Plant Cell Monogr (7)
E. Waigmann · M. Heinlein: Viral Transport in Plants
DOI 10.1007/7089_2006_101/Published online: 13 February 2007
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Spread Throughout the Plant: Systemic Transport of Viruses

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Abstract Viral long distant transport is an essential step for systemic infection. Because the process involves different types of highly differentiated vascular-associated cells, the virus systemic movement is regulated differentially at each tissue interface. In this chapter, we review current knowledge about viral systemic transport process in non-Arabidopsis hosts. We especially focus on viral and host factors participating in viral systemic transport. We also briefly overview the effect of RNA silencing, the host innate immunity, on viral systemic movement.

1 Introduction

After replication and accumulation at the local infection sites, viruses have to travel to uninfected, systemic tissues via the vasculature in order to establish systemic infection. This process, which involves entering into, traveling through, and exiting from the vasculature into uninfected tissue, is collectively termed "systemic movement". Systemic movement is not just an aggregation of numerous cell-to-cell movement processes as occurs in local infection; rather, cell-to-cell and systemic movement are two different modes of viral translocation. During systemic movement, the virus crosses several different types of cells, including mesophyll (MS), bundle sheath (BS), vascular parenchyma (VP), and companion cells (CC), as well as sieve elements (SE) of the vascular system. The involvement of so many different types of tissues and cells in systemic movement stands in stark contrast to local movement, which occurs between only relatively few tissues, such as MS and epidermis, or within uniform population of the cells of the same tissue. Due to the involvement of a number of different cells, systemic movement is expected to be more complex than local movement at the molecular level. Moreover, the rates of the two types of movement are quite different. Generally, local movement is a relatively slow process (e.g., 5–15 μm/h, see Gibbs 1976), presumably restricted by the rate of viral replication. In contrast, long-distance movement through the vascular system is rather rapid (e.g., 50–80 mm/h (see Gibbs 1976), as it occurs with the flow of photoassimilates and, in some

Table 1 Virus–host combinations that develop limited virus systemic movement[a]

Virus	Host	Inoculated leaves ME	BS	CC	SE	Systemic leaves SE	CC	BS	ME	Refs.
CCMV	Resistant soybean strains	⇒|								Goodrick et al. 1991
CMV	Transgenic plants expressing CMV replicase	⇒	⇒|							Wintermantel et al. 1997
TAV Florida strain	Cucumber	⇒	⇒|							Thompson et al. 1998
CP-less TMV	*N. tabacum*	⇒	⇒|							Ding et al. 1996
RCNMV with mutant MP	*N. edwardsonii* and cowpea	⇒	⇒|							Wang et al. 1998
Move through internal/external phloem										
BGMV	*N. benthamiana*		⇒	⇒	⇒	⇒	⇒	⇒		Morra et al. 2000; Qin et al. 2001
AbMV	Bean		⇒	⇒	⇒	⇒	⇒	⇒		Levy et al. 2003
	Susceptible hosts		⇒	⇒	⇒	⇒	⇒	⇒		Barker 1987; Barker 1989
Move only through external phloem										
PLRV	Resistant hosts		⇐	⇐	⇐	⇐	⇐	⇐		Barker et al. 1986; Derrick et al. 1992, 1997
PepMoV-FL	Resistant pepper strain		⇐	⇐	⇐	⇐	⇐	⇐		Guerini et al. 1999

[a] In susceptible hosts, the indicated viruses, except for PLRV, move from non-vascular ME to different vascular-associated and vascular cell types (BS, VP, CC, SE) of the inoculated leaves and, through SE, to systemic leaves, which they first enter through the vasculature and then unload into ME. In restrictive hosts, viral movement is arrested at different cellular boundaries, which usually are specific for a host-viral strain combination as shown in this Table. Arrows mark the cell types between which the indicated viral strain can move in the corresponding restrictive host, and solid vertical lines indicate the cell boundaries at which the viral movement is arrested

cases, does not require viral replication (Susi et al. 1999; Wintermantel et al. 1997). Moreover, these two types of movement require different sets of viral proteins, suggesting that local and systemic movements utilize different host molecular machineries, especially during entrance to and traffic through the plant intercellular connections, plasmodesmata (PD). The involvement of highly specialized host tissues and multiple viral factors in systemic transport has impeded direct experimental approaches, such as protein microinjection or transient gene expression by microbombardment, to study this transport process at the molecular level. Therefore, the viral systemic movement has been studied by analyzing the accumulation of viral product in systemic leaves: a lower accumulation of viral product in remote tissue may be attributed to either impaired systemic movement of the virus or systemic acquired resistance (SAR). The possibility of SAR can be eliminated by confirming the establishment of secondary infection of the same viral strain in systemic leaves.

This experimental design, however, cannot always define the true reason for the SAR-independent lack of viral product in systemic tissue. This is because plant innate immune response to viral infection often includes RNA silencing, which many viruses counter by encoding RNA silencing suppressors (Baulcombe 2002, 2004; Bisaro 2006; Marathe et al. 2000; Moissiard et al. 2004; Qu et al. 2005; Scholthof 2005; van der Boogaart et al. 1998; Voinnet 2001; Wang et al. 2005). Since viral RNA silencing suppressor is encoded by the moving virus itself, it presumably functions most efficiently at the infection front, where cell-to-cell movement of the virus into uninfected tissue takes place. Therefore, it is difficult to distinguish the reasons for inefficient viral accumulation: is it impaired viral movement, or inhibited viral accumulation based on inefficient viral suppression of RNA silencing at the infected site?

In this chapter, we review current knowledge about the viral and host factors participating in viral systemic movement in non-Arabidopsis host plants, and demonstrate how studies of restriction of systemic movement in specific hosts (Table 1) is used to define cellular boundaries that represent barriers for viral movement. We also briefly describe viral and host factors that were once assumed to be involved in viral translocation during systemic movement, but were then revealed to be involved in suppression of host RNA silencing of the virus.

2
Viral Factors Required for Systemic Movement

Viral systemic movement in a non-Arabidopsis host is often studied using host–virus combinations that show defective systemic accumulation (reviewed in Ueki et al. 2006; Waigmann et al. 2004). Systemic infection of a viral

strain can be restricted in certain hosts, while closely related strains can infect the same host systemically (Ueki et al. 2006; Waigmann et al. 2004). In many cases, the differences in systemic movement can be attributed to sequence variation(s) in a viral factor(s) required for viral systemic movement (Ueki et al. 2006, and references therein; Waigmann et al. 2004). These observations demonstrate that the viral factors are indeed involved in systemic movement, possibly via close interaction with the host machinery (Ueki et al. 2006, and references therein; Waigmann et al. 2004). In addition, compatibilities of these viral factors to the host machinery may define the host susceptibility to the viral strain. Table 1 summarizes several examples of the virus–host combinations that result in limited viral movement.

Among these viral systemic movement factors, some are not actually required for the translocation of viral genome during systemic infection, but for suppression of RNA silencing. For example, the 126-kDa tobamovirus protein and potyvirus HC-Pro have long been designated "systemic movement factors", and it is only recently that these two factors were found to actually be viral suppressors for RNA silencing; as such they do not aid in the movement itself, but in the accumulation of virus.

In this section, we discuss viral factors that are assumed to aid in the translocation process per se during long-distance movement. The involvement of RNA silencing suppressors for systemic movement is discussed in Sect. 5.

2.1
Movement Proteins

The term "movement protein" (MP) is normally used for viral factors that are required for local, cell-to-cell movement. However, in some cases, these factors are also required for the viral systemic transport, possibly exercising an additional function(s) to enable systemic movement. For example, the BR1 and BL1 MPs of bipartite geminiviruses, such as *Bean common mosaic virus* (BCMV), *Tomato golden mosaic virus* (TGMV), and *African cassava mosaic virus* (ACMV), aid in systemic transport (Jeffrey et al. 1996; Schaffer et al. 1995; von Arnim et al. 1993), and triple gene block protein 1 (TGBp1) MPs of hordeiviruses and potexviruses, which function during cell-to-cell movement, are required for systemic infection as well (Kalinina et al. 2001; Lough et al. 2001; Solovyev et al. 1999). Similarly, *Cucumber mosaic virus* (CMV) MP is involved in both local and systemic movement of the virus (De Jong et al. 1995; Kaplan et al. 1997; Li et al. 2001; Sanz et al. 2000; Takeshita et al. 1998). In many of these cases, however, the local and systemic MP activities can be uncoupled. For instance, point mutations in *Red clover necrotic mosaic virus* (RCNMV) MP prevent the virus systemic movement, while they do not affect its cell-to-cell movement (Wang et al. 1998). Moreover, MPs of some luteoviruses, such as *Bean yellow dwarf virus* (BYDV) and *Potato leaf roll virus*

(PLRV), are involved in systemic transport (Chay et al. 1996; Lee et al. 2002), possibly by associating with the specialized deltoid-shaped PD that connect CC with SE in some hosts (Schmitz et al. 1997). In other, less restrictive hosts, MP is not required for the vascular transport of luteoviruses, e.g., PLRV and *Beet western yellow luteovirus* (BWYV) (Lee et al. 2002; Ziegler-Graff et al. 1996). These results suggest that luteoviral MPs may, at least in part, determine the host specificity of the viral systemic movement.

2.2 Coat Proteins

Coat protein (CP) is a viral factor known to be required for systemic movement of the vast majority of viral species, including tobamoviruses (e.g., *Tobacco mosaic virus* (TMV) (Dawson et al. 1988; Holt et al. 1991; Osbourn et al. 1990; Saito et al. 1990; Siegal et al. 1962; Takamatsu et al. 1987)), dianthoviruses (e.g., RCNMV (Vaewhongs et al. 1995; Xiong et al. 1993) and *Carnation ring spot virus* (CRSV) (Sit et al. 2001)), tombusviruses (e.g., *Tomato bushy stunt virus* (TBSV) (Desvoyes et al. 2002; Scholthof et al. 1993), *Cucumber necrosis virus* (CuNV) (McLean et al. 1993), and *Cymbidium ring spot virus* (CymRSV) (Dalmay et al. 1992; Huppert et al. 2002)), geminiviruses e.g., *Maize streak virus* (MSV) (Boulton et al. 1989; Boulton et al. 1993; Liu et al. 2001; Liu et al. 1999), TYLCV (Noris et al. 1998), BYDV (Liu et al. 1998), *Beet mild curly top virus* (Soto et al. 2005), *Bean golden mosaic virus* (BGMV) (Pooma et al. 1996) and TGMV (Brough et al. 1988; Gardiner et al. 1988) alfamoviruses, e.g., *Alfalfa mosaic virus* (AMV) (Spitsin et al. 1999; van der Kuyl et al. 1991), cucumoviruses (e.g., CMV (Takeshita et al. 1998; Taliansky et al. 1995)), bromoviruses (e.g., *Brome mosaic virus* (BMV) (Rao et al. 1996)), luteoviruses (e.g., BWYV (Mutterer et al. 1999; Ziegler-Graff et al. 1996)), potexviruses (e.g., *White clover mosaic virus* (Lough et al. 2001), *Potato virus X* (PVX) (Santa Cruz et al. 1998)), and potyviruses (e.g., *Tobacco etch virus* (TEV) (Dolja et al. 1994, 1995), *Tobacco vein mottling virus* (TVMV) (Lopez-Moya et al. 1998)), and *Pea seed-borne mosaic virus* (PSbMV) (Andersen et al. 1998). Consistent with the role of viral CPs in systemic movement, the occurrence of encapsidated particles of diverse viruses, for example, TMV (Ding et al. 1996; Esau et al. 1967), *Cucumber green mottle mosaic tobamovirus* (CGMMV) (Simon-Buela et al. 1999), BWYV (Esau et al. 1972a,b), BYDV (Gill et al. 1975; Jensen 1969), CMV (Blackman et al. 1998), and PLRV (Schmitz et al. 1997; Shepardson et al. 1980), in the vasculature or vascular fluid obtained from infected plants suggests that these viruses move through the vascular system as assembled virions.

However, encapsidation may not be a prerequisite for the systemic transport of many other viruses, because several viral strains that lack encapsidation activity are still able to move systemically. For example, umbraviruses, which do not produce CP, move systemically, possibly by forming a ribonucle-

oprotein complex between the viral genomic RNA and a viral protein encoded by ORF3 (Taliansky et al. 2003b). Furthermore, even in a number of viruses that produce CP, elimination of this protein (e.g., in RCNMV (Xiong et al. 1993), TBSV (Scholthof et al. 1995), CuNV (McLean et al. 1993), and TGMV (Brough et al. 1988; Gardiner et al. 1988)) or disruption of its encapsidation capacity in CP mutants (e.g., in *Cowpea chlorotic mottle bromovirus* (CCMV) (Schneider et al. 1997) and CymRSV (Dalmay et al. 1992; Huppert et al. 2002)) does not abolish systemic infection of some hosts. On the other hand, the systemic movement ability of some CP mutants of several viruses, such as TMV (Culver et al. 1995; Dawson et al. 1988), CRSV (Sit et al. 2001), RCNMV (Xiong et al. 1993), TGMV (Pooma et al. 1996), and TEV (Dolja et al. 1994; Dolja et al. 1995), is impaired, although they retain their encapsidation activity. Therefore, in many virus–host combinations, the CP function in viral encapsidation can be uncoupled from its function in systemic movement. The latter function of CP may involve interaction with and modification of host factors, in order to facilitate the systemic traffic of viral components.

In addition to their major CP component, some viral capsids contain minor constituents that may also play a role in the systemic movement. For example, the capsid of the luteovirus BWYV consists of two protein species: a major 22-kDa component, p3, and a minor 74-kDa component, the read-through protein (RT) p74; p74 is synthesized by suppressing translational termination of p3, which allows the translation to continue to the adjacent ORF5, thereby producing an additional read-through protein domain (RTD) (Bahner et al. 1990; Brault et al. 1995; Filichkin et al. 1994; Martin et al. 1990; Wang et al. 1995). BWYV mutants that do not produce RTD are still encapsidated, forming virions, but they exhibit reduced systemic infection in *Nicotiana clevelandii*, suggesting that the RTD is required, by as yet unknown mechanism, for efficient systemic transport of the virus (Mutterer et al. 1999).

2.3 VPg of Potyviruses

Another viral factor involved in systemic movement is the potyvirus viral genome-linked protein (VPg), which is covalently attached to the 5′ end of viral genomic RNA and is essential for viral replication activity (reviewed in Revers et al. 1996; Urcuqui-Inchima et al. 2001). VPg has been identified as a factor required for systemic movement and as a host-range determinant in TEV strains for *Nicotiana tabacum* (Schaad et al. 1996, 1997), and in *Potato virus A* (PVA) strains for *Nicanda physaloides* and potato plants (Räjamaki et al. 1999, 2002, 2003). In addition, a point mutation in *Turnip mosaic virus* (TuMV) VPg, substituting phenylalanine at position 12 with methionine, impairs local and systemic movement of TuMV in *Nicotiana benthamiana* and Arabidopsis, demonstrating that this amino acid residue of VPg is crucial for systemic movement of the virus (Dunoyer et al. 2004).

How does VPg facilitate systemic transport? VPg has been shown to interact with the eukaryotic initiation factor-4E (eIF4E) in vitro and in planta (Leonard et al. 2000, 2004; Robaglia et al. 2006; Schaad et al. 2000; Wittmann et al. 1997), and eIF4E has been shown to move from cell to cell (Gao et al. 2004b). Therefore, it is tempting to speculate that the potyvirus VPg interacts with endogenous eIF4E, such that the host factor aids the virus movement (see also Sect. 3.4). Since VPg binds covalently to the 5′ end of viral genome RNA, eIF4E-VPg-viral genome may move cell to cell as a complex after the replication in single cells. In addition to eIF4E aiding VPg to mediate viral movement, VPg may modulate the biochemical activity of eIF4E by increasing the binding affinity of eIF4E to another initiation factor, eIF4G, and reducing it toward mRNA cap (Michon et al. 2006).

Additional, novel potyvirus VPg-interacting proteins (PVIPs), which have a PHD finger-like cysteine-rich domain (Schindler et al. 1993), have been identified from pea, Arabidopsis and *N. benthamiana* (Dunoyer et al. 2004, see also Sect. 3.5). Possible involvement of the VPg–PVIPs interaction during the systemic infection process suggests that PVIPs may represent another class of host factors involved in the potyviral systemic movement process (Dunoyer et al. 2004).

Thus, although VPg is involved in the replication process (reviewed in Revers et al. 1996; Urcuqui-Inchima et al. 2001), it may also function as a crucial factor for the viral systemic movement process. Because no evidence exists to suggest that VPg facilitates viral movement by impairing host resistance, e.g., RNA silencing, VPg may represent a bona fide movement factor required for systemic translocation of the virus. In contrast, HC-Pro was once assumed to be a systemic movement factor, and was later revealed to be a RNA silencing suppressor (Kasschau et al. 2001).

2.4
Umbravirus ORF3 Proteins

Umbraviruses are unusual in that they do not encode a conventional CP and, thus, do not form true viral particles in infected tissues (reviewed in Robinson et al. 1999). Nevertheless, umbraviruses rapidly establish systemic movement in compatible hosts. One of the viral factors, the ORF3 protein of *Groundnut rosette virus* (GRV), supports long-distance transport of both GRV RNA and the genomic RNA of a CP-less mutant of an unrelated virus, TMV (Ryabov et al. 1999). In addition, when the GRV ORF3 protein is expressed from chimeric TMV in place of TMV CP, designated TMV(ORF3), it binds the TMV(ORF3) RNA and facilitates its transport through the host plant vasculature, demonstrating that the GRV ORF3 protein can systemically translocate heterologous RNA molecules, presumably in the form of ribonucleoprotein complexes (Taliansky et al. 2003a). Moreover, chimeric TMV strains expressing the ORF3-encoded proteins from other umbraviruses, such as *Pea ena-*

tion mosaic virus-2 (PEMV-2) and *Tobacco mottle virus* (TMoV), instead of TMV CP, move systemically in *N. benthamiana* and *N. clevelandii*, but not in *N. tabacum* (Ryabov et al. 2001b). Because *N. benthamiana* and *N. clevelandii* are systemic hosts for PEMV-2, TMoV, and TMV, whereas *N. tabacum* is a systemic host only for TMV and not for the two umbraviruses (Ryabov et al. 2001b), the ORF3 protein may also determine the host specificity of the systemic transport process. While the mechanism underlying this ORF3 protein function is still unclear, simple protection of the viral RNA from cellular nucleases may not play a major role in establishing the host range of systemic transport because the ORF3 protein–RNA complexes are stable in cell extracts of both *N. benthamiana*, in which the ORF3 protein supports systemic infection, and *N. tabacum*, in which it does not (Ryabov et al. 2001b).

Electron microscopic studies have shown that in vivo, within infected cells, binding of the ORF3 protein to RNA produces filamentous ribonucleoprotein particles with a helical structure, albeit not as uniform as classical virions (Taliansky et al. 2003a). In vitro, the ORF3 protein forms oligomers and binds RNA, consistent with its RNA-binding activity in vivo (Taliansky et al. 2003a). The ORF3 protein–RNA complexes are detected in all types of cells and are abundant in phloem-associated ones, especially in CC and immature SE (Taliansky et al. 2003a); this accumulation of ORF3 protein within the host plant vasculature is consistent with biological role of this protein as a facilitator of umbraviral systemic transport.

In addition, a recent study has shown that when transiently expressed, umbravirus ORF3 protein is targeted to nuclei, preferably nucleoli (Kim et al. 2004). The relationship of this newly discovered cellular localization of ORF3 to its function as a systemic movement factor has yet to be clarified.

3 Host Factors Involved in Systemic Movement

Besides the effectors encoded by the genome of the invading virus, the process of systemic movement involved host cell components, which often directly interact with the viral factors during movement. To date, several host factors involved in viral systemic movement have been identified from *Arabidopsis thaliana* using reverse genetic analysis. However, due to lack of genomic sequence information, our knowledge about plant factors involved in viral systemic movement in many non-Arabidopsis plant species is quite limited.

3.1 Pectin Methylesterase (PME)

PME has been identified as a cell-wall protein that interacts with tobamovirus MP in the course of cell-to-cell movement (Chen et al. 2000; Dorokhov et al.

1999). The role of PME in viral systemic movement has also been demonstrated using antisense suppression of its gene in tobacco plants, which preferentially occurs within the vascular tissues (Chen et al. 2003). TMV accumulation in uninoculated leaves of these PME-antisense plants is significantly delayed, indicating impaired systemic transport of this virus. Since no differences were detected in the vascular loading and unloading of a fluorescent solute between the PME-antisense plants and wild-type tobacco, PME is not involved in the phloem transport of solutes (Chen et al. 2003). Immunofluorescence confocal microscopy analysis demonstrated that, in the PME-antisense plants, TMV virions enter the host vasculature but fail to exit into uninoculated non-vascular tissues (Chen et al. 2003). Therefore, in the PME-antisense plants, TMV unloading from the vasculature is significantly impaired, whereas its loading into the tissue remains intact. The mechanism by which the MP–PME interaction affects the viral movement remains unknown, but it has been suggested that PME, via biochemical modification of pectins, may loosen the cell wall around PD, allowing the PD to open more easily (Boevink et al. 2005), or it may simply help transport MP to or anchor it at the cell wall, potentially in the vicinity of PD (Boevink et al. 2005; Chen et al. 2000).

3.2
cdiGRP, Callose, and *β*-1,3-Glucanase

Besides proteins required for the movement process, the host plants produce factors that negatively regulate viral systemic movement; one such factor is a cadmium-induced glycine-rich protein (cdiGRP), discovered in tobacco plants (Ueki et al. 2002). Identification of cdiGRP was based on the observations that systemic movement of tobamoviruses, such as TVCV and TMV, is blocked in tobacco plants pretreated with low concentrations of the heavy metal cadmium, while local virus movement in these plants is not affected (Citovsky et al. 1998; Ghoshroy et al. 1998). This inhibitory effect of cadmium ions is tobamovirus-specific because systemic movement of TEV was not impaired by the same treatment (Ghoshroy et al. 1998); interestingly, however, cadmium treatment also inhibited the systemic spread of RNA silencing in *N. tabacum* and *N. benthamiana* plants (Ueki et al. 2001). Cadmium-induced inhibition of systemic viral spread occurs by a SAR-independent mechanism because it is also observed in *NahG*-expressing transgenic plants (Citovsky et al. 1998) which are unable to accumulate salicylic acid and develop SAR (Gaffney et al. 1993). Immunodetection of tobamoviral CP revealed that the virus accumulates in the vasculature of uninoculated, systemic leaves but not in the surrounding MS cells, indicating that, in cadmium-treated plants, the spreading virus enters, but does not exit, the host plant vascular system (Citovsky et al. 1998).

The cdiGRP cDNA was isolated by a PCR-based subtraction cloning strategy as a tobacco gene whose expression was induced by a low concentration

of cadmium ions whereas high, toxic amounts of cadmium did not induce the *cdiGRP* gene (Ueki et al. 2002). Cadmium-induced expression of cdiGRP is tissue-specific, with the protein found mainly in the cell walls of the plant vascular bundle. Importantly, constitutive expression of cdiGRP in transgenic plants significantly reduces tobamoviral systemic movement in the absence of cadmium, whereas antisense suppression of cdiGRP allows the virus to spread systemically, even in cadmium-treated plants (Ueki et al. 2002). cdiGRP does not restrict viral movement directly. Instead, this protein induces – by an as-yet unknown mechanism – callose accumulation on PD on the cell walls of the phloem; these callose deposits, in turn, most likely reduce viral transport from the phloem into the surrounding non-vascular cells (Ueki et al. 2002). Callose is a 1,3-β-D-glucan (Stone et al. 1992) deposited at the collar region of the PD (Northcote et al. 1989). Degradation of callose is thought to increase PD permeability (Botha et al. 2000; Northcote et al. 1989), whereas its deposition is believed to restrict intercellular transport (Bucher et al. 2001; Delmer et al. 1993; Iglesias et al. 2000) by relaxing or constricting, respectively, of the PD collar sphincter. Thus, callose may represent a polysaccharide plant cell wall component that restricts viral systemic movement through PD, most likely by reducing PD permeability.

A cdiGRP-interacting protein, GrIP, was identified from a *N. tabacum* cDNA library by two-hybrid screening using cdiGRP as bait (Ueki et al. 2005). Like cdiGRP, GrIP is expressed in vascular tissue and accumulates in the cell wall (Ueki et al. 2005). Interestingly, accumulation of cdiGRP protein and callose, with or without the cadmium ion treatment, was enhanced in GrIP-overexpressing transgenic plants relative to wild-type plants, demonstrating that GrIP is involved in the regulation of cdiGRP expression/accumulation (Ueki et al. 2005). Since the levels of the *cdiGRP* mRNA were not affected by GrIP expression, GrIP must regulate the accumulation of the cdiGRP protein at the post-transcriptional level (Ueki et al. 2005). Because GrIP binds to cdiGRP in vitro and in vivo, this GrIP–cdiGRP interaction may stabilize and/or help cell wall targeting of cdiGRP (Ueki et al. 2005). Therefore, GrIP, together with cdiGRP and callose, may control PD transport in *N. tabacum*.

The amount of callose in the cell walls is directly controlled by the balance of two opposing enzymatic activities: callose synthase, which produces callose, and β-1,3-glucanase, which hydrolyzes it (Kauss 1985, 1996). Plant callose synthases are still poorly characterized, whereas β-1,3-glucanases have been better studied. Plant β-1,3-glucanases are grouped into three classes according to their structure (reviewed in Beffa et al. 1996a; Leubner-Mezger et al. 1999). Class I β-1,3-glucanases are basic proteins localized in the vacuole of MS and epidermal cells; class II and III β-1,3-glucanases are acidic isoforms secreted into the cell walls. Class II β-1,3-glucanases include the pathogenesis-related (PR) proteins PR2, PR N, and PR O, and class III consists of a single member, PR-Q′ (reviewed in Beffa et al. 1996a; Leubner-Mezger et al. 1999). By virtue of their ability to regulate the amount of callose, which then restricts intercellular

transport, β-1,3-glucanases can be regarded as cellular factors controlling viral movement. Indeed, TMV infection of tobacco plants elevates β-1,3-glucanase activity, which presumably enables more efficient viral movement (reviewed in Beffa et al. 1996a). Conversely, antisense suppression of β-1,3-glucanase in Nicotiana species results in increased callose deposits in the cell wall (Beffa et al. 1996b), reduced PD permeability (Iglesias et al. 2000), and delayed local and systemic movement of such viruses as TMV, *Tobacco necrosis virus*, and PVX (Beffa et al. 1996b; Iglesias et al. 2000). Similarly, overexpression of the β-1,3-glucanase coding sequence from a TMV-based vector facilitates viral movement, whereas antisense expression of the same sequence delays viral movement in the inoculated leaf (Bucher et al. 2001). Thus, induction of callose accumulation by an abiotic stimulus, e.g., cadmium ions via cdiGRP (Ueki et al. 2002), or by antisense suppression of β-1,3-glucanases (Beffa et al. 1996b; Iglesias et al. 2000), negatively regulates systemic and/or cell-to-cell transport of plant viruses. Potentially, GrIP, cdiGRP, β-1,3-glucanases, and callose represent a multicomponent system that controls PD transport by constricting/relaxing the callose sphincter at the collar regions of PD.

3.3
Tomato Mosaic Virus CP-Interacting Protein-L (IP-L)

A tobacco protein that interacts with tobamoviral CP in vitro, *Tomato mosaic virus* (ToMV) CP-interacting protein-L (IP-L), was identified by screening a tobacco cDNA library using the yeast two-hybrid system (Li et al. 2005). The isolated cDNA was identical to an elicitor-responsive protein from *N. tabacum*, and was also highly homologous to senescence-related proteins from tomato and pepper (Li et al. 2005). The *IP-L* gene expression is markedly increased by inoculation of ToMV and PVX (Li et al. 2005). Importantly, when expression of *IP-L* was suppressed by virus-induced gene silencing (VIGS) utilizing a PVX-based vector in *N. benthamiana* plants, infection of ToMV was significantly delayed, demonstrating that a high level of IP-L is required for efficient systemic infection in the host (Li et al. 2005). Though the molecular mechanism of its action has not been elucidated, IP-L may represent a factor that enhances viral systemic movement, and/or the process of viral replication. Since tobamoviral CP is involved in systemic movement (Dawson et al. 1988; Holt et al. 1991; Osbourn et al. 1990; Saito et al. 1990; Siegal et al. 1962; Takamatsu et al. 1987), the CP-interacting IP-L may be involved in viral systemic movement as well.

3.4
Eukaryotic Initiation Factor-4E (eIF4E)

eIF4E binds specifically to the 5′-CAP structure of mRNA to initiate the translation process in the host cell cytoplasm (Robaglia et al. 2006). The eIF4E

proteins from a compatible host plant interact with potyviral VPg in vitro and in vivo (Leonard et al. 2000, 2004; Robaglia et al. 2006; Schaad et al. 2000; Wittmann et al. 1997). Moreover, recent studies have suggested that incompatibilities of host eIF4E isoforms with potyviral VPg may underlie naturally occurring host resistance to potyvirus by restricting its systemic movement. For example, the *pvr2* locus in pepper, which confers recessive resistance to strains of *Potato virus Y* (PVY), corresponds to the host *eIF4E* gene (Ruffel et al. 2002). Consistent with this idea, PVX-based transient expression of *eIF4E* from a susceptible pepper host (Yolo Wonder strain) restores the systemic movement of PVY in a resistant host (Yolo Y strain) (Ruffel et al. 2002). A similar observation was obtained from an analysis of lettuce resistance to *Lettuce mosaic virus* (LMV) (Nicaise et al. 2003): when *eIF4E* from susceptible, tolerant, and resistant hosts were sequenced, variations in the sequence were found near the predicted CAP-recognition pocket of the protein (Nicaise et al. 2003). Transient expression of eIF4E from the susceptible host, in this case by simultaneous expression of the protein and the virus from a recombinant LMV vector with the *eIF4E* sequence inserted between *LMV-P1* and *LMV HC-Pro* genes, restored systemic infection of the virus in the resistant host, again demonstrating that eIF4E from a susceptible host is sufficient to complement the systemic infection of the virus in a resistant host (Nicaise et al. 2003). Moreover, eIF4E sequence variations have been demonstrated to underlie the resistance mechanism of several pea strains to *Pea seed-borne mosaic virus* (PSbMV) (Gao et al. 2004a,b). These results demonstrate that the compatibility between the host factor and VPg may be crucial to efficient potyviral systemic infection. Because PVY replication occurs in isolated protoplasts from a resistant pepper strain with a *pvr2* genotype, which carries a mutated *eIF4E* gene, the viral resistance based on the incompatibility between the viral VPg and host eIF4E may not be exclusively due to impaired viral replication (Arroyo et al. 1996). Moreover, eIF4E itself can move from cell to cell (Gao et al. 2004b), suggesting that this host protein binds to the viral VPg, which in turn covalently associates with the 5′-CAP structure of the viral genome, and aids VPg in the translocation of the viral genome complex to the neighboring cells, possibly by interacting with and gating PD (see also Sect. 2.3).

Whether the eIF4E–VPg interaction defines the potyviral systemic mobility, rather than cell-to-cell movement, is still uncertain. As described in Sect. 2.3, potyviral VPg may act as a host-range determinant by limiting the viral systemic movement. Based on this idea, the molecular interactions that involve VPg and determine the virus systemic mobility should occur specifically during the systemic translocation, rather than during the cell-to-cell movement process. Potentially, the host eIF4E that participates in the potyviral systemic infection is specifically expressed in vascular-associated tissues, and thus is involved in viral systemic movement via the vasculature. Alternatively, eIF4E may be involved mainly in the cell-to-cell movement, while

another, as yet unknown, host factor(s) facilitates long-distance translocation via its interaction with the viral VPg.

3.5 Potyvirus VPg-Interacting Protein (PVIP)

PVIP is another host protein that may interact with potyviral VPg in the processes of cell-to-cell and viral systemic movement. PVIP was identified by yeast two-hybrid screening of a pea cDNA library with PSbMV VPg as bait (Dunoyer et al. 2004). The PVIP protein has no homology to any proteins with known function, and appears to be plant-specific (Dunoyer et al. 2004). A small family of genes in Arabidopsis (*AtPVIP*s) and a gene from *N. benthamiana* (*PVIPnb*) exhibited a homology to the pea *PVIP* (*PVIPp*) gene at the protein level (Dunoyer et al. 2004). These proteins display differential interactions with VPgs from different strains of potyvirus; VPg from PSbMV, TuMV, and LMV interacts with AtPVIP1, AtPVIP2, PVIPp, and PVIPnb, whereas VPg from TEV, *Cowpea mosaic virus* (CPMV), *Tomato black ring virus* (TBRV), and *Grapevine fan leaf virus* (GFLV) does not (Dunoyer et al. 2004). Within the (Wintermantel et al. 1997) amino acid residues of TuMV VPg, deletion of the 66 N-terminal residues abolishes interaction of the VPg with AtPVIPs, demonstrating the involvement of this VPg domain in the interaction with AtPVIPs (Dunoyer et al. 2004). When the sequence of this VPg domain was compared in different potyviruses, several amino acid variations were found. When, based on this information, the phenylalanine residues at position 12 within TuMV VPg was substituted with methionine to mimic the sequence of TEV VPg, the interaction of TuMV VPg with AtPVIP1, AtPVIP2, PVIPp, and PVIPnb in a two-hybrid system was abolished, indicating that this amino acid residue is crucial for the specificity of the virus–host VPg–PVIP interaction (Dunoyer et al. 2004). Importantly, the mutant TuMV strain with the phenylalanine-to-methionine substitution in VPg displayed a significant delay in its local and systemic infection of *N. benthamiana*, suggesting the involvement of the TuMV VPg–PVIPnb interaction the infection process (Dunoyer et al. 2004). Moreover, RNAi suppression of the *AtPVIP1* and *AtPVIP2* genes dramatically inhibited TuMV systemic infection and disease symptom development, demonstrating the importance of these host factors for the viral infection (Dunoyer et al. 2004).

4 Cellular Route for Viral Systemic Movement

Viral systemic movement comprises six major consecutive steps:

1. Virus translocation from MS to BS cells
2. Penetration into the VP through the BS

3. Entry into the phloem CC/SE complex (or, for some viruses, into xylem-associated cells from the VP, see Sect. 4.4)
4. Rapid transport to systemic uninfected plant organs through the phloem SE (or xylem, in some cases)
5. Unloading from the CC/SE complex into uninfected VP
6. Egress from the VP through BS cells and into the MS cells of systemic plant organs

Some of the boundaries between the different cell types involved in these steps of the systemic transport can block translocation of some viral strains (Table 1), demonstrating that these boundaries can serve as natural barriers for those viruses. Moreover, viruses can enter the host vasculature through both major and minor veins whereas they exit the vasculature only from the major veins (Cheng et al. 2000; Santa Cruz et al. 1998), suggesting that the process of virus unloading may be more restrictive than that of virus loading (see also sections Sect. 4.1 and Sect. 4.5). This notion is supported by the observations that PME and cdiGRP/cadmium treatments restrict tobamoviral systemic movement by blocking the viral egress from, but not entry into, the vasculature (Chen et al. 2003; Citovsky et al. 1998; Ghoshroy et al. 1998; Ueki et al. 2002) (see Sect. 3.1 and Sect. 3.2).

For their movement through the host vasculature, plant viruses are thought to take the same route that the plant utilizes for transport of its photoassimilates (Leisner et al. 1993a,b). Tracking radioisotope-labeled sucrose and low molecular weight fluorescent dye in host plants has demonstrated that photoassimilates are transported from lower, fully expanded leaves (source) to the upper, young leaves (sink) (Leisner et al. 1993b; Oparka et al. 2000; Roberts et al. 1997; Santa Cruz et al. 1999). Similarly, tracking systemically moving viruses, such as *Cauliflower mosaic virus* (CaMV) (Leisner et al. 1993c) or GFP-expressing recombinant tobamoviruses and PVX (Cheng et al. 2000; Santa Cruz et al. 1998), showed that these viruses, and presumably many others, move through the phloem from source leaves to sink tissues.

Viruses can enter two structurally different types of phloem – the internal and the external phloem – for their upward and downward movement, respectively (for details, see Andrianifahanana et al. 1997; Cheng et al. 2000; Guerini et al. 1999). Having entered the phloem SE, viruses move in two opposite directions: upward to the sink leaves and downward to the roots. The upward movement occurs significantly faster than the downward spread (Andrianifahanana et al. 1997; Cheng et al. 2000) (see also Sect. 3.4). As a consequence, sink leaf tissues represent the major and preferential target for viral systemic movement.

4.1 Leaf Veins Utilized for Viral Entry Into and Exit out of the Vascular System

The leaf veinal system is classified into major (classes I-III) and minor veins (classes IV and smaller) (Roberts et al. 1997). GFP-expressing recombinant TMV was used to define the routes for vascular invasion of viruses in the source leaves of *N. benthamiana*: viral loading occurred both in minor (classes IV and V) and in major veins (classes III and larger). Thus, all vein classes function equally as gateways for TMV entry into the vasculature of the source leaves (Cheng et al. 2000). In contrast, virus unloading appears to be more selective. First, the virus unloads from major veins, but not from minor ones. Second, virus unloading patterns change during the course of development, i.e., during the sink-to-source transition. In tobacco leaves, this transition occurs basipetally, from apex to base, so that the apical part of the leaf starts functioning as a source when the basal part is still a sink (Roberts et al. 1997; Turgeon 1989). In such transitioning leaves, GFP-expressing recombinant PVX and TMV are unable to unload into the source portions of the leaf (Cheng et al. 2000; Roberts et al. 1997). The sink-to-source transition events probably alter leaf vasculature at the molecular level (van Bel et al. 2003a,b), and these developmental changes presumably block, by an as yet unknown mechanism, the ability of the virus to exit the minor veins of the leaf vasculature.

Interestingly, in the dicotyledonous *N. benthamiana* plant, the specific patterns of virus unloading and vein involvement mirror those of the fluorescent solute carboxyfluorescein (CF), although viral unloading occurs considerably slower than that of the much smaller CF (Roberts et al. 1997). Parallels in the unloading of viruses and solutes have also been found in monocotyledonous plants, such as barley, in which unloading patterns of CF and GFP-expressing recombinant *Barley stripe mosaic virus* (BSMV) display a striking similarity, with both CF and the virus exiting major longitudinal veins, but not transverse veins (Haupt et al. 2001). Therefore, in both dicots and monocots, the virus appears to hijack the physiological route that the plants have evolved for export of photoassimilates from source to sink tissues.

4.2 Invasion of the Vasculature Across the BS/VP Boundary

During the vascular-invasion process, the first cell type that the virus encounters is the BS. Presumably, viruses enter BS cells by a cell-to-cell movement mechanism whereas viral transport from the BS into the VP occurs by a different pathway. Indeed, TMV MP, which is sufficient to gate PD in nonvascular tissues (Ding et al. 1992; Tomenius et al. 1987; Waigmann et al. 1994), accumulates within the PD between the BS and VP, but does not increase the permeability of PD at this intercellular boundary (Ding et al. 1992). Thus, the

BS/VP boundary in the inoculated leaf may represent the first barrier encountered by viruses during their long-distance movement.

Illustrating the biological relevance of the BS/VP barrier, viral transport can be specifically blocked at this cellular interface in some hosts. For example, CCMV systemic movement is arrested in the BS cells of a resistant cultivar of soybean, and this restriction is responsible for the resistant phenotype (Goodrick et al. 1991). Transgenic tobacco plants that overexpress CMV replicase do not support systemic CMV infection due to the block in viral translocation from BS to the VP (Wintermantel et al. 1997). Similarly, in cucumber cotyledons, a chimeric cucumovirus strain expressing the CP of the Florida strain of *Tomato aspermy virus* (TAV), whose systemic movement is restricted in cucumber plants, accumulates in the BS cells but is not observed in the VP (Thompson et al. 1998). These data suggest that the PD at the BS/VP boundary are equipped with a restrictive mechanism(s) that blocks the systemic movement of incompatible viral strains.

4.3
Entry into the CC/SE Complex Across the VP/CC and/or BS/CC Boundaries

Once in the VP, plant viruses proceed into the CC/SE complex. To this end, they must first enter the CC, crossing the VP/CC boundary. The existence of this boundary is inferred from experiments with a CP-less TMV mutant which is able to cross the BS/VP boundary and accumulate in the VP, but remains excluded from the CC (Ding et al. 1996). Interestingly, the mutant TMV strain with truncated CP, which is deficient in encapsidation activity, still shows systemic movement, suggesting that the CP exerts an unknown function for entrance into the CC from the VP, possibly by interacting with specific host machinery at this intercellular boundary (Ding et al. 1996) (see also Sect. 2.2). Moreover, point mutations in RCNMV MP prevent viral systemic movement, whereas cell-to-cell movement of the mutants remains unaffected; since these systemic-movement-defective mutants accumulate only at low levels in the CC/SE complex in inoculated leaves, their systemic movement most likely is arrested because of impaired virus loading into or accumulation within the CC/SE complex (Wang et al. 1998).

In some cases, such as in minor leaf veins in the Nicotiana species, virus may load into the CC/SE complex directly from BS cells that contact CC and are not separated from them by VP, as in major veins of many plant species (Ding et al. 1995; Santa Cruz et al. 1998). In the case of PVX infection in *N. benthamiana*, viral CP, which is known to be required for the cell-to-cell movement, is found associated with PD at the BS/CC and BS/VP interfaces, but not at the VP/CC interface (Santa Cruz et al. 1998). This may suggest that the virus preferentially enters the CC directly from the BS cells in minor veins in Nicotiana, bypassing the VP (Santa Cruz et al. 1998). Collectively, these observations suggest that the VP/CC boundary (and/or BS/CC bound-

ary, in some cases) in the inoculated leaf represents the second barrier to viral systemic movement.

4.4
Viral Movement Across the CC/SE Boundary and Through the Conduit

In the phloem CC/SE complex, the virus passes from CC into SE, where it presumably utilizes pressure-driven flow of photoassimilates for rapid long-distance movement to systemic sink leaves. Since the enucleated SE are perforated at both longitudinal ends, they provide an unrestricted and uninterrupted path for the long-distance transport of various macromolecules and solutes throughout the plant. Since some viruses do not require replication for their efficient systemic movement (Susi et al. 1999; Wintermantel et al. 1997) and are transported systemically as virions (see Sect. 2.2), these viruses may move through SE in, at least partially, an encapsidated form. Other viruses, such as PVA and *Sweet potato chlorotic stunt virus* (SPCSV), may undergo decapsidation and replication while moving systemically because they are susceptible to RNA silencing during this transport, implying the exposure of the viral genome and replication within the components of the CC/SE complex (Germundsson et al. 2006; Kreuze et al. 2005). Interestingly, in minor leaf veins in *N. benthamiana*, PVX virion, which is required for viral cell-to-cell movement in most tissues, localizes within PD at the BS/VP and BS/CC, but not at the CC/SE boundary (Santa Cruz et al. 1998). Thus, at the CC/SE interface, unlike at others where PVX takes the form of a virion to pass, the virus may use a non-virion transport intermediate (Santa Cruz et al. 1998), suggesting that the CC/SE boundary in the inoculated leaf represents the third potential barrier to systemic movement of, at least, some viruses.

Although the size-exclusion limit of PD at the CC/SE boundary is larger than at other boundaries (Kempers et al. 1993, 1997), these PD may still need to be modified by the viral movement factors; indeed, MPs of several plant viruses, such as PLRV and CMV, localize to the PD at the CC/SE boundary (Blackman et al. 1998; Hofius et al. 2001; Schmitz et al. 1997), presumably modulating these channels for viral passage. Similarly, PVX may utilize a viral component(s) other than the CP/virion to enlarge the PD and enter SE (Santa Cruz et al. 1998). Furthermore, GFP-tagged CMV expressed from a CC-specific promoter of *Commelina yellow mottle virus* (ComYMV) is transported into the SE, indicating its ability to gate the PD that connect these cells; this transport is specific because dimeric GFP, which is also expressed from the ComYMV promoter, remains confined to CC (Itaya et al. 2002). Consistent with the MP role during viral transport from CC into SE, in CMV-infected *N. clevelandii* CMV virions are found in SE but not in CC, suggesting that CMV genomes translocate into the SE as MP–RNA complexes and form virions only within the SE (Blackman et al. 1998).

Although most viruses are known to use the phloem for systemic movement, some, such as *Rice yellow mottle virus* (RYMV) (Opalka et al. 1998) and CGMMV (Moreno et al. 2004), have been reported to move through xylem components. Accumulation of *Soilborne wheat mosaic virus* (SBWMV) has also been demonstrated in the xylem, suggesting the involvement of this tissue in SBWMV systemic movement (Verchot et al. 2001).

As described in Sect. 4, long-distance transport proceeds at different rates and in two directions: upward movement is faster, and downward movement is slower (Andrianifahanana et al. 1997; Cheng et al. 2000). Tracing the movement of TMV, *Pepper mottle potyvirus* (PepMoV), and PLRV demonstrated that these two movement modes occur through structurally different types of phloem – external and internal (Barker et al. 1986; Cheng et al. 2000; Derrick et al. 1992, 1997; Guerini et al. 1999). The external and internal phloem in the transport veins of petioles and stems of such plant families as *Solanaceae, Cucurbitaceae* and others derive from the abaxial (facing away from the axis of the plant and located on the underside of the leaf) and adaxial (facing toward the axis of the plant and located on the upper side of the leaf) phloem, respectively, of the major leaf veins (Cheng et al. 2000; Turgeon 1989). In *N. benthamiana* inoculated with a GFP-expressing recombinant strain of TMV, GFP fluorescence is detected in the external phloem and external phloem-associated cells of the stem internode below the inoculated leaf, and exclusively in the internal phloem and internal phloem-associated cells of the stem internode above the inoculated leaf. These two opposing venues of viral transport are almost independent because only little traffic is detected between the internal and external phloem of the stem (Cheng et al. 2000). Similarly, systemic infection of pepper plants by the Florida isolate of PepMoV (PepMoV-FL) follows a defined pattern of downward movement through the external phloem and upward movement through the internal phloem (Andrianifahanana et al. 1997), whereas the virus-resistant pepper cultivar *Capsicum annuum* cv. Avelar allows downward movement of PepMoV-FL through the external phloem, but restricts upward movement through the internal phloem, resulting in young stem tissues that are virus-free (Guerini et al. 1999). Finally, differential involvement of the internal and external phloem in viral systemic movement was also shown using PLRV-resistant potato plants in which virus is restricted to the internal phloem, whereas both internal and external phloem display PLRV accumulation in the susceptible potato plants (Barker et al. 1986; Derrick et al. 1992, 1997). Thus, plant viruses may move to the roots, downward from the inoculated leaf, through the abaxial phloem of leaves and external phloem of petioles and stems, but utilize the adaxial leaf phloem and internal petiole and stem phloem for their upward movement to the sink leaves.

4.5
Virus Unloading from the Phloem into Systemic Organs

For most viruses, unloading from the phloem into the surrounding non-vascular tissues of systemic, uninoculated organs and propagation in these tissues is the last step in establishing efficient systemic infection. One exception to this rule are "phloem-limited" viruses that are confined to the vascular components and do not appear in systemic MS tissues, such as luteoviruses (Mayo et al. 1996; Smith et al. 1999), some, but not all (Michelson et al. 1997; Morra et al. 2000; Rogers et al. 2001), bipartite (Morra et al. 2000; Qin et al. 2001) and monopartite geminiviruses (Rojas et al. 2001), bipartite closteroviruses (Wisler et al. 1998), and others. These viruses may be limited to the phloem because of their blocked unloading into systemic non-vascular tissues, limited replication in these tissues after unloading and/or because some of them do not encode a bona fide MP (Briddon 2003). These phloem limitations can be removed by coinoculation of a second virus, which provides viral functions that the phloem-limited virus lacks and that are required for infection of non-vascular tissues; studies of the mechanisms underlying this in-*trans* complementation can provide useful insights into the molecular causes of phloem limitation.

For example, luteoviruses, following direct injection into phloem cells by aphids, spread within the phloem but do not leave the host vasculature, although they can replicate in protoplasts derived from non-vascular tissues (reviewed in Mayo et al. 1996; Smith et al. 1999). Coinfection of *N. clevelandii* or *N. benthamiana* with a mixture of PLRV luteovirus and an unrelated PVY potyvirus results in a higher titer of PLRV and its more frequent occurrence within MS cells (Barker 1987, 1989), suggesting that potyviral factors facilitate phloem unloading of PLRV. The potyviral determinants that alleviate luteoviral phloem limitation have not been identified; however, they probably do not include HC-Pro, the potyviral RNA silencing suppressor, because transgenic *N. benthamiana* plants expressing PVA HC-Pro do not promote the occurrence of luteoviruses in MS cells (Savenkov et al. 2001).

The phloem limitation of PLRV may derive from a combination of the host RNA silencing against this virus and other, as yet uncharacterized, processes. This notion is based on the observations that phloem unloading of PLRV into MS tissues is induced following coinoculation by a cucumovirus CMV(ORF4) strain, which is a chimeric CMV expressing the ORF4-encoded GRV MP instead of CMV MP, but not by a mutated CMV(ORF4) with blocked expression of the viral RNA silencing suppressor 2b (Ryabov et al. 2001a). On the other hand, PLRV spread beyond the phloem was promoted, via an unknown mechanism, by coinoculation with PEMV-2, but not with CMV, TMV, PVY, PVX, some of which encode viral RNA silencing suppressors (Ryabov et al. 2001a). Moreover, a recombinant PVX that expresses GRV MP did not rescue PLRV movement, suggesting that the RNA silencing suppressor of PVX

and MP of GRV are unable to allow PLRV unloading from the vasculature (Ryabov et al. 2001a). Thus, PLRV may be restricted to the phloem by a coalescence of two factors: lack of ability to unload from the phloem per se, and failure to accumulate in the MS due to the host defense reactions.

In the case of bipartite geminiviruses, BGMV remains largely confined to the vascular tissues of *N. benthamiana* whereas several other bipartite geminiviruses, such as *Cabbage leaf curl virus* (CabLCV), TGMV, unload into the surrounding MS (Morra et al. 2000; Qin et al. 2001). When BGMV is coinoculated with TGMV, it gains the ability to infect MS cells, suggesting that inoculation with TGMV alleviates BGMV phloem limitation (Morra et al. 2000). The TGMV factors that allow systemic BGMV infection include a *cis*-acting, non-coding TGMV BRi element upstream of the BR1 (formerly BR1) ORF and at least one of the two *trans*-acting factors, the AL2 protein and the BR1/BL1 proteins (formerly BR1/BL1) encoded by DNA-B of the virus (Morra et al. 2000). A later study suggested that AL2, in association with host factors, acts through the BRi region to enhance the TGMV BR1 gene expression (Qin et al. 2001). Since the BGMV genome also encodes BR1 and BL1, which represent geminiviral MPs, phloem limitation of BGMV may in fact not be caused by the lack of movement function per se. Instead, it may be based on tissue incompatibility of viral gene expression and the resulting low levels of BGMV BR1/BL1 production, insufficient for allowing movement into MS cells. Similarly, DNA-B of a non-phloem limited *Bean dwarf mosaic virus* (BDMV) overcame the phloem limitation of *Abutilon mosaic virus* (AbMV) in bean (*Phaseolus vulgaris*) whereas, in the reciprocal combination, DNA-B of AbMV failed to confine DNA-A of BDMV within the phloem (Levy et al. 2003). Thus, AbMV DNA-B, which encodes the BR1/BL1 geminivirus movement factors, is not the sole determinant of phloem limitation of the AbMV while BDMV DNA-A likely encodes additional determinants important for BDMV movement beyond the phloem (Levy et al. 2003).

Another example of complementation of viral systemic transport by coinoculation with an unrelated virus is restoration of movement of the potyvirus isolate PepMoV-FL through the internal phloem of *C. annuum* cv. Avelar plants by cucumovirus strain CMV-KM (Guerini et al. 1999). In this host, PepMoV-FL does not move within the internal phloem at all, as opposed to just being restricted in phloem unloading into MS (Guerini et al. 1999). CMV-KM truly promotes PepMoV-FL's phloem movement because it does not enhance PepMoV-FL accumulation in plant protoplasts, indicating that the presence of CMV-KM does not simply block the host cell defense reactions against PepMoV-FL (Guerini et al. 1999). Also, systemic spread of a long-distance-movement-deficient M strain of CMV (M-CMV) in zucchini squash (*Cucurbita pepo*) was rescued by coinoculation with either *Zucchini yellow mosaic potyvirus* strain A (ZYMV-A) or its attenuated variant ZYMV-AG (Choi et al. 2002). In this case, however, it is unclear whether the rescuing potyvirus provided a bona fide movement function or the RNA silencing-

suppressing activity of its HC-Pro protein (reviewed in Revers et al. 1996; Urcuqui-Inchima et al. 2001).

To date, two host factors have been identified that may affect viral unloading: PME and cdiGRP (Chen et al. 2003; Ueki et al. 2002). As described in detail in sections Sect. 3.1 and Sect. 3.2, PME is required for the systemic transport of tobamoviruses whereas cdiGRP negatively regulates this process. Both proteins appear to affect viral unloading into the non-vascular tissues, such that reduced levels of PME expression or elevated levels of cdiGRP expression in the tobacco vasculature "trap" the virus within the phloem of the systemic leaves (Chen et al. 2003; Ueki et al. 2002). These observations suggest that viral systemic movement may be a directional process employing different molecular pathways for entry into and exit out of the host plant phloem. Moreover, the differences in vascular loading and unloading of plant viruses are also evident from the afore described (see Sect. 4.1) observations of functional equivalence of different vein classes for virus entry and their lack of equivalence for its exit (Cheng et al. 2000). Thus, macromolecular transport into the plant vasculature may occur by a relatively loosely regulated process, whereas transport out of the vasculature may be more selective and/or tightly regulated (see also Sect. 4).

Interestingly, some plant organs, such as the apical shoot meristem, appear to restrict the movement of viruses, such as TMV, and remain permanently virus-free (Cheng et al. 2000). Recent studies have demonstrated that this phenomenon may not be due to blocked viral entrance into the area, but, instead, may be based on suppression of viral replication in the restrictive tissues by the host RNA silencing defense response (Xie et al. 2001) (see also Sect. 5).

5
The Effect of RNA Silencing, the Host Innate Immunity, on Viral Systemic Movement

Typically, the ability of a virus to move systemically is assessed by measuring the levels of viral proteins and/or genomes in systemic tissues. The absence of viral products in uninoculated, systemic leaves, with a normal level of local accumulation in the inoculated leaf, is presumed to be based on SAR and/or blocked systemic movement. Traditionally, when the possible involvement of SAR and hypersensitive reactions can be ruled out, the absence of virus in systemic leaves is postulated to be due to a block in viral systemic movement. However, recent progress in understanding molecular mechanisms of the plant innate immune response by RNA silencing and its inhibition by RNA silencing suppressors encoded by many plant viruses (reviewed in Baulcombe 2001; Baulcombe 2002, 2004; Bisaro 2006; Dunoyer et al. 2005; Scholthof 2005; Soosaar et al. 2005) has revealed that some of the seemingly "blocked viral

systemic movement" is more likely to represent a blocked viral accumulation due to RNA silencing rather than inhibition of the viral transport per se, and that the normal viral spread often requires suppression of RNA silencing by the virus.

For example, the CMV 2b (Soards et al. 2002) and TBSV p19 proteins (Scholthof et al. 1995) modulate viral spread by counteracting RNA silencing (Soards et al. 2002; Voinnet et al. 1999). In fact, many viral factors, once thought of as determinants of local and/or systemic movement, have been revealed to function as RNA silencing suppressors. Specifically, PVX p25, one of the TGB proteins required for cell-to-cell movement (Angell et al. 1996; Beck et al. 1991), also acts as an RNA silencing suppressor, the function that is required for efficient local spread of PVX (Bayne et al. 2005). Also, the TMV 126-kDa protein was considered to be a host range determinant that restricts viral systemic movement. For example, in *N. tabacum* cv. Xanthi nn, TMV Holm's masked strain (TMV-M) accumulates only at low levels in vascular tissues of the inoculated and uninoculated systemic leaves, whereas the TMV-U1 strain – which differs from TMV-M mainly in the sequence of its 126-kDa protein (Shintaku et al. 1996) – accumulates to high levels in both types of leaves of the same host (Ding et al. 1995; Nelson et al. 1993). Based on these data, the attenuated symptoms were attributed to a combination of low replication efficiency and suppression of viral systemic movement, and the 126-kDa protein was implicated in these effects (Chen et al. 1996; Ding et al. 1995; Nelson et al. 1993). Recently, however, the 126-kDa protein has been shown to suppress RNA silencing in *N. tabacum* and *N. benthamiana*, indicating that the lack of TMV-M movement is most likely due to this viral strain's weaker ability to suppress host RNA silencing (Ding et al. 2004).

Another example of functional reassessment of viral protein activity from a systemic movement factor to RNA silencing suppressor is the potyviral HC-Pro protein. HC-Pro was originally found to be involved in polyprotein processing (Carrington et al. 1989), long-distance movement (Cronin et al. 1995; Kasschau et al. 1997; Klein et al. 1994), and efficient replication at the single-cell level (Kasschau et al. 1997). A later study demonstrated correlation of the systemic mobility and replication efficiency of TEV HC-Pro mutants with their capacity for suppression of RNA silencing, suggesting that HC-Pro functions as an RNA silencing suppressor, and that this function is responsible for the HC-Pro effects on viral movement (Kasschau et al. 2001).

Furthermore, RNA silencing may also be responsible for exclusion of apical meristems from viral infection The RNA silencing is mediated by the host RNA-dependent RNA polymerases (RDRs), such as RDR1, which is involved in host defense against TMV and PVX (Xie et al. 2001), and RDR6, which has been implicated in host resistance against cucumoviruses (Mourrain et al. 2000). When GFP-expressing PVX was inoculated on a *N. benthamiana* line with RNAi-silenced RDR6, the virus invaded apical meristems, which remained largely virus-free in the wild-type plants (Schwach et al. 2005). These

results demonstrated that the meristem exclusion, once assumed to be due to a "transport barrier" for viral invasion (Foster et al. 2002), is actually based, at least in part, on the host RNA silencing activity. Indeed, growing, meristematic regions of plants are thought to represent strong photosynthetic sinks and, by implication, preferred transport destinations of RNA silencing signals (Schwach et al. 2005).

6 Concluding Remarks

Viral systemic movement in non-Arabidopsis hosts is often studied using, as experimental systems, virus–host combinations that show limited systemic viral infection (see Table 1 for examples of such combinations). In most cases, this type of host resistance is very specific, i.e., the host is resistant to a few specific isolates of a virus, but not to other closely related strains. One possible explanation for this specificity is that the host plant cannot tolerate alterations in its intercellular transport machinery that are dramatic enough to impede the movement of a wide spectrum of viruses. In other words, viruses may have evolved to "pirate" the fundamental intercellular transport pathways that are essential for the physiology of the host plant itself for their own spread, making it impossible for the host to completely block these venues of viral spread.

The process of viral systemic movement has long since attracted the attention of many plant biologists and virologists; yet, its detailed molecular mechanisms and pathways remain obscure. Based on the information reviewed in this chapter, two main gaps in our understanding of viral systemic movement are immediately clear: except for very few cases, host factors that participate in the movement per se have not been identified, and the molecular events that allow viruses to cross PD within and between different tissues and cell types are unknown. The main reason for this lack of knowledge may be the complexity of the system. The involvement of different types of unique vascular-associated cells and more than one viral factor for movement complicates experimental approaches. Moreover, the involvement of host defense, such as RNA silencing that is not directly related to transport through PD, in the process of viral systemic accumulation is liable to cause misinterpretation of the experimental results. Segregation of viral systemic "movement" from the overall systemic infection process – which is the sum total of replication, movement, host defense, and viral counter-defense reactions – is vital for elucidating the systemic translocation process.

Acknowledgements The work in our laboratory is supported by grants from the NIH, NSF, USDA, BARD, BSF, and CDR-USAID to V.C.

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