The Informosome-like Virus-Specific Ribonucleoprotein (vRNP) May Be Involved in the Transport of Tobacco Mosaic Virus Infection

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A new type of informosome-like virus-specific ribonucleoprotein (vRNP) differing from mature tobacco mosaic virus (TMV) particles in buoyant density and structure was found in TMV-infected cells (Yu. L. Dorokhov, N. M. Alexandrova, N. A. Miroshnichenko, and J. G. Atabekov, 1983, Virology 127, 237-252). Two groups of TMV ts mutants were used to discover whether there is a correlation between the vRNP formation and systemic spreading of virus infection (transport) over the infected plant. The first group of mutants (Nill18, flavum) contains a ts mutation in the coat protein gene but are capable of systemic spreading at nonpermissive temperature (tr transport); the second group of mutants (Nill2619, Lsl) cannot spread systemically at restrictive temperature (ts transport). It is shown that vRNP can be produced at restrictive temperature by tr-transport mutants but not by ts-transport mutants. The latter can produce vRNP only at a permissive temperature. The role of vRNP in long-distance transport of the virus infection is supported by two other observations: (a) upper leaves that were maintained at 5°C accumulate potentially infective material and material with the properties of vRNP but not virus particles and (b) plants that were simultaneously infected with Lsl and Nill18 at a nonpermissive temperature exhibited long-distance transport and vRNP. These results also implicate coat protein in long-distance transport. It is suggested that vRNPs are novel types of virus-specific particles that are involved in both cell-to-cell and long-distance transport of TMV infections.

INTRODUCTION

It has been recently shown (Dorokhov et al., 1983a) that virus-specific informosome-like ribonucleoprotein (vRNP) particles are produced in TMV-infected plants. vRNP differed from mature TMV particles in buoyant density, in electron microscopic appearance, and in that the vRNP was more sensitive to ribonuclease. The vRNP particles appear to be TMV specific because they are synthesized in the presence of actinomycin D (AMD) and contain RNAs identified as genomic and a set of the intermediate-size (I class) subgenomic RNAs (Dorokhov et al., 1983a). It was also shown (Dorokhov et al., 1983b) that aurintricarboxylic acid, a well-known inhibitor of protein synthesis, markedly stimulates both the synthesis of TMV-specific subgenomic RNAs and vRNP production. EDTA treatment of TMV-induced polyribosomes releases vRNP, suggesting its involvement in translation; pulse-chase experiments showed that vRNP does not serve as a precursor for the mature virion (Dorokhov et al., 1983b). It has been suggested by Dorokhov et al. (1983a) that TMV vRNP particles are involved in the systemic spreading (transport) of infection over the plant, i.e., that vRNP serves as a transport form of the virus genetic material.

TMV mutants have been used in the present work to find out whether there is a correlation between vRNP formation and the systemic spreading of virus infection. The first group of mutants (Nill18, flavum)
contain a ts mutation in the coat protein gene but are capable of systemic spreading at nonpermissive temperature (33°C) (Jockusch, 1964, 1966a, b; Wittman-Liebold et al., 1965), i.e., they are temperature resistant (tr) in the transport function. Therefore Ni118 and flavum can be further designated as "ts coat protein; tr transport." The second group of mutants (Ni2519, Ls1) cannot spread systemically at restrictive temperature (ts transport). Ls1, a ts mutant of tomato TMV strain L (Nishiguchi et al., 1978, 1980), produces tr coat protein, but is unable to spread from cell to cell at restrictive temperature (i.e., it can be designated as "tr coat protein; ts transport"). Ni2519, a ts mutant of TMV tr-strain A14, produced tr coat protein and can replicate RNA at nonpermissive temperature (Jockusch, 1968; Bosch and Jockusch, 1972). Two different functions, i.e., virus assembly (owing to the ts properties of the genomic RNA molecule) and spreading of virus from cell to cell, are ts in Ni2519 (Atabekov and Taliansky, 1960; Taliansky et al., 1982a, b; Kaplan et al., 1982). Ni2519 is further designated as "tr coat protein, ts transport, ts assembly."

The long-distance movement of virus infection through the plant has been studied under the differential-temperature-treatment (DTT) conditions (Dawson et al., 1975; Dorokhov et al., 1981) in which lower leaves of a tobacco plant are mechanically inoculated with TMV and kept at a temperature (25°C) that permits normal virus replication whereas upper leaves are placed into a cold chamber (5°C), whereas the rest of the plant was maintained at 25°C. The plants were under continuous lighting of 1000 lx. The upper uninoculated leaves were kept at 3-5°C throughout the DTT and labeling. Prior to labeling, the plants with four to six mechanically inoculated lower leaves were subjected to DTT for 72-96 hr. During DTT these plants were labeled through the stems with 1 mCi/ml [3H]uridine (2 mCi/plant), 100 µg/ml AMD (1 mg), and 100 µg/ml rifampicin (1 mg), (Sigma, USA) or with 100 µCi/ml of a 14C-amino acid mixture (0.2 mCi) and also AMD and rifampicin at a concentration of 100 µg/ml. Healthy plants maintained under the DTT conditions were labeled in the same way. After 24-hr labeling, the upper leaves were cut off and homogenized in the cold in the presence of the extracting buffer solution as described previously (Dorokhov et al., 1983a).

Biochemical testing of the complementation of long-distance transport using TMV mutants Ls1 and Ni118. The lower leaves of tobacco plants were mechanically inoculated with (a) Ls1, (b) Ni118, and (c) a mixture of Ls1 and Ni118, after which the plants were kept for 2 days at 25°C and then for 2 days at 33°C. To detect vRNP, samples of lower (mechanically inoculated) and upper (uninoculated) leaves were labeled with [3H]uridine (50 µCi/ml) in the presence of AMD (100 µg/ml) for 18 hr at 33°C. After labeling the samples were homogenized and vRNP was isolated as described elsewhere (Dorokhov et al., 1983a).
RESULTS

Analysis of vRNP Produced by Different TMV Strains

The first series of experiments (Figs. 1-4) showed vRNP formation in tobacco infected with different TMV \textit{ts} mutants. For Nil18 and \textit{flavum} (\textit{ts} coat protein, \textit{tr} transport), it can be seen from Figs. 1 and 2 that vRNP (buoyant density, 1.38-1.43 g/cm\textsuperscript{3}) and TMV (buoyant density about 1.32 g/cm\textsuperscript{3}) are produced at permissive temperature (25°) and can be seen after different times of labeling. On the other hand, no virus material can be found at nonpermissive (33°) temperature because of coat protein denaturation. The only virus-specific material (labeled in the presence of AMD) was represented by vRNP. No such material could be observed in the healthy control (Figs. 1C and F).

Lsl (\textit{ts} transport, \textit{tr} coat protein) and Ni2519 (\textit{ts} transport, \textit{tr} coat protein, \textit{ts} assembly), TMV mutants \textit{ts} in the transport function, produced vRNP only at permissive temperatures (Figs. 3A and C; Figs. 4A and B). Little, if any, labeled material with the buoyant density similar to that of vRNP could be isolated from plants infected at 33° with TMV mutants temperature sensitive in the transport function.

Transport of vRNP to the Upper Uninoculated Leaves of Tobacco Plants from the Lower Leaves Systemically Infected with TMV under the DTT Conditions

Recently we have shown that under the DTT conditions the potentially infective vRNP could be isolated from plants infected at 33° with TMV mutants temperature sensitive in the transport function.
vRNP formed in the TMV-infected cells of the lower leaves was transported to the upper leaves, assuming no synthesis in tissue maintained at 5°. However, it cannot be excluded that free viral RNA was transported along the stem of the infected plant and in the upper leaves it complexed with protein. To test this, the above experiment was carried out with 14C-amino acids instead of [3H]uridine. Most of the radioactivity in the upper leaves of an inoculated plant occurred in the material with a buoyant density of 1.40 g/cm³ (Figs. 5C and D), with a less prominent zone at 1.46 g/cm³. These experiments support the notion, but do not prove, that vRNP, rather than free RNA, was transported and accumulated in the upper leaves.

Numerous experiments of this type have been performed. The material with the buoyant density similar to that of vRNP (1.36-1.47 g/cm³) was invariably found in the upper leaves un inoculated and systemically infected at 5° under the DTT conditions. Practically no nucleoprotein could be detected in the control (upper leaves of the healthy plant under DTT).

These results led us to suppose that material is accumulated in the vascular system and in the basal areas of upper un inoculated leaves (Dorokhov et al., 1981).

Figure 5 shows the results of experiments on the detection of vRNP in the uninoculated upper tobacco leaves of plants systemically infected with TMV under the DTT conditions. Tobacco plants with TMV-infected lower leaves were subjected to DTT for 72 hr and then labeled with [3H]uridine through the stem in the presence of AMD and rifampicin for 24 hr. A considerable amount of 3H-labeled vRNP could be revealed in the upper uninoculated leaves (Fig. 5A). No material with the buoyant density of vRNP could be found in control experiments with healthy plants labeled under the same conditions in the presence of the antibiotics (Fig. 5B).

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Complementation of the Long-Distance Transport of Viral Infection

It is known that all the TMV mutants defective in the coat protein spread within the inoculated leaves but usually cannot move into the upper and lower uninoculated leaves (Siegel et al., 1962). There are grounds for believing that the coat protein in the transport form (vRNP) is needed to protect viral RNAs during the long-distance transport. To test this suggestion, experiments were run on the complementation of the long-distance transport of viral infection. To this end, plants were simultaneously infected with two TMV mutants: Lsl (ts in transport but tr in the coat protein) and Ni118 (ts in the coat protein and in long-distance transport but tr in short-distance transport).

We found considerable amount of $^{3}H$-labeled vRNP in the upper leaves of tobacco plants mixedly infected with Ni118 and Lsl; no such material was seen in control singly infected with Ni118 or Lsl (Fig. 6).

In the present experiments, vRNP found in the lower leaves of plants simultaneously infected with Lsl and Ni118 contain the coat protein and are more resistant to RNase than those of Ni118 (data not shown). It should be noted that the viral RNA was considerably more sensitive to ribonuclease attack in the coat-protein-free vRNP of Ni118 than in vRNP of *vulgare*, which contains the coat protein (Dorokhov et al., 1983a).

**DISCUSSION**

The existence of TMV mutants *ts* in spreading over the infected plant suggests that some particular virus-specific (i.e., coded by the virus genome) protein(s) are responsible for the transport function. Recently it has been shown by Talianisky *et al.* (1982c, d) that the transport function can be complemented by a temperature-resistant helper TMV strain in dually infected plants. The molecular mechanism of this function is obscure. It is possible

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**Fig. 6.** Biochemical testing of the complementation of long-distance transport between TMV mutants Lsl and Ni118. Lower leaves of tobacco plants were inoculated with (A) Lsl, (B) Ni118, and (C) Lsl + Ni118, and the plants were kept for 2 days at 25°C and then for 2 days at 33°C. Samples of lower inoculated and upper uninoculated leaves were labeled with $[^{3}H]$uridine (50 μCi/ml) in the presence of AMD (100 μg/ml) for 18 hr at 33°C, and vRNP was isolated as in Dorokhov *et al.* (1983a). (A–C) CsCl gradient diagrams of vRNP synthesized in the lower leaves inoculated with (A) Lsl, (B) Ni118, and (C) Lsl + Ni118; (E–G) the same for upper uninoculated leaves of plants infected with (E) Lsl, (F) Ni118, and (G) Lsl + Ni118. (D, H) Healthy controls.
that a TMV-specific transport protein somehow affects the infected cell (for example, modifies the plasmodesmata or increases their number) (Wieringa-Brants et al., 1981; Sulzinski and Zaitlin, 1982) and/or performs direct complexing with TMV RNA.

vRNP is produced at 25 and 33° by TMV mutants (Ni118 and flavum) temperature resistant in spreading infection from cell to cell (tr in transport function). On the contrary, no (or very little) vRNP can be produced by ts mutants in the transport function (Ls1 and Ni2519) at restrictive temperatures (Figs. 3 and 4). This correlation between the blockage of the transport function and the absence of vRNP at restrictive temperature allows one to suggest that vRNP is involved in the transport of virus genetic material from cell to cell. It was suggested that some virus-specific protein(s) should be responsible for the transport of TMV genetic material (genomic and/or subgenomic RNAs) upon the movement of infection over the plant (Atabekov and Morozov, 1979; Leonard and Zaitlin, 1982). It seems possible that this transport protein(s) can be structural component(s) of vRNP. Further, it can be speculated that the transport protein(s) produced by TMV mutants which are ts in transport function is nonfunctional at high temperature and therefore is incapable of being incorporated into vRNP. However, this would not necessarily cause the disappearance of vRNP from the infected cell since vRNP contains not only virus-specific but also host-cell proteins (Dorokhov et al., 1983a). The reason for the absence of vRNP from plants infected with TMV ts-transport mutants seem to be more complicated than just the inability of the transport protein to form a complex with TMV-specific RNAs.

DTT is actually the only technique of relatively synchronous infection of a plant with the virus in vivo (Dawson et al., 1975; Dorokhov et al., 1981). The potentially infective material, termed previously the "infectious entity," moves under the DTT conditions into and spreads about in the basal areas of upper uninoculated leaves kept at low temperature, and then into the distal areas of these leaves and the basal areas of the lower uninoculated leaves (Dorokhov et al., 1981).

The presence in the upper uninoculated tobacco leaves of the nucleoprotein material (labeled with either [3H]uridine or [14C]-amino acids) having the buoyant density similar to that of vRNP (Fig. 5) allows one to suggest that vRNP is preformed at 25° in lower inoculated leaves and moves to upper uninoculated leaves under the DTT conditions. Thus we assume that vRNP serves as a universal transport form of the virus genetic material in the long-distance movement of virus infection.

TMV mutants defective in the coat protein spread from cell to cell but usually remain within the inoculated leaves. The long-distance spreading of the infection from the lower inoculated leaves to the upper ones occurs extremely slowly (Siegel et al., 1962). The deep suppression of the long-distance transport is characteristic of all TMV mutants defective in the coat protein. It was supposed that the long-distance transport of the unstable form is impossible because the viral RNA is more readily degraded in the vascular system than in the parenchymal cells (Siegel et al., 1962). The situation changes radically on assuming that the transport form is not free RNA but vRNP.

Dorokhov et al. (1983a) recently showed that the coat protein is a major structural component of vRNP formed by TMV vulgar and by TMV ts mutants at permissive temperature. On the other hand, the coat protein was shown to be absent from the vRNP of TMV ts coat protein mutants at nonpermissive temperature. It is important to note that the lack of the coat protein in vRNP is not accompanied by a loss of short-distance transport of these mutants, i.e., the presence of the coat protein is not crucial for the short-distance transport. Conversely, there are grounds for believing that the coat protein in the transport form (vRNP) is needed to protect viral RNAs during the long-distance transport. To test this suggestion, experiments were run on the complementation of the long-distance transport of viral infection. To this end, plants were simultaneously infected with
Ls1 and Ni118. It was expected that during mixed infection Ls1 will make the tr coat protein which will be incorporated into the vRNP of the ts mutant Ni118 instead of its homologous coat protein at 33°. As a result, the Ni118 RNA will be protected from RNase attack in mixed vRNP and will therefore be able to participate in long-distance transport.

In agreement with this suggestion, we found considerable amounts of 3H-labeled vRNP in the upper leaves of tobacco plants mixedly infected with Ni118 and Ls1: no such material was seen in controls singly infected with Ni118 or Ls1 (see Fig. 6).

It should be borne in mind that under such conditions the complementation could be reciprocal: first, it was expected that the mixed Ni118 vRNP having acquired the Ls1 coat protein would be involved in long-distance transport, and second, it was less possible but not excluded that vRNP of Ls1 itself could spread in the presence of Ni118. However, in any case the results presented in Fig. 6 imply that vRNP formed in the lower leaves is under such experimental conditions transported to the upper leaves, i.e., that vRNP is involved in the long-distance transport.

REFERENCES


plants: In some resistant species virus is confined to a small number of initially infected cells. *Virology* 121, 12–19.


