of tubule-forming and virion-transporting viruses like CPMV (Carvalho et al. 2004), CaMV (Citovsky et al. 1991), AMV (Schoumacher et al. 1992a,b) BMV (Jansen et al. 1998) and TSWV (Soellick et al. 2000) are

able to bind nucleic acids in a sequence non-specific manner in vitro or to fix GTP (Carvalho et al. 2004; Li and Palukaitis 1996) like TMV (Citovsky et al. 1990; Li and Palukaitis 1996). These common biochemical properties may reflect structural similarities between members of the 30K superfamily as already suggested by Melcher (2000). However, despite the recent attempts at resolving the three-dimensional structure of TMV MP (Brill et al. 2000, 2004), the first crystal structure of a MP within the 30K superfamily is still being awaited.

## 3
## Tubules in the Transport of *Bromoviridae*

In the family *Bromoviridae*, studies on cell-to-cell movement have mainly been focused on AMV, BMV, CMV and *Prunus necrotic ringspot virus* (PNRSV, genus *Ilarvirus*). For BMV, CP is required for cell-to-cell and long-distance movement (Rao and Grantham 1995, 1996; Schmitz and Rao 1996), although in some hosts, limited cell-to-cell movement occurs if high concentrations of inoculum are applied (Flasinski et al. 1995). Despite the fact that tubules have never been detected in infected tissues, the presence of tubules containing BMV particles extending from transfected protoplasts (Kasteel et al. 1997) together with results from mutational analyses (Okinaka et al. 2001; Schmitz and Rao 1996) support the notion that BMV moves cell-to-cell in the form of virions. For CMV, all five of the proteins encoded by the three genomic RNAs affect the movement of the virus (Palukaitis and Garcia-Arenal 2003). However, the 3a protein encoded by CMV RNA 3 is considered to be the primary movement protein (Canto et al. 1997; Kaplan et al. 1995; Nagano et al. 1997) and the CP has been proven to be required for cell-to-cell movement (Suzuki et al. 1991), even in the epidermis of inoculated leaves (Canto et al. 1997). However, the ability to form virions is not a prerequisite for cell-to-cell movement (Kaplan et al. 1998). In addition, the capacity of the 3a MP of CMV to bind RNA as initially demonstrated by Li and Palukaitis (1996) appears to be essential to promote virus movement (Palukaitis and Garcia-Arenal 2003). Thus, despite the ability of the MP to generate tubules on the surface of protoplasts (Canto and Palukaitis 1999), it is assumed that CMV moves as a ribonucleoprotein complex (Palukaitis and Garcia-Arenal 2003).

The transport mechanism of AMV, which requires RNA3-encoded proteins MP and CP, has been reported to share characteristics with those of both TMV and CPMV (Kasteel et al. 1997; Sánchez-Navarro and Bol 2001). Thus, while CP mutants defective in virion assembly are still able to move cell-to-cell (Sánchez-Navarro and Bol 2001), virus particles are clearly detected within tubules protruding from wild-type AMV-infected protoplasts (Kasteel et al. 1997). Also, the capacity of the MP to promote cell-to-cell and

systemic movement of the virus in plants was shown to be strictly correlated with the ability of the MP to assemble into tubules (Sánchez-Navarro and Bol 2001). More precisely, the 44 C-terminal amino acids of the MP were shown by mutational analyses to be dispensable for tubule assembly and cell-to-cell movement of AMV. Nevertheless, this C-terminal domain is still able to confer specificity to the transport process, as it allowed BMV MP to promote AMV movement upon replacement of the C-terminal domain of the MP of BMV by that of AMV MP (Sánchez-Navarro and Bol 2001). To gain further insight in such transport specificity, additional chimeric RNA 3 mutants with the AMV MP gene replaced by the corresponding MP gene of PNRSV, BMV, CMV, TMV or CPMV were recently tested (Sanchez-Navarro et al. 2006). It appeared from this survey that all RNA 3 hybrids carrying the extended C-terminal 44 aa of AMV MP were functional and that this region is able to interact specifically with AMV virus particles in vitro. Remarkably, the replacement of the CP gene in RNA 3 by a mutant gene encoding a CP defective in virion formation did not affect cell-to-cell transport of the chimeras with a functional MP, thereby clearly demonstrating that virus particles are not required for the cell-to-cell movement mediated by the MP of either AMV, BMV, CPMV or TMV. The most likely explanation for this phenomenon would be that the two mechanisms described in the 30K superfamily could represent two variants of the same viral transport system, where the C-terminus of the MP could be adapted to recognize the cognate CP. It is likely that the same rule applies also to non-*Bromoviridae* such as CPMV, GFLV and CaMV. Strikingly, the C-terminal part of the MP of all these viruses is involved in coat protein recognition and binding. For CPMV it was shown that the MP C-terminus is located on the inside of the tubule (van Lent et al. 1991), thus in close proximity to the virus particles. Incorporation of virions into the tubule was disturbed with a C-terminal deletion mutant of the MP, giving rise to "empty" tubules (Lekkerkerker et al. 1996), i.e., tubules without virus particles. Furthermore, Carvalho et al. (2003) showed specificity of MP binding to CPMV virions, but not to capsids of BMV, TMV or of the related Comoviruses *Cowpea severe mosaic virus* (CPSMV) and *Red clover mottle virus* (RCMV) (Carvalho et al. 2003). Moreover, in blot overlay assays the MP specifically bound to only one, the large, of the two CPMV coat proteins. GFLV movement is likely also to be governed by a specific interaction between tubule and virions, as suggested by the results obtained with chimeric constructs between GFLV and the closely related *Arabis mosaic virus* (ArMV). Virus spread only occurred when the 9 C-terminal residues of the MP were of the same viral origin as the coat protein (Belin et al. 1999). As already mentioned, in the case of CaMV, a C-terminal mutant MP was identified that kept its ability to form tubules, but was unable to support virus movement, suggesting that the ten last C-terminal amino acids of the MP are involved in interactions with the virus particles (Thomas and Maule 1995a).

## 4
## Intracellular Trafficking Pathways and Mechanisms of Tubule Assembly

With the new possibilities offered by the green fluorescent protein (GFP) and its variants to investigate protein trafficking in living cells, attention has turned to the question of how intracellular transport and targeting of MP to plasmodesmata may occur. Viruses that infect plants have developed a variety of strategies to move from cell to cell and are heavily dependent on endogenous host transport systems during movement, as with regard to all other aspects of their life cycles. Pioneering work with TMV MP has demonstrated a close association of the MP with multiple host components. Several models for the movement of TMV have been proposed by which the viral RNA together with the MP could be transported to plasmodesmata in association with the endoplasmic reticulum, microtubules and microfilaments (for recent reviews see Boevink and Oparka 2005; Heinlein 2002b; Heinlein and Epel 2004; Lucas 2006). However, the precise involvement of the cytoskeleton and endomembrane system in the spread of TMV infection remains a subject of intense studies (see Waigmann et al. 2007, in this volume). Even the unlikely hypothesis that cytoplasmic streaming could support the rapid diffusion of infectious TMV ribonucleoprotein complexes to plasmodesmata cannot be ruled out under present circumstances (Boevink and Oparka 2005). Concerning the smaller family of viruses that employ the tubule-guided movement, the analysis of their trafficking mechanisms has focused essentially around CPMV, GFLV, CaMV and AMV.

The use of cytoskeletal inhibitors like Latrunculin B (inhibits the assembly of actin filaments) and Oryzalin (inhibits the assembly of microtubules) as well as inhibitor of secretion like Brefeldin A (BFA), (Nebenführ et al. 2002; Ritzenthaler et al. 2002) have been particularly helpful to unravel the targeting mechanisms of several tubule-forming MPs. Thus, it was shown in protoplasts transiently expressing a MP-GFP fusion of CPMV and CaMV, that neither a functional secretory pathway nor an intact cytoskeleton is required for MP targeting to the plasma membrane (Huang et al. 2000; Pouwels et al. 2002). However, BFA severely inhibited tubule formation, suggesting that vesicle transport is needed for tubule formation or more likely that BFA interfered with the targeting of an essential host protein to the plasma membrane (Huang et al. 2000; Pouwels et al. 2002). For AMV, it was also shown that MP transport to the cell wall and tubule assembly do not rely on an intact cytoskeleton (Huang et al. 2001a), but data on the role of the endomembrane system are not yet available. Support for a stepwise process in CPMV movement was provided by additional mutational analyses of the MP (Carvalho et al. 2004; Pouwels et al. 2003). It is suggested that the MP would first diffuse from the place of synthesis to the plasma membrane as a dimer where it then accumulates in punctuate structures before assembling into tubules, possibly in a similar manner to microtubules as suggested from the

GTP-binding capacity of the MP (Carvalho et al. 2004; Pouwels et al. 2003, 2004). During this assembly process, virions are specifically included within tubules (Fig. 4). It is not known yet whether MP and virions are cotransported along the same pathway or whether they use different routes that converge at the entry of tubules. However, it has been proposed that in the adjacent cell, the tubule destabilizes, thereby releasing the virions for further infection (Pouwels et al. 2003). A similar process may also apply to the intra- and intercellular movement of GFLV as demonstrated using GFP:MP fusion protein in tobacco epidermal or BY-2 cells (Figs. 1C and 3C), although in contrast to CPMV, CaMV, and AMV, a role for microtubules and secretion in the assembly of tubules is established (Laporte et al. 2003). The use of a polarized system (stably transformed tobacco BY-2 cells) for GFLV analyses versus the analysis of MP expression in protoplasts for CaMV, CPMV and AMV, could well account for some of the observed discrepancies. Indeed, for GFLV, treatment with Oryzalin alone or together with Latrunculin B did not abolish tubule formation within cross walls of BY-2 cells, but resulted in their addi-

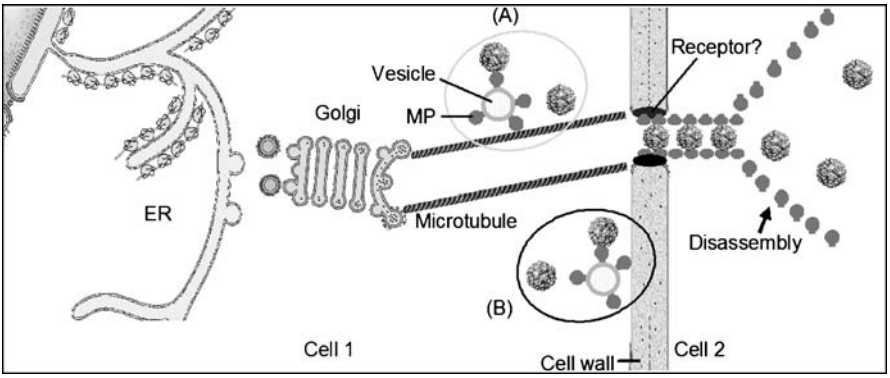


**Fig. 4** Schematic representation of the intracellular trafficking and cell-to-cell movement steps during infection with tubule-forming viruses. After penetration and decapsidation of virion particles within the initially infected cell (Cell 1), the viral genome is translated and replication starts. Following virion assembly, virions are transported to plasmodesmata. It remains to be determined whether virions are co-transported with MP. Two basic mechanisms of MP-targeting to plasmodesmata have been described for different viruses. One transport model (*A – grey circle*), which is exemplified by GFLV, suggests that MP is transported on Golgi-derived secretory vesicles along microtubules (Laporte et al. 2003). The other mechanism (*B – black circle*) applies, for example, to CPMV and involves the transport of MP first to the plasma membrane and then to plasmodesmata (Carvalho et al. 2004; Pouwels et al. 2003, 2004). It remains to be determined whether a specific receptor for MP is implied in the mentioned mechanisms and where it is located. Once at the cell periphery at sites probably related to plasmodesmata, MP self-assembles into tubules by which viral particles move from cell to cell. The release of virions in the non-infected cell may be mediated by tubule disassembly (Cell 2). Elements of the figure are not drawn to scale

tional assembly at ectopic sites (Laporte et al. 2003), a phenomenon that may not be visible when working with protoplasts due to changes in the tubulin cytoskeleton upon protoplast preparation (Tylicki et al. 2003). Concerning the involvement of the secretory pathway in GFLV tubule formation, BFA treatment did not abolish tubule formation but severely decreased their numbers (Laporte et al. 2003), as also observed for the tubules formed by CPMV and CaMV MPs (Huang et al. 2000; Pouwels et al. 2002). However, contrarily to CPMV, GFLV MP was strongly redistributed to the cytoplasm upon BFA treatment, suggesting that the MP could traffic along the secretory pathway in a manner similar to membrane-bound protein cargo (Fig. 4). This hypothesis is further supported by the intrinsic membrane properties of the GFLV MP and its ability to physically interact with the cytokinesis-specific syntaxin KNOLLE (Heese et al. 2001; Laporte et al. 2003; Lauber et al. 1997). A way to address this issue more precisely than simply by using BFA, which has multiple cellular targets (Nebenführ et al. 2002), would be to use specific inhibitors of secretion such as dominant negative mutants of the Arf1 and Sar1 GTPases involved in the COPI and COPII vesicular trafficking pathways, respectively (daSilva et al. 2004; Takeuchi et al. 2000, 2002; Xu and Scheres 2005; Yang et al. 2005). In addition, analysis of the interactions between viral MPs and host factors should provide further insight into the movement process. For TMV several MP-interacting host proteins have been identified, such as tubulin (Ashby et al. 2006; Heinlein et al. 1995), actin (McLean et al. 1995), pectin methylesterase (Chen et al. 2000; Dorokhov et al. 1999), KELP, a putative transcriptional co-activator that modulates host gene expression during pathogenesis, (Matsushita et al. 2001), calreticulin (Chen et al. 2005) or the microtubule-associated protein MPB2C (Kragler et al. 2003) (for recent reviews see Boevink and Oparka 2005; Lucas 2006; Oparka 2004; Waigmann et al. 2004) (also see Waigmann et al. 2007, in this volume).

For tubule-forming viruses also, host factors with affinity for the MP have been found. In a yeast two-hybrid screening with the MP of TSWV, Soellink and coworkers found interactions with DnaJ-like chaperones (Soellick et al. 2000). These proteins have functions including protein transport in organelles and the regulation of the chaperone heat-shock protein Hsp70. Remarkably, the latter protein is also involved in the translocation of Closteroviruses (Alzhanova et al. 2001). In addition, TSWV MP was shown to bind proteins with homologies to myosin and kinesin, suggesting an involvement of molecular chaperones in the attachment of TSWV nucleocapsids to the cytoskeleton for subsequent intracellular trafficking (von Bargen et al. 2001). A yeast two-hybrid screen led to the identification of a vesicular-associated membrane protein (VAMP) termed MPI7 that binds CaMV (Huang et al. 2001b). In sequence, MPI7 is related to mammalian Rab acceptor proteins (PRA1), a family of proteins binding Rab GTPases and vSNARE, components implicated in the regulation and proper delivery of transport vesicles (Nebenführ 2002). The protein was localized to punctuate spots at the cell periph-

ery, probably representing plasmodesmata, and in vivo association between the MP and MPI7 was confirmed by fluorescence resonance energy transfer (FRET) (Huang et al. 2001b). Using a GST-pull down approach, CaMV MP was also recently shown to bind the virion-associated protein (VAP) through a C-terminal coiled-coil domain (Stavolone et al. 2005). Immunogold electron microscopy revealed that the VAP and viral movement protein colocalize on CaMV particles within plasmodesmata (Stavolone et al. 2005). Thus, although not proven, the CaMV MP together with VAP and possibly virions might interact to transport vesicles via MPI7 during their delivery to plasmodesmata, in a similar manner to GFLV (Fig. 4). The same transport mechanism could also apply to CPMV, as it was shown that its MP binds GTP and that this binding is required for MP targeting and tubule formation (Carvalho et al. 2004). Although no GTPase activity could be demonstrated for the MP, the GTP-binding activity may become significant if the "grab a Rab" model proposed by Oparka for selective transport of MP to the plasmodesmata is considered (Oparka 2004). Rab GTPases, which play a role in specificity of vesicle transport (Nebenführ 2002; Rutherford and Moore 2002), could carry the MP together with a cargo vesicle to the plasma membrane and at the same time, by GTP hydrolysis, could provide the molecular switch to start MP polymerization. At the plasmodesmata, specific interactions between v-SNARE (soluble N-ethylmaleimide-sensitive factor adaptor protein receptors) and t-SNARE complexes then make the vesicles fuse with the plasma membrane. The vesicles could even transport necessary enzymes for cell wall degradation to enlarge the plasmodesmatal channel or to form secondary channels for virus transport.

Better understanding of the vesicular transport pathways involved in secretion and endocytosis as well as of cytoskeleton-driven transport mechanisms should soon provide new ideas and details about the molecular mechanisms and routes employed by MP and virions for their delivery to the plasma membrane and plasmodesmata. Similarly, resolving the *modus operandi* of plasmodesmatal cell-to-cell transport through the identification of novel plasmodesmal proteins is currently under progress. Altogether, these approaches will converge to provide new hints to the manner by which viruses hijack and modify these pores.

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