Advances in
VIRUS RESEARCH

Edited by

KARL MARAMOROSCH· FREDERICK A. MURPHY

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VOLUME 30

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PREFACE

With Volume 30, the editors of *Advances in Virus Research* will be Karl Maramorosch, Frederick A. Murphy, and Aaron J. Shatkin. In the past three decades, since its publication was initiated, the field of virology has grown enormously, and will continue to do so. Forthcoming volumes will fill the needs of virologists by providing articles on molecular virology and by increasing the scope of subject coverage. The newly created international Advisory Board will assist the editors in accomplishing this task.

Max A. Lauffer, the late Kenneth M. Smith, and Fredrick B. Bang were responsible for making *Advances in Virus Research* the most influential review publication in the field of virology. Dr. Lauffer can leave the editorial chair with the assurance that this publication, which has earned international respect under his guidance, will continue its leading role in the field.

Karl Maramorosch
Frederick A. Murphy
Aaron J. Shatkin

MAX A. LAUFFER

MAX A. LAUFFER

On the occasion of his retirement in the spring of 1984, a group of distinguished biologists and biophysicists gathered at a symposium commemorating the many contributions of Max A. Lauffer to our knowledge of virus structure and function. All the participants had been influenced to some degree by his ideas. Among the activities transferred to others on his retirement was the coeditorship of *Advances in Virus Research,* a position he held since its founding in 1953. With the late Kenneth M. Smith, he planned the first volume which set the tone for this serial publication. One has only to leaf through the pages of a few volumes of *Advances in Virus Research* to be aware of his influence. It is fitting that acknowledgment of his many contributions be made here.

Studying the structure and function of viruses has been at the forefront of Dr. Lauffer's research efforts from the beginning of his professional life. His ideas stemmed from the conviction that something possessing such profound biological activity as a virus could be described in terms of physics and chemistry. Although a common viewpoint today, this was by no means apparent then. Dr. Lauffer has also been attracted to the intriguing question of the role of water in life processes. In 1958, he and his colleagues observed that under certain conditions protein subunits from tobacco mosaic virus assembled into rods at room temperature and disassembled at the temperature of ice. The assembly being apparently reversible indicates that the assembly of the protein coat of the virus is an entropically driven reaction. In biology, such reactions are interesting because they can be understood only in terms of an interaction with solvent, the most likely candidate being water. In 1965 he and his colleagues reported that indeed sufficient water is released on polymerization to account for the driving entropy. There are many other entropically driven reactions in biology; they all seem to function in situations in which structures must be assembled and disassembled readily. In 1975, he published a book, "Entropy-Driven Processes in Biology," in which much of this information is collated. His interest in this area continues.

Max Augustus Lauffer, Jr., is the son of Max Augustus and Elsie May (Keiper) Lauffer. He was born on September 2, 1914 in Dauphin County, Pennsylvania. His grandfather, Gottlieb Lauffer, came to this country from Switzerland in 1885 and finally settled in Middletown, Pennsylvania. To this day, Dr. Lauffer maintains the family farm there. He traveled all the way from Middletown to State College to attend Pennsylvania State University, graduating with a B.S. degree in 1933 at the tender age of

nineteen. A year later, he received an M.S. degree, and then went to the University of Minnesota, where he received a Ph.D. degree in 1937. He then went to the Rockefeller Institute for Medical Research, now The Rockefeller University, where he became a fellow, assistant, and then associate. He was associated with Wendell Stanley, and began his career-long research in structure–function relationships in viruses, especially tobacco mosaic virus. A year later, 1938, he published six papers; the number has now grown to more than 180. In 1944 he went to the University of Pittsburgh, first to the physics department as an associate professor and then as professor of biophysics in 1947. In 1949 he founded the department of biophysics at the University of Pittsburgh, one of the earliest of such departments, and chaired it until 1956. Between 1956 and 1963, he was dean of the Division of Natural Sciences. From 1963 until his retirement, he held the Andrew Mellon chair in biophysics, and is currently Andrew Mellon Professor Emeritus. In that year also, 1963, until 1967, he again assumed the chairmanship of the Biophysics Department. In 1967 that department joined the Department of Microbiology forming the Department of Biophysics and Microbiology, which Dr. Lauffer chaired from 1971 to 1975.

Dr. Lauffer participated in many professional activities apart from the University of Pittsburgh and the Rockefeller Institute. In the summer of 1941, he was a special lecturer in chemistry at Stanford University; Priestly Lecturer at Pennsylvania State College in 1946; Gehrmann Lecturer at the University of Illinois College of Medicine in 1951; and visiting professor at the University of Bern, Switzerland in 1952; at the Max-Planck Institüt for Virus Research, Tubingen, Germany from 1965 to 1966; and at the University of the Philippines, Quezon City, in 1967. He also served there as a Rockefeller Foundation consultant. He was a member of the teaching staff in physiology at the Woods Hole Marine Biological Laboratory from 1953 to 1956. He served intermittently between 1947 and 1968 as consultant to the U.S. Army Biological Laboratories, Ft. Detrick, Maryland. He was a member from 1960 to 1964 and vice-chairman from 1963 to 1964 of the institutional research grants committee of the American Cancer Society; a member from 1961 to 1963 of the program-project committee of the National Institute of General Medical Sciences; and a member of the National Advisory General Medical Sciences Council of the National Institutes of Health from 1963 until 1967. He was a director of the Health Research and Services Foundation, Pittsburgh, from 1975 to 1981 and chairman of its research advisory committee from 1964 to 1967. In 1952 Dr. Lauffer was invited by Mr. Kurt Jacoby, then vice-president of Academic Press, to become coeditor with Kenneth M. Smith of *Advances in Virus Research,* established at the time

as the first review series in the field of virology. Dr. Lauffer continued as coeditor until his retirement in 1984.

In addition to his *Advances in Virus Research* editorial activities, he was a member of the board of editors of *Archives of Biochemistry and Biophysics* from 1944 to 1954 and of the *Biophysical Journal* from 1960 to 1964, and editor of the *Biophysics Journal* from 1969 to 1973.

Dr. Lauffer was the recipient of the Eli Lilly Research Award in biochemistry in 1945; the Pittsburgh Award of the American Chemical Society in 1958; the Outstanding Achievement Award, University of Minnesota, in 1964; and he was one of the founders of the Biophysical Society and its president in 1961.

He was a member of the board of Christian Education, U.P. Church, United States, from 1963 to 1972; of the council on Church Study and Society of U.P. Church, United States, from 1963 to 1971, and its chairman from 1971 to 1974; and a trustee of the College of Wooster from 1967–. He has been a member of the World Federalist Association for about 35 years, and is currently vice-president of the Pittsburgh chapter. He is also and has been several times previously a member of the Session of Southminster U.P. Church, Pittsburgh.

Dr. Lauffer is married to Erika (Erskine) and has four children: Edward William, Susan Keiper, Max Erskine, and John Erskine.

It is apparent that Max A. Lauffer's influence will be felt for a long time to come, not only by readers of *Advances in Virus Research* but also by his many students, colleagues, and associates, by the scientific community at large, and, indeed, by the human community.

Charles L. Stevens
Karl Maramorosch
for the Editors

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ADVANCES IN VIRUS RESEARCH, VOL. 30

AN UNUSUAL REPLICATION STRATEGY OF AN ANIMAL IRIDOVIRUS

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I. Introduction

Frog virus 3 (FV3) belongs to the family Iridoviridae (Fenner and Gibbs, 1983) which comprises several vertebrate and invertebrate viruses. The characteristic features of an iridovirus are (1) polyhedral symmetry, (2) large (approximately 170 kilobase pairs), linear, double-stranded DNA genome, and (3) a cytoplasmic site of replication. The best studied member of the Iridoviridae is frog virus 3 (FV3), isolated by Granoff *et al.* (1966) from a renal adenocarcinoma of the leopard frog, *Rana pipiens*. Although FV3 has no relevance to the formation of the tumor, it has many interesting features that made it a worthy object of study in its own right. In this review we will mainly focus on the unusual structure of the FV3 genome and its unique replication strategy. For details of virus-specific RNA and protein synthesis and their regulation, the reader is referred to a recent review by Willis *et al.* (1985).

II. Structure of FV3

A. The Virion

1. Morphology

The intracellular virus measures 120 × 130 nm in diameter (Granoff, 1969). The extracelular particle delimited by plasma membrane (acquired during budding) measures 160 to 200 nm in diameter. The membrane is not needed for infectivity since unenveloped particles are infectious (Willis and Granoff, 1974). However, enveloped virus is more infectious on a particle to particle basis (Braunwald *et al.*, 1979). The enveloped FV3 particles have a buoyant density of 1.28 g/cm^3 and unenveloped particles 1.32 g/cm^3 (Morris *et al.*, 1966).

FV3, like other iridoviruses, possesses icosahedral symmetry. This was established by electron microscopy using negative-staining and freeze-etching techniques (Stoltz, 1973; Tripier and Kirn, 1973; Tripier *et al.*, 1975). The icosahedral lattice is composed of capsomeres measuring 6 to 8 nm (Murti *et al.*, 1984). The capsid has a skew symmetry with a triangulation number of 133 or 147 (Darcy-Tripier *et al.*, 1984). The capsomeres are closely packed with a center-to-center spacing of 7.2 nm (Darcy-Tripier *et al.*, 1984). Beneath the icosahedral lattice there is an inner membrane composed of lipids and proteins. The inner membrane contains transmembrane proteins as revealed by freeze-etching studies (Darcy-Tripier *et al.*, 1984). This membrane is essential for infectivity since lipid solvents render virions noninfectious (Willis and Granoff, 1974). The virus core below the membrane is composed of DNA and proteins and appears as a long convoluted filament (Darcy-Tripier *et al.*, 1984).

2. Composition

The nonenveloped virion contains 9% lipid, and the infectivity of the virus is destroyed by either ether or phospholipase A (Willis and Granoff, 1974). The amounts of the various phospholipids in virion particles differ from that of the host cell membrane in that there is little sphingomyelin, no cholesterol, and the ratios of phosphatidylserine plus phosphatidylinositol to phosphatidylcholine are reversed, suggesting *de novo* synthesis of viral membrane in infected cells. The envelope lipids from virus particles released by budding have not been examined, but one would expect that they resemble the plasma membrane of the host.

FV3 proteins are customarily designated by their molecular weights with the prefix ICP (infected cell protein) or VP (viral structural protein) attached. However, because FV3 proteins have been examined under dif-

ferent electrophoretic conditions, the molecular weight reported for individual FV3 proteins varies, making it difficult to compare data from different investigators. For example, the molecular weight of the major capsid protein is variously reported as 48,000 (Silberstein and August, 1976a; Aubertin *et al.*, 1980) and 55,000 (Elliott and Kelly, 1980; Willis *et al.*, 1977). Using two-dimensional gel electrophoresis, Elliott *et al.* (1980) detected 29 viral structural proteins.

FV3 proteins do not undergo detectable posttranslational processing. No evidence for glycosylation, sulfation, or cleavage from precursors has been obtained (Elliott and Kelly, 1980). However, 10–15 phosphoproteins, ranging in size from 10,000 to 114,000 are found in virions or within infected cells (Aubertin *et al.*, 1980; Elliott and Kelly, 1980), and a protein kinase able to phosphorylate these and other viral proteins has been isolated and purified from virions (Silberstein and August, 1976a,b). The role of protein kinase in FV3 replication is no known, but Aubertin *et al.* (1980) point out that only the virion core proteins are phosphorylated *in vivo*, and suggest that the degree of phosphorylation may determine whether or not a protein becomes associated with the nucleocapsid.

FV3 virions contain at least six enzymatic activities (reviewed in Goorha and Granoff, 1979). Two of these, nucleotide phosphohydrolase (Vilagines and McAuslan, 1971) and the pH 5 endodeoxyribonuclease (Aubertin *et al.*, 1971) are found in viral cores, whereas protein kinase (Silberstein and August, 1973), endoribonuclease, and the pH 7.5 endodeoxyribonuclease are external, i.e., they are solubilized by 0.5% NP40–50 m*M* 2-mercaptoethanol (Kang and McAuslan, 1972). The sixth activity, a protein phosphatase, has not been localized (Silberstein and August, 1973). The FV3 protein kinase (MW 44,000) has been purified to homogeneity by Silberstein and August (1976a) and has been shown to comprise 0.4% of the virion protein.

The distribution of viral proteins within purified virions has been examined by both biophysical and biochemical techniques. Neutron scattering and controlled degradation of virions using nonionic detergent and pronase demonstrated that the virus is composed of four concentric domains: (1) a central, spherical core consisting of the DNA and associated proteins; (2) an intermediate lipid membrane containing VPs 63 and 44 and probably other proteins; (3) an outer icosahedral shell consisting mainly of VP 48; and (4) a viral envelope derived from the host cell membrane and containing VP 58 (Cuillel *et al.*, 1979; Aubertin *et al.*, 1980; Darcy-Tripier *et al.*, 1982; Robach *et al.*, 1983). Neutralization studies using monospecific serum (Aubertin *et al.*, 1981) and monoclonal antibodies (Chinchar *et al.*, 1984) confirmed the external location of the major capsid protein (VP 48), and suggested that ICP 38 was also externally located.

B. The Genome

1. Size and Composition

FV3 virions contain a single, linear, double-stranded DNA genome with a GC content of 53% (Smith and McAuslan, 1969; Houts *et al.*, 1970). The molecular weight of FV3 DNA was first estimated as 130×10^6 on the basis of velocity sedimentation and electron microscopy (Smith and McAuslan, 1969; Houts *et al.*, 1970) but Kelly and Avery (1974) have reported a lower estimate (100×10^6) based on reannealing kinetics of DNA and velocity sedimentation of DNA after careful removal of all structural proteins. Recent measurements of the contour lengths of purified FV3 DNA gave a value of 51.00 ± 5.60 μm in length or a calculated molecular weight of $98.28 \pm 10.58 \times 10^6$ (Murti *et al.*, 1982). DNA hybridization studies have shown no sequence homology between FV3 and other iridoviruses (Kelly and Avery, 1974).

2. Methylation

An unusual attribute of FV3 DNA is its high degree of methylation. Willis and Granoff (1980) found that over 20% of the deoxycytosine residues — apparently every deoxycytosine in the sequence CpG — was methylated at the 5 carbon position. Although the initial rounds of viral DNA synthesis takes place in the host cell nucleus (Goorha, 1982), methylation occurs only after this DNA is transported to the cytoplasm (Willis and Granoff, 1980). Methylation therefore is a postreplicative event; pulse-chase experiments indicated that methylation of the DNA is completed within an hour of its synthesis (Goorha, unpublished results). There are four possible functions for methylated bases in DNA: (1) protection against endonucleases, e.g., restriction-modification, (2) DNA replication, (3) recombination and repair, and (4) control of gene expression. Recent work favors the idea that the methylation of FV3 DNA may protect it from viral endonucleases; Goorha *et al.* (1984) found that the unmethylated DNA of an azacytidine-resistant FV3 mutant is degraded *in vivo* by a nuclease associated with infection by wild-type FV3. However, these data do not exclude other possible functions for DNA methylation.

3. Sequence Organization

FV3 DNA lacks inverted repeats of the kind seen in herpesviruses nor does it have cross-linked termini such as those observed in poxvirus genomes (Murti *et al.*, 1982). The DNA also lacks proteins covalently bound to the molecular ends such as those observed in adenoviruses (Murti *et al.*, 1982). However, FV3 genome contains a sequence organization unique to