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The structure of nucleoprotein cores released from adenovirions

Michael E.Vayda, Alan E.Rogers* and S.J.Flint+

Department of Biochemical Sciences, Princeton University, Princeton, NJ 08544, USA

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ABSTRACT

The morphology, protein composition and DNA organization of nucleoprotein core complexes isolated from type 5 adenovirions have been examined by electron microscopy and biochemical techniques. The morphology of such core structures is in some ways strikingly similar to that exhibited by cellular chromatin. 'Native' core preparations contain compact and less highly-folded forms: the latter appear as thick fibres, 150-300Å in diameter. Upon exposure to 0.4M NaCl, adenovirus cores undergo a transition to a beaded string form, reminiscent of nucleosomes. Of the three arginine-rich proteins, polypeptides V, VII and μ present in 'native' cores, only polypeptide VII remains associated with viral DNA in the presence of 0.4M NaCl. We therefore conclude that the nucleosome-like beads are constructed solely of polypeptide VII. The results of micrococcal nuclease digestion experiments suggest that polypeptide VII is sufficient to protect some 100-300bp of adenoviral DNA.

INTRODUCTION

The human adenovirus genome, linear, double-stranded DNA of $20\text{-}23 \times 10^6$ daltons (1,2) is packaged within icosahedral virions in a nucleoprotein structure termed the core. Such cores, first identified by electron microscopy (3), can be released from the outer shell of capsomers by exposure of purified virions to such denaturants as acetone or pyridine (4-7). Unlike papovavirions (8-10), adenovirions contain no cellular histones. Rather, the viral DNA is associated with two virus-specific, arginine-rich proteins, V and VII (6, 8, 11-13). Polypeptide VII, the major core protein, appears to be present at 1170 to 1350 molecules per virion (13, 15). This protein is rich in arginine (23%) and alanine (19%) residues, but deficient in lysine (11, 15, 16). Polypeptide VII is synthesized as a 20,000 dalton precursor, pVII (17, 18), whose N-terminal twenty amino acids are removed by proteolytic cleavage during the final phases of virion maturation (18, 19-21). A third arginine-rich virion protein, designated μ, has also been described (22): this polypeptide, apparent molecular weight about 4,000 daltons, possesses an extraordinary amino acid composition, comprising 54% arginine and 13% histidine (22). Neither the precise location nor the role of this protein

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have been determined.

It seems obvious that core-associated polypeptides must mediate packaging of adenoviral DNA within virions. Nevertheless, the details and consequences of these DNA-protein interactions are not understood (for example 23, 24). It is, moreover, controversial whether adenoviral DNA in core particles is afforded the same kind of protection from nuclease attack as cellular DNA packaged by nucleosomes. Corden and colleagues (24) reported that digestion of adenoviral cores with micrococcal nuclease yields rather discrete DNA products of 200 and 1800 base-pairs. Others have failed to reproduce this result, observing only viral DNA smaller than that generated by micrococcal nuclease digestion of cellular chromatin (25, 26). In one case, however, parallel digestions of viral nucleoprotein present in nuclei at 3 or 6 hrs. after infection (25) did yield viral DNA fragments of 145 and 185 nucleotides. The ability of micrococcal nuclease to liberate defined DNA fragments when infected cell nuclei are digested has also been described by others (27, 28, see Discussion). The apparent difference in the microccocal nuclease sensitivity of intranuclear adenoviral DNA compared to that exhibited when it is present in cores has led some authors to suggest that the core proteins are replaced by cellular histones once the viral genome enters the infected cell nucleus (25, 27, 28). Direct evidence that supports this proposal has yet to be collected.

It is of considerable interest to establish the composition and nature of intracellular nucleoproteins in which the adenoviral genome might reside during different periods of the infectious cycle. Unfortunately, our knowledge of both the proteins with which adenoviral DNA interacts once it enters the cell nucleus and the relationship (if any) of intranuclear to virion nucleoprotein structure is scanty. In this paper, we present the results of an examination of the structure of adenoviral cores by electron microscopy and biochemical methods: these data provide a more detailed picture of core structure than available hitherto and thus establish a base line from which to describe intracellular nucleoprotein(s) containing adenoviral DNA.

MATERIALS AND METHODS

Cells and Virus HeLa cells were maintained at a density of $2.5\text{-}5.0\times10^5$ cells/ml in suspension culture in SMEM (GIBCO) supplemented with 3 to 5% calf serum, (Flow labs) and 2mM glutamine. Adenovirus type 5 (Ad5) was propagated and purified as described previously (29, 30). To prepare radioactively labelled virions, HeLa cells were infected with Ad5 at a multiplicity of 40pfu/cell. After incubation at 37°C for 14 hours, infected cells were collected by centrifugation and resuspended in 1/10th the original culture volume of RPMI without arginine or without methionine (Selectamine-RPMI GIBCO

or Amersham) or of SMEM, containing 1mCi per 100 ml ^{3}H-Arginine (15-30 Ci/mmol; NEN), ^{35}S-Methionine (345 Ci/mmol; Amersham) or ^{3}H-Thymidine (50-80Ci/mmol; NEN), respectively, at approximate concentrations of 100mM, 10mM or 75mM, respectively. Four hours after addition of the labelled compound, cultures were diluted 5-fold with SMEM and maintained at 37°C until harvested 46 hours after infection.

Isolation of Adenovirus Core Complexes Type 5 adenovirus cores were obtained by a procedure very similar to that described by Prage et al., (7) and dialyzed overnight at room temperature against 10mM Tris-HCl pH 7.4 containing 0.05mM EDTA or purified by sedimentation at 30,000rpm and 4°C for 2 hours in 15-25% (w/v) sucrose gradients containing 10mM Tris pH 7.4 and 0.05-0.2mM EDTA in a Beckman SW41 rotor. Fractions containing adenovirus cores were identified by optical density at 260 nm., the presence of ^{3}H-thymidine or ^{3}H-arginine or by blotting, then were pooled and dialyzed as described previously. Salt disruption was achieved by addition of 5M NaCl to the final concentration desired.

Examination of Adenovirus Core Preparations in the Electron Microscope. Carbon support films were prepared by evaporation of carbon onto freshly cleaved mica discs and transferred to 200 mesh copper grids as described by Thach et al, (31). Films were made hydrophilic by glow discharge not more than 30 minutes before the spreading of samples. Virion cores were visualized by negative staining with 2% (w/v) uranyl acetate in 30% (v/v) ethanol, or after rotary shadowing with platinum/palladium. Examinations were made in a Phillips EM400 electron microscope.

Nuclease Digestions Digestions with DNAase I (Sigma) were performed at 25°C in 10mM Tris-HCl, pH7.4 containing 10mM $MgCl_2$, 100 µg/ml BSA and various NaCl concentrations as indicated. The rate of DNAase I digestion in the presence of increasing concentrations of NaCl was determined using viral DNA labelled in vivo with ^{3}H thymidine or ^{32}P orthophosphate and purified from virions by pronase digestion and phenol extraction as described by Petterson and Sambrook (32). Such calibration curves were then used to choose the DNAase I concentrations necessary to achieve identical rates of digestion of deproteinized DNA regardless of the ionic strength. Comparisons between the rate of digestion of deproteinized viral DNA and DNA within core structures were then made at identical DNAase I concentrations, appropriate for the salt concentration employed. Reactions were terminated by addition of 5% trichloroacetic acid (TCA) and precipitable material was collected on glass fiber filters, which were dried and counted in Econofluor (NEN).

Digestions with micrococcal nuclease were performed in 10mM Hepes pH 7.0 containing 85mM KCl, 1mM $MgCl_2$, 1mM $CaCl_2$, 0.01 or 0.6M NaCl and 5.5% (w/v) sucrose for 5 minutes at 25°C with varying enzyme concentrations, as indicated in Figure legends. Reactions were stopped by addition of EDTA and EGTA, each to 20mM. The samples were immediately heated at 60°C for 3 minutes. Following pronase digestion and phenol extraction, the DNA products were subjected to electrophoresis in 1.8% agarose gels cast in 20mM Tris-HCl pH 8.3 containing 1mM EDTA and 25mM sodium acetate at 40 volts for 16 hrs, and transferred to nitrocellulose by the technique of Southern (33) using 16x SSC as transfer medium (1x SSC is 0.15M NaCl; 0.015 M sodium citrate, 1mM EDTA, pH 6.8). Following hybridization with nick-translated (34) adenoviral DNA or fragments generated by restriction endonuclease digestion at specific activities of $0.7-2\times10^{8}$ cpm/µg, the blots were washed essentially as described by Lawn et al (35) and exposed to Kodak XAR-5 film for appropriate periods of time. Gels to which ^{3}H-thymidine-labelled DNA products had been applied were prepared for exposure to Kodak XAR-5 film as described in the next paragraph.

SDS-Polyacrylamide Gel Electrophoresis Samples to be analyzed for the presence of labelled polypeptides were denatured by boiling for 10 minutes in 0.1% (w/v) SDS; 5mM β-mercaptoethanol and 5% glycerol, and subjected to

electrophoresis through discontinuous polyacrylamide gels as described by Laemmli (36). When electrophoresis was complete, gels were washed for 1 hour in a solution of 5% methanol and 7% acetic acid, soaked in En^{3}Hance (NEN), and then in water for about 3 hrs (until gels swelled to their original dimensions), dried and exposed to Kodak XAR-5 film at -80°C.

RESULTS

Electron Microscopy of Adenovirus Cores.

Purified adenovirus type 5 virions, labelled in vivo with ^{3}H-thymidine, were exposed to 10% pyridine according to the method of Prage et al, (7) and examined in the electron microscope either directly or after sedimentation in 15-25% sucrose gradients. These conditions of preparation and spreading invariably yielded a variety of core structures, examples of which are shown in Figure 1. The approximately spherical, compact cores with a beaded or

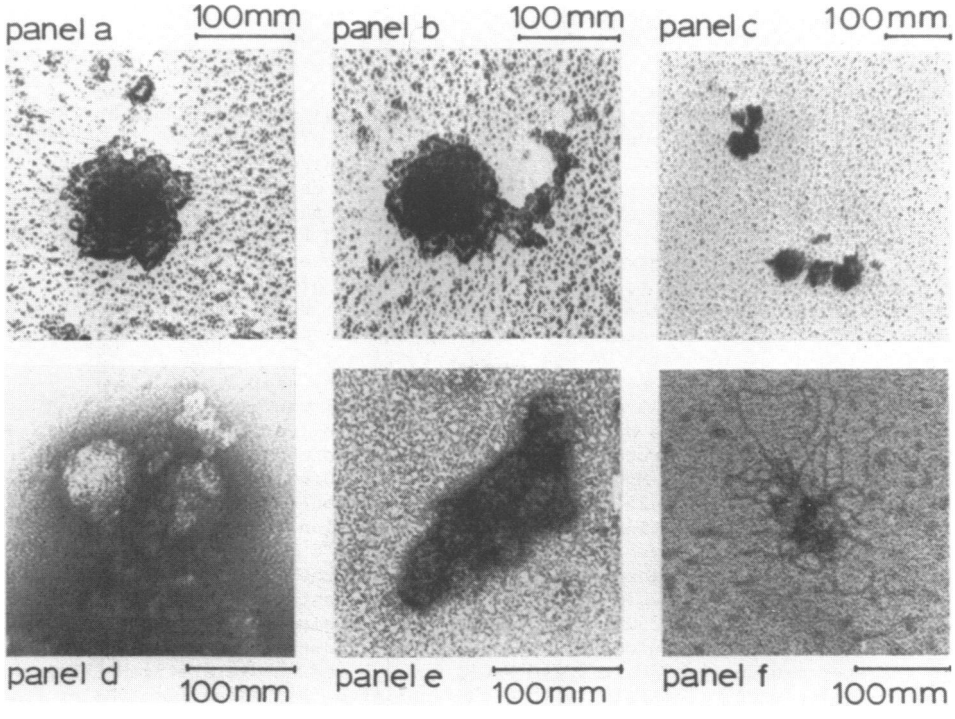

Figure 1: Examples of structures seen during electron microscopic examination of adenovirus type 5 cores. Purified type 5 adenovirions were disrupted with 10% pyridine and examined in the electron microscope after shadowing (panels a, b, c and f) or negative staining (panels d and e). Panels a to e show examples of structures seen in all preparations arranged in a series corresponding to increased unfolding. Panel f illustrates the typical structure seen when purified virions were exposed to 0.5% (w/v) sodium deoxycholate for 3 minutes at 58°C.

granular surface (Figure 1, panels a and b) are very similar to those seen in previous experiments (5, 12, 23, 37, 38), as are compact structures from which strings or loops radiate (Figure 1 panel b; 23, 37, 38). The diameter of shadowed core particles, 125±25 nm based on 99 examples, is somewhat larger than the diameter of the virion of 87nm measured by Brown and colleagues (23). This difference may reflect some flattening or loosening of the core structure: Brown and colleagues (23) have, for example, reported a diameter of 167nm for negatively-stained pyridine cores.

Examples of the more unfolded forms (Figure 1e) are shown at higher magnification in Figure 2: these are typical of all examined and were present

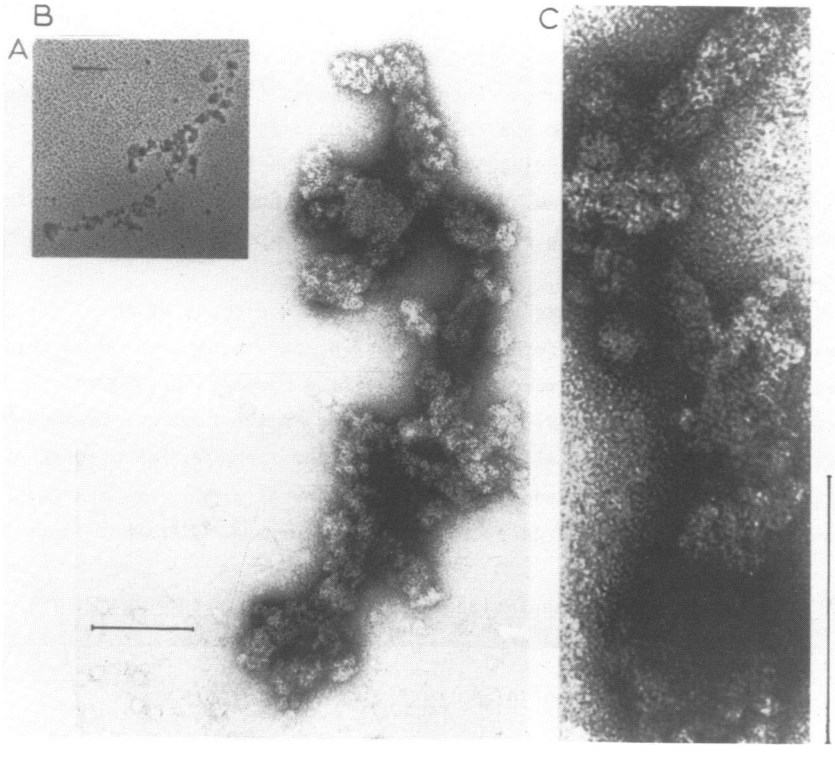

Figure 2: Electron micrographs of adenovirus cores that display a fibrous morphology. Cores, purified from ^{3}H-thymidine-labelled virions by sedimentation in neutral sucrose gradients following pyridine disruption in the absence (A and B) or presence (C) of 0.1% formaldehyde, were shadowed with platinum palladium (A) or negatively stained with 2% uranyl acetate (B and C). The bars indicate 100nm.

in all core preparations. They exhibit a folded appearance in which roughly spherical particles, approximately 10nm in diameter, comprise a thick fibre which varies in width from 160Å to 300Å and in contour length from 600 to 800 nm. These images suggest that the core structure can be described as a helical ribbon, composed of two strands of the "knobby" particles, 9-10nm in diameter. When this description is used as the basis for calculation of the volume of such core particles, ($V= \pi r^2 l$ where r = 100Å and l = 600-800nm, these measurements), then such fibres can be estimated to occupy $1.9\text{-}2.5 \times 10^5$ nm^3. This value (which must be an overestimate for the core fibre does not entirely occupy the space of a solid cylinder of these dimensions (Figure 2)) is in reasonable agreement with the volume of the spherical space available within the capsid shell ($V= 4/3\ \pi r^3$ where r, the internal diameter of the virion, is taken as 75nm (23 and our measurements)), 2.2×10^5 nm^3, into which the core must be packed. This description is also consistent with the presence of 'rod-like' elements reported by Nermut (38) and the 21nm (210Å) particles described by Brown and colleagues (23): in the more compact forms described by these authors adjacent segments of the folded double fibre would appear as units of about 20nm. Indeed, the image shown in panel e of Figure 1 is very reminiscent of those reported by Brown and colleagues (Figure 8 of reference 23).

It was difficult to make an absolutely accurate count of the representation of unfolded forms for in most preparations some cores were aggregated, whereas others were broken: the less compact forms were, nevertheless, typical of core preparations made in the presence of 10mM NaCl, and represented a substantial fraction of the total population of discrete structures, 20-25%, when randomly-chosen sections of grids were scored. The non-uniform distribution of compact and looser cores in different fractions of

TABLE 1: Approximate representation of different core structures in fractions from 15-30% sucrose gradient

Fraction[+]	% Total				Number Examined
	Compact	Rosette	Partially Unfolded	Open Fibre	
Core Peak	5.6	48.2	30.8	15.3	195.
Shoulder	0	27.5	23.5	48.3	149.

Discrete forms observed in grid areas selected at random were placed in one of the four classes listed which were defined in terms of the structures shown in Figure 1. Thus, 'Compact' = Figure 1a, 'Rosette' = Figure 1b plus structures from which more loops radiate, 'Partially Unfolded' = Figures 1c or 1d, 'Open Fibre' = Figures 1e and 2.

[+]These fractions correspond to fractions 15 and 18 of the gradient shown in Figure 5.

sucrose gradients, summarized in Table 1, provides strong evidence that the more unfolded particles are not artifacts of the spreading conditions employed. Rather, they appear to represent nucleoproteins that begin to unfold during core preparation and, therefore, might be considered intermediates in the pathway from fully compact cores to those radiating long loops comprising DNA, seen when cores are exposed to such detergents as sodium deoxycholate (Figure 1 panel f; 37) or aged at 4°C (23).

The fibrous appearance of partially-unfolded type 5 adenovirus cores, especially apparent in Figure 2 is reminiscent of that of the approximately 200-300Å diameter thick chromatin fibre (39, 40). When incubated with 25 μgm/ml DNAase I at 25°C, about 48% of ^{3}H-labelled Ad5 core DNA was rendered acid-soluble in 7 minutes, compared to 78% of deproteinized DNA digested under identical conditions (Figure 3). These values are quite similar to those obtained when similar experiments were performed with cellular chromatin (41, 42). Moreover, DNAase I attacked viral DNA in cores at a slower rate than it

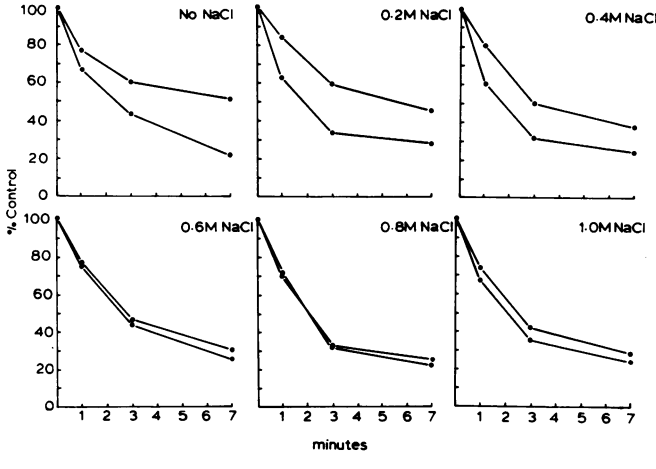

Figure 3: Sensitivity of adenovirus cores and deproteinized DNA to DNAase I digestion. Ad5 cores and deproteinized DNA, labelled with ^{3}H-thymidine, were incubated in parallel at DNA concentrations of 3.3 μg/ml with DNAase I for the periods indicated. The concentration of DNAase I used at each [NaCl] shown was chosen to normalize activity upon a deproteinized Ad5 DNA substrate, (see Materials and Methods). The amount of TCA-precipitable material remaining after digestion is expressed as a percentage of the input, viral DNA. Some variation in the amount of DNA digested in a 7 minute reaction was observed: the amounts of DNA remaining acid insoluble were 23%, 27%, 24%, 26%, 25% and 23% in 0, 0.2M, 0.4M, 0.6M, 0.8M and 1.0M NaCl, respectively. The difference in percentage of input DNA in cores compared to naked DNA resistant to DNAase I was 28, 20, 14 and 4% in 0, 0.2M, 0.4M and 0.6M NaCl, respectively. In all parts of the figure ●-● and 0-0 represent digestion of cores and deproteinized DNA, respectively.

did deproteinized DNA (Figure 3). The sensitivities of core and deproteinized Ad5 DNA to DNAase I were also compared in the presence of NaCl, under conditions designed to compensate for the increasing concentrations of NaCl (see Materials and Methods). The profiles of digestion of core and deproteinized Ad5 DNA were very similar at 0.6M-1.0M NaCl. At intermediate salt concentrations (0.2M and 0.4M in Figure 3), some protection of core DNA was reproducibly observed, although the rate and extent of digestion were increased compared to 'native' cores. Thus, exposure of core particles to increasing concentrations of NaCl appears to induce a gradual transition that renders their DNA more sensitive to nuclease attack.

The morphological consequences of such a transition were therefore examined by electron microscopy. Panels A, B and D of Figure 4 show a selection of typical molecules observed when Ad5 cores were examined after

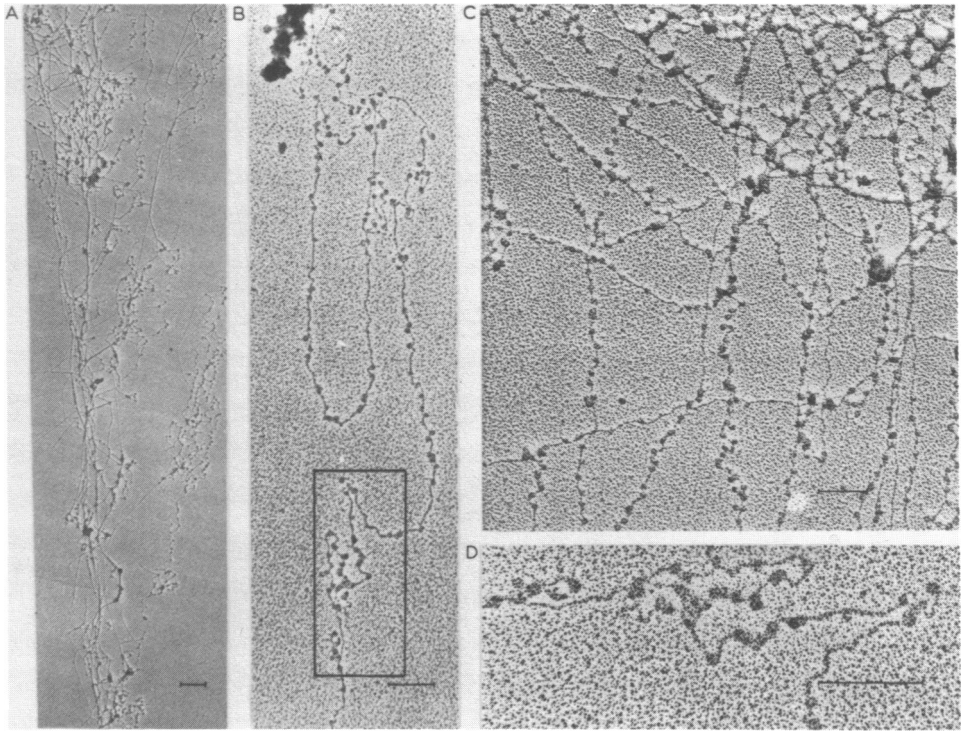

Figure 4: Electron micrographs of salt-disrupted adenovirus core particles. Type 5 adenovirus cores were exposed to 0.4M NaCl (panels A, B and D) or 1.0M NaCl (panel C) for 15 minutes at 20°C, spread for electron microscopy and negatively-stained. Panel D shows a higher magnification of the nucleoprotein strand boxed in panel B. In all parts, the bar indicates 100nm.

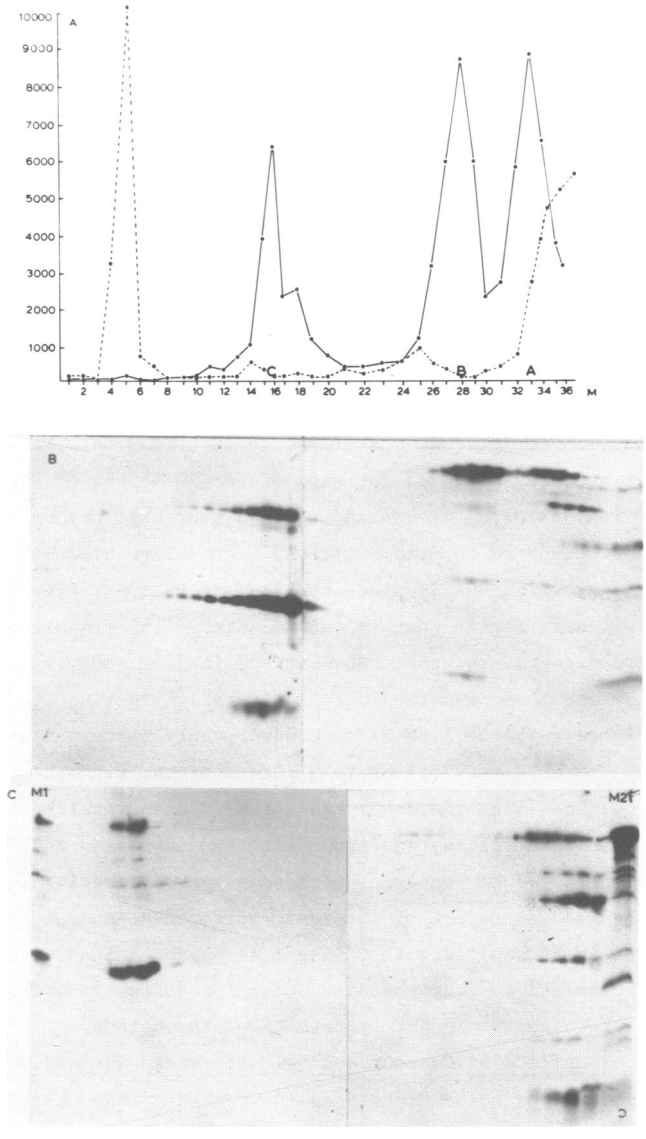

Figure 5: Protein composition of native and salt-disrupted Ad5 cores. Ad5 virions, labelled with ^{3}H-arginine, were disrupted in 10% pyridine and 0.01M (panel A, solid line and panel B) or 0.6M NaCl (panel A, dashed line and panel C) and centrifuged in 15-30% (w/u) sucrose gradients containing the appropriate [NaCl]. The 0.6M NaCl gradient was poured over a cushion of 1.75gm. cc^{-1}. CsCl. Aliquots of each fraction were counted and the remainder electrophoresed in 15% polyacrylamide gels. ^{14}C-labelled proteins of 92kd (68kd), 43kd, 30kd and 12.5kd (NEN) were applied to track M in panel B and ^{3}H-arginine and ^{35}S-methione-labelled Ad5 virions to tracks M1 and M2 in panel C.

exposure to 0.4M NaCl. It can be seen from the examples shown in panel A of Figure 5 that much of the material was very tangled. Discrete molecules, like that shown in Figure 4B, were seen, albeit more rarely. The tangles probably resulted from aggregation of individual viral genomes via interactions among the 'sticky' terminal protein molecules (43, 44). Be that as it may, the most striking feature of these salt-disrupted core particles is their beaded appearance, which is reminiscent of nucleosomal, cellular DNA (45-47). The 'beads' with which adenoviral DNA was associated appeared roughly spherical, exhibited a mean diameter of 9.5±0.5nm, and presumably correspond to the 9-10nm subunits comprising the thick fibres shown in Figure 2. The fibres running between beads displayed a diameter of 23±4 Å and must, therefore, correspond to naked DNA. The distances between 'beads' were very variable, typically from 9.5 to 132.7nm, that is about 28 to 390 bp. The interbead DNA length most frequently measured corresponded to 30-45 bp. of DNA. Although protein-free regions of viral DNA and regions that have not unfolded were present in cores exposed to 0.4M NaCl, the beaded form predominated. Under these conditions, viral DNA is protected against DNAase I digestion (Figure 3) although to a lesser degree than in native cores. The transition to complete sensitivity to digestion of core DNA observed in 0.6M NaCl (Figure 3) was not accompanied by any obvious morphological alteration, nor was any seen up to 1.0M NaCl (Figure 4C). At 1.5M NaCl, however, only protein-free DNA, aggregated by presumed terminal protein interactions, was observed (not shown). At 0.2M NaCl, on the other hand, the unfolded, thick-fibre forms predominated, an observation consistent with the increased DNAase I-sensitivity compared to 'native' cores seen in the experiment shown in Figure 3. The gradual nature of the ionic strength-dependent transition to a structure that is as sensitive to DNAase I as deproteinized viral DNA, therefore, appears to reflect the disruption (or destabilization) of a hierarchy of protein-protein and protein-DNA interactions.

Polypeptide Composition of Compact and Salt-Disrupted Adenovirus Cores

Type 5 adenovirus cores were released from purified, ^{3}H-arginine labelled virions and sedimented in 15-25% sucrose gradients. Aliquots of each fraction were then electrophoresed in SDS-polyacrylamide gels, with the results shown in Figure 5. 'Native' cores gave three peaks of ^{3}H-arginine-labelled material, designated A, B and C in Figure 5A. Peak A, fractions 33-36, contained polypeptides II, III, and IV, and apparently smaller quantities of polypeptides IIIa and XI. Peak B, fractions 25-29, was, on the other hand, enriched in polypeptides II, VI, IX and a polypeptide of apparent molecular

weight 55,000 daltons, probably IVa_2. Thus peaks A and B must correspond to the penton subassembly and the capsid face assembly, respectively (7). A separate complex of polypeptides II and VI was also present in fractions 31-33, partially separated from pentons. The sedimentation of viral DNA in fractions 11-19 (data not shown) identified peak C as the core complex. Intensely-labelled polypeptides, exhibiting apparent molecular weights of 48,000, 18,000 and about 6,000 daltons, were present in the peak C (Figure 5B): pyridine-released adenovirus cores therefore contain the polypeptide designated as μ by Hosakawa and Sung (23), as well as the previously described core proteins V and VII. Peak C included a shoulder of more slowly-sedimenting material, which appears enriched for polypeptide VII and depleted in polypeptide μ . Interestingly, more slowly-sedimenting fractions of the core peak also contained a higher proportion of the more unfolded core structures (see Table 1).

When ^{3}H-arginine labelled virions were exposed to 0.6M NaCl, only two peaks of labelled material were seen (Figure 5A): the first comprised the polypeptides of disrupted capsid assemblies that have barely entered the gradient (Figure 5C), whereas the second (which was caught on a cushion of 1.75 gm.cc^{-1} CsCl) contained all the viral DNA, as determined by blotting (data not shown). Its high rate of sedimentation can be ascribed to the aggregation induced when cores are exposed to NaCl concentrations of 0.4M or greater (see Figure 4). Polypeptide VII was present only in those fractions that contained viral DNA (Figure 5C). Conversely, no polypeptide μ and only trace amount of polypeptide V remained in association with Ad5 DNA after exposure of cores to 0.6M NaCl. The core peak shown in Figure 5B also contained small amounts of other viral polypeptides, such as II, IV and VI. These polypeptides are not present in cores prepared in the presence of 10mM NaCl (Figure 5B) and most probably reflect the presence of a small quantity of intact virions (800S) which would be caught on the CsCl cushion: virions could also be the source of the small quantities of polypeptide V observed in these fractions. We conclude that the transition of adenovirus cores from compact structures, including highly-folded thick fibres, to 'beads-on-a-string' is accompanied by the loss of polypeptides V and μ. The 'beads' like those shown in Figure 3 must therefore comprise polypeptide VII alone, a conclusion in accord with its previously-reported, tight association with adenoviral DNA (14, 23).

Micrococcal Nuclease Digestion of Adenovirus cores.

'Native' or salt-disrupted Ad5 cores were digested with micrococcal

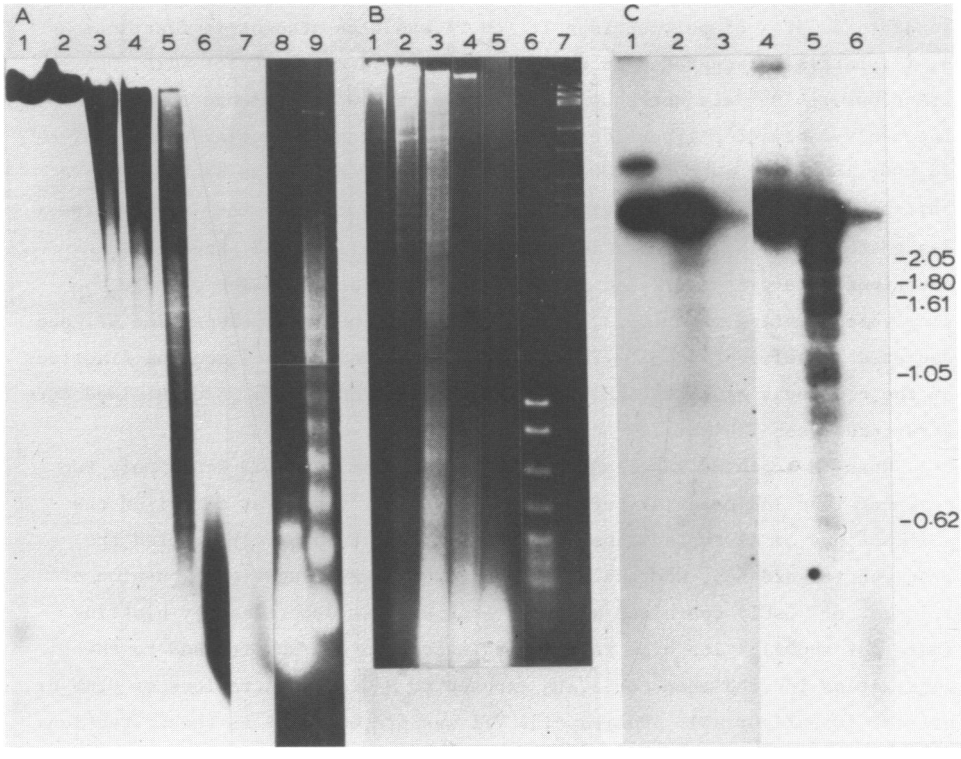

Figure 6: Micrococcal nuclease digestion of salt-treated cores and deproteinized adenoviral DNA. In all experiments shown, micrococcal nuclease (MN) digestions were for 5' at 20°C and the DNA products of digestion were deproteinized before electrophoresis in 1.8% agarose gels. Panel A: ^{3}H-thymidine-labelled Ad5 cores were digested with 0, 100, 500, 2500, 7500 or 37,500 units of MN per A_{260} unit of DNA in 0.6M NaCl, tracks 1 to 6, respectively. Tracks 1 to 6 were obtained by a 16hr. exposure of the fluorogram. Track 7 is a 3hr. exposure of track 6. Tracks 8 and 9 show the products of digestion of pBR322 DNA with HpaII and of HeLa cell chromatin with 100 units MN per A_{260} unit, respectively. Panel B: Unlabelled, deproteinized Ad5 DNA was digested as for tracks 1-5 of panel A, tracks 1 to 5, respectively. Tracks 6 and 7 show HpaII digested pBR322 DNA and Ad5 DNA, digested with BglII, respectively. The gel was photographed after staining with ethidium bromide. Panel C: Unlabelled, Ad2 DNA (tracks 1-3) or Ad2 cores (tracks 4-6) were incubated in 0.6M NaCl, dialyzed into .01M Tris-HCl, pH7.4 containing 1mM EDTA and 0.01M NaCl and digested with 0, 0.25 and 3.0 units of MN per A_{260} unit. The DNA products were deproteinized and digested with SmaI before electrophoresis. Blots made from the gels were hybridized to nick-translated DNA of the fragment 91.9 to 93.5 units (576 base pairs) generated by SmaI plus KpnI digestion of cloned HindIII fragment F (89.5 to 97.0 units) of Ad2 DNA.

nuclease under the conditions listed for each reaction in the legends to Figures 6 and 7. Typical DNA products obtained when cores were exposed to 0.4M NaCl are shown in panel A of Figure 6: limited micrococcal nuclease digestion generated a very complex set of DNA fragments, from 8.8kb to less than 1.0kb in length. The products of the most extensive digestions (tracks 6 and 7, panel A, Figure 6) were heterogeneous, low molecular weight DNA fragments. Deproteinized Ad5 DNA also yielded a large population of high molecular weight DNA fragments when digested under identical conditions (panel B of Figure 6) or in 0.01M NaCl (not shown). As can be seen in Figure 6 and the summary given in Table 2, the sets of fragments of greater than 0.5kb generated from salt-treated cores or deproteinized Ad5 DNA were very similar. This conclusion also holds when much smaller regions of the viral genome were examined by the indirect end-labelling technique (48, 49), as shown for the region 91.9 to 98.3 units in panel C of Figure 6: in this case micrococcal nuclease digestion of salt-treated cores or deproteinized DNA generated the same set of DNA fragments, the most prominent of which were 1.8, 1.35, 1.2 and 0.8kb in length. We, therefore, conclude that these larger fragments are the result of sequence-specific cleavage of the enzyme (50-52) and thus that viral DNA is quite accessible to the nuclease in salt-disrupted cores, a conclusion that is quite consistent with the presence of extensive regions of protein-free DNA (Figure 3). Nevertheless, the low molecular weight DNA

TABLE 2: DNA fragments generated by micrococcal nuclease digestion of Ad5 Cores

Deproteinized Ad5 DNA	Salt Cores	Deproteinized Ad5 DNA	Salt Cores	Deproteinized Ad5 DNA	Salt Cores
8.60	8.80	2.85	2.90	1.32	1.30
7.40	7.40	2.70	2.75	1.26	1.25
6.80	6.80	2.60	2.60	1.15	1.15
6.40	6.40	2.51			1.13
5.95	6.00		2.45		1.07
5.70		2.35	2.35	0.97	0.97
4.70	4.70	2.30	2.30	0.88	0.88
	4.40	2.15	2.15	0.76	0.77
4.25	4.20	2.10		0.67	0.65
	3.95	2.00	2.00	0.57	0.57
3.80	3.80	1.90	1.90	0.51	0.50
3.70		1.83	1.80	0.45	0.44
3.60	3.60		1.70		0.39
3.20	3.25	1.65		0.32	0.32
3.15		1.46	1.45		0.25
3.04		1.38	1.40		

The lengths, in kb., of the DNA fragments generated upon micrococcal nuclease digestion of deproteinized Ad5 DNA and salt-disrupted cores (Figure 6 and other similar experiments) are listed.

generated from salt-disrupted cores by micrococcal nuclease digestion is visibly larger than that released from deproteinized, viral DNA, 130 to 450 nucleotides compared to 90 to 290 nucleotides (compare tracks 6 and 3 in panel C of Figure 6).

Two quite different results were obtained when 'native' cores were digested with micrococcal nuclease. The first, shown in panel A of Figure 7

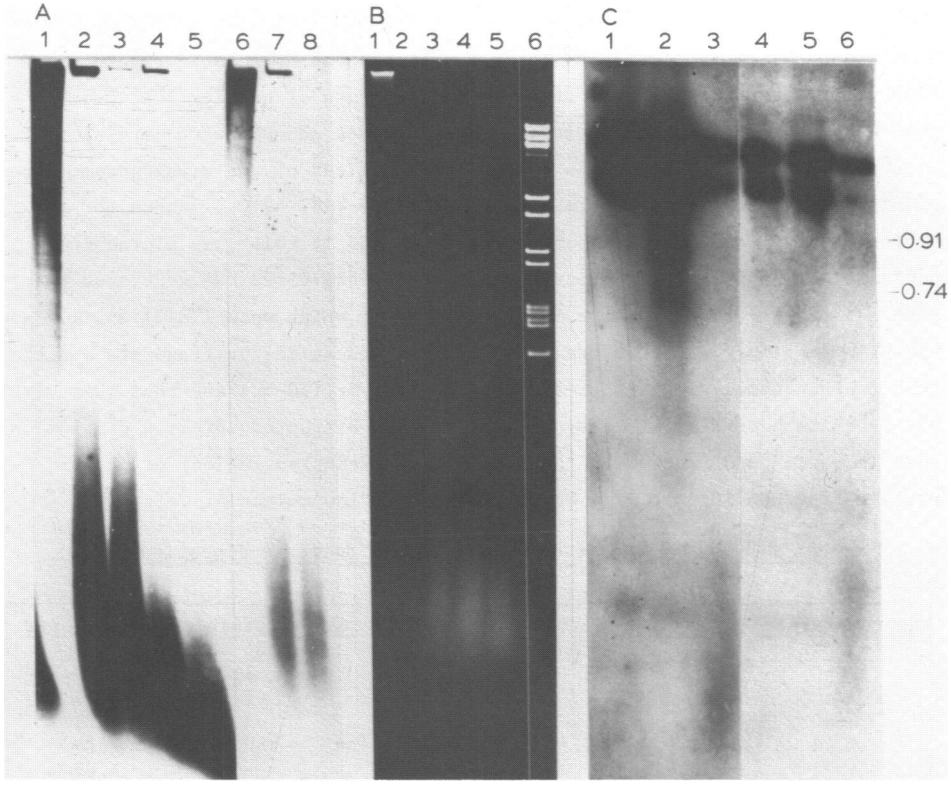

Figure 7: Micrococcal nuclease digestion of 'native' adenovirus cores. Panel A: ^{3}H-thymidine labelled Ad5 cores were incubated with 3 (tracks 1 and 6), 15 (tracks 2 and 7), 75 (tracks 3 and 8), 375 (track 4) or 1675 (track 5) units MN per A_{260} unit. The fluorogram was exposed for 16hrs. (tracks 1 to 5) or for 3hrs. (tracks 6 to 8). Panel B: Unlabelled Ad5 cores were incubated with 0, 0.5, 2.5, 12.5 or 62.5 units MN per A_{260} unit in 0.01M NaCl and the DNA products visualized after staining with ethidium bromide, tracks 1 to 5, respectively. Ad5 DNA fragments generated by BglII cleavage are shown in track 6. Panel C: Unlabelled deproteinized Ad2 DNA (tracks 1, 2 and 3) or Ad2 cores (tracks 4, 5 and 6) were incubated with 0, 0.25 and 3.0 units of MN per A_{260} unit. DNA products that hybridize to the viral DNA fragment 96.0 to 97.0 units (366bp) generated by BglII plus HindIII digestion of cloned HindIII fragment F were identified after BglII digestion as described in the legend to Figure 6.

closely resembled that consistently seen with salt-treated cores. (Compare panel A of Figure 6 with panel A of Figure 7, which is taken from the same agarose gel.) More extensive digestion of 'native' cores released low molecular DNA very similar to that generated upon digestion of salt-treated cores (compare tracks 5 and 6, panel A, Figure 7 with track 7, panel A, Figure 6). Such low molecular weight DNA, which included more discrete fragments of 0.25 and 0.40kb (tracks 7 and 8, panel A, Figure 7) has been observed in all experiments we have performed with native (and salt-treated) cores. By contrast, the release of high molecular weight DNA fragments has been variable: a result like that shown in panel A of Figure 9 has been observed in only two of the dozen or so digestions we have performed. In all others, like those shown in panels B and C of Figure 7, no discrete, high molecular weight bands were seen. Rather, the predominant products of digestion were small. Thus, in the experiment shown in panel C, digestion of deproteinized Ad2 DNA yielded specific DNA fragments of 0.91, 0.74, 0.65, 0.56 and 0.47kb that hybridize to a probe spanning the 475bp region 96.0 to 97.3 units in the Ad2 genome. Upon further digestion, these were converted to small DNA fragments on the order of 90 to 265 nucleotides. By contrast, DNA of some 130 to 350 nucleotides was generated when 'native' cores were digested. As this was the pattern most frequently observed when 'native' cores were exposed to micrococcal nuclease, we conclude that packaging of viral DNA in cores renders the sequences at which the nuclease prefers to cleave inaccessible. Those 'native' core preparations in which sequence-specific micrococcal nuclease cleavage was observed presumably contained predominantly unfolded structures (Figure 1; 23).

DISCUSSION

The results presented in this paper establish a number of structural analogies between adenovirus core nucleoprotein and cellular chromatin. Cores released from purified virions at low ionic strength unfold readily from a compact, spherical structure (Figures 1a and b) to a thick fibre, whose average diameter is approximately 200 Å (Figures 1e, and 2), and which displays a morphology like that of the chromatin thick fibre (39, 40). Careful scrutiny of this form of adenovirus core nucleoprotein suggests that it is composed of roughly spherical subunits, 9-10nm in diameter. Adenovirus cores that have been exposed to concentrations of NaCl of 0.4M or greater are less resistant to DNAase I digestion (Figure 3) and appear as a "beaded-string" (Figure 4) in which the diameter of the "beads" corresponds to

that of the subunits that appear to comprise more condensed forms. Such morphological similarities of Ad5 core nucleoprotein to cellular chromatin are all the more striking for the very different nature of the respective DNA-associated proteins.

We have established that 'native' core complexes contain not only polypeptides V and VII, but also polypeptide : the cosedimentation of polypeptide μ, with both viral DNA and polypeptide VII in gradients like that shown in Figure 6 (all three peak in fraction 16 in this example) leaves no doubt that polypeptide μ is a normal constituent of adenovirion cores. The relatively low level of this protein, about 125 copies/virion (22), its low molecular weight (22 and Figure 6) and its low methionine content, about 2% (22), would all contribute to its previous oversight when core polypeptides were examined. Salt-disrupted cores, by contrast, contain only DNA and polypeptide VII (Figure 6C). Thus, the 10nm particles must comprise only this polypeptide: cellular nucleosomes comprise the four core histones, H2A, H2B, H3 and H4, of which at least H3 and H4 appear to be required to package cellular DNA in a nucleosome-like array (53-55).

Viral DNA fragments some 130-400bp in length are released when salt-disrupted or 'native' cores are digested with micrococcal nuclease. Despite their heterogeneity, such fragments must reflect association of polypeptide VII with viral DNA, for the corresponding products of digestion of deproteinized DNA are 90-290bp. The conclusion that polypeptide VII can protect DNA in adenovirus cores has been confirmed by the isolation of discrete nucleoprotein complexes that contain both viral DNA and polypeptide VII after extensive micrococcal nuclease digestion of 'native' or salt-disrupted cores (unpublished observations). Any analogy between adenoviral and cellular DNA-protein interactions must, however, end here, for the products of micrococcal nuclease digestion of adenovirus cores in no way resemble the relatively homogeneous, 146bp. fragments bound to nucleosomes (see 56, 57 for reviews). Rather, they appear as a heterogeneous population in which more discrete bands can usually be discerned (Figures 6 and 7). The picture is not noticeably different when relatively small regions of the adenoviral genome are examined (panel C, Figures 6 and 7). This result might be an accurate reflection of the organization of polypeptide VII assemblies along the adenoviral genome. On the other hand, artifacts induced by the preparative procedures we have employed, including loss or slippage of proteins, cannot be discounted. Indeed, the relatively low degree of protection afforded against DNAase I digestion in 0.4M NaCl might argue that

salt-disrupted cores are not all that stable when maintained under these conditions.

Comparison of previous digestion studies of adenovirus cores with the data presented here is complicated: different workers have examined cores in pentonless virions (24-26) or released after pyridine treatment (25, this paper). More importantly, control digestions of deproteinized DNA were not included previously. Nevertheless, examination of all published data does reveal one consensus: the major products of micrococcal nuclease digestion of cores are heterogeneous and smaller than cellular nucleosomal DNA (24-26, Figures 6 and 7). The experiments presented here emphasize that no significance can be attached to higher molecular weight products of digestion of cores, such as the 1.8kb. fragment described by Corden and colleagues (24) unless it is established beyond doubt that they do not merely reflect sequence specific cleavage by the nuclease (Figures 6 and 7, Table 2). Moreover, several products of sequence-specific cleavage are less than 1kb in length (Table 2) and might be identified as oligomers of a repeating unit. Heterogeneous, low molecular weight DNA, apparently indistinguishable from that released from cores, has also invariably been observed among the products of digestion of intranuclear viral DNA at early times after infection (25, 26, 27, 28, our unpublished results). The more discrete fragments that several authors have reported from 3hr. after infection (25, 27, 28) are so similar to those of cellular nucleosomes that it is difficult to escape the conclusion that they are released from viral DNA associated with cellular histones: integration of up to one-third of the infecting viral DNA into the cellular genome can occur in the absence of viral DNA replication (58-60). The lag in appearance of nucleosome-sized viral DNA noted by Tate and Philipson (25), as well as the high multiplicities of infection employed (25, 27, 28), are consistent with the notion that integrated viral DNA sequences are the source of such discrete viral DNA fragments.

'Native' adenovirus core preparations contain polypeptides V and μ, whereas the disrupted, beaded structures do not (Figure 6 and 7). Nevertheless, it is not yet possible to ascribe defined roles to these proteins for we cannot exclude the possibility that the structural transition is a consequence of a disruption of interactions between individual polypeptide VII assemblies. Cores that lack polypeptide V, obtained by exposure of virions to sarkosyl, exhibit a compact, 'knobby', structure (23) so it seems unlikely that polypeptide V plays a crucial role in formation of the more condensed core structures. Whether sarkosyl cores retain polypeptide

μ is not known. The extraordinary composition of polypeptide μ, 54% arginine, 13% histidine, 10% glycine, 10% alanine and 5% serine (23) suggests, however, that it must be a DNA-binding protein. Except for its relatively high histidine content, the amino acid content of polypeptide μ is remarkably similar to of mammalian protamines (61, 62) as is its size. It is therefore tempting to speculate that polypeptide μ plays an important role in condensation of the adenoviral DNA for packaging into virions, when the genome is, of course, transcriptionally quiescent. Clearly, much further work, including elucidation of the manner in which polypeptide μ interacts with viral DNA in 'native' cores, and of the nucleoprotein structure of transcribed forms of the viral genome, is needed to establish the validity of such an hypothesis.

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+To whom correspondence should be addressed.
*Present address: Department of Biochemistry and Biophysics, University of California, Parnassus Street, San Francisco, CA 94117, USA

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