Comprehensive Virology

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Foreword

The time seems ripe for a critical compendium of that segment of the biological universe we call viruses. Virology, as a science, having only recently passed through its descriptive phase of naming and numbering, has probably reached that stage at which relatively few new—truly new—viruses will be discovered. Triggered by the intellectual probes and techniques of molecular biology, genetics, biochemical cytology, and high-resolution microscopy and spectroscopy, the field has experienced a genuine information explosion.

Few serious attempts have so far been made to chronicle these events. This comprehensive series, which will comprise some 6000 pages in a total of about 22 volumes, represents a commitment by a large group of active investigators to analyze, digest, and expostulate on the great mass of data relating to viruses, much of which is now amorphous and disjointed and scattered throughout a wide literature. In this way, we hope to place the entire field in perspective as well as to develop an invaluable reference and sourcebook for researchers and students at all levels. This series is designed as a continuum that can be entered anywhere but which also provides a logical progression of developing facts and integrated concepts.

The first volume contains an alphabetical catalogue of almost all viruses of vertebrates, insects, plants, and protists, describing them in general terms. Volumes 2–5 deal primarily, though not exclusively, with the processes of infection and reproduction of the major groups of viruses in their hosts. Volume 2 deals with the simple RNA viruses of bacteria, plants, and animals; the togaviruses (formerly called arboviruses), which share with these only the feature that the virion's RNA is able to act as messenger RNA in the host cell; and the reoviruses of animals and plants, which all share several structurally singular features, the most important being the double-strandedness of their multiple RNA molecules. This grouping, of course, has only slightly more in its favor than others that could have been or indeed were considered.

Volume 3 addresses itself to the reproduction of all DNA-containing viruses of vertebrates, a seemingly simple act of classification, even though the field encompasses the smallest and the largest viruses known.

The reproduction of the larger and more complex RNA viruses represents the subject matter of Volume 4. These share the property of lipid-rich envelopes with the togaviruses included in Volume 2. They share as a group, and with the reoviruses, the presence of enzymes in their virions and the need for their RNA to become transcribed before it can serve messenger functions.

Volume 5 attends to the reproduction of DNA viruses in bacteria, again ranging from small and simple to large and complex.

Aspects of virion structure and assembly of many of these viruses will be dealt with in the following series of volumes, while their genetics, the regulation of their development, viroids, and coviruses will be discussed in subsequently published series. The last volumes will concentrate on host–virus interactions, and on the effects of chemicals and radiation on viruses and their components. At this juncture in the planning of *Comprehensive Virology*, we cannot foresee whether certain topics will become important aspects of the field by the time the final volumes go to press. We envisage the possibility of including volumes on such topics if the need arises.

It is hoped to keep the series at all times up to date by prompt and rapid publication of all contributions, and by encouraging the authors to update their chapters by additions or corrections whenever a volume is reprinted.

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* See note on page xiv.

NOMENCLATURE OF ANIMAL VIRUS GROUPS

A strong tendency has become evident in recent years to give virus groups names that have more or less self-evident meanings, referring to some structural characteristic of a given group of viruses. Thus the names *picornaviridiae* and *togaviridiae* have become officially recognized for groups of animal viruses, notwithstanding the fact that also very many plant and bacterial viruses are small-RNA viruses and many viruses of other groups contain "mantles" (togas). The term "togaviruses" appeared preferable to the older term "arboviruses" because of the latter's derivation and lack of identifying character. In the same spirit *oncornaviruses* appears justified, even though not all members of the class are truly oncogenic (and "RNA tumor viruses" is no better in this regard), and similar limitations hold for the terms *rhabdoviruses* and *myxoviruses*. The only exception to this terminology based on structure would appear to be the official sanction of the term *reoviridiae*. This word has no meaning to the uninitiated and is erroneous in the eyes of those who know its meaning, since the plant virus members of this group lack a respiratory-enteric system and are not orphans. *Diplornaviruses* is a good descriptive term for these viruses, since they share the characteristic feature of double-stranded RNA, and the fact that there exist double-stranded-RNA viruses that do not belong to this group is as irrelevant as the fact that the term "picornaviridiae" does not include all small-RNA viruses. However the International Committee of Virus Nomenclature appears to favor the term "reoviridiae," as used by Prof. Joklik.

H. F.-C.

CHAPTER 1

Reproduction of RNA Bacteriophages

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1. INTRODUCTION

Studies on virus replication have been greatly facilitated by the discovery in 1961 of a small RNA-containing bacteriophage (Loeb and Zinder, 1961). The RNA coliphage represents a biological system uniquely reduced to its simplest form. Unlike all other bacterial viruses, the RNA phage does not contain DNA, but rather RNA as its sole genetic material. As a consequence, the RNA must serve a dual function both as a template for nucleic acid synthesis and as a messenger for virus-specific protein synthesis. Due to the size of the genome and the limited number (three) of gene products, it has been possible to elucidate the biological processes of replication and translation as well as the mechanisms by which these events are controlled. Over the past several years, considerable progress has been made in this respect, and though by no means complete, our knowledge of the RNA bacteriophage has become quite extensive. We have attempted to summarize below some of the more recent contributions toward our understanding of the molecular biology of virus replication.

Several reviews on specific aspects of RNA bacteriophages have

been published: on replication, Lodish (1968*a*), Erikson (1968), and Stavis and August (1970); on translation, Kozak and Nathans (1972) and Sugiyama *et al.* (1972); on RNA sequence analysis, Gilham (1970); on RNA structure and function, Weissmann *et al.* (1973) and Fiers (1973); on assembly, Hohn and Hohn (1970); and on RNA viruses in general, Zinder (1974).

2. PROPERTIES OF THE RNA PHAGE

2.1. Properties of the Particles

Infection of susceptible bacteria by RNA phages yields 10^3–10^4 viral particles per cell. These particles are composed of a single molecule of RNA, 180 molecules of coat protein, and one molecule of the maturation (A) protein. The RNA genome contains only three genes, those for the coat protein, the maturation protein, and a protein subunit of the phage RNA polymerase. All of the *Escherichia coli* RNA phages that have been isolated are similar in structure and properties. These phages are among the smallest and genetically simplest infectious, self-replicating organisms known.*

An extensive study of over 30 *E. coli* RNA phages has shown that they are all similar and fall into three or possibly four serological groups (Scott, 1965; Watanabe *et al.*, 1967; Sakurai, 1968). Most of the commonly studied phage (f2, MS2, R17, M12, fr, and FH5) are in the same group and the coat protein sequences of these phages differ from each other by only a few amino acids The Qβ phage is in another group and, although similar in size and many physical properties, has certain distinctive properties, including an RNA genome that appears to be slightly larger than that of other viruses (Boedtker, 1971).

The diameter of these particles is 20–27 nm. They sediment at 75–84 S and have a density of 1.42–1.47 g/ml (Paranchych and Graham, 1962; Enger *et al.*, 1963; Hofschneider, 1963; Marvin and Hoffmann-Berling, 1963; Davern, 1964*a*; Gesteland and Boedtker, 1964; Strauss and Sinsheimer, 1963; Overby *et al.*, 1966). Their particle weight is between 3.6 and 4.2×10^6, a value that agrees with the sum of the molecular weights of the individual components. As determined by X-ray analysis, R17 has a diameter of 27 nm, with an outer shell 3–4 nm thick and a hollow core of about 3 nm in diameter (Fishbach *et al.*, 1965).

* Editor's note: The applicability of the term "organism" to a virus may well be questioned [H. F.-C.].

Of the normal products of a bacterial lysate, only a small proportion of the phage particles, 10–20%, produce an infective center. This can be explained either by unsuccessful infection of the bacterium by normal phage or by the possibility that many of the phages are defective in some manner. One type of particle with known low infectivity has been detected as having a buoyant density slightly lower than normal, and is thus termed an L particle (Rohrmann and Krueger, 1970). These L particles appear to have a normal ratio of protein to RNA. They contain A protein and their RNA is infectious in spheroplasts. It is thought that the decreased buoyant density reflects a structural modification in the capsid surface. Another form of defective particle is one that lacks maturation protein. These particles were recognized as products of infection of nonpermissive bacteria by RNA phages which contained an amber mutation in the maturation protein cistron of the genome (Heisenberg and Blessing, 1965; Lodish *et al.*, 1965; Heisenberg, 1966; Argetsinger and Gussin, 1966; Tooze and Weber, 1967). Such particles also appeared after infection of cultures deprived of histidine at a time when polymerase but not maturation protein was synthesized. Under these conditions, defective particles lacking maturation protein were produced (Kaerner, 1969, 1970). These defective particles cannot be distinguished from normal phage by electron microscopy. However, they do not adsorb normally to the bacterial host, and the RNA of the phage is sensitive to attack by RNase (Argetsinger and Gussin, 1966; Heisenberg, 1966). The evidence suggests that in the absence of maturation protein the RNA fails to be packaged correctly and as a consequence protrudes from the particle.

2.2. Physical Properties of the RNA

Each virus particle contains one molecule of single-stranded RNA. The RNA of f2 and similar phages has a molecular weight of $1.1–1.3 \times 10^6$ and a sedimentation coefficient of about 27 S (in 0.1M NaCl) (Strauss and Sinsheimer, 1963; Gesteland and Boedtker, 1964; Overby *et al.*, 1966; Marvin and Hoffmann-Berling, 1963; Mitra *et al.*, 1963; Boedtker, 1971). The $Q\beta$ RNA appears to be slightly larger, with a molecular weight of 1.5×10^6. The four bases are present in nearly equimolar amounts. The RNA is a linear structure since both 5′ and 3′ termini have been detected (*vide infra*). Sedimentation of the RNA under a variety of conditions (Strauss and Sinsheimer, 1968) has failed to show evidence for the potential formation of ring structures.

The radius of gyration, reported as 16 nm (in 0.2M NaCl)

(Strauss and Sinsheimer, 1963) and 19 nm (in 0.1M NaCl) (Gesteland and Boedtker, 1964), together with the sedimentation coefficient, indicates a compact structure. Thermal denaturation profiles, slow reactivity with formaldehyde (Strauss and Sinsheimer, 1963), and analysis of nucleotide sequences (*vide infra*) indicate extensive hydrogen bonding throughout the molecule. From the change in absorbance upon reaction with formaldehyde, the helical fraction of the RNA was estimated to be about 70% (Mitra *et al.*, 1963; Boedtker, 1967). The RNA probably has a specific secondary structure since limited digestion with ribonuclease at low temperature gives specific cleavage products (Bassel and Spiegelman, 1967; Min Jou *et al.*, 1968; Spahr and Gesteland, 1968; Gould *et al.*, 1969; Thach and Boedtker, 1969).

RNA phages that infect *Pseudomonas* (Feary *et al.*, 1963; Bradley, 1966) and *Caulobacter* (Schmidt and Stanier, 1965; Shapiro and Bendis, 1974) have also been isolated.

2.2.1. Primary Structure

Work on the primary structure of the genome of RNA bacteriophages has been quite extensive in recent years. Much of the sequence analysis has been done on RNA from R17 (Adams *et al.*, 1969*a*; Steitz, 1969*a*; Sanger, 1971), MS2 (Fiers *et al.*, 1971), and Qβ (Weissmann *et al.*, 1973), although f2 and M12 RNA have also been studied (Webster *et al.*, 1969; Thirion and Kaesberg, 1970). In addition, small molecules such as Qβ 6 S RNA (Banerjee *et al.*, 1969*a*) and several classes of variant RNA (Bishop *et al.*, 1968; Kacian *et al.*, 1971) synthesized *in vitro* by the Qβ RNA polymerase (replicase, synthetase) have also been analyzed. As a result of the labors of several groups, over 30% of the genome, or more than 1000 nucleotides of R17 and MS2, and about 15% of Qβ have been sequenced. Of more critical value, however, is that the known sequences include the oligonucleotides at both 5′ and 3′ termini as well as at least part of the cistrons of all three phage-coded proteins. The method of nucleotide sequence analysis essentially involves the specific enzymic cleavage of intact molecules of RNA purified from phage or of RNA fragments synthesized *in vitro* by phage polymerase under limiting conditions (Billeter *et al.*, 1969). Enzymic hydrolysis is usually accomplished by use of the following: T_1 ribonuclease, which specifically cleaves 5′ bonds after Gp residues; U_2 ribonuclease, which splits at purine residues; the carbodiimide method, in which the reagent specifically reacts with guanine and uridine residues and renders the latter resistant

to ribonuclease A; ribonuclease A, which splits at cytosine and uridine residues. Separation and sequence analysis of the enzyme digests are generally performed according to the methods developed by Sanger and his colleagues and involve two-dimensional ionophoresis in 8M urea and thin-layer DEAE homochromatography. The detailed techniques of purification, fractionation, and cleavage of the RNA as well as separation and isolation of the polynucleotides have been detailed (Steitz, 1969*a*; Gilham, 1970; Sanger, 1971) and will not be described here.

2.2.1(a). 5′ Terminus

The 5′ terminus of the RNA from all RNA bacteriophages analyzed thus far is pppGp (DeWachter *et al.*, 1968*a,b*; Glitz, 1968; Roblin, 1968*a,b*; Watanabe and August, 1968*a*). The serologically related phages MS2 and R17 have identical sequences at the 5′ ends for at least the first 125 bases (Adams *et al.*, 1972), whereas f2 has identical sequences for at least the first 74 residues (Ling, 1971) (Table 1). Even RNA from Qβ, which is serologically and chemically distinct, contains 5′-terminal sequences homologous to those of group I phage RNA (Adams *et al.*, 1972). These similarities, however, cannot be related directly to the protein-coding capacity of the RNA. Translation does not occur at or near the 5′ terminus since the initial sequences do not contain either of the formylmethionine codons AUG or GUG necessary for initiation of protein synthesis (DeWachter *et al.*, 1968*c*; DeWachter and Fiers, 1969; Adams and Cory, 1970; Ling, 1971). In the group I phages, even though AUG and GUG codons appear in preceding residues, translation does not begin until the 130th nucleotide. In Qβ, the first AUG codon is found at the 62nd nucleotide and it is the initiation signal for the translation of the first cistron (Billeter *et al.*, 1969).

As no specificity of base is required, the conservation of primary structure for a long sequence at the 5′ terminus is surprising. It has been suggested by several workers (Adams and Cory, 1970; DeWachter *et al.*, 1971*a*) that the similarities in sequences may not be fortuitous. Adams and Cory (1970) have hypothesized that the homology of sequence is evidence for the evolution of the RNA phages from a common prototype and that perhaps the 5′-terminal sequence was retained because it may have advantages in natural selection. It was suggested, for instance, that the 5′-terminal sequence protects the molecule from exonucleolytic attack (Kuwano *et al.*, 1970) by its capacity to form tight hydrogen-bonded loops (Fig. 1). In addition, as

TABLE 1

5′-Terminal Sequences in Bacteriophage RNA

RNA	5′ Terminus[a]
f2[b]	pppGGGUGGGACCCCUUUCGGGGUCCUGCUCAACUUCCUGUCGAGCUAAUGCCAUUUUUAAUGUCUUUAGCGAGACG...
R17[c]	pppGGGUGGGACCCCUUUCGGGGUCCUGCUCAACUUCCUGUCGAGCUAAUGCCAUUUUUAAUGUCUUUAGCGAGACG...
Qβ[d]	pppGGGGGACCCCCCUUUAGGGGGUCAC(ACACCUC)AGCAGUACUUCACUGAGUAUAAGAGGACAUAUG...
Qβ minus strand[e]	pppGGGAGGAGAGAGGGCAAAGCAGAUCCCCCUCUCACUCGUAAGAGUAAUUGUG...
Qβ 6 S[f]	pppG...
Qβ "variant" MV–1[g]	pppGGGAU...

[a] The sequences in parentheses are as yet uncertain.
[b] Ling (1971).
[c] Adams and Cory (1970), Adams *et al.* (1972).
[d] Billeter *et al.* (1969).
[e] Goodman *et al.* (1970).
[f] Banerjee *et al.* (1969*a*).
[g] Kacian *et al.* (1971).