Production of the tobacco mosaic virus (TMV) transport protein in transgenic plants is essential but insufficient for complementing foreign virus transport: a need for the full-length TMV genome or some other TMV-encoded product

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We have reported previously that tobamoviruses enable the transport of red clover mottle comovirus (RCMV) in tobacco plants normally resistant to RCMV. Here we show that RCMV transport does not take place in transgenic tobacco plants (line To-4) producing the 30K transport protein of tobacco mosaic virus (TMV), whereas the transport of the TMV Ls1 mutant, the cell-to-cell movement of which is temperature sensitive, is complemented in these plants. However, RCMV transport is observed when these transgenic plants are infected with both RCMV and TMV Ls1 at the non-permissive temperature (33 °C). It is suggested that (i) the hypothetical modification of transgenic plant plasmodesmata by the TMV 30K transport protein can specifically mediate the cell-to-cell movement of the homologous virus (TMV), but is insufficient to mediate RCMV transport; (ii) the presence of the full-length TMV genome or a certain TMV-encoded product(s) besides the 30K protein is essential for complementation of the RCMV transport function. The possibility that line To-4 might provide enough 30K protein to complement TMV Ls1 but not RCMV cannot be ruled out. During double infection the mutant 30K protein may, in concert with the wild-type 30K protein, provide the transport function for RCMV.

It is generally recognized that cell-to-cell movement of a plant virus in an infected plant requires a particular virus-encoded transport function. Virus-specific transport proteins (TPs) are thought to be actively involved in expressing this transport function (for reviews see Hull, 1989; Atabekov & Taliansky, 1990). With tobacco mosaic virus (TMV), there is strong evidence that the 30K protein is responsible for this process (Moshi et al., 1987; Deom et al., 1987). Comparison of the amino acid sequences of the putative TPs of different plant viruses reveals substantial variability in their structure, although certain sequence similarities allow plant viruses to be tentatively divided into several groups (Hull, 1989; Atabekov & Taliansky, 1990). The structural diversity among the TPs probably reflects the host specificity of the plant viruses, but also indicates that there may be several mechanisms by which they mediate cell-to-cell movement.

TMV infection moves from cell to cell through plasmodesmata. The structure of these channels apparently does not allow the virus to pass and must therefore be modified, probably by a virus-encoded TP. Although the precise mode of action of the TMV 30K protein is obscure, it has been suggested that it interacts with a putative host factor(s) near or in the plasmodesmata, thereby increasing their permeability (Wolf et al., 1989; Atabekov & Taliansky, 1990; Deom et al., 1990; Atkins et al., 1991). Tobamoviruses are believed to move through modified plasmodesmata in the form of virus-specific ribonucleoproteins (vRNP), structurally different to virus particles (Dorokhov et al., 1984).

Quite a different transport mechanism appears to be used by comoviruses, although the putative 58K/48K transport protein of cowpea mosaic virus (CPMV, the type member of the comovirus group) has some similarity to the TMV 30K protein (Meyer et al., 1986), and, moreover, the 58K/48K TP of another comovirus [red clover mottle virus (RCMV)] has been found associated with plasmodesmata (Shanks et al., 1989), similarly to TMV TP. It has been suggested that, unlike those of tobamoviruses, CPMV TP is effective only for virus particles (Wellink & van Kammen, 1989), and that CPMV moves through specific tubular structures generated after comovirus infection (van Lent et al., 1990). Despite the differences in the transport mechanisms of tobamoviruses and comoviruses, the former have re-
recently been shown to complement the transport of RCMV in tobacco plants which do not support the transport of RCMV alone (Malysheenko et al., 1988, 1989). Moreover, in cowpea plants sunnhemp mosaic tobamovirus complements the transport of RCMV B-RNA (Malysheenko et al., 1988), which does not encode either the transport (58K/48K) protein or the coat protein (these genes are located on the M-RNA of RCMV) and is therefore unable to pass from cell to cell alone (Rezelman et al., 1982). It appears that the TP of a helper tobamovirus can promote transport not only of its own genome, but also of the genome of a helper-dependent comovirus (which naturally uses a different mechanism).

Thus, the presence of a functionally active TP encoded by a helper tobamovirus is a prerequisite for complementation of RCMV transport. However, the question is bound to arise whether this is sufficient or whether some other products or functions need to be provided by the complete helper virus.

To answer this question, experiments on complementation between TMV and RCMV were performed using transgenic tobacco plants expressing the TMV 30K protein. TMV 30K protein gene cDNA to TMV strain U1 RNA was synthesized by priming using a synthetic oligonucleotide complementary to nucleotides 5745 to 5766 of TMV RNA. This cDNA contained additional nucleotides which, when in the double-stranded form, were inoculated with the helper tobamovirus which confers resistance to TMV 30K protein the Lsl mutant failed to complement RCMV; however, RCMV accumulation and transport were observed when such plants were infected with both RCMV and TMV Lsl (Table 1). In some experiments
the dependent virus (RCMV) as well as TMV Ls1 could be detected not only within the inoculated leaves but also in non-inoculated, systemically infected ones. Thus, systemic spread of RCMV was promoted by the TMV mutant in transgenic plants producing the temperature-resistant 30K TP.

One can suggest that primary modification of plasmodesmata by the TMV 30K TP (Wolf et al., 1989; Atkins et al., 1991) is necessary but insufficient for comovirus transport, and that transport of a comovirus (RCMV) requires some additional TMV-specific factor(s). However, from these data we can conclude only that the spread of RCMV from cell to cell in tobacco plants becomes possible after or concurrently with the transport of TMV; the minimal set of conditions has not been determined. It is also unclear whether the entity transported in this case is the comovirus particle or just naked RCMV RNA non-specifically trapped by the TMV vRNP.

We cannot rule out the possibility that line To-4 might provide enough 30K protein to complement TMV Ls1, but not RCMV. During the double infection, the temperature-sensitive 30K protein may, in combination with the wild-type 30K protein, provide the transport function for RCMV.

References


Table 1. Accumulation and transport of RCMV in transgenic tobacco plants containing the TMV 30K protein gene in the presence or absence of TMV Ls1*

<table>
<thead>
<tr>
<th>Plant line</th>
<th>Production of the 30K protein</th>
<th>Helper virus</th>
<th>Inoculated leaves†</th>
<th>Non-inoculated leaves§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Expt. 1</td>
<td>Expt. 2</td>
</tr>
<tr>
<td>To-4</td>
<td>+</td>
<td>Ls1</td>
<td>180</td>
<td>1060</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>None</td>
<td>≤ 20</td>
<td>≤ 20</td>
</tr>
<tr>
<td>Tk-1</td>
<td>−</td>
<td>Ls1</td>
<td>≤ 20</td>
<td>≤ 20</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>None</td>
<td>≤ 20</td>
<td>≤ 20</td>
</tr>
</tbody>
</table>

* All experiments were done at 33°C (non-permissive temperature for TMV Ls1 cell-to-cell transport).
† Assayed 7 days after inoculation.
§ Assayed in upper systemic (non-inoculated) leaves 25 days after inoculation of lower leaves.
SHORT COMMUNICATION


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