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Comprehensive Virology

11

Regulation and Genetics

Plant Viruses

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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 passed only recently 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 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, and 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.

Volume 1 contains an alphabetical catalogue of almost all viruses of vertebrates, insects, plants, and protists, describing them in general terms. Volumes 2–4 deal primarily, but 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.

Volume 3 addresses itself to the reproduction of all DNA-containing viruses of vertebrates, encompassing the smallest and the largest viruses known. The reproduction of the larger and more complex RNA viruses is the subject matter of Volume 4. These viruses share the property of being enclosed in lipoportein membranes, as do the togaviruses included in Volume 2. They share as a group, along with the reoviruses, the presence of polymerase enzymes in their virions to satisfy the need for their RNA to become transcribed before it can serve messenger functions.

Volumes 5 and 6 represent the first in a series that focuses primarily on the structure and assembly of virus particles. Volume 5 is devoted to general structural principles involving the relationship and specificity of interaction of viral capsid proteins and their nucleic acids, or host nucleic acids. It deals primarily with helical and the simpler isometric viruses, as well as with the relationship of nucleic acid to protein shell in the T-even phages. Volume 6 is concerned with the structure of the picornaviruses, and with the reconstitution of plant and bacterial RNA viruses.

Volumes 7 and 8 deal with the DNA bacteriophages. Volume 7 concludes the series of volumes on the reproduction of viruses (Volumes 2–4 and Volume 7) and deals particularly with the single- and double-stranded virulent bacteriophages.

Volume 8, the first of the series on regulation and genetics of viruses, covers the biological properties of the lysogenic and defective phages, the phage-satellite system P 2–P 4, and in-depth discussion of the regulatory principles governing the development of selected lytic phages.

Volume 9 provides a truly comprehensive analysis of the genetics of all animal viruses that have been studied to date. These chapters cover the principles and methodology of mutant selection, complementation analysis, gene mapping with restriction endonucleases, etc. Volume 10 also deals with animal cells, covering transcriptional and translational regulation of viral gene expression, defective virions, and integration of tumor virus genomes into host chromosomes.

The present volume deals with the considerable advances in methodology and understanding that have greatly broadened the general interest of molecular biologists in plant viruses in recent years. This volume represents the only one of the Comprehensive Virology series to deal exclusively with plant viruses, although their replication and assembly are covered in Volumes 2 and 5. The first two chapters focus on the nature of multicomponent viruses or coviruses that carry a

subdivided genome distributed over separate virus particles. The third chapter concerns itself with the classical and simplest case of satellitism in viruses (a parasite of a parasite) and with other cases of defective viruses that are not completely functional. The fourth chapter deals with the mode and regulation of the first event after RNA virus infection, the translation strategy of multigenic viral RNAs. The fifth chapter describes the advantages and uses of mesophyll protoplasts in the study of plant virus replication and its regulation. Finally there is an authoritative chapter on the remarkable nature of viroids, disease causing self-replicating RNA molecules which are too small to code for proteins and may act by causing the activation of pathogenic genes carried within the host's genome.

Volume 12 will cover the general properties of special virus groups of invertebrates, algae, fungi, and bacteria. Volume 13 will be the last of the series on structure and assembly, dealing with general principles of virion structure, phage assembly, and the complete structural analyses of small RNA and DNA phage nucleic acids and proteins. Volume 14 will deal with the general properties of newly characterized groups of vertebrate viruses, such as, for example, arena-, corona-, and bunyaviruses.

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CHAPTER 1

Plant Covirus Systems: Three-Component Systems

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1. INTRODUCTION

Recent developments have brought together viruses which were not previously supposed to bear any relationship except that they were simple RNA-containing plant viruses. These viruses are the spherical bromoviruses, once thought to have a very small genome (Bockstahler and Kaesberg, 1961); the small spherical cucumber mosaic virus and its relatives, which, in contrast to bromoviruses, are transmitted by aphids; the aphid-transmitted alfalfa mosaic virus, which is one of the very few small viruses with bacilliform particles; and a number of structurally not very well studied viruses among which are tobacco streak virus and citrus leaf rugose virus, which seem to have in common the possession of spherical virions of different size.

The reason for handling these viruses in one chapter is the nature of their genome. Electrophoresis in polyacrylamide gel has shown that their virions contain three RNA species with molecular weights not far from 10^6 (Fig. 1). These RNA species are in separate capsids. Infectivity studies with separated RNA species or with nucleoprotein species containing these RNAs have indicated that all three RNAs are needed for infection. With some of these viruses, these indications are much stronger than with others.

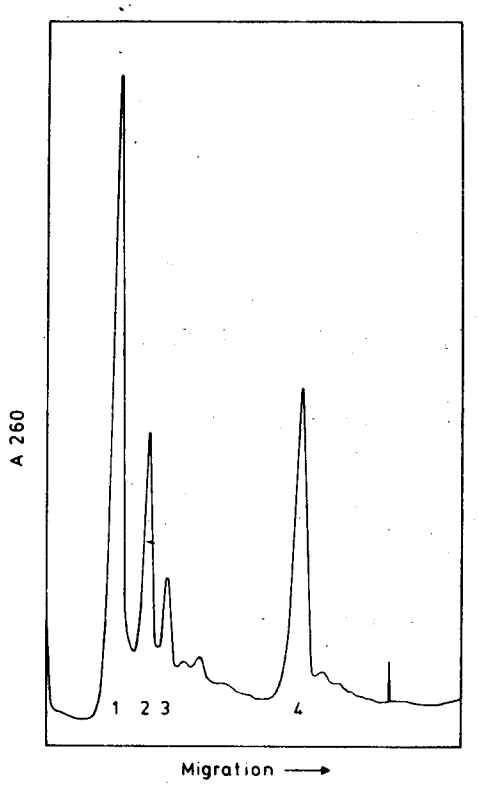

Fig. 1. Characteristic pattern of the RNAs of a plant virus with a tripartite genome (AMV) as revealed by electrophoresis in 3% polyacrylamide gel. The three peaks at the left represent the three parts of the genome. They are designated as RNAs 1, 2, and 3. Their molecular weights are not far from 10^6. RNA 4 at the right has a molecular weight of about 0.3×10^6 and comprises a cistron for the coat protein. With heterocapsidic viruses, this RNA or its translation product is needed to start an infection. Minor RNAs migrating between RNAs 3 and 4 or faster than RNA 4 are often found. With AMV they are called X- and Z-RNAs, respectively.

A further step in the argumentation in favor of a tripartite genome of these viruses was that genetic markers could be exchanged by exchanging RNA or nucleoprotein species in the test tube.

Finally, it could be demonstrated with alfalfa mosaic and brome mosaic viruses that the three RNAs have at least partly unique base sequences and that three species of double-stranded RNA occur in infected cells.

The preparations of plant viruses with tripartite genomes often contain more than three RNA species. One of these, a small RNA with a molecular weight of about 0.3×10^6, occurs very reproducibly and sometimes in appreciable amounts (Fig. 1). It is encapsidated either separately or together with the smallest genome part. With bromoviruses, cucumber mosaic virus, and alfalfa mosaic virus, it has been shown that this RNA directs in an efficient way the synthesis of viral coat protein in *in vitro* translation systems. When unfractionated RNA preparations are carefully freed from this RNA, no change in infectivity occurs in the case of bromo- and cucumoviruses, but in the case of the other viruses mentioned above infectivity is completely lost.

With the latter viruses, infectivity can be restored by adding the small RNA or by adding a small amount of its translation product, the coat protein. Apparently, with these viruses, the coat protein is necessary to start an infection. It has to be assumed that the gene for the coat protein in the genome is not available for translation, as it is in the monocistronic messenger RNA.

On the basis of the above, we can divide the tripartite genomes into protein-independent and protein-dependent genomes. Viruses of the former group have their genome parts in identical capsids and will be called here "isocapsidic viruses." Viruses of the latter group will be called "heterocapsidic viruses" since they have bacilliform or spherical capsids of different size. Apparently, the capsids are fitted to the size of the genome parts. It seems as if the coat protein is adapted to a biological function rather than to a very stringent role in particle structure. At this moment it cannot be stated with certainty whether capsid heterogeneity is always correlated with protein dependency, but for reasons of convenience we will maintain the division between isocapsidic and heterocapsidic viruses throughout this chapter.

Thus the grouping of the viruses we will handle is as shown in Table 1.

Jones and Mayo (1975) showed that BRLV is serologically related to TSV. That CLRV and the related CVV are heterocapsidic is inferred from an electron micrograph given by Garnsey (1975). Lister and Saksena (1976) have measured different particle size classes with preparations of NRSV. These authors point out that several other

TABLE 1

Grouping of Viruses with Tripartite Genomes

Brome mosaic virus (BMV) Cowpea chlorotic mottle virus (CCMV) Broad bean mottle virus (BBMV)	Bromoviruses	Isocapsidic viruses
Cucumber mosaic virus (CMV) Tomato aspermy virus (TAV) Peanut stunt virus (PSV)	Cucumoviruses	
Alfalfa mosaic virus (AMV) Tobacco streak virus (TSV) Black raspberry latent virus (BRLV) Citrus leaf rugose virus (CLRV) Citrus variegation virus (CVV) Elm mottle virus (EMotV) *Prunus* necrotic ringspot virus (NRSV) Lilac ring mottle virus (LRMV)		Heterocapsidic viruses

viruses may be grouped with TSV; thus Tulare apple mosaic virus (TAMV) has been shown by them to have similar particle classes and RNA species. Infectivity is greatly enhanced when particle classes are combined. Furthermore, TAMV is serologically related to CLRV (Lister, Gonsalves, and Garnsey, unpublished). Preparations of EMotV contain quasispherical particles which sediment in a sucrose density gradient in three bands. Infectivity is mainly associated with the fastest-sedimenting component, but addition of the slower-sedimenting components enhanced the infectivity about threefold (Jones and Mayo, 1973). NRSV is classified by Fulton (1968) with apple mosaic virus and rose mosaic virus in a group called ILAR viruses (isometric, labile, ringspot). Four centrifugal components comparable with those of NRSV and TAMV were detected by Lister and Saksena (1976) in preparations of apple mosaic virus. Preparations of lilac ring mottle virus (LRMV) consist of two centrifugal components. The two components have the same buoyant density but can be separated by polyacrylamide gel electrophoresis suggesting difference in particle size (Huttinga and Mosch, 1976). In the electron microscope, rather irregularly shaped isometric particles are seen (Van der Meer *et al.*, 1976).

There are indications that the genome of barley stripe mosaic virus is also tripartite (Lane, 1974*b*). However, the molecular weights of the three RNAs from this rod-shaped virus are around 1.25×10^6, and there is apparently no RNA of molecular weight 0.3×10^6.

2. STRUCTURE AND COMPOSITION

The properties of AMV and of the bromoviruses have been extensively described in review articles by Hull (1969) and Lane (1974*a*), respectively. The structure of CMV has been the subject of studies by Kaper and Geelen (1971) and Kaper (1972), whereas Habili and Francki (1974*a*,*b*) have compared the structural properties of CMV and TAV. We will restrict ourselves here to the most important and most recent data. Emphasis will be on those structural properties which are related to the leitmotiv of this chapter, the tripartite genome.

2.1. Isocapsidic Viruses

The bromoviruses have icosahedral capsids of 26 nm diameter built from 180 identical protein subunits of molecular weight 20,000

(Lane, 1974*a*). CMV has the same structure (Finch *et al.*, 1967), but the diameter of the particles is somewhat larger and the subunit molecular weight is close to 25,000 (Hill and Shepherd, 1972; Van Regenmortel *et al.*, 1972; Habili and Francki, 1974*a*). Bromovirus particles undergo swelling at pH values above 6.0 and become sensitive to ribonuclease and proteolytic enzymes in the swollen state (Lane, 1974*a*; Pfeiffer and Hirth, 1975). Cucumoviruses do not show such a swelling phenomenon but are nevertheless sensitive to ribonuclease (Francki, 1968; Habili and Francki, 1974*b*; Mink, 1975). At alkaline pH, CMV undergoes drastic structural changes (Kaper and Geelen, 1971).

All isocapsidic viruses are dissociated into protein and RNA by sodium dodecylsulfate (SDS) and high salt concentrations, which led Kaper (1972, 1973) to postulate that the structure of these viruses is mainly stabilized by protein–RNA interactions rather than by protein–protein interactions. Under proper conditions, the viruses can be reassembled from salt-prepared protein and RNA (Bancroft, 1970; Kaper and Geelen, 1971; Lane, 1974*a*) (see also chapter by P. P. Hung in Vol. 6 of this series).

The four main RNA species of bromo- and cucumoviruses have molecular weights of about 1.1, 1.0, 0.8, and 0.3 $\times$ 10^6. Somewhat higher and lower values have also been reported for the three large RNAs (Lane, 1974*a*; Kaper and Waterworth, 1973; Peden and Symons, 1973; Habili and Francki, 1974*a*; Kaper and Re, 1974). Since RNAs 3 and 4 are encapsidated together, there are only three types of virions. These three types of virions have nearly identical particle weights and sediment as single species with *s* values of about 86 S and 99 S in the case of bromo- and cucumoviruses, respectively (Lane, 1974*a*; Van Regenmortel, 1967; Mink *et al.*, 1969; Stace-Smith and Tremaine, 1973). The virion types can be partly resolved by equilibrium density gradient centrifugation. Density differences are more pronounced in bromoviruses than in cucumoviruses (Lane, 1974*a*; Lot and Kaper, 1973). This is probably due to the fact that in several strains of CMV an additional small RNA (RNA 5, of molecular weight 0.11 $\times$ 10^6, Kaper and West, 1972) is present. All nucleoprotein fractions from an RbCl density gradient contained about the same amount of RNA 5. Apparently this RNA is encapsidated with a variety of other RNAs (Lot and Kaper, 1976*a*,*b*).

Nucleotide sequence information is available only for the RNAs of BMV. From analysis of octa- and nonanucleotides of complete pancreatic ribonuclease digests of the separated RNAs, it follows that RNAs 1, 2, and 3 have at least partly unique nucleotide sequences. RNA 4 yielded two octanucleotides and three nonanucleotides, which