Analysis of local and systemic spread of the crucifer-infecting TMV-Cg virus in tobacco and several Arabidopsis thaliana ecotypes

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Abstract. The crucifer-infecting tobacco mosaic virus, TMV-Cg, infects Arabidopsis thaliana (L.) Heynh. efficiently without causing severe symptoms. The systemic spread of TMV-Cg in Arabidopsis was evaluated in 14 ecotypes. Five days after inoculation, TMV-Cg was detected in apical leaves of 8 out of 14 ecotypes. As expected, the spread of TMV-Cg in the ecotypes tested was considerably faster than that of tobacco mosaic virus (TMV-U1). To study the participation of viral proteins in the TMV-Cg-induced infection, a complete genomic cDNA of TMV-Cg was cloned. The role of TMV-Cg movement protein in systemic spread was tested with a hybrid virus, constructed from the TMV-U1 genome and the TMV-Cg movement protein gene. Contrary to expectations, the systemic spread of this hybrid in Arabidopsis was similar to that of TMV-U1. The failure of the hybrid virus to spread at rates similar to those of TMV-Cg was not due to restrictions in local movement. In tobacco (Nicotiana tabacum L.), the hybrid virus spread efficiently and induced systemic mosaic symptoms characteristic of TMV-U1. The TMV-Cg cDNA clone provides an attractive tool to study virus–host interactions.

Introduction

The establishment of a systemic viral infection in plants is a complex process that requires specific interactions between plant and virus factors. During this process, the virus enters a cell, replicates in its cytoplasm, moves locally to adjacent cells and finally, spreads systemically to distant leaves through the vascular tissue. The use of the common strain of the tobacco mosaic virus (TMV-U1), which belongs to the solanaceous-infecting group of tobamovirus, has shown that viral proteins are required for the establishment and spread of infection in tobacco plants (Deom et al. 1987; Mushi et al. 1987; Arce-Johnson et al. 1995, 1997).

Currently, the crucifer Arabidopsis thaliana is the preferred plant species for genetic and molecular studies, owing to its short lifecycle and compact genome, which has been completely sequenced. The study of viral infection in this species requires a virus that is able to spread systemically, but that does not kill the plant in the process. The crucifer-infecting subgroup of tobamovirus meets these requirements in Arabidopsis (Lartey et al. 1997). This subgroup includes TMV-Cg (Lartey et al. 1997), Cr-TMV (Dorokhov et al. 1994), oilseed rape mosaic virus (ORMV) (Aguilar et al. 1996), and turnip vein clearing virus (TVCV) (Lartey et al. 1996).

TMV-Cg was initially isolated from naturally infected garlic and it multiplies systemically in Arabidopsis ecotypes Columbia, C-24 and Po-1 without inducing any detectable resistance reaction in the plant (Ishikawa et al. 1991; Pereda et al. 2000). The genome of TMV-Cg consists of a unique positive single-strand RNA molecule of 6303 nucleotides (Yamanaka et al. 1998). It encodes the four proteins characteristic of the tobamovirus group (Goel et al. 1982). Two of these proteins are involved in viral replication, one of approximately 130 kDa with predicted methyltransferase and RNA helicase motifs and the other, a read-through protein of about 180 kDa containing RNA replicase motifs (Ishikawa et al. 1986). A 30-kDa movement protein (MP) required for cell-to-cell spread (Deom et al. 1987; Mushi et al. 1987) and a 17-kDa coat protein (CP) are also encoded. The TMV-Cg genome has a sequence overlap between the 3 terminal portion of the MP gene and the 5 terminus of the CP gene (Yamanka et al. 1998), a feature characteristic of the crucifer-infecting subgroup (Dorokhov et al. 1994; Aguilar et al. 1996; Zaitlin 1999).

Abbreviations used: CP, coat protein; dpi, days post-inoculation; ELISA, enzyme-linked immunosorbent assay; MP, movement protein; ORF, open reading frame; TMV, tobacco mosaic virus.
TMV-Cg and TMV-U₁ are closely related viruses but they exhibit very different behaviors in Arabidopsis. In previous studies, we detected TMV-Cg earlier, and in greater amounts, than TMV-U₁ in leaves of ecotype Po-1 (Pereda et al. 2000). Since variability in the virus–host interaction among different Arabidopsis ecotypes has been observed (Martin et al. 1997), we wanted to study the systemic spread of TMV-U₁ and TMV-Cg in several ecotypes. In this study, we cloned the complete sequence of TMV-Cg and evaluated its capacity to infect a collection of 14 ecotypes of Arabidopsis thaliana. The availability of the cloned TMV-Cg genome now makes it possible to study the participation of the different virus proteins in each phase of the infection. The role of TMV-Cg MP in systemic virus spread was studied with the use of a hybrid virus constructed for this purpose. The hybrid was tested in Arabidopsis, and also in TMV-sensitive and -resistant tobacco plants.

Materials and methods
Cloning of TMV-Cg

TMV-Cg was isolated from systemically infected tobacco plants [Nicotiana tabacum (L.) cv. Xanthi nn]. First-strand cDNA was synthesized with avian myeloblastosis virus reverse transcriptase as described by Holt et al. (1991), using the primer Cg1ST: 5’-GGATC- CGGTAAACCTGGGCCTACCCGGGTTA-3’. This sequence is complementary to the 3’ end of TMV-Cg from nucleotides 6284 to 6303 (GenBank database, accession number D38444) with flanking BstEII and BamHI sites (underlined sequences). Polymerase chain reaction (PCR) was performed on this template using the long and accurate PCR method of Barnes (1994). We used the first-strand primer (Cg1ST) and the forward (sense-strand) primer T₁Cg2: 5’-TACGCTGCAGTAATACGACTCACTATAGG-3’. This sequence matched the first 23 nucleotides of the MP in TMV-Cg genome. A 2021 nucleotide fragment was digested with PstI and ligated to the complementary sites in pBS-Cg. The cloned DNA was sequenced to confirm fidelity of the amplification. pTMV-NA, which contains full-length TMV-U₁ cDNA sequence, but which includes NdeI and BstEII restriction sites adjacent to the start and stop codons of the MP in TMV-U₁ genome (Deom et al. 1994), was used as a vector. The MP sequence was excised from pBS-Cg using the NdeI and BstEII sites and subsequently ligated to the complementary sites in pTMV-NA, thus, creating the hybrid U₁-MPCg.

In vitro transcription and inoculation of plants

To conduct transcription experiments, TMV-Cg, TMV-U₁, and U₁-MPCg cDNA clones were linearized using BstEII and KpnI, respectively. In vitro transcription was performed in the presence of 5 mM 7-methylguanosine (cap) and bovine serum albumin (BSA, final concentration of 100 µg L⁻¹) using the Promega (Madison WI) Riboprobe T₇ system. After 1 h of incubation at 37°C, 100 µL of 20 mM phosphate buffer was added. Tobacco plants (cv. Xanthi nn) were mechanically inoculated in one leaf with the whole transcription reaction. Infection symptoms were monitored, and systemically infected tobacco plants were used as a source of progeny viruses. TMV-U₁, TMV-Cg, and U₁-MPCg were purified from systemically infected tobacco plants in 20 mM phosphate buffer (pH 7.4).

Tobacco plants, cvv. Xanthi NN (resistant genotype carrying the N resistance gene), Xanthi nn and 2005 (transgenic NN tobacco that expresses MP of TMV-U₁, Deom et al. 1991), were grown in the greenhouse. Two-month-old plants were mechanically inoculated on two leaves with 100 µL of virus, diluted to 10 ng µL⁻¹. For the local movement experiment, resistant plants (NN genotype) were maintained for 2 d at 32°C before inoculation with the three viruses, and shifted to 25°C at 4 and 12 h post-inoculation. Arabidopsis thaliana (L.) Heynh. ecotypes Be-0, Bla-2, Btg-2, Bur-0, C-24, Col-0, Hae-1, Ler-0, Mt-0, Nie-0, Nso-0, Po-1, Uk-4 and Wei-0 were grown in vitro in Gamborg’s B5 solid medium. The growth chamber was adjusted to 23 ± 2°C with a 16 h photoperiod. Four- to six-week-old plants were mechanically inoculated on three rosette leaves. Sterile cotton swabs dusted with carborundum and dipped into the viral suspension (3 ng µL⁻¹ virus in 20 mM phosphate buffer) were rubbed on the leaf surface. A pool of 12 plants of each ecotype was inoculated in two different experiments.

Viral coat protein detection in virus infected plants

Antibodies against TMV-Cg and TMV-U₁ were developed in rabbits. TMV-Cg was detected by enzyme-linked immunosorbent assay (ELISA) with TMV-Cg antiserum at 1:500 dilution and alkaline phosphatase anti-rabbit conjugated IgG at 1:20000 dilution as the second antibody (Pereda et al. 2000). ELISA assays to detect TMV-U₁ and U₁-MPCg were made using anti-TMV-U₁ commercial antibodies from Agdia Inc. (Elkhart, IN), following the manufacturer’s instructions. Coat proteins were also detected by western blots. For western blot analysis, 50 µg of fresh Arabidopsis leaves from the pool of 12 inoculated plants, or tobacco leaf discs (20 mm diameter) were used. Proteins were extracted in 100 µL of extraction buffer (125 mM Tris–HCl pH 6.8, 0.1% SDS and 20% v/v glycerol) and insoluble material was removed by centrifugation. 15 µL of each protein sample were denatured at 95°C, separated on 15%
acrylamide–SDS gels and transferred to a nitrocellulose membrane. Anti-virus serum at 1:1000 and alkaline phosphatase anti-rabbit IgG at 1:20000 dilution were used to detect coat proteins.

Leaf skeleton hybridization

Arabidopsis leaves were harvested at different stages of infection, and prepared for hybridization as described by Larney et al. (1997). Briefly, chlorophyll was removed from leaves using 95% ethanol and proteins were digested with proteinase K treatment at 45°C for 3 h. Leaves were incubated in a prehybridization solution for 2 h and hybridization was carried out overnight at 42°C. A 32P probe of 518 base pairs (bp) corresponding to the TMV-U1 CP gene or a 495-bp probe corresponding to the TMV-Cg CP gene was used. Leaves were washed twice at 42°C in 2.5×SSC and rinsed in distilled water. Leaves were then carefully mounted on nitrocellulose membranes and autoradiographed.

Results and discussion

Evaluation of the systemic spread of TMV-Cg in Arabidopsis ecotypes

The general capacity of TMV-Cg to infect Arabidopsis was tested by inoculating 14 ecotypes with TMV-Cg (Table 1). The wild-type strain, TMV-U1, was also inoculated to compare the systemic spread of both viruses in the different ecotypes. Plants were inoculated with virions on the rosette leaves at the time of initiation of growth of the inflorescence stem. This made it possible to distinguish between local spread in the inoculated leaf and systemic movement through vascular tissues. The use of in vitro-grown plants ensured a plant environment with a high relative humidity, preventing the inoculated leaves from drying.

TMV-Cg, as revealed by the presence of its CP, was detected in the inoculated rosette leaves of all ecotypes tested, at 3 days post-inoculation (dpi). At 5 dpi, a consistent CP band was detected in western blots (Table 1). TMV-Cg spread rapidly from inoculated leaves to apical leaves. It was detected 5 dpi in uninoculated apical leaves of the ecotypes Be-0, Bla-2, Btg-2, C-24, No-0, Po-1 and Wei-0. TMV-Cg CP was consistently detected in the apical leaves of all ecotypes tested at 9 dpi (Table 1).

The most efficient systemic spread of TMV-Cg was found in ecotypes Bla-2, C-24, No-0 and Po-1, which showed the highest CP accumulations as detected by western blots (not shown). During the first week after inoculation, most plants did not show symptoms of infection. Subsequently, some plants developed mild symptoms, and at 15 dpi, about half of the plants of different ecotypes showed curving of rosette leaves and some stunting. Mt-0, the ecotype displaying the shortest lifecycle under the growing conditions used, showed red–violet coloring in leaves, stunting, and death of most plants by 15 dpi.

Our results show that TMV-Cg spreads systemically and accumulates to high levels in all Arabidopsis ecotypes studied, without causing severe infection symptoms in most of those ecotypes. This is of great significance because it allows the study of virus–host interactions at different stages of plant development. Maximal accumulation in apical leaves was reached at 15 dpi in most ecotypes, similar to previously reported assays for TMV-Cg in Col-0 (Ishikawa et al. 1991). These results indicate that TMV-Cg replication, cell-to-cell movement and systemic spread are highly efficient processes in all Arabidopsis ecotypes studied.

The systemic spread of TMV-U1 in Arabidopsis ecotypes was less efficient, and slower, than that of TMV-Cg. Despite the fact that TMV-U1 was present 5 dpi in the inoculated leaves of all ecotypes tested (Table 1), this virus was only detected for the first time in the apical leaves of most ecotypes 15 dpi. The virus spread more rapidly in ecotypes

Table 1. TMV-Cg and TMV-U1 spread in different Arabidopsis ecotypes

<table>
<thead>
<tr>
<th>Ecotypes</th>
<th>Inoculated leaf</th>
<th>TMV-Cg</th>
<th>Apical leaf</th>
<th>Inoculated leaf</th>
<th>TMV-U1</th>
<th>Apical leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Be-0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Bla-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Btg-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bur-0</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>C-24</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Col-0</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hs-0</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>Ler-0</td>
<td>+</td>
<td>nt</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Mt-0</td>
<td>+</td>
<td>nt</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nie-0</td>
<td>+</td>
<td>nt</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>No-0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Po-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Uk-4</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Wei-0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>
Btg-2 and UK-4, reaching the apical leaves 9 dpi (Table 1). In Po-1, TMV-U1 was detected at 12 dpi (not shown). The apical leaves of ecotype Col-0 were positive for TMV-U1 at 15 dpi. No hypersensitive response was evident and no transcripts of the pathogenesis related protein PR-1 were detected (not shown). Our results are consistent with several studies that indicate that Arabidopsis ecotypes. This possibility has been suggested in several studies involving Arabidopsis ecotypes and viruses such as the oilseed rape mosaic tobamovirus (Martín et al. 1997), the tobacco etch virus (Mahajan et al. 1998), and TMV-U1 (Dardick et al. 2000).

**Local and systemic spread of the U1-MPCg hybrid virus in tobacco**

The differential spread of TMV-U1 and TMV-Cg in Arabidopsis may be explained by differences in the viral proteins of these two tobamoviruses. In particular, it is conceivable that differences in the structure of the movement proteins could account for differential rates of spread; therefore, we used the hybrid U1-MPCg to study the participation of TMV-Cg movement protein in viral spread. The hybrid virus was tested firstly in tobacco, the natural host of TMV-U1.

To eliminate possible differences in the rates of replication between the three viruses, tobacco BY-2 protoplasts were transfected with transcripts of the hybrid U1-MPCg, and the wild-types TMV-U1 and TMV-Cg, obtained by in vitro transcription. These preliminary experiments consistently showed similar rates of replication for the three viruses as estimated by CP accumulation (not shown). This suggests that replication of the hybrid virus was not affected by the changes imposed upon the last six amino acids of the replicase (Fig. 1). This result is in accordance with the previous demonstration that the last 16 nucleotides of the replicase and the sequence within the MP ORF are not essential for TMV-U1 replication, or for MP subgenomic RNA synthesis (Grdzelishvili et al. 2000).

Local movement of TMV-Cg, TMV-U1 and the hybrid U1-MPCg, was evaluated in Xanthi NN tobacco plants infected with the corresponding RNA transcripts. As expected, each of the three viruses gave rise to hypersensitive local lesions. Although TMV-U1 always appeared to produce a larger number of lesions than TMV-Cg and the hybrid, this was not statistically significant (Table 2). As a negative control, the mutant virus TMV-U1ΔMP, from which the MP ORF was deleted (Gafny et al. 1992), was used. Plants infected with TMV-U1ΔMP did not develop local lesions (Table 2). Similar results were obtained when the plants were infected with viral particles isolated from infected plants.

Differences in size of the local lesions are generally a reflection of the rate of cell-to-cell spread and viral replication (Hilf and Dawson 1993; Fenczik et al. 1995). Since we found small differences in local lesion size between the hybrid and TMV-U1 after 12 h of N gene inactivation (Table 2), we concluded that the hybrid has a slightly slower local movement than TMV-U1. Local lesions induced by TMV-Cg were of an intermediate size that was not different from the other two viruses. The lesions of the three viruses

**Table 2. Number and size of local lesions in Xanthi NN tobacco leaves inoculated with viral transcripts of TMV-Cg, TMV-U1 and the hybrid U1-MPCg**

<table>
<thead>
<tr>
<th></th>
<th>TMV-Cg</th>
<th>TMV-U1</th>
<th>U1-MPCg</th>
<th>U1-ΔMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>75 ± 20a</td>
<td>92 ± 17a</td>
<td>83 ± 23a</td>
<td>0</td>
</tr>
<tr>
<td>Size (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-48</td>
<td>1.2 ± 0.4a</td>
<td>1.4 ± 0.3ab</td>
<td>0.9 ± 0.2a</td>
<td>–</td>
</tr>
<tr>
<td>12-48</td>
<td>1.4 ± 0.3ab</td>
<td>2.0 ± 0.3b</td>
<td>1.1 ± 0.3a</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic representation of the cloned cDNA of TMV-Cg, TMV-U1 and the hybrid virus U1-MPCg. Replicase (126/183), movement protein (MP) and coat protein (CP) are indicated in boxes. Upper arrows indicate lambda phage T7 promoter and bottom arrows indicate subgenomic RNA promoters. The genomic sequences of the replicase end and the MP N-terminus are indicated. In the U1-MPCg hybrid the last six amino acids of the carboxyl end of the 183-kDa protein were modified to express the wild-type TMV-Cg movement protein. The putative viral assembly origin (AO) is shown.
were larger and of similar size when they were measured 72 h after a shift to 25°C (not shown).

Systemic movement was evaluated in sensitive Xanthi nn tobacco plants inoculated with isolated virions. Unexpectedly, TMV-Cg infection induced a few lesions in the inoculated leaf at 4 dpi (not shown). The symptoms induced by TMV-Cg were characterized by necrotic stripes in medial and apical leaves that intensified over time, with deformity of upper leaves by 20 dpi (Fig. 2). In contrast, the hybrid U1-MPCg induced the mosaic symptom and wrinkling of upper leaves characteristic of TMV-U1 (Fig. 2). The systemic spread of U1-MPCg in tobacco was compared with those of TMV-Cg and TMV-U1 by ELISA (Fig. 3A) and western blots (not shown). The accumulation rates of the three viruses were similar in the inoculated leaves, reaching a maximum 5 dpi. Systemic spread of TMV-Cg was much slower than that of TMV-U1, while U1-MPCg spread systemically at an intermediate rate. All three viruses reached maximum systemic accumulation between 11 and 13 dpi.

The efficiency of the local and systemic spread of the hybrid U1-MPCg in tobacco indicates that, as expected, the movement protein is being transcribed and complements TMV-U1 movement function. U1-MPCg viral particles isolated from infected tobacco plants exhibited the characteristic size and morphology of the tobamoviruses under electronic microscopy (not shown). This suggests that the functional TMV-Cg assembly origin is located in the MPCg RNA sequence as indicated in Fig. 1. In fact, it has been previously suggested that the crucifer-infecting tobamovirus subgroup, like the solanaceous-infecting subgroup, has its assembly origin in the MP reading frame (Lartey et al. 1996). In contrast, the group infecting cucurbits and legumes has the assembly origin in the CP gene (Lartey et al. 1996).

Local and systemic movement of the hybrid U1-MPCg in Arabidopsis

The spread of the hybrid virus U1-MPCg, compared with TMV-Cg and TMV-U1, was evaluated in the Arabidopsis ecotype Col-0, which permits only delayed movement of TMV-U1. ELISA analyses showed that all three viruses were present in the inoculated leaves, with TMV-U1 accumulation being lower than that of the other two viruses (Fig. 3). However, only TMV-Cg moved systemically in this ecotype. The fact that U1-MPCg failed to spread systemically

Fig. 2. Symptoms observed 20 dpi in sensitive tobacco plants (cv. Xanthi nn) infected with TMV-Cg (A) and U1-MPCg (B). A control (mock-inoculated) plant also is shown (C). TMV-Cg induces wrinkling, mosaic and necrotic spots. The hybrid virus U1-MPCg induces leaf wrinkling and mosaic, resembling symptoms characteristic of TMV-U1.
indicates that the presence of TMV-Cg MP sequence in the hybrid virus was not sufficient for an efficient systemic invasion of the plant. In our study, the maximal accumulation of TMV-Cg in apical leaves occurred at 15 dpi, which was similar to the results of Ishikawa et al. (1991). We also tested the spread of the hybrid virus U1-MPCg in Btg-2, an ecotype that permits rapid systemic spread of TMV-U1. As in the Col-0 ecotype, spread of U1-MPCg in Btg-2 was similar to that of TMV-U1 (not shown). Our results are similar to the results of Zhang et al. (1999), who replaced TMV-U1 movement and coat proteins with the corresponding proteins of the crucifer-infecting turnip vein-clearing virus (TVCV). These authors failed to detect systemic movement of the chimeric viruses in turnip plants.

Since both TMV-Cg and TMV-U1 were detected in the inoculated leaves, but only TMV-Cg efficiently established a systemic infection in leaves of all Arabidopsis ecotypes, we wondered if a restriction in the local movement of U1-MPCg was linked to its failure to spread systemically. To study local virus movement, in situ hybridization experiments were conducted in the inoculated leaves to detect viral CP RNA of U1-MPCg and TMV-U1. On inoculated leaves, both viruses behaved similarly to TMV-Cg; they began to move from infected cells initially, and then to neighboring tissues (Fig. 4). Circular foci, 2–3 mm in diameter, were seen at 3 dpi. The fact that these foci grew in size and spread throughout the inoculated leaf by 10 dpi, indicates that all three viruses were capable of efficient cell-to-cell spread. Thus, our results do not support the hypothesis that a restriction in local movement underlies the failure of U1-MPCg and TMV-U1 to spread systematically in Arabidopsis.

It has been proposed that the immunity of most plant species to viruses is due to compatibility requirements between the viral proteins and the host factors present in the plant. It is possible that active defense mechanisms, such as post-transcriptional gene silencing, are always operating in the plant, and that the only virus/host combinations leading to infection are those in which the virus can overcome these mechanisms (Waterhouse et al. 2001). Several lines of evidence indicate that RNA silencing is a general anti-viral defense mechanism in plants, and it has been demonstrated that certain plant viruses encode proteins that suppress this silencing (Vance and Vaucheret 2001; Mlotshwa et al. 2002). The delayed spread of TMV-U1 into systemic tissues could be due to a silencing response of Arabidopsis. However, we were able to detect viral RNA in inoculated leaves (Fig. 4) and in apical leaves (not shown) from 15 dpi until the plants became senescent. A different active response may also be operating, since a proteolytic cleavage of MP has been detected during the TMV-U1 infection of Arabidopsis (Hughes et al. 1995; Dardick et al. 2000).

The failure of U1-MPCg and of TMV-U1 to spread systemically in the Arabidopsis ecotypes used in this study may result from obstacles in the entrance to, transport through, or exit of the viruses from the plant vascular system. An Arabidopsis mutant that restricts systemic movement of the TVCV and TMV-L tobamoviruses has been reported (Lartey et al. 1998). This mutant is thought to lack a key factor for the entrance of the virus into vascular tissue. In this respect, the participation of at least two genes in modulation of susceptibility to TMV-U1 has been described in Arabidopsis (Dardick et al. 2000). The interaction between host factors and viral proteins is currently a topic of great interest. Recently, unconventional functions for viral proteins have been postulated. For instance, the replicase–helicase domain has been reported to participate in viral spread (Hirashima and Watanabe 2001). In the case of tomato bushy stunt virus it is likely that coat protein enables efficient unloading of assembled virions from the vascular tissue (Qu and Morris 2002). We have initiated genetic studies designed to detect host factors related to the delay of TMV-U1 movement in Arabidopsis ecotypes with different levels of susceptibility to the virus.
Acknowledgments

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Fig. 4. (A) Local and systemic spread of the viruses TMV-U1, U1-MPCg and TMV-Cg in Arabidopsis. Viral coat protein RNA accumulation was detected by leaf skeleton hybridization. Inoculated leaves at 5 and 10 dpi show the local spread of the three viruses. (B) Apical leaf of U1-MPCg-inoculated plant at 10 dpi (that looks identical to TMV-U1-infected plants at this time) without coat protein RNA signal, and apical leaf of TMV-Cg-infected plants at 10 dpi. (C) Control (mock-inoculated) leaf.


