COMPLEMENTARY REGIONS TO TOBACCO MOSAIC VIRUS RNA IN HOST DNA

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SUMMARY- TMV RNA has been found to hybridize with both nuclear and chloroplast tobacco DNAs. Chloroplast DNA contains an appreciable greater proportion of complementary sequences to TMV RNA than does nuclear DNA. The data suggest that only a small portion of TMV RNA is homologous with a highly redundant DNA species.

Ever since the demonstration by Doi and Spiegelman (1) of the absence of host DNA nucleotide sequences complementary to MS2 bacterial virus RNA, it has been assumed that this might be a general phenomenon, applicable to a variety of host DNA-viral RNA systems. Experimental results are presented in this paper which indicate that complementary sequences for tobacco mosaic virus (TMV) RNA are present in host DNA, particularly in chloroplast DNA.

METHODS

Nuclei were prepared from tobacco leaves (2) and DNA was extracted from them by suspending them in BPES buffer (3) adding sodium dodecyl sulfate to 1%. The suspension was incubated for 3 hours at 50° in the presence of 1 mg/ml Pronase (Calbiochem) followed by overnight incubation at 37°. Fifty µg/ml RNase were added and the preparation dialyzed at 50° against several changes of BPES buffer. The dialyzate was extracted several times with phenol and then with a 24:1 mixture of chloroform and isoamyl alcohol. The DNA was spooled after addition of 2 volumes of ethanol and resuspended in 0.1 BPES. It was then further purified by preparative isopycnic centrifugation in CsCl (4). Chloroplast DNA was prepared as described by Whitfeld and Spencer (5) with a final purification step involving passage of the DNA through a Sepharose 4B (Pharmacia) column and collection of material appearing in the exclusion volume. Tritium labeled TMV RNA was prepared by excising small

tobacco leaves which had been inoculated with TMV 3 days previously and incubating these leaves for 2 days under fluorescent lamps in 15 cm petri dishes each containing 0.5 mC ^{3}H-uridine. TMV was isolated from these leaves (6) and the RNA extracted according to Mandeles and Bruening (7). Tritium-labeled tobacco leaf RNA was prepared as previously described (2). For the hybridization experiments, alkali-denatured DNA was immobilized on B-6 S & S membrane filters (8) and each membrane was incubated in 1 ml 2 x SSC containing 0.1% sodium dodecyl sulfate and appropriate amounts of RNA at 68° for periods indicated in the text. The membranes were then washed (9) and counted in a liquid scintillation spectrometer. Blank membranes as well as membranes embedded with bacterial DNAs (Escherichia coli and Micrococcus lysodeikticus) retained very few counts (0 - 7 cpm) after incubation with the highest concentrations of RNA used in these experiments. It was found that the sodium dodecyl sulfate additive was essential to obtain low counts on control membranes without DNA, particularly after incubation with TMV RNA. TMV RNA was subjected to electrophoresis on polyacrylamide gels for 4 hours (10). The gels were cut into 1.05 mm slices on dry ice and allowed to dry for 2 days at room temperature. The slices were then digested with 0.2 ml 30% H_2O_2 at 50° for 24 hours and counted in 15 ml of a scintillation cocktail containing 1 part Triton X-100 (Rohm and Haas) to 2 parts toluene.

RESULTS AND DISCUSSION

It can be seen from Fig. 1A, which presents the results of a saturation-hybridization experiment, that a considerable proportion of tobacco chloroplast DNA is complementary to TMV RNA. The data are transformed in Fig. 1B to yield a curve whose slope gives the reciprocal of the saturating-hybridization value (11). For this experiment the value proves to be 7.1%. That is, at least 7% of the chloroplast DNA contains sequences which are complementary to TMV RNA. In other experiments values of 3.4 and 3.6 were obtained. We cannot account for the variability of our data but, nevertheless, it is clear that an appreciable proportion of chloroplast DNA is complementary to TMV RNA.

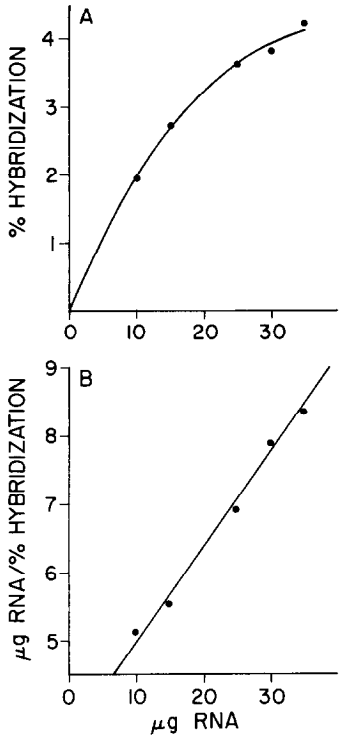

Fig. 1

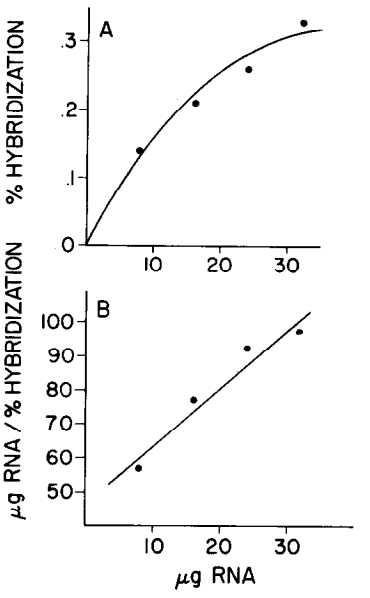

Fig. 2

Fig. 1 Hybridization of TMV RNA to tobacco chloroplast DNA. Membranes containing 1 μg DNA were incubated with different concentrations of RNA (specific activity=17,000 cpm/μg) for 23 hours. A. Saturation curve; B. Plot of data to yield a curve whose slope is the reciprocal of the saturation-hybridization value.

Fig. 2 Hybridization of TMV RNA to tobacco nuclear DNA. Membranes containing 1.5 μg DNA were incubated with different concentrations of RNA (specific activity=16,000 cpm/μg) for 18 hours. A and B as in Fig. 1

Tobacco nuclear DNA also contains sequences which are complementary to TMV RNA (Fig. 2), although in lower proportion (in this case 0.59%) than is the case for chloroplast DNA.

Although it is clear that TMV RNA participates in molecular hybridization reaction with host DNA, the nature of the saturation curves are peculiar in that much higher concentrations of TMV RNA than of ribosomal RNA are required to approach saturation. Fig. 3 presents a saturation curve of tobacco leaf ribosomal RNA with chloroplast DNA to illustrate this point and similar data

of the hybridization of leaf RNA to nuclear DNA are given by Matsuda et al. (2). A comparison of the abscissas in Figure 1 and 3 shows that about a 35 times greater concentration of TMV RNA than of ribosomal RNA is required to achieve about the same degree of saturation.

In order to account for the required high concentration of TMV RNA, we suggest that only a small portion of TMV RNA is homologous with a highly redundant sequence present in both chloroplast and nuclear DNAs. If this hypothesis is correct, we can estimate that about 1 - 5% of the TMV RNA actually participates in the hybridization reaction.

We have considered two other possible explanations for the experimental results: 1) many TMV particles might contain host RNA rather than TMV RNA, and 2) host RNA constitutes a minor contaminant in TMV RNA preparations. If the first proposal were true, the data would suggest that a mixture of messenger RNAs, transcribed primarily from chloroplast DNA, are encapsidated with TMV protein. This seems unlikely because partial nucleotide sequence analyses indicate that TMV RNA preparations are largely homogeneous (12, 13, 14). In order to explore whether proposal 2 could account for the data, several TMV RNA preparations were analyzed by polyacrylamide gel electrophoresis. A typical result is shown in Fig. 4 where it can be seen that the bulk of the radioactivity appears in a narrow region which is coincident with TMV RNA. It does not appear likely, therefore, that host RNA contamination can account for the hybridization data because of the size homogeneity of the preparation. This possibility cannot be ruled out absolutely, however, because some radioactivity does appear in the gel in regions other than that occupied by unbroken TMV RNA. It is clear that ribosomal RNA contamination cannot account for the results because about a 10 x greater proportion of chloroplast DNA is complementary to TMV RNA than to ribosomal RNA.

If we conclude that there is partial homology between host DNA and viral RNA, then the significance of this phenomenon for the infection process remains to be determined. It does not appear likely that host DNA acts as a

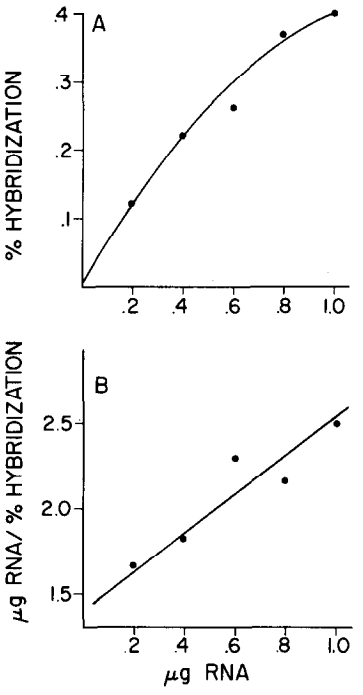

Fig. 3

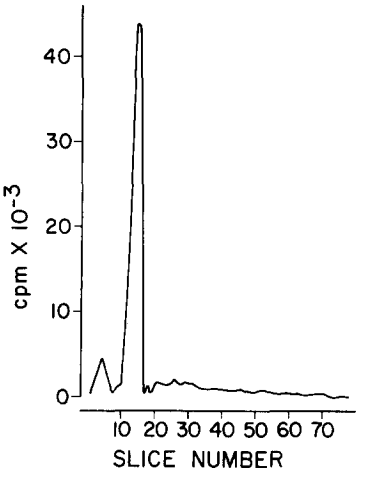

Fig. 4

Fig. 3 Hybridization of tobacco leaf ribosomal RNA to tobacco chloroplast DNA. Membranes containing 2 μg DNA were incubated with different concentrations of RNA (specific activity=61,000 cpm/μg) for 19 hours. A and B as in Fig. 1.

Fig. 4 Electrophoresis of ^{3}H-TMV RNA on polyacrylamide gel. Direction of electrophoresis is left to right.

template for viral RNA biosynthesis because Actinomycin D does not interfere with virus replication (15). It has been established recently that TMV infection interferes with chloroplast function in several important respects (16,17) and we suggest that the complementary portion of TMV RNA may serve as a blocking agent for some host DNA function(s). One observation in support of this idea is the severe yellow symptom induced in inoculated leaves by defective strain infection in contrast to absence of symptoms in non-defective strain infection (18,19). Viral RNA of a defective strain does not become encapsidated with coat protein and so may remain a more effective agent for blocking host DNA function.

ACKNOWLEDGMENTS

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