JOURNAL OF VIROLOGY, Nov. 1985, p. 439–448
0022-538X/85/110439-10$02.00/0
Copyright © 1985, American Society for Microbiology
Vol. 56, No. 2

Molecular Composition of the Adenovirus Type 2 Virion

JAN VAN OOSTRUM AND ROGER M. BURNETT*

Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received 28 May 1985/Accepted 7 August 1985

The representation of the different structural polypeptides within the adenovirus virion has been accurately determined, and the particle molecular weight has been derived. A stoichiometric analysis was performed with [^{35}S]methionine as a radiolabel, and analytical sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to separate the polypeptides. The recently available sequence of the adenovirus type 2 genome was used to determine the number of methionines in each polypeptide. The resulting relative representation was placed on an absolute scale by using the known number of hexon polypeptides per virion. The analysis provides new information on the composition of the vertex region, which has been the subject of some controversy. Penton base was found to be present in 60 copies, distributed as pentamers at each of the 12 vertices. Three fiber monomers were associated with one penton base to form the penton complex. Polypeptide IX was present in 240 copies per virion and 12 copies per group-of-nine hexons, supporting a model proposed earlier for the distribution of this protein. The location of polypeptide IX explains the dissociation of the virus outer capsid into groups-of-nine hexons. The penton base was microheterogeneous, and the relative amounts suggest that the symmetry mismatch, which occurs within the penton complex between base and fiber, is resolved by the synthesis of penton base polypeptides from two closely spaced start codons.

Adenovirus is the name for a family of related viruses, the first of which was isolated by Rowe et al. (46). The virion is icosahedral (26) and contains at least nine different structural proteins. The structural polypeptides have been labeled II to IX on the basis of decreasing molecular weight as revealed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (35). Polypeptides II, III, and IV correspond to hexon, penton base, and fiber, which are the major constituents of the outer capsid of the virion. Polypeptide IIIa has been assigned to the vertex region (20). The viral DNA is associated with polypeptides V and VII to form a nuclear core organized as 12 spherical subunits, each in contact with a vertex of the icosahedral outer capsid (12). The core organization has recently been confirmed by ion etching and electron microscopy (38). Both polypeptides VI and VIII are associated with hexons, and polypeptide VI also has a high affinity for DNA (48). Polypeptide IX is associated with hexons and has a stabilizing effect on the capsid (17). Models summarizing current views on the spatial organization of the various structural polypeptides in the virion are contained in recent reviews by Philipson (43) and Pettersson (42).

The outer capsid consists of 252 morphological units formed from the major coat proteins (26). Pentons lie at the 12 vertices of the icosahedral capsid, and 240 trimeric hexons form the 20 facets and 30 edges (22). Dissociation of the capsid releases planar groups-of-nine hexons (GONs), which form the major part of the capsid (50). The subunit composition of penton, a complex of penton base and fiber, has recently been investigated by using physical techniques, to reveal a trimeric base and a dimeric fiber (18, 19). This result for the fiber agrees with structural predictions from the amino acid sequence (24). However, the results are inconsistent with previous studies indicating a pentameric base and trimeric fiber (43) and require two symmetry mismatches. One of these would occur between the fiber and penton base, and the second would occur between the penton base and its surrounding five peripentonal hexons.

One nonstructural protein and two structural proteins of adenovirus have been crystallized. Microcrystals of fiber were first observed in 1971 (36), but three-dimensional crystals suitable for X-ray diffraction have been obtained only recently (18). A fragment of the nonstructural 72,000-molecular-weight DNA-binding protein has also been crystallized (53). Hexon was first crystallized by Pereira et al. (40), and a structural analysis of adenovirus type 2 (Ad2) hexon is under way in our laboratory. A 0.6-nm envelope model (14, 14a) has revealed how trimeric hexon molecules form a close-packed capsid (13, 13a), and the polypeptide chain has recently been traced within a 0.29-nm resolution electron density map (M. M. Roberts, J. L. White, M. G. Grütter, and R. M. Burnett, submitted for publication).

The capsid model was based on a comparison of electron micrographs of virions and GONs with the crystallographic model. Recent results, obtained from image analysis of electron micrographs of quarter-capsids, viral fragments, each containing a penton and five facets of 12 hexons, have confirmed that all facet hexons lie on a small p3 net (54). The current investigation was undertaken to test a model for the distribution of polypeptide IX within the capsid (13), which explains capsid dissociation by the location of this capsid-stabilizing protein. An earlier determination of the absolute number of polypeptide IX molecules in the virion by using [^{14}C]valine (11) relied on the assumption that the valine residues were distributed within polypeptides in direct proportion to their length. The recent availability of the DNA sequence of the complete genome of Ad2 (1, 3, 45) now permits an accurate determination of the stoichiometric relationship of all virion polypeptides. [^{35}S]methionine was used as a radiolabel to obtain a cost-efficient, highly specific radioactivity in all polypeptides. The polypeptides were separated on linear SDS-polyacrylamide gradient gels, and their total radioactivity was then normalized by using their methionine content. Hexon was used to place the relative values on an absolute scale, as it is the only protein for which all the vital information on subunit composition, copy num-

* Corresponding author.

ber per virion, and sequence, has been firmly established (1, 25).

MATERIALS AND METHODS

Cells and virus. KB cells were grown to a density of 2.5×10^5 cells per ml in Spinner Eagle medium supplemented with 5% calf serum and then infected with Ad2 wild-type virus at a multiplicity of 20 PFU per cell. The HeLa plaque assay (55) was used to determine the number of PFU. The suspension culture was kept at 37°C for 44 h before the cells were harvested by centrifugation at 1,500 × *g* for 25 min.

[^{35}S]methionine-labeled virus. Ad2 wild type (20 PFU per cell) was used to infect 2.5×10^5 KB cells per ml of Spinner Eagle medium supplemented with 5% calf serum. At 8 h postinfection the cells were sedimented by centrifugation at 800 × *g* for 10 min and resuspended in 9/10 of the original volume in the same medium, but lacking methionine. After incubation for 20 min to deplete the residual endogenous methionine, 1/10 volume of the standard medium supplemented with 150 μl of [^{35}S]methionine (1,000 Ci/mmol) was added to give a radioactivity of 4.8 μCi/ml in the growth medium. Replication was continued at 37°C until 44 h postinfection. The level of [^{35}S]methionine was monitored and maintained above 2 μCi/ml by the addition of labeled and unlabeled methionine, in the same ratio as before, to ensure a constant supply throughout the growth phase.

Preparation of virus. Virus was prepared as described by Lawrence and Ginsberg (33), except that the cells were disrupted by freezing and thawing. The harvested cells were washed twice with 150 mM NaCl in 10 mM phosphate buffer (pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride. After being frozen and thawed four to six times, the cells were treated with DNase at 37°C, the suspension was centrifuged at 1,500 × *g* for 10 min, and the supernatant was collected. The solution was then extracted three times with equal volumes of Freon 113 (E. I. du Pont de Nemours & Co., Inc.), and the final aqueous phase was layered on a CsCl block gradient of density 1.2 to 1.4 g/ml. The viral band was collected after centrifugation for 3 h at 4°C at 100,000 × *g* in a Beckman SW 27.1 rotor. To obtain a good separation of mature virions from earlier forms and defective particles, the virus was subjected to a second centrifugation. The virus, after 1:1 dilution with 1 mM EDTA in 10 mM Tris hydrochloride (pH 8.1), was layered on top of a linear CsCl gradient of 1.2 to 1.4 g/ml and centrifuged for 16 h as described above. The virus, at a density of 1.345 g/ml, was collected by puncturing the bottom of the tube and carefully removing the visible band of virus.

Preparation of GONs. The preparative procedure was that of Russell et al. (47) as modified by Boulanger et al. (10). Virus was dialyzed against 150 mM NaCl in 10 mM Tris hydrochloride containing 1 mM phenylmethylsulfonyl fluoride (pH 8.3). Sodium deoxycholate was added to a final concentration of 0.5% (wt/vol), and the solution was incubated at 57°C for 60 to 90 s until a sudden clearance of the bluish opaque virus solution occurred. The solution was layered onto a linear 10 to 30% glycerol gradient resting on a 60% glycerol cushion. After centrifugation for 2 h at 4°C in a Beckman SW 41 rotor at 100,000 × *g*, the bottom of the tube was punctured and 0.5-ml fractions were collected. A sedimentation profile was obtained from the optical density for unlabeled virus or from the radioactivity for labeled virus. Fractions containing GONs were pooled and stored at −20°C.

Preparation of the penton complex. Ad2 was dialyzed against 5 mM Tris-maleate buffer containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride (pH 6.3) for 18 h at 4°C (32). The slightly opaque solution was centrifuged in a Beckman Airfuge at 50,000 × *g* for 30 min at room temperature. The supernatant, which contained the majority of the peripentonal hexons together with the pentons, was collected and used immediately.

Analytical SDS-polyacrylamide gel electrophoresis. All gels were made with the buffer system described by Laemmli (31), with a fixed ratio of acrylamide to bisacrylamide of 30:0.8. The flat gels, of thickness 1.5 mm, were prerun for 20 min before the sample was loaded. Samples were mixed with a small amount of buffer, consisting of 0.128 M Tris hydrochloride containing 4% SDS, 0.2 M 2-mercaptoethanol, and 20% glycerol (pH 6.7), and boiled for at least 3 min at 100°C. The gels were run at a constant current of 20 mA at 4°C.

All gels were run with equal amounts of protein in each of five lanes. Linear 10 to 17.5% acrylamide gradient gels, 120 mm long (short gels), were used for the analysis of GONs. Longer gels (220 mm) run for 16 h were used for the analysis of the complete virus. Short gels with a gradient of 10 to 14% were used to resolve the high-molecular-weight region of the viral polypeptides. These were also run for 16 h, which resulted in the loss of all polypeptides with a molecular mass smaller than that of polypeptide V (approximately 45,000 daltons). The short gel system was used to analyze the penton complex.

All gels were stained with 0.2% Coomassie brilliant blue R-250 in 50% methanol–3.5% glacial acetic acid for 2 h and destained in 25% methanol–7% glacial acetic acid. For autoradiography, gels were soaked in Autofluor (National Diagnostics), an image enhancer, for 1 h and dried under vacuum. Kodak X-ray film was exposed to the dried gels for 2 to 48 h.

Scintillation counting of polyacrylamide gels. Destained gels were sliced into 1.15-mm pieces with an electrical gel slicer (Hoefer Scientific Instruments). Each slice was transferred to a glass scintillation vial containing 0.9 ml of 50% hydrogen peroxide. After incubation for 48 to 72 h at 47°C to dissolve the gels, the vials were cooled and 13 ml of Biofluor (New England Nuclear Corp.) was added to each. Each sample was counted for 10 min in a Beckman LS-100 liquid scintillation counter with a fully open window. Serial dilutions of highly radioactive samples were used to test for a nonlinear response, indicating that quenching was occurring in the samples. Without complete hydrolysis of the polyacrylamide, the residual material gave this effect.

It should be noted that disappearance of solid polyacrylamide, as judged by eye, does not necessarily indicate complete hydrolysis. It was essential to use a 50% solution of hydrogen peroxide, as a 30% solution was unable to completely dissolve all slices. Incubation with 50% hydrogen peroxide for 24 to 48 h, depending on the polyacrylamide concentration, resulted in the disappearance of visible polyacrylamide, but incubation for a further 24 h was required to ensure complete dissolution and no resultant quenching. The recovery of radioactivity from the gels was better than 95%. No difference in recovery was found between gels that were stained and destained as usual and gels sliced immediately after electrophoresis without staining. This indicated that there was no loss of protein from the gels or quenching due to binding of dye.

Analysis of gel profiles. The radioactivity of each gel was analyzed with the aid of a computer program. The radioactivity of the individual slices for each lane was entered into a computer file, and the raw data were plotted to provide a

TABLE 1. Stoichiometric analysis of the Ad2 virion

Polypeptide type (no. of methionines)	Radioactivity (cpm) for lane:					No. of copies[a]
	1	2	3	4	5	
II (28)	1,081,086	1,071,399	1,038,464	1,058,327	1,029,957	720 ± 7[b]
III (11.1)[c]	33,367	31,339	33,302	33,423	31,467	56 ± 1
IIIa (11)	45,350	42,146	41,647	41,291	43,902	74 ± 1
IV (12)	22,775	22,345	20,688	21,234	21,701	35 ± 1
V (6)	50,055	49,524	48,607	49,108	49,186	157 ± 1
VI (3)	55,012	53,932	51,418	54,008	54,570	342 ± 4
VII (3)	130,765	125,467	129,151	132,888	135,982	833 ± 19
VIII (3)[d]	34,175	33,727	31,862	32,987	33,311	211 ± 2
IX (2)	26,510	26,945	24,894	25,831	25,151	247 ± 2

[a] The average number of polypeptides per virion and its standard deviation are obtained from the five measurements.
[b] The copy number for hexon is assumed to be 720. The standard deviation was estimated from the variation in the observed radioactivity, assuming that identical samples were loaded in each lane.
[c] An average value is used for penton base as described in the text.
[d] Three methionines have been assumed for polypeptide VIII for reasons discussed in the text.

direct record. Contiguous slices defining the radioactivity peaks were assigned to each known viral polypeptide. Slices preceding and trailing each peak were assigned to background for that peak. Each background could contain a variable number of slices, so that an average value could be obtained from as many slices as the distance between the peaks permitted. The integrated count was determined for each polypeptide by subtracting the area under a sloping base line from the total area of the peak. The base line was drawn between the averaged background values preceding and trailing the peak. The radioactivity in the hexon band was divided by the number of methionine residues in the hexon polypeptide and then by the assumed copy number of 720 to obtain a value for the radioactivity incorporated per methionine residue.

The number of copies for any other polypeptide was then obtained by dividing its integrated count by its number of methionines and the overall count per methionine. Five lanes were run in a gel, and each lane was analyzed and normalized to its hexon band separately. The copy numbers were then averaged, and the standard deviations were determined. Since this procedure involved the assumption that 720 copies of the hexon polypeptide were always present, a standard deviation could be estimated for hexon by assuming that equal quantities of protein had been applied to each lane. Adjustments to the initial assignments of peak and background were usually necessary, both to correct input errors and to adjust the assignments. Correct assignments were reflected in improved standard deviations. The total count was determined for each lane and compared with that expected from the amount of sample loaded on the gel. Recoveries of 95 to 100% were found.

Comparative fingerprinting. The two major penton bands from the SDS-polyacrylamide gel were analyzed by the method of Cleveland et al. (16). The protein bands were incorporated in the stacking part of a 13% SDS-polyacrylamide gel and partially digested with 0.005, 0.01, or 0.05 μg of *Staphylococcus aureus* protease V8 (Miles Laboratories) during a 30-min period without current. After completion of the electrophoresis, the gel was fixed, impregnated with Autofluor, and dried, and a 36-h exposure made on X-ray film.

RESULTS

SDS-polyacrylamide gel electrophoresis. Although our original goal was to determine the copy number of polypeptide IX within the virion, the study was extended to all the structural polypeptides reliably known to be present in mature virions (Table 1). Two experimental factors required consideration before an accurate determination could be made. SDS-polyacrylamide gel electrophoresis normally resolves only a limited number of well-spaced polypeptides and so does not simultaneously separate all the viral structural polypeptides, which range from 110,000 to 14,000 daltons. Hexon is the only reliable internal standard, but has a high molecular weight, a high copy number, and a large number of methionines. This enhanced the difficulty in obtaining accurate values for polypeptides with a small mass difference and low methionine content.

After some experimentation, a 220-mm linear polyacrylamide gradient gel (10 to 17.5% gradient) was found to resolve all polypeptides adequately (Fig. 1). Nine polypeptides could be clearly identified (II to IX and IIIa). The very low amount of other polypeptides observed indicates that our virus preparation almost exclusively contained mature virions (43). Virus and GONs, isolated by the methods described above, also have been used for a structural analysis of protein-protein interactions in the adenovirus capsid by using electron microscopy (54; J. van Oostrum, P. R. Smith, M. Mohraz, and R. M. Burnett, manuscript in preparation). Virus and GONs were found almost exclusively as complete assemblies, containing 240 or 9 trimeric hexon molecules, respectively. In this investigation, we have analyzed only those proteins that have been clearly identified as belonging to the mature form. The only significant additional peaks, apart from those labeled in Fig. 1, were two immediately following the hexon peak and two between IV and V. The last two are most probably IVa_1 and IVa_2 (21). The separation of polypeptides VIII and IX was particularly sensitive to the running conditions and to the amount of sample loaded. These polypeptides have diffuse bands, as their molecular weights are low. The difficulty was compounded by the sensitivity of their copy numbers to any small change in the assigned radioactivity, since their peak heights were low. Polypeptide IX has a particularly unfavorable ratio of methionine with respect to the hexon (28:2). Careful attention to the amount of sample loaded on the gels and to the electrophoresis conditions eventually resulted in a resolving power illustrated by the separations shown in Fig. 1. A relatively low protein concentration (Fig. 1a) gave the best separation of the polypeptides together with adequate radioactivity in each protein peak (Fig. 1b). Table 1 indicates that the precision in the determination of copy numbers from five lanes of a typical gel of the complete virion was 1 to 3%.

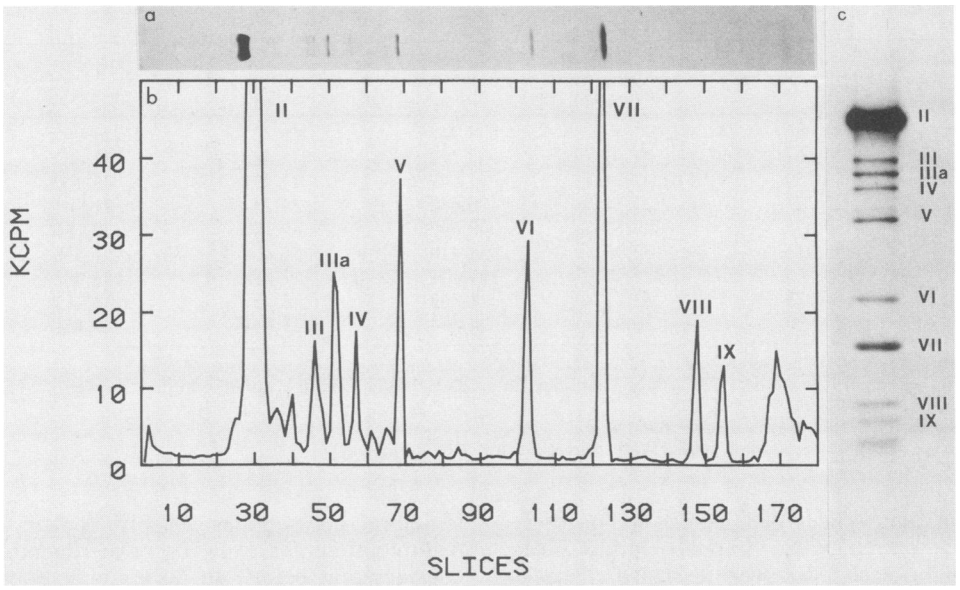

FIG. 1. Separation of [^{35}S]methionine-labeled polypeptides from Ad2 virions in a 220-mm linear 10 to 17.5% SDS-polyacrylamide gradient gel. (a) One lane of the Coomassie brilliant blue-stained gel; (b) distribution of radioactivity in 1.15-mm slices cut from the gel shown in panel a; (c) autoradiogram of a similar gel at a higher protein concentration than that optimal for separation but more clearly showing the various viral polypeptides. The three polypeptide III bands are not resolved in the autoradiogram owing to both the higher protein concentration loaded on the gel and to the spreading associated with autoradiography. Roman numerals indicate the viral polypeptides whose presence in the mature virion is unambigious and for which we have determined the copy number.

The absolute error in the stoichiometric analysis is probably somewhat higher than this measure of the experimental reproducibility.

Stoichiometric analysis. The complete DNA sequence for Ad2 (1, 3, 45) enables the derivation of a reliable distribution for all the amino acid residues in the virus-encoded polypeptides for the first time. However, two potential problems exist in obtaining the number of methionines from the DNA sequence. These are the possible existence of introns and the variable presence of the methionine specified by the start codon at the N terminus of the polypeptide product.

Except for polypeptide IX, all mRNAs for the structural polypeptides are processed, with a tripartite leader sequence spliced to each coding region. The latter is apparently colinear with the DNA sequence for all the structural polypeptides (37, 49), although this has only been rigorously demonstrated for the hexon gene (29). The presence of the initiator AUG codon-specified methionine as the N-terminal residue is usually established by N-terminal sequence analysis. However, acetylation of the α-amino group of the N-terminal residue in virtually all adenovirus proteins has prevented standard sequence analysis for polypeptides II (30), III (9), IV (5), V (3), and IX (5). This problem does not arise for polypeptides VI (2), VII (3, 51), VIII (39), or IIIa (8), which arise from precursors as a result of cleavage by an endopeptidase during maturation. For the former group, the question arises whether the methionine specified by the initiator AUG is removed on acetylation.

Cell-free protein synthesis, with and without inhibition of N-terminal acetylation, has been used by Anderson and Lewis (5) to obtain labeled adenoviral polypeptides. The N-terminal sequences reveal that the methionine encoded by the initiator AUG codon is removed for the hexon and polypeptide IX, whereas it is retained for the fiber. These results are in accord with the observations of Jörnvall (28) and Bloemendal (6) that methionine removal on acetylation depends on the identity of the second residue. Methionine is removed if the second residue is alanine, serine, or occasionally glycine or threonine. Methionine is retained if the next residue is glutamic acid, aspartic acid, or lysine.

The results of the analysis and relevant information concerning each polypeptide are given in this section. The amino acid composition of the translated gene product was obtained by using a program package for sequence analysis (44). The Ad2 DNA sequence was kindly provided by R. J. Roberts, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. The molecular weights of the mature polypeptides were calculated (27) after allowing for possible removal of the N-terminal methionine and subsequent acetylation and any proteolytic cleavage (Table 2). The molecular weights do not reflect possible changes in mass from glycosylation or phosphorylation.

Polypeptide II (hexon). Hexon is probably the best-characterized molecule in the virion (13), which contains 240 hexon trimers. The N terminus is an acetylated alanine (30). Both protein and DNA sequencing reveal 28 methionine residues (1, 29) among the 967 amino acids in the polypeptide of molecular mass 109,077 daltons. The known value of 720 polypeptide chains per virion was used as the internal standard.

Polypeptides III (penton base) and IV (fiber). The translated DNA sequence of the penton base gave a molecular mass of 63,296 daltons, which is considerably lower than earlier estimates of 85,000 daltons from SDS-polyacrylamide gels (43). The results of an N-terminal analysis (9) are unclear. Although the penton complex (penton base and fiber) did not appear to contain a free N-terminal amino acid, a low incidence of proline as the terminal residue in penton base was attributed to proteolytic digestion. The first residue after the initiator methionine in penton base is a glutamine, and so we cannot predict with confidence by using the rules described earlier whether the initiator methionine is retained. Gels of freshly prepared virus show that penton base

TABLE 2. Characteristics of the structural polypeptides

Polypeptide type (location)	Precursor derived	N-terminal sequence (mature polypeptide)	No. of residues	Molecular mass (daltons)
II (hexon)	No	Ac-Ala-Thr-Pro	967	109,077[a]
III (penton base)	No	Ac-Met-Gln-Arg[b]	571	63,296[a]
IIIa (vertex region)	Yes	Gly-Leu-Asn	566	63,287
IV (fiber)	No	Ac-Met-Lys-Arg	582	61,960[a]
V (core)	No	Ac-Ser-Lys-Arg[b]	368	41,631[a]
VI (hexon associated?)	Yes	Ala-Phe-Ser	217	23,449
VII (core)	Yes	Ala-Lys-Lys	174	19,412
VIII (hexon associated?)	Yes	Ala-Ala-Gln[c]	134[c]	14,539
IX (groups-of-nine)	No	Ac-Ser-Ala-Asn	139	14,339[a]

[a] The molecular mass includes 42 daltons for the N-terminal acetyl group.
[b] The sequences for these polypeptides have not been determined experimentally, but are derived from the DNA sequence. We have assumed that the methionine is present in polypeptide III and absent in polypeptide V.
[c] The sequence of polypeptide VIII has not been determined, but is derived as described in the text.

runs as three faint, closely spaced bands under the conditions used in our experiment (Fig.1). Virus stored for several days produced a pattern of two closely spaced bands at the positions of the first and third initial bands (Fig. 2). The difference in molecular mass between the final two bands was approximately 1,000 daltons. Comparative fingerprinting with *S. aureus* V8 protease (16) was performed to confirm that there was a high degree of sequence homology in the two bands (data not shown). The N-terminal sequence of penton base, Met-Gln-Arg-Ala-Ala-Met-Tyr-Glu-Glu-Gly-Pro-Pro-Pro, shows a methionine in position 6 and prolines at positions 11, 12, and 13. The second methionine is derived from an AUG in the DNA sequence, which could provide an alternative starting codon. This suggests that the different penton base bands could arise by translation from alternative start codons. That derived from the first AUG is presumably acetylated, in accord with the observation of a blocked N terminus (9). We assume that the second polypeptide is not acetylated and is exposed to the action of an aminopeptidase, eventually blocked by the unusual triplet of prolines. This scheme explains the time course from the three-band to the two-band pattern, the latter indicating the completion of peptidase action on the polypeptide obtained by translation from the second start codon. Although we cannot exclude alternative explanations for the variable length of the polypeptide, such as premature termination of translation, this model explains all the observations.

The total radioactivity in the penton base region of the gels was always constant and independent of the number of bands. If our explanation of the observed phenomenon is correct, then the total number of methionine residues allocated to the penton base must be corrected for the partial loss of the N-terminal methionine. It is unclear whether there are 12 or 11 residues in the full-length polypeptide, as it is not known whether the initial methionine is retained. The short polypeptide would contain only 10 methionines as a result of the elimination of the methionine at position 6. The ratio of the longer to the shorter of the final two bands was approximately 2:3. The maximum and minimum number of methionines within the two bands is therefore 10.8 and 10.4, respectively. We estimate that preparations showing three bands could have an additional 0.3 methionine. This is contributed by the methionine within the intermediate polypeptide, assuming a distribution of 1:1.5:1.5. Since the overall range of values is 10.4 to 11.1, the maximum possible error from the uncertainty is less than 10%. The exact value chosen within the range has little effect on the final value for the representation of penton base in the virion. Since our gels were generally run with fresh virus, and since we have assumed that the N-terminal methionine is retained, we have used the upper limit of 11.1 methionines for all the calculations; this results in a lower limit for the representation of the penton base.

The fiber DNA sequence encodes for 582 residues and contains 12 methionines, including the acetylated methionine specified by the initiator AUG codon (5). The virion contains 35 polypeptides, each with a molecular mass of 61,960 daltons.

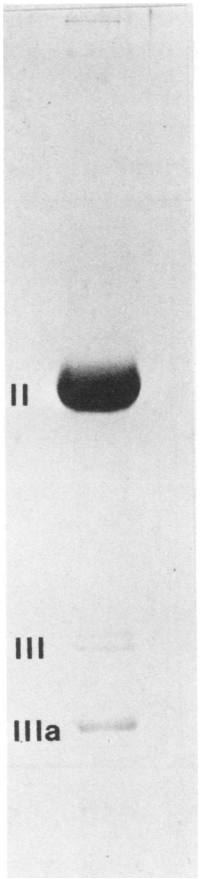

FIG. 2. Separation of the high-molecular-weight viral proteins on a 120-mm linear 10 to 14% SDS-polyacrylamide gradient gel.

TABLE 3. Stoichiometric analysis of the groups-of-nine from Ad2

Polypeptide type (no. of methionines)	Radioactivity (cpm) for lane:					No. of copies
	1	2	3	4	5	
II (28)	204,615	159,390	116,970	80,391	37,365	27.0
IX (2)	6,381	4,626	3,472	2,543	1,199	11.6 ± 0.2

Polypeptide IIIa. Polypeptide IIIa is derived from a precursor molecule after cleavage in the N-terminal region (8). The mature protein contains an N-terminal glycine (34). In the translated DNA sequence, the first glycine occurs at residue 20. The loss of the first 19 residues would create the observed difference in molecular weight between the precursor and the mature polypeptide. The latter has 566 residues, including 11 methionines, with a molecular mass of 63,287 daltons. The virion contains 74 copies of polypeptide IIIa.

Polypeptides V and VII. Polypeptide V is incorporated into the core without modification, whereas polypeptide VII is derived from a precursor. The N-terminal sequence of polypeptide V is Met-Ser-Lys-Arg-Lys (3). Assuming that the initiator methionine is eliminated on acetylation of the serine, six methionines will be present in the 368 residues.

The other core protein, polypeptide VII, is better characterized (3, 51). An N-terminal alanine is found in the mature polypeptide, with the cleavage site located between residues 24 and 25 (Gly-Ala), counting from the initiator AUG-encoded methionine. The resulting chain contains 174 residues, of which 3 are methionines.

Polypeptides VI and VIII. Polypeptide VI is derived from a precursor molecule by removal of 33 residues from the N terminus (2). Cleavage occurs between residues 33 and 34 (Gly-Ala), leaving three methionines in the mature polypeptide of 217 residues.

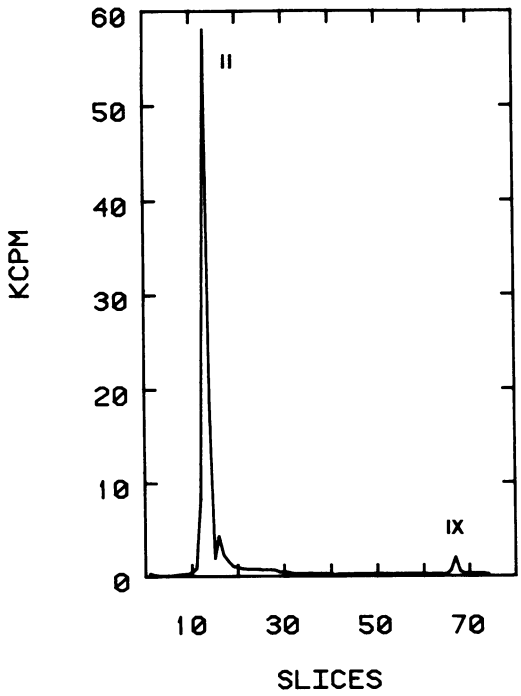

FIG. 3. Separation of the polypeptides from GONs on a 120-mm linear 10 to 17.5% SDS-polyacrylamide gradient gel.

Polypeptide VIII is derived from a precursor (39), but little is known about the cleavage site or sites. A virus-encoded endopeptidase with a specificity for Gly-Ala bonds has been implicated in the processing of precursor forms of polypeptides VI, VII, and VIII (52). The precursor of polypeptide VIII contains three such Gly-Ala bonds. A double cleavage by the endopeptidase could remove residues 1 to 22, containing three methionines, and the methionine-free C terminus from residues 157 to 227. This would create a polypeptide with 134 residues of 14,539 daltons, containing three methionines. Our gels are consistent with this hypothesis, as they show polypeptide VIII to have a molecular mass of about 15,000 daltons and to exhibit somewhat more radioactivity than polypeptide IX with slightly less protein.

Polypeptide IX. The gene sequence for polypeptide IX was one of the first available (4). The N-terminal methionine is removed, and the following serine is acetylated (5). There are two methionines in the polypeptide of 139 residues. The analysis of the complete virion indicated that the total content is 247 copies of polypeptide IX. Analysis of GONs revealed that each contains an average of 11.6 copies of polypeptide IX (Table 3). Even with high concentrations of GONs, the radioactivity profiles show that only these two protein species are present (Fig. 3), in agreement with results of earlier studies (21).

DISCUSSION

Stoichiometric analysis. The presence of 240 trimeric hexons within the virion and its manner of dissociation have long been established (43). Electron microscopy of virus purified by the described methods, which were designed to obtain a homogeneous sample, showed the virions to be almost exclusively intact. An internal experimental control was provided by the determination of the number of copies for polypeptide IX, both for the GON and for the virion. The excellent agreement of the value for 20 GONs (232) with that for the virion (247) indicates that little or no loss of individual hexons occurred from the purified assemblies. We are therefore confident that our assumption of 720 hexon polypeptides per virion is valid. Hexon is one of the most abundant proteins and has by far the largest mass and methionine content. The high radioactivity required in the hexon band to obtain adequate signals in the other bands resulted in smearing of the hexon peak (Fig. 1). This made it somewhat difficult to determine the exact amount of radioactivity in the hexon band for gels of whole virus, as clear assignments of the peak and background positions were difficult. Although the same effect was observed in gels of GONs, these contain only hexon and polypeptide IX (Fig. 3), and good background assignments could be made.

The absolute error in the analysis of virions depends on the radioactivity of hexon, which is dependent on the choice of its background. An estimate of this error was obtained by assigning maximum and minimum values to hexon. The maximum value was obtained by including the two small trailing peaks in the total count (Fig. 1) and eliminating the

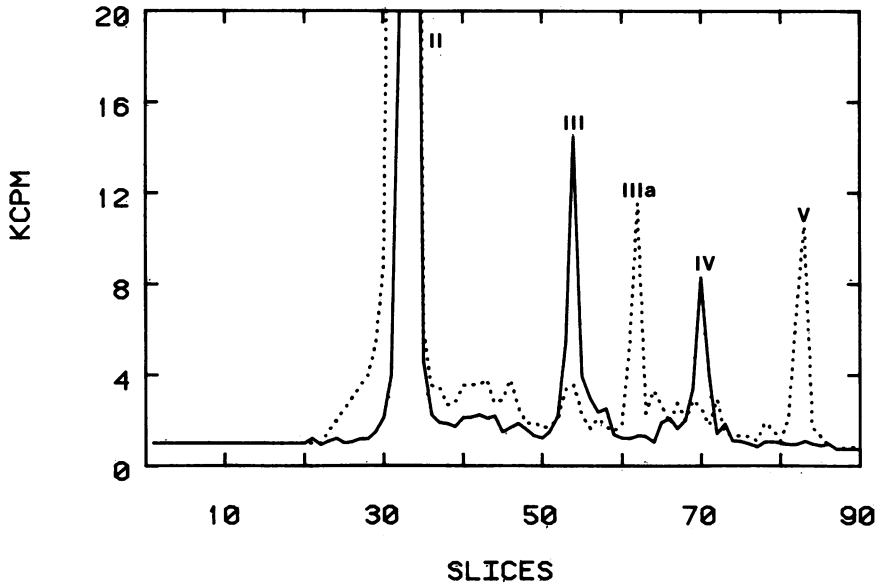

FIG. 4. Linear 10 to 14% SDS-polyacrylamide gradient gels showing the distribution of polypeptides within the two components resulting from removal of the vertex region of adenovirus. The solid line indicates the presence of vertex polypeptides II, III, and IV in the supernatant. The dotted line shows the polypeptides in the residual virus. The two lines in the graph are not to scale and thus do not reflect the relative representation of the two sets of polypeptides in the complete virion.

background correction. A minimum value was created by using the slices between the major hexon peak and the first trailing peak for the background assignment. The discrepancy between these two estimates and that obtained after applying the background correction in the usual way was at most 5%. The two small peaks trailing the major hexon peak have been treated as hexon components, as they appear in all our preparations of virions and GONs. The total radioactivity in these two peaks is only about 2% of that contained in the major hexon peak. The overall error in the experiment can be estimated in another way, from the constraint that certain polypeptides should be present in known multiples of integer factors determined by the symmetry. The deviations observed for penton base (56 versus 60), fiber (35 versus 36), and polypeptide IX (247 versus 240) are all less than 7%. The two estimates of the error are in good agreement. In our analysis, we assigned individual background values for each polypeptide by applying a sloping correction based on the values immediately preceding and following its peak. This treatment gave rise to a somewhat higher background correction in the congested area including polypeptides III, IIIa, and IV. Although this could result in a slight underestimate for the values of these polypeptides, the results for polypeptides III and IV were very close to multiples of 12, consistent with the location of the penton complex at the 12 vertices.

Polypeptides III and IV. Since the penton complex lies at the 12 vertices, the results clearly show that penton base is pentameric and that the fiber is trimeric. To confirm this result, we removed penton from the virus by dialysis against Tris-maleate buffer (pH 6.3). The complex was then analyzed as described above (Fig. 4). Since only bands from the peripentonal hexons, penton base, and fiber were present, this gel was intrinsically more accurate. A ratio of 1.73:1 was obtained for penton base to fiber, confirming the assigned ratio of 60:36 (1.67:1).

Our results do not support the conclusions of Devaux et al. (18, 19) that penton is trimeric and fiber is dimeric. They deduced that the penton base is trimeric from an observed molecular mass of 246,000 daltons from neutron-scattering measurements by using an apparent molecular mass of the penton base subunit determined from SDS-polyacrylamide gel electrophoresis. The gene sequence shows that the correct value is 63,296 daltons, a substantial deviation from an apparent molecular mass of 80,000 to 85,000 daltons. The corrected molecular mass would give a tetrameric base. The molecular mass of fiber was measured at around 156,000 daltons from neutron scattering and 161,000 daltons from hydrodynamic measurements. The physical measurements were thus unable to distinguish between a dimeric and a trimeric fiber when the correct subunit mass of 61,960 daltons was used (19). A similar ambiguity existed in studies on small crystals of fiber by using X-ray powder diffraction and electron microscopy. Although the evidence pointed toward a hexagonal or trigonal space group, an orthorhombic group could not be excluded. The measured crystal density indicated a dimeric fiber within the hexagonal and trigonal space groups or a trimer in an orthorhombic group (18).

The earlier model of a trimeric penton base in association with a dimeric fiber would result in a total penton mass of 313,808 daltons from the DNA sequence. This is far below the previously observed range of 362,000 to 660,000 daltons (19, 43). Our description of a pentameric penton base with a trimeric fiber leads to a mass of 502,360 daltons, which is within the observed range. Our conclusion that the fiber is trimeric also requires a reevaluation of the β-sandwich dimeric model of Green et al. (24).

Knowledge of the DNA sequence has enabled us to reevaluate previously published data from radiolabeling experiments. Most of the previous analyses were performed with [^{14}C]valine on the assumption that the distribution of valine is uniform and thus proportional to the molecular weight. The ratio of valines in a pentameric penton base and a trimeric fiber is 2.02:1, calculated from the DNA sequence. The ratio observed by Boudin et al. (9) was 2.06:1, which is

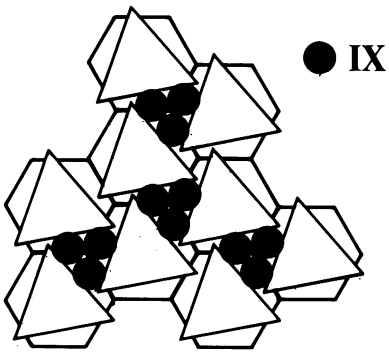

FIG. 5. Model of the distribution of protein IX within a GON, redrawn with the modifications described in the text from Burnett (13).

in excellent agreement. However, if penton base were a trimer and fiber were a dimer, a ratio of 1.82:1 would be obtained. Boudin and Boulanger (7) used antibodies against Ad2 penton to isolate this component and found a value of 0.63 for the ratio of methionine in fiber and penton base. This correlates well with the theoretical ratio of 0.65 for a pentameric penton base and a trimeric fiber.

The band splitting of the penton base in high-resolution gels was unexpected and so the experiments were repeated and extended to Ad5. In both cases, multiple bands were found. Akusjärvi and Persson (2) demonstrated that mRNA belonging to the L-2 family in an in vitro translation system gave rise to a novel polypeptide with an apparent molecular mass of 80,000 daltons, running slightly faster than penton base in SDS-polyacrylamide gels. The 80,000-dalton polypeptide was shown to be almost identical to penton base by tryptic fingerprinting. In preliminary experiments, we found a ratio of approximately 2:3 for the upper versus the lower band of penton base (Fig. 2). We hypothesize that the two polypeptides are derived from two closely spaced, in-phase initiation codons. The result casts some light on the symmetry mismatch between the trimeric fiber and the pentameric penton base, which has always been conceptually difficult to explain. It is possible that fiber can only bind to the shorter version of the penton base polypeptide. The trimeric fiber would then bind three copies of the shorter penton base polypeptide, and the resultant partial complex would be completed by the addition of two full-length penton base polypeptides. Further investigation is required to explore this idea.

Polypeptide IIIa. Polypeptide IIIa is present with 74 copies per virion, which is somewhat greater than the previous estimate of 60 copies (8). Using the exact number of valine residues available from the DNA sequence, we recalculated the copy number from the original data (8) and found values for polypeptide IIIa ranging from 66 to 71 copies per virion, comparable with our findings. Polypeptide IIIa was not released with the peripentonal hexons and the penton after dialysis against Tris-maleate buffer (Fig. 4). This result is in contrast to earlier results and indicates that polypeptide IIIa has more affinity for the remaining capsid than for the vertex proteins. Furthermore, this result rules out the suggestion that polypeptide IIIa is positioned between the peripentonal hexons and the penton (8, 21). It is possible that polypeptide IIIa connects the vertex region to the core, with 6 polypeptides per vertex and 72 per virion.

Polypeptide IX. The analysis shows that polypeptide IX is present with 247 copies in complete virions and 11.6 copies in GONs. These values indicate that 12 copies of polypeptide IX are present in each of the GONs within the 20 facets, and that none is present elsewhere in the viral capsid. This result is in accord with the essential features of a model for the distribution of polypeptide IX within the capsid (13). The original model contained a trimer of polypeptide IX in each of the four large cavities between hexons within the outer surface of the groups-of-nine. Additional polypeptides were present at symmetry-related monomer-binding sites. These additional sites were necessary for consistency with the available stoichiometric information, but only the trimers were assumed to play a role in capsid stabilization.

The modified model showing the distribution of polypeptide IX in a GON is illustrated in Fig. 5. Protein IX is buried within the large, but not the small, cavities. The cavities are created by hexon molecules symmetrically arranged on each facet (13, 54). A complete facet of the icosahedron requires the addition of three peripentonal hexons to the GON, which will create three additional small cavities but no additional trimer-binding sites. The arrangement confers stability upon the hexons within a facet, but does not stabilize the peripentonal hexons or those at the capsid edges. The distribution explains the preferential loss of peripentonal hexons on disruption of the virion under mild conditions, resulting in the release of GONs. Furthermore, the model accounts for the nonrandom disruption pattern of GONs observed by Pereira and Wrigley (41). A detailed analysis of the role of polypeptide IX in the architecture of adenovirus will be published elsewhere (R. M. Burnett, manuscript in preparation).

Previous determinations of the representation of the structural polypeptides have been summarized by Philipson (43), who tabulated data from several investigations involving a variety of techniques (8, 11, 21). Our results show some differences with the earlier data. There is good agreement for the major capsid proteins (hexon, penton base, and fiber), but protein IIIa is more abundant than found previously. Lower-molecular-weight proteins, from polypeptides V to IX, show the most striking differences. Our values for these polypeptides are consistently lower than those found previously: 157 versus 180 for polypeptide V; 342 versus 450 for polypeptide VI; 833 versus 1,070 for polypeptide VII; and 247 versus 280 for polypeptide IX.

The data also permit a determination of the minimum molecular mass of the complete adenovirus particle. We have used the distribution of polypeptides II, III, IIIa, IV, V, VI, VII, VIII, and IX from Table 1, modified by the appropriate architectural constraints for polypeptides II, III, IV, and IX. The molecular masses were calculated from the gene sequences (Table 2) and the distribution of nucleotides in the 35,937 base pairs of DNA. We have also included the two copies of the terminal DNA-binding protein of mass 55,000 daltons (15). Agreement with values obtained previously (23) is excellent for the nucleic acid component of 22.2 $\times 10^6$ daltons (23×10^6) and somewhat less good for the protein component of 126.5×10^6 daltons (150×10^6). The total particle mass is 148.7×10^6 daltons, of which 85% is protein. This value does not include the low-molecular-weight polypeptides, observed by SDS-polyacrylamide gel electrophoresis, which are thought to be remnants of precursor polypeptides after their maturation by proteolytic cleavage (43). If the particle mass is calculated from the precursor polypeptides instead of those found in the mature virion, the protein component is increased by 5.7×10^6 daltons.

Without a model for the architecture of adenovirus, the absolute numbers of polypeptides present in the virion

cannot be derived from stoichiometric data. However, the data impose a powerful constraint on possible architectural models, and when a model has been confirmed by the stoichiometric results an exact value can be given for the copy number. Examples are the penton complex at the vertex and polypeptide IX within the facets. It is not possible to derive the exact copy number per virion for proteins that are not restricted by the overall or local symmetry of the virion. We regard the representation of polypeptides II, III, IV, and IX in the outer capsid of adenovirus as established by the current architectural model. Thus, 240 trimeric hexons, 12 pentons consisting of a pentameric penton base and a trimeric fiber, and 240 copies of protein IX comprise a symmetrical outer capsid.

ACKNOWLEDGMENTS

We thank H. S. Ginsberg and P. I. Freimuth, Department of Microbiology, Columbia University, New York, N.Y., for their assistance in the production of adenovirus. R. J. Roberts, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., kindly made available to us his computer file containing the complete Ad2 sequence.

The investigation was supported by Public Health Service grant AI-17270 from the National Institute of Allergy and Infectious Diseases, grant PCM-84-18111 from the National Science Foundation, and by an Irma T. Hirschl Career Scientist Award to R.M.B.

LITERATURE CITED

1. **Akusjärvi, G., P. Aleström, M. Pettersson, M. Lager, H. Jörnvall, and U. Pettersson.** 1984. The gene for the adenovirus 2 hexon polypeptide. J. Biol. Chem. **259**:13976–13979.
2. **Akusjärvi, G., and H. Persson.** 1981. Gene and mRNA for precursor polypeptide VI from adenovirus type 2. J. Virol. **38**:469–482.
3. **Aleström, P., G. Akusjärvi, M. Lager, L. Yeh-kai, and U. Pettersson.** 1984. Genes encoding the core proteins of adenovirus type 2. J. Biol. Chem. **259**:13980–13985.
4. **Aleström, P., G. Akusjärvi, M. Perricaudet, M. B. Mathews, D. F. Klessig, and U. Pettersson.** 1980. The gene for polypeptide IX of adenovirus type 2 and its unspliced messenger RNA. Cell **19**:671–681.
5. **Anderson, C. W., and J. B. Lewis.** 1980. Amino-terminal sequence of adenovirus type 2 proteins: hexon, fiber, component IX, and early protein 1B-15K. Virology **104**:27–41.
6. **Bloemendal, H.** 1977. The vertebrate eye lens: a useful system for the study of fundamental biological processes on a molecular level. Science **197**:127–138.
7. **Boudin, M.-L., and P. Boulanger.** 1981. Antibody-triggered dissociation of adenovirus penton capsomer. Virology **113**:781–786.
8. **Boudin, M.-L., J.-C. D'Halluin, C. Cousin, and P. Boulanger.** 1980. Human adenovirus type 2 protein IIIa. II. Maturation and encapsidation. Virology **101**:144–156.
9. **Boudin, M.-L., M. Moncany, J.-C. D'Halluin, and P. A. Boulanger.** 1979. Isolation and characterization of adenovirus type 2 vertex capsomer (penton base). Virology **92**:125–138.
10. **Boulanger, P. A., C. Devaux, and M.-H. Loucheux-Lefebvre.** 1978. Comparative optical properties of free and assembled hexon capsomeres of human adenovirus type 2. FEBS Lett. **85**:52–56.
11. **Boulanger, P., P. Lemay, G. E. Blair, and W. C. Russell.** 1979. Characterization of adenovirus protein IX. J. Gen. Virol. **44**:785–800.
12. **Brown, D. T., M. Westphal, B. T. Burlingham, U. Winterhoff, and W. Doerfler.** 1975. Structure and composition of the adenovirus type 2 core. J. Virol. **16**:366–387.
13. **Burnett, R. M.** 1984. Structural investigations on hexon, the major coat protein of adenovirus, p. 337–385. *In* A. McPherson and F. A. Jurnak (ed.), Biological macromolecules and assemblies, vol. 1. Virus structures. John Wiley & Sons, Inc., New York.

13a. **Burnett, R. M.** 1985. The structure of the adenovirus capsid. II. The packing symmetry of hexon and its implications for viral architecture. J. Mol. Biol. **185**:125–143.

14. **Burnett, R. M., M. G. Grütter, and J. L. White.** 1981. The molecular structure of hexon, the major coat protein of adenovirus; its placement and its interactions in the icosahedral viral capsid, p. 865–868. *In* M. Balaban (ed.), Structural aspects of recognition and assembly in biological macromolecules, vol. 2. International Science Services, Rehovot, Israel.

14a. **Burnett, R. M., M. G. Grütter, and J. L. White.** 1985. The structure of the adenovirus capsid. I. An envelope model of hexon at 6 Å resolution. J. Mol. Biol. **185**:105–123.

15. **Challberg, M. D., S. V. Desiderio, and T. J. Kelly, Jr.** 1980. Adenovirus DNA replication in vitro: characterization of a protein covalently linked to nascent DNA strands. Proc. Natl. Acad. Sci. USA **77**:5105–5109.
16. **Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
17. **Colby, W. W., and T. Shenk.** 1981. Adenovirus type 5 virions can be assembled in vivo in the absence of detectable polypeptide IX. J. Virol. **39**:977–980.
18. **Devaux, C., C. Berthet-Colominas, P. A. Timmins, P. Boulanger, and B. Jacrot.** 1984. Crystal packing and stoichiometry of the fibre protein of adenovirus type 2. J. Mol. Biol. **174**:729–737.
19. **Devaux, C., M. Zulauf, P. Boulanger, and B. Jacrot.** 1982. Molecular weight of adenovirus serotype 2 capsomers: a new characterization. J. Mol. Biol. **156**:927–939.
20. **Everitt, E., L. Lutter, and L. Philipson.** 1975. Structural proteins of adenoviruses. XII. Location and neighbour relationship among proteins of adenovirion type 2 as revealed by enzymatic iodination, immunoprecipitation and chemical cross-linking. Virology **67**:197–208.
21. **Everitt, E., B. Sundquist, U. Pettersson, and L. Philipson.** 1973. Structural proteins of adenoviruses. X. Isolation and topography of low molecular weight antigens from the virion of adenovirus type 2. Virology **52**:130–147.
22. **Ginsberg, H. S., H. G. Pereira, R. C. Valentine, and W. C. Wilcox.** 1966. A proposed terminology for the adenovirus antigens and virion morphological subunits. Virology **28**:782–783.
23. **Green, M.** 1970. Oncogenic viruses. Annu. Rev. Biochem. **39**:701–756.
24. **Green, N. M., N. G. Wrigley, W. C. Russell, S. R. Martin, and A. D. McLachlan.** 1983. Evidence for a repeating cross-β sheet structure in the adenovirus fibre. EMBO J. **2**:1357–1365.
25. **Grütter, M. G., and R. M. Franklin.** 1974. Studies on the molecular weight of the adenovirus type 2 hexon and its subunit. J. Mol. Biol. **89**:163–178.
26. **Horne, R. W., S. Brenner, A. P. Waterson, and P. Wildy.** 1959. The icosahedral form of an adenovirus. J. Mol. Biol. **1**:84–86.
27. **IUPAC Inorganic Chemistry Division Commission on Atomic Weights.** 1974. Atomic weights of the elements 1973. Pure Appl. Chem. **37**:589–603.
28. **Jörnvall, H.** 1975. Acetylation of protein N-terminal amino groups: structural observations on α-amino acetylated proteins. J. Theor. Biol. **55**:1–12.
29. **Jörnvall, H., G. Akusjärvi, P. Aleström, H. von Bahr-Lindström, U. Pettersson, E. Appella, A. V. Fowler, and L. Philipson.** 1981. The adenovirus hexon protein: the primary structure of the polypeptide and its correlation with the hexon gene. J. Biol. Chem. **256**:6181–6186.
30. **Jörnvall, H., H. Ohlsson, and L. Philipson.** 1974. An acetylated N-terminus of adenovirus type 2 hexon protein. Biochem. Biophys. Res. Commun. **56**:304–310.
31. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
32. **Laver, W. G., N. G. Wrigley, and H. G. Pereira.** 1969. Removal of pentons from particles of adenovirus type 2. Virology **39**:599–605.
33. **Lawrence, W. C., and H. S. Ginsberg.** 1967. Intracellular uncoating of type 5 adenovirus deoxyribonucleic acid. J. Virol.

1:851–867.
34. **Lemay, P., M.-L. Boudin, M. Milleville, and P. Boulanger.** 1980. Human adenovirus type 2 protein IIIa. I. Purification and characterization. Virology **101**:131–143.
35. **Maizel, J. V., Jr., D. O. White, and M. D. Scharff.** 1968. The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A, and 12. Virology **36**:115–125.
36. **Mautner, V., and H. G. Pereira.** 1971. Crystallization of a second adenovirus protein (the fibre). Nature (London) **230**:456–457.
37. **Nevins, J. R., and S. Chen-Kiang.** 1981. Processing of adenovirus nuclear RNA to mRNA. Adv. Virus Res. **26**:1–35.
38. **Newcomb, W. W., J. W. Boring, and J. C. Brown.** 1984. Ion etching of human adenovirus 2: structure of the core. J. Virol. **51**:52–56.
39. **Öberg, B., J. Saborio, T. Persson, E. Everitt, and L. Philipson.** 1975. Identification of the in vitro translation products of adenovirus mRNA by immunoprecipitation. J. Virol. **15**:199–207.
40. **Pereira, H. G., R. C. Valentine, and W. C. Russell.** 1968. Crystallization of an adenovirus protein (the hexon). Nature (London) **219**:946–947.
41. **Pereira, H. G., and N. G. Wrigley.** 1974. In vitro reconstruction, hexon bonding and handedness of incomplete adenovirus capsid. J. Mol. Biol. **85**:617–631.
42. **Pettersson, U.** 1984. Structural and nonstructural adenovirus proteins, p. 205–270. *In* H. S. Ginsberg (ed.), The adenoviruses. Plenum Publishing Corp., New York.
43. **Philipson, L.** 1983. Structure and assembly of adenoviruses. Curr. Top. Microbiol. Immunol. **109**:1–52.
44. **Pustell, J., and F. C. Kafatos.** 1982. A convenient and adaptable package of DNA sequence analysis programs for microcomputers. Nucleic Acids Res. **10**:51–59.
45. **Roberts, R. J., K. E. O'Neill, and C. T. Yen.** 1984. DNA sequences from the adenovirus 2 genome. J. Biol. Chem. **259**:13968–13975.
46. **Rowe, W. P., R. J. Huebner, L. K. Gillmore, R. H. Parrott, and T. G. Ward.** 1953. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exp. Biol. Med. **84**:570–573.
47. **Russell, W. C., K. McIntosh, and J. J. Skehel.** 1971. The preparation and properties of adenovirus cores. J. Gen. Virol. **11**:35–46.
48. **Russell, W. C., and B. Precious.** 1982. Nucleic-acid binding properties of adenovirus structural polypeptides. J. Gen. Virol. **63**:69–79.
49. **Sharp, P. A.** 1984. Adenovirus transcription, p. 173–204. *In* H. S. Ginsberg (ed.), The adenoviruses. Plenum Publishing Corp., New York.
50. **Smith, K. O., W. D. Gehle, and M. D. Trousdale.** 1965. Architecture of the adenovirus capsid. J. Bacteriol. **90**:254–261.
51. **Sung, M. T., M. A. Lischwe, J. C. Richards, and K. Hosokawa.** 1977. Adenovirus chromatin. I. Isolation and characterization of the major core protein VII and precursor pro-VII. J. Biol. Chem. **252**:4981–4987.
52. **Tremblay, M. L., C. V. Déry, B. G. Talbot, and J. Weber.** 1983. In vitro cleavage specificity of the adenovirus type 2 proteinase. Biochim. Biophys. Acta **743**:239–245.
53. **Tsernoglou, D., A. D. Tucker, and P. C. van der Vliet.** 1984. Crystallization of a fragment of the adenovirus DNA binding protein. J. Mol. Biol. **172**:237–239.
54. **van Oostrum, J., and R. M. Burnett.** 1984. Architecture of the adenovirus capsid. Ann. N.Y. Acad. Sci. **435**:578–581.
55. **Williams, J. F.** 1970. Enhancement of adenovirus plaque formation on HeLa cells by magnesium chloride. J. Gen. Virol. **9**:251–255.