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 (Meshe 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 stucture 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-
ciently been shown to complement the transport of RCMV in tobacco plants which do not support the transport of RCMV alone (Malyshenko et al., 1988, 1989). Moreover, in cowpea plants sunnhemp mosaic tobamovirus complements the transport of RCMV B-RNA (Malyshenko 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, generated a EcoRI site. After synthesis the second strand DNA was cut with EcoRI (TMV cDNA has an additional natural EcoRI site at nucleotide 4254) and cloned into the EcoRI site of plasmid pUC19. Then, the TMV sequence from the plasmid obtained (pUC30K) was excised with Eco31.I (which cleaves at TMV nucleotide 4745) and EcoRI. The fragment obtained (TMV nucleotides 4745 to 5766), including the 30K TP gene, was ligated into the pRT104 plant expression vector (Topfer et al., 1987) at the XhoI and EcoRI sites in the polylinker region between the 35S promoter of cauliflower mosaic virus and the polyadenylation signal of the nopaline synthase gene. The HindIII fragment of the resulting plasmid, pRT30K, containing the chimeric gene, was inserted into the HindIII site of the pBIN19 plasmid of the binary vector system (Bevan, 1984). The final plasmid, pBIN30K, contained the gene for neomycin phosphotransferase II, which confers resistance to the antibiotic kanamycin, and the gene encoding the 30K protein of TMV U1; it was conjugated into Agrobacterium tumefaciens LA4404, which was used as a non-oncogenic helper strain in the binary vector system. Leaf discs of Nicotiana tabacum cv. Petit Habana SR1 were inoculated with the A. tumefaciens thus obtained. Transformed plant cells were selected for kanamycin resistance and regenerated into plants as described by Horsch et al. (1985), yielding five different transgenic lines.

Deom et al. (1987) have shown that transgenic tobacco plants expressing the TMV 30K protein gene can complement a TMV mutant, Ls1, which is temperature-sensitive for the cell-to-cell movement function (Nishiguchi et al., 1977, 1980). Therefore, we tested the ability of all our transgenic plant lines to support the transport of TMV Ls1 at the non-permissive temperature of 33 °C. The leaves of rooted cuttings were mechanically inoculated with TMV Ls1 (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 Ls1 was exhibited by line To-4 (2100 ng/g of TMV Ls1 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 Ls1 mutant at 33 °C, but this effect was not reproducible, and the accumulation of TMV Ls1 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 Ls1 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 Ls1. 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 Ls1 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 Ls1 (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


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