TABLE I
RADIAL ORGANISATION OF PROTEIN AND DNA IN NATIVE ADENOVIRUS AND H_2ts112

Shell radii	Native virus		H_2ts112	
0–303	DNA	22.2×10^6	DNA	4.4×10^6
	Protein	22.3×10^6	Protein	16×10^6
303–348	Protein	30.9×10^6	Protein	12×10^6
348–420	Protein	83.7×10^6	Protein	92×10^6

high density in the outer protein shell of the mutant implies a movement of protein on maturation to the native virus. X-ray small-angle scattering from the the mutant particle showed that the small piece of encapsidated DNA, unlike that of the native particle DNA, does not give rise to the 1/29 $Å^{-1}$ maximum (4) and is therefore not ordered in a similar manner. Considering the extremely icosahedral shape of the virus as seen in the electron microscopy, we can convert the spherical shell model into an equivalent scattering icosahedron (4) which gives us a measure of the overall real particle dimensions. For both the mutant and native virus this yields a distance between fivefold vertices of 520 Å. This value is confirmed by electron-microscopy measurements from specimens in amorphous ice that give 515 ± 15 Å, corresponding to a water content of ~0.3 g/g of virus, which is reasonable. The distance between fivefold axes also yields a hexon-hexon distance ~103 Å which agrees well with values obtained from isolated GON either by x-rays (98–100 Å) or by EM in amorphous ice (101 Å). They are larger than expected from a close packing of the hexons, which would correspond to ~89 Å. This implies that another protein, perhaps protein IX, plays a role in the capsid integrity. Indeed, in image reconstructions of GON we see rather tenuous contacts between adjacent hexons. Colby and Shenk (6) observed, however, that the mutant H_5dl313, which lacks protein IX, is able to form rather fragile but complete capsids. We do not know the dimensions of these capsids and must conclude that this observation merely shows that close packing is possible, and that a looser packing, perhaps modulated by protein IX, is found in the native virus.

Received for publication 3 May 1985.

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PROGRESS IN UNDERSTANDING ADENOVIRUS ARCHITECTURE

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The icosahedral adenovirus virion (Fig. 1) is formed from at least 10 different polypeptides and has a total molecular mass of 149,000 d.[1] The detailed architecture is being explored by x-ray crystallography, electron microscopy, and biochemistry (1). A 2.9 Å electron density map of the major coat protein, hexon, has been obtained for adenovirus type 2. The molecular envelope (2) reveals that, although trimeric, hexon has a basal region exhibiting pseudo-hexagonal symmetry. An upper triangular region, directed outward when the molecule is incorporated into the capsid, provides a recognizable feature for the determination of hexon organization using the electron microscope. The three-dimensional structure determination was recently completed,[2] and the chain tracing reveals 859 of the 967 amino acids. The base is formed from three pairs of nonidentical domains, whereas three identical domains shape the top. The majority of the missing amino acids, and the regions of heterologous sequence, lie within the hexon tops and thus the outer capsid surface.

A model for the overall arrangement of hexons in adenovirus has been derived (3), and confirmed using the

[1]van Oostrum, J., and R. M. Burnett. 1985. Molecular composition of the adenovirus type 2 virion. Manuscript submitted for publication.

[2]Roberts, M. M., J. L. White, M. G. Grutter, and R. M. Burnett. 1985. Three-dimensional structure of adenovirus hexon. Manuscript in preparation.

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Volume 49 January 1986 22–24

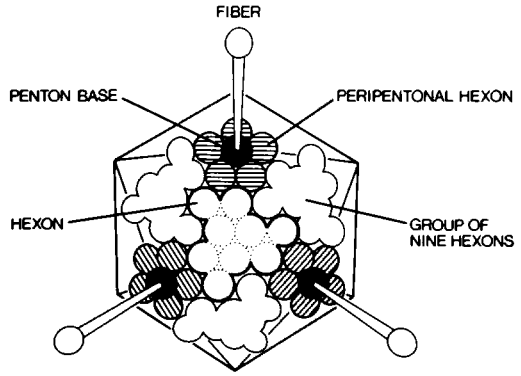

FIGURE 1 A schematic view of adenovirus indicating the major external proteins. The order of dissociation is: peripentonal hexons; penton complex (base and fiber); groups-of-nine; core. Illustration by John Mack (1).

known molecular morphology to determine the orientation of hexons from high-resolution electron micrographs of various capsid fragments (4). A quartet of hexons forms the asymmetric unit of an icosahedral hexon shell (Fig. 2), which can be closed by the addition of pentons at the 12 vertices. If the hexon trimer is taken as a complex structure unit, its interactions in the four topologically distinct environments are very similar, with conservation of bonds for at least four of the six interhexon contact faces. The crystal-like construction explains the flat facets and sharp edges characteristic of adenovirus. Expansion of the crystalline facets by using one additional topologically related environment permits the construction of larger "adenovirus-like" capsids of any size (Fig. 3).

Dissociation of the icosahedral adenovirus under mild conditions results in the loss of the pentons, located at each of the 12 vertices, with their five surrounding peripentonal hexons. The remaining 180 hexons, which form the major

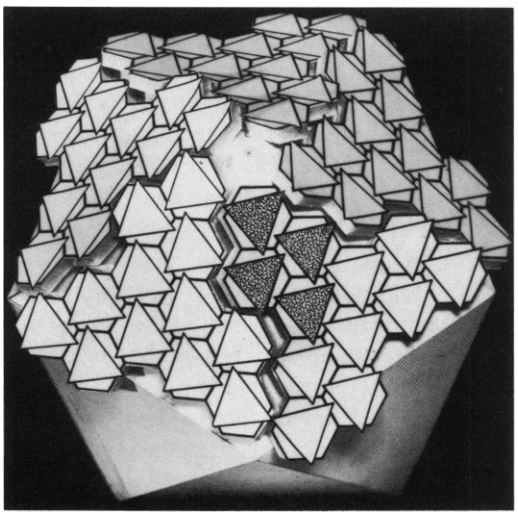

FIGURE 2 Our model for hexon packing in adenovirus. An asymmetric unit of the icosahedral hexon shell is shaded. Although each hexon environment is topologically distinct, the set of six hexon contacts shows many identities.

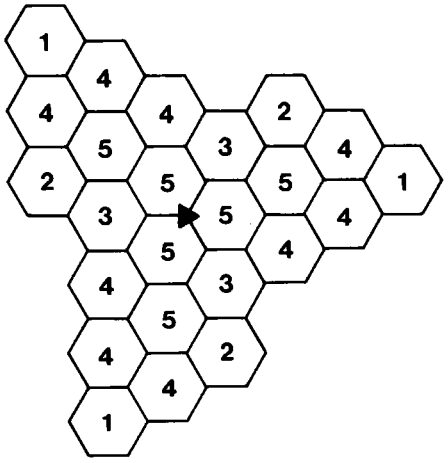

FIGURE 3 A hypothetical viral facet with twice the number of hexons (3). Hexons 1 to 4 are in identical environments to those in the adenovirus shell. Hexon 5 lies in a different, but topologically related, position. Capsids of any size can be constructed using only these five hexons.

part of the facets, dissociate as 20 groups-of-nine (Fig. 1). A model for the location of adenovirus polypeptide IX as a capsid cement (1) explains its dissociation pattern and that of the groups-of-nine (Fig. 4). We have used radiolabeling to obtain an accurate absolute determination of the molecular composition of the virion.[1] The representation of different proteins is consistent with our capsid model, and shows further that the penton base is a pentamer and the fiber a trimer. Two slightly different penton base polypeptides are present in the ratio of 2:3. This suggests that the symmetry mismatch between fiber and base is overcome by binding of fiber only to the three shorter penton base polypeptides.

The construction of adenovirus illustrates how a faceted impenetrable protein shell can be formed while highly conserved intermolecular bonding is maintained. The design uses the geometry of an oligomeric structure unit and symmetry additional to that of the icosahedral point

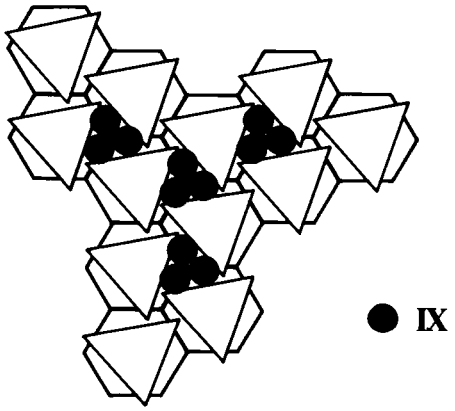

FIGURE 4 The proposed distribution of polypeptide IX within the capsid. The capsid "mortar" stabilizes the group-of-nine and affects its disruption pattern, but does not affect the bonding of peripentonal hexons.

group to achieve close-packing within both facet and edge. This contrasts with the continuously curved capsid model of Caspar and Klug (5), in which the polypeptide is usually assumed to be the structure unit. Here, the number of quasiequivalently related bonding configurations required for the structure unit tends to infinity as the capsid increases in size.

Our results for adenovirus suggest that larger units than the polypeptide always should be considered as possible structure units when evaluating viral architecture. Large, weakly-interacting building blocks permit accurate assembly into a highly restricted set of related locations. Stability is later conferred by the addition of a different structural component. This new approach casts light, for example, on the controversial demonstration of an all-pentameric polyoma capsid (6), and on the dissociation of the small plant viruses (7). In polyoma, one class of 60 pentamers bonds identically to give an icosahedral shell, with another class of 12 pentamers completing the capsid. In the small plant viruses, one set of intersubunit interactions within the hexameric clusters is almost identical to that of the pentamers. In addition, the clusters move apart as units when calcium is removed.

The investigation was supported by National Institutes of Health (AI-17270), National Science Foundation (PCM-8418111), and an Irma T. Hirschl Career Scientist Award to R. M. Burnett.

Received for publication 9 May 1985.

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DNA INJECTION APPARATUS OF PHAGE P22

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During the assembly of double stranded DNA phages, DNA molecules are translocated from the cytoplasm into preformed procapsid shells, and condensed into a tightly wound state (Earnshaw and Casjens, 1980). At a later stage in the life cycle the process reverses, and the chromosome is released from the capsid and injected into the cytoplasm of a new host cell. At the heart of these processes is the unique vertex that morphologically forms the head/tail junction (Murialdo and Becker, 1978). This vertex is assembled as the very first stage of capsid assembly, serving first as the initation complex for procapsid assembly (Black and Showe, 1983).

We will refer to this vertex as the DNA translocating or portal vertex. A feature of these vertices in phages T4, λ, and $\phi 29$ is a special "portal protein" species, which forms a 12-fold ring at the vertex (Bazinet and King, 1985).

In the mature phage the portal vertex is located at a junction marked by symmetry mismatch. The tails of all known double-stranded DNA phages have sixfold rotational symmetry, while the capsid vertices have fivefold symmetry. Hendrix (1978) has proposed that this mismatch is a fundamental aspect of the enzymatic machinery of DNA packaging, and reflects the need for a rotation of some structure within the specialized vertex during DNA translocation.

In the Hendrix model the symmetry mismatch between pentameric and hexameric structures provides the metastable states permitting rotation. If there is rotation at this vertex, the portal proteins probably play a central role (Bazinet and King, 1985).

P22 is a double-stranded DNA phage infecting *Salmonella typhimurium*, whose cell attachment and DNA injection apparatus is simple in comparison with phages T4 and λ. It has been the subject of extensive genetic and biochemical investigation, and its structure and assembly pathway is understood in considerable detail (King and Casjens, 1974; Thomas et al., 1982; Fuller and King, 1982). The procapsid of P22 is assembled through the copolymerization of two major proteins, the coat and scaffolding subunits, and four minor proteins (Fuller and King, 1982). After shell completion, the portal vertex of the procapsid interacts with a complex of DNA packaging proteins and replicating DNA (Earnshaw and Casjens, 1980). After initation of DNA packaging, but before its completion, all of the scaffolding subunits exit from the procapsid, and the coat protein lattice expands and stabilizes forming the mature capsid (King and Casjens, 1974). At the termination of packaging a headful of DNA is cut from the replicating concatemeric DNA, releasing the packaging proteins.

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Volume 49 January 1986 24–26