Reciprocal function of movement proteins and complementation of long-distance movement of Cymbidium mosaic virus RNA by Odontoglossum ringspot virus coat protein

Prabha Ajjikuttira,1 Chiang-Shiong Loh1 and Sek-Man Wong1,2

1Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore
2Temasek Life Sciences Laboratory, 1 Research Link, Singapore 117604, Singapore

Correspondence
Sek-Man Wong
dbswsm@nus.edu.sg

INTRODUCTION

Of several orchid-infecting viruses worldwide, the potexvirus Cymbidium mosaic virus (CymMV) and the tobamovirus Odontoglossum ringspot virus (ORSV) are the most prevalent. The occurrence of these two orchid viruses results in significant economic losses to the orchid industry, caused by stunted growth and reduction in flower size and quality. Additionally, CymMV and ORSV are estimated to have coinfected about 14% of cultivated orchids worldwide (Wong et al., 1994). However, CymMV and ORSV belong to different taxonomic groups of plant viruses and have obvious differences in genome organization and sequence arrangement. For viruses to infect plants successfully, they must move from the initial sites of infection into surrounding cells and over long distances by using the pre-existing pathways within the plant. Virus-encoded proteins facilitate the movement of viruses through plants.

Movement proteins (MPs) facilitate the cell-to-cell movement and coat proteins (CPs), in addition to encapsidating viral RNA, have been shown to facilitate systemic movement of some viruses (Carrington et al., 1996). Movement of CymMV is aided by proteins expressed from three overlapping open reading frames (ORFs), designated the triple-gene block (TGB). Proteins encoded by the TGB are TGB1 (26 kDa), TGB2 (13 kDa) and TGB3 (10 kDa) (Wong et al., 1997). The ORSV MP is expressed from a single ORF (Ryu & Park, 1995). The CPs of CymMV and ORSV are each expressed from their respective single ORFs. The MPs of potexviruses and tobamoviruses facilitate cell-to-cell movement, whilst the CPs help in long-distance movement of virions (Deom et al., 1987; Meshi et al., 1987; Beck et al., 1991; Angell et al., 1996). In addition, potexviruses have been shown to require CP for cell-to-cell movement (Chapman et al., 1992).

Despite differences in sequences and genome organization, taxonomically distinct viruses are known to display complementarity in the transport function (Fuentes & Hamilton, 1991; Ziegler-Graff et al., 1991; Richins et al.,...
1993; Taliansky et al., 1993; Giesman-Cookmeyer et al., 1995; Cooper et al., 1996; Solovyev et al., 1996; Morozov et al., 1997; Lauber et al., 1998; Ryabov et al., 1999a; Tamai et al., 2003). The CPs of potex- and potyviruses also display complementarity in the movement process (Fedorkin et al., 2000). The best-studied examples of complementation are those involving the TMV MP in relation to MPs of other viruses. The TMV MP is known to support cell-to-cell movement of Cucumber mosaic virus (CMV) (Cooper et al., 1996), Barley stripe mosaic virus (BSMV) (Solovyev et al., 1996), Potato virus X (PVX) (Morozov et al., 1997) and Bean necrotic yellow vein virus (BYNYV) (Lauber et al., 1998). The hordeivirus BSMV, potexvirus PVX and benuvirus BYNYV (Petti et al., 1990; Beck et al., 1991; Pringle, 1997) contain the TGB (Morozov et al., 1989), which is required for virus movement, whereas viruses like CMV and TMV contain a single MP gene. The TMV MP supports cell-to-cell and long-distance movement of movement-deficient CMV in Nicotiana benthamiana (Rao et al., 1998), but was only able to support cell-to-cell movement of movement-deficient CMV in Nicotiana tabacum cv. Xanthi (Cooper et al., 1996). TGBp1 is believed to be the MP of potexviruses (Lough et al., 1998, 2000) and TMV MP has been shown to complement the cell-to-cell movement of a TGB1-deficient PVX (Fedorkin et al., 2001). In this work, we investigated the complementation of cell-to-cell and long-distance movement functions between CymMV and ORSV provided by the viral MPs and CPs.

**METHODS**

**PCR and cloning of MPs and CPs of CymMV and ORSV.** The templates used for amplifying the MP and CP genes of CymMV and ORSV were pCT10 (Yu & Wong, 1998b) and pOT2 (Yu & Wong, 1998a), respectively. The CymMV MP genes are encoded by the TGB between nt 4333 and 5478. The 5’ primer (CymMV-5’-TGB1-f) was 5’-GGTCTAGAATGGACAGCTAGCTTAGT-3’, which represents CymMV nt 4333–4361 (underlined) and contains a unique 5’-flanking XbaI site (shown in italics). The 3’ primer (CymMV-3’-TGB3-r) was 5’-GGGATCCCTATTTTTAAAATTTTTG-3’, which represents CymMV nt 5460–5478 (underlined) and contains a unique 5’-flanking BamHI site (shown in italics). The CymMV TGB gene fragment used to generate the TGB transgenic plants contained ORFs TGB1, TGB2 and TGB3, in order to have the same overlap-

**Electroporation of plasmids into Agrobacterium tumefaciens LBA4404.** The recombinant plasmids were introduced into A. tumefaciens by electroporation using a BTX Electro Cell Manipulator 600. To prepare electropo-competent cells, previously prepared glycerol stocks were streaked onto MG/L plates (Chilton et al., 1974) and the cells were allowed to grow at 28°C for 24 h. Cells were scraped off the plate, vortexed in 15% sterile glycerol for 10 min, centrifuged at 14 000 g for 2 min and snap-cooled in liquid nitrogen. Recombinant plasmid (1 µl) was added to 40 µl thawed electropo-competent cells that were aliquoted into a pre-chilled 0-2 cm gap cuvette P/N 620 (BTX) and the mixture was incubated on ice for 10 min. Electroporation was carried out at 2-5 kV, 129 Ω, 1-44 kV. The electro- porated cells were plated onto LB agar plates supplemented with 125 µg streptomycin ml⁻¹ and 50 µg kanamycin ml⁻¹ and incubated at 28°C for 48 h for selection of transformant colonies.

**Transformation of N. benthamiana and regeneration of transgenic plants.** A cell suspension of A. tumefaciens was prepared by inoculating the colonies harbouring the recombinant plasmids into 2 ml LB medium containing 125 µg streptomycin 1⁻¹ and 50 mg kanamycin 1⁻¹. The culture was incubated for 16 h at 28°C on a rotary shaker maintained at 240 r.p.m. The OD₆₆₀₅₅ of the suspension was read and the concentration of the cells was adjusted to 1 × 10⁶ cells ml⁻¹ with LB medium. Sterile leaf discs from in vitro-cultured N. benthamiana were co-cultured with the A. tumefaciens suspension. The leaf discs were cultured on MS solid medium

![Fig. 1. Schematic representation of the genes of interest cloned in the vector pBI121. The genes of interest were: CymMV TGB123 (a), ORSV MP (b), CymMV CP (c) and ORSV CP (d).](image-url)
(Murashige & Skoog, 1962) and 4–6 cm tall shoots that regenerated in 4–6 weeks were harvested. Four putative transgenic lines were obtained.

**Molecular analysis of transgenic plants.** Genomic DNA (0.5 µg) extracted from putative transgenic plants (F0) was subjected to PCR to detect the presence of the respective MP and CP transgenes, using gene-specific primers (Table 1). Putative transformants were sub-cultured on LB medium every 2 weeks. Shoots were rooted *in vitro* on basal MS solid medium and subsequently transferred to a greenhouse for hardening. For the CymMV TGB1 and CymMV CP transgenic lines, 7 out of 24 and 26 out of 31 individually transformed lines, respectively, that showed a band of the expected size by PCR screening were selected for further experiments. For the ORSV MP and ORSV CP transgenic lines, 10 out of 20 and 7 out of 23 individually transformed lines, respectively, that showed a band of the expected size by PCR were used in further experiments. The presence of transgene from the putative CP transgenic plants was confirmed by Southern blot analysis. Seeds from one line carrying two copies of the transgene for ORSV CP and one carrying a single copy for CymMV CP were sown to generate the F1 generation. PCR was carried out to detect the F1 transgenes in the seedlings prior to use in the complementation tests. For the MP transgenic plants, F1 seedlings were similarly obtained and PCR-tested before use. Transgene expression was confirmed by Northern blot analysis. Data for screening and confirmation of transgenic lines are presented in the Supplementary Figure (available in JGV Online).

**Generation of digoxigenin (DIG)-labelled cDNA and cRNA probes for Southern and Northern blot analyses.** Minus-strand RNA probes for the MPs and CPs of CymMV and ORSV were prepared. The CymMV TGB and ORSV MP probes were complementary to nt 4333–5478 and 4807–5718, respectively. Minus-strand RNA probes for the CymMV and ORSV CPs were complementary to nt 5481–6152 and 5721–6197, respectively. A DIG RNA labelling kit (Roche Diagnostics) was used to generate the probes.

### Table 1. Primers used in the study

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<td>ORSV-3′-MP-r</td>
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<td><strong>Primer sets used to construct the point mutations</strong></td>
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RESULTS

Replication and infectivity of mutants in protoplasts and plants

Four mutants were constructed, each carrying a modified start codon of the MP or CP genes of CymMV or ORSV. The start-codon mutations introduced were ATG to ATC in the full-length cDNA clones of p18Cy13 of CymMV and pOT2 of ORSV.

Northern blot analysis of total RNAs isolated from protoplasts showed that both genomic and subgenomic RNA of the CymMV and ORSV mutants were replicated (Fig. 2a, b). Northern blot analysis showed that neither genomic nor subgenomic RNAs of the CymMV and ORSV MP and CP mutants were detected in inoculated and systemic leaves of N. benthamiana plants [21 days post-infection (p.i.)] that were inoculated with *in vitro* transcripts of mutants, but were detected readily in positive-control plants inoculated with *in vitro* transcripts of p18Cy13 and pOT2 (Fig. 2c, d). These results suggest that p18Cy13inaTGB1 and pOT2inaMP were unable to move from cell to cell and long distance. Northern blots verified that p18Cy13inaCP and pOT2inaCP were unable to move systemically.

Complementation of the movement function of p18Cy13inaTGB1 by transgenic plants expressing ORSV MP

RT-PCR using RNA extracted from leaves of non-transgenic plants inoculated with infectious transcripts of p18Cy13 produced an expected-size band of 757 bp (Fig. 3a, lane C). In non-transgenic plants inoculated with *in vitro* transcripts of p18Cy13inaTGB1, no band was amplified from either inoculated or systemic leaves (Fig. 3a, lanes under NTG). A distinct band of approximately 757 bp was amplified from the inoculated leaves, but not from systemic leaves, of transgenic plants expressing the ORSV MP gene (Fig. 3a, lanes under TG). To confirm the RT-PCR results, the RNA was probed with *in vitro* transcripts of pCymMV CP. Both genomic and subgenomic CymMV RNAs were detected from inoculated, but not systemic, leaves of ORSV MP transgenic plants (Fig. 3b, lanes under TG). Non-transgenic plants inoculated with *in vitro* transcripts of p18Cy13inaTGB1 did not yield any signals from either inoculated or systemic leaves (Fig. 3b, lanes under NTG). These results indicated that the ORSV MP was able to support cell-to-cell movement, but not systemic movement, of p18Cy13inaTGB1.

Complementation of movement function of pOT2inaMP by transgenic plants expressing CymMV TGB1

Non-transgenic plants inoculated with *in vitro* transcripts of pOT2 showed mosaic symptoms by 18 days p.i. on both the inoculated and systemic leaves, but not with pOT2inaMP transcripts. RT-PCR of RNA extracted from
plants inoculated with pOT2 transcripts showed a prominent band of the expected size of 472 bp (Fig. 3c, lane C). For non-transgenic plants inoculated with pOT2inaMP transcripts, no RT-PCR band was amplified from either inoculated or systemic leaves (Fig. 3c, lanes under NTG). Total RNA extracted from CymMV TGB1 transgenic plants inoculated with pOT2inaMP transcripts yielded a 472 bp RT-PCR band from inoculated leaves, but not from systemic leaves (Fig. 3c, lanes under TG). Northern blot analysis confirmed the results (Fig. 3d). Both genomic and subgenomic RNAs were detected from the RNA of non-transgenic plants inoculated with pOT2 (Fig. 3d, lane C) and from inoculated, but not systemic, leaves of CymMV TGB1 transgenic plants inoculated with pOT2inaMP transcripts (Fig. 3d, lanes under TG). As expected, ORSV viral RNA was not detected from either inoculated or systemic leaves of non-transgenic plants inoculated with pOT2inaMP transcripts (Fig. 3d, lanes under NTG). These

![Fig. 2](http://vir.sgmjournals.org) 1547

Complementation of MPs and CPs of CymMV and ORSV

Fig. 2. Northern blot analysis to test the replication [(a) and (b), in protoplasts] and infectivity [(c) and (d), in plants] of p18Cy13 and pOT2 mutants, respectively, in N. benthamiana. Protoplasts isolated from leaves were electroporated with in vitro transcripts of mutants of p18Cy13inaTGB1, p18Cy13inaCP, pOT2inaMP and pOT2inaCP. RNA was extracted from protoplasts 48 h post-electroporation. CymMV and ORSV represent in vitro transcripts of p18Cy13 and pOT2. inaTGB and inaCP represent in vitro transcripts of p18Cy13inaTGB1 and p18Cy13inaCP, respectively.

![Fig. 3](http://vir.sgmjournals.org) 1547

Analysis of complementation of p18Cy13inaTGB1 in ORSV MP transgenic (TG) [(a) and (b)] and pOT2inaMP in CymMV TGB1 TG [(c) and (d)] N. benthamiana plants by RT-PCR [(a) and (c)] and Northern blot analysis [(b) and (d)]. M represents 1 kb DNA marker (Gibco-BRL); N represents negative control [total RNA extracted from non-TG (NTG) plants]; C represents positive control (total RNA from CymMV-infected plants); TG represents transgenic plants carrying the ORSV MP or CymMV TGB1 transgene; I and S represent inoculated and systemic leaves, respectively.
results showed that CymMV TGB1 could support cell-to-cell, but not systemic, movement of MP-defective ORSV.

Absence of complementation of pOT2inaCP by transgenic plants expressing CymMV CP

At 21 days p.i., no symptoms of ORSV infection were observed on either inoculated or systemic leaves of transgenic CymMV CP plants inoculated with pOT2inaCP transcripts. Non-transgenic plants inoculated with pOT2 transcripts showed symptoms of infection at about 18 days p.i. on both inoculated and systemic leaves. RT-PCR showed an expected-size band of 542 bp in the inoculated leaves of CymMV CP transgenic plants and non-transgenic control plants inoculated with pOT2inaCP transcripts, but not in their systemic leaves. This indicated that the CymMV CP in the transgenic plants was unable to complement the systemic spread of pOT2inaCP (Fig. 4a, lanes under NTG and TG, respectively).

To confirm the above results, a mixture of in vitro transcripts of p18Cy13 and pOT2inaCP was inoculated onto non-transgenic healthy seedlings of N. benthamiana at the eight-leaf stage. RNA extracted from inoculated and systemic leaves harvested at 21 days p.i. was subjected to RT-PCR to amplify the ORSV CP gene. Positive controls for the RT-PCR were total RNA extracted from N. benthamiana plants infected with in vitro transcripts of pOT2.

and negative controls were total RNA extracted from mock-inoculated leaves of N. benthamiana (Fig. 4b, lanes C and N, respectively). The expected 542 bp band was detected in the inoculated, but not systemic, leaves of doubly inoculated plants (Fig. 4b, lanes under DI). This result confirmed that the CymMV CP was unable to support the systemic movement of the CP-deficient clone pOT2inaCP.

![Fig. 4. RT-PCR analysis of complementation of mutant pOT2inaCP in N. benthamiana. (a) CymMV CP transgenic (TG) plants; (b) plants doubly inoculated with in vitro transcripts of p18Cy13+pOT2inaCP. M represents 1 kb DNA marker (Gibco-BRL); N represents negative control (total RNA extracted from non-TG plants); C represents positive control (total RNA extracted from CymMV-infected plants); TG represents transgenic plants carrying the CymMV CP transgene; DI represents double inoculation; I and S represent inoculated and systemic leaves, respectively. Arrows indicate the 542 bp PCR product of the ORSV CP gene.](image-url)

![Fig. 5. Analysis of complementation of mutant p18Cy13inaCP inoculated onto ORSV CP transgenic N. benthamiana plants. (a) RT-PCR of ORSV CP transgenic (TG) plants. M represents 1 kb DNA marker (Gibco-BRL); N and NTG represent negative control (total RNA extracted from non-TG plants); C represents positive control (total RNA extracted from CymMV-infected plants); TG represents transgenic plants carrying the ORSV CP transgene. (b) Absence of CymMV infection in ORSV CP TG plants inoculated with p18Cy13inaCP. Non-TG plants (lane C) infected with CymMV were used as a positive control. (c) Western blot analysis of ORSV CP TG plants inoculated with in vitro transcripts of p18Cy13inaCP. Total proteins were blotted onto a PVDF membrane and probed with CymMV CP-specific antiserum. C represents positive control (total proteins extracted from CymMV-infected plants); TG represents transgenic plants carrying the ORSV CP transgene; I and S represent inoculated and systemic leaves, respectively; M represents BenchMark PreStained protein ladder (Invitrogen). Asterisks represent the PCR product of the 757 bp CymMV CP gene.](image-url)
Complementation of p18Cy13inaCP by transgenic plants expressing ORSV CP

No visible symptoms of CymMV infection were observed in inoculated or systemic leaves of transgenic plants expressing the ORSV CP gene or in non-transformed control plants inoculated with in vitro transcripts of p18Cy13inaCP by 21 days p.i. In non-transformed plants inoculated with p18Cy13 transcripts, typical symptoms of CymMV infection were observed as small white streaks on systemic and inoculated leaves by 18 days p.i. Control plants were non-transformed plants inoculated with in vitro transcripts of p18Cy13inaCP or p18Cy13. RT-PCR showed the expected-size band of 757 bp (indicated by an asterisk) in the inoculated and systemic leaves of ORSV CP transgenic plants inoculated with p18Cy13inaCP transcripts (Fig. 5a, lanes under TG). In non-transformed plants inoculated with p18Cy13inaCP transcripts, the RT-PCR product was detected in inoculated leaves, but not in systemic leaves (Fig. 5a, lanes under NTG). Sequencing of the 757 bp PCR product confirmed that p18Cy13inaCP RNAs containing the modified start codon were present in the systemic leaves. This indicated that N. benthamiana plants carrying the ORSV CP transgene were capable of complementing movement of the CymMV CP-defective mutant p18Cy13inaCP.

In tissue-printing analyses, RNA derived from p18Cy13inaCP was unable to move in inoculated, non-transformed plants (Fig. 6a), but could move from cell to cell (Fig. 6b) in ORSV CP transgenic plants. CymMV CP RNA was also detected in the systemic leaves of ORSV CP transgenic plants (Fig. 6e). This indicated that the ORSV CP was able to complement the transport of CP-deficient CymMV genome.

Immunoblots showed that CymMV CP was not detected in either the inoculated or systemic leaves of ORSV CP transgenic plants (Fig. 6h, k). Western blot analysis of

![Image of RNA and immuno-leaf-blot analyses of complementation of mutant p18Cy13inaCP in ORSV CP transgenic (TG) N. benthamiana. (a–f) RNA leaf blot. Mock-inoculated onto non-TG plant [(a) and (d)]; ORSV CP TG plant inoculated with in vitro transcripts of p18Cy13inaCP [(b) and (e)]; non-TG plant inoculated with CymMV as a positive control [(c) and (f)]. Imprinted membranes were probed with a CymMV CP (–) RNA probe. (g–l) Immunoblot analyses. Non-TG plant inoculated with in vitro transcripts of p18Cy13inaCP [(g) and (j)]; ORSV CP TG plants inoculated with in vitro transcripts of p18Cy13inaCP [(h) and (k)]; non-TG plant inoculated with in vitro transcripts of p18Cy13 [(i) and mock-inoculated plant (l)]. Tissue-printed membranes were probed with CymMV CP-specific antiserum. Panels IL and SL represent inoculated and systemic leaves, respectively.](http://vir.sgmjournals.org)
these leaves yielded similar results, indicating that the p18Cy13inaCP transcripts were unable to produce CymMV CP (Fig. 5c, lanes under TG). Similar results also indicated that the p18Cy13inaCP was unable to produce CP (Fig. 6g, j). CymMV RNA probably moved unencapsidated in ORSV CP transgenic plants as a virus-replication complex (VRC) (Kawakami et al., 2004). These results are consistent with those reported in the complementation of PVX CP mutants by potyviral CPs, where proteins of PVX CP mutants could not be detected from transgenic plants expressing the Potato virus Y (PVY) CP (Fedorkin et al., 2000).

To address the question of whether the p18Cy13inaCP RNA was encapsidated by the ORSV CP, TEM was carried out to look for the presence of assembled virions. Virus particles were absent in inoculated and systemic leaves of test ORSV CP transgenic plants inoculated with p18Cy13inaCP transcripts. Wild-type CymMV and ORSV particles were found in non-transgenic control plants inoculated with in vitro transcripts of p18Cy13 and pOT2, respectively (data not shown).

To test whether crude sap extracts of ORSV CP transgenic plants that complemented movement of in vitro transcripts of p18Cy13inaCP could cause CymMV infection, crude sap extracts of these plants (prepared by homogenizing the systemic leaf) were inoculated onto non-transgenic N. benthamiana plants. Plants infected with CymMV were used as a control. The experimental samples showed no RT-PCR band, but the control samples clearly showed a 757 bp band at 21 days p.i. (Fig. 5b, lanes TG and C, respectively). The crude sap extracts of p18Cy13inaCP-infected ORSV CP transgenic plants were unable to infect non-transgenic N. benthamiana plants systemically.

**DISCUSSION**

The proteins encoded by the TGB of potexviruses are essential for cell-to-cell movement of these viruses (Beck et al., 1991; Angell et al., 1996). In addition, the CP is also required for cell-to-cell transport (Chapman et al., 1992). The tobamovirus MP is essential for cell-to-cell movement, whilst the CP facilitates long-distance movement of the viruses (Deom et al., 1987; Meshi et al., 1987). Deletion constructs of MP genes of cDNA clones of CymMV (nt 4339–5355 deleted) and ORSV (nt 4952–5241 deleted) showed neither cell-to-cell nor systemic movement when inoculated onto N. benthamiana (Soh, 2000). Introduction of mutations within the TGB of White clover mosaic virus did not induce symptoms or spread in either systemic or local lesion hosts, indicating that all TGB proteins are involved in cell-to-cell spread (Beck et al., 1991), but TGBp1 is an absolute requirement for the cell-to-cell movement (Lough et al., 1998). Inactivation of the CP gene in another full-length cDNA clone of CymMV pCT11 produced no systemic infection of CymMV in N. benthamiana (Yu, 1999) and was therefore in agreement with the infection produced by p18Cy13inaCP. The results of the pOT2inaCP assay are similar to those of the inactivation of the start codon of the CP of pOT2, which did not produce systemic infection of ORSV in N. benthamiana (Yu & Wong, 1998a).

The results presented indicate that the ORSV MP was able to facilitate cell-to-cell transport of p18Cy13inaTGB1. The ORSV MP does not require CP for cell-to-cell movement; hence, in the transgenic plants, this MP enabled the cell-to-cell movement of p18Cy13inaTGB1. For successful complementation of long-distance movement, the ORSV MP transgene required an ORSV-like CP, which, being unavailable, was unable to complement the long-distance transport of p18Cy13inaTGB1. In BSMV, a virus with TGB organization, the replacement of the TGB with a TMV MP allowed the resulting hybrid virus to infect the inoculated leaves in a host-dependent manner (Solovyev et al., 1996). These results indicate that tobamovirus MPs are able to support the movement of BSMV. Similarly, by using the co-bombardment approach, cell-to-cell movement of a 25 kDa MP-defective full-length cloned PVX genome is restored by 35S constructs expressing the MP gene of a tobamovirus (Tomato mosaic virus and crucifer tobamovirus) or Red clover necrotic mosaic virus (Morozov et al., 1997). The cell-to-cell movement of PVX mutants could be complemented efficiently by the tobamovirus Sunn-hemp mosaic virus (SHMV) in cowpea, suggesting that the SHMV MP can substitute functionally for the MPs and CP of PVX (Atabekov et al., 1999). Successful trans-complementation of BNYVV with TMV P30 has also been documented (Lauber et al., 1998). Our results are consistent with the idea that tobamovirus MP is able to support cell-to-cell, but not long-distance, movement of movement-deficient viruses that possess a TGB organization.

We have shown that the TGB1 transgenic plants were able to complement the cell-to-cell movement of pOT2inaMP transcripts. In the latest model, potexvirus TGBp1 is speculated to bind to virions or a ribonucleoprotein complex that exists as CP binding to viral RNA. This complex is anchored to the endoplasmic reticulum, where TGBp2 and TGBp3 interact with TGBp1 to move the complex from cell to cell (Verchot-Lubicz, 2005). A recent paper showing that TMV moves across plasmodesmata through VRCs (Kawakami et al., 2004) supports the notion of the existence of a viral-movement complex.

Thus, it is possible that the CymMV TGB requires recognition of CymMV CP to bind and form a functional ribonucleoprotein complex for long-distance movement. Therefore, the TGB1 transgenic plants were unable to complement the long-distance movement of pOT2inaMP. TGB-containing viruses are categorized into two groups, based on their requirement for the CP to affect cell-to-cell movement. The hordei- and benyviruses do not require CP for cell-to-cell movement (Quillet et al., 1989; Petty & Jackson, 1989), whereas the potexviruses require a CP to potentiate cell-to-cell movement (Chapman et al., 1992;
Forster et al., 1992; Baulcombe et al., 1995). Recent work on the TMV MP, which is not capable of modifying plasmodesmata or trafficking between cells without an assisting cell protein named NCAPP1 (Lee et al., 2003), suggests that viral MPs interact with some uncharacterized cell proteins that are required for cell-to-cell movement. Therefore, it is possible that, during virus evolution, the potexvirus TGB2 and TGB3 mimic components of the host in cell-to-cell movement. Our work has successfully analyzed the possibility of reciprocal complementation between the MPs of two orchid-infesting viruses, CymMV and ORSV, despite their differences in MP organization. In movement-complementation experiments using the double-inoculation approach in barley plants, the cell-to-cell movement of BSMV was facilitated by TMV (Malyshenko et al., 1989) and TMV movement was demonstrated to be mediated by BSMV (Hamilton & Dodds, 1970). The TMV-like MPs alter the plasmodesmatal size-exclusion limit (PD SEL) (Carrington et al., 1996). Similarly, among the TGBs of potexviruses, TGBp1, the protein encoded by TGB1, is capable of interacting with PD and increasing the PD SEL (Morozov & Solovyev, 2003). Therefore, in the reciprocal complementation between the MPs of CymMV and ORSV, the PD-gating activities of the two MPs expressed in transgenic plants are likely to have aided in the complementation process. Our results are consistent with the above observations.

In CP complementation between PVX and PVY, the PVX CP-deficient mutant is able to move from cell to cell, but not long distances, in PVY CP transgenic plants (Fedorkin et al., 2000). The cell-to-cell movement of a PVX CP movement-deficient mutant can be restored by potyviral CPs of Potato virus A and PVY in transient co-bombardment experiments. In that study, the question of whether PVX CP transgenic plants can support the cell-to-cell movement of PVY CP mutants was not addressed. As CymMV and PVX both belong to the potexvirus group, this result has similarity to our study, where ORSV CP in transgenic plants can support the long-distance movement of CymMV CP RNA. It can be speculated that the potexvirus CP can be complemented by other CPs, notably those of PVY and ORSV. A chimeric TMV with Alfalfa mosaic virus (AMV) CP is able to systemically infect plants that are resistant to TMV (Spitsin et al., 1999). The host range of TMV is altered by incorporating the AMV CP. This phenomenon suggests a possibility that coinfection by CymMV and ORSV may expand the host range of orchid varieties that otherwise would not have been infected by either virus individually. In another example, a chimeric TMV with a replacement of the CP gene by Groundnut rosette virus ORF3 is able to move rapidly through the phloem, despite low levels of virus RNA accumulation in infected cells (Ryabov et al., 1999b). Our results showed that the ORSV CP was able to complement the inactivated CymMV CP mutant. The p18Cy13inaCP transcripts were able to move in inoculated and systemic leaves of transgenic ORSV CP plants (Fig. 6b, e), implying that the ORSV CP was able to assist long-distance trafficking of an unrelated viral RNA. However, it could not functionally replace the CymMV CP in a manner to enable the formation of mature virions.

There was no CymMV CP detected in Western blots (Fig. 5c), suggesting that there was probably no trans-encapsidation by ORSV CP. Therefore, neither heterologous virion formation nor encapsidation of the inactivated p18Cy13inaCP by ORSV CP occurred. It is likely that CP complementation occurs because the ORSV CP is able to bind to p18Cy13inaCP transcripts. These results show that the movement process of the CymMV does not depend on the formation of virus particles. They also suggest that the CPs of tobamo- and potexviruses may display complementary activities in the viral-movement process in mixed infections that occur naturally. The complementary role of the ORSV CP to aid the long-distance movement of CymMV RNA is unlikely to be due to heterologous encapsidation. mRNA may be stored in the cytoplasm of higher eukaryotes in the form of masked messenger ribonucleoprotein (masked mRNP) particles, which are inactive in translation (Spirin, 1994), or as VRCs (Kawakami et al., 2004). The CymMV CP RNA long-distance movement aided by ORSV CP is likely to have resulted from the formation of masked mRNPs or VRCs of CymMV. Hence, CymMV CPs could not be translated, although CymMV RNA was detected in systemic leaves. Synergism in replication of CymMV and ORSV in orchid protoplasts has been reported previously (Hu et al., 1998). We have now observed a synergistic effect resulting in increased symptom severity in plants coinfected with CymMV and ORSV (unpublished observations). Complementation between CymMV and ORSV may result in enhancement of virus movement in coinfected plants, causing synergism. This study will lead to further understanding of the interactions between CymMV and ORSV and manifestation of disease-symptom development.

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