Evidence that Potato Leafroll Virus RNA is Positive-stranded, is Linked to a Small Protein and Does Not Contain Polyadenylate

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SUMMARY

RNA extracted from particles of potato leafroll virus (PLRV) infected tobacco mesophyll protoplasts. Treating the RNA with proteinase K did not abolish its infectivity. In messenger-dependent rabbit reticulocyte lysate, PLRV RNA induced the synthesis of specific polypeptides: a major product of mol. wt. 71,000 but no product the size of coat protein. PLRV RNA is therefore positive-stranded. A genome-linked protein (apparent mol. wt. 7000) was detected in preparations of PLRV RNA but no polyadenylate sequence was found. These features may prove to be characteristic of luteoviruses.

Although the luteovirus group includes several economically important viruses (Rochow & Duffus, 1981), there is relatively little information about their genome nucleic acids or replication strategy. This is because the concentration of virus particles in plants is low, and it has been difficult to obtain the amounts needed for biochemical tests. The introduction of an improved method of purifying the particles of two luteoviruses (Takanami & Kubo, 1979a), which increases the yield about fivefold, has enabled their particles and particle components to be characterized in more detail. Potato leafroll virus (PLRV) particles contain single-stranded RNA of mol. wt. about $2 \times 10^6$ (Rowhani & Stace-Smith, 1979; Takanami & Kubo, 1979b), and are serologically related to those of several other luteoviruses (Roberts et al., 1980). In this paper we describe the infectivity and translation products of PLRV RNA, and the results of tests for genome-linked protein and for polyadenylate sequences.

Particles of a Scottish isolate of PLRV were purified from infected leaves of potato cv. Cara or Physalis floridana as described by Tamada & Harrison (1980). RNA was extracted by suspending pellets of virus particles in 2% (w/v) SDS in 1 mM-EDTA, 0.01 M-Tris-HCl pH 9, heating at 60 °C for 10 min and then mixing with an equal volume of a mixture of water-saturated phenol and m-cresol (9:1, v/v), containing 0.1% 8-hydroxyquinoline. The aqueous phase was re-extracted with the phenol mixture, and RNA was precipitated by adding 2.5 vol. ethanol, then dissolved in 0.5% SDS, 0.15 M-sodium acetate pH 6, and re-precipitated with ethanol. Before use as inoculum, or in translation experiments, the RNA was dissolved in 0.01 M-tris–HCl, 0.05 M-NaCl pH 7.6, precipitated again with ethanol, and the RNA precipitate was washed with absolute ethanol before dissolving it in water.

Although PLRV infectivity cannot be assayed by mechanical inoculation of test plants, Kubo & Takanami (1979) infected tobacco protoplasts by inoculation with purified preparations of PLRV particles. We tested the infectivity of PLRV RNA by inoculating protoplasts, which were isolated from leaves of Nicotiana tabacum cv. Xanthi as described by Kubo et al. (1975), with the RNA at 50 to 100 μg/ml, using the polyethylene glycol method described by Maule et al. (1980). The washed protoplasts were cultured for 2 days at 21 °C in continuous light of 3000 lux. Samples of $10^5$ protoplasts were then triturated in 1 ml 2% polyvinylpyrrolidone + 0.05% Tween-20 + 0.15 M-NaCl in 0.02 M-phosphate buffer pH 7.4, and their PLRV antigen content was assayed by enzyme-linked immunosorbent assay as described by Tamada & Harrison (1980).
PLRV RNA infected the protoplasts but the yield of PLRV antigen differed considerably between experiments, with a maximum equivalent to 6 μg virus particles per 10⁶ protoplasts. When the RNA was treated with 0.2 mg/ml proteinase K for 16 h at 37 °C as described by Harrison & Barker (1978), it remained infective for protoplasts.

To test whether it acts as an mRNA, 0.6 to 1.2 μg PLRV RNA in 2 μl water was mixed with 10 μl rabbit reticulocyte lysate (nuclease-treated, messenger-dependent; Amersham International) and 1 μl (45 μCi) [35S]methionine (600 Ci/mmol; Amersham International); no attempt was made to optimize the system. After incubation at 30 °C for 60 min followed by treatment with ribonuclease A and EDTA (Shih et al., 1978), the radioactive polypeptides were analysed by electrophoresis in 8% polyacrylamide gels using a discontinuous buffer system (Laemmli, 1970). An autoradiogram of the translation products is illustrated in Fig. 1(a). A major translation product of mol. wt. 71 000 was obtained consistently. In some experiments minor products, the largest of mol. wt. about 125 000, were visible. No PLRV-specific product was evident in the position of coat protein either in these experiments or when [3H]lysine was used instead of [35S]methionine. Although electrophoresis in polyacrylamide–agarose gels indicated that the only major RNA component was of mol. wt. about 1.9 × 10⁶, the preparations may have contained an active mRNA as a minor
Table 1. Specific radioactivities of $^{125}$I-labelled RNA

<table>
<thead>
<tr>
<th>Source of RNA</th>
<th>Buffer</th>
<th>Pronase</th>
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<tbody>
<tr>
<td>Potato leafroll virus</td>
<td>39857†</td>
<td>22736</td>
</tr>
<tr>
<td>Tomato black ring virus</td>
<td>26569</td>
<td>1018</td>
</tr>
<tr>
<td>Tobacco mosaic virus</td>
<td>1030</td>
<td>1326</td>
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</tbody>
</table>

* Samples of about 5 µg RNA were incubated for 16 h at 37 °C in 0.5% SDS + 0.15 M NaCl + 0.015 M sodium citrate pH 7.7 with or without Pronase (Koch-Light) at 1 mg/ml. RNA was recovered by repeated precipitation in ethanol.

† Ct/min/µg RNA, obtained by counting in Biofluor.

To test for a protein linked to PLRV RNA, the RNA preparations were iodinated with Na$^{125}$I and chloramine T, and the iodinated product was separated from non-covalently bound iodide by sedimentation to near equilibrium in gradients of caesium trichloroacetate followed by treatment in formamide and sedimentation in sucrose gradients containing SDS (Mayo et al., 1979a). PLRV RNA thus treated was not obviously degraded (Fig. 1b); it was much more radioactive than similarly treated RNA from tobacco mosaic virus and had a specific radioactivity more like that of RNA from tomato black ring virus (Table 1). When $^{125}$I-labelled RNA from PLRV was treated with Pronase or proteinase K (Harrison & Barker, 1978) and recovered by precipitation with ethanol, its specific radioactivity decreased to about half that of buffer-treated RNA, whereas the specific radioactivity of $^{125}$I-labelled RNA from tobacco mosaic virus was not decreased and that of $^{125}$I-labelled RNA from tomato black ring virus was decreased about 25-fold (Table 1). This suggests that PLRV has a genome-linked protein, that some amino acid residues remain attached to PLRV RNA after Pronase treatment, as found for similarly treated poliovirus RNA (Nomoto & Imura, 1979), and that these include iodotyrosine. When $^{125}$I-labelled RNA from PLRV (about 10 µg) was treated with 1 µg ribonuclease A (Sigma) for 1 to 2 h at 30 °C, and 9 vol. acetone was then added, about 95% of the $^{125}$I was precipitated; when the precipitate was analysed by electrophoresis in 20% polyacrylamide gels (Mayo et al., 1979a), the $^{125}$I migrated as one broad band (Fig. 1c). The mobility was slightly less than that of the genome-linked protein of tomato black ring virus, and the mol. wt. was estimated to be about 7000 using CNBr fragments of myoglobin (mol. wt. markers, BDH) as markers. When $^{125}$I-labelled RNA from PLRV was treated with Pronase before nuclease treatment and gel electrophoresis, no bands were visible in the autoradiograms, presumably because the protease-resistant $^{125}$I-labelled peptide was too small to be retained in the gels.

Two approaches were used to test for polyadenylate sequences in PLRV RNA. In the first, the RNA was used as a template for reverse transcriptase with oligo(dT) as a primer (Kacian & Myers, 1976), and incorporation of $[^3H]$dTTP into polynucleotides was measured essentially as described by Fraenkel-Conrat (1976). PLRV RNA did not stimulate incorporation. For example, in one experiment 4 pmol dTTP was incorporated when PLRV RNA (50 µg/ml) was used as template compared to 3 pmol in a reaction without template. In contrast, 426 pmol was incorporated when tomato black ring virus RNA, which contains poly(A) (Mayo et al., 1979b), was used as template at the same concentration. In the second approach, PLRV RNA was mixed with oligo(§H)dT) in annealing conditions and the proportion of the radioactivity becoming resistant to S1 nuclease was measured (Tracy &
Kohne, 1980). No reaction was detected with 1 μg PLRV RNA whereas less than 1 ng poly(A) always gave a positive result.

The results show that PLRV resembles the majority of plant viruses in that the RNA in its particles is positive-stranded, being both infective and a messenger RNA. PLRV RNA is bound to a small protein but remains infective when this protein is degraded by protease. In this respect, PLRV resembles cowpea mosaic virus (Stanley et al., 1978) rather than the nepoviruses, which have genome-linked proteins that influence infection (Harrison & Barker, 1978; Mayo et al., 1979a). However, unlike both cowpea mosaic virus and the nepoviruses, PLRV RNA does not contain poly(A). Southern bean mosaic virus RNA has a genome-linked protein and lacks poly(A), but loses its infectivity when treated with proteinase K (Veerisetti & Sehgal, 1980) and, when it is translated in vitro, coat protein is found in the products (Salerno-Rife et al., 1980). PLRV RNA therefore has a novel combination of biochemical features, which may prove to be characteristic of the luteovirus group as a whole.

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REFERENCES


Short communications


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