Cell-to-cell movement of potato virus X involves distinct functions of the coat protein

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Complementation of movement-deficient potato virus X (PVX) coat protein (CP) mutants, namely PVX.CP-Xho lacking the 18 C-terminal amino acid residues and PVX.ΔCP lacking the entire CP gene, was studied by transient co-expression with heterologous proteins. These data demonstrated that the potyvirus CPs and both the major and minor CPs of beet yellows closterovirus could complement cell-to-cell movement of PVX.CP-Xho but not PVX.ΔCP. These data also indicated that the C-terminally truncated PVX CP lacked a movement function which could be provided in trans by the CPs of other filamentous viruses, whereas another movement determinant specified by some region outside the most C-terminal part of the PVX CP could not be complemented either by potyvirus or closterovirus CPs. Surprisingly, the CP of spherical cocksfoot mottle sobemovirus rescued all of the PVX CP movement functions, complementing the spread of PVX.CP-Xho and, to a lesser extent, PVX.ΔCP. Both these mutants were also rescued by the tobacco mosaic virus (TMV) movement protein (MP). To shed light on the movement function of PVX CP, attempts were made to complement PVX.CP-Xho by a series of TMV MP mutants. An internal deletion abolished complementation, suggesting that the internal region of TMV MP, which includes a number of overlapping functional domains important for cell-to-cell transport, provides an activity complementing movement determinant(s) specified by the C-terminal region of PVX CP.

Introduction

Plant virus transport systems recruit and modify normal plant intra- and intercellular pathways for movement of viral proteins and nucleic acids (see Leisner, 1999; Lazarowitz & Beachy, 1999; Lazarowitz, 1999; Lee et al., 2000; Jackson, 2000, for recent reviews). Different plant viruses have some common principles of cell-to-cell movement. However, the exact nature of their transport systems varies between virus taxa (Mushegian & Koonin, 1993; Carrington et al., 1996; Morozov & Solovyev, 1999; Lazarowitz, 1999; Leisner, 1999). Virus proteins involved exclusively in cell-to-cell transport, which are dispensable for genome replication, encapsidation, vector transmission and other functions, are termed movement proteins (MPs). In some cases, viral proteins of at least two other types can be involved in virus movement, namely the coat protein (CP) and the replication-associated proteins (Carrington et al., 1996, 1998; Weiland & Edwards, 1996; Deom et al., 1997; Leisner, 1999). Many plant viruses, for example, representatives of the families Bromoviridae, Comoviridae, Caulimoviridae and Bunyaviridae and genera Tobamovirus, Dianthovirus and Tombusvirus, have evolved single MPs. In spite of the significant sequence divergence between these MPs and the different mechanisms of plasmodesmata (PD) modification, they have common sequence motif(s) also found in the well-studied MP of tobacco mosaic virus (TMV) (Mushegian & Koonin, 1993; Leisner, 1999; Bertens et al., 2000; Melcher, 2000; Rhee et al., 2000). On the other hand, members of the family Closteroviridae and genera

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Potexvirus, Hordeivirus, Carnovirus, Necrovirus and Begomovirus code for two or more MPs (Leisner, 1999; Lazarowitz & Beachy, 1999; Morozov & Solovyev, 1999; Alzhanova et al., 2000).

The MPs of potex-, carla-, pomo-, plclu-, beny- and hordeiviruses are encoded by a module of three partially overlapping genes (triple gene block, TGB) (Morozov et al., 1989, 1999; Carrington et al., 1996; Herzog et al., 1998; Lough et al., 1998; Morozov & Solovyev, 1999; Leisner, 1999). The TGB-encoded MPs are referred to as TGBp1, TGBp2 and TGBp3, according to the position of the respective gene in the TGB. The TGBp1 proteins contain an NTPase/helicase domain with sequence motifs similar to those in the DNA helicases of superfamily 1 (Koonin & Dolja, 1993), an ATP/GTPase domain and have RNA-binding activities in vitro (Rouleau et al., 1994; Kalinina et al., 1996; Donald et al., 1995, 1997; Bleykasten et al., 1996; Lough et al., 1998; Morozov et al., 1999; Wung et al., 1999). Intact NTPase motifs are required for the functional competence of TGBp1 in vivo (Donald et al., 1995; Bleykasten et al., 1996; Angell et al., 1996; Erhardt et al., 2000). TGBp2 and TGBp3 are the membrane proteins that are co-targeted to the cell periphery and influence TGBp1 sorting to cell wall-associated punctate bodies (PD and areas surrounding the PD) (Erhardt et al., 1999, 2000; Solovyev et al., 2000; Yang et al., 2000).

Participation of virus-encoded NTPases has also been demonstrated for the transport systems of plant poty- and closteroviruses (Roberts et al., 1998; Carrington et al., 1998; Agranovsky et al., 1997, 1998; Peremyslov et al., 1999; Alzhanova et al., 2000). Potyvirus CI protein, which is also essential for genomic RNA replication (Lain et al., 1991; Carrington et al., 1998), represents an RNA helicase/NTPase of superfamily 2 (Koonin & Dolja, 1993), whereas the closterovirus HSP70-like proteins are related to a large family of cell chaperones involved in the energy-dependent processes of protein folding and transport (Agranovsky et al., 1997). Apart from having NTPase as a key MP, potex-, poty- and closteroviruses share the following features: their particles are flexuous filaments and their CPs are involved in the cell-to-cell movement of virus infection (Chapanan et al., 1992; Forster et al., 1992; Dolja et al., 1995; Rojas et al., 1997; Santa Cruz et al., 1998; Alzhanova et al., 2000; Robertson et al., 2000). It could be speculated that potyviral CI, closteroviral HSP70-like and TGBp1 proteins generate reversible energy-dependent conformational changes in the filamentous viruses or virion-like ribonucleoproteins (RNPs) that are required for the trafficking of viral genomes through the PD (Roberts et al., 1998; Santa Cruz et al., 1998; Lough et al., 1998; Carrington et al., 1998; Medina et al., 1999; Morozov et al., 1999; Peremyslov et al., 1999; Alzhanova et al., 2000). Direct evidence for the ability of the potato virus X (PVX) TGBp1 NTPase to induce conformational changes in homologous virions in vitro, converting them into a translation-competent form, has recently been reported (Atabekov et al., 2000). In closteroviruses, it has also been found that binding of HSP70-like proteins to virions is probably required for the infectivity of virus particles (Napuli et al., 2000; Satyanarayana et al., 2000).

Potexviral CP is believed to be responsible for the formation of movement-competent complexes with viral RNA, either virions or virion-like RNPs containing both the CP and TGBp1 proteins (Lough et al., 1998, 2000; Santa Cruz et al., 1998; Atabekov et al., 2000). These complexes are speculated to be targeted to PD by the TGBp2/TGBp3 proteins (Lough et al., 1998; Solovyev et al., 2000). Similarly, it is proposed that the potyviral CP has a direct role in the formation of CP-RNA transport complexes (perhaps by encapsidation), which are specifically guided to and through PD by conical deposits formed by the potyviral CI protein (Rodriguez-Cerezo et al., 1997; Carrington et al., 1998; Medina et al., 1999). Interestingly, similar structures containing filamentous virions are also found in closterovirus-infected cells (Pinto et al., 1988; Medina et al., 1999), providing another parallel to cell-to-cell movement of different viruses with long flexuous virions.

To test the possible functional compatibility between individual components of potex-, poty- and closterovirus transport systems, we have previously used transient complementation assays. In these experiments, plant leaves were co-inoculated by microprojectile bombardment with the cauliflower mosaic virus (CaMV) 35S promoter-driven cDNAs of movement-deficient PVX derivatives and vectors expressing genes of potex-, poty- and closterovirus movement-associated proteins (Morozov et al., 1997, 1999; Agranovsky et al., 1998; Fedorkin et al., 2000). It was shown that in transient complementation tests, cell-to-cell movement deficiency of the PVX CP C-terminally truncated frameshift mutant could be rescued by the co-expressed potyvirus CP genes. Additionally, the PVX CP mutant was able to move from cell to cell in transgenic plants expressing potyvirus CP; however, the PVX RNA was not encapsidated by the potyvirus CP (Fedorkin et al., 2000). These data suggest that the CPs of potex- and potyviruses share some movement-associated function(s) that are not related to genome encapsidation. This conclusion has been confirmed by recent data demonstrating that cell-to-cell movement and encapsidation functions of white clover mosaic potexvirus CP can be separated: the movement-deficient C-terminally truncated CP mutant is still able to form virions (Lough et al., 2000).

To further understand the role(s) of CPs in virus movement, we performed transient complementation experiments in which two cell-to-cell movement-defective PVX mutants (with either a frameshift mutation in the CP gene or a deletion of this gene) were tested for their capacity to be complemented by the major and minor CPs of beet yellows closterovirus (BYV), the CP of cocksfoot mottle sobemovirus (CMV) and the TMV MP and its non-functional derivatives. Our data demonstrate that the PVX CP has at least two movement-associated functions, one of which can be complemented by the CPs of
unrelated viruses and non-functional deletion mutants of the TMV MP.

Methods

Construction of recombinant clones. All recombinant DNA procedures were carried out by standard methods (Sambrook et al., 1989). E. coli, strains DH5α, XLI-Blue and SURE were used for cloning of recombinant constructs. pRT-PVX.Cp, pRT-PVACp, pRT-PVY.Cp, pPVX.GUS-Xho, pPVX.GFP.ΔCp and pPVX.GUS-Bsp recombinant constructs were described previously (Morozov et al., 1997; Fedorkin et al., 2000). To construct clones with BYV CP genes under the control of the CaMV 35S promoter, the BYV-specific insert was excised with NcoI and PvuII from pRT-60-24h(−), a pQE-based expression vector with the insert representing the BYV genome between nt 12919 and 13883, which was then cloned between the NcoI and NrdI sites of pRT-BYV.65K to replace the BYV 65 KDa (65K) gene (Agranovsky et al., 1998). The resulting plasmid, pRT-BYV.65K, contained the gene for the BYV p22 major CP (p24). This construct was then cloned between the NcoI and NrdI sites of pRT-BYV.65K to replace the BYV 65 KDa (65K) gene (Agranovsky et al., 1998). The resulting plasmid, pRT-BYV.65K, contained the gene for the BYV p24 minor CP under the control of the CaMV 35S promoter and the p24 translational enhancer of PVX (Smirnyagina et al., 1991). To obtain a similar construct for the BYV p22 major CP gene (pRT-BYV.22K), the PCR product obtained with primers oligo 22 and oligo 1 (Agranovsky et al., 1994) was digested with BglII and XhoI and cloned into the BglII/XhoI-digested pRT-BYV.24K to replace the p24 insert. The CaMV CP gene cDNA was amplified by PCR using a full-length CfMV clone (Russian isolate; Ryabov et al., 1996; kindly made available by S. Zavriev, Institute of Agricultural Biotechnology, Moscow, Russia). The primers used for PCR were: CfMV-5′-CP-forward, 5′ CCAGGATCCGAATGTAGGG-TAGAGAAGGACGACAAACAA, containing an underlined BamHI site and corresponding to nt 3089–3120 of the CfMV genome (base numbering refers to Mäkinen et al., 1995) and CfMV-3′-CP-reversed, 5′ GGCTCTAGAGGTGTGTACCTCCCTGACTCTCGACTAC, containing an underlined XhoI site and complementary to nt 3853–3881 of the CfMV (Norwegian isolate; Mäkinen et al., 1995). The resulting PCR product was digested with BamHI and XhoI and cloned into a similarly digested pRT-100 (Toepfer et al., 1987) downstream of the 35S promoter. Referring to the amino acid numbering of the TMV vuglare strain MP (Goel et al., 1982), the mutants had the following deletions in the TMV 30K MP-coding sequence: NT96, aa 1–106; CT11, aa 258–268; CT33, aa 236–268; CT86, aa 198–268; and DELA, aa 130–186. To obtain pRT-TMV.388, pRT-TMV.CT11 was digested with Bsp120I, blunt-ended by Klenow fragment and religated. To construct pRT-TMV.27K, pRT-TMV.30K was digested with EcoRI and BglII, blunt-ended by Klenow fragment and religated. In pRT-TMV.CT86, 15 non-viral C-terminal extra amino acids (AEGPWNLQKKSLCSS) were added and in variant pRT-TMV.388, 10 non-viral C-terminal extra amino acids (PAHGTYRRSR) were added. In pRT-TMV.147, the 30K MP C-terminal amino acids DSEATVAEEDSF were replaced by the non-viral amino acids EAMSPKTIRFNCSSQA. To obtain pPVX.GFP-Xho, pPVX.GFP (Fedorkin et al., 2000) was partially digested with XhoI, filled in by Klenow enzyme and religated. To construct pRT-dsRed, the Apol–XhoI fragment of pDsRed1-1 (Clontech), containing the gene of the red fluorescent protein, was cloned into similarly digested pRT-100 (Toepfer et al., 1987).

Particle bombardment and virus movement detection. Particle bombardment of detached Nicotiana benthamiana leaves was performed using the flying-disk method with a high-pressure helium-based PDS-1000 apparatus (Bio-Rad) as described by Morozov et al. (1997). Replication and movement of PVX.GUS was monitored by histochemical detection of glucuronidase (GUS) expression (Jefferson, 1987). Samples were infiltrated with the colorimetric GUS substrate modified to limit the diffusion of the intermediate products of the reaction (De Block & Debrouwer, 1992). After overnight incubation at 37 °C, the leaves were fixed in 70% ethanol and examined by light microscopy. Green fluorescence protein (GFP) was detected using a Zeiss Axioscope 20 fluorescence microscope (excitation filter BP 450–490, chromatic beam splitter FT 510 and either long pass emission filter LP 520 or band pass filter HQ 535/50x).

Fig. 1. PVX wt clone and movement-deficient mutants: PVX.GUS-Bsp, PVX.GFP.ΔCp, PVX.GFP-Xho, PVX.GUS-Xho. Open boxes indicate genes. Areas of the coding regions disabled by frameshift mutations are indicated as dashed boxes. Molecular masses of the encoded proteins are indicated in kDa. 165K, RNA polymerase; 25K, 12K, 8K, triple gene block movement proteins; CP, coat protein; fs-25K, fs-CP, frameshift mutations in TGBp1 and CP, respectively.
Fig. 2. Transient complementation of cell-to-cell movement of the transport-deficient PVX genomes by different CP and MP constructs by particle bombardment of *N. benthamiana* leaves. Histochemical detection of GUS activity performed 3 days p.b. of the leaf with pPVX.GUS-Xho (a), co-bombardment of pPVX.GUS-Xho with pRT-BYV.22K (b), pPVX.GUS-Xho with pRT-BYV.24K (c), and pPVX.GUS-Xho with pRT-CIMV.CP (d). Fluorescence of GFP (e, bar 50 µm) and dsRed (f) detected 3 days after co-bombardment of pPVX.GFP-Xho, pRT-CIMV.CP and dsRed. (g) Superimposition of the images in (e) and (f). Fluorescence of GFP (h, bar 50 µm) and dsRed (i) detected 3 days after co-bombardment of pPVX.GFP.ΔCP, pRT-CIMV.CP and dsRed. (j) Superimposition of the images in (h) and (i). Scale bar in (a) represents 6 mm.
Results and Discussion

Complementation of cell-to-cell transport of movement-deficient PVX CP mutants by closterovirus or sobemovirus CPs

We have recently reported that cell-to-cell movement of PVX.GUS-Xho, a PVX CP frameshift mutant lacking 18 C-terminal amino acid residues (Fig. 1), can be trans-complemented by potyviral CPs (Fedorkin et al., 2000). In the present study, the CPs of the filamentous closterovirus BYV and the spherical sobemovirus CfMV were tested in the co-bombardment assay for their ability to rescue the movement deficiency of the PVX CP mutant. In controls, histochemical detection of GUS activity in N. benthamiana leaves revealed that at 3 days post-infection (p.i.), PVX.GUS-Xho was confined to individual cells or to small groups of cells (Fig. 2a and Table 1; cf. Chapman et al., 1992, Fedorkin et al., 2000). When N. benthamiana leaves were co-bombarded with pPVX.GUS-Xho and the 35S promoter plasmids pRT-BYV.22K, pRT-BYV.24K or pRT-CfMV.CP, which encode the major and minor CPs of BYV and the CP gene of CfMV, respectively, GUS staining revealed infection foci several-fold larger than those in the negative control (Fig. 2b–d and Table 1). Similar levels of rescued virus movement have been observed for co-bombardment of pPVX.GUS.Xho with the homologous PVX CP gene (Table 1; Fedorkin et al., 2000). Therefore, the CPs of a clostero- and a sobemovirus, similar to the potyvirus CP (Fedorkin et al., 2000), were able to complement cell-to-cell movement of the PVX CP frameshift mutant, indicating that these proteins could provide a movement function that was lost by truncated PVX CP.

We have previously shown that the frameshift mutant PVX.GUS-Xho behaved differently from the PVX.GFP.CP mutant in which the CP gene was removed completely (Fedorkin et al., 2000). To use the same reporter gene for both PVX CP mutants and to analyse virus movement at the cell level, we constructed a GFP-tagged PVX mutant encoding the same frameshift mutation as PVX.GUS-Xho (Fig. 1). As expected, PVX.GFP.Xho showed a lack of cell-to-cell movement in bombarded N. benthamiana leaves and pRT-PVX.CP complemented cell-to-cell movement of PVX.GFP.CP and PVX.GFP.Xho with similar efficiency (data not shown). As with the GUS-tagged PVX mutant PVX.GUS.Xho, pRT-BYV.22K, pRT-BYV.24K, pRT-CfMV.CP, pRT-PVY.CP and pRT-PVY.CP were able to rescue the movement defect of PVX.GFP.Xho and were therefore similar to pRT-PVX.CP (Fig. 2e and Table 2). The growth rate of the infection foci was similar in these experiments. For example, GFP fluorescence was detected for the major 22K BYV CP in 4–57 ± 0.25 epidermal cells at 2 days post-bombardment (p.b.), 9 ± 3 ± 1.37 cells at 3 days p.b. and 18 ± 10 ± 1.34 cells at 4 days p.b.; for CfMV CP, fluorescence was in 4 ± 20 ± 0.60 epidermal cells at 2 days p.b., 10 ± 65 ± 1.47 cells at 3 days p.b. and 18 ± 89 ± 0.94 cells at 4 days p.b. (Table 2 and data not shown). At later stages (6–8 days p.b.), infection foci often coalesced and the underlying palisade mesophyll cells gave a strong fluorescent signal making a precise estimation of their size difficult (data not shown).

In contrast, cell-to-cell movement of pPVX.GFP.CP could not be complemented by either pRT-BYV.22K or pRT-

Table 1. Complementation of GUS-tagged movement-deficient PVX derivatives in co-bombardment assays

The mean diameters (μm) of infected foci detected 3 days p.b. by GUS staining are indicated.

<table>
<thead>
<tr>
<th>Complementing plasmid</th>
<th>pPVX.GUS-Xho</th>
<th>pPVX.GUS-Bsp</th>
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<tbody>
<tr>
<td>pPVX.GUS-Xho</td>
<td>57 ± 11.9</td>
<td>420 ± 25.5</td>
</tr>
<tr>
<td>pPVX.GUS-Bsp</td>
<td>420 ± 25.5</td>
<td>60 ± 12.6</td>
</tr>
<tr>
<td>pRT-PVX.CP</td>
<td>255 ± 23.1</td>
<td>ND</td>
</tr>
<tr>
<td>pRT-BYV.22K</td>
<td>240 ± 15.7</td>
<td>ND</td>
</tr>
<tr>
<td>pRT-BYV.24K</td>
<td>279 ± 25.5</td>
<td>ND</td>
</tr>
<tr>
<td>pRT-CfMV.CP</td>
<td>232 ± 26.4</td>
<td>ND</td>
</tr>
<tr>
<td>pRT-TMV.30K</td>
<td>265 ± 17.3</td>
<td>375 ± 22.3</td>
</tr>
<tr>
<td>pRT-TMV.27K</td>
<td>245 ± 24.7</td>
<td>59 ± 12.7</td>
</tr>
<tr>
<td>pRT-TMV.NT96</td>
<td>270 ± 19.9</td>
<td>61 ± 10.1</td>
</tr>
<tr>
<td>pRT-TMV.147</td>
<td>390 ± 22.9</td>
<td>340 ± 12.2</td>
</tr>
<tr>
<td>pRT-TMV.CT11</td>
<td>379 ± 18.4</td>
<td>328 ± 11.3</td>
</tr>
<tr>
<td>pRT-TMV.CT33</td>
<td>159 ± 15.4</td>
<td>62 ± 10.5</td>
</tr>
<tr>
<td>pRT-TMV.CT86</td>
<td>60 ± 10.9</td>
<td>58 ± 10.5</td>
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<tr>
<td>pRT-TMV.388</td>
<td>62 ± 12.2</td>
<td>58 ± 13.3</td>
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<tr>
<td>pRT-TMV.DEL4</td>
<td>59 ± 13.5</td>
<td>60 ± 11.7</td>
</tr>
</tbody>
</table>

ND, Not determined.

Table 2. Complementation of GFP-tagged movement-deficient PVX CP mutants in co-bombardment assays

Average number of epidermal cells in infection foci detected 3 days p.b. by GFP fluorescence is indicated.

<table>
<thead>
<tr>
<th>Complementing plasmid</th>
<th>Movement-deficient PVX CP mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pPVX.GFP.Xho</td>
</tr>
<tr>
<td>pRT-TMV.30K</td>
<td>10 ± 63 ± 1.06</td>
</tr>
<tr>
<td>pRT-PVX.CP</td>
<td>12 ± 50 ± 1.35</td>
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<tr>
<td>pRT-BYV.22K</td>
<td>10 ± 21 ± 1.02</td>
</tr>
<tr>
<td>pRT-BYV.24K</td>
<td>9 ± 52 ± 1.37</td>
</tr>
<tr>
<td>pRT-CfMV.CP</td>
<td>10 ± 65 ± 1.47</td>
</tr>
<tr>
<td>pRT-PVY.CP</td>
<td>10 ± 22 ± 1.54</td>
</tr>
<tr>
<td>pRT-PVY.CP</td>
<td>10 ± 97 ± 1.82</td>
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</table>

ND, No movement.
BYV.24K (Table 2). Similarly, pPVX.GFP.ΔCP was not complemented by pRT-PVX.CP (Fedorkin et al., 2000 and Table 2). However, pRT-CfMV.CP was able to complement movement of PVX.GFP.ΔCP. In this case, GFP fluorescence was detected in small groups of six to seven cells (Fig. 2h and Table 2) and were therefore smaller than those in complementation of pPVX.GFP-Xho by pRT-CfMV.CP (Fig. 2e and Table 2). In contrast to complementation by the homologous CP, the size of the infection foci in the case of CfMV CP did not increase after 3 days p.i. (data not shown). To provide evidence that we were observing limited virus movement and not diffusion of GFP from the initially infected cells, the plasmid pRT-dsRed was added to the mixture of plasmids pRT-CfMV.CP and pPVX.GFP-Xho or pPVX.GFP.ΔCP precipitated onto the metal particles for co-bombardment. pRT-dsRed carries the gene for the red fluorescent protein from Discosoma species (Clontech) cloned under the control of the 35S promoter. This approach demonstrates that the limited virus spread observed in the case of complementation of PVX.GFP-Xho or PVX.GFP.ΔCP by pRT-CfMV.CP occurs in the absence of dsRed diffusion (Fig. 2e–j). Co-bombardment with pRT-dsRed was routinely used in other complementation experiments to label the initially infected cell and to confirm that virus cell-to-cell movement rather than GFP leakage had occurred.

The CPs of potex-, poty- and clostero-viruses are distantly related (Koonin & Dolja, 1993) and the major and minor clostero-virus CPs, like those of potex- and poty-viruses, are necessary for virus cell-to-cell movement (Alzhanova et al., 2000). These data, and results from previous work (Fedorkin et al., 2000), thus indicate that viral CPs in three taxonomic groups of filamentous viruses share some activities that are necessary for cell-to-cell movement. Importantly, both the clostero- and potyvirus CPs are able to complement PVX.Xho but not PVX.ΔCP (Tables 1 and 2). Therefore, these heterologous CPs require the C-terminally truncated PVX CP to complement cell-to-cell movement. Presumably, the truncated PVX CP lacks a movement function that may be provided non-specifically by the CPs of other filamentous viruses in complementation experiments, whereas another movement determinant is provided by the N-terminal PVX CP region retained in the C-terminally truncated mutant CP-Xho. Interestingly, small C-terminal deletions in both the major and minor clostero-virus CPs resulted in a movement-deficient phenotype, suggesting C-terminal localization of some movement determinants similar to potex- and potyvirus CPs (Alzhanova & Dolja, 2000).

In spherical plant viruses, the requirement of CPs for cell-to-cell movement in plants has been reported (Kaplan et al., 1998; Lin & Heaton, 1999; Tenllado & Bol, 2000). In particular, the CP is one of three proteins required for transport of sobemovirus infections (Sivakumar et al., 1998). As the CP of sobemovirus CfMV complemented not only pPVX.GUS(GFP)-Xho but also pPVX.GFP.ΔCP (Table 2), although to a lesser extent, one could conclude that the CP of spherical sobemo-virus differs from the CPs of filamentous poty- and clostero-viruses in its ability to complement the whole range of movement functions provided by the PVX CP. Our evidence that the CfMV CP can replace virus-specific movement determinant(s) contained in the region outside the most C-terminal part of the PVX CP indicates that the mechanism of movement complementation could be different in experiments with the CPs of filamentous viruses and CfMV.

Complementation of cell-to-cell transport of PVX CP mutants by TMV MP and its derivatives

To shed light on the movement function associated with the C-terminal region of the PVX CP, an attempt was made to complement cell-to-cell movement of PVX.CP-Xho by a series of mutants of TMV 30K MP, where a number of functional domains have already been identified and mapped (Citovsky et al., 1992, 1993; Gafny et al., 1992; Mushegian & Koonin, 1993; Waigmann et al., 1994; Berna, 1995; Haley et al., 1995; Hughes et al., 1995; Kahn et al., 1998; Brill et al., 2000; Chen et al., 2000).

It has been previously shown that the MP of tomato mosaic tobamovirus (ToMV) was able to transiently complement a movement-deficient PVX derivative with mutated TGBp1 and a double PVX mutant with both a truncated TGBp1 and the frameshift mutation CP-Xho (Morozov et al., 1997; Atabekov et al., 1999). By using different TMV 30K mutants in complementation analysis, we hoped that the disabled movement function of the truncated PVX CP might be restored by the 30K deletion mutants, although non-functional as MPs, retaining definite functional domain(s) that complement the domain(s) missing in the truncated PVX CP.

In preliminary control experiments, the expression vector pRT-TMV.30K, carrying the TMV MP gene under control of the 35S promoter, was co-bombarded with pPVX.GUS-Xho and pPVX.GUS-Bsp. As expected, the MP of TMV, similar to that of tobamovirus ToMV (Morozov et al., 1997), complemented movement deficiency of both PVX derivatives with truncated TGBp1 and CP genes (Table 1). When pRT-TMV.30K was co-bombarded with pPVX.GFP.Xho, GFP fluorescence was detected in 5±46±0·51 epidermal cells at 2 days p.b., 10·63±1·06 cells at 3 days p.b. and 40±16±2·41 cells at 4 days p.b. (Table 2 and data not shown), demonstrating faster cell-to-cell movement than in complementations with CPs and probably reflecting highly efficient movement of the TMV MP between cells. These data agreed with our previous conclusion that tobamovirus MPs could functionally replace the transport system of PVX (Atabekov et al., 1999). Similarly, the MPs of groundnut rosette umbravirus were able to potentiate cell-to-cell movement of PVX with deletions of both TGB genes and CP (Ryabov et al., 1998).

In further experiments, expression vectors carrying different deletion mutants of the TMV 30K gene were individually co-bombarded with pPVX.GUS-Xho. GUS stain-
Complementation of PVX CP movement function

Complementation of the movement-deficient PVX derivatives by TMV 30K and its mutants. (a) Functional domains mapped in the TMV 30K MP. SEL, Region responsible for increasing PD size exclusion limit (Waigmann et al., 1994); CW, region of targeting to cell wall (Berna, 1995); RBD A and B, RNA-binding domains (Citovsky et al., 1992); F, region controlling protein folding (Citovsky et al., 1992); T, region of targeting to PD and/or the cytoskeleton (Kahn et al., 1998); PME, region of binding to pectin methylesterase (Chen et al., 2000). (b) Cell-to-cell movement complementation of PVX.GUS-Xho mutant and PVX.GUS-Bsp in co-bombardment assays with the plasmids indicated on the right. Deletions in the 30K MP are shown. Grey shading in the construct pRT-TMV.147 indicates non-viral residues replacing the MP sequence. The internal deletion in pRT-TMV.DEL4 is indicated by the line.

Fig. 3. Complementation of the movement-deficient PVX derivatives by TMV 30K and its mutants. (a) Functional domains mapped in the TMV 30K MP. SEL, Region responsible for increasing PD size exclusion limit (Waigmann et al., 1994); CW, region of targeting to cell wall (Berna, 1995); RBD A and B, RNA-binding domains (Citovsky et al., 1992); F, region controlling protein folding (Citovsky et al., 1992); T, region of targeting to PD and/or the cytoskeleton (Kahn et al., 1998); PME, region of binding to pectin methylesterase (Chen et al., 2000). (b) Cell-to-cell movement complementation of PVX.GUS-Xho mutant and PVX.GUS-Bsp in co-bombardment assays with the plasmids indicated on the right. Deletions in the 30K MP are shown. Grey shading in the construct pRT-TMV.147 indicates non-viral residues replacing the MP sequence. The internal deletion in pRT-TMV.DEL4 is indicated by the line.

Step-wise truncation of the TMV 30K MP from the C terminus by 33, 71 and 73 amino acids (mutants TMV.CT33, TMV.CT86 and TMV.388, respectively) resulted in partial inhibition (TMV.CT33) and then in complete loss of complementation (TMV.CT86 and TMV.388) (Table 1) as the deletions entered the internal region containing the domains responsible for cell wall targeting, increase in size exclusion limit (SEL) (Kahn et al., 1998; Citovsky, 1999; Leisner, 1999; Rhee et al., 2000) and probably endoplasmic reticulum membrane binding (Brill et al., 2000). In contrast, deletion of 11 residues from the C terminus of TMV MP (mutant TMV.CT11) gave rise to an enhancement of complementation as compared to wt MP (Fig. 3 and Table 1). To test whether this effect was due to the deletion of specific C-terminal MP residues, we used an additional frameshift mutant, TMV.147, where the last 12 amino acids of TMV MP were replaced by a 15-residue-long sequence encoded by another reading frame (Fig. 3). Like TMV.CT11, TMV.147 showed enhanced level of pPVX.GUS-Xho complementation (Table 1). This effect could be explained by recent data suggesting that specific phosphorylation of Ser and Thr residues located in the last 10 amino acids of the TMV MP has a role in temporal regulation of its movement activity (Citovsky, 1999; Rhee et al., 2000; Waigmann et al., 2000). Importantly, the mutants TMV.147 and TMV.CT11, like wt TMV MP, were both able to complement movement deficiency of not only PVX.GUS-Xho but also PVX.GUS-Bsp with a truncated TGBp1 gene (Fig. 3 and Table 1). These data indicate that these mutants were functionally similar to the wt TMV MP in their ability to replace the transport system of PVX, as was suggested by Atabekov et al. (1999).

N-terminal deletions of 26 and 108 residues (mutants TMV.27K and TMV.NT96, respectively) (Fig. 3) had little effect on pPVX.GUS-Xho complementation (Table 1). Importantly, these TMV MP mutants were unable to complement cell-to-cell movement of the TGBp1-deficient PVX derivative PVX.GUS-Bsp (Fig. 3 and Table 1) and pPVX.GFP.ΔCP (data not shown). Although TMV.27K and TMV.NT96 mutants do not function like the wt TMV MPs, they are still able to provide certain functional domain(s) required to trans-complement deficient movement function of the C-terminally truncated PVX CP. Both these mutants lack several important MP functional regions, but retain the main nucleic acid-binding domains and the regions responsible for cell wall targeting and SEL increase (Fig. 3) (Kahn et al., 1998; Citovsky, 1999; Leisner, 1999).

The mutant with an internal deletion (TMV.DEL4) was unable to complement the PVX CP frameshift mutant (Table 1). TMV.DEL4 corresponds to the previously described mutant ‘del-4’, which had defects in PD SEL augmentation, binding to the cell wall receptor pectin methylesterase (PME) and a partial defect in RNA binding (Waigmann et al., 1994; Karpova et al., 1997; Dorokhov et al., 1999; Chen et al., 2000). Thus, the fact that the internal deletion affects the TMV MP's ability to
complement the C-terminally truncated PVX CP suggests that this region of the TMV 30K, which includes a number of overlapping functional domains, is responsible for complementation of the movement function specified by the truncated region of PVX CP.

The following model can be proposed to explain complementation of the PVX CP mutants by the unrelated single MPs. The complementing functional MP can form movement-competent RNPs with PVX genomic RNA that move intra-cellularly to the cell wall regions enriched in PD (Chen et al., 2000) and can be then transported through the PD, which might be pre-modified by TGB proteins or complementing foreign MPs (specific PD receptors bound and the PD dilated) (Lough et al., 1998; Kragler et al., 2000). This process needs no specific interactions with TGB proteins. The partially functional TMV MP mutants, like the wt 30K protein, might form RNPs, which include PVX RNA, and traffic them to dilated PD, in this case, by TGB proteins. The inability of TMV.DEL4 to complement PVX.GUS-Xho (Table 1) may represent an additional piece of evidence for a role of partially functional TMV MP derivatives in complementation of the truncated PVX CP. It is known that TMV.DEL4 MP has a lower efficiency of RNP formation and is incapable of binding the cell wall receptor PME (Chen et al., 2000). Also taking into account the fact that certain viral non-MPs (most probably incapable of PME-binding) with high RNA-binding ability cannot complement PVX.GUS-Xho movement (Fedorkin et al., 2000), it is tempting to speculate that cell wall targeting is at least one of the functions absent from the C-terminally truncated PVX CP.

However, another question remains to be solved: what is the functional activity, which is still retained in the truncated CP, required for supporting PVX.GUS(GFP)-Xho movement in complementation experiments with poty- and closterovirus CPs or TMV MP mutants?

Note added in proof. A potential clue to the understanding of PVX CP movement-related functions was found in two recent articles on the TMV cellularly to the cell wall regions enriched in PD (Chen et al., 2000) and can be then transported through the PD, which might be pre-modified by TGB proteins or complementing foreign MPs (specific PD receptors bound and the PD dilated) (Lough et al., 1998; Kragler et al., 2000). This process needs no specific interactions with TGB proteins. The partially functional TMV MP mutants, like the wt 30K protein, might form RNPs, which include PVX RNA, and traffic them to dilated PD, in this case, by TGB proteins. The inability of TMV.DEL4 to complement PVX.GUS-Xho (Table 1) may represent an additional piece of evidence for a role of partially functional TMV MP derivatives in complementation of the truncated PVX CP. It is known that TMV.DEL4 MP has a lower efficiency of RNP formation and is incapable of binding the cell wall receptor PME (Chen et al., 2000). Also taking into account the fact that certain viral non-MPs (most probably incapable of PME-binding) with high RNA-binding ability cannot complement PVX.GUS-Xho movement (Fedorkin et al., 2000), it is tempting to speculate that cell wall targeting is at least one of the functions absent from the C-terminally truncated PVX CP.

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References


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