Final Report
ERDA Contract E (11-1)-2384

Studies of the Genetic Material of Some
RNA Viruses

by

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This constitutes the final report of work performed with partial support of ERDA Contract E (11-1)-2384. The progress and accomplishments of this project have been truly remarkable, particularly during the past three years primarily because of technological developments which permit experimental approaches not hertofore possible.

The principle investigator is appreciative of the financial support and encouragement provided him by ERDA and its predecessor agency, the AEC, and at the same time deplores the changing policies which increasingly narrow the mission orientation of the research the agency is prepared to support. What is lost sight of is the foundation upon which all practical technological advances are based; the information and concepts gained through non-directed research. It is well and good for each mission-oriented agency to take the attitude that we are only here to milk fundamental knowledge for the common good, not to develop fundamental knowledge--that is someone else's business. This attitude, apparently now adopted by ERDA, is shortsighted in my opinion and cannot but help to impede progress towards its goal, whatever that happens to be at the moment.

Eucaryotic organisms differ from procaryotic organisms in their translation machinery. Whereas procaryotes can readily translate several individual proteins from a single polycistronic RNA, eucaryotes, as far as is known, can only make a single translational start on messenger RNA and, as a consequence, synthesize and translate monocistronic, rather than polycistronic, RNAs. The RNA containing viruses that have eucaryotic hosts are therefore presented with the problem of how to get their genetic information translated because it is, of necessity, multi-genic. The multi-genic nature of viral RNA derives from the necessity of having coding sequences for a minimum of at least one viral protein and a RNA replicating enzyme or part of one. Most viral genomes actually contain additional informational sequences. Several translational strategies to deal with the problem have evolved among the eucaryotic RNA containing viruses. Among these are:

a) The poliovirus model--a polyprotein is synthesized which is split into the functional virus proteins by specific proteolytic cleavage.

b) The split genome--a number of RNA containing viruses have genomes consisting of a number of RNA molecules,

rather than having all of the genetic information contained in a molecule. Current evidence indicates that the double-stranded RNA Reo type viruses and the negative stranded orthomyxo (flu) viruses have genomes that consist of a number (6-12) of monocistronic RNAs all encapsidated in single virus particles. The plant single plus stranded split genome viruses are unique in having the 2 to 3 pieces of their genomes separately encapsidated so that more than one virus particle is required for successful inoculation. In a well studied case, the alfalfa mosaic virus genome is composed of three molecules of RNA, two of which are monocistronic and the third dicistronic. During infection the dicistronic RNA molecule is processed to produce a highly efficient monocistronic RNA for capsid protein.

c) The tobacco mosaic virus strategy. I have been concerned with the translational strategy of tobacco mosaic virus which has an undivided genome. During the course of the ERDA supported contract we have determined that a LMC (low molecular weight RNA component-250,000 d) appears in infected tissue. This RNA strand was determined to have the same nucleotide sequence and polarity as a portion of the TMV RNA. This LMC was extracted and purified

for characterization and upon using it to stimulate a wheat germ derived cell-free protein synthesizing system was found to be a monocistronic messenger RNA for the viral capsid protein. The conclusion is reached that during infection the TMV RNA is processed into at least one monocistronic messenger RNA. Suggestive evidence was obtained that still other monocistronic RNAs may be produced during the infection process but that their appearance might be transient. The decision was made, therefore, to explore this matter further in the protoplast system where synchronous infection occurs and the time course of events during infection can be more easily followed.

A reliable tobacco protoplast system for study of the events of viral infection was developed in my laboratory as a modification of other published procedures. One of the problems that had to be overcome was the definition of cultural conditions that would favor the uptake and incorporation of exogenously supplied nucleic acid and protein precursors so that meaningful, easily interpretable experiments could be done. It was found that appropriate pre-treatment of plant tissue would lead to uniformly excellent protoplast preparations and that

incubation of the protoplasts in the salts of either Murishige and Skoog's or Nagata and Takebe's culture media would favor incorporation of exogenously supplied radioactive precursor. The time and rate of synthesis of virus specific protein was followed as a function of time after inoculation. It was found in agreement with others that infected cells synthesize three new proteins with nominal molecular weights of 160,000, 140,000 and 17,500 daltons. Synthesis of these proteins is first detected between 5 and 7 hours after inoculation at which time the 140,000 protein is synthesized more rapidly than the other two. This situation obtains until 9 hours of inoculation at which time the synthetic rate of the smallest protein, the viral capsid protein, overtakes that of the 140,000 protein. The rate of viral capsid protein synthesis continues to increase rapidly so that at 24 hours it accounts for 50% of the total protoplast protein synthesis and at 48 hours, 70%. As a consequence of infection, therefore, the protoplasts are turned into factories for synthesis of viral capsid protein. An interesting feature of our data is that it appears that normal cellular protein synthesis is not reduced during the massive synthesis of capsid protein but, rather, that capsid protein synthesis

is in addition to, not at the expense of, cellular protein synthesis. This conclusion is based on the observation that incorporation of labelled protein precursor into acid insoluble product increases rather dramatically along with capsid protein synthesis. It is a tentative conclusion because it assumed that the intracellular pool specific activity of protein precursor is constant and this has not been determined.

At the present time efforts are actively underway to determine how long it takes for newly synthesized capsid protein to be incorporated into virus particles. For this purpose a non-SDS 3-20% low cross-linked polyacrylamide gel was devised into which both virus and free capsid protein can electrophoretically migrate to different positions. Although these experiments are not yet complete, the results obtained so far are surprising in indicating that appreciable time elapses (hours) between synthesis of protein and assembly of virions. Experiments to determine the time and rate of virus-specific nucleic acid species should provide evidence for our current working hypothesis that viral protein is synthesized in considerable amount before viral nucleic acid synthesis and that the rate of viral RNA replication is the limiting

factor in the rate of virion assembly. The performance of these interesting experiments will have to rely on funding from agencies other than ERDA because of ERDA's narrow view of what is important.

Among other research findings supported by this contract are:

1. Strains of the plant virus, TMV, fall into groups. Within a group there is no appreciable nucleotide sequence divergence between the strain RNAs. Between groups there is no detectable sequence homology!

2. A study was initiated into the molecular biology of replication of the POTY plant virus group. Among the information so far made available from this study is the following:

- a. Tobacco etch virus (a POTY virus) RNA terminates at the 3' end in a poly A sequence. An interesting and puzzling feature of this phenomenon is that only one half of the viral RNA molecules have polyA, the other half do not. Both types of RNA are, however, equally infectious.

- b. Synthesis of viral specific proteins (of which there are 5) is inhibited by chloramphenicol. This is quite different from the situation

with other known plant viruses and indicates that translation of POTY virus RNA takes place with the aid of chloroplastic, rather than with cytoplasmic, ribosomes. If this proves to be so, it would be the first recorded instance of such a phenomenon.

c. The synthesis of virus specific RNA is inhibited by timely application of actinomycin D. This is contrary to the situation in TMV infected tissue and may indicate the involvement of host DNA in the replicative process.

3. A study has recently been initiated into the possibility of developing a plant virus derived RNA plasmid to act as a carrier for the introduction of maternally inherited favorable genetic information into plants. The prospects for doing this look excellent. Initial development in progress plans the use of the short RNA of the split genome tobacco rattle virus as a carrier of zein messenger RNA into tobacco protoplasts.

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