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The Molecular Biology of Adenoviruses 1

30 Years of Adenovirus Research 1953—1983

Edited by Walter Doerfler

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With 69 Figures

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Preface

A puzzling epidemiological problem was the driving force behind the discovery of human adenoviruses by Wallace Rowe and his colleagues 30 years ago. The development of a plaque assay for poliomyelitis virus in 1953 led us to the threshold of quantitative virology, and in the same year the double-helical structure of DNA was discovered and became a cornerstone of molecular biology.

The potential of adenoviruses as research tools in the molecular and cellular biology of eukaryotic cells was recognized as early as the late 1950s and early 1960s by several investigators. Structural and biochemical studies dominated the early years. In 1962, some of the adenoviruses were the first human viruses shown to be oncogenic in experimental animals. Thus adenovirology offered the investigator the entire gamut of host cell interactions, productive and abortive, as well as transformed and tumor cell systems. The possibilities that adenoviruses afforded for the study of the molecular biology and genetics of eukaryotic cells were fully realized in the late 1960s and the 1970s.

Over many years, adenoviruses have proved to be a very successful model for research in molecular biology, facilitating the recognition and development of new principles in biology that have turned out to be generally applicable. Work on the icosahedral structure of the virion, the functional organization of the viral genome, a novel mode of DNA replication, problems of viral (foreign) DNA insertion into the host chromosome, the concept of transforming genes, the splicing of RNA, inverse correlations between DNA methylation and gene expression, and many other aspects attest to the viability of this model system. Recently, effects of adenovirus type 12 transformation on the class I major histocompatibility system of the host have been documented. Considering the complexity of the tumor problem, important parameters still remain to be discovered, and the model

of transforming viral or cellular genes will perhaps have to be refined and modified. The effects of adenovirus infection on amplifications and rearrangements of host genes are just beginning to be recognized. Recently discovered nucleotide sequence homologies between the E1A region of adenovirus type 12 and certain oncogenes raise tantalizing questions.

The role of adenoviruses in studies on the molecular biology of eukaryotes has occasionally been compared with that of bacteriophage lambda in investigations of prokaryotes. Some of the basic features of the organization and expression of the viral genome are still unknown, although the entire nucleotide sequence of human adenovirus type 2 has become available.

Adenoviruses will be used in the future to work out the detailed mechanisms and controls of some of the main reactions in molecular biology, and in that important role may indeed resemble that of bacteriophage lambda.

The main topics of adenovirus research have been repeatedly summarized in many excellent reviews. The three volumes in this series, The Molecular Biology of Adenoviruses, provide summaries of current research as well as more formal reviews.

I thank the editors of the series Current Topics in Microbiology and Immunology for inviting me to be guest editor, and I am indebted to all the contributors to the three volumes for submitting their manuscripts on time. I am particularly grateful to Prof. DIETRICH GÖTZE and MARGA BOTSCH at Springer-Verlag, Heidelberg, and to PETRA BÖHM and BIRGIT KIERSPEL in Köln for their careful and painstaking work.

Köln, December 1983 WALTER DOERFLER

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Structure and Assembly of Adenoviruses

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1 Introduction

Adenoviruses, this year celebrating the 30th anniversary of their discovery by man, are still in the foreground of scientific interest. With their multiple genes they are in fact one of the outstanding model systems for studying gene expression and its control in mammalian cells, as evidenced in numerous reviews (PHILIPSON et al. 1975; DOERFLER 1977; GINSBERG 1979; PHILIPSON 1979; NEVINS and CHEN-KIANG 1981; FLINT and BROKER 1981). The advances in the biochemistry and molecular biology of these viruses have not yet been balanced by equal progress in ultrastructural research of the virion itself and its assembly in the cell, although the architectural problems have recently received attention (PEREIRA and WRIGLEY 1974; EVERITT et al. 1975; BROWN et al. 1975; CORDEN et al. 1976; BURNETT et al. 1978; NERMUT 1980). Although adenoviruses are recovered from several hosts, including among others humans, monkeys, mice, and birds, with distinct but small differences in single polypeptides, the virion itself is of remarkably uniform morphology (Fig. 1). They all have an icosahedral shell and the capsid is built up from 252 capsomers, comprising 240 hexons, each surrounded by six neighboring capsomers, and 12 pentons at the vertices of the icosahedron each surrounded by five peripentonal hexons (VALENTINE and PEREIRA 1965; GINSBERG et al. 1966). Inside the capsid is a nuclear core containing the viral DNA and at least three viral proteins. The adenoviruses have been classified as viruses with an icosahedral nucleocapsid, but since the viral DNA is associated with viruscoded core proteins, the term nucleocapsid should probably be reserved for the core, and in analogy with other viruses the outer shell should be called the capsid. The human adenoviruses have for obvious reasons been investigated in more detail than those from other species. On the basis of biochemical, immunological, morphological and biological criteria they have been divided into seven subgenera, previously called subgroups. Each subgenus contains one or several serotypes, now referred to as species, separated on the basis of neutralization with type-specific antisera. Table 1 shows the basis for the current classification.

2 Architecture of the Virion

The icosahedral shape of the capsid leads to some confusion in the estimates given for the size of the virion. Diameter values of 60–70 nm have been published but it is not always clear whether these refer to the edge-to-edge distance or to the vertex-to-vertex distance. The dimensions of an icosahedron can always be calculated from the length of its edge (MATTERN 1969). The size for the edge of the capsid has been measured to be 43 nm and the edge-to-edge distance then corresponds to 70 nm and the vertex-to-vertex distance to 82 nm. These calculations give a diameter of the virion along the fivefold symmetry axis of around 73 nm (NERMUT 1975).

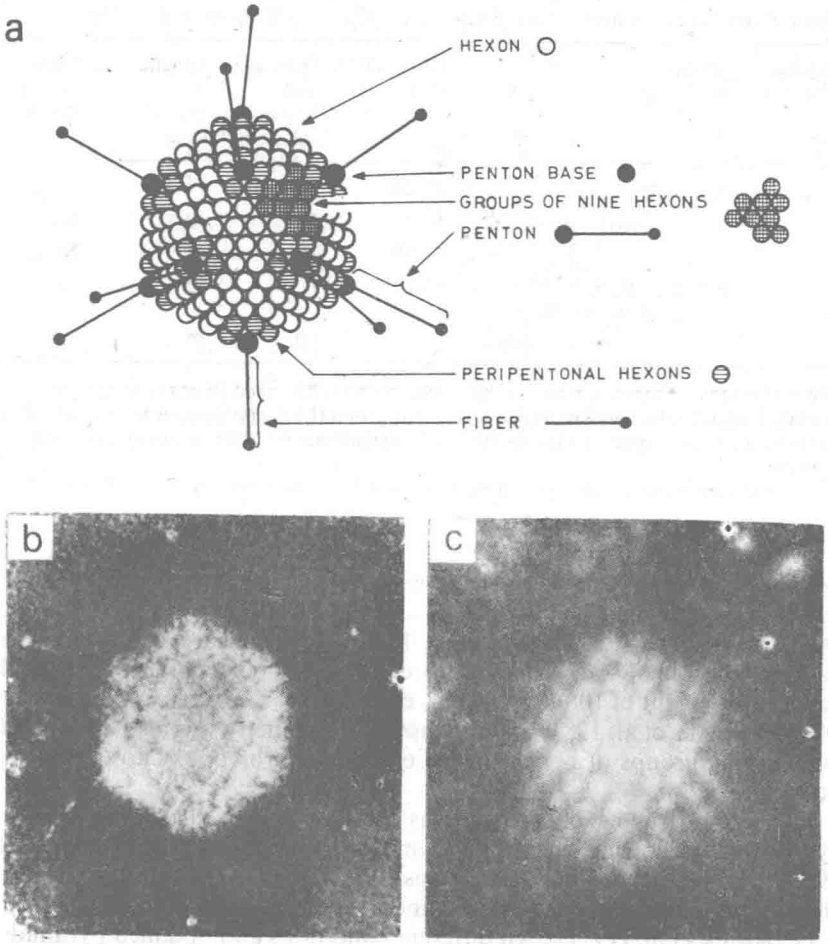


Fig. 1 a–c. Structure of the adenovirus capsid. **a** The icosahedral outline of the adenovirus capsid and the location of various components. [PHILIPSON and PETTERSSON (1973)]. **b.** Electron micrograph showing the Ad5 virion contrasted with sodium silicotungstate (VALENTINE and PEREIRA 1965). Note the antenna-like fiber protruding from each vertex. Figure kindly provided by Dr. W. RUSSELL, Mill Hill, London). **c** Electron micrograph of Ad5 virus contrasted with sodium silicotungstate, showing the regular icosahedral symmetry of adenoviruses. (HORNE et al. 1959)

The major subunit of the icosahedral shell of the virion is the hexon, which has attracted extensive attention because it is produced in large amounts in the infected cell, is soluble in the native form, and can be examined in the electron microscope. Electron microscopy (VALENTINE and PEREIRA 1965) and low-angle X-ray diffraction (TEJG-JENSEN et al. 1972) of

Table 1. Classification of human adenoviruses. [Adapted from WIGAND et al. (1982)]

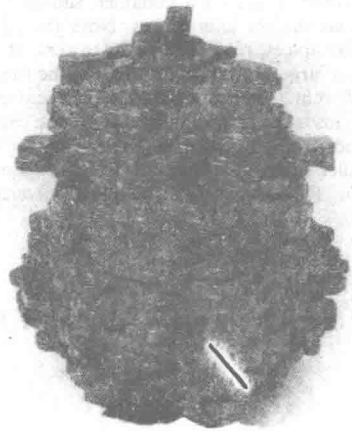
Subgenus[a] (subgroup)	Species (serotypes)	GC in DNA (%)	Hemagglutination subgroup[b]	Length of fibers (nm)	Oncogenicity in vivo[c]
A	12, 18, 31	47–49	IV	28–31	High
B	3, 7, 11, 14, 16, 21, 34, 35	50–52	I	9–11	Weak
C	1, 2, 5, 6	57–59	III	23–31	None
D	8, 9, 10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36, 37	57–60	II	12–13	None
E	1	57	III	17	None

[a] Two additional subgenera, F and G, have recently been described (WADELL et al. 1980)
[b] Group I agglutinates monkey erythrocytes and group II rat erythrocytes in a tight lattice forming a complete pattern. Groups III and IV agglutinate rat erythrocytes in an incomplete pattern
[c] Almost all nononcogenic serotypes have been shown to transform rodent cells in vitro

the hexons defined their basic shape and dimensions. They were originally described as spherical bodies with a central hole (VALENTINE and PEREIRA 1965) or hexagons (PETTERSSON et al. 1967). Biochemical work established that each hexon of Ad2 contains three identical polypeptides, each with a molecular weight of 108000 (MAIZEL et al. 1968a; GRÜTTER and FRANKLIN 1974; JÖRNVALL et al. 1981) and the threefold symmetry was also established by analyzing groups of hexons in the electron microscope (CROWTHER and FRANKLIN 1972).

The adenovirus hexon protein was the first animal virus protein to be crystallized. Following the original method described by PEREIRA et al. (1968), purified hexons dialyzed against 0.8 *M* KH_2PO_4 form a precipitate which is gradually converted into tetrahedral crystals. FRANKLIN et al. (1971) and CORNICK et al. (1971) used different conditions and obtained pyramidal crystals. Structural studies of both crystal types revealed that they had cubic symmetry and the space group $P2_13$. The length of the cubic cell is 10.99 nm and each unit cell contains four hexons. Since there are three asymmetrical units in the cell there must be three crystallographic asymmetrical units per hexon. The cylinder axis of the hexon is parallel to the axis of threefold symmetry. Consequently, there are three structural units symmetrically arranged around the cylinder axis of the hexon, which was also confirmed by electron microscopy (CROWTHER and FRANKLIN 1972). Attempts to reconstruct the hexon from electron-microscopic images indicated that different portions of the hexons have different symmetries (NERMUT 1975; NERMUT and PERKINS 1979).

Recent structural studies by X-ray crystallography (BURNETT et al. 1978; BERGER et al. 1978; BURNETT, 1983) have provided a more detailed 3D model of the hexon suggesting that it is a holotriangular prism (Fig. 2) with the following principal features: the lowest 1-nm portion of the hexon facing



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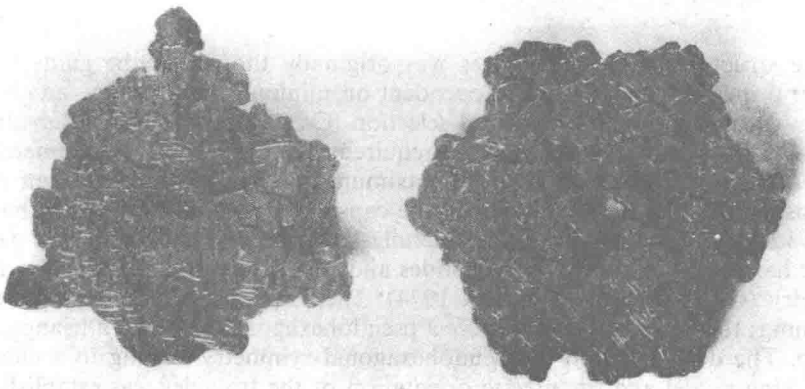

b

Fig. 2a, b. A model of hexon based on an electron density map at 0.6 nm resolution. **a** The intact trimer, illustrating the dense base and the more open top. **b** The model has been split open to emphasize the different features of the top and base. The triangular top (*left*), seen from above, shows the three towers surrounding a small hole and reveals the true threefold symmetry of the hexon trimer. The array of towers gives the adenovirus its spiky appearance since they are on the outside of the capsid. The base (*right*), seen from below, reveals the pseudo-hexagonal symmetry which allows the hexons to pack close together in the capsid. The large cavity in the base, which narrows in the middle to emerge between the towers at the top, is also seen. Figure kindly provided by Dr. R. BURNETT, Columbia University, New York)

the DNA core measures 7.5 nm in diameter and has an axial hole with a diameter of 3.5 nm. The middle portion from 1–5.2 nm is hexagonal with an 8.9-nm side, and the top portion from 5.2–11.6 nm facing the outside of the virion is triangular with a 7.5 nm side. The top triangle is twisted by around 30° relative to the middle hexagon. The centre of the channel is very narrow at the middle of the hexon but widens in both directions, especially toward the base. The height of the hexon is 11.6 nm. The hexons can be released from the virus in groups of nine, nonamers, each corresponding to one of the triangular facets of the icosahedron (Fig. 3). These nonamers have been useful in clarifying the polarity of the hexon, the majority showing a left-handed structure on hydrophobic carbon films but a right-handed structure on hydrophilic positively charged carbon films (NERMUT 1980). Thus the internal surface of the hexon is predominantly hydrophobic,

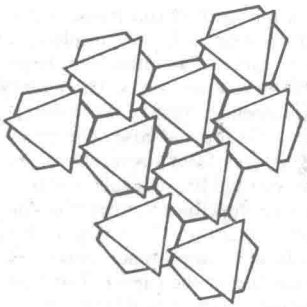

Fig. 3. A schematic model for a nonamer, showing the asymmetric hexon-hexon interactions. Note the offset of the triangular apices, representing the towers, at the top. This gives a large central cavity between the towers from three different hexon molecules at the centre of the nonamer. This is the arrangement seen on the capsid facet, as distinct from the other possible arrangement where the smaller cavity is at the centre. (Figure kindly provided by Dr. R.M. BURNETT, Columbia University, New York)

whereas the top is negatively charged at physiological pH, as would be expected if the hexon interacted with the core on the inside and gave the particle a negative charge on the outside.

2.1 Organization of the Virus Capsid

The structure of animal viruses was originally thought to be guided by two principles, one physical, dependent on minimum free energy, and one biological, dependent on natural selection (CASPAR 1966). The icosahedral capsid in adenovirus follows the requirements for the 5′3′2′ symmetries of an icosahedron, and therefore maximum bonding strength between the capsomers should give stability to the capsid. The hexon would then have six identical binding sites and hopefully six structural subunits. However, the hexon has only three polypeptides and shows threefold rotational symmetry (GRÜTTER and FRANKLIN 1974). The present model solves this dilemma; the hexon appears to have a pseudohexagonal base and a triangular top. The determination of pseudohexagonal symmetry leading to a close-packing model and the precise orientation of the triangles was established with crystallographic techniques (BURNETT, personal communication).

The interactions between the nonamers and the peripentonal hexons surrounding each of the pentons are, however, weaker than those within the nonamers. The penton-hexon interaction is obviously electrostatic, since dialysis at pH 6.3–6.5 causes release of pentons. Recent physicochemical data suggest that the penton base, like the hexon, contains only three polypeptides (DEVAUX et al. 1982). The minimum energy principle cannot therefore apply in the peripentonal region, and an additional linker protein between the penton base and the peripentonal hexons must provide additional stability. Protein IIIa may be a candidate for this linker, since it is probably present in five copies per vertex region (EVERITT et al. 1975; BOUDIN et al. 1980).

The fiber is highly asymmetric, consisting of a rod with a terminal knob (Fig. 1). The diameter of the rod is around 2 nm and the diameter of the knob around 4 nm. The length of the fiber varies among adenovirus subgen-

era (Table 1), and is 9–13 nm for subgenera B and D and 23–31 nm for subgenera A and C (NORRBY 1969b). Ad4 in subgenus E has 17-nm-long fibers. Avian adenoviruses, except for one species, have two fibers of different lengths extending from different sites on the penton base (LAVER et al. 1971; GELDERBLOM and MAICHLE-LAUPPE 1982). Dimers of fibers have been encountered but it is unclear whether they have a physiological role (WADELL and NORRBY 1969a; NORRBY et al. 1969b). Fibers from Ad5 have been crystallized (MAUTNER and PEREIRA 1971) and structural studies are currently under way (GREEN et al. 1983; DEVAUX et al. 1983). Conformational studies suggested that the native fiber is mainly in a β-pleated sheet configuration (BOULANGER and LOUCHEUX 1972). Inspection of the amino acid sequence revealed a repeating motif of 15 residues organized in two short β-strands and β-bends (GREEN et al. 1983). The fiber unit has been thought to consist of three probably identical polypeptide chains (SUNDQUIST et al. 1973a) but recent data suggest a dimer structure (DEVAUX et al. 1983). The fiber obviously functions as the attachment organelle for the virus at the plasma membrane of the cell at the initial phase of infection (PHILIPSON et al. 1968).

The general principle for building the adenovirus capsid therefore involves close packing of proteins, with morphological symmetry greater than the true molecular symmetry, in a capsid with icosahedral symmetry. Several additional proteins are, however, required in building the capsid of the adenoviruses, as became evident from the detailed analysis of the protein composition of the particles.

2.2 The Core

The adenovirus genome, which is a linear double-stranded DNA of around $20–23 \times 10^6$ daltons (GREEN et al. 1967; VAN DER EB et al. 1969), is packaged within the icosahedral capsid in a nucleoprotein structure called the core. The cores first identified by electron microscopy (EPSTEIN 1959) can be released from the outer shell by exposure of purified virions to one of several denaturants such as heat, desoxycholate, and pyridine (RUSSELL et al. 1971; PRAGE et al. 1970). After freeze fracturing the particles often fracture between the capsid and the core (BROWN et al. 1975; NERMUT 1978). If so, the edge of the cores can be measured, and values of 34 nm have been reported (NERMUT 1978). When the cores are isolated in solution without the capsid, they are not rigid enough to maintain an icosahedral shape. Freeze-fracture studies also suggest that the nucleoprotein is surrounded by a protein shell, but the protein covering the cores has not been identified. It has tentatively been suggested that protein V, the so-called minor core protein, might form a protein shell around the nucleoprotein containing the major core protein VII (NERMUT 1980). The adenovirus nucleoproteins do not contain cellular histones as do papova virions (ROBLIN et al. 1971; FREARSON and CRAWFORD 1972); the viral DNA is instead associated with

two virus-encoded basic proteins V and VII. A third arginine-rich core protein called μ with a molecular weight of around 4000 has recently been described (HOSAKAWA and SUNG 1976). Neither the precise location nor the role of this protein in the core has been determined.

2.3 Organization of the Nucleocapsid

The core-associated polypeptides probably mediate the packaging of adenovirus DNA within the virions, but the adenovirus DNA in core particles appears not to be protected from nuclease attack in a way similar to cellular DNA packaged in nucleosomes. CORDEN et al. (1976) reported that digestion of adenovirus cores with micrococcal nuclease yields discrete DNA products in a ladder arrangement from 200 to 1800 bp. Others have failed to reproduce this result, observing only protected viral DNA which was smaller than that obtained from cellular chromatin (TATE and PHILIPSON 1979; BROWN and WEBER 1980). Native core preparations are, however, compact, with the structure of a thick fiber of around 30 nm in diameter (VAYDA et al. 1983) which displays a morphology similar to that of chromatin fibers. These thick fibers contain polypeptides V and VII and also polypeptide μ, and all three proteins sediment together with released cores. When the cores are exposed to 0.5 *M* NaCl, proteins V and μ are released and the cores become at the same time less resistant to nuclease digestion and appear in the electron microscope as a beaded string similar to cellular nucleosomes. Although there is disagreement concerning a ladder arrangement of the protected DNA sequences at low concentrations of nucleases (CORDEN et al. 1976; TATE and PHILIPSON 1979; VAYDA et al. 1983), a consensus exists that polypeptide VII protects around 100–150 bp of viral DNA. The virus protein VII may therefore form a protein particle about which the DNA is wound in a similar fashion to nucleosomes. The diameter of such a filament would be about 9 nm provided there are three protein subunits of 2.5–3 nm in diameter per turn. Protein VII would then provide a helical core structure which is packed in a more condensed form with the protein V and the μ polypeptide. Finally, a terminal DNA binding protein is covalently attached to each 5′ end of the DNA but this cannot influence the structure since it is only present in two copies per viral genome.

3 Protein Composition

The adenovirus particle was originally claimed to be composed of two kinds of proteins, hexons and pentons, in addition to the core, but when the adenovirus proteins were analyzed by SDS-PAGE (MAIZEL et al. 1968a; MAIZEL 1971), a considerable complexity was revealed and it is now agreed