STRUCTURE AND SYNTHESIS OF SMALL VIRUSES
AND THEIR COMPONENT PARTS

Three Year Summary Progress Report

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PROGRESS REPORT ABSTRACT

We have continued during the past three years to study the biological properties of brome mosaic virus (BMV) as a model illustrative of multicomponent viruses in general. We have developed an in vitro protein synthesizing system from wheat germ which translates the BMV RNAs very efficiently. BMV 4, the smallest of the BMV RNAs, is not required for infectivity but serves as an excellent messenger for the coat protein cistron. Although RNA 3 also contains the coat protein cistron it serves best to induce the synthesis of a larger protein designated protein 3A. It appears an interrelationship exists in vivo between RNA 3 and 4 involving the regulation of coat protein synthesis. RNA 1 and 2 also serve as templates in the wheat system directing the synthesis of several large polypeptides. We have also established that the wheat system is effective in translating virus messengers with host ranges markedly different from BMV.

The BMV RNAs have also been shown to have tRNA-like character in that they are all chargeable with tyrosine. We have studied this reaction and it is clear that the charging and messenger capabilities of the BMV RNAs are independent.

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1. Introduction

This report describes research supported by the Energy Research and Development Administration under Contract No. AT(11-1)-1633 for the period February 16, 1972, to February 15, 1975. This contract supported about 12% of the research conducted in the laboratory of the principal investigator, Paul Kaesberg, and he devoted approximately 20% of his time to this project. The contract supported approximately 50% of the salary of Dr. D. S. Shih, a post-doctoral research associate, during each of the past three years. Two students, Ann Palmenberg and Joseph Moosic, have done research related to this project, although they have not received any salary support. Dr. L. Andrew Ball who is now Assistant Professor at the University of Connecticut was supported as a post-doctoral research associate in an earlier contract; his work was finished and published under the present contract. One post-doctoral research associate, Jeffrey Davies, has done research related to this project but did not receive salary support. He is now a staff member (Assistant Scientist) at the University of Wisconsin.

The overall objective of this research has been to study the structure and synthesis of small viruses with the expectation that the results would be relevant to viruses in general and to the cells in which they are replicated. In the past it has been useful to study viruses that are easy to handle, but which have properties in common with viruses that are medically or economically important. Probably the viruses now in most need of study are the multi-component viruses--viruses whose genetic information is divided among several nucleic acids. These include viruses implicated in infectious diseases (influenza), cancer (leukoviruses), and economically important plant viruses.

As a more specific objective, we proposed 3 years ago, in our Renewal Application and Progress Report, that we begin studies of brome mosaic virus, as a model, multicomponent virus and, in particular, that we investigate the messenger capability of BMV RNA in the then newly discovered wheat embryo cell-free protein synthesizing system.

2. Main Research Accomplishments

Our earlier research showed that BMV is a multicomponent virus consisting of 3 virions which, together, are required for infectivity. Virion A contains BMV RNA1, Virion B contains BMV RNA2 and Virion C contains BMV RNA3 and BMV RNA4. The viral RNAs are, themselves, infectious. However, RNAs 1, 2 and 3 are sufficient for infectivity. RNA4 is not needed (Lane and Kaesberg, Nature 232, 40, 1971).

Even though RNA4 is not needed for infectivity it is a product of the infection because it is contained in Virion C. Hence the information for its synthesis must be contained in one of the other viral RNAs. We have proved that RNA4 sequences are contained in RNA3 (Shih, Lane and Kaesberg, J. Mol. Biol. 64, 353, 1972). To do this, the viral RNAs were digested enzymatically and the products of digestion were compared. Selected products were sequenced. All of the sequenced products from RNA4 were also products of RNA3.

Our complementation studies have shown that BMV RNA3 contains the information for synthesis of the viral coat protein. We have developed an in vitro protein synthesizing system from wheat germ that translates BMV RNAs (Shih and Kaesberg, Proc. Nat. Acad. Sci. 70, 1799, 1973). We have shown that BMV RNA4, as well as RNA3, contains the coat protein cistron

and moreover serves as an excellent messenger for it. RNA3 is a di-cistronic messenger, and serves best to induce synthesis of a protein designated protein 3A. Only when BMV RNA3 is broken down does it serve to induce synthesis of coat protein. We believe that this interrelationship between BMV RNAs 3 and 4 is an important factor in the in vivo regulation of coat protein synthesis.

The BMV RNAs mimic tyrosine transfer RNA in that they are chargeable with tyrosine under conditions in which the transfer RNA is charged (Hall, Shih and Kaesberg, *Biochem. J.* 129, 969, 1972). We have characterized this reaction; we have measured its fidelity, efficiency, and specificity.

The wheat germ system has now become exceedingly useful and popular throughout the world. We have shown that the system can translate a variety of messages, even polycistronic messages from RNA phages (Davies and Kaesberg, *J. Virol.* 12, 1434, 1973). We have defined some of the properties of the phage RNA translation including its correct termination even in the case of amber mutations which induce premature termination.

The BMV RNAs have also become popular test messengers in a variety of animal and bacterial systems. We have shown that RNA4 is translated efficiently and correctly in a cell-free protein synthesizing system derived from mouse L cells. (Ball, Minson and Shih, *Nature New Biology* 246, 206, 1973).

Our studies of modified BMV RNA show that its messenger and charging abilities are independent (Shih, Kaesberg and Hall, *Nature* 249, 353, 1974). Thus the charging reaction must have a biological role other than its possible involvement in protein synthesis. Our studies indicate that its primary function relates to replication of the viral RNA.

3. Specific Objectives for Future Research

Our overall objective continues to be the study of the structure and synthesis of small viruses as stated in Part 1 of this report. In the last 3 years we have emphasized studies of the biological characteristics of the multicomponent virus BMV. We will continue our overall objective and the emphasis of the last 3 years of our work.

Specifically our objective for the next 3 years may be stated as follows:

We have a cell-free system from a eukaryotic organism--wheat embryo--that can translate a homologous messenger--brome mosaic viral RNA--with great fidelity and efficiency. We plan to simplify and further characterize the system. We want to determine what factors affect fidelity and efficiency. We will use the system to determine what information exists in brome mosaic virus--which we regard as a simple, model virus illustrative of multicomponent viruses in general. The Renewal Application accompanying this report describes how we propose to implement the above objectives.

4. Personnel Trained

Post-doctoral Research Associates

Dr. L. A. Ball (tenure on this project completed)

Dr. J. Davies (tenure on this project completed)

Dr. D. S. Shih (tenure as a postdoc completed, advanced to rank of Assistant Scientist)

Graduate Students

Ann Palmenberg (in residence)

J. P. Moosic (in residence)

5. Bibliography

The following papers were published during the period of this report:

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(C00-1633-23) Ball, L. A. and Paul Kaesberg. A polarity gradient in the expression of the replicase gene of RNA phage QB. *J. Mol. Biol.* 74, 547 (1973).

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(C00-1633-31) Davies, J. and Paul Kaesberg. Translation of virus messenger RNA synthesis of bacteriophage QB proteins in a cell-free extract from wheat embryo. *J. Virology* 12, 1434 (1973).

(C00-1633-32) Ball, L. A. and P. Kaesberg. Cleavage of the N-terminal formylmethionine residue from a bacteriophage coat protein in vitro. *J. Mol. Biol.* 79, 531 (1973).

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(C00-1633-36) Shih, D. S., P. Kaesberg and T. C. Hall. Messenger and aminoacylation functions of brome mosaic virus RNA after chemical modification of 3' terminus. *Nature* 249, 353 (1974).

(C00-1633-37) Davies, J. W. and Claudia Benike. Translation of virus mRNA: synthesis of bacteriophage PP7 proteins in cell-free extracts from Pseudomonas aeruginosa. Virology 61, 450 (1974).

(C00-1633-38) Davies, J. W. and P. Kaesberg. Translation of virus mRNA: protein synthesis directed by several virus RNAs in a cell-free extract from wheat germ. J. gen. Virol. 25, 11 (1974).

(C00-1633-39) Davies, J. W., P. Kaesberg and T. O. Diener. Potato spindle tuber viroid. XII. An investigation of viroid RNA as a messenger for protein synthesis. Virology 61, 281 (1974).

(C00-1633-40) Hall, T. C., R. K. Wepperrich, J. W. Davies, L. G. Weathers and J. S. Semancik. Functional distinctions between the ribonucleic acids from citrus exocortis viroid and plant viruses: cell-free translation and aminoacylation reactions. Virology 61, 486 (1974).

6. Significance of This Research

There are three principal motives for studying small viruses as proposed in this application: 1) It is believed that study of such viruses will contribute to overall knowledge of virus structure and function and that this should ultimately lead others to find means of controlling infectious and metabolic diseases. 2) Small viruses represent a genetic relationship between a small number of easily identifiable proteins and a single nucleic acid. These studies may contribute to an explanation of genetic phenomena in chemical and physical terms.

Ten or fifteen years ago plant viruses and large bacteriophage were the only viruses available in amounts and purity to serve as suitable test objects for structure and synthesis studies. These viruses were excellent models for the subsequent elegant investigations with polio virus, influenza and other medically important viruses.

Similarly the discovery of small phages marked the period of intense and successful work in molecular biology in which these viruses and their component parts were used to elucidate mechanisms of protein and nucleic acid synthesis.

The study of multicomponent viruses is certainly now of prime importance--as exemplified by the great interest in such multicomponent viruses as influenza, tumor viruses and the economically important plant viruses. We believe it is important to work with a simple, model, multicomponent system like BMV.

The study of protein synthesis in eukaryotes is also now of great interest. With BMV and wheat embryo extracts, we have one of the few cell-free protein synthesizing systems that work.

7. Present Division of Federal Support

In addition to receiving research support from this Energy Research and Development Administration contract, Professor Paul Kaesberg is principal investigator of an NIH grant (CA-15613) in the net amount of \$76,189 for the first year of a five year award which started February 1, 1974. He also shares an NIH grant (AC-01466) with Professor W. W. Beeman in the net amount of \$79,169 for the fourth year of a five year grant which started in March, 1971. Professor Kaesberg is a Research Career Investigator of the Public Health Service and his salary is paid by them and the University of Wisconsin.