Molecular Virology

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OUTLINE STUDIES IN BIOLOGY

Editor's Foreword

The student of biological science in his final years as an undergraduate and his first years as a graduate is expected to gain some familiarity with current research at the frontiers of his discipline. New research work is published in a perplexing diversity of publications and is inevitably concerned with the minutiae of the subject. The sheer number of research journals and papers also causes confusion and difficulties of assimilation. Review articles usually presuppose a background knowledge of the field and are inevitably rather restricted in scope. There is thus a need for short but authoritative introductions to those areas of modern biological research which are either dealt with in standard introductory textbooks or are not dealt with in sufficient detail to enable the student to go on from them to read scholarly reviews with profit. This series of books is designed to satisfy this need.

The authors have been asked to produce a brief outline of their subject assuming that their readers will have read and remembered much of a standard introductory textbook of biology. This outline then sets out to provide by building on this basis, the conceptual framework within which modern research work is progressing and aims to give the reader an indication of the problems, both conceptual and practical, which must be overcome if progress is to be maintained. We hope that students will go on to read the more detailed reviews and articles to which reference is made with a greater insight and understanding of how they fit into the overall scheme of modern research effort and may thus be helped to choose where to make their own contribution to this effort.

These books are guidebooks, not textbooks. Modern research pays scant regard for the academic divisions into which biological teaching and introductory textbooks must, to a certain extent, be divided. We have thus concentrated in this series on providing guides to those areas which fall between, or which involve, several different academic disciplines. It is here that the gap between the textbook and the research paper is widest and where the need for guidance is greatest. In so doing we hope to have extended or supplemented but not supplanted main texts, and to have given students assistance in seeing how modern biological research is progressing, while at the same time providing a foundation for self help in the achievement of successful examination results.

J. M. Ashworth, Professor of Biology, University of Essex.

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Contents

1 Introduction

1.1 Historical development of molecular virology

Viruses have occupied a central position in molecular biology ever since its development as an independent discipiine. Indeed, molecular biology itself largely developed out of the pioneer studies of Delbrück, Luria and Hershey, who realized, in the late 1930's, that bacterial viruses (bacteriophages, often abbreviated to phages) had properties which made them uniquely suitable as a model system for an attack on one of the then outstanding problems of biology, the definition of the gene in physical and chemical terms. The favourable properties of these viruses include the rapidity of their growth, their ease of assay, and the availability of easily scored genetic markers. Taken together, this means that quantitative genetic experiments can be done very quickly. The small size of viruses also suggested that their structure would be simple, which, in turn, led to the belief that this kind of system would be suitable for biochemical and physiological studies.

During the next two decades a small group of phage workers uncovered a series of fundamental principles which laid the foundations of modern virology and which had far-reaching effects on biological research in general. These studies established the basic pattern of virus replication, confirmed the identification of the nucleic acid molecule as the genetic material, and led to fundamental advances in the understanding of gene structure. An important factor during this period was the concentration of effort on a limited number of phages, notably the *Escherichia coli* phages T2 and T4. At the same time Lwoff and his colleagues were studying phage λ, a temperate phage of *E. coli*, work which was to lead to equally fundamental observations on the regulation of macromolecular synthesis.

The study of animal and plant viruses has its origins in the latter half of the 19th century and was largely initiated by workers in medical, veterinary and agricultural disciplines. Many of their practical successes owe little to molecular biology, stemming instead from those approaches successful in combating other parasites, such as vector control and the breeding of resistant varieties of plants. The introduction of new tissue culture techniques in the early 1950's was, however, an event crucial in the development of animal virology, both as an applied subject and as a branch of molecular biology. The development by Dulbecco of a simplified assay for the titration of animal viruses based on the standard plaque assay method used with phage was another crucial event at this time.

The development of animal virology owes much to concepts derived from phage studies and, to a lesser extent, plant viruses. However, in recent years many important features of animal viruses have been described which have no bacteriophage parallels. These include the phenomenon of virion maturation by budding from cell transformation and reverse transcription.

1.2 What is a virus?

A recent definition of the term virus (Luria and Darnell, 1968) runs 'Viruses are entities whose genome is an element of nucleic acid, either DNA or RNA, which reproduces inside living cells and uses their synthetic machinery to direct the synthesis of specialized particles, the virions, which contain the viral genome and transfer it to other cells'.

Viruses differ from other obligate intracellular parasites (such as Rickettsiae, and the Psittacosis group of organisms) in several fundamental respects, including:

(1) Virus particles (virions) contain only one type of nucleic acid: this can be either DNA or RNA.

(2) Virus-specified proteins are synthesized using host ribosomes.

(3) Viruses multiply by independent synthesis of their constituent parts which are then assembled to reconstitute new virus particles, rather than by growth and division.

Virus particles consist essentially of nucleic acid (the virus genome) surrounded by a protein coat. The function of the coat is to protect the nucleic acid from the harsh extracellular environment, to facilitate its entry into host cells, and, in many animal viruses, to play an important role in the initiation of virus macromolecular synthesis during the early part of infection. The structure of virus particles varies enormously in complexity. Many plant viruses, for example, contain a single small RNA molecule packaged in a coat made up of many identical copies of a single protein, whereas some animal virus particles have coats made up of multiple copies of at least 30 different proteins surrounding an extremely long DNA molecule. Likewise the size of virus particles also varies considerably from one type to another. A starting point in most schemes of virus classification is the chemical nature of the virus genome; thus viruses are grouped into those with DNA genomes and those with RNA genomes, and these groups are further subdivided into viruses with single- and double-stranded genomes.

Three major groups of viruses can be distinguished on the basis of host specificity; viruses of bacteria and blue-green algae, plant viruses, and animal viruses. Many members of these groups show specialized features connected with the problem of gaining entry into and replicating in their particular host cells. A good example of features restricted to one of these groups is the complex structure that many bacteriophages have evolved to overcome the problem of introducing their nucleic acids into their hosts through the barrier of the tough, rigid, bacterial cell wall. Animal viruses do not encounter such a barrier and plant viruses enter cells in different ways.

1.3 Titration of viruses

Quantitative analysis demands methods for the determination of the numbers of virus particles in a sample. Many methods are available. Some of these assay virus particles directly e.g. electron microscopy and haemagglutination of red blood cells, whereas others measure the infectious titre of a virus stock. These two types of method may not give the same titre since not all virions may be infectious. For phages there is generally a one to one correspondence between physical and infectious particles; for animal viruses the infectious titre is usually lower than the number of particles. A typical phage assay involves mixing a suitable dilution of virus (e.g. containing 100–200 infectious particles) with a concentrated suspension of bacteria (about 10^8 cells) suspended in molten agar held at 45°C. This mixture is poured over the surface of a petri plate containing solidified nutrient agar, to form a thin layer which soon hardens, thus immobilizing the phage and bacteria. The plate is incubated to permit multiplication of the bacteria which forms a confluent film of cells

over the agar surface, except where an infectious phage particle has been deposited. At this site the virus infects a cell and multiplies within it. The crop of a hundred or so progeny liberated from this cell infect adjacent bacteria which in turn produce further virus. Thus a local chain-reaction develops which after a few hours is visible as a clearing in the otherwise dense lawn of confluent bacteria. This clear zone is known as a plaque. By counting the number of plaques a direct estimate of the number of infectious virus particles is obtained. The plaque assay for animal viruses developed by Dulbecco is basically similar. Virus is added to a sheet of tissue culture cells growing in liquid medium on the flat bottom of a dish. After allowing the virus to adsorb to the cells, a layer of molten agar containing nutrient medium is poured over the cells and allowed to harden. This prevents free diffusion of virus through the medium. As with the phage assay, local areas of virus growth, each starting from one infected cell, are produced in the cell sheet. As cell death rather than cell lysis is the common end result of animal virus infections, plaques are usually detected by staining with dyes which are only taken up by living cells; the plaques stand out as colourless areas against a stained background of uninfected cells.

1.4 Virus–host interactions

Cell death is a common end result of virus infection. Many bacteriophages, for example, only escape from the host after cell lysis, and these viruses have evolved special mechanisms to break down the cell wall and membrane when virus growth is completed. Cells infected with many animal viruses do not lyse at the end of the virus growth period, and a contributory factor to their death is that during infection these viruses selectively and irreversibly turn off host DNA, RNA and protein synthesis in favour of their own macromolecular synthesis. Many bacteriophages also turn off host macromolecular synthesis and some even cause the breakdown of host components to provide building blocks for their own synthesis. Productive infections of cells without cell death do occur, however. The viruses causing this type of infection have evolved methods of virion release which do not cause irreversible cell damage. Many animal viruses, for example, are released from the cell membrane by a budding process, during which the virus incorporates a small piece of modified cell membrane as one of its structural components. This type of infection is compatible not only with cell survival but also with simultaneous growth of the infected cell and virus production.

In chronological order the events which take place during the virus growth cycle are:

(1) Adsorption of the virion to the cell surface.

(2) Entry of the virus nucleic acid (bacteriophages) or whole or part of the virion (animal viruses) into the cell.

(3) Transcription and translation of viral mRNA from the virus genome.

(4) Genome replication.

(5) Assembly of progeny virions and their release from the cell.

The whole process is known as the virus growth cycle. The growth cycles of bacteriophages are usually short, being measured in minutes, whereas animal viruses have much longer growth cycles (Fig. 1.1). This is probably related to the faster metabolic processes found in bacteria, which, of course, have much shorter growth cycles than eukaryotic cells.

Not all virus infections lead to progeny production. Some of the reasons for this are self-evident, including host resistance (host-virus relationships are often highly specific, e.g. poliovirus will not infect non-primate cells because they lack surface receptors for virus adsorption). In other situations, however, the virus genome may enter the cell and remain

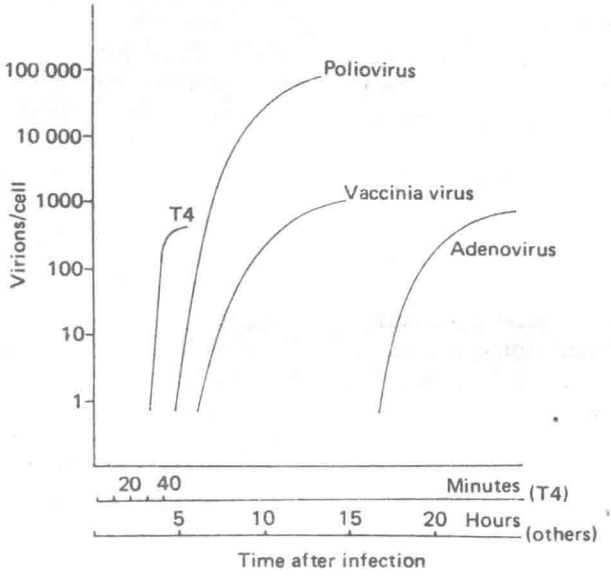

Fig 1.1 Growth curves of animal and bacterial viruses.

there, sometimes for many cell generations, without concomitant virus production. The classical example of this type of host-virus interaction is the lysogeny of bacteria by bacteriophages. Here, after infection, the virus DNA inserts itself into and becomes covalently linked to the bacterial chromosome. An efficient mechanism under virus control then ensures that this situation is maintained until certain environmental conditions occur, when the virus genome is excised from the host chromosome and virion production and cell lysis occur.

Many animal viruses can also integrate their nucleic acid into the host cell genome. With many DNA viruses this may happen during the course of a productive infection. Rarely, and usually only under special conditions in the laboratory, integration of the DNA of these viruses may be accompanied by striking changes in cell morphology and growth patterns (transformation); such cells may cause tumours in susceptible hosts (Chapter 5). Many types of RNA tumour virus, however, transform cells efficiently and continue to grow in the transformed cells. The genome of these cells contain covalently linked DNA copies of the RNA virus genome. Latency as exhibited by herpiviruses is another example of the long-term association of virus and host cell.

2 The virion

2.1 Principles of virion construction (Table 2.1)
Two basic patterns of virus structure can be recognized: (a) protein subunits arranged in a spherical shell with cubic symmetry (a crystallographic term indicating the relationship of subunits to each other) and (b) protein subunits arranged with helical symmetry. In both cases the protein subunits surround the nucleic acid molecule to form a structure known as a nucleocapsid. Helical nucleocapsids of animal viruses are always enveloped in a membrane which contains virus proteins and host cell lipids; many plant viruses and some bacteriophages exist as naked helical nucleocapsids. Only one group of viruses with cubic symmetry, the herpesviruses, is enveloped. In two groups of enveloped viruses (the togaviruses and the RNA tumour viruses) the arrangement of the protein subunits of the nucleocapsid is not known. In many groups of viruses with cubic symmetry, the spherical shell is not in direct contact with the nucleic acid but encloses another protein structure containing the viral genome; this is known as the virus core.

Some very large viruses, including the T-even bacteriophages and the poxviruses, have been classified as 'complex', the assumption being that they have neither cubic nor helical symmetry. It now seems likely that the head of T-even bacteriophages is made up of protein subunits arranged with cubic symmetry. The arrangement of protein subunits in the core of poxviruses is unclear at present.

2.2 Special features of virion anatomy

2.2.1 Particles with cubic symmetry

All viruses with this basic structure that have been critically examined have icosahedral symmetry. (An icosahedron is a symmetrical polyhedron with 12 vertices and 20 faces, each an equilateral triangle which in turn may be further subdivided into small equilateral triangles.) Caspar and Klug have shown that this type of construction is the only general way in which spherical shells may be constructed from large numbers of identical protein subunits. Although it is impossible to put more than 60 identical subunits on the surface of a sphere in such a way that each is identically arranged. many viruses with icosahedral symmetry have more than 60 subunits in their shell. Caspar and Klug resolved this puzzle by invoking the principle of quasi-equivalence which allows the formation of these shells if the bonds holding the subunits together are deformed in slightly different ways in different parts of the shell. It is also possible to construct such icosahedra by using more than one kind of structural subunit. Subunits are often arranged in groups of 5 (pentons) and groups of 6 (hexons) in the shell; the adenovirus is a good example of a virus with this type of structure (Fig. 2.1). This is a well characterized virus in structural terms, largely because its structural subunits are soluble under mild conditions, a property not shown by the structural subunits of most other viruses. The adenovirus subunits can be readily purified from infected cells where they are made in large

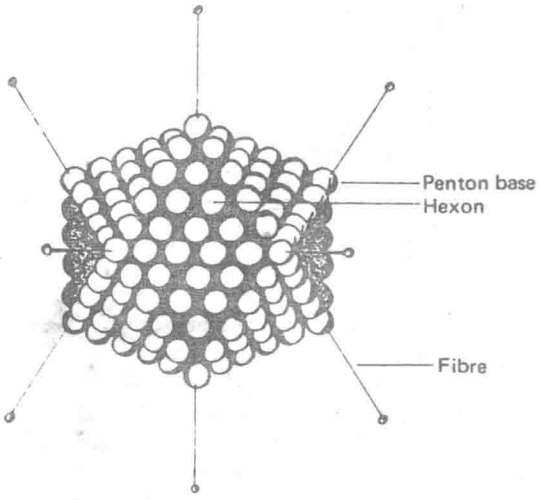

Fig. 2.1 Adenovirus – drawing of the virion.

amounts. The virus shell (capsid) is comprised of 252 structural subunits (capsomers), 240 being hexons with 6 neighbours, and a molecular weight of about 350 000 and 12 being pentons having 5 neighbours and a molecular weight of 400 000–515 000. These occur at the vertices of the icosahedron, and consist of a base and a fibre with a terminal knob projecting from the surface of the virus. If the virus is disrupted by strong detergents or urea the hexons are released in two forms, those neighbouring pentons being solubilized as singles and those forming the triangular facets of the icosahedron being released as groups of 9 (nonamers). Contained within the virus is a core containing a duplex DNA molecule (molecular weight 20-25 x 10^6) associated with protein. Further complexities of structure are revealed when the polypeptide composition of the structural subunits is examined. Hexons appear to be made up of 3 identical polypeptides (molecular weight 120 000); fibres are also trimers, the subunit polypeptide having a molecular weight of 60 000–65 000. Penton bases are difficult to work with but appear to be made of a number of identical polypeptides, molecular weight 70 000. The core contains two polypeptides (molecular weights 17 000 and 45 000) present in multiple copies.

The long penton fibres with their terminal knob are a feature unique to the adenovirus group. Distinguishing features of other icosahedral viruses include the number of structural subunits in the outer shell, which varies from 12 (bacteriophage ϕX174) to 812 (Tipula iridescent virus), their diameter, which varies from about 20 nm (parvoviruses) to 130 nm (Tipula iridescent virus), and the nature of the nucleic acid contained within the shell. This may be double stranded DNA (papova-, adeno-, herpes- and Tipula iridescent viruses), single-stranded DNA (bacteriophage ϕX174, parvoviruses), single-stranded RNA (picornaviruses, bacteriophage Qβ and its relatives, and many plant viruses) or double-stranded RNA (reoviruses). It is possible that many tailed bacteriophages have a head constructed with icosahedral symmetry; these viruses present so many other specialized features that they will be described in detail later.

Reoviruses stand out from the other icosahedral viruses in many ways. For example, they are the only group with double-stranded virion RNA, and their genomes are fragmented, each virion containing a number of different pieces of unequal sized RNA. In addition they are the only icosahedral viruses known which possess two shells, one surrounding the other. The inner shell contains the viral RNA and is known as the core It contains a transcriptase which synthesizes single-stranded RNA from the double-stranded template. Transcriptases from other viruses with icosahedral symmetry have not been described; this finding is probably connected with the observation that reoviruses are the only icosahedral viruses from which infectious nucleic acid has not been extracted.