RNA-dependent RNA polymerase of tobacco plants
(central dogma of molecular biology/RNA replicase/tobacco mosaic virus infection/tobacco necrosis virus infection/RNA nucleotidyltransferase)

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ABSTRACT  Several properties of RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) active fractions obtained from tobacco plants, be they uninfected, infected with tobacco mosaic virus, or infected with tobacco necrosis virus, were compared. By the seven criteria tested, the RNA-dependent RNA polymerase from these three sources behaved the same, although its activity is greatly but variably stimulated by the two virus infections. It thus appears probable that these two viruses do not code for this enzyme, but rely for their replication on their ability to stimulate production of a host enzyme. The conclusion that cells contain RNA replicating capability represents a modification of the central dogma of molecular biology.

In previous studies of the RNA-dependent RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) from Turkish tobacco infected with tobacco necrosis virus (TNV), we have shown evidence that a lower activity of similar nature occurred in uninfected plants (1, 2). Similar observations concerning uninfected tobacco have been reported from other laboratories (3–6). We have now designed an experimental approach to further test the working hypothesis that virus infection only stimulates the production of such a host enzyme, rather than directing the synthesis of a virus-specific enzyme. To this end we decided to use the two viruses TNV and tobacco mosaic virus (TMV) in a tobacco variety where they give the same necrotic response, i.e., Nicotiana tabacum, var. Xanthi nc. We followed the procedures of enzyme extraction and partial purification previously reported for Turkish tobacco (1, 2), using these plants uninfected and after 2 or 3 days of infection. Both the membrane-bound Triton X-solubilized and the cytoplasmic soluble enzyme were compared in regard to their behavior upon ammonium sulfate fractionation, glycerol gradient centrifugation, isoelectric focusing, DEAE-Sephadex chromatography, as well as their activity with different concentrations of Mg2+ and Mn2+, and with different templates.

RESULTS
As far as the amounts of enzyme in the three groups of plants are concerned, we consistently find considerably more activity in TNV- than in TMV-infected plants; that was previously also found to be the case with Turkish tobacco even though there TMV causes systemic infection. Healthy plants show much less enzyme than infected, and the enzyme is largely cytoplasmic, also in contrast to the infected plants, in which the bound and soluble activities are in the same range (Table 1). The data in this table also show that, in regard to effects of Mg2+ concentration and added RNA on enzyme activity, TMV-infected resembles uninfected plant tissue.

Concerning the nature of the "six enzymes" investigated (bound and cytoplasmic, from uninfected, TMV-, and TNV-infected leaves), to the extent tested by the seven criteria listed above, no differences could be detected; most of the data therefore are not shown in detail.

The highest activity upon stepwise ammonium sulfate fractionation was always precipitated between 0.33 and 0.4 saturation; the Triton X-100-"solubilized" enzyme fractions, which retain more or less endogenous template, show some lesser but significant activity at lower saturation.

Upon glycerol gradient centrifugation, performed as previously described (1), the template-free enzyme from all six sources shows the same sharp peak about 20% down the gradient (tube 20–19 of 25), suggesting a molecular weight of about 400,000 (between gamma globulin and catalase, which, however, are only 3–4 tubes apart in this 10–30% gradient); any enzyme–template complex activity is found at about the middle of the gradient.

Upon DEAE-Sephadex fractionation, performed according to Clark et al. (7) but with linear ammonium chloride elution, the activity peaks sharply at 0.28 M salt concentration for the soluble enzyme from the three sources (Fig. 1). Upon isoelectric focusing (9) (ampholyte pH range 3.5–11 in a gradient of 5–20% sucrose), the activity of ammonium sulfate-fractionated "soluble" and "solubilized" "healthy" and "infected" enzyme is found to give a single sharp peak at pH 5.1 ± 0.2 with low values below pH 4 and above pH 6. Surprisingly, this is so regardless of the level of RNA content and dependence of the fraction tested.

Varying the divalent metal contents during the test shows an optimum at 5 mM Mg2+, and a lower optimum for Mn2+ at 1 mM for all three DEAE-Sephadex-fractionated "soluble" enzymes. The higher enzyme activities at low Mg2+ (5 mM as compared to the 25 mM previously used; see refs. 1 and 2) are also found for the crude "soluble" enzymes (see Table 1). The dependence on added RNA is, however, more absolute at 25 mM Mg2+—again for the enzyme from the various sources—for both crude (Table 1) and purified enzyme.

Another similarity is noted when template specificity, or the lack thereof, is studied. All the enzymes are stimulated by all the RNAs tested as templates, turnip yellow mosaic virus (TYMV) RNA being usually the most active; there was no evident relationship between the inoculated virus and the effectiveness of its RNA as template (see Table 2).

Abbreviations: TNV, tobacco necrosis virus; TMV, tobacco mosaic virus; TYMV, turnip yellow mosaic virus.

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Table 1. RNA polymerase activities of crude fractions obtained from virus-infected and uninfected leaves

<table>
<thead>
<tr>
<th>Plants†</th>
<th>Assay conditions</th>
<th>3H]UMP incorporation, cpm†</th>
<th>Solubi-</th>
<th>Bound</th>
<th>Soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg2+ mM</td>
<td>RNA ‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNV-infected</td>
<td>5 †</td>
<td>-</td>
<td>2344</td>
<td>2029</td>
<td>785</td>
</tr>
<tr>
<td>(45 g)</td>
<td>25 †</td>
<td>+</td>
<td>3554</td>
<td>2238</td>
<td>2980</td>
</tr>
<tr>
<td>TMV-infected</td>
<td>5 †</td>
<td>-</td>
<td>200</td>
<td>25</td>
<td>541</td>
</tr>
<tr>
<td>(44 g)</td>
<td>25 †</td>
<td>+</td>
<td>334</td>
<td>61</td>
<td>1966</td>
</tr>
<tr>
<td>Uninfected</td>
<td>5 †</td>
<td>-</td>
<td>114</td>
<td>24</td>
<td>107</td>
</tr>
<tr>
<td>(56 g)</td>
<td>25 †</td>
<td>+</td>
<td>168</td>
<td>56</td>
<td>367</td>
</tr>
</tbody>
</table>

* Extraction of leaves and assays were performed as usual (1, 2); data are given for 3 μl of enzyme fraction. The 16,000 × g pellet, taken up in 1/10 of the plant weight of standard extraction buffer [0.01 M pH 8.1 Tris-Cl/0.01 M KC1/0.025 M NH4Cl/10% (vol/vol) glycerol, 0.1% (vol/vol) mercaptoethanol], “bound enzyme,” was treated with 1/10 volume of 10% (vol/vol) Triton X-100 at 0° for 1 h and then centrifuged at 30,000 × g; the supernate represents the “solubilized enzyme.” The original 30,000 × g supernate was centrifuged at 100,000 × g to remove virus and the supernate was precipitated with 50% saturated ammonium sulfate; the precipitate, taken up in the same buffer, was redissolved between 15% and 35% ammonium sulfate saturation (2), and the final precipitate was taken up in 1/10 of the plant weight (“soluble enzyme”).

† The same number of equivalent leaves, diminished in weight as the result of virus infection.

‡ Ten micrograms of turnip yellow mosaic virus (TYMV) RNA.

§ Conditions of solubilization of the bound enzyme, developed for the TMV-stimulated enzyme, are apparently less efficient for TMV-infected and healthy plant tissue.

Table 2. Effect of various RNAs on RNA polymerase activity of “soluble enzyme” from virus-infected and uninfected plants

<table>
<thead>
<tr>
<th>RNA (10 μg)</th>
<th>TNV-infected</th>
<th>TMV-infected</th>
<th>Uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>94</td>
<td>116</td>
<td>263</td>
</tr>
<tr>
<td>TNV</td>
<td>558</td>
<td>376</td>
<td>381</td>
</tr>
<tr>
<td>TMV</td>
<td>610</td>
<td>445</td>
<td>455</td>
</tr>
<tr>
<td>TYMV</td>
<td>968</td>
<td>906</td>
<td>1084</td>
</tr>
<tr>
<td>Brome mosaic</td>
<td>739</td>
<td>564</td>
<td>554</td>
</tr>
<tr>
<td>Phage Q8</td>
<td>691</td>
<td>569</td>
<td>466</td>
</tr>
<tr>
<td>Yeast</td>
<td>593</td>
<td>403</td>
<td>469</td>
</tr>
</tbody>
</table>

* “Soluble enzyme” was prepared as in Table 1, then purified by DEAE-Sephadex chromatography (see Fig. 1). Enzyme assays were in presence of 25 mM Mg2+. With 5 mM Mg2+, 2- to 5-fold higher incorporations are obtained, with the same patterns throughout (TYMV > TMV, TNV, etc.), but incorporations without RNA are relatively higher.

† Increase above zero-time control.

** DISCUSSION **

Previous studies have indicated that infection of barley with brome mosaic virus (BMV) stimulates production of an enzyme which, in contrast to the above enzymes, responds better to the RNA of the bromovirus group than to other RNAs (10). Recent studies with alfalfa mosaic virus-infected tobacco have indicated that the resultant RNA-dependent RNA polymerase also behaves in various respects differently from the above-described TNV- and TMV-stimulated tobacco enzyme (5, 6).

Furthermore, there exists good, though circumstantial, evidence that bromovirus and alfalfa mosaic virus RNAs carry a gene for a 35,000-dalton protein on RNA component 3, which is related to RNA replicase function (11–13). It is therefore here proposed as a working hypothesis that these viruses with a total genome of almost 3 × 10⁶ daltons do code for a component of their replicase, while TNV and TMV of 1.4 and 2 × 10⁶ dalton RNA content lack such a component and only stimulate the production, or increase the activity, of an RNA-dependent RNA polymerase preexisting in tobacco. Indications are that such enzymes occur in all the green plant leaves investigated. Whether this seeming correlation between photosynthetic RNA replicating capability is purely coincidental remains to be determined. It appears also possible that the lack of RNA-dependent RNA polymerase activities frequently reported for healthy cells, be they cucumber cotyledons or mammalian cells, is due to enzyme levels being below the detection or acceptance level of the method or the investigator. The few positive reports regarding the presence of RNA replicating activity in animal cells (e.g., refs. 14, 15) have been questioned, and were not further substantiated by the authors.

The possibility of a cryptic or latent RNA virus infection in seemingly healthy plants must also be considered as cause for the existence of an RNA replicase. This can, however, be ruled out on logical grounds, for one would not expect such a virus-specific enzyme to be greatly increased, without change in properties, by infection with other virulent viruses.

Data of the type presented here thus seem to signify an RNA replicating capability in normal cells which requires that the central dogma of molecular biology now be schematically represented as

\[
\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein},
\]

with the solid arrows indicating the mainstream of events. Additional evidence that this enzyme makes RNA complementary to any template will be reported elsewhere.

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