

## Identification of Plant Virus Movement-Host Protein Interactions

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Z. Naturforsch. **56c**, 669–679 (2001); received March 1, 2001

Plant Viruses, Cell-to-Cell Transport, Protein-Protein Interaction

After the discovery of ‘movement proteins’ as a peculiarity of plant viruses and with the help of novel methods for the detection and isolation of interacting host proteins new insights have been obtained to understand the mechanisms of virus movement in plant tissues. Rapid progress in studying the molecular mechanisms of systemic spread of plant infecting viruses revealed an interrelation between virus movement and macromolecular trafficking in plant tissues. This article summarizes current explorations on plant virus movement proteins (MPs) and introduces the state of the art in the identification and isolation of MP interacting host proteins.

### Introduction

The number of proteins which a plant attacking virus needs for a successful invasion of its host can be narrowed down to i.) a nucleic acid binding protein (nucleocapsid protein), specifically encapsidating the viral genome, ii.) a DNA polymerase (*Geminiviridae*), reverse transcriptase (*Caulimoviridae*) or RNA-dependent RNA polymerase (for multiplication and transcription of RNA virus genomes), and iii.) a movement protein (MP), essential for intra- and intercellular motion of the virus to enable systemic infection of the whole plant. Whereas nucleocapsid proteins and virus-encoded polymerases are components in both animal and plant infecting viruses, the MPs manifest a specific feature of plant viruses.

An intriguing example which hints a correlation between the adaptation of viruses to plants concomitant with the appearance of a virus gene encoding a movement function is given within the *Bunyaviridae* family (Fig. 1). This virus family consists of five genera, four of them exclusively infecting vertebrates or insects (Elliott, 1996). The fifth genus, denominated *Tospovirus*, is closely related to the other four genera in its genome structure and in the morphology of the virions, however, the M segment of the *Tospovirus* genomic RNA is bearing an additional open reading frame (Kormelink *et al.*, 1992). Later it was shown that a non-

structural protein, termed NSm, is translated from this cistron and that this protein represents the MP of the *Tospoviruses* (Kormelink *et al.*, 1994). It is still a matter of discussion whether the appearance of this specified additional cistron opened the door to attack organisms of the plant kingdom during the evolution of the *Bunyaviridae*.

Following the indication that MPs are one important key for viruses to successfully attack plants, the main question ascended how a plant virus MP interacts with cellular functions to provoke a systemic infection of the organism. With the discovery of a plant virus MP indispensable for systemic spread, firstly shown for the 30 kDa protein encoded by tobacco mosaic virus (TMV) in the laboratories of Atabekov and Zaitlin in 1982 (as reviewed in Pennazio *et al.*, 1999), a direct relation between virus transport and plasmodesmata, small microchannels connecting plant cells, was decisively validated. Molecular and genetic experiments corroborated the close relationship between plasmodesmal transport and plant virus MPs, e.g. by localization of MPs at plasmodesmata or by microinjection studies showing that MPs influence the size exclusion limit of the microchannels (for review: Lucas and Gilbertson, 1994; Carrington *et al.*, 1996; Koll and Büttner, 2000).

Systemic movement of plant viruses does not only involve cell-to-cell transport from the site of infection into the neighboring cells, but for long dis-



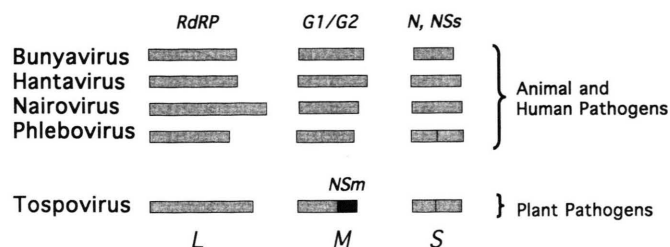


Fig. 1. Schematic drawing of the *Bunyaviridae* genome organization (according to Elliott, 1996).

Each of the five genera consists of three RNA segments which are entitled L (large), M (medium), and S (small), depicted as grey bars. Proteins encoded in minus-sense orientation on the respective segments are: RdRP, a RNA dependent RNA polymerase; G1/G2, a glycoprotein precursor and N, the nucleocapsid protein. NSs, a nonstructural protein, is encoded in plus-sense orientation by *Phlebo-* and *Tospoviruses*. Also in plus-sense orientation, the extra NSm open reading frame is exclusively arranged on the M segment of *Tospoviruses* (shown as a dark grey bar).

tance movement the general opinion is that viruses have to take advantage of phloem transport for a successful invasion of the whole plant. Possible roles of plant virus encoded proteins during the passage into and out of the sieve elements in minor veins and the transition of viral genomes or virus particles through different cell layers are still a matter of discussion (Nelson and van Bel, 1998). Sieve elements and companion cells act as 'traffic control centers' of the phloem (Oparka and Turgeon, 1999) in a way that, species specific, symplastic or apoplastic loading of photoassimilates occurs. Symplastic loaders connect phloem parenchyma and sieve element accompanying cells by numerous plasmodesmata, while apoplastic loaders apparently have no plasmodesmal connection (Fig. 2). Consequently, viruses attacking apoplast loading species (i.e. most herbaceous plants; Gamalei, 1989) have to circumvent this barrier. Actually, Ding *et al.* (1998) reported that some viruses exploit plasmodesmata between sieve elements and the vascular parenchyma in minor veins to gain direct access to the phloem, rather than entering the companion cells. A family of key chaperones was postulated to be involved in macromolecular transport in the phloem parenchyma – companion cell – sieve element complex of apoplastic loaders (Oparka and Turgeon, 1999), and therefore it can be speculated whether virus MPs or other virus encoded proteins interfere with the proposed chaperone activity (Crawford and Zambryski, 1999).

### Designated movement proteins of plant viruses

As described above, plant virus MPs facilitate cell-to-cell translocation of the viral genome,

which could be transported either as virions or some form of MP-nucleic acid complex (Carrington *et al.*, 1996; Ding *et al.*, 1999). In the recent past it turned out that for a successful systemic infection of a host plant, viruses have developed different strategies, involving one or more virus encoded gene functions which obviously recruit host proteins for intercellular transport. The following paragraphs therefore briefly summarize the present knowledge of movement mechanisms of distinct plant virus taxa.

### *Carla-, Hordei- and Potexviruses*

Members of these genera characteristically express proteins denominated TGBp1, TGBp2 and TGBp3 from tightly linked or occasionally overlapping open reading frames appearing at the 3' end of the respective genomic RNA (specified as the 'triple gene block', TGB). It has been shown that all three proteins can act as MPs (Gilmer *et al.*, 1992). TGBs have been divided into two classes (Solov'yev *et al.*, 1996). For viruses belonging to TGB class I (*Hordeiviruses* and beet necrotic yellow vein virus (BNYVV), a tentative *Furovirus*) it has been shown that viral RNA itself is the infectious entity, presumably in complex with viral MPs (Erhardt *et al.*, 2000, and references therein). Conversely, viruses of TGB class II (members of the *Potexvirus* genus), move from cell to cell as virions (Santa Cruz *et al.*, 1998). Experiments by Erhardt *et al.* (2000) lead to promising insights of the interconnections of the three TGB-proteins P42, P13 and P15 of BNYVV in TGB mediated transport. In this work, the jellyfish green fluorescent protein (GFP) was translation-

ally linked to the N-terminus of P42. This chimaeric protein was able to drive viral cell-to-cell movement and localized to punctate bodies apposed to cell walls of *Chenopodium quinoa*. The GFP-P42 punctate body formation was depending on the expression of P13 and P15 during the infection and co-localized with callose, which suggests that P42 is associated with plasmodesmata. It can be speculated that the three TGB proteins of BNYPV perform protein-protein interactions, maybe sequentially to transfer P42 to plasmodesmata, however, other viral proteins as well as cellular proteins may also be required for a successful transport of the virus.

*Closteroviridae*

The beet yellows virus (BYV) encoded homolog to cellular heat-shock proteins (Hsp70h) was observed in plasmodesmata and co-localized with vi- rions present in the cytoplasm of infected cells (Medina *et al.*, 1999), firstly indicating a connec- tion between viral intra- and intercellular trans- port mechanisms and molecular chaperones. A re- cent genetic analysis of cell-to-cell movement of BYV unveiled that all together five viral proteins (three dedicated MPs and two structural proteins, denominated the ‘quintuple gene block’) potenti-

ate intercellular transport of the virus (Alzhanova *et al.*, 2000).

*Cucumo-, Diantho- and Tobamoviruses*

The seemingly best studied examples for plant virus movement proteins are provided by tobacco mosaic virus (TMV) and red clover necrotic mo- saic virus (RCNMV). Both viruses require an about 30 kDa large MP in order to move from cell to cell. Functional features of the TMV MP can be summarized as i.) it binds TMV genomic RNA, ii.) it interacts with cytoskeletal elements, possibly to facilitate intracellular transport, iii.) it increases the size exclusion limit of mesophyll plasmodes- mata, and iv.) its interference with plasmodesmata is negatively regulated by phosphorylation (Waig- mann *et al.*, 2000; for a recent review: Rhee *et al.*, 2000). For cell-to-cell movement of TMV is well accepted that the TMV MP actively and specifi- cally alters plasmodesmal permeability very likely utilizing endogenous pathways for macromolecu- lar transport between plant mesophyll cells. Studies regarding long distance transport of TMV revealed a crucial role of the TMV coat protein, indicating that additional or alternative transport functions of the host are seduced for systemic spread of the virus (for review: Goshroy *et al.*,

Table I. Compilation of currently identified movement-host protein interactions.

<b>Virus movement protein</b>	<b>Examined plant species</b>	<b>Interacting protein</b>	<b>Initial method of isolation</b>
Tobacco mosaic virus (TMV) 30 K	Tobacco	Pectin methylesterase (also interacting with CaMV <sup>a</sup> and TVCV movement protein) <sup>b</sup>	Renatured western blot and microsequencing YTHS
Cucumber mosaic virus (CMV) 2b	Tobacco	2bip; similar to unknown protein from <i>A. thaliana</i> <sup>c</sup>	YTHS
Tomato spotted wilt virus (TSWV) NSm	<i>A. thaliana</i>  Tobacco Tomato	Myosin-kinesin like protein <sup>d</sup> , Protein of unknown function <sup>d</sup> DnaJ like protein <sup>e</sup> DnaJ like protein <sup>f</sup> DnaJ like protein <sup>d</sup>	YTHS

With the help of the yeast two-hybrid system (YTHS), a genetic assay for *in vivo* detections of protein-protein interactions in *Saccharomyces cerevisiae*, movement-host protein interactions have been identified. The DnaJ like proteins found to interact with NSm are characterized by an N-terminal J-domain and therefore are related to molecular chaperones shown to be involved in protein folding and protein transport.

<sup>a</sup> CaMV, cauliflower mosaic virus; TVCV, turnip vein clearing virus. <sup>b</sup> Dorokhov *et al.*, 1999; Chen *et al.*, 2000. <sup>c</sup> Ham *et al.*, 1999. <sup>d</sup> von Bargen, 2001. <sup>e</sup> Kellmann *et al.*, 2000. <sup>f</sup> Soellick *et al.*, 2000.

1997). In order to search for host proteins involved in MP-mediated virus transport, cell wall associated pectin methylesterases have been identified as interaction partners (Table I). A possible role of this interaction during TMV movement will be discussed below.

Two proteins have been determined as MPs of cucumber mosaic virus (CMV). Properties of the CMV encoded 3a protein resemble those of the TMV MP, e.g. the protein is targeted to plasmodesmata and, correspondingly, traffics associated with a ribonucleoprotein complex into the vasculature (Blackman *et al.*, 1998; Ding, B. *et al.*, 1995). The CMV 2b protein has been proposed to be involved in long distance movement, because a mutant lacking the corresponding open reading frame was unable to spread systemically in cucumber plants (Ding, S. W. *et al.*, 1995). In favor of studying the intrinsic components of long distance movement, 2b was taken as a bait in yeast two-hybrid interaction trap experiments, and recently, an interacting protein from tobacco has been isolated (Table I).

### *Geminiviridae*

Viruses of this family possess a single stranded, circular DNA genome which replicates in the nuclei of infected host cells. Consequently, for intra- and intercellular movement of such genomes, two transport functions have to be arranged: one crossing the nuclear envelope and the second passing the cell wall. Indeed, geminiviruses encode two MPs denominated BR1 and BL1 which act in a cooperative manner to transport the viral genome (for review: Lazarowitz, 1992). For the phloem-limited squash leaf curl virus (SqLCV) it was shown that BL1 is able to directly interact with BR1 (Sanderfoot and Lazarowitz, 1995). When the proteins are individually expressed in tobacco protoplasts or insect cells, BL1 is localized to the periphery and BR1 to nuclei, however, when co-expressed in either cell type, BL1 re-localized BR1 from the nucleus to the cell periphery. Further investigations of the function of BL1 unveiled that in SqLCV infected pumpkin leaves BL1 is associated to unique tubules which were observed in procambial cells and minor veins (Ward *et al.*, 1997). It is not clear whether BL1 is incorporated into the wall of the tubule or is being transported

by it. Subcellular fractionation studies suggested that the tubules were derived from the endoplasmic reticulum (ER), implying that SqLCV recruits the ER as a conduit for cell-to-cell movement in a developmental manner. Several features of the BL1 induced tubules appear to distinguish them from tubules reported for *Tospoviruses*, *Como-* and *Caulimoviridae* (see below), e.g. the BL1 induced tubules can be seen to cross the walls of procambial cells and project into the cytoplasm on either side, whereas the tubules described for *Tospoviruses* and *Comoviridae* appear to project into the cytoplasm from only one side of the mesophyll cell wall (Ward *et al.*, 1997 and references therein). However, for both types of tubules it is proposed that they serve as a conduit for the transport of viral nucleic acid or virions, and a generally asked question is whether cellular proteins are associated with virus-induced tubules and whether host proteins are needed to force the virus particles through these channels.

### *Luteoviruses*

The viruses of this genus are transmitted by aphids usually leading to an inoculation of the sieve element – companion cell complex (Fig. 2). Tacke *et al.* (1991) identified an internal initiation of an open reading frame nested within the coat protein gene of potato leafroll virus (PLRV) and found that the translated 17 kDa protein has a single-stranded nucleic acid binding activity. *In situ* localization of the 17 kDa protein revealed intrinsic properties to bind to plasmodesmata and that the protein is limited to the phloem (Schmitz *et al.*, 1997; Sokolova *et al.*, 1997), suggesting a specific role in transport mechanisms through deltoid-shape plasmodesmata connecting the sieve element – companion cell complex (Fig. 2). Therefore, the 17 kDa protein is providing a useful tool for a better understanding of phloem-specific proteins and their possible capabilities in long distance movement (for review: Oparka and Santa Cruz, 2000).

### *Potyviruses*

Intercellular movement of *Potyviruses* still remains an enigma. *Potyvirus* genome expression is accomplished through the translation of the viral RNA into a polyprotein that is cleaved into at



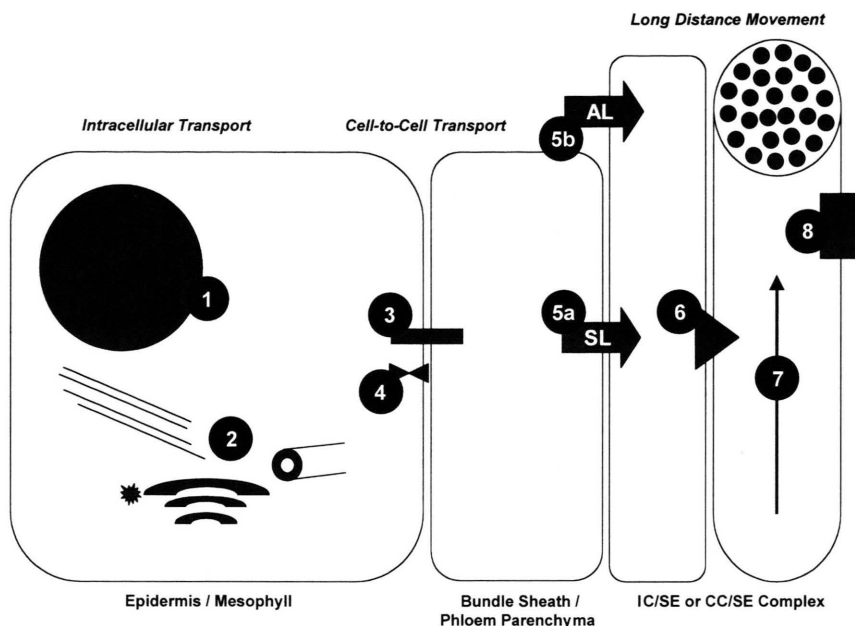


Fig. 2. Different cell layers proposed to be passed by plant viruses to achieve systemic movement employing the phloem system.

Infective virus particles have to move intracellularly to the plasmamembrane and the cell wall. Following cell-to-cell movement, the virus has to cross bundle sheath and phloem parenchyma cells to finally reach the companion cell-sieve element complex for long distance movement. In favor of an action of movement- or other virus encoded proteins, putative sites of host proteins which could interact with viral proteins are serially numbered and depicted as black circles. 1: nuclear pore complex (only required for DNA viruses); 2: cytoskeleton, vesicular transport systems and cytoplasmic proteins; 3: mesophyll-mesophyll or mesophyll-bundle sheath cell connecting primary or secondary plasmodesmata; 4: cell wall located proteins or enzymes ('receptors'); 5a: plasmodesmata adjoining phloem parenchyma- and intermediary cells (regarding plants with a symplastic loading configuration); 5b: transport systems involved in an apoplastic loading configuration adjoining phloem parenchyma and companion cells; 6: deltoid shaped plasmodesmata (pore-plasmodesma units) facilitating macromolecular transport between companion cells and sieve elements; 7: phloem specific proteins and 8: transport facilities involved in phloem unloading.

Abbreviations: AL, apoplastic loading configuration; SL, symplastic loading configuration; IC, intermediary cells; CC, companion cells; SE, sieve elements.

least eight viral proteins (Dougherty and Carrington, 1988). In contrast to most other viruses, the *Potyvirus* do not encode a dedicated MP. Nonetheless, it has been shown that cell-to-cell and long distance transport requires an assembly-competent nucleocapsid protein. Additionally, the NIa protein, which provides catalytic functions for replication and polyprotein processing, and the helper component-proteinase (HC-Pro) have been shown to be essential in long distance movement, however, it is not known whether they interact directly with transport processes or indirectly effect on the host plant for successful virus movement (Cronin *et al.*, 1995; Rojas *et al.*, 1997). Recently, alanine scanning of the cylindrical inclusion body protein (CI) revealed that it also has a cell-to-cell move-

ment function which can be genetically separated from its role during RNA replication (Carrington *et al.*, 1998).

#### *Tospoviruses; Bromo-, Como- and Caulimoviridae*

The common feature of viruses belonging to these taxa is that they very likely perform cell-to-cell movement by a tubule based mechanism (for review: Carrington *et al.*, 1996). A MP dependent induction of tubular structures has been reported for representatives of the *Bromoviridae* (Zheng *et al.*, 1997; Jansen *et al.*, 1998), and for cowpea mosaic virus (CPMV) and tomato spotted wilt virus (TSWV) it has been shown that the tubules are composed of the respective MPs and presumably

cellular constituents (van Lent *et al.*, 1990, 1991; Goldbach and Peters, 1996). Electron microscopy suggests that the tubules project unidirectionally into one cell and replace the desmotubulus of targeted plasmodesmata. It is tempting to assume that TSWV moves from cell to cell along the tubules as non-enveloped nucleocapsids, because the diameter of the MP induced tubules (40–50 nm) is large enough for a passage of the infectious virus particles (Storms *et al.*, 1995).

Strikingly, the MPs of CPMV, TSWV and cauliflower mosaic virus (CaMV) can induce tubular structures in protoplasts of plant- and insect cells, implying that these MPs may interact with cellular components that are conserved across the boundaries of the animal and plant kingdoms (Huang *et al.*, 2000 and references therein). An analysis of partially purified tubules derived from CPMV infected plant protoplasts revealed that beside the MP and the nucleocapsid protein of the virus no other proteins could be detected, suggesting that host proteins are not a major structural component of the tubules (Kasteel *et al.*, 1997). This, however, does not rule out the possibility that host proteins are in any way functionally involved in the process of tubule formation or during an active transport of the virus through these conduits. Host proteins interacting with NSm, the MP of TSWV, have been identified recently (Table I), and their role in the systemic movement of the virus is discussed below.

### Interactions between movement- and host proteins

Viral MPs are well suited probes for studying intra- and intercellular trafficking of macromolecules in plant tissues (Lazarowitz and Beachy, 1999), and direct interactions between MPs and plant proteins have been proposed. The investigations of MPs encoded by plant attacking viruses as described above revealed that MPs are usually associated with plasmodesmata. Two classes of MPs can be distinguished: those which bind viral nucleic acids and are able to enhance the size exclusion limit of the attacked plasmodesma, leading to a passage of the virus between mesophyll cells, and those MPs which mainly alter the ultrastructure of plasmodesmata by inducing tubular structures, proposing that intercellular transport of the

virus is facilitated by these conduits (for review: Koll and Büttner, 2000). Comparison of secondary structure predictions based on MP amino acid sequences revealed core structures of many plant virus MPs, and from these alignments an overall secondary structure prediction was generated (Melcher, 2000). A '30K superfamily of viral movement proteins', based on properties of the TMV 30 kDa MP, was intended, including also plant phloem proteins as well as the tubule forming MPs, however, the latter assemble in a separated branch of the protein family.

Recent analysis focussing on the identification and isolation of MP interacting plant proteins lead to promising results which could allow more insight into the understanding of plasmodesmal function (Jackson, 2000). Evidence accumulates that viral MPs indeed use endogenous plant pathways for intra- and intercellular trafficking of macromolecules. Beside plasmodesmata, the cytoskeleton also appeared to be involved in virus transport (Heinlein *et al.*, 1995; McLean *et al.*, 1995). These findings are well taken since at least mesophyll cell connecting plasmodesmata were found to be associated with actin filaments (Ding *et al.*, 1996). Furthermore, from analyses on the interference between the 30 kDa MP of TMV and tubulins there is strong evidence that viral RNA transport is driven by dynamic processes of microtubule-polymerization (Boyko *et al.*, 2000).

Screening for MP interacting proteins lead to the isolation of proteins shown to directly interact with MPs derived from TMV, CMV and TSWV (Table I). Using the TMV 30 kDa MP as a probe in renatured western blot analysis followed by affinity chromatography, plant pectin methylesterase (PME) has been identified as a crucial host component involved in TMV movement (Dorokhov *et al.*, 1999; Chen *et al.*, 2000). Furthermore, PME was also shown to interact with the MPs of turnip vein clearing virus (TVCV) and CaMV in experiments employing the yeast two-hybrid system (YTHS). The YTHS represents a genetic assay for *in vivo* detections of protein-protein interactions (for review: Fields and Sternglanz, 1994). What could be the answer to the question how PME, an enzyme which is involved in cell wall turnover, facilitates virus movement? The unprocessed PME polypeptide carries an ER translocation signal and therefore the enzyme could be des-

tined to be transferred to the cell wall. Presumably, MP binding to PME results in a 'piggyback' transport of the MP to the cell wall and presumably to plasmodesmata through the ER secretory pathway (Chen *et al.*, 2000).

In order to determine how 2b, a MP encoded by CMV, mediates virus long distance transport, the YTHS was employed to search for 2b interacting host proteins. A protein entitled 2bip (2b-interacting protein) was isolated from tobacco. The deduced amino acid sequence of the protein revealed similarities to a protein of unknown function of *Arabidopsis thaliana* (Ham *et al.*, 1999). The transcript of 2bip was detected in leaves, stems, roots and flower organs of tobacco to nearly identical levels. Interestingly, preliminary data suggest a localization of 2bip at the nucleus, indicating that the 2b-2bip interaction could rather play a role in transcriptional regulation of host genes during CMV replication than during long distance movement.

Similarly, the MP of TSWV (NSm) was used as a bait in YTHS experiments and three host proteins could be identified as interaction partners, i.e. a protein of unknown function, a myosin-kinesin like protein and a DnaJ like protein (Soellick *et al.*, 2000; Kellmann *et al.*, 2000; von Bargaen, 2001). DnaJ (Hsp40) proteins belong to a family of molecular chaperones and operate together with Hsp70 chaperones in a variety of cellular functions like protein folding, protein transport and activation of various transcription and replication factors (for review: Cheetham and Caplan, 1998). Whether the identified NSm-host protein interactions occur simultaneously or sequentially in different plant organs or tissues during the systemic spread of TSWV remains to be elucidated. The amino acid sequence of the myosin-kinesin like protein also shows lower similarity to interaptins, desmoplakins and golgins, suggesting that it is part of an intracellular vesicular transport system in which intermediate filaments, the actin cytoskeleton and the *trans* golgi system are involved. Therefore it can be speculated that, according to the TMV MP-PME interaction, the interconnection between NSm and the myosin-kinesin like protein permits intracellular movement of TSWV nucleocapsids utilizing the cytoskeleton or vesicle sorting systems. Because NSm belongs to the group of tubular-forming MPs, the question arises if there is

a connection between the three NSm-interacting host proteins and the induction or composition of the tubules. To answer this question, it would be helpful to conclude the destined function of these host encoded proteins, to study their localization in the plant cell or in different plant organs, to follow the expression patterns of the corresponding genes after inoculation and to perform reverse genetics with respective host species. At the moment, putative mechanisms can only be proposed on the basis of amino acid sequences, i.e. by search for homologies to polypeptides whose functions have already been described.

#### *Involvement of molecular chaperones in plant virus movement*

The interaction between the TSWV MP (NSm) and DnaJ like proteins has been independently determined for proteins derived from *A. thaliana*, tobacco and tomato (Table I). The three DnaJ like proteins exhibit a striking similarity to each other (Fig. 3) and carry a typical J-domain at their N-termini (Buckau and Horwich, 1998). With a closer look on alignments which integrate the more 'archetypical' DnaJ chaperones which are well characterized for their function in the heat shock response acting together with DnaK and GroEL, it appeared that, except for the common J-domain, the group of DnaJ like proteins which interacts with NSm forms a subclass within the DnaJ protein family (von Bargaen, 2001). A peculiarity of the polypeptides encoded by the clones A39, M541 and T19/8 is that they lack DnaJ typical cysteine-rich domains which were found to bind zinc ions (Martinez-Yamout *et al.*, 2000), instead, the NSm interacting DnaJ like proteins are comprised of lysine-rich domains.

In the past years it was manifested that DnaJ chaperones exhibit a range of functions, including a role in protein import into the ER, mitochondria and peroxisomes. Several models concerning intra- and intercellular transport of macromolecules postulated the activity of chaperones (e.g. Kragler *et al.*, 1998; for review: Jackson, 2000), where they are thought to partially unfold a protein before its translocation through plasmodesmata. Virus encoded homologs to Hsp70 chaperones have been found in the genome of *Closteroviridae* and were recently shown to be indispensable for cell-to-cell

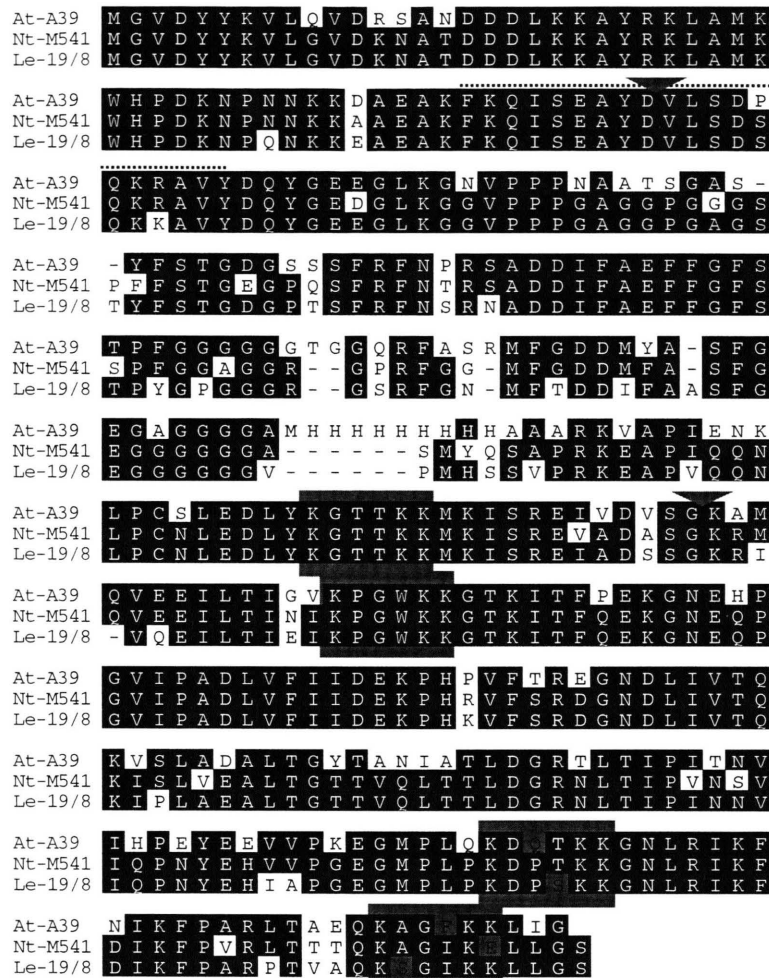


Fig. 3. Alignment of DnaJ like proteins interacting with NSm.

Molecular chaperones of the DnaJ (Hsp40) family were found to be involved in cellular functions including protein folding and protein transport. Amino acid sequences of DnaJ like proteins from *A. thaliana* (At-A39; acc. no. AL021749), tobacco (Nt-M541; acc. no. AF191497) and tomato (Le-19/8; acc. no. AJ295232), shown to interact with the TSWV encoded NSm movement protein, were aligned using the clustal method of the MegAlign program (DNASTar Inc.). Amino acids which match the consensus sequence are dark-boxed, dashes indicate gaps introduced for optimal alignment. The dotted line marks the conserved J-domain of the proteins. Four lysine enriched domains bearing the core motif K-X-X-X-K-E/K are underlayed in grey boxes. Grey arrowheads mark conserved positions of introns determined for the *At-A39* and *Le-19/8* gene.

transport (Peremyslov *et al.*, 1999). With the identification of DnaJ like chaperones as MP interacting proteins further evidence can be presented which corroborates that molecular chaperones are seduced by plant viruses for a successful infection. In contrast, the dominant *RTM2* gene encoding a protein consisting of a domain resembling small heat shock proteins appeared to be necessary for the restriction of long distance movement of tobacco etch

virus (TEV, a member of the *Potyvirus* genus) in several *A. thaliana* ecotypes (Whitham *et al.*, 2000). Nonetheless, in analogy to the identified MP-DnaJ interactions and the crucial role of the Hsp70h protein for the movement of *Closteroviridae*, the *RTM2* mediated restriction of TEV trafficking has been discussed to be the result of pathogen recognition of the plant, followed by signaling to induce a TEV restrictive state in the phloem.



### Concluding remarks and outlook

For many plant infecting viruses, MPs have been identified which are indispensable for systemic spread throughout the host plant. The MPs do not exhibit a high amount of amino acid sequence similarity, except when secondary structure predictions of the polypeptides are compared, a consensus domain pattern can be deduced (Melcher, 2000). In many cases, MPs have been located at mesophyll cell connecting plasmodesmata and/or have been shown to induce or build tubular structures in the cytoplasm. Often, viruses depend on more than one protein carrying a movement function, e.g. for long distance translocation of TMV or PVX, presence of the respective coat protein is indispensable (Ghoshroy *et al.*, 1997; Fedorkin *et al.*, 2001). Additional movement functions can be encoded either by the virus itself (e.g. the 'quintuple gene block' of the *Closteroviridae*, Alzhanova *et al.*, 2000), or a solely dedicated MP of a virus genome recruits cellular proteins to carry out its dedicated function, e.g. the 30 kDa MP of TMV or the NSm protein of TSWV.

In spite of being intensively studied, most of the steps involved in virus translocation across the cell wall either by MP affected dilatation of plasmodesmata or by modifying plasmodesmal ultrastructure after MP-induced formation of tubuli are not well understood. At the end, virus trafficking into and out of the vascular tissues still remains not

unimportantly obscure because plant virus movement to some extent depends on the sink-source transitions in a growing host plant, making a defined analyses of vascular virus movement more difficult (Oparka *et al.*, 1999). In the process of identifying host genes that are essential for virus movement, biochemical, cellular and genetic approaches are now on the way to shed more light on molecular virus-host protein interactions. With current methods so far used to localize the MPs in plant tissues or to isolate corresponding host factors, there is strong evidence that the ER, the cytoskeleton and molecular chaperones are involved at least in intracellular plant virus trafficking (Peremyslov *et al.*, 1999; Reichel *et al.*, 1999; Boyko *et al.*, 2000; Kellmann *et al.*, 2000; Soellick *et al.*, 2000; Whitham *et al.*, 2000). The utilization of virus MPs as a tool together with rapid progress in the development of plant 'proteomics' will help to get more insight into cell-to-cell and long distance movement of own and foreign macromolecules.

### Acknowledgements

Work in our own laboratory was supported by a grant of the Deutsche Forschungsgemeinschaft (KE 690). Many thanks to Susanne von Bargen, Martina Paape and Birgit Piechulla for helpful discussions and critical reading of the manuscript.

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