CURRENT REVIEW

Plasmodesmata: Gateways to Local and Systemic Virus Infection

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As channels that provide cell-to-cell connectivity, plasmodesmata are central to the local and systemic spread of viruses in plants. This review discusses the current state of knowledge of the structure and function of these channels and the ways in which viruses bring about functional changes that allow macromolecular trafficking to occur. Despite the passing of two decades since the first identification of a viral movement protein that mediates these changes, our understanding of the relevant molecular mechanisms remains in its infancy. However, viral movement proteins provide valuable tools for the modification of plasmodesmata and will continue to assist in the dissection of plasmodesmal properties in relation to their core roles in cell-to-cell communication.

It is now well established that viruses exploit plasmodesmata (PD) as cell-to-cell symplastic connections to achieve spreading local and systemic infection in host plants. These channels theoretically provide access to most tissues and their use leads to familiar patterns of infection. These patterns are frequently revealed in the distribution of virus symptoms and can be visualized in real time as sites of virus accumulation when using a virus that expresses a fluorescent marker protein (Fig. 1A). The principles underlying these patterns define two modes of virus movement, local and systemic, which can be differentiated genetically with respect to the host and the virus. In both cases, however, PD provide the essential pathways for infection. Described briefly, from the point of initial entry to the tissue, viruses spread from cell to cell through PD to create a local infection focus until they encounter the tissues of the vasculature, at which point they are loaded into the phloem elements for translocation to distal tissues. Transport in the phloem is passive and follows the phloem transport path from source to sink tissues, where the virus is unloaded from the veins to infect surrounding cells. The developmental transition of photosynthetic tissues from sink to source means that, during the early stage of infection, only partial invasion of the leaf is seen, being restricted to the proximal region that had not yet transitioned to “source” status when the virus arrived. All subsequent young leaves are infected during the early stage of

development and show complete infection. Young leaves may be infected either from newly developed phloem or by virus spread cell to cell from adjacent tissues. Access of viruses to the nonvascularized meristematic tissues occurs but is more frequently restricted by a defense reaction based upon RNA silencing (Martin-Hernandez and Baulcombe 2008). The patterns of root infection follow similar principles. During the later stages of infection, the amount of virus accumulation (and gene expression) may also be restricted by RNA silencing.

PD are tightly controlled channels that normally allow the passive diffusion of small, soluble molecules but restrict the passage of macromolecular structures, as represented by virus particles or virus genomes. Therefore, it is logical that viruses have evolved mechanisms to adapt the functional properties of PD to permit the spread of infection. This adaptation is manifest as the action of virus-encoded movement proteins (MP) that lead to altered PD flux and the cell-to-cell transport of viruses or their genomes. The nature of virus MP and the way they directly or indirectly chaperone the infectious agent to and through PD will be a significant feature of this review, although this can only be fully appreciated within the context of knowledge about PD structure, composition, and function and the relationship that PD hold with intracellular trafficking pathways.

PD: structure and function.

The structure and function of PD have been fully reviewed elsewhere (Lucas et al. 2009; Maule 2008). Briefly, they are plasma membrane-lined channels that traverse the cell wall between adjacent cells (Fig. 1B). They are formed during cytokinesis when a component of the endoplasmic reticulum (ER) becomes trapped in the developing phragmoplast. The ER forms a central axial membranous element, termed the desmotubule. The space between the plasma membrane and the desmotubule is called the cytoplasmic sleeve and, from electron micrographs, is seen to be interrupted by proteinaceous spokes that create a network of microchannels through which small molecules are judged to diffuse. Therefore, PD have plasma membrane, ER membrane, and soluble phase continuity across the new cell wall, providing opportunities for soluble and membrane-associated molecules to translocate from cell to cell. The extracellular cell wall domain surrounding PD is also distinctive, having callose deposits close to the neck aperture and pectin-rich areas in the central region (Fig. 1B). PD are present at the interfaces of all symplastically connected cells but become structurally and functionally modified to create specific symplastic domains (Ehlers and van Bel 2010; Oparka and Turgeon 1999); for example, distinguishing different inter-

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cellular boundaries in the vasculature including in extremis for the creation of phloem element sieve pores.

Our current knowledge of the molecular composition of PD is still very limited. Very few proteins have been shown definitively to be located at PD, although a significantly larger number may have a transient or indirect association (Maule 2008). Although it is speculation, reference to the analogous functions of the nuclear pore complex in macromolecular transport might suggest that there will be many tens or hundreds of proteins associated with the structure and function of PD. There is

evidence for cytoskeletal components (actin, myosinVIII, centrin, and tropomyosin) (Faulkner et al. 2009), a kinase capable of phosphorylating *Tobacco mosaic virus* (TMV) MP, a family of receptor-like proteins (PDL1-8) (Thomas et al. 2008), β -1,3-glucanase, a family of callose-binding proteins (PDCB) (Simpson et al. 2009), a reversibly glycosylated polypeptide associated with Golgi and PD (RGP2), lipid raft-associated remorin (Raffaele et al. 2009a), and ER-associated calreticulin all being directly associated with the structure (Maule 2008). Interestingly, these collectively represent the various structural

Fig. 1. A, Still image from time-lapse movie of the development of *Tobacco rattle virus* (TRV) infection in *Nicotiana benthamiana* as revealed from the virus-driven expression of green fluorescent protein (GFP). In this image, the virus-inoculated leaf is marked with an arrow. *N. benthamiana* plants were inoculated with TRV capable of expressing GFP from modified RNA2 (MacFarlane and Popovitch 2000). Photographic images were taken every 30 min under UV light for a period of 2 to 6 days postinfection. Plants were grown under a photoperiod of 16 h of light and 8 h of darkness for the duration of the experiment (published with permission from the John Innes Centre). **B,** Cartoon of a simple plasmodesma showing the general organization and the proposed locations of proteins shown to be associated with the structure. **C through E,** Models for virus translocation through plasmodesmata. **C,** Epel (upper) and Verchot-Lubicz (lower) models for triple gene block (TGB)-mediated movement of *Potato virus X*. The Epel model hinges upon the pathogenesis induction of β 1,3-glucanase and its secretion to the wall for degradation of callose at the plasmodesmata (PD), and increased PD gating to allow the passage of the TGB-vRNA complex (Epel 2009). The Verchot-Lubicz model is based upon the formation of a TGBp1+2+3 docking complex at PD for the binding and translocation of virus particles into neighboring cells (Verchot-Lubicz et al. 2005). **D,** Actin-severing model for the action of the movement proteins of *Cucumber mosaic virus* and *Tobacco mosaic virus*, where degradation of actin in PD releases tensional constraints and relaxes the size exclusion limit (Su et al. 2010). The diagram also incorporates the concept of β 1,3-glucanase induction and callose degradation (Epel 2009). **E,** Involvement of the PD-located protein (PDL) family members as potential receptors for the formation of tubules in PD, where PDL become associated with the basal section of the growing tubule (Amari et al. in press).

domains within the PD. Hence, components of the cytoskeleton, RGP2, and the kinase probably occur within the cytoplasmic sleeve; calreticulin is an ER luminal protein; PDLF is a type I integral membrane protein of the plasma membrane; and β -1,3-glucanase and PDCB are associated with the extracellular face of the plasma membrane to interact with callose (β -1,3-glucan) at the neck region. The presence of remorin is important because it indicates that the plasma membrane within PD may contain lipid raft microdomains. These support the presence of glycosylphosphatidylinositol (GPI)-anchored proteins, such as PDCB, that are typically associated with lipid raft microdomains (Grennan 2007).

Regulation of molecular flux through PD and the roles of the components listed above remains a matter of conjecture. Most of the attention has focused on physical constriction of the PD aperture by callose deposition in the near-wall (Chen and Kim 2009; Epel 2009; Hofmann et al. 2010) or on the importance of actin, probably surrounding the desmotubule, which could place tensional constraints upon the PD structure to limit molecular flux (Ding et al. 1996; Su et al. 2010; White et al. 1994).

Callose production is regulated by a large family of callose synthases and β -1,3-glucanases. Callose synthases operate within the context of larger molecular complexes (Verma and Hong 2001), implicating yet further proteins in regulating callose synthesis. Recently, it has been shown that mutations in the callose synthase *GSL8* led to an increased PD size exclusion limit (SEL), allowing the diffusion of the cell autonomous protein *SPEECHLESS* to neighboring cells (Guseman et al. 2010). On the other hand, *Arabidopsis* knockout lines for the PD-located β -1,3-glucanase *AtBG_pap* showed increased callose at PD and reduced PD permeability (Levy et al. 2007a and b). Collectively, these proteins and their paralogues provide huge potential for specificity with respect to tissues and induced responses. The lack of single mutant phenotypes for many of these genes, however, suggests that their functions are at least partially redundant.

The observation that overexpression of PDCB (which has callose-binding activity but no known catalytic activity) or RGP2 (which has no known function) increased callose accumulation at PD and reduced molecular flux (Simpson et al. 2009; Zavaliev et al. 2010) shows that regulation of callose accumulation may be significantly more complicated than currently proposed. This is reinforced by the finding that other intracellular and extracellular signals seem to play a role in this regulation. Reactive oxygen species (ROS) induced by treatment with oxidants or by a defective maintenance of cell redox homeostasis have been shown to increase callose concentration at PD or to modulate intercellular transport (Benitez-Alfonso and Jackson 2009; Stonebloom et al. 2009). This may be important in the response to stresses and for some developmental transitions where cell-to-cell communication is critical. For example, exposure to aluminum (Jones et al. 2006; Sivaguru et al. 2000) and cadmium (Ueki and Citovsky 2005) induce ROS production and callose deposition at PD, reducing intercellular movement of symplastic dyes and viral MP. During some developmental processes, such as leaf transition to senescence and fiber cell elongation in cotton, a reduction in PD SEL by callose deposition correlates with an increase in intracellular ROS and in the activity of ROS-scavenging enzymes (Jongebloed et al. 2004; Li et al. 2007; Ruan et al. 2004). More direct evidence of this regulatory mechanism is provided by the study of mutants in the synthesis of tocopherol (the main antioxidants in leaves) or in ROS-detoxifying enzymes (e.g., the thioredoxin *GAT1*). These mutants accumulate increased callose at PD, which reduces symplastic transport and results in severe de-

velopmental phenotypes such as accelerated senescence and impaired meristem development (Abbasi et al. 2009; Benitez-Alfonso et al. 2009).

From the predicted impact of callose on flux through PD, we might expect a correlated impact on virus infection, and this is, indeed, the case. Hence, increasing callose turnover in transgenic tobacco expressing specific antisense RNA to β -1,3-glucanase delayed the spread of TMV (Beffa et al. 1996). Conversely, overexpression of the gene from a TMV vector increased the size of necrotic lesions of the virus (Bucher et al. 2001). Furthermore, overexpression of some viral MP—for example, TMV MP, (Olesinski et al. 1996), *Tomato spotted wilt virus* (TSWV) MP (Rinne et al. 2005), and *Potato leaf roll virus* (PLRV) MP (Kronberg et al. 2007)—led to increased accumulation of callose, altered carbohydrate partitioning, and altered plant phenotypes, reminiscent, in part, of virus-infection phenotypes. Interestingly, plants overexpressing RGP2 and showing increased callose accumulation show similar phenotypes to those described above for plants transgenic for viral MP. In contrast to the latter plants, however, the RGP2 transgenics showed a decrease in cell-to-cell movement of TMV (Zavaliev et al. 2010). RGP2 is a member of the ^CIRGP that accumulate as large homomultimeric complexes of approximately 400 kDa in the cytoplasmic sleeve of PD (De Pino et al. 2007; Dhugga 2006), perhaps pointing to a physical blockage of the channel.

With respect to actin, treatment of tissue with the chemical disruptors of actin fibers was found to increase PD SEL (Ding et al. 1996) and to increase the size of the PD pore at the neck region of PD (White et al. 1994). This has functional implications because MP seem to interact with actin filaments and mediate virus spreading to neighboring cells (Heinlein et al. 1995; McLean et al. 1995; Su et al. 2010). There is also evidence that TMV MP increases the cross-sectional dimension of the desmotubule by swelling of the ER lumen, regulating the transport by diffusion of TMV replication complex in the ER-desmotubule continuum (Guenoune-Gelbart et al. 2008).

An additional level of flux control was shown recently where PD at the base of tobacco trichomes showed selectivity for the direction of trafficking and, further, selective specificity in that TMV could move whereas diffusion of green fluorescent protein (GFP) was restricted, even when viral MP was present (Christensen et al. 2009a and b). Currently, there is no explanation for how this might be achieved. Hence, we have a picture of PD as dynamic but highly regulated structures that respond to intra- and extracellular stimuli to modulate the passage of large and small molecules from cell to cell and through the vasculature.

Virus MP.

Virus MP provide the means for viruses to exploit inter- and intracellular trafficking pathways: in the case of viroids, the movement determinants are provided by secondary RNA structures (Wang and Ding 2010) that presumably interact with host proteins. The “colors and flavors” of MP have been reviewed extensively (Lucas 2006; Melcher 2000; Taliansky et al. 2008). Briefly, they can be divided into a few broad classes based upon functional properties (Fig. 1C through E). i) MP that show RNA-binding properties and mediate the translocation of the viral ribonuclear protein complexes (RNP) without obvious structural reorganization of PD (e.g., TMV 30K). Many of these proteins, alone or as part of the infection, are themselves trafficked through PD and may accumulate within the channel. ii) MP that bring about a structural reorganization of the PD channel through the formation of tubules that provide the conduit for the movement of virus particles or RNP from cell to cell (e.g., *Cowpea mosaic virus*

[CPMV] MP). iii) A loose group of subsidiary proteins defined genetically as MP but that have functions more aligned to intracellular trafficking of viral components to PD (e.g., *Potexvirus* TGBp2 and TGBp3); these may or may not show the ability to bind to viral nucleic acids.

Despite the functional commonalities of MP in mediating virus spread through PD, there is increasing evidence that the subtleties of specific virus movement may differ, even between quite closely related viruses. Nevertheless, it is the case that viruses that move from cell to cell as RNP have MP that bind nonspecifically to single-stranded (ss)RNA, an activity not widely demonstrated for MP of viruses moving as particles. Nevertheless, these generalizations are also complicated by sometimes oversimplistic definitions of MP function. Classically, the term has been used for proteins that are necessary for spreading infections but which have little impact on replication in single cells when mutated. However, the multifunctional nature of many viral proteins means that subsidiary movement functions may also be associated with other proteins. For example, the 126-kDa replicase protein from TMV was shown to have a role in movement (Hirashima and Watanabe 2001, 2003), and the P3 protein from *Cauliflower mosaic virus* (CaMV) was shown to bridge the interaction between the viral MP (P1) and the coat protein (CP) (Kobayashi et al. 2002); P3 also supports aphid transmission of the CaMV (Stavallone et al. 2005). The multifunctional nature of the proteins also means that MP may show alternative functions; for example, the 25-kDa MP of *Potato virus X* (PVX) also shows silencing suppressor activity (Bayne et al. 2005).

More systematically, MP can be divided into several large evolutionary groupings based upon functional properties and predicted secondary structures (Melcher 2000). This review will focus on recent work relating to the largest “30K family” group, to the family of triple-gene-block (TGB) MP, and to work related to *Closterovirus* and *Potyvirus* spp. The 30K group is a large family, a subset of which is represented by the proteins that form physical tubules, which contain and translocate virus particles through PD. These are distinct from other TMV 30K-like MP that form RNP and do not form tubules. An alternative functional classification of MP based on whether they transmit virus particles or RNP has also been proposed (Scholthof 2005).

30K family.

Evidence to support the concept of the 30K family of MP has come from complementation experiments using transgenic plants expressing *Alfalfa mosaic virus* (AMV) RNAs 1 and 2. These plants complement the replication of incoming cognate RNA3, which expresses MP and CP, and GFP to allow monitoring of viral cell-to-cell movement. AMV MP has a C-terminal domain of 44 amino acids (A44) that interacts with cognate CP or virus particles but is dispensable for tubule formation and AMV movement. Also, AMV CP has a C terminus dispensable for AMV cell-to-cell movement but necessary for virus encapsidation (Sanchez-Navarro and Bol 2001; Tenllado and Bol 2000). AMV RNA3 movement still occurs when both MP and CP are deleted of their C-terminal domains, indicating that AMV RNA3 could also be translocated through tubules as a viral RNP complex (Sánchez-Navarro and Bol 2001; Sánchez-Navarro et al. 2006). In the complementation system, replacement of AMV MP with TMV MP showed RNA3 movement whether or not the AMV A44 domain was retained. In this case, movement must again have occurred as a viral RNP complex (Sánchez-Navarro et al. 2006). When MP from *Brome mosaic virus*, *Cucumber mosaic virus* (CMV), CPMV, *Grapevine fanleaf virus* (GFLV), CaMV, or *Prunus necrotic ringspot virus* were used, movement was recorded but only upon addi-

tion of the A44 domain. Because this list includes viruses from within and outside (CMV) the 30K-tubule-forming group, it strengthens the broader concept of the 30K family. With the exception of CaMV MP, all these modified MP retained their capacity to promote RNA3 local movement whether or not AMV CP was competent for encapsidation (Sanchez-Navarro et al. 2006, 2010). This was taken to indicate that caulimoviral MP mediated only particle movement, whereas tubule-forming MP from RNA viruses promoted the transport of RNA3 as a viral RNA-CP complex. The unique limitation applying to CaMV may provide a necessary protection to the genomic DNA in the PD, an environment designed for the non-cell autonomous transport of RNA (Sanchez-Navarro et al. 2010).

This conservation of function for tubule-forming 30K family MP is in accordance with analyses of their organization. Hence, although they show little direct sequence relatedness, common functional domains for CaMV, CPMV, AMV, GFLV, and TSWV MP have been defined by mutagenesis (Belin et al. 1999; Huang et al. 2001; Lekkerkerker et al. 1996; Li et al. 2009; Thomas and Maule 1995). Generally, the core of the protein is required for tubule formation while the C terminus is dispensable for this function but necessary for the interaction with cognate CP (via P3 in the case of CaMV) (Carvalho et al. 2003; Stavallone et al. 2005).

Although the majority of the viruses represented in the 30K group appear to have single MP, *Geminivirus* spp. separately encode functions that transfer the ssDNA genome from the nucleus to PD and for translocation through PD (Jeske 2009). Interestingly, in the assay described above, complementation of AMV movement with *Geminivirus* MP was not successful, perhaps questioning the inclusion of the latter within the 30K group (Sanchez-Navarro et al. 2010).

TGB family.

Hordei-like (*Benyvirus*, *Hordeivirus*, *Pecluvirus*, and *Pomovirus*) and potex-like (*Alexvirus*, *Carlavirus*, *Foveavirus*, and *Potexvirus*) virus groups encode three proteins named TGBp1, TGBp2, and TGBp3 (TGB proteins) (class I and class II TGBp [Jackson et al. 2009]). There is a significant body of work that analyses the cell biological and biochemical properties of these proteins. Generally, it is now clear that the functional principles of the TGB proteins differ between the two groups despite organizational and some sequence similarity. Briefly, a difference between the hordei-like and potex-like viruses lies in the need for the CP for movement, with only the former group moving from cell to cell in its absence. In this case, viral RNAs complexed with TGBp1 are assumed to represent the mobile infectious unit (Lim et al. 2008), where TGBp1 is substituting for the CP seen in class II virus infections. TGBp1 is a sequence-nonspecific RNA-binding protein with NTPase and RNA helicase domains and, for class II, has the capacity to modify PD SEL and to traffic alone to neighboring cells. Class I TGBp1 is much larger than for class II through a large N-terminal addition that has additional RNA-binding domains, which may substitute for CP binding in the class II viruses. TGBp2 and TGBp3 are transmembrane proteins that span the ER membrane to establish a physical presence in both the ER lumen and the cytoplasm. The emerging picture for the collective processes mediated by class I TGBp is for TGBp1 to establish a direct interaction with TGBp3 by association with TGBp2 and trafficked to PD. The presence of TGBp2 strengthens the association with PD and may increase the PD SEL even though, alone, it is not targeted to this site (Tamai and Meshi 2001). However, this picture needs to be qualified by the observation that the detail of TGBp interactions and their consequences for subcellular targeting can be affected by the ratios of the respective proteins (Lim et al. 2008, 2009).

Tail structures and filamentous virus movement.

An interesting property of class II TGBp1 is its association with the filamentous PVX particle to form a polar “tail” structure visible by atomic force microscopy (AFM) and by immunolabeling (Karpova et al. 2006). This structure is reminiscent of similar structures observed for *Closterovirus* and *Potyvirus* spp. It has been proposed that these polar structures on filamentous viruses may represent a common mechanism for the polar translocation of virus particles that could allow destabilization of the particle for RNA translation in the neighboring cells (Taliany et al. 2008). In the case of *Closterovirus* spp., the tail is a complex structure involving several proteins with roles in virus movement (Alzhanova and Hruby 2007; Dolja et al. 2006). In addition to the CP and a further nonstructural protein, p6, a minor coat protein (CPm), p64, and HSP70h have all been shown to be necessary for cell-to-cell movement; p20 is necessary for systemic movement. The tail structure is formed from CPm, p64, p20, and HSP70h. Tailless virions result when the ATPase domain of HSP70h is inactivated and virions cannot be translocated through PD. Hsp70h has been shown to localize autonomously to PD and, as an integral part of the virion, has the potential to target the virion to PD for translocation. For cell-to-cell movement, p6 is the exception, in that it is not associated with the particle but becomes associated with ER. Using AFM, tail structures have also been visualized for *Potyvirus* spp. In this case, the RNA 5'-proximal end of the particle was immunolabeled for the virus-genome-linked protein (VPg) (Puustinen et al. 2002) and helper component protein (HC-Pro) (Torrance et al. 2006). *Potyvirus* spp. differ from many other viruses in that they have no dedicated MP but use several proteins that have additional roles in virus multiplication (Revers et al. 1999). Mutagenesis experiments have implicated CP, VPg, and the cylindrical inclusion protein (CIp) in cell-to-cell movement, and HC-Pro, VPg, and the newly identified PIPO protein (Wen and Hajimorad 2010) in systemic movement. More recent work (Wei et al. 2010) has shown that the N terminus of the *Turnip mosaic virus* P3 protein fused to PIPO (P3N-PIPO) is located at PD where it interacts with CIp to initiate the formation of inclusions. The existence of threads of CIp passing through PD supported the view that CI may be directly involved in virus translocation through the channel. The simplest interpretation of the CP requirement is that encapsidated viral RNA is the mobile *Potyvirus* infectious unit. This, however, has not been proven, although the close association of virus particles with CI located over PD apertures might support this view. Following microinjection, CP has also been shown to mediate the movement of viral RNA from cell to cell, and CP accumulates in PD during infection. HC-Pro had similar activity and may also be important in local *Potyvirus* spp. movement.

Host proteins interacting with MP.

Key to obtaining a clear understanding of the processes mediated by viral MP will be the identification of interacting proteins. A range of host proteins have been shown to interact with viral MP (Maule 2008) (Supplementary Table S1). Of these, only the PDL proteins have been shown to have positive attributes with respect to virus movement. By interacting specifically with MP of viruses (e.g., GFLV and CaMV) employing tubule-guided movement, PDL provide a PD docking platform or the catalyst for the assembly of MP into tubules, thereby allowing the passage of virions from cell to cell (Amari et al. in press). The molecular mechanism leading to the physical reconfiguration of the PD, including displacement of the desmotubule, remains to be established.

TMV MP has been studied most widely for its interaction with host proteins (Harries et al. 2010). Hence, it has been

shown to interact with cytoskeletal elements actin and tubulin (Heinlein et al. 1995; McLean et al. 1995; Su et al. 2010), microtubule end-binding protein (EB1) (Brandner et al. 2008), γ -tubulin (Boyko et al. 2007), microtubule-associated protein MP-binding protein 2C (MPB2C) (Kragler et al. 2003), calreticulin (Chen et al. 2005), pectin methyl esterase (Dorokhov et al. 1999), PD-associated protein kinase (Lee 2008; Lee et al. 2005), DnaJ co-chaperone for HSP70 (Shimizu et al. 2009), and transcriptional coactivators (MBF1 and KELP, shown for the related *Tomato mosaic virus* MP) (Matsushita et al. 2002; Sasaki et al. 2009). Many of the more recent findings here relate to MP-associated intracellular trafficking (Harries et al. 2010) (discussed below). Of the others, the PD-associated protein kinase may be necessary for MP phosphorylation shown to be important for trafficking activity (Lee et al. 2005). MP association with transcriptional co-activators may be connected with altered host gene expression following infection; KELP has been implicated in plant defense responses. Overexpression of KELP reduced virus movement probably by interfering with MP targeting to PD (Sasaki et al. 2009). Interaction with DnaJ is interesting because similar interactions exist for a wider range of MP (e.g., *Potato virus Y* [PVY] [Hofius et al. 2007] and TSWV [von Bargon et al. 2001]) and, together with the findings related to *Closterovirus* HSP70h, might indicate a general role for HSP70 chaperones in virus movement. For PVY, overexpression of the DnaJ domain protein led to delayed cell-to-cell spread of the infection (Hofius et al. 2007).

An important recent finding was that remorin (REM), associated with lipid raft microdomains in PD, was able to bind in vitro and in vivo with PVX TGBp1 (Raffaele et al. 2009a). As for many of the interacting proteins identified, remorin acts as an antagonist of virus movement. It was proposed (Raffaele et al. 2009a) that remorin may negatively influence viral trafficking by preventing the increase in SEL mediated by PVX TGBp1 (Angell et al. 1996). It will be interesting to see whether this effect is specific to PVX or more generally applicable to viruses with TGB proteins or even less-related viruses. In this respect, it is important to note that the group of REM controlling PVX movement are also differentially regulated during plant development (Raffaele et al. 2009b) and during plant-microbe interactions (Campo et al. 2008; Rocco et al. 2008; Widjaja et al. 2009), and share physical similarities to MP (Reymond et al. 1996). This group of REM also interacts with symbiotic receptors present in plasma membrane surrounding the bacteria *Sinorhizobium meliloti* in symbiotic nodules of *Medicago truncatula*, thereby controlling infection and release of rhizobia into the host cytoplasm (Lefebvre et al. 2010).

Intracellular trafficking to PD.

It is difficult to separate the subcellular processes involving virus MP that are positively associated with the forward trafficking of viruses from later processes reflecting the need, for example, for MP turnover and recycling or, indeed, from secondary functions of the MP, such as RNA silencing. Logically, it might be expected that the former would be found at the boundary of infection where the virus is moving into previously uninfected cells, and that cells located behind the infection front will reveal later events. Unfortunately, the precise front of infection is often very difficult to identify. Relevant in this context is the trafficking of PLRV MP, which trafficked to PD via interactions with actin and the ER but, in sink tissues where the MP was subject to rapid turnover, was more dependent on the microtubule network (Vogel et al. 2007). Similarly, the use of chemical inhibitors may not distinguish between early and late events or direct versus indirect effects. In addition, it is not always clear to what extent viruses have evolved very individual relationships with the cell biological machin-

ery for intracellular trafficking. Hence, a comparison of different viruses for their dependence on an intact actin network for sustained movement showed that TMV, PVX, and *Tomato bushy stunt virus* were dependent whereas *Turnip vein-clearing virus* (TVCV), a virus from the same genus as TMV, was not. This difference in actomyosin requirement for movement is correlated with the capacity of the 126 kDa from TMV to associate with microfilaments, a property that is not attributed to the equivalent protein from TVCV (Harries et al. 2009b). An important outcome from this work is that viruses appeared to have evolved differently in their requirements for intracellular trafficking in a manner not correlated with predicted phylogeny. Despite these reservations, the study of the intracellular behavior of viral MP has revealed some important clues as to where and how MP chaperone virus complexes to PD prior to translocation to neighboring cells (Harries et al. 2010).

Models for virus trafficking through PD.

In the absence of a good understanding of the physical nature of PD and of their regulation, many of the models describing how viruses might actually traffic from cell to cell necessarily remain rather speculative. We know, however, that PD provide a selection of physical environments in the form of cytoplasmic channels and membrane surfaces for molecular trafficking. A summary of the current models of virus movement through PD is presented in Figure 1C through E.

Sambade and Heinlein (2009) and Epel (2009) highlight the potential importance of the surface of the ER, represented by the desmotubule, as the pathway for movement of TMV and PVX and, certainly, MP for both these viruses show ER association in the near cytoplasm (discussed above). Interestingly, large, even particulate, TMV virus replication complexes have been reported to move through PD (Kawakami et al. 2004). These complexes include MP, the 126-kDa replicase-associated protein, and viral RNA. These massive structures, or even individual virus RNP, far exceed the SEL of PD. Based upon evidence in favor of a positive correlation between β -1,3-glucanase gene expression and virus invasion (Elvira et al. 2008; Gorovitz et al. 2007), Epel (2009) also invokes a virus replication-induced β -1,3-glucanase which is directed to PD to reduce callose in the wall and increase the SEL. For PVX infection, he also proposes that TGBp2 and TGBp3 complexes serve as a protein raft transporting the vRNA-TGBp1 complex by diffusion on the ER membrane through PD. He goes further in suggesting that ER association might activate a defense response seen, in part, as the induction of β -1,3-glucanase, and that the TGB proteins (probably TGBp3) target these pathogenesis-related (PR) protein enzymes in vesicles to the cell wall (Fig. 1C).

Also addressing *Potexvirus* sp. movement, Verchot-Lubicz (2005) proposed a complementary model (Fig. 1C) that integrates CP-dependent movement of viral RNA with the control of viral RNA translation and the suppression of host defenses. She envisaged three phases. i) Early phase: TGBp1 moves to PD where it increases the SEL. It also recruits TGBp2 and TGBp3 associated with ER membranes to form a membrane-bound complex called “docking complex” at PD and moves all three proteins into cells ahead of the infection front. In this location, TGBp1 suppresses plant defense based on RNA silencing, providing the virus with a spatial or temporal advantage for tissue infection. ii) Middle phase: the docking complex recruits CP-ssRNA RNP to TGBp1 for translocation through PD. iii) Late phase: interaction of TGBp1 with TGBp2 and TGBp3 in the next cell releases viral RNA from TGBp1 for translation; the original state of the channel is restored by TGBp1 sequestration with free CP.

Very recent excellent work (Su et al. 2010) proposed an alternative mechanism that connects the concept of actin-constraint

of PD SEL and virus movement (Fig. 1D). Notwithstanding the importance of actin for virus spread (Genoves et al. 2010; Harries et al. 2009a, b, and c; Hofmann et al. 2009), they show that the MP of CMV and TMV probably operate similarly in binding G- and F-actin in vitro. In vivo, this leads an inhibition of actin polymerization and severing of F-actin. They reconcile this apparent contradiction by proposing that the sequestration of MP to the cytoplasmic ER or to PD prevents actin depolymerization in the cytoplasm but allows it to occur in or near the PD, leading to increased SEL. How or whether this relates to a parallel reduction in callose was not investigated. It was suggested from this study that the regulation of actin structure by as-yet-unidentified endogenous actin-binding proteins may be involved in the movement of other macromolecules between plant cells, and that MP might mimic such endogenous mechanisms to increase PD SEL.

Tubule-based movement of viruses through PD is clearly very different structurally and mechanistically. Tubule formation results in the apparent displacement of the desmotubule to create a physical “tube” for viruses to move to neighboring cells (Fig. 1E). The tubules have the properties of homo-oligomers formed by direct interaction of MP monomers (Amari et al. in press; Kasteel et al. 1993). The finding that tubule-forming MP of GFLV and CaMV interact with PDL proteins as receptors at the PD (Amari et al. in press) is a major advance in our understanding of this process. What is not yet clear, however, is whether virus particles or RNP move through a static tubule or whether tubules are built with integral virus particles to propel the infection to the next cell. The former hypothesis seems mechanistically less favorable because it implies a driving force allowing particles to move autonomously inside the tubule cavity. This force could theoretically be provided by downstream displacement by viral particles entering the tubules. However, this does not explain why a CPMV MP mutant with tubule-forming capacity and capable of incorporating particles into tubules fails to promote virus movement (Pouwels et al. 2003). Thus, the most likely mechanism remains treadmilling with MP and virions being coassembled at one end of the tubules and disassembly at the other end, resulting in the release of the particles. This mechanism could also prevail for the tubule-guided transport of viral RNA-CP complexes as hypothesized for AMV (Sanchez-Navarro et al. 2006, 2010).

Systemic movement.

To take advantage of the vasculature for long-distance or systemic movement, viruses must cross several unique intercellular boundaries via PD. Hence, access to the phloem from the mesophyll necessitates traversing bundle sheath cells, phloem parenchyma, and companion cells. The form, number, and distribution of PD at each of these boundaries can differ (Ehlers and van Bel 2010; Fitzgibbon et al. 2010; Turgeon and Wolf 2009) and may present the virus with particular barriers to movement, which may be distinguishable genetically. Hence, for viroids, the separate phases of movement can be distinguished on the basis of RNA structural motifs required for local and systemic movement as distinct from those required for replication (Zhong et al. 2008). Plant genetic variants defective in systemic movement of specific viruses often have the interface between the bundle-sheath cells and phloem parenchyma as the barrier to infection, pointing to distinct PD functions at this boundary. Also, most but not all viruses move systemically as virus particles even though the CP may be dispensable for local movement (Robinson 1992). It is possible that this reflects the unfavorable environment for nonencapsidated RNAs in the phloem, although many other nonviral RNAs appear to be translocated through the phloem, probably as RNP (Buhtz et al. 2010). The *Umbravirus* sp. *Groundnut*

rosette virus, which has no encoded CP, specifically recruits a host nuclear protein (fibrillarin), making RNP complexes necessary for phloem translocation (Kim et al. 2007). Intriguingly, *Potato virus A* (PVA; genera *Potyvirus*), which does encode a CP needed for virus movement, also forms complexes between viral proteins and fibrillarin (Rajamäki and Valkonen 2009).

Unloading from the phloem is probably also a distinct process, occurring primarily from class III veins in the case of TMV and PVX (Cheng et al. 2000; Roberts et al. 1997) and from all classes for PVA (Rajamäki and Valkonen 2003). How the detail of the processes of phloem loading and unloading differ is not clear. However, PVX TGBp1 and CP fused to GFP and expressed in the phloem failed to unload to the surrounding tissues unless, for a low proportion of plants, PVX infection was also present (Mekuria et al. 2008). This indicated that other viral factors may be necessary for movement across the relevant interfaces. Interestingly, this unloading was quite specific, not mediating the movement of 2× GFP. This contrasted with TMV infection, which allowed the unloading of 2× GFP but not of the PVX reporter proteins. Apparently, rather than exhibiting a nonspecific increase in the PD SEL, cellular unloading interfaces also carry specificity. With respect to a mechanism, it was also interesting that the restraint on phloem unloading was also relaxed in the presence of protease inhibitors whether PVX was present or not, pointing to protein turnover being important in maintaining the restrictions on phloem unloading (Mekuria et al. 2008).

The phloem exerts physiological and physical constraints upon virus systemic movement, leading to the consistent patterns of virus infection seen in Figure 1A. It is also assumed that mass flow in phloem sieve elements is affected by the physical dimensions of the pores in the sieve plate (Mullendore et al. 2010). Although sieve pores are derived from modified PD, the role of callose in determining the physical properties of the pore is not completely clear. The potential for controlling systemic virus movement by regulating the physical dimensions of the sieve pores has not, to our knowledge, been investigated.

Movement of RNA silencing signals as a determinant of systemic infection.

It has long been suspected (Kobayashi and Zambryski 2007; Vogler et al. 2008) and recently demonstrated (Dunoyer et al. 2010a and b; Molnar et al. 2010) that small interfering RNAs (siRNAs), formed as part of RNA silencing-mediated defense, move in the symplastic continuum provided by PD. Evidence that siRNAs move through PD also comes from the complementation for non-cell autonomous silencing in defective *Turnip crinkle virus* (TCV) infections by ectopic expression of one of the small TCV MP (p9) (Zhou et al. 2008). In addition, Kobayashi and Zambryski (2007) have shown correlations between the degree in the spread of silencing and opening of the PD aperture as measured by the movement of GFP (27 kDa) and 2× GFP (54 kDa), and Vogler and associates (2008) have shown that the zone of extended silencing is enhanced in the presence of the TMV MP. However, in this case, an MP mutant that was defective with regard to normal TMV MP subcellular associations and PD targeting and for transport of viral RNA, but retained RNA binding and cell-to-cell trafficking ability, produced a similar effect (Vogler et al. 2008). This suggests that the principles that allow viral RNA and siRNA movement may differ.

Hence, the scenario exists whereby the success of virus invasion becomes a race between the efficiencies of virulence and defense operating at the interface between infected and noninfected cells. The balance in the race is tipped in favor of the virus through the evolution of virus-encoded suppressors of RNA silencing. Like small RNAs, some suppressors have been shown to act non-cell autonomously (e.g., *Potyvirus* HC-

Pro) (Rojas et al. 1997) and even some MP have been shown to carry suppressor activity (e.g., PVX TGBp1) (Bayne et al. 1995), serving both purposes of virus movement and combat of host defense. Virus-related small RNAs are formed as the product of the action of plant-encoded, Dicer-like enzymes on regions of double-stranded (ds) virus-specific RNA (Alvarado and Scholthof 2009). This yields short, 21- to 24-nucleotide dsRNAs, one strand of which becomes the guide RNA for sequence-specific cleavage or translational inhibition of target viral RNAs through the action of plant-encoded Argonaute (AGO) proteins (Alvarado and Scholthof 2009). Interestingly, non-cell autonomous properties of siRNAs seems to relate to the movement of ds RNA duplexes rather than ss-siRNAs or their complexes with AGO proteins (Dunoyer et al. 2010b). There are, however, examples of mRNAs (ssRNA) being able to move from cell to cell (Lucas et al. 2009) and nonencapsidated viral RNA move as ssRNP complexes in line with the ssRNA-binding activity of many MP. Also, some MP have RNA helicase activity (e.g., TGBp1) and two plant RNA helicases have been identified in relation to functions that contribute to PD-trafficking (Kobayashi et al. 2007; Stonebloom et al. 2009). Therefore, it is possible that ds-siRNAs may be denatured prior to transit through PD.

In recent work, Shi and associates (2009) used TCV lacking a CP gene and, therefore, defective for both movement and silencing suppression. By complementing silencing suppression with homologous and heterologous RNA virus suppressors, it was shown that RNA suppression was necessary for RNA genome amplification but that cell-to-cell movement was not a direct consequence of silencing suppression (Shi et al. 2009). Interestingly, many viral RNA-silencing suppressors were originally classified as proteins required for systemic movement (Scholthof 2005), pointing to the balance between virulence and defense as a key determinant in the outcome of systemic movement of the virus. In certain cases, suppressor activity appears to determine tissue specificity, especially with respect to meristematic tissues (Martin-Hernandez and Baulcombe 2008).

Future prospects.

It is two decades since TMV 30K was first identified as a MP that interacted with PD. Although significant progress has been made in the identification of the many varieties of MP, it remains surprising that we still understand so little about the mechanism of virus translocation through PD. The recent work on actin severing by CMV and TMV MP (Su et al. 2010) perhaps comes closest to revealing a mechanism that explains the increase in SEL required for virus transport but, even here, we have no knowledge of the motive force that carries the RNP through the PD channel. Similarly, for the tubule-forming MP, the identification of PDL as MP “receptors” (Amari et al. in press) makes a significant forward step; however, again, key questions remain. Fundamentally, what is needed is a much greater understanding of the nature of PD constituents and how they convey non-cell autonomy of a wide range of metabolites and informational molecules.

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LITERATURE CITED

Abbasi, A. R., Saur, A., Hennig, P., Tschiersch, H., Hajirezaei, M., Hofius, D., Sonnewald, U., and Voll, L. M. 2009. Tocopherol deficiency in

- transgenic tobacco (*Nicotiana tabacum* L.) plants leads to accelerated senescence. *Plant Cell Environ.* 32:144-157.
- Alvarado, V., and Scholthof, H. B. 2009. Plant responses against invasive nucleic acids: RNA silencing and its suppression by plant viral pathogens. *Semin. Cell Dev. Biol.* 20:1032-1040.
- Alzhanova, D., and Hruby, D. E. 2007. A host cell membrane protein, golgin-97, is essential for poxvirus morphogenesis. *Virology* 362:421-427.
- Amari, K., Boutant, E., Hofmann, C., Schmitt-Keichinger, C., Fernandez-Calvino, L., Didie, P., Lerich, A., Mutterer, J., Thomas, C., Heinlein, M., Mély, Y., Maule, A. J., and Ritzenthaler, C. Plasmodesmal proteins that mediate movement of plant viruses. *PLoS Pathog.* In press.
- Angell, S. M., Davies, C., and 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 glauca*. *Virology* 216:197-201.
- Bayne, E. H., Rakitina, D. V., Morozov, S. Y., and Baulcombe, D. C. 2005. Cell-to-cell movement of *Potato Potexvirus X* is dependent on suppression of RNA silencing. *Plant J.* 44:471-482.
- Beffa, R. S., Hofer, R. M., Thomas, M., and Meins, F. 1996. Decreased susceptibility to viral disease of beta-1,3-glucanase-deficient plants generated by antisense transformation. *Plant Cell* 8:1001-1011.
- Belin, C., Schmitt, C., Gaire, F., Walter, B., Demangeat, G., and Pinck, L. 1999. The nine C-terminal residues of the grapevine fanleaf nepovirus movement protein are critical for systemic virus spread. *J. Gen. Virol.* 80:1347-1356.
- Benítez-Alfonso, Y., and Jackson, D. 2009. Redox homeostasis regulates plasmodesmal communication in *Arabidopsis* meristems. *Plant Signal. Behav.* 4:655-659.
- Benítez-Alfonso, Y., Cilia, M., Roman, A. S., Thomas, C., Maule, A., Hearn, S., and Jackson, D. 2009. Control of *Arabidopsis* meristem development by thioredoxin-dependent regulation of intercellular transport. *Proc. Natl. Acad. Sci. U.S.A.* 106:3615-3620.
- Boyko, V., Hu, Q., Seemanpillai, M., Ashby, J., and Heinlein, M. 2007. Validation of microtubule-associated *Tobacco mosaic virus* RNA movement and involvement of microtubule-aligned particle trafficking. *Plant J.* 51:589-603.
- Brandner, K., Sambade, A., Boutant, E., Didier, P., Mely, Y., Ritzenthaler, C., and Heinlein, M. 2008. *Tobacco mosaic virus* movement protein interacts with green fluorescent protein-tagged microtubule end-binding protein 1. *Plant Physiol.* 147:611-623.
- Bucher, G. L., Tarina, C., Heinlein, M., Di Serio, F., Meins, F., and Iglesias, V. A. 2001. Local expression of enzymatically active class I beta-1,3-glucanase enhances symptoms of TMV infection in tobacco. *Plant J.* 28:361-369.
- Buhtz, A., Pieritz, J., Springer, F., and Kehr, J. 2010. Phloem small RNAs, nutrient stress responses, and systemic mobility. *BMC Plant Biol.* 10:64-64.
- Campo, S., Manrique, S., Garcia-Martinez, J., and San Segundo, B. 2008. Production of cecropin A in transgenic rice plants has an impact on host gene expression. *Plant Biotechnol. J.* 6:585-608.
- Carvalho, C. M., Wellink, J., Ribeiro, S. G., Goldbach, R. W., and van Lent, J. W. M. 2003. The C-terminal region of the movement protein of *Cowpea mosaic virus* is involved in binding to the large but not to the small coat protein. *J. Gen. Virol.* 84:2271-2277.
- Chen, M. H., Tian, G. W., Gafni, Y., and Citovsky, V. 2005. Effects of calreticulin on viral cell-to-cell movement. *Plant Physiol.* 138:1866-1876.
- Chen, X.-Y., and Kim, J.-Y. 2009. Callose synthesis in higher plants. *Plant Signal. Behav.* 4:489-492.
- Cheng, N.-H., Su, C.-L., Carter, S. A., and Nelson, R. S. 2000. Vascular invasion routes and systemic accumulation patterns of *Tobacco mosaic virus* in *Nicotiana benthamiana*. *Plant J.* 23:349-362.
- Christensen, N., Tilsner, J., Bell, K., Hammann, P., Parton, R., Lacomme, C., and Oparka, K. 2009a. The 5' cap of *Tobacco mosaic virus* (TMV) is required for virion attachment to the actin/endoplasmic reticulum network during early infection. *Traffic* 10:536-551.
- Christensen, N. M., Faulkner, C., and Oparka, K. 2009b. Evidence for unidirectional flow through plasmodesmata. *Plant Physiol.* 150:96-104.
- De Pino, V., Boran, M., Norambuena, L., Gonzalez, M., Reyes, F., Orellana, A., and Moreno, S. 2007. Complex formation regulates the glycosylation of the reversibly glycosylated polypeptide. *Planta* 226:335-345.
- Dhugga, K. 2006. Golgi glucan synthases. Pages 114-122 in: *The Science and Lore of the Plant Cell Wall: Biosynthesis, Structure and Function*, T. Hayashi, ed. BrownWalker Press, Boca Raton, FL, U.S.A.
- Ding, B., Kwon, M. O., and Warnberg, L. 1996. Evidence that actin filaments are involved in controlling the permeability of plasmodesmata in tobacco mesophyll. *Plant J.* 10:157-164.
- Dolja, V. V., Kreuze, J. F., and Valkonen, J. P. T. 2006. Comparative and functional genomics of closteroviruses. *Virus Res.* 117:38-51.
- Dorokhov, Y. L., Makinen, K., Frolova, O. Y., Merits, A., Saarinen, J., Kalkinen, N., Atabekov, J. G., and Saarma, M. 1999. A novel function for a ubiquitous plant enzyme pectin methylesterase: the host-cell receptor for the tobacco mosaic virus movement protein. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 461:223-228.
- Dunoyer, P., Brosnan, C. A., Schott, G., Wang, Y., Jay, F., Alioua, A., Himber, C., and Voinnet, O. 2010a. An endogenous, systemic RNAi pathway in plants. *EMBO (Eur. Mol. Biol. Organ.) J.* 29:1699-1712.
- Dunoyer, P., Schott, G., Himber, C., Meyer, D., Takeda, A., Carrington, J. C., and Voinnet, O. 2010b. Small RNA duplexes function as mobile silencing signals between plant cells. *Science* 328:912-916.
- Ehlers, K., and van Bel, A. J. 2010. Dynamics of plasmodesmal connectivity in successive interfaces of the cambial zone. *Planta* 231:371-385.
- Elvira, M. I., Galdeano, M. M., Gilardi, P., Garcia-Luque, I., and Serra, M. T. 2008. Proteomic analysis of pathogenesis-related proteins (PRs) induced by compatible and incompatible interactions of *pepper mild mottle virus* (PMMoV) in *Capsicum chinense* L-3 plants. *J. Exp. Bot.* 59:1253-1265.
- Epel, B. L. 2009. Plant viruses spread by diffusion on ER-associated movement-protein-rafts through plasmodesmata gated by viral induced host beta-1,3-glucanases. *Semin. Cell Dev. Biol.* 20:1074-1081.
- Faulkner, C. R., Blackman, L. M., Collings, D. A., Cordwell, S. J., and Overall, R. L. 2009. Anti-tropomyosin antibodies co-localise with actin microfilaments and label plasmodesmata. *Eur. J. Cell Biol.* 88:357-369.
- Fitzgibbon, J., Bell, K., King, E., and Oparka, K. 2010. Super-resolution imaging of plasmodesmata using 3D-structured illumination microscopy (3D-SIM). *Plant Physiol.* 153:1453-1463.
- Genoves, A., Navarro, J. A., and Pallas, V. 2010. The intra- and intercellular movement of *Melon necrotic spot virus* (MNSV) depends on an active secretory pathway. *Mol. Plant-Microbe Interact.* 23:263-272.
- Gorovits, R., Akad, F., Beery, H., Vidavsky, F., Mahadav, A., and Czosnek, H. 2007. Expression of stress-response proteins upon whitefly-mediated inoculation of *Tomato yellow leaf curl virus* in susceptible and resistant tomato plants. *Mol. Plant-Microbe Interact.* 20:1376-1383.
- Grennan, A. K. 2007. Lipid rafts in plants. *Plant Physiol.* 143:1083-1085.
- Guenoun-Gelbart, D., Elbaum, M., Sagi, G., Levy, A., and Epel, B. L. 2008. *Tobacco mosaic virus* (TMV) replicase and movement protein function synergistically in facilitating TMV spread by lateral diffusion in the plasmodesmal desmotubule of *Nicotiana benthamiana*. *Mol. Plant-Microbe Interact.* 21:335-345.
- Guseman, J. M., Lee, J. S., Bogenschütz, N. L., Peterson, K. M., Virata, R. E., Xie, B., Kanaoka, M. M., Hong, Z. L., and Torii, K. U. 2010. Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in *Arabidopsis* CHORUS (GLUCAN SYNTHASE-LIKE 8). *Development* 137:1731-1741.
- Harries, P. A., Palanichelvam, K., Bhat, S., and Nelson, R. S. 2008. *Tobacco mosaic virus* 126-kDa protein increases the susceptibility of *Nicotiana tabacum* to other viruses and its dosage affects virus-induced gene silencing. *Mol. Plant-Microbe Interact.* 21:1539-1548.
- Harries, P. A., Palanichelvam, K., Yu, W., Schoelz, J. E., and Nelson, R. S. 2009a. The *Cauliflower mosaic virus* protein P6 forms motile inclusions that traffic along actin microfilaments and stabilize microtubules. *Plant Physiol.* 149:1005-16.
- Harries, P. A., Park, J. W., Sasaki, N., Ballard, K. D., Maule, A. J., and Nelson, R. S. 2009b. Differing requirements for actin and myosin by plant viruses for sustained intercellular movement. *Proc. Natl. Acad. Sci. U.S.A.* 106:17594-17599.
- Harries, P. A., Schoelz, J. E., and Nelson, R. S. 2009c. Covering common ground: F-actin-dependent transport of plant viral protein inclusions reveals a novel mechanism for movement utilized by unrelated viral proteins. *Plant Signal. Behav.* 4:454-456.
- Harries, P. A., Schoelz, J. E., and Nelson, R. S. 2010. Intracellular transport of viruses and their components: utilizing the cytoskeleton and membrane highways. *Mol. Plant Microbe Interact.* 23:1381-1393.
- Heinlein, M., Epel, B. L., Padgett, H. S., and Beachy, R. N. 1995. Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* 270:1983-1985.
- Hirashima, K., and Watanabe, Y. 2001. Tobamovirus replicase coding region is involved in cell-to-cell movement. *J. Virol.* 75:8831-8836.
- Hirashima, K., and Watanabe, Y. 2003. RNA helicase domain of tobamovirus replicase executes cell-to-cell movement possibly through collaboration with its nonconserved region. *J. Virol.* 77:12357-12362.
- Hofius, D., Maier, A. T., Dietrich, C., Jungkuntz, I., Bornke, F., Maiss, E., and Sonnewald, U. 2007. Capsid protein-mediated recruitment of host DnaJ-like proteins is required for *Potato virus Y* infection in tobacco plants. *J. Virol.* 81:11870-11880.
- Hofmann, C., Niehl, A., Sambade, A., Steinmetz, A., and Heinlein, M. 2009. Inhibition of *Tobacco mosaic virus* movement by expression of an actin-binding protein. *Plant Physiol.* 149:1810-1823.
- Hofmann, J., Youssef-Banora, M., de Almeida-Engler, J., and Grundler, F. M. W. 2010. The role of callose deposition along plasmodesmata in

- nematode feeding sites. *Mol. Plant-Microbe Interact.* 23:549-557.
- Huang, M., Jongejans, L., Zheng, H. Q., Zhang, L., and Bol, J. F. 2001. Intracellular localization and movement phenotypes of *Alfalfa mosaic virus* movement protein mutants. *Mol. Plant-Microbe Interact.* 14:1063-1074.
- Jackson, A. O., Lim, H. S., Bragg, J., Ganesan, U., and Lee, M. Y. 2009. Hordevirus replication, movement, and pathogenesis. *Annu. Rev. Phytopathol.* 47:385-422.
- Jeske, H. 2009. Geminiviruses. TT viruses—the still elusive human pathogens. *Curr. Top. Microbiol. Immunol.* 331:185-226.
- Jones, D. L., Blancaflor, E. B., Kochian, L. V., and Gilroy, S. 2006. Spatial coordination of aluminum uptake, production of reactive oxygen species, callose production and wall rigidification in maize roots. *Plant Cell Environ.* 29:1309-1318.
- Jongebloed, U., Szederkenyi, J., Hartig, K., Schobert, C., and Komor, E. 2004. Sequence of morphological and physiological events during natural ageing and senescence of a castor bean leaf: sieve tube occlusion and carbohydrate back-up precede chlorophyll degradation. *Physiol. Plant.* 120:338-346.
- Karpova, O. V., Zayakina, O. V., Arkhipenko, M. V., Sheval, E. V., Kiselyova, O. I., Poljakov, V. Y., Yaminsky, I. V., Rodionova, N. P., and Atabekov, J. G. 2006. *Potato virus X* RNA-mediated assembly of single-tailed ternary 'coat protein-RNA-movement protein' complexes. *J. Gen. Virol.* 87:2731-2740.
- Kasteel, D., Wellink, J., Verver, J., Vanlent, J., Goldbach, R., and Vankammen, A. 1993. The involvement of *cowpea mosaic virus-M* RNA-encoded proteins in tubule formation. *J. Gen. Virol.* 74:1721-1724.
- Kawakami, S., Watanabe, Y., and Beachy, R. N. 2004. *Tobacco mosaic virus* infection spreads cell to cell as intact replication complexes. *Proc. Natl. Acad. Sci. U.S.A.* 101:6291-6296.
- Kim, S. H., MacFarlane, S., Kalinina, N. O., Rakitina, D. V., Ryabov, E. V., Gillespie, T., Haupt, S., Brown, J. W. S., and Taliansky, M. 2007. Interaction of a plant virus-encoded protein with the major nucleolar protein fibrillarin is required for systemic virus infection. *Proc. Natl. Acad. Sci. U.S.A.* 104:11115-11120.
- Kobayashi, K., and Zambryski, P. 2007. RNA silencing and its cell-to-cell spread during *Arabidopsis* embryogenesis. *Plant J.* 50:597-604.
- Kobayashi, K., Otegui, M. S., Krishnakumar, S., Mindrinos, M., and Zambryski, P. 2007. INCREASED SIZE EXCLUSION LIMIT2 encodes a putative DEVH box RNA helicase involved in plasmodesmata function during *Arabidopsis* embryogenesis. *Plant Cell* 19:1885-1897.
- Kobayashi, K., Tsuge, S., Stavolone, L., and Hohn, T. 2002. The *Cauliflower mosaic virus* virion-associated protein is dispensable for viral replication in single cells. *J. Virol.* 76:9457-9464.
- Kragler, F., Curin, M., Trutnyeva, K., Gansch, A., and Waigmann, E. 2003. MPB2C, a microtubule-associated plant protein binds to and interferes with cell-to-cell transport of *Tobacco mosaic virus* movement protein. *Plant Physiol.* 132:1870-1883.
- Kronberg, K., Vogel, F., Rutten, T., Hajirezaei, M. R., Sonnewald, U., and Hofius, D. 2007. The silver lining of a viral agent: increasing seed yield and harvest index in *Arabidopsis* by ectopic expression of the *potato leaf roll virus* movement protein. *Plant Physiol.* 145:905-918.
- Lee, J.-Y. 2008. Phosphorylation of movement proteins by the plasmodesmal-associated protein kinase. *Methods Mol. Biol.* 451:625-639.
- Lee, J. Y., Taoka, K., Yoo, B. C., Ben-Nissan, G., Kim, D. J., and Lucas, W. J. 2005. Plasmodesmal-associated protein kinase in tobacco and *Arabidopsis* recognizes a subset of non-cell-autonomous proteins. *Plant Cell* 17:2817-2831.
- Lefebvre, B., Timmers, T., Mbengue, M., Moreau, S., Herve, C., Toth, K., Bittencourt-Silvestre, J., Klaus, D., Deslandes, L., Godiard, L., Murray, J. D., Udvardi, M. K., Raffaele, S., Mongrand, S., Cullimore, J., Gamas, P., Niebel, A., and Ott, T. 2010. A remorin protein interacts with symbiotic receptors and regulates bacterial infection. *Proc. Natl. Acad. Sci. U.S.A.* 107:2343-2348.
- Lekkerkerker, A., Wellink, J., Yuan, P., vanLent, J., Goldbach, R., and vanKammen, A. V. 1996. Distinct functional domains in the cowpea mosaic virus movement protein. *J. Virol.* 70:5658-5661.
- Levy, A., Erlanger, M., Rosenthal, M., and Epel, B. L. 2007a. A plasmodesmata-associated beta-1,3-glucanase in *Arabidopsis*. *Plant J.* 49:669-682.
- Levy, A., Guenoune-Gelbart, D., and Epel, B. L. 2007b. Beta-1,3-glucanases: plasmodesmal gate keepers for intercellular communication. *Plant Signal. Behav.* 2:404-407.
- Li, H. B., Qin, Y. M., Pang, Y., Song, W. Q., Mei, W. Q., and Zhu, Y. X. 2007. A cotton ascorbate peroxidase is involved in hydrogen peroxide homeostasis during fibre cell development. *New Phytol.* 175:462-471.
- Li, W. M., Lewandowski, D. J., Hilf, M. E., and Adkins, S. 2009. Identification of domains of the *Tomato spotted wilt virus* NSm protein involved in tubule formation, movement and symptomatology. *Virology* 390:110-121.
- Lim, H. S., Bragg, J. N., Ganesan, U., Lawrence, D. M., Yu, J. L., Isogai, M., Hammond, J., and Jackson, A. O. 2008. Triple gene block protein interactions involved in movement of *Barley stripe mosaic virus*. *J. Virol.* 82:4991-5006.
- Lim, H. S., Bragg, J. N., Ganesan, U., Ruzin, S., Schichnes, D., Lee, M. Y., Vaira, A. M., Ryu, K. H., Hammond, J., and Jackson, A. O. 2009. Subcellular localization of the *Barley stripe mosaic virus* triple gene block proteins. *J. Virol.* 83:9432-9448.
- Lucas, W. J. 2006. Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. *Virology* 344:169-184.
- Lucas, W. J., Ham, L. K., and Kim, J. Y. 2009. Plasmodesmata—bridging the gap between neighboring plant cells. *Trends Cell Biol.* 19:495-503.
- MacFarlane, S. A., and Popovich, A. H. 2000. Efficient expression of foreign proteins in roots from tobamovirus vectors. *Virology* 267:29-35.
- Martin-Hernandez, A. M., and Baulcombe, D. C. 2008. Tobacco rattle virus 16-kilodalton protein encodes a suppressor of RNA silencing that allows transient viral entry in meristems. *J. Virol.* 82:4064-4071.
- Matsushita, Y., Miyakawa, O., Deguchi, M., Nishiguchi, M., and Nyunoya, H. 2002. Cloning of a tobacco cDNA coding for a putative transcriptional coactivator MBF1 that interacts with the *Tomato mosaic virus* movement protein. *J. Exp. Bot.* 53:1531-1532.
- Maule, A. J. 2008. Plasmodesmata: structure, function and biogenesis. *Curr. Opin. Plant Biol.* 11:680-686.
- McLean, B. G., Zupan, J., and Zambryski, P. C. 1995. *Tobacco mosaic virus* movement protein associates with the cytoskeleton in tobacco cells. *Plant Cell* 7:2101-2114.
- Mekuria, T., Barnunusinghe, D., Payton, M., and Verchot-Lubicz, J. 2008. Phloem unloading of *Potato virus X* movement proteins is regulated by virus and host factors. *Mol. Plant-Microbe Interact.* 21:1106-1117.
- Melcher, U. 2000. The '30K' superfamily of viral movement proteins. *J. Gen. Virol.* 81:257-266.
- Molnar, A., Melynyk, C. W., Bassett, A., Hardcastle, T. J., Dunn, R., and Baulcombe, D. C. 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* 328:872-875.
- Mullendore, D. L., Windt, C. W., Van As, H., and Knoblauch, M. 2010. Sieve tube geometry in relation to phloem flow. *Plant Cell* 22:579-593.
- Olesinski, A. A., Almon, E., Navot, N., Perl, A., Galun, E., Lucas, W. J., and Wolf, S. 1996. Tissue-specific expression of the *tobacco mosaic virus* movement protein in transgenic potato plants alters plasmodesmal function and carbohydrate partitioning. *Plant Physiol.* 111:541-550.
- Oparka, K. J., and Turgeon, R. 1999. Sieve elements and companion cells—traffic control centers of the phloem. *Plant Cell* 11:739-750.
- Pouwels, J., Kornet, N., van Bers, N., Guighelaar, T., van Lent, J., Bisseling, T., and Wellink, J. 2003. Identification of distinct steps during tubule formation by the movement protein of *Cowpea mosaic virus*. *J. Gen. Virol.* 84:3485-3494.
- Puustinen, P., Rajamäki, M. L., Ivanov, K. I., Valkonen, J. P. T., and Mäkinen, K. 2002. Detection of the potyviral genome-linked protein VPg in virions and its phosphorylation by host kinases. *J. Virol.* 76:12703-12711.
- Raffaele, S., Bayer, E., Lafarge, D., Cluzet, S., Retana, S. G., Boubekour, T., Leborgne-Castel, N., Carde, J. P., Lherminier, J., Noirot, E., Satiat-Jeunemaitre, B., Laroche-Traineau, J., Moreau, P., Ott, T., Maule, A. J., Reymond, P., Simon-Plas, F., Farmer, E. E., Bessoule, J. J., and Mongrand, S. 2009a. Remorin, a solanaceae protein resident in membrane rafts and plasmodesmata, impairs *Potato virus X* movement. *Plant Cell* 21:1541-1555.
- Raffaele, S., Bayer, E., and Mongrand, S. 2009b. Upregulation of the plant protein remorin correlates with dehiscence and cell maturation: a link with the maturation of plasmodesmata? *Plant Signal. Behav.* 4:915-919.
- Rajamäki, M. L., and Valkonen, J. P. T. 2003. Localization of a potyvirus and the viral genome-linked protein in wild potato leaves at an early stage of systemic infection. *Mol. Plant-Microbe Interact.* 16:25-34.
- Rajamäki, M. L., and Valkonen, J. P. T. 2009. Control of nuclear and nucleolar localization of nuclear inclusion protein A of picorna-like *Potato virus A* in *Nicotiana* species. *Plant Cell* 21:2485-2502.
- Revers, F., Le Gall, O., Candresse, T., and Maule, A. J. 1999. New advances in understanding the molecular biology of plant/potyvirus interactions. *Mol. Plant-Microbe Interact.* 12:367-376.
- Reymond, P., Kunz, B., Paul-Pletzer, K., Grimm, R., Eckerskorn, C., and Farmer, E. E. 1996. Cloning of a cDNA encoding a plasma membrane-associated, uronide binding phosphoprotein with physical properties similar to viral movement proteins. *Plant Cell* 8:2265-2276.
- Rinne, P. L. H., van den Boogaard, R., Mensink, M. G. J., Kopperud, C., Kormelink, R., Goldbach, R., and van der Schoot, C. 2005. Tobacco plants respond to the constitutive expression of the tospovirus movement protein N with a heat-reversible sealing of plasmodesmata that impairs development. *Plant J.* 43:688-707.
- Roberts, A. G., Santa Cruz, S., Roberts, I. M., Prior, D. A. M., Turgeon, R., and Oparka, K. J. 1997. Phloem unloading in sink leaves of *Nicotiana*

- benthamiana*: comparison of a fluorescent solute with a fluorescent virus. *Plant Cell* 9:1381-1396.
- Robinson, D. J. 1992. Detection of tobacco rattle virus by reverse transcription and polymerase chain-reaction. *J. Virol. Methods* 40:57-66.
- Rocco, M., Corrado, G., Arena, S., D'Ambrosio, C., Tortiglione, C., Sellaroli, S., Marra, M., Rao, R., and Scaloni, A. 2008. The expression of tomato prosystemin gene in tobacco plants highly affects host proteomic repertoire. *J. Proteomics* 71:176-185.
- Rojas, M. R., Zerbin, F. M., Allison, R. F., Gilbertson, R. L., and Lucas, W. J. 1997. Capsid protein and helper component proteinase function as potyvirus cell-to-cell movement proteins. *Virology* 237:283-295.
- Ruan, Y. L., Xu, S. M., White, R., and Furbank, R. T. 2004. Genotypic and developmental evidence for the role of plasmodesmal regulation in cotton fiber elongation mediated by callose turnover. *Plant Physiol.* 136:4104-4113.
- Sambade, A., and Heinlein, M. 2009. Approaching the cellular mechanism that supports the intercellular spread of *Tobacco mosaic virus*. *Plant Signal. Behav.* 4:35-38.
- Sambade, A., Brandner, K., Hofmann, C., Seemanpillai, M., Mutterer, J., and Heinlein, M. 2008. Transport of TMV movement protein particles associated with the targeting of RNA to plasmodesmata. *Traffic* 9:2073-2088.
- Sanchez-Navarro, J. A., and Bol, J. F. 2001. Role of the *Alfalfa mosaic virus* movement protein and coat protein in virus transport. *Mol. Plant-Microbe Interact.* 14:1051-1062.
- Sanchez-Navarro, J. A., Herranz, M. C., and Pallas, V. 2006. Cell-to-cell movement of *Alfalfa mosaic virus* can be mediated by the movement proteins of Ilar-, bromo-, cucumo-, tobamo- and comoviruses and does not require virion formation. *Virology* 346:66-73.
- Sanchez-Navarro, J., Fajardo, T., Zicca, S., Pallas, V., and Stavelone, L. 2010. Caulimoviridae tubule-guided transport is dictated by movement protein properties. *J. Virol.* 84:4109-4112.
- Sasaki, N., Ogata, T., Deguchi, M., Nagai, S., Tamai, A., Meshi, T., Kawakami, S., Watanabe, Y., Matsushita, Y., and Nyunoya, H. 2009. Over-expression of putative transcriptional coactivator KELP interferes with *Tomato mosaic virus* cell-to-cell movement. *Mol. Plant Pathol.* 10:161-173.
- Scholthof, H. B. 2005. Plant virus transport: motions of functional equivalence. *Trends Plant Sci.* 10:376-382.
- Shi, Y., Ryabov, E. V., van Wezel, R., Li, C., Jin, M., Wang, W., Fan, Z., and Hong, Y. 2009. Suppression of local RNA silencing is not sufficient to promote cell-to-cell movement of *Turnip crinkle virus* in *Nicotiana benthamiana*. *Plant Signal. Behav.* 4:15-22.
- Shimizu, T., Yoshii, A., Sakurai, K., Hamada, K., Yamaji, Y., Suzuki, M., Namba, S., and Hibi, T. 2009. Identification of a novel tobacco DnaJ-like protein that interacts with the movement protein of *Tobacco mosaic virus*. *Arch. Virol.* 154:959-967.
- Simpson, C., Thomas, C., Findlay, K., Bayer, E., and Maule, A. J. 2009. An *Arabidopsis* GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking. *Plant Cell* 21:581-594.
- Sivaguru, M., Fujiwara, T., Samaj, J., Baluska, F., Yang, Z. M., Osawa, H., Maeda, T., Mori, T., Volkmann, D., and Matsumoto, H. 2000. Aluminum-induced 1-3-beta-D-glucan inhibits cell-to-cell trafficking of molecules through plasmodesmata. A new mechanism of aluminum toxicity in plants. *Plant Physiol.* 124:991-1005.
- Stavelone, L., Villani, M. E., Leclerc, D., and Hohn, T. 2005. A coiled-coil interaction mediates *Cauliflower mosaic virus* cell-to-cell movement. *Proc. Natl. Acad. Sci. U.S.A.* 102:6219-6224.
- Stonebloom, S., Burch-Smith, T., Kim, I., Meinke, D., Mindrinos, M., and Zambryski, P. 2009. Loss of the plant DEAD-box protein ISE1 leads to defective mitochondria and increased cell-to-cell transport via plasmodesmata. *Proc. Natl. Acad. Sci. U.S.A.* 106:17229-17234.
- Su, S., Liu, Z., Chen, C., Zhang, Y., Wang, X., Zhu, L., Miao, L., Wang, X.-C., and Yuan, M. 2010. *Cucumber mosaic virus* movement protein severs actin filaments to increase the plasmodesmal size exclusion limit in tobacco. *Plant Cell* 4:1373-87.
- Taliansky, M., Torrance, L., and Kalinina, N. O. 2008. Role of plant virus movement proteins. *Methods Mol. Biol.* 451 :33-54.
- Tamai, A., and Meshi, T. 2001. Cell-to-cell movement of *Potato virus X*: the role of p12 and p8 encoded by the second and third open reading frames of the triple gene block. *Mol. Plant-Microbe Interact.* 14:1158-1167.
- Tenllado, F., and Bol, J. F. 2000. Genetic dissection of the multiple functions of *Alfalfa mosaic virus* coat protein in viral RNA replication, encapsidation, and movement. *Virology* 268:29-40.
- Thomas, C. L., Bayer, E. M., Ritzenthaler, C., Fernandez-Calvino, L., and Maule, A. J. 2008. Specific targeting of a plasmodesmal protein affecting cell-to-cell communication. *Plos Biol.* 6:180-190.
- Thomas, C. L., and Maule, A. J. 1995. Identification of structural domains within the cauliflower mosaic virus movement protein by scanning deletion mutagenesis and epitope tagging. *Plant Cell* 7:561-572.
- Torrance, L., Andreev, I. A., Gabrenaite-Verhovskaya, R., Cowan, G., Makinen, K., and Taliansky, M. E. 2006. An unusual structure at one end of potato potyvirus particles. *J. Mol. Biol.* 357:1-8.
- Turgeon, R., and Wolf, S. 2009. Phloem transport: cellular pathways and molecular trafficking. *Annu. Rev. Plant Biol.* 60:207-221.
- Ueki, S., and Citovsky, V. 2005. Identification of an interactor of cadmium ion-induced glycine-rich protein involved in regulation of callose levels in plant vasculature. *Proc. Natl. Acad. Sci. U.S.A.* 102:12089-12094.
- Verchot-Lubicz, J. 2005. A new cell-to-cell transport model for Potexviruses. *Mol. Plant-Microbe Interact.* 18:283-290.
- Verma, D. P. S., and Hong, Z. L. 2001. Plant callose synthase complexes. *Plant Mol. Biol.* 47:693-701.
- Vogel, F., Hofius, D., and Sonnewald, U. 2007. Intracellular trafficking of *Potato leafroll virus* movement protein in transgenic *Arabidopsis*. *Traffic* 8:1205-1214.
- Vogler, H., Kwon, M. O., Dang, V., Sambade, A., Fasler, M., Ashby, J., and Heinlein, M. 2008. *Tobacco mosaic virus* movement protein enhances the spread of RNA silencing. *PLoS Pathog.* 4:e1000038. Published online.
- von Barga, S., Salchert, K., Paape, M., Piechulla, B., and Kellmann, J. W. 2001. Interactions between the *Tomato spotted wilt virus* movement protein and plant proteins showing homologies to myosin, kinesin and DnaJ-like chaperones. *Plant Physiol. Biochem.* 39:1083-1093.
- Wang, Y., and Ding, B. 2010. Viroids: small probes for exploring the vast universe of RNA trafficking in plants. *J. Integr. Plant Biol.* 52:28-39.
- Wei, T., Zhang, C., Hong, J., Xiong, R., Kasschau, K. D., Zhou, X., Carrington, J. C., and Wang, A. 2010. Formation of complexes at plasmodesmata for potyvirus intercellular movement is mediated by the viral protein P3N-PIPO. *PLoS Pathog.* 6:e1000962.
- Wen, R. H., and Hajimorad, M. R. 2010. Mutational analysis of the putative pipo of *Soybean mosaic virus* suggests disruption of PIPO protein impedes movement. *Virology* 400:1-7.
- White, R. G., Badelt, K., Overall, R. L., and Vesik, M. 1994. Actin associated with plasmodesmata. *Protoplasma* 180:169-184.
- Widjaja, I., Naumann, K., Roth, U., Wolf, N., Mackey, D., Dangl, J. L., Scheel, D., and Lee, J. 2009. Combining subproteome enrichment and Rubisco depletion enables identification of low abundance proteins differentially regulated during plant defense. *Proteomics* 9:138-147.
- Zavaliev, R., Sagi, G., Gera, A., and Epel, B. L. 2010. The constitutive expression of *Arabidopsis* plasmodesmal-associated class I reversibly glycosylated polypeptide impairs plant development and virus spread. *J. Exp. Bot.* 61:131-142.
- Zhong, X. H., Archual, A. J., Amin, A. A., and Ding, B. 2008. A genomic map of viroid RNA motifs critical for replication and systemic trafficking. *Plant Cell* 20:35-47.
- Zhou, Y., Ryabov, E., Zhang, X. M., and Hong, Y. G. 2008. Influence of viral genes on the cell-to-cell spread of RNA silencing. *J. Exp. Bot.* 59:2803-2813.

AUTHOR-RECOMMENDED INTERNET RESOURCE

John Innes Center plant virus time lapse video:
www.youtube.com/watch?v=ZMPnUrNeEVs