Electron Microscopy of Proteins

Volume 5

Viral Structure

edited by
JAMES R. HARRIS

and
ROBERT W. HORNE

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Foreword

The development of electron microscopy and virology has been closely associated and it is appropriate that a volume in the series "Electron Microscopy of Proteins" should be devoted to viruses. We see how the structures of viruses have been probed and described by methods of increased sophistication in electron microscopy and other areas including those of data processing. What is particularly noticeable from the chapters is the wide range of complexity from the simple to complex viruses superimposed on the general themes of helical and icosahedral symmetry. The structure of the flexuous viruses described in the book is certainly simpler than that of the bacterial viruses, for example, but there is no enormous difference in a physical sense in the way structures of the mature particles are solved and regarded although special cases do occur. For example, the mismatch in symmetry between the capsid and tail connector in some bacterial viruses—dramatized in "A Tail of Two Symmetries"—is an unusual arrangement. What is of enormous difference is the complexity of the events and pathways leading to the assembly of final products which are a reflection of these pathways. We know the theoretical advantages of helical and icosahedral symmetry and we must continue to ask ourselves how and why structures are assembled as they are. It is encouraging to note that this area of virology, in which a variety of approaches are combined with the power of modern electron microscopy, is providing a fascinating and integrated view of the complexity of processes as well as of structures and their precursors or derivatives. That the inclusion body of a polyhedrosis virus may be considered as a modification of the double shell of a reovirus is an intriguing idea—among many in this book. However, in spite of all the morphological complexities, the basic chemical mechanisms underlying them are limited.

In terms of application, it has long been realized that electron microscopy is a useful technique which can give answers of a practical nature

quickly and the refinements deriving from our basic understanding of viruses and their associated forms and methods to detect them are of considerable consequence to virologists and others. It is clear that many aspects of virology and electron microscopy continue to go hand-in-hand and the particulars of these provided by the expert authors of this volume offer stimulating and enjoyable reading to those of us who are interested in viruses and their structure.

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Preface

Viral Structure, Volume 5 of the series 'Electron Microscopy of Proteins', departs from the pattern established in the earlier volumes of the series, in that a single specialization is now covered rather than the more diverse subject matter included in the earlier volumes. In fact, this returns to the original plan of the Editors, which proposed subject-orientated volumes, but which for technical reasons relating to the availability of completed manuscripts and the avoidance of excessive delay in publication was not adopted.

The present book contains nine chapters which deal with animal, plant and bacterial viruses. A loose division can be made between those chapters which place considerably more emphasis on the fundamental biophysical aspects and image analysis and those which are more biologically applied, but this is by no means clear-cut. In general, the chapters place emphasis upon electron microscopical data rather than upon techniques, although the chapter on immunoelectron microscopy contains a considerable amount of information on specimen preparation. Some chapters do present a more personal interpretation than others, but overall, the book contains authoritative, up-to-date and broadly based reviews of the various topics covered.

All the topics included are presented by scientists who are actively involved and internationally known in their respective fields of study. The first two chapters deal with bacteriophage structure and morphogenesis and are followed by detailed presentations on adenoviruses and influenza virus. The chapter on filamentous plant viruses forms a link between these more fundamental chapters and those following which are slightly more biomedically applied. These are hepatitis viruses, reoviruses, the immunoelectron microscopy of plant viruses, and herpes viruses. Despite the breadth of coverage outlined above, a number of important and interesting topics have inevitably been omitted. Fortunately, viruses such as

TMV and the polyoma viruses have received extensive coverage elsewhere in the literature, but other areas such as the immunoelectron microscopy of animal and bacteriophage viruses, and viral nucleoproteins and nucleic acids have been lost due to authors backing out too late in the production schedule for alternative authors to be commissioned. It is hoped, nevertheless, that the range of subject matter included will be of sufficient interest and that it is presented in sufficient depth to render the book of general value to virologists.

The electron microscope has made an increasing contribution to virology since the instrument became widely available to biologists in the 1950s. There must be currently few virology laboratories that do not make use of this facility, and the study of viruses by those interested in the high-resolution TEM techniques and image analysis has helped to push forward important avenues of investigation. Although atlases of viral ultrastructure are available, the aim of the present volume has been to include, where appropriate, extensive discussion of the more biochemical, immunological and biophysical aspects alongside the electron microscopical data. It is the combination of electron microscopy with other technical approaches for the study of biological materials that underlines the strength, directness and value of this approach, points which are not always apparent if electron microscopical data is considered in isolation from that of other approaches.

We would like to thank the staff of Academic Press, Inc., in London and Orlando for their enthusiastic support for the continuation of the series and for their help throughout the production of this volume. The support and interest shown by Dr. J. F. Harrison, Director of the North East Thames Regional Transfusion Centre, Brentwood, U.K. is gratefully acknowledged.

October 1985

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Contents

1. The Structure of Bacteriophage T7

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ELECTRON MICROSCOPY OF PROTEINS, 5
VIRAL STRUCTURE

I. INTRODUCTION

Since the inception of molecular biology, studies based on the "complex", dsDNA-containing bacteriophage systems have been of central importance to the development of contemporary molecular genetics as well as to virology in general. Moreover, investigations concerned with the structure and assembly of these phage particles have assumed a major role in relation to the elucidation of biosynthesis at its most basic noncovalent level—macromolecular assembly.

Over the past few years, many aspects of the progress in this area have been reviewed, including the pathways of assembly reactions (Casjens and King, 1975; Murialdo and Becker, 1978), the packaging of DNA into viral particles (Black, 1981; Earnshaw and Casjens, 1980), the regulatory mechanisms governing assembly and the linkage between structure and function (Hohn and Katsura, 1977; Kellenberger, 1980; King, 1980; Black and Showe, 1983; Carrascosa, Chapter 2 of this volume). In large part, these reviews have emphasized results obtained in the T-even, lambda and P22 phage systems. A comparable structurally oriented coverage has not been given for bacteriophage T7 (Studier, 1972; Krueger and Schroeder, 1981), a viral system which combines several attractive features as an object of study. Its genome, comprising about 50 structural genes, has been sequenced in its entirety (Dunn and Studier, 1983), facilitating detailed correlation between the functional properties of the various T7 proteins and their underlying molecular biology. Its assembly pathway has been characterized in detail (Serwer, 1976; Serwer *et al.*, 1983). The isometric shape of the T7 capsid (and related particles) lends itself to the integration of structural studies based on low-angle diffraction from concentrated suspensions of particles with those based on electron microscopic observations. In this context, the diffraction data obtained from fully hydrated specimens afford a consistency check against the eventuality of dehydration artifacts incurred in preparing specimens for electron microscopy. Moreover, T7 is the most completely characterized and hence archetypal member of an extensive family of bacteriophages (Hausmann, 1976; Serwer *et al.*, 1983; Mertens and Hausmann, 1982), which

have in common close structural similarities and patterns of gene expression.

It is the purpose of this chapter to summarize the present state of understanding of T7 structure while emphasizing the contributions made by various electron microscopic approaches and, in some instances, identifying salient problems for further study. Functional aspects of the bacteriophage T7 structure will be discussed and compared with analogous phenomena in other viral systems. Regulatory mechanisms will also be considered, with a view to distinguishing tactics from strategy in the overall assembly pathway, i.e. those mechanisms that are peculiar to T7 as distinct from those which are more general.

II. PROPERTIES OF THE VIRION AND OF VIRUS-RELATED PARTICLES

A. Morphological Observations

T7 phage particles represented by several different electron microscopy techniques are portrayed in Figs. 1 to 3. Scanning electron microscopy (SEM) of gold/palladium-coated virions adsorbed to bacterial envelopes demonstrates the characteristic polyhedral shape of the T7 capsid (Fig. 1). This interpretation is confirmed by the appearance of negatively stained specimens viewed in the conventional transmission electron microscope (CTEM) (Fig. 2). Also revealed in such images is the stubby T7 tail attached to a single vertex: the protruding portion of tail is ~23 nm in length and tapers from an apparent width of ~21 nm close to the point at which it joins the capsid, to ~9 nm at its extremity. Also visualized under favorable staining conditions (Fig. 2) are the filamentous tail fibers which are attached around the proximal portion of the tail (cf. Sections III.B and III.C).

Additional features may be inferred from dark-field scanning transmission electron microscope (STEM) images of unstained virions prepared by freeze-drying (Fig. 3). In these images, the intensity of the signal (represented here as brightness, Fig. 3a) is proportional to specimen mass (Crewe and Groves, 1974) over a substantial range of thickness (Steven *et al.*, 1983b). The high maximum density projected by the virion makes it difficult to convey all density variations of interest within the overall dynamic range. Low-density features and modulations are represented to greater advantage after computer processing (e.g. Fig. 3b), whereby the dynamic range is compressed by effecting a background subtraction calculated as the locally averaged density. Thus Fig. 3b reveals with particu-

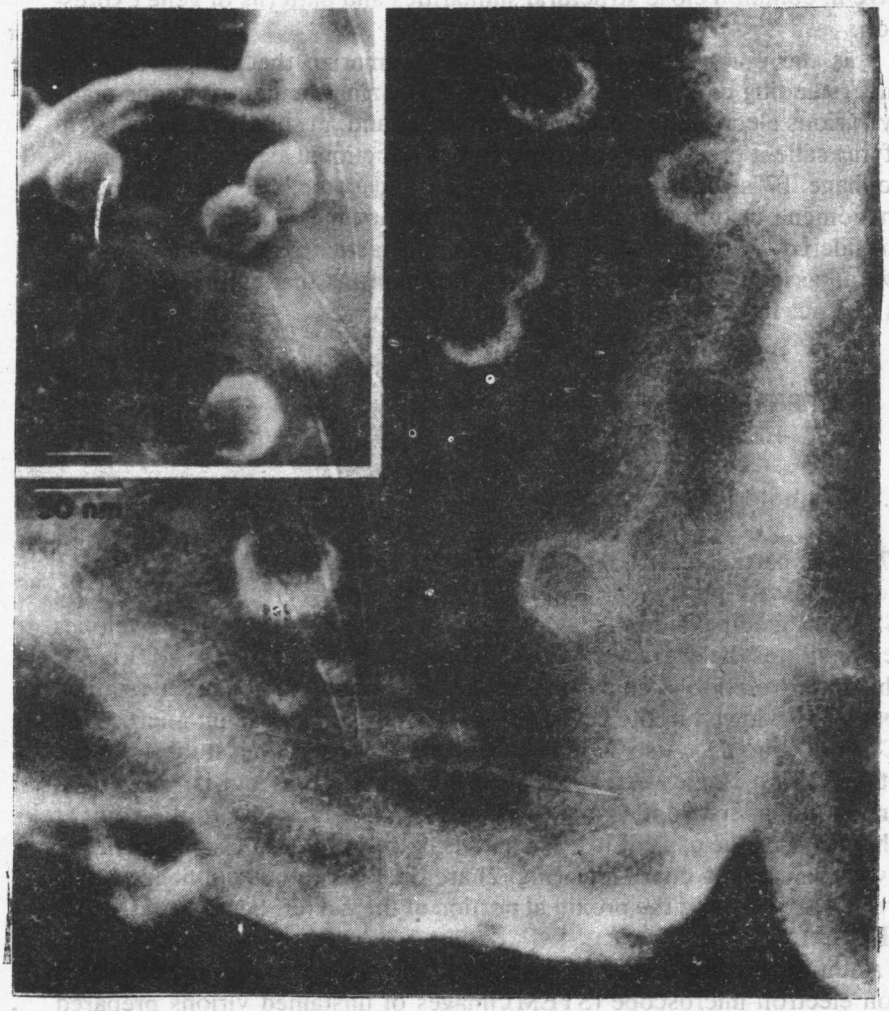

Fig. 1. Bacteriophage T7 virions adsorbed to *E. coli* bacteria, imaged by low-loss scanning electron microscopy after preparation by critical point drying followed by coating with gold/palladium. (Adapted from Panessa-Warren and Broers, 1979.)

lar clarity the tails as well as the tail fibers. With this specimen, the latter appear mainly to have been detached from the virions upon adsorption to the carbon film. This image also indicates the presence of spherical concentrations of density (B in Fig. 3b) within the polyhedral capsids, which presumably represent the condensed configuration of the packaged genomes (cf. Williams and Richards, 1974).

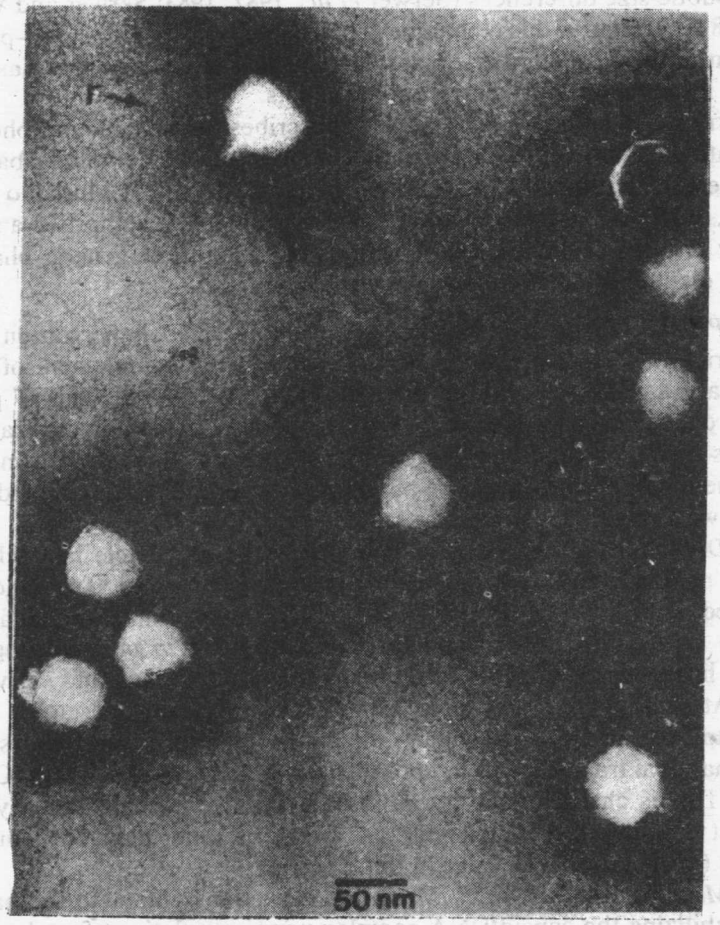

Fig. 2. Bacteriophage T7 virions imaged by bright-field conventional transmission electron microscopy after negative staining with uranyl acetate. On most particles, the stubby conical tail attached to one vertex of the polyhedral capsid is visible. In some cases, the filamentous tail fibers (F) are also visualized. (Courtesy of R. C. Williams, University of California, Berkeley.)

B. Assembly Pathway

In the case of phage T7, characterization of distinct types of virus-related particles has been greatly assisted by the refinement of agarose gel electrophoresis (Serwer, 1983). In this technique, particles are sorted primarily according to surface charge and diameter, and it has been possible to obtain good resolution between particle types that are distinguished by

rather subtle size differences (Serwer *et al.*, 1982, 1983; Serwer and Watson, 1982). Similarly, this technique has helped to clarify precursor–product relationships among these particles and to characterize them as assembly intermediates or aberrant by-products.

In terms of overall organization, T7 subscribes to the same morphogenetic pathway followed by all similarly complex dsDNA-containing bacteriophages characterized to date (Casjens and King, 1975; Murialdo and Becker, 1978; Carrascosa, Chapter 2 of this volume). It comprises a specifically ordered sequence of interactions in which three general phases may be distinguished as follows:

(1) *Prohead assembly:* In prohead assembly, the polymerization of a geometrically correct precursor particle with its full complement of the major capsid protein (gp10) but devoid of DNA is effected. The T7 prohead is called Capsid I (Serwer, 1976). Regulation of its polymerization involves form-determining interactions between gp10 and gp9, a major constituent of Capsid I in whose absence particles are not assembled, as well as with other core proteins.

(2) *DNA packaging:* Capsid I serves as the receptacle into which DNA is packaged from a concatemeric replicating complex. The genome packaged ($\sim 26 \times 10^6$ daltons) is linear, with a short terminal redundancy (Ritchie *et al.*, 1967; Dunn and Studier, 1983). An *in vitro* packaging system has been established for the T7 system (Masker and Serwer, 1982). At some point between the completion of Capsid I assembly and the completion of DNA packaging, the capsid undergoes an irreversible conformational transformation, entering into the "expanded" state (Capsid II). This is characterized by an increase in size accompanied by an apparent thinning of the shell wall, as well as by other physicochemical changes (cf. Section IV.B later in this chapter).

(3) *Maturation reactions:* Maturation reactions complete the pathway, stabilizing the capsid/DNA complex upon completion of packaging and finally yielding an infectious, fully equipped virion. With T7, this phase includes tail assembly, which takes place *in situ* upon the special-

Fig. 3. Bacteriophage T7 virions imaged without staining by dark-field scanning transmission electron microscopy after preparation by freeze-drying. In the unprocessed image (a), high values of projected specimen density are represented by bright tones and conversely. (Courtesy of J. J. Dunn and J. F. Hainfeld, Brookhaven National Laboratory, Long Island, New York.) The very wide dynamic range of projected densities spanned in these images has been compressed in (b) by digital image processing. This procedure more clearly outlines tails and renders visible some tail fibers (F), as well as ball-shaped concentrations of density (B) within the polyhedral capsids. Presumably, these represent the configuration of the packaged DNA.