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Studies of the Genetic Material of
Some RNA Viruses

Progress Report

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Abstract

A program has been initiated to look and identify virus specific monocistronic messenger RNAs in extracts of plant virus infected tissue. In order to ensure identification of such messenger RNAs, should they exist, efforts are being made to increase the sensitivity of response of a wheat germ derived cell-free protein synthesizing system to added RNA template. The results are promising and they will continue.

A series of tobacco mosaic virus strains have been tested for similarity of genome nucleotide sequence similarity to the UI strain by a molecular hybridization technique. The results indicate that some of the tested strains are closely related to the UI strain in nucleotide sequence and that others are completely unrelated.

Progress for the nine months since the inception of this support agreement is not remarkable because of laboratory setup time at a new institution, recruitment and training of laboratory personnel and peculiar technical difficulties encountered in attempting to reproduce laboratory procedures in a new setting. However, the initial problems have now been overcome and we have laid the groundwork for significant experimentation during the coming year. Work has been initiated on two problems and progress to date is listed below.

1. Strategy employed for translation of the plant virus genome -

The background for this problem together with literature citations is set forth in the renewal proposal which accompanies this progress report. Briefly, it derives from the question of how viral RNA, which presumably contains several cistrons, is translated in a eucaryotic host whose protein synthesizing apparatus is believed to translate primarily monocistronic messenger RNA. Evidence from Brome Mosaic and Alfalfa Mosaic virus indicates that at least for capsid protein, monocistronic messenger RNA is split from a larger genomic component. An experimental program has been initiated to investigate whether other plant viruses generate a monocistronic messenger RNA for coat protein by "genome fracturing" and whether monocistronic messengers might not

also be generated by a similar process for other genes present in the plant virus genome.

The initial approach is based on the observation that extracts of tobacco mosaic virus tissue contain fragments of viral RNA of about the size that one would postulate for monocistronic coat protein messenger RNA. The experiment is designed to determine whether this fragment acts as template for an in vitro protein synthesizing system derived from wheat germ and if so, does it act as template for a recognizable product (capsid protein?). Because the viral RNA fragments are present in tissue extracts in low concentration (they have been recognized so far only as a radioactively labelled component) we have initiated a program to increase the sensitivity of the wheat germ system to stimulation by added RNA template. Starting with the system described by Davies and Kaesberg (J. Virology, 1972, 12:1434), the parameters have been adjusted to the point where substantial response is observed to the addition of less than a microgram of tobacco mosaic virus RNA. Our results to date indicate that it is not unlikely that the system can be made even more sensitive by further manipulation and we are in the progress of doing this before testing size fractionated RNA extracts of infected tissue for the presence of unique messenger activity.

2. Classification of tobacco mosaic virus strains according to nucleotide sequence similarity.

A comparison of the nucleotide sequence similarity of strains of tobacco mosaic virus is made possible by the ability to extract and purify the double-stranded (DS) form of TMV RNA, half of which is composed of the complementary strand to TMV RNA. In the experiments performed to date the DS form of the U1 strain of TMV has been mixed with a ten-fold excess of radioactively-labelled TMV RNA and a 150-fold excess of unlabelled RNA extracted from several different TMV strains. The mixture has been heated in order to melt the DS RNA and reannealing has been allowed to proceed after addition of appropriate amount of salt. Ribonuclease-resistant radioactivity is then measured to determine the similarity of nucleotide sequence of the challenge RNA to U1 RNA. Radioactivity is high in the case where challenge RNA does not interfere with annealing of labelled U1 RNA to the complementary strand derived from DS RNA and it is low when the challenge RNA is similar to U1 RNA.

The results of one such experiment are as follows:

0.1 μ g U1 DS RNA mixed with 1 μ g 3 H-U1 RNA and 15 μ g of RNA from the following strains:

	Ribonuclease resistant CPM
none	583
U1	59
U2	591
J14D1	71
06-67	80
CV3 (Berkeley)	536
CV3 (Japan)	582
CV4 (Berkeley)	586
CV4 (Czech)	587
GT AMV	413
YA	82
HRG	557
dahlemense	568

It can be seen from these results that some of the strains compete with the annealing of labelled U1 RNA from which it may be concluded that their nucleotide sequence is similar to that of U1. Other strains exhibit no competition from which it may be concluded that their nucleotide sequence differs appreciably from that of U1, so much so that one wonders whether they can be considered members of the same virus species.

These experiments will be repeated and extended by making use of DS RNAs from several other strains. The object is to confirm these results by cross-comparisons and to determine the number of nucleotide sequence groups into which "strains of TMV" may be classified.

3. I have co-authored a rather lengthy and comprehensive review dealing with the replication of the small RNA containing plant viruses. This will appear shortly in a series called Comprehensive Virology to be published by Plenum Press. I am attaching only one copy because of its bulk in type written form. Additional copies will follow as soon as I receive the galleys.