Movement of a Barley Stripe Mosaic Virus Chimera with a Tobacco Mosaic Virus Movement Protein


A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russia; and *Institute of Biochemistry and Plant Virology, Biologische Bundesanstalt für Land- and Forstwirtschaft, Messeweg 11/12, D-38104 Braunschweig, Germany

Received September 28, 1995; accepted January 12, 1996

The tobacco mosaic virus (TMV) 30K movement protein (MP) gene was inserted into a full-length cDNA clone of barley stripe mosaic virus (BSMV) RNAβ replacing the triple gene block (TGB). The resulting recombinant ND-MPT genome, consisting of infectious wt transcripts of BSMV RNAs α and γ, together with the hybrid RNAβ transcript, was inoculated onto test plants to study the functional compatibility between the BSMV TGB-adapted genetic system and the tobamovirus transport gene. ND-MPT infected the inoculated leaves of Nicotiana benthamiana and Chenopodium amaranticolor, which are common hosts for the parental viruses; the size, growth rate, and morphology of local lesions on C. amaranticolor were influenced by the foreign MP gene. However, the hybrid virus failed to infect barley, N. tabacum (var. Samsun), and N. clevelandii, the selective hosts. Thus, the TMV MP was able to functionally substitute for the BSMV TGB-coded MPs, i.e., the 30K MP functioned independently of any other BSMV sequences. However, the TMV MP gene promoted the cell-to-cell movement in a host-dependent manner.

INTRODUCTION

The cell-to-cell translocation of a plant virus genome is a function of both the viral and the host genomes, in which virus-coded movement protein(s) (MPs) and host-coded components are involved (for reviews, see Atabekov and Taliansky, 1990; Maule, 1991; Citovsky and Zambryski, 1993; Deom et al., 1992; Lucas and Gilbertson, 1994).

The strategies for coding and expression of the cell-to-cell transport function (TF) vary substantially between different virus groups. For example, tobamoviruses contain a single 30K MP gene in a unipartite genome (Deom et al., 1987). Unlike the single-MP-coding viruses, the genomes of hordeiviruses (Petti et al., 1990; Jackson et al., 1991), furoviruses (Gilmer et al., 1992), and potexviruses (Beck et al., 1991) contain the so-called triple gene block (TGB) (Morozov et al., 1989) and require three MPs to express their TF.

Much evidence points to the conclusion that viruses belonging to different taxonomic groups are able to complement each other's TF, i.e., that the MPs coded by unrelated plant viruses are functionally interchangeable (Ziegler-Graff et al., 1991; Taliansky et al., 1993; Fuentes and Hamilton, 1991; Richins et al., 1993). In particular, there was complementation of tobacco mosaic virus (TMV) transport in barley by barley stripe mosaic virus (BSMV) (Hamilton and Dodds, 1970), and the transport of BSMV was facilitated by TMV in tobacco (Malyshenko et al., 1989). The rod-shaped BSMV has a tripartite genome consisting of three RNAs designated α, β, and γ. RNAβ contains four ORFs, the 5’-proximal coat protein (CP) gene, and the TGB, collectively encoding the TF. The CP is dispensable for the replication and systemic movement of BSMV, whereas mutations in the TGB eliminate movement (Petty and Jackson, 1990). Here, we replaced the TGB in BSMV ND18 RNAβ with the TMV 30K MP gene and assayed the ability of the resulting hybrid to infect the host and nonhost plants of the parental viruses.

MATERIALS AND METHODS

Plant material

Nicotiana tabacum, N. benthamiana, and Chenopodium amaranticolor were grown in a greenhouse under natural light conditions supplemented with sodium halide lamps (16-hr photoperiod). Hordeum vulgare “Black Hulles” plants were maintained in growth chambers (16-hr light/8-hr dark periods at 25°C).

cDNA clones

Full-length cDNA clones of the ND18 strain of BSMV capable of producing infectious in vitro transcripts were
kindly provided by A. O. Jackson, University of California, Berkeley (Petty et al., 1988, 1989).

First strand cDNA was synthesized from the TMV-U1 RNA using AMV reverse transcriptase (Promega) and the 3'-primer d(5'-AACACTTTTACAGGGTAAA) complementary to the TMV-U1 30K gene nucleotides 82–102 (positions 4984–4504 in TMV genome) (Goelet et al., 1982). The reaction mix (20 μl) contained 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 10 mM magnesium chloride, 1 mM dNTPs, 20 units of RNasin (Promega), and cDNA product was heat denatured at 95°C for 5 min. The dsDNA was generated and amplified in a reaction containing 50 pmol of 5'-primer d(5'-GGGccatggCTCTAGTTGTTAAAG-GAAAAG), which represents TMV 30K gene nucleotides 1–25 (positions 4903–4927 in TMV genome) and contains a unique 5'-flanking Ncol site (in lowercase), 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 5 mM magnesium chloride, and 3 units of Taq polymerase in a final volume of 60 μl. All PCRs were 20 cycles of 1 min at 94°C, 1 min at 53°C, and 2 min at 72°C. The resulting amplified DNA fragment, containing an internal BglII site (position 4967 in TMV genome) and 5'-flanking Ncol site, was isolated with the QIAEX gel extraction kit (QIAGEN).

The TMV-U1 MP gene cDNA clone pKS-30K was previously generated to include flanking EcoRI and BamHI sites at the 5'- and 3'-termini, respectively (Ivanov et al., 1994).

Generation of BSMV RNAβ hybrid cDNA clones

pND-MPT (Fig. 1) was created by substituting the TMV 30K gene for the BSMV TGB in a two-step cloning procedure. First, the PCR fragment containing the 5'-terminal part of the TMV 30K gene was digested with Ncol and BglII (Fig. 1) and ligated into an Ncol–BglII-cleaved BSMV RNAβ cDNA clone (Pettij and Jackson, 1990) to generate pND-30K5 (not shown). The pKS-30K (see above) was digested with BglII and BamHI to produce a DNA fragment containing most of the TMV MP gene. This fragment was ligated into the BglII-cleaved pND-30K5. The resulting clones were tested for the proper insert orientation by restriction enzyme mapping.

The deletion mutant clone pND-Δ58 was made by digestion of the wt cDNA clone of RNAβ with Ncol and Sall (Pettij and Jackson, 1990), filling in the cohesive ends and recircularizing the plasmid by blunt-end ligation.

In vitro transcription and RNA inoculation

Infectious wt and chimeric BSMV transcripts were synthesized from full-length clones linearized with MluI for RNAs α and γ, and with SpeI for wt and chimeric RNAβ. Transcription with T7 RNA polymerase (Promega) and inoculation of plants were performed as described previously (Petty et al., 1989; Petty and Jackson, 1990).

Following inoculation, plants were maintained in growth chambers. Symptom formation was observed daily for up to 15 days. The necrotic lesion symptoms were scored visually and recorded by photography.

Detection of BSMV infection

Leaf disks excised from individual leaves, or disks combined from five to six leaves, were ground in 50 mM sodium phosphate, pH 7 (0.2 ml buffer per 100 mg of tissue). The unfractionated homogenate was mixed with an equal volume of a 2× SDS–polyacrylamide gel electrophoresis sample buffer (Laemmli, 1970) followed by boiling for 2 min and fractionation in a 15% gel by PAGE. After electrophoresis, proteins were transferred to nitrocellulose membranes (Schleicher and Schuell) and detected with BSMV antiserum (kindly provided by V. K. Novikov). Immunosorbent electron microscopy (ISEM) (Mline and Lesemann, 1984) was adapted for analysis of BSMV particles in homogenates of C. amaranticolor leaves. The grids were treated for 5 min with antisera to BSMV diluted 1:1000, incubated with extracts of local lesion tissues for 2 days, washed, and negatively stained with 1% uranyl acetate. For immunodecoration experiments, anti-BSMV serum (collection of BBA, Braunschweig) diluted 1:50, was used.

Analysis of RNA

Total cellular RNA was extracted by SDS–phenol–LiCl mixture as described (Verwoerd et al., 1989). To perform slot-blot hybridizations, total RNA preparations were immobilized on a nylon membrane using “Slot Blot,” Model PR648 (Hoefer Scientific Instruments). Northern blot analysis was conducted as described previously (Solovyev et al., 1994). The filters were probed with 32P-labeled cDNA prepared with a random deoxyhexamers cDNA labeling kit (Boehringer) on the isolated cloned DNA fragments specific for the TMV MP gene or BSMV 58K MP and CP genes as described in the manufacturer's protocol.

To determine the nucleotide sequence of the progeny of chimeric genomic RNAs, the DNA was amplified from total RNA preparation by RT–PCR (see above). The first strand synthesis primer d(5'-CCCgtaccATCAGATT-GAATGATCTGA) was complementary to positions 3059–3084 in the 3'-UTR of BSMV RNAβ (Gustafson and Armour, 1986) and contained a flanking KpnI site (in lowercase letters), whereas the second strand primer d(5'-GAATtgattCTTCCAGATGCGAGGAAG) represented the 3'-end of the BSMV CP gene (positions 654–681) and also contained a KpnI site. The resulting PCR-generated DNA fragment was digested with KpnI and cloned into the KpnI site of the pBluescript II (SK) cloning vector (Stratagene). Complete sequencing of the virus-specific DNA inserts was performed by using the dideoxynucleotide method and Erase-a-Base kit (Promega).
RESULTS

Replacement of the BSMV TGB with the TMV MP gene

For construction of the recombinant clone pND-MPT, the cDNA fragment encompassing the TMV-U1 MP gene (starting with the initiation codon and ending with the terminator followed by a polylinker-derived 7-nt sequence) was inserted in two steps into an NcoI-BglII-cleaved BSMV-ND18 RNAβ cDNA clone (Fig. 1). In the final construct (pND-MPT), the BSMV βb and βd genes were replaced by the TMV 30K gene that was followed, in a different reading frame, by the 3'-terminal part of the βc gene and the 3'-UTR of BSMV genome (Figs. 1A and 1B). The TMV-specific insertion in pND-MPT was confirmed for integrity by dideoxynucleotide sequencing.

Cell-to-cell movement characteristics of the BSMV-TMV hybrid in N. benthamiana

Although the natural systemic hosts of BSMV are monocotyledonous grasses, in contrast to TMV, which has dicotyledonous hosts, both viruses are able to systemically infect N. benthamiana (Jackson et al., 1991). In accordance with previous studies (Petty and Jackson, 1990; Petty et al., 1990, 1994), infectious transcripts of the wt BSMV-ND18 cDNA clones produced typical necrotic lesions on C. amaranticolor and systemic symptoms on N. benthamiana and H. vulgare 'Black Hullers.' The mixture of RNAα and RNAγ transcripts alone, or RNAα, RNAγ, and the deletion mutant pND-Δ58 RNAβ transcripts, infected neither C. amaranticolor nor N. benthamiana. The mutant pND-Δ58 with the N-terminal one-third of the 58K MP (βb protein) deleted was analogous to the β-2.0 mutant of Petty and Jackson (1990). On the other hand, when N. benthamiana leaves were inoculated with the chimeric RNAβ (ND-MPT) transcripts in the presence of wt BSMV-ND18 RNAα and RNAγ transcripts, the hybrid virus accumulated in the inoculated leaves to levels comparable to those of wt BSMV-ND18, as determined by Western blot analysis (Fig. 2). The symptoms of infection (mild chlorosis) induced on the inoculated leaves by the hybrid virus appeared later than in wt BSMV infection.
FIG. 2. Immunodetection of the BSMV coat protein in N. benthamiana leaves infected with one of the following: wt BSMV transcripts (wtBSMV); a mixture of wt BSMV RNA α and γ transcripts with the hybrid RNAβ transcript (ND-MPT); a mixture of wt BSMV RNA α and γ transcripts with the transcripts of the RNAβ deletion variant (ND-Δ58). Mock indicates mock-inoculated leaves. Position of the coat protein (CP) from purified BSMV particles is indicated.

and were not observed on uninoculated leaves. In line with this, no BSMV CP was detected in the noninoculated leaves by Western blotting (data not shown).

To examine hybrid virus RNA accumulation, total RNA preparations isolated from the N. benthamiana leaves inoculated with wt BSMV and ND-MPT were subjected to slot-blot and Northern blot hybridization analyses with TMV MP, BSMV βb, and CP gene-specific probes (Figs. 3 and 4). Supporting the results of Western blot analysis, the presence of the TMV 30K gene sequences was detected only in leaves inoculated with ND-MPT (Fig. 3) but not in the noninoculated leaves, whereas parental viruses BSMV-ND18 and TMV caused systemic infections. To rule out the cross-hybridization between wt and hybrid genomes, parallel hybridization reactions were performed with probes specific for the BSMV 58K protein and TMV 30K MP genes (Fig. 3). The results confirmed that the virus accumulation observed in ND-MPT-infected leaves was not due to contamination with wt BSMV RNAβ (Fig. 3). It should be emphasized that in hybrid virus infections, only chimeric RNAβ (which was shorter than wt BSMV RNAβ) was detected by Northern blotting with BSMV CP gene-specific probe (Fig. 4).

Maintenance of the TMV MP gene insert in the chimeric RNA progeny

It has been shown that the MP genes undergo sequence modifications in monocot- and dicot-specific bromovirus hybrids (Mise et al., 1993; Schneider and Allison, 1993). Total RNA extracted from the ND-MPT-inoculated leaves of N. benthamiana exhibiting a strong Western blot signal (Fig. 2) was reverse transcribed and PCR amplified. The entire 30K MP gene sequence within the chimeric RNAβ was amplified with BSMV-specific primers. The DNA fragment encompassing the TMV MP gene flanking by the BSMV RNAβ sequences (Fig. 1) was cloned and sequenced. Comparison of the sequences of ND-MPT progeny revealed two base substitutions leading to amino acid changes in the MP [Thr(18) to Ser, and Asn(228) to Ser] relative to the wild-type TMV-U1 sequence (Goelet et al., 1982). These mutations are unlikely to have a dramatic effect on the TMV MP function, because they occurred in nonconserved regions (Melcher, 1990).

Inability of the hybrid virus to infect the parental virus differential hosts

Barley and the members of Solanaceae (N. tabacum var. Samsun, N. clevelandii) are differential hosts for infection by BSMV and TMV, respectively (Jackson et al., 1991; Dawson and Hilf, 1992). To examine the contribution of the TMV 30K MP gene to the host specificity, 96 barley, 40 N. tabacum, and 12 N. clevelandii plants were inoculated with ND-MPT transcripts. No BSMV CP was detected in barley, tobacco, and N. clevelandii leaves inoculated with ND-MPT, whereas wt BSMV-ND18 transcripts infected 60–70% of inoculated barley leaves, and ND-MPT transcripts infected 50% of inoculated N. benthamiana leaves.
Induction of the necrotic lesions on C. amaranticolor by the hybrid virus

BSMV-ND18 caused large necrotic lesions on C. amaranticolor by 4 days postinfection (dpi) (Fig. 5A; Petty et al., 1989, 1994) and complete collapse by 7–9 dpi. The ND-MPT-induced local lesions appeared by 10 dpi; the size and morphology of lesions in ND-MPT infection (Fig. 5C) differed from those produced by wt BSMV-ND18, being more similar to the wt TMV-induced lesions (Fig. 5B). The TMV local lesions were first visible by 2–3 dpi and continued to enlarge for at least 4 more days, whereas the ND-MPT-induced lesions did not enlarge.

Unexpectedly, our attempts to passage the ND-MPT hybrid virus from the C. amaranticolor local lesions to healthy C. amaranticolor plants were unsuccessful, whereas the wt BSMV-ND18 could be readily passaged, producing necrotic reaction on C. amaranticolor. Moreover, no CP could be detected by Western blot analysis in the ND-MPT-specific lesions, in contrast to wt BSMV- and TMV-specific necrotic spots (data not shown). The level of the signal in Northern slot-blot analysis with 30K-specific probe was at least several orders of magnitude weaker in lesions containing the hybrid virus than for wt TMV infection (data not shown), despite the similar sizes of TMV and ND-MPT lesions. Consistent with this, ISEM revealed that accumulation of the BSMV rod-like particles in the ND-MPT-induced lesions was approximately 1000–10,000 times lower than in the lesions induced by wt BSMV. It is important to note that particles were rarely detected in the C. amaranticolor leaves inoculated with the movement-deficient BSMV mutant ND-ΔS8 control. The virus particles in C. amaranticolor produced by chimeric constructs were strongly decorated by BSMV antiserum, and the particle length distribution was generally similar in the hybrid virus and wt BSMV infections. However, the proportion of shorter particles (modal length, 75–95 nm) was somewhat larger from infections by the hybrid virus than by wt BSMV (data not presented). This is consistent with the shorter size of the ND-MPT RNAβ species in comparison to RNAβ of the wt BSMV (Fig. 1).

DISCUSSION

There is now ample evidence for the complementation of cell-to-cell TF of transport-deficient plant viruses by unrelated helper viruses in doubly infected plants (for review, see Atabekov and Taliansky, 1990). Taking into account that the genetic elements, other than MP genes, can affect the cell-to-cell movement and long-distance spread (Allison et al., 1988; Watanabe et al., 1987; Nejidat et al., 1991; Jackson et al., 1991; Gal-on et al., 1994; Deom et al., 1994; De Jong and Ahlquist, 1995) such traditional double-infection experiments cannot be interpreted unequivocally in terms of functional interchangeability of the MPs coded by the partner viruses.

In this study, the recombinant BSMV was used to examine the functional interchangeability of cell-to-cell TF coded by two viruses having no sequence similarity between their MPs (BSMV and TMV). It can hardly be assumed that the biochemical functions of the TGB-coded proteins completely coincide with those coded by monocistronic transport genes with which TGB MPs do not have structural or sequence similarity. However, the BSMV TGB MPs can be replaced by a single TMV MP coded by the hybrid virus in the inoculated N. benthamiana leaves (Figs. 2–4). Unlike the parental viruses, the hybrid BSMV failed to infect N. benthamiana systemically. The inability of ND-MPT to systemically infect N. benthamiana might reflect some fundamental differences in the BSMV and TMV transport systems. For example,
it may concern the contribution of the TMV CP, but not BSMV CP, in long-distance movement (Dawson and Hilf, 1992; Jackson et al., 1991). Obviously, the failure of the hybrid virus to infect N. benthamiana systemically was not due to reduction of the rate of its accumulation in inoculated leaves, since the level of ND-MPT accumulation was similar to that of wt BSMV (Fig. 2).

Although the ND-MPT recombinant directed sufficient cell-to-cell spread in inoculated leaves of N. benthamiana, it was unable to infect either barley or N. tabacum (var. Samsun) (and N. clevelandii), selective hosts of BSMV and TMV, respectively. These data indicate that in common hosts the MP may function independently of any other virus-encoded protein(s) to promote the hybrid virus cell-to-cell movement. However, in selective hosts some kind of host-specific adaptation of MP genes and, probably, other genomic elements (e.g., replicase gene) is needed for the hybrid virus cell-to-cell movement. In other words, the hybrid BSMV is presumably able to replicate and produce the TMV MP in primarily inoculated cells, yet nonetheless fails to move from cell to cell in the selective hosts. This seems to contradict the results of the movement complementation experiments in plants doubly inoculated with TMV and BSMV: the cell-to-cell movement of BSMV in barley was facilitated by TMV (Malyshenko et al., 1989), and TMV movement in barley was mediated by BSMV (Hamilton and Dodds, 1970).

One might assume that in certain virus–host combinations the presence of a specific active MP in the cell per se is a necessary but insufficient condition to ensure an efficient cell-to-cell spread of the hybrid virus coding for this MP. In line with this assumption, it has been shown that production of the TMV MP is essential but insufficient by itself for complementation of foreign virus (comovirus) transport (Taliansky et al., 1992). In particular, the blockage or inhibition of the hybrid virus cell-to-cell spread in selective hosts may be due to the lack of a hypothetical host-specific or virus-specific factor(s) or/and virus-induced host protein(s) in the hybrid-infected cells. When viruses with monocistronic transport genes were used for recombination, the foreign MPs in the resulting hybrid (and reassortant; Allison et al., 1988) viruses could efficiently substitute for one another in common hosts for both parental viruses (Nejidat et al., 1991; De Jong and Ahlquist, 1992; Hilf and Dawson, 1993; Jackson et al., 1991; Mise and Ahlquist, 1995; Gieszman-Cookmayer et al., 1994; Fenczik et al., 1995). Similar to the BSMV hybrid ND-MPT, the hybrid viruses of monocistronically adapted brome mosaic virus and dicot-adapted cowpea chlorotic mottle virus failed to support systemic infection and they also supported little or no cell-to-cell movement in the inoculated leaves of selective hosts of the parental viruses (Mise et al., 1993; Mise and Ahlquist, 1995). As shown by Fenczik et al. (1995), the replacement of the TMV MP gene by that of Odontoglossum ringspot tobamovirus allowed the hybrid to move systemically in orchids, whereas this hybrid virus was localized in the inoculated tobacco leaves (Hilf and Dawson, 1993). It should be noted that the hybrid was able to systemically infect N. benthamiana, a common systemic host for both viruses (Hilf and Dawson, 1993).

BSMV and TMV produce necrotic lesions of distinct morphology on inoculated leaves of C. amaranticolor (Figs. 5A and 5B). It is noteworthy that the ND-MPT, where the TGB was replaced with the TMV 30K MP gene, produced necrotic lesions similar in size and morphology to those of TMV (Fig. 5C). Thus, the lesion phenotype was influenced by the foreign MP gene inserted into BSMV RNA/β. This is in general agreement with the observations that the MPs may play a role in symptoms induction (Nejidat et al., 1991; Deom et al., 1994; Rao and Grantham, 1995; De Jong and Ahlquist, 1995). In contrast to wt BSMV, the BSMV hybrid containing the 30K TMV MP gene accumulated within the local lesions in extremely low amounts. This observation suggests that even negligible amounts of the ND-MPT recombinant can mediate sufficient cell-to-cell spread of the virus and subsequent host response to form the visible TMV-type lesions in C. amaranticolor.

Taken together, our results show that the MP genes of distantly related plant viruses (hordei- and tobamoviruses) can substitute for each other in certain virus–host systems, and the MP genes may contribute to the lesion phenotype produced in a common host for both parental viruses.

ACKNOWLEDGMENTS

We are grateful to Dr. A. O. Jackson for providing the BSMV infectious cDN clones and Dr. A. A. Agranovsky for help in preparing the paper. This work was supported by INTAS Grant INTAS-93-0989.

REFERENCES


Deom, C. M., Shaw, M., and Beachy, R. N. (1987). The 30-kilodalton