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REVIEW

Cell-to-cell movement of three genera (+) ss RNA plant viruses

Katarzyna Otulak · Grażyna Garbaczewska

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Abstract The current investigations of three genera plant virus cell-to-cell movement were presented. Viruses reveal different local transport strategies, but all of them are the results of virus factors–host components interactions. The *Tobacco mosaic virus* (TMV) does not require capsid protein for translocation through plasmodesmata but 30 K movement protein participates in this process. It was found direct or indirect TMV movement proteins host partners in *Tobamovirus* movement like: pectin methylesterase, movement protein binding 2C, chaperones or cytoskeleton components and endoplasmatic reticulum membranes. The *Potex*- and *Potyvirus* cell-to-cell movement is closely related to replication network. The PVX capsid protein and triple gene block protein system are responsible for efficient local transport. *Potyviruses* move through the plasmodesmata by involving viral encoded proteins but not specific movement proteins. While the *Potyvirus* is the biggest known plant virus genus, host components participating in or regulating directly its plasmodesmata-movement are still not clear.

Keywords Cell-to-cell movement · Transport · Plasmodesmata · *Tobamovirus* · *Tobacco mosaic virus* · *Potexvirus* · Triple gene block · *Potyvirus*

Abbreviations

CI	Cytoplasmic inclusion protein, *Potyvirus* helicase
CP	Capsid protein
CRT	Calreticulin
eIF4E, eIF4G	Eukaryotic translation factors
ER	Endoplasmatic reticulum
ERdt	Endoplasmatic reticulum desmotubule
M complex	Movement complex
ML	Middle lamela
MP	Movement protein
Mt	Microtubules
MPB2C	Movement protein binding 2C
NCAP	Non-cell autonomous protein
NTR	Non-translated region
ORF	Open reading frame
PD	Plasmodesmata
PM	Plasma membrane
PME	Pectin methylesterase
PVX	*Potato virus X*
PVY	*Potato virus Y*
RNP	Ribonucleoprotein complex
SEL	Size exclusion limit
STP	Single-tailed particles
TGB	Triple gene block
TMV	*Tobacco mosaic virus*
vs	Vesicle

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K. Otulak (✉) · G. Garbaczewska
Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences-SGGW, Nowoursynowska 166, 02-776 Warsaw, Poland
e-mail: katarzyna_otulak@sggw.pl

Introduction

The basis for effective virus infection in host-plants tissues is the replication of pathogen genetic material. Infection

spreads due to two-phase virus transport process. The initial stage is the transport from the place of inoculation to neighboring cells, which is called local or cell-to-cell transport (Oparka 2004). In the second stage called long-distance or systemic transport (van Bel 2003), the pathogen penetrates inside vascular tissues and transports to uninfected cells. Irrespective of virus genus, the two mentioned types of transport require activation of two "agents"—host proteins and virus components.

In plant tissues cell-to-cell communication is possible due to plasmodesmata (PD). PD specific ultrastructure and size exclusion limit (SEL) were well described (Ding et al. 1992; Lucas 2006), but PD molecular composition is still unclear and its potential components remain under investigation (Waigmann et al. 2004). SEL limited to 1 kDa is a barrier for the pathogen effective translocation to neighboring cells. There are two strategies of transporting through PD—the virus spreads as a particle or viral genetic material is transported in the form of ribonucleoprotein complexes (RNP). The process consists of three events:

1. Interaction between potential docking complex and PD,
2. Induction of PD microchannel increasing,
3. Fixation of inner translocation system in adjacent cell cytoplasm.

What is indispensable in these events, irrespective of virus group, is protein or virus protein which participates in transport—movement protein (MP) (Lucas and Lee 2004; Verchot-Lubicz 2005; Chen and Kim 2006; Lucas 2006). MPs are able to "mediate" in protein and vRNP translocation across PDs (Lucas et al. 1993). Numerous studies show that macrocomplexes movement follows the control pattern characteristic for non-cell autonomous protein (NCAP) molecules (Lucas 1995; Haywood et al. 2002). Detailed studies of RNA plant virus local transport mechanisms took place in the '90s. The subject of MP proteins/host-plant components interaction remains under extensive study and is very much up-to-date, also due to new information on proteins constituting PD complex and on relation between cellular structures and PD in translocation process.

The object of this paper is to present the strategies of cell-to-cell movement of three genera (+) ss RNA plant viruses:

1. Model genus *Tobamovirus*, represented by *Tobacco mosaic virus* (TMV),
2. *Potexvirus*, coding characteristic sequences TGB [*Potato virus X* (PVX)],
3. The largest *genus Potyvirus*, represented by *Potato virus Y* (PVY).

Tobamovirus MP: Family 30 K Proteins

Tobacco mosaic virus can move through PD in the form of virions, which was confirmed in the studies by Esau and Cronshaw (1967), Gibbs (1976), Weintraub et al. (1976). 30 kDa protein, which enables TMV cell-to-cell transport, was identified as interacting with PD components. Also it was stated that in the presence of MP TMV, PD molecular size exclusion limit (SEL) increases from 1 kDa to ca 10 kDa in tobacco transgenic lines, where 30 kDa protein expressions took place (Wolf et al. 1989). The family 30 k proteins is the largest group of viral movement protein. They are characterized by weak sequence resemblance but they have one conservative motive $LXDX_{50–70}G$ (Koonin et al. 1991). Secondary structure analysis showed that the core region is build from 4 α-helis (α-A-D) and 7 β-structures (β-1-7). MP TMV core region is surrounded by two domains binding single-stranded nucleic acid (Citovsky et al. 1992) and domain engaged in PD SEL increase (Boyko et al. 2000). It is claimed that the C-terminal region is a regulating agent for the functional domains. The experiments conducted by Waigmann et al. (2000) and Citovsky and Zambryski (1993) proved that in the MP TMV C-terminal area are located three phosphorylation sites, which regulate MP biological activity. Studies by Brill et al. (2000) showed that two MP regions are resistant to digestion by trypsin and they contain two highly hydrophobic domains between 58 and 85 amino acid residues as well as 145 and 175. Such characteristic of two MP TMV domains enables us to categorize this protein as integral membrane protein (Reichel et al. 1999). The MP TMV C-terminal area from 250 to 268 amino acid residues was highly sensitive to trypsin treatment. In view of that it was shown that two MP TMV potential transmembrane domains "spin" the membrane to form U-shaped protein conformation while the N- and C-terminal regions are exposed to cytosol (N_{cyt}–C_{cyt} topology) (Brill et al. 2000).

MP TMV and its partners in movement

Direct transport of the virus genome from infected to neighboring healthy cells is realized through binding MP protein with virus nucleic acid and moving of this complex through PD. MP TMV was the first movement protein that showed binding with single-stranded (ss) RNA or DNA (Citovsky et al. 1990). Complexes of MP TMV–nucleic acid are estimated to measure 1.5–3.5 nm in diameter, which is compatible with 3.2–4.3 nm SEL-broadened PD (Waigmann et al. 1994; Kiseylova et al. 2001). The MP TMV mutation analysis indicated that activity of nucleic acid single strand is present in domains between amino acid radical 112–185 and 186–268 movement protein

(Citovsky et al. 1992). The MP TMV binding to nucleic acid is not restricted only to specific nucleotide sequences (Atabekov et al. 1999). However, it was not proved how the tobacco mosaic virus MP locates virus RNA in an infected cell. It was suggested that MP with very strong affinity to single-stranded nucleic acid attaches to any ss RNA or ss DNA. It should be stressed that TMV replication and TMV proteins translation partly overlap "viral factories", which is why it is highly probable that MP TMV may easily acquire RNA TMV immediately during synthesis de novo (Heinlein et al. 1998; Brill et al. 2000). It was claimed that MP TMV forms complexes with endogenous cellular RNA during expression in bacterial cells. RNA TMV forms ribonucleoprotein complexes (vRNP) in infected plant cells. MP–virus genome complexes form usually during advanced transport process—they are called M-complex (movement complex). In this manner, the virus genome in the form of both RNA and DNA is protected against host cells' nucleases and creates a system, which facilitates the transfer and is compatible with PD transfer function (Kiseloyva et al. 2001). The M-complex formation of MP TMV and RNA TMV in vitro is non-replicating and non-translating in in vitro conditions and isolated protoplasts. Such inhibition suggests that M-complexes are partly non-capsidated during transfer across PD (Karpova et al. 1997). Such decapsidation may take place during the phosphorylation of MP TMV by protein kinases connected to the cell wall (Karpova et al. 1999).

During infection, pathogens adapt host cellular processes to their needs. MP proteins "build in" cell-to-cell transports pathways so that the virus can effectively spread its genome. While MP biological activity is scrutinized and relatively well known, MP partners in host-plant have not been fully examined yet. Also in this area, TMV gives us the most data. MP TMV is the first movement protein for which several interacting host proteins were isolated and identified. MP TMV cooperates with 38 kDa cell wall protein isolated from tobacco leaves. On the basis of sequence analysis it was stated that it is cell wall pectin methylesterase (PME) (Dorokhov et al. 1999; Chen et al. 2000). The mechanism adopted for PME participation in TMV cell-to-cell transfer still remains unclear. One possibility is that PME plays the role of plant cell receptor to MP TMV (Dorokhov et al. 1999). PME immunolocalization in tobacco tissues indicated the presence of this protein in cell wall also in PD (Chen et al. 2000). Due to PME binding, MP TMV is docked to the host cell wall, if it takes place near PD, the movement is initiated. In case when binding PME to the cell wall is found in an area without PD, the movement is blocked and MP TMV is degraded or transferred to cytoplasm. PME may also participate in MP TMV transport to host plant ER. MP use ER membranes to transport from the place of synthesis to PD (Heinlein et al. 1998), where ER is also present. TMV movement proteins may be transferred to ER with the use of PME due to translocation signal to ER (Gaffe et al. 1997). According to these analyses, PME molecules may bind ER membranes, which is why they are able to interact with MP TMV and attach them to ER from the cytoplasm while conducting the transport to the cell wall. PME can be activated when MP TMV is accumulated beside the cell wall.

The concept assuming active participation of ER membranes in TMV cell-to-cell transport is called ER sliding model (Guenoune-Gelbart et al. 2008). It is suggested that newly synthesized MP TMV is incorporated into ER membranes and binds replicated (+) ss vRNA (Fig. 1). What is formed is a complex connected to endomembranes, which may also trigger virus replication–the forming of a replication complex (Asurmendi et al. 2004). In line with the given assumptions, MP can either directly or indirectly change properties of the wall surrounding PD, which can lead to PD broadening. Possibly it is connected with transport to ER with the use of cytoskeleton or ER vesicles which contains MP-vRNA complex moving across PD (Kawakami et al. 2004; Boevink and Oparka 2005; Lin et al. 2005; Wright et al. 2007, Sambade et al. 2009). When MP is not present, PD ER is stationary (Heinlein and Epel 2004). If MP is incorporated in ER, cell-to-cell transport takes place via MP-vRNA complex diffusion to PD desmotubules lipid matrix under the action of concentration gradient between healthy and infected cells (Lippincott-Schwartz et al. 2000; Runions et al. 2006). The MP presence in ER or in ER vesicles as well as the relation with microtubules (Heinlein et al. 1995) and microfilaments (McLean et al. 1995) suggests that elements of the cytoskeleton are an important component engaged in the transport to PD and in cell-to-cell movement of the viral ribonucleic complex (Mitra et al. 2003). Actins and myosins are present inside PD and are responsible for the ER membrane translocation (Blackman and Overall 1998; Yokota et al. 2009). Gillespie et al. (2002) and Heinlein and Epel (2004) postulated that PD broadening in induced MP may enable an active transport of vesicles from ER (with MP-vRNA) due to microfilaments. It was estimated that cell-to-cell transport of the vesicles (with virus complex) with the cytoskeleton involved in I-stage infection cells reaches 160 nm/s (Kawakami et al. 2004). The movement of the viral replication complex from the place of I-stage infection to adjacent cells is spotted within 20 h, whereas from the place of II-stage infection to adjacent cells within 4 h (Kawakami et al. 2004). Similar effects were noticed in MP TMV (MP:GFP) movement—observed after around 24 h after the construct injection.

Guenoune-Gelbart et al. (2008) updated TMV cell-to-cell movement model. During initial stages of infection, the plant reacts by accumulating callose and PD blocking

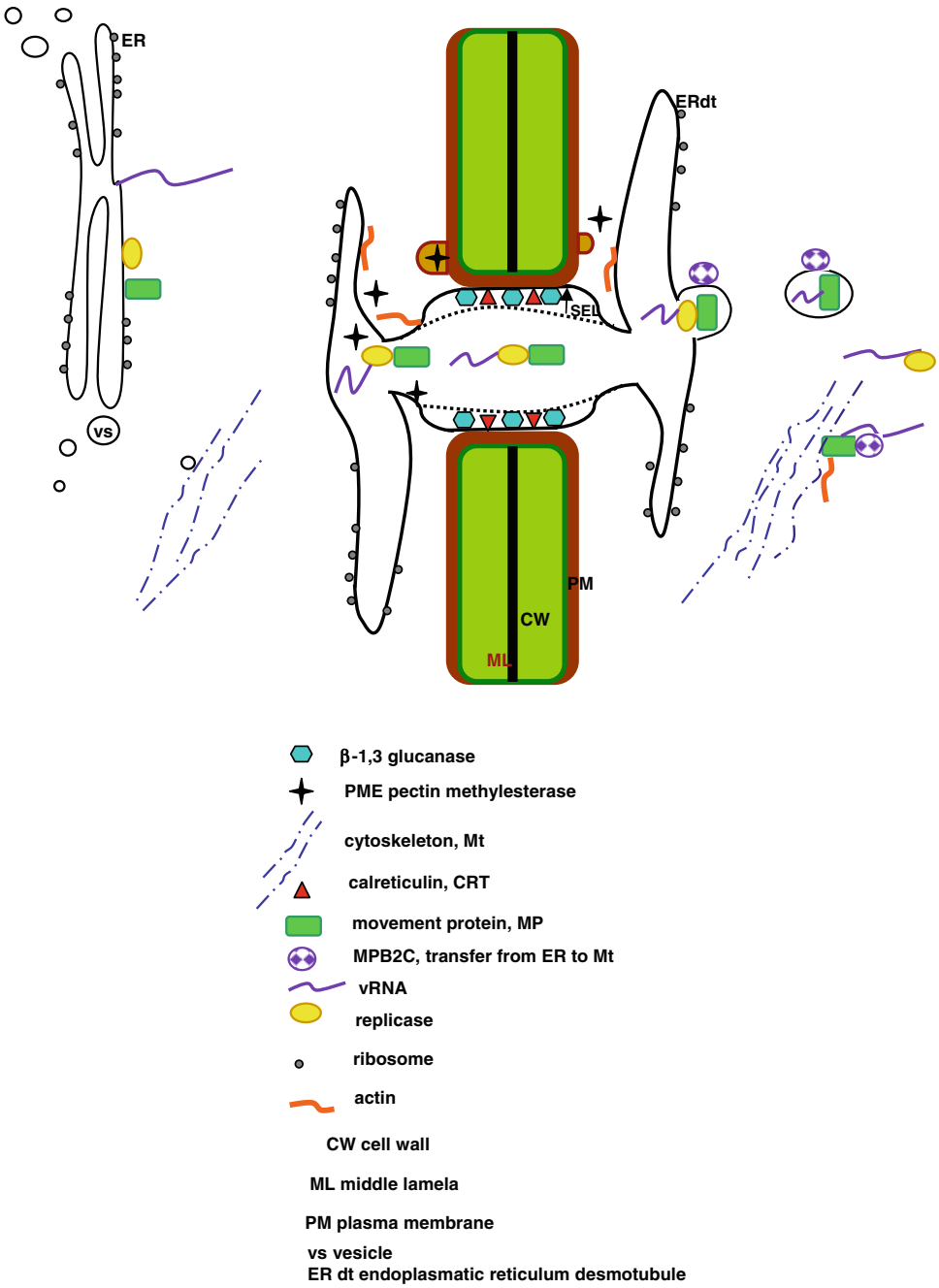

Fig. 1 *Tobacco mosaic virus* cell-to-cell movement. TMV MP binds and incorporates in ER and it binds vRNA. Replication and MP function synergistically by providing β-1,3-glucanase to the cell wal near PD. The β-1,3-glucanase limits callose accumulation near PD. The MP-vRNA-replication complexes diffuse into ER-desmotubule membranes. Cytoskeleton participate in TMV MP-vesicles movement to ER in PD. The MPB2C is a receptor in TMV MP transfer from ER to cytoskeleton triggering. Calcium binding protein—localized in PD—calreticulin is also engaged in TMV transport between ER and PD

(I-stage infection). MP TMV in synthesis binds with and enters ER (Mas and Beachy 1999; Brill et al. 2000), and it binds vRNA (Citovsky et al. 1992, Sambade et al. 2009). It was suggested that replication is also joined into the complex (II-stage infection) (Heinlein et al. 1998; Liu et al. 2005). Such complex may spread cell-to-cell to reach ER-desmotubules in the form of vesicles (Peremyslov et al. 1999; Liu et al. 2005) or through diffusion to ER membranes. Probably ER vesicles move to PD along actin filaments (Kawakami et al. 2004). The newest Hofmann et al. (2009) findings are consistent with the model that MP or MP/vRNP particles trafficking is rather mediated by ER (primarily) than involved in direct interaction with actin filaments. The PD-targeted MP transport and associated viral RNA occurs by diffusion in the ER membranes. The actin–myosin system with actin-binding factors may control this pathway. This system is able to support or slow down the transport of membrane-embedded protein complexes.

Tagami and Watanabe (2007) showed that secretive pathway/track ER-Golgi apparatus is rather not involved in supplying VRC (viral replicating complex) to PD or in

virus cell-to-cell movement. Replication and MP function synergistically possibly by providing β-1,3-glucanase I-class to the cell wall near PD (due to ER vesicles) (Fridborg et al. 2003). I-class β-1,3-glucanase limits callose accumulation near PD which is induced by viral infection due to the diffusion of MP-vRNA-replication complex in ER-desmotubules membranes (Fig. 1) (Gillespie et al. 2002; Kawakami et al. 2004; Liu et al. 2005). Guenoune-Gelbart et al. (2008) suggested that the cytoskeleton is not directly involved. It functions as ER stabilizing factor (Heinlein et al. 1998) or it makes ER vesicles with β-1,3-glucanase reach the cell wall near PD (Fridborg et al. 2003). Components of the cytoskeleton have their share in cell-to-cell movement of vesicles with MP to ER in PD (Oparka 2004).

Stressed was an indirect role in cell-to-cell transport not only of the cytoskeleton but also of the proteins cooperating with the cytoskeleton. Kragler et al. (2003) stressed the specific interaction between MP TMV and microtubule associated protein in tobacco tissues—MPB2C (movement protein binding 2C). This endogenous plant agent may play a role of a negative effector in MP TMV cell-to-cell movement (Gillespie et al. 2002). Waigmann et al. (2004) postulated only indirect involvement of MPB2C in TMV cell-to-cell transport. They concluded that the protein might participate in the late stages of TMV infection, which could take place in the area right behind the front face of the spreading infection. The interaction with MPB2C may be induced in order to eliminate MP from host-cell cytoplasm. Potentially, MPB2C can be a receptor, which triggers MP transfer from ER to microtubules where MP forms stable complexes (Boyko et al. 2000). Curin et al. (2007) claimed that over-expression of MPB2C in *N. benthamiana* plants caused changes in MP-TMV location because of redistribution from PD to microtubules. In plants in which MPB2C was suppressed, the presence of microtubules associated MP-TMV was highly reduced and the presence of MP TMV near PD was increased as compared with unmodified plants. It was postulated that MPB2C might act as a decisive factor, which determines the balance between PD and TMV-MP microtubular location. Perhaps TMV-MP/MPB2C interaction is regulated during the infection spread as MP TMV microtubular accumulation is controlled in time and space. Host agent, MPB2C is not vital for TMV cell-to-cell movement or for systemic spreading. It is only needed for effective accumulation of TMV-MP in microtubules and it determines the subcellular location of TMV-MP.

What was also underlined was the involvement of host cell proteins of the type chaperones in TMV cell-to-cell movement. Chen et al. (2005) suggested calreticulin engagement in TMV translocation between ER and PD. Calreticulin is a protein, which was localized in PD due to N-terminal signal peptide (Baluska et al. 1999; Michalak et al. 1999). Chen et al. (2005) showed that calreticulin interacts with TMV MP and over-expression of this protein in transgenic plants determines TMV relocation from PD to microtubules and thus cell-to-cell transport of the virus. Potential role of calreticulin in PD capacity was discussed also by Boevink and Oparka (2005), who underlined the ability of PD to modify thanks to MP TMV–CRT cooperation by changes in cell wall structure.

Potexvirus cell-to-cell movement determinants

Unlike *Tobamovirus*, members of *Potexvirus* genera need capsid protein for cell-to-cell transport the role of MP is filled by three types of proteins encoded by virus RNA. On the basis of these properties, Scholthof (2004) classified this type of plant virus transport to the so-called Type II (see Table 1). Potexvirus have single-stranded (+) ss RNA coding 5 open reading frame (ORF) (Verchot-Lubicz et al. 2007). First ORF codes viral replication, central region is made up from three overlapping ORFs known as triple gene block (TGB). Proteins coded by TGB transcripts are required for cell-to-cell transport (Verchot-Lubicz 2005). Final ORF is virus capsid protein, engaged not only in assembling the particles but also in cell-to-cell transport (Huisman et al. 1988; Santa Cruz et al. 1998).

Potexvirus code two proteins associated with ER: TGBp2 and TGBp3 (Mitra et al. 2003; Ju et al. 2005). They are the core of the movement process and so far no direct connection with replication process has been found. Two structures RNA stem-loop 5′SL1 and 5′SL2 are necessary for PVX replication (Miller et al. 1998, 1999). 5′SL1 is a multifunctional element, involved in virus replication, cell-to-cell transport and virions assembling. Host proteins may identify 5′SL1 by capsidation of viral particles, they can promote translation of replication of genome RNA (Kwon et al. 2005). A series of deletion mutation was used to

Table 1 Plant viruses classiffication based on movement proteins types according to Scholthof (2004) with modifications

Classification	Coat protein	Number of MPs	Example
TYPE I	Not required	1	*Tobamovirus*
		2	*Carmovirus*
		3	*Hordeivirus*
TYPE II	Required	1	*Cucumovirus*
		2	*Potyvirus*
		3	*Potexvirus*
		1–2	*Geminivirus*
TYPE III	Particles	1 and tubules	*Comovirus*
		3–4 (and 2CPs)	*Closterovirus*

identify a segment of RNA responsible for cell-to-cell movement of PVX. Deletion in the first 107 nt of PVX genome (on 5′SL1) eliminated the movement of this pathogen, which showed that 5′NTR (non-translated region) is an element engaged in both transport and replication (Lough et al. 2006). In case of Potexvirus there is subgenome synthesis of RNA, CP may bind to 5′SL1, moving the host agent. Capsid protein is needed for cell-to-cell transport as this region hides RNA from virus replication during the transfer to adjacent cells (Kwon and Kim 2006). There was an experiment conducted regarding the effect of TGBp1 on assembling the particles and their movement. Particles, which contained TGBp1 on one virion tail, are called STP (single-tailed particles, Fig. 2b) (Karpova et al. 2006). The RNA of PVX with STP is a form fit for translation (Karpova et al. 2006), while PVX virions do not have that quality, possibly, TGBp1 associated with virions at early stages of infection cause destabilization of translation. When the virus enters the cell, CP phosphorylation may trigger translation, and then it initiates the virus infection cycle. When PVX spreads cell-to-cell, TGBp1 may function as a promoter causing STP transfer to adjacent cells (Atabekov et al. 2000; Rodionova et al. 2003).

Verchot-Lubicz (2005) distinguished events in PVX cell-to-cell movement. During early events it is TGBp1 that sets inside PD (Verchot-Lubicz 2005) and not capsid protein as it was assumed before (Rouleau et al. 1995; Oparka et al. 1999). TGBp1 can increase the size exclusion limit. Some TGBp1 molecules move to neighboring cells, where they blocked RNA silencing ahead of virus infection. Other molecules TGBp1 bind with TGBp2 or TGBp3 and formed in that way complexes move across PDs. TGBp1/TGBp2 or TGBp1/TGBp3 complexes may transfer to adjacent cells where they act as a blockage of silencing and other types of host defense response. It may play an important role in initiating virus transport. In neighboring cells, TGBp2 and TGBp3 bind with ER membrane near PD, which creates a docking complex (Fig. 2a). In the middle events, TGBp1-PVX virions or ribonucleoprotein complexes move across PD. The ribonucleoprotein complex containing TGBp1, capsid protein and viral RNA moves across PDs. Basing on structural analysis of TGBp1-PVX complex described by Kiselyova et al. (2003), we still do not know its nature. In the late events, TGBp1-PVX or TGBp1-CP-RNA is associated with ER membrane by adjacent cell docking complex. The complex is built from joined together oligomers TGBp1 in TGBp2 and TGBp3. The TGBp1 presence in oligomers form unwinds PVX virions or the ribonucleoprotein complexes, making viral RNA available for translation (Fig. 2b) (Verchot-Lubicz 2005). Virus replication in the neighboring cell follows translation. This membrane-bound movement complex also might be a center for replication of viral RNA following translation. Some free CP displaces TGBp1 within the PD, restoring the PD to its resting state. TGBp1 might be recycled to provide either more round of RNA transport or to spread beyond the infection front suppressing RNA silencing.

This model for *Potexvirus* links viral RNA translation and counter defense with virus transport. These models suggest that PD transport of viral nucleic acids does not occur in isolation from other events in the infection cycle. What happens to the nucleic acid after it moves through the PD might determine the mechanisms by which it is transported from cell to cell.

TGB cooperating system

TGBp1 is a multifunctional protein needed for cell-to-cell movement of *Potexvirus*. TGBp1 provokes PD expansion (guiding the transfer of the virus and other molecules between cells). It shows the activity of RNA helicase and it may also be a component of vRNP complex (Yang et al. 2000; Howard et al. 2004). Moreover, TGBp1 is a suppressor of RNA silencing (Voinnet et al. 2000). It was proposed that TGBp1 interacts with RDR6, which participates in the production of short interfering RNA (siRNA) (Qu et al. 2005; Schwach et al. 2005; Xie and Guo 2006). RDR6, DCL4 and HEN1 are agents required for initiating and maintaining the silencing of virus-induced gene in developmental tissues. DCL4 produces 21 nt viral siRNA, amplified by RDR6 (Dunoyer et al. 2005; Blevins et al. 2006; Deleris et al. 2006). TGBp1 mutations, which block its activity also stop virus transport, which indicates that these processes are associated (Bayne et al. 2005). TGBp1 *Potexvirus* blocks 21 nt siRNA amplification or it inhibits RDR6–this question still remains unclear. However, its role as a silencing suppressor protects the replicating virus from being a target for RNA silencing.

TGBp2 and TGBp3 *Potexvirus* are ER-binding proteins. The analysis of amino acidic sequence showed that TGBp2 has 2 transmembrane domains and TGBp3 one N-terminal domain (Krishnamurthy et al. 2003; Mitra et al. 2003). Mutation disrupting membrane associations of these proteins also inhibit virus movement, indicating that ER association is important (Krishnamurthy et al. 2003). GFP to TGBp3 fusion and introducing a construct into a PVX genome in protoplasts of inoculated plants resulted in constructs fluorescence mainly in small granular vesicles and in ER (Ju et al. 2005). Granular vesicles accumulate on actin filaments, which suggest that they may move along the cytoskeleton to PD. Studies conducted with the use of an electron microscope showed that these are vesicles from ER induced by GFP-TGBp2. Vesicular structures

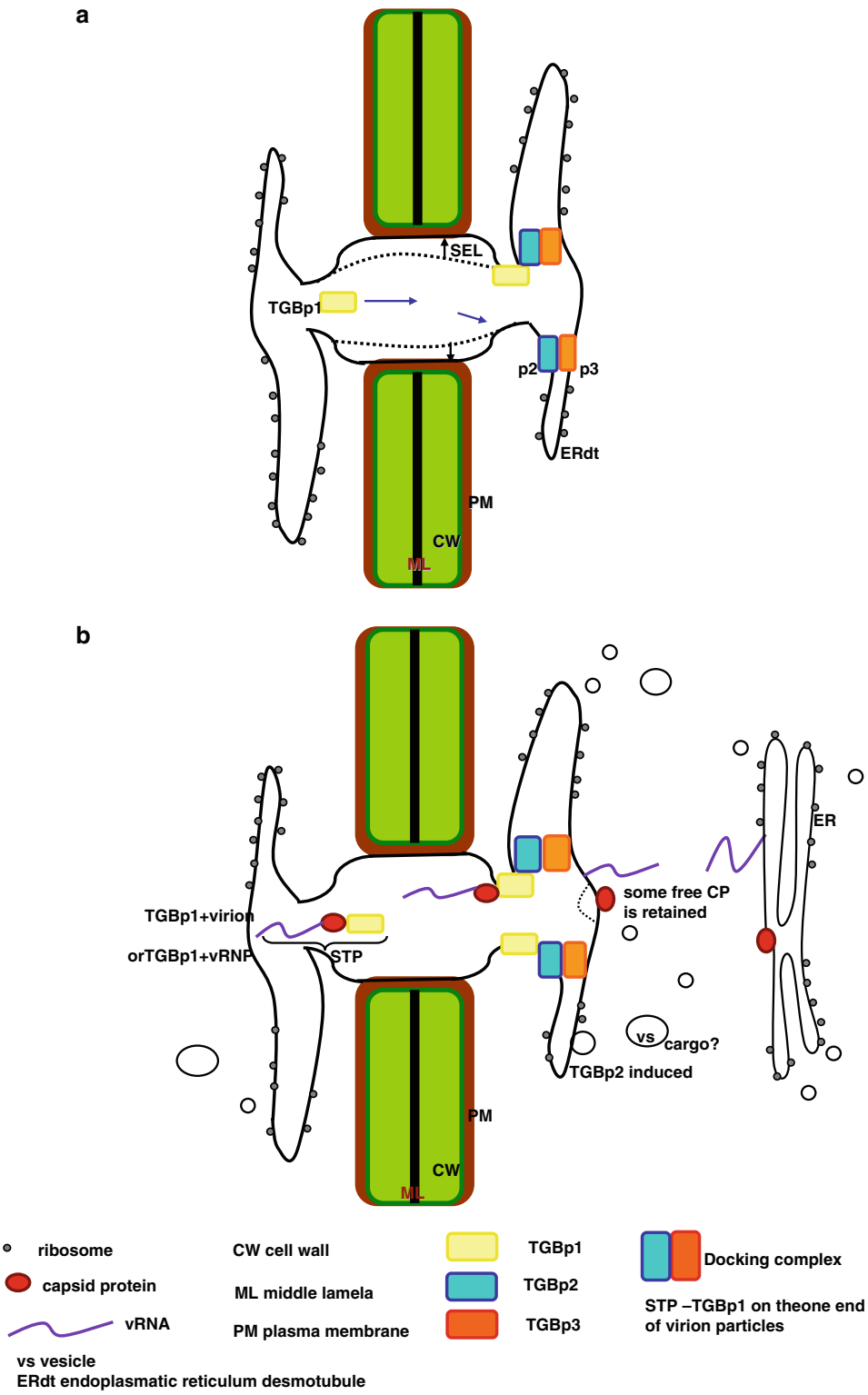

Fig. 2 *Potato virus X* local transport. **a** Early stage: preludium to virus move. The TGBp1-PVX moves itself and is able to increase SEL. TGBp1 binds TGBp2 or TGBp3 and forms complexes move across PD. These complexes may transfer to adjacent cells. They initiate virus transport. TGBp2 withTGBp3 bind ER membranes and create docking complexes. **b** Late stage: virions or RNP complexes moves across PD. The TGBp1-PVX virons or RNP complexes (TGBp1 + CP + vRNA) are associated with ER membranes by docking complexes. TGBp1 presence in oligomers form unwind PVX virions or RNP complexes, making vRNA available for translation

contained ribosomes, were immunoactive from GFP and BiP antigens (chaperon protein present in ER). Deletion of conservative amino acids in TGBp2 central region (located between 2 transmembrane domains) blocked the accumulation of GFP-TGBp2 in small, granular vesicles and inhibited cell-to-cell movement of the virus (Ju et al. 2007). Such data indicate that vesicular structures induced by TGBp2 PVX are necessary for virus movement (Ju et al. 2007). In tobacco plants, where the construct GFP-TGBp3 was subject to expression, fluorescence was connected

mainly with ER (Krishnamurthy et al. 2003). When plasmid containing GFP-TGBp3 was subject to co-expression with PVX, fluorescence was observed in granular vesicles, similar to those induced by TGBp2 (Schepetilnikov et al. 2005). It is possible that TGBp2 can direct TGBp3 to the same ER vesicles during viral infection. Studies prove that TGBp2 and TGBp3 sometimes coexist (Zamyatnin et al. 2002). So far there has not been provided any data which would show whether TGBp2-related vesicles contain TGBp1, vRNA, CP or virus-like particles. In addition, there is no direct evidence that would confirm a direct mutual interaction between TGB and CP PVX. There is scarce evidence to support the idea that all four proteins and vRNA form a transport-complex. The more we learn about the role of particular proteins, the more questions arise about how such different types of activity can cooperate in promoting cell-to-cell trafficking of viral RNA.

Some scholars suggest that TGBp1, TGBp2, and TGBp3 form a membrane-associated complex, which moves along ER and across PD. Another view is that the activity of *Potexvirus* transport proteins and CP can rather cooperate in time and space then directly form a single complex (Verchot-Lubicz 2005). Data that CP is accumulated inside PD and that TGBp1 gates PD suggest that these two proteins can act independently of TGBp2 and TGBp3 in order to regulate changes in PD aperture (Verchot-Lubicz et al. 2007). Virions or viral RNA can be transported to PD during later stages of infection inside the cell. If TGBp2 and TGBp3 coordinate the transport of TGBp1-CP-vRNA complex along ER into PD (Morozov and Solovyev 2003; Lucas 2006), available data illustrating that vesicles inducing TGBp2 PVX are vital for cell-to-cell transfer (Ju et al. 2007) bring to mind a question about the contents of these vesicles. Do they transfer infection agents from cell to cell? Model in which vesicles transport viral RNA to PD seems to be rather in opposition to the theory on the movement of TGBp1-CP-vRNA complex along ER to PD. It is also possible that TGBp2-induced vesicles play a role in modulating the ER stress responses (Ju et al. 2005) or other events in the virus life cycle, thereby enabling virus cell-to-cell movement.

Potyviruses components of transport networks

The genus *Potyvirus* doesn't code specific movement proteins. The *Potyvirus* movement involves viral proteins that perform additional roles in the virus life cycle (Carrington et al. 1996). The *Potyvirus* are positive-strand RNA viruses that encode ten mature proteins through a polyprotein expression strategy (Riechmann et al. 1992). Cell-to-cell transport requires an assembly component capsid protein (Dolja et al. 1994, 1995) suggesting that intercellular transport involves virion formation. The *Potyvirus* are classified as secondary movement type (II) regarding the engagement of CP protein in cell-to-cell transport and a need for several additional proteins (in case of PVY, two) to act as MP (see Table 1, Scholthof 2004).

The potyviral CP is a three-domain protein with variable N- and C-terminal regions exposed and a particle surface and a conserved core domain that interacts with viral RNA (Shukla and Ward 1989). Dolja et al. (1994; 1995) produced mutants in the CP-core region TEV-GUS. All of these were defective in cell-to-cell movement and in virion assembly. This mutational analysis also showed that the N-terminal domain of the CP has an accessory role in this movement process since mutants with this domain removed slow cell-to-cell movement in inoculated leaves.

Eagles et al. (1994) and Klein et al. (1994) have implicated the CI protein, an RNA helicase required for genome replication, in *Potyvirus* cell-to-cell movement. In electron microscopy studies CI protein is seen to form aggregates (pinwheels or cylindrical inclusions) in the cytoplasm of infected cells. These inclusions are seen positioned over the plasmodesmatal aperture (Fig. 3) (Revers et al. 1999). Two alanine-scanning mutants (CI-AS) with substitutions affecting the N-terminal region of CI protein TEV (*Tobacco etch virus*) were defective in intercellular movement (Carrington et al. 1998). Carrington et al. (1998) also suggested that the N-terminal region of CI protein provides a critical replication–independent movement function. The *Potyvirus* RNA replication requires the helicase-associated activities, while cell-to-cell movement requires a non-replication function involving the N-terminal region and possibly helicase activities. In combination with ultrastructural analyses and genetic data Carrington et al. (1998) results support a model in which *Potyvirus* CI protein interacts directly with PD and capsid protein within ribonucleoprotein complexes to facilitate *Potyvirus* cell-to-cell movement. Ultrastructural research of tissues at an early stage of infection with immunogold labeling of specific *Potyvirus* proteins, have supported the role CI in local transport with cooperation in CP (Rodriguez-Cerezo et al. 1997; Roberts et al. 1998). Observation of young tobacco leaves infected with TVMV (*Tobacco vein mottling virus*; Rodriguez-Cerezo et al. 1997) or cells during advanced PSbMV (*Pea seed-borne mosaic virus*) infection in pea cotyledons (Roberts et al. 1998) showed that cytoplasmic inclusions immunolabeled for CI protein and CP were attached to the plasma membrane, close to or over the plasmodesmal openings. The plasmodesmal aperture contained CP and also viral RNA during TVMV tobacco infection. Behind PSbMV infection front cytoplasmic inclusions were no longer associated with the cell wall or with CP. At an advanced stage of infection cytoplasmic inclusions accumulated as the characteristic pinwheels in

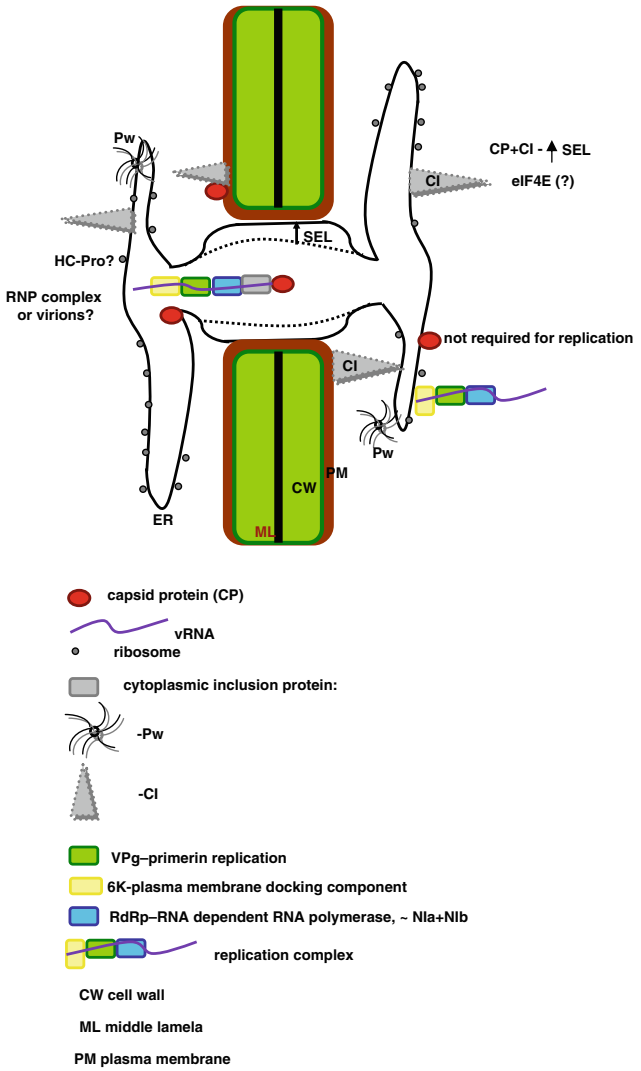

Fig. 3 *Potato virus Y* cell-to-cell movement. The PVY CP acts as MP. In *Potyvirus* local transport CI protein cooperates with CP in SEL increasing and often were attached to palsma membrane, close to PDs. The *Potyvirus* cytoplasmic inclusions (pinwheels or laminated inclusions) can function to position viral complexes through PDs. The role of the HC-Pro protein is not clear in cell-to-cell transport (probably interact with CP in SEL increasing). Does *Potyvirus* move as a virions, RNP complex or maybe as a replication complex ?—direct evidence is still lacking. Interacting host components are still not known

the cytoplasm (Roberts et al. 1998). Both studies suggested that *Potyvirus* cytoplasmic inclusions could function transiently to transfer viral complexes through PD.

Some genetic analyses indicate the involvement of the HC-Pro protein in cell-to-cell transport, but its direct role in this process is not clear (Cronin et al. 1995; Kasschau et al. 1997). The TEV HC-Pro mutant appeared to move from cell-to-cell less efficiently than the native/natural virus (Kasschau et al. 1997), whereas TVMV HC-Pro mutant was unable to spread in inoculated leaves (Klein et al. 1994). HC-Pro proteins together with CP were shown to increase plasmodesmal SEL and to promote viral RNA movement from cell to cell, whereas CI and NIa proteins did not induce these effects. These studies show that at least two potyviral proteins, CI and CP, cooperate and could be considered movement proteins. From Carrington's model (1998) CI protein may direct intracellular translocation of the viral transport complex, which includes the capsid protein. CP may interact with PD to increase the SEL. CI protein may function to position the viral complex for translocation through CI structures into the PD and to adjacent cells (Fig. 3) (Revers et al. 1999). Strong correlation between virion assembly and for cell-to-cell movement (Dolja et al. 1994, 1995) and also fibrillar material (similar to PSbMV particles) may be taken as an indication that *Potyviruses* move intracellular as a virions, but direct evidence is still lacking.

It is interesting that *Potyvirus* is the biggest known plants virus *genus*, but the knowledge about interacting host components is not clear. Recently, a potential link between virus accumulation and cell-to-cell movement was identified when the eukaryotic translocation factors eIF4E and eIF(iso)4E were shown to aid in virus cell-to-cell movement (Gao et al. 2004). These observations correspond with earlier studies where plant mutants with eIF4E exhibit limited virus spread (Arroyo et al. 1996). It has been speculated that *Potyvirus* intracellular movement may occur via an interaction of eIF4E with eIF4G, which then binds microtubules (Lellis et al. 2002).

Conclusion

It is important to realize that the host proteins may function in the virus infection process in translocation and/or replication and also in cell-to-cell movement (Nelson and Citovsky 2005). Viruses reveal different movement strategies with specific viral and host components interacting in efficient, functional transport events. Most of the viruses are transported as nucleoprotein complexes, others as virions, so taxonomically different viruses can use similar strategies, but some use more than one. The inherent flexibility of plant viruses to adjust the nature of the transported material to specific circumstances a property, that is probably of key importance for adaptation to new host. In our review several processes are described, for which the precise mechanism is unknown, therefore many fascinating challenge still lie ahead. Probably studies of the functional role of identified host factors could have predominant influence on plant virus movement. This knowledge should substantially improve our understanding of how the virus and the host partner together cooperate in movement process.

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