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

M. E. Taliansky, S. I. Malyshenko, I. B. Kaplan, O. A. Kondakova and J. G. Atabekov*

Department of Virology and A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, U.S.S.R.

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 (Mesity 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-
The leaves of rooted cuttings were mechanically inoculated with TMV Lsl (70 μg/ml) and 5 days later the amount of virus in inoculated leaves was determined by direct double antibody sandwich (DAS) ELISA (Clark & Adams, 1977) with antibodies to TMV, using serial dilutions of the virus as concentration standards and the Tk-1 plant line (transformed with pBIN19 alone) as a control. The highest efficiency in complementing TMV Lsl was exhibited by line To-4 (2100 ng/g of TMV Lsl accumulation in infected leaf tissue, ≤20 ng/g in control), suggesting that this line produced a functionally active 30K protein. Data from immunoblot analysis using antibodies against an 11 amino acid oligopeptide corresponding to the carboxy terminus of the TMV U1 30K protein (not shown) confirmed that this line produced the TMV 30K protein; it comigrated with the 30K TP produced in virus-infected leaf tissue. The plant line To-1 also complemented the Lsl mutant at 33 °C, but this effect was not reproducible, and the accumulation of TMV Lsl in this case was never higher than 200 ng/g leaf tissue. The other three transgenic lines (To-1, To-2 and To-5) did not complement TMV Lsl at all. In accordance with these data, immunoblot analysis revealed only trace (if any) amounts of 30K protein in lines To-1, To-2, To-3 and To-5. Therefore, To-4 was chosen as being most suitable for subsequent cell-to-cell transport complementation experiments.

The leaves of rooted cuttings were inoculated with 500 μg/ml RCMV alone (control) or with a mixture of 500 μg/ml RCMV and 70 μg/ml TMV Lsl. All experiments were done at 33 °C. Accumulation of RCMV in inoculated and non-inoculated leaves was assessed by DAS ELISA. The antiserum used in these experiments was generated against a purified preparation of RCMV. As expected, in the control Tk-1 plants producing no 30K protein the Lsl mutant failed to complement RCMV at the non-permissive temperature (Table 1); this was in accord with the idea that a functional TMV 30K protein is essential for complementing the comovirus movement function. However, transgenic To-4 plants, which did produce the temperature-resistant TMV 30K protein, also 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 Lsl 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 Lsl, 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 Lsl*

<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>Expt. 1</td>
<td>Expt. 2</td>
<td>Expt. 3</td>
</tr>
<tr>
<td>To-4</td>
<td>+</td>
<td>Lsl</td>
<td>≤ 20</td>
<td>≤ 20</td>
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<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Tk-1</td>
<td>-</td>
<td>Lsl</td>
<td>≤ 20</td>
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<tr>
<td></td>
<td>None</td>
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</tbody>
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* All experiments were done at 33 °C (non-permissive temperature for TMV Lsl cell-to-cell transport).
† ELISA data averaged for three or four plants.
‡ Assayed 7 days after inoculation.
§ Assayed in upper systemic (non-inoculated) leaves 25 days after inoculation of lower leaves.

References


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