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Nucleosome-Like Structural Subunits of Intranuclear Parental Adenovirus Type 2 DNA

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The intranuclear structure of parental adenovirus 2 DNA was studied using digestion with micrococcal nuclease as a probe. When cultures were infected with ^{32}P-labeled virions, at a multiplicity of 3,000 particles per cell, 14 to 21% of parental DNA penetrated the cell and reached the nucleus. Of this parental DNA, 60% could be solubilized by extensive digestion with micrococcal nuclease. The nuclease-resistant fraction contained viral deoxyribonucleoprotein monomers and oligomers. These nucleosome-like structures contained DNA fragments which are integral multiples of a unit-length DNA of approximately 185 base pairs. The monomeric DNA is similar in length to the unit-length DNA contained in cellular nucleosomes. However, the viral oligomers are slightly smaller than their cellular counterparts. DNA-DNA hybridization demonstrated that all segments of the viral genome, including those expressed as mRNA only at late times, are represented in the nucleosomal viral DNA. The amount of early intranuclear viral chromatin was proportional to multiplicity of infection up to multiplicities of 4,000 particles per cell. However, viral transcriptional activity did not increase in direct proportion to the amount of viral chromatin. Maximum accumulation of intranuclear viral chromatin was achieved by 3 h after infection. The intranuclear parental viral chromatin remained resistant to nuclease digestion even at late times in infection, after viral DNA replication had begun.

Adenovirus type 2 replication requires a series of steps which result in the transfer of virion DNA to the nucleus of the infected cell. Studies of virus penetration and uncoating have demonstrated that the final steps in this process occur in the nucleus, approximately 1 to 2 h after infection (24). Although purified virion DNA can be infectious (13, 27) and the virion DNA has been reported to be free of viral proteins after uncoating (24), it seems unlikely that naked viral DNA normally serves as template for transcription. It is now well established that eucaryotic genes are packaged in chromatin structures identified as nucleosomes (28). Nucleosome oligomers and monomers can be generated by controlled digestion of nuclei or chromatin by micrococcal nuclease (18). Quantitative analysis has demonstrated that each nucleosome contains about 185 to 200 base pairs of DNA consisting of a nuclease-resistant core of 140 base pairs wrapped around an octomer of the four histones H2A, H2B, H3, and H4. This core is connected to the adjacent core by a DNA segment of 15 to 100 base pairs (18), the linker DNA, which is more susceptible to digestion than core DNA.

Similar nucleosome structures have been detected in papovavirus DNA, both in virions and in intracellular structures present late in productive infection (15, 25, 35). A similar structure has been proposed for the virion cores of adenovirus 2 DNA (7). It has been suggested that the virion DNA (23×10^6 daltons) consists of 180 "nucleosomes," each containing about 200 base pairs of DNA wrapped around six copies of the arginine-rich virion polypeptide VII. Additional data suggest that late in productive infection newly replicated viral DNA is also packaged in a chromatin structure (7).

We have studied the structure of intranuclear parental adenovirus 2 DNA in cultures infected with virions containing radioactive viral DNA. Using micrococcal nuclease to study DNA structure, we have demonstrated that the parental intranuclear DNA is in a chromatin-like structure. The relationship of these structures to multiplicity of infection (MOI), the time course of infection, and viral transcription has been examined.

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MATERIALS AND METHODS

Cell culture and virus purification. KB cells were grown in suspension cultures as described previously (10). Stocks of ^{32}P-labeled adenovirus 2 were prepared as described (8), and virus was purified by cesium chloride centrifugation (10). Virus was used within 14 days after purification. The concentration of ^{32}P-labeled adenovirus 2 preparations was determined from the optical density at 260 nm based on the equation, 1 optical density unit at 260 nm = 1×10^{12} virus particles (24).

Isolation of nuclei. Suspension culture KB cells were infected at a MOI of 3,000 particles per cell with radioactive virus or 100 PFU/cell with nonradioactive virus, except where otherwise indicated. After 1 h of adsorption, cells were diluted to 3×10^5 cells per ml in Joklik's minimum essential medium supplemented with 5% horse serum. Unless indicated otherwise, cultures were usually harvested 3 h after the beginning of adsorption, and the cells were washed twice with cold phosphate-buffered saline.

All steps in harvesting cells or purifying nuclei were performed at 0 to 4°C. The following three methods were used to prepare nuclei.

(i) Isotonic buffer. Washed cells were resuspended at a concentration of 3×10^6 cells per ml in 0.01 M Tris-hydrochloride, pH 7.5, 0.15 M NaCl, 0.0015 M $MgCl_2$, and 0.05% Triton X-100 (Sigma Chemical Co.). After 15 min at 0°C, cells were disrupted with 10 strokes of a tightly fitted pestle in a Dounce homogenizer. The nuclei were pelleted at $600 \times g$ for 5 min at 4°C.

(ii) Hypotonic buffer. Washed cells were resuspended at a density of 3×10^6 cells per ml in 0.01 M Tris-hydrochloride, pH 7.4, 0.01 M NaCl, 0.0015 M $MgCl_2$, and 0.05% Triton X-100. After 5 min at 4°C, the swollen cells were lysed in a Dounce homogenizer with 20 strokes of a loosely fitted pestle. The nuclei were pelleted at $600 \times g$ for 5 min at 4°C.

(iii) Sucrose buffer. Washed cells were resuspended at a concentration of 10^7 cells per ml in lysis buffer (0.3 M sucrose, 0.01 M Tris-hydrochloride, pH 7.9, 0.001 M $CaCl_2$, and 0.5% Nonidet P-40 [Shell Chemical Co.]) (30). Cells were lysed at 4°C in a Dounce homogenizer as in method ii. Nuclei were collected by low-speed centrifugation and washed two times with lysis buffer and twice with digestion buffer (0.3 M sucrose, 0.001 M Tris-hydrochloride, pH 7.9, and 0.1 mM $CaCl_2$). In some experiments with isolated nuclei, digestion buffer contained 1.0 mM $CaCl_2$, which increased the rate of digestion by micrococcal nuclease but did not alter the pattern of fragment release with respect to degree of solubility.

Procedure iii was used routinely for isolation of nuclei before micrococcal nuclease digestion. Each batch of nuclei prepared by the above methods was found intact and essentially free of cytoplasmic contamination by phase-contrast microscopy. All nuclear preparations were used immediately for further experiments.

Isolation of chromatin. KB cells labeled for 72 h with [^{3}H]thymidine (final concentration, 0.2 μCi/ml) were washed with phosphate-buffered saline as described above (method iii). Chromatin was prepared from the nuclear pellet as described by Hancock (17), except that 10 mM Tris-hydrochloride, pH 8.0, replaced phosphate buffer. The chromatin pellet was washed twice with 10 ml of 1 mM Tris-hydrochloride–0.5 mM EDTA (pH 8.0) and twice with digestion buffer. After the final wash the chromatin pellet was disbursed in digestion buffer by shearing in a Dounce homogenizer fitted with a tight pestle. The DNA concentration was adjusted to 300 to 500 μg/ml with digest buffer.

Micrococcal nuclease digestion of nuclei. Nuclei from cultures infected with ^{32}P-labeled virus were prepared by method iii and then resuspended in digestion buffer to give a DNA concentration of about 300 μg/ml, as determined by the optical density reading (at 260 nm) of an aliquot diluted in 5 M urea and 2 M NaCl (30). The digestion was carried out at 37°C with micrococcal nuclease (Worthington Biochemicals Corp.; 0.1 U/μg of DNA). Aliquots were removed at various times, and the amount of labeled DNA rendered acid soluble was determined by precipitation with 10% trichloroacetic acid. In some experiments solubilization in perchloric acid was determined as described by Lohr et al. (22).

When DNA was to be analyzed by electrophoresis, the digestion was stopped by addition of EDTA, NaCl, and pancreatic RNase A (2). The material was digested for 30 min at 37°C and then treated with lauryl sarcosine (0.5% final concentration) and 160 μg of proteinase K per ml, followed by phenol extraction (33). When deoxyribonucleoproteins (DNP) were to be analyzed by electrophoresis, the digestion was halted by the addition of EDTA to a 10 mM final concentration. The nuclear suspension was held on ice for 60 min, sheared, and cleared by centrifugation for 10 min at 10,000 rpm (Sorvall R2B, SS34 rotor). The supernatant was used as a source of nucleosome monomers and oligomers (3).

Gel electrophoresis of DNA and DNP. Nuclear DNA purified after micrococcal nuclease digestion and adenovirus 2 DNA digested with endo R·*Sma*I (26) were resolved on either 1.4% agarose slab gels (0.3 by 18 by 16 cm) or 2.0% agarose tube gels (0.7 by 12 cm) as described previously (10). Electrophoresis was performed overnight at 50 V at room temperature for slab gels and 8 h at 1.5 mA per tube for cylindrical gels. ^{32}P-labeled viral DNA was visualized by autoradiography of gels covered with plastic wrap and exposed on Kodak X-omat R film (XR-5). The slices (1 mm) from cylindrical gels were hydrolyzed at 80°C in 0.4 ml of 30% H_2O_2 containing 1% concentrated NH_4OH for at least 4 h, neutralized with 1 M acetic acid, and counted in 5 ml of Formula-963 (New England Nuclear). DNP was analyzed in 3.5% acrylamide–0.5% agarose gels (34). Electrophoresis was conducted in the cold at 20 mA for 6 h. After electrophoresis, gels were prepared for fluorography (4, 20). ^{32}P-labeled DNA fragments generated by micrococcal nuclease digestion of nuclei from infected cells were hybridized to *Sma*I adenovirus 2 DNA fragments according to a method described previously (29), and the hybrids were visualized by autoradiography.

In vitro RNA synthesis by isolated nuclei. Purified nuclei were assayed for RNA synthetic capacity

as previously described (37, 38), using 500 μCi of [^{3}H]UTP (New England Nuclear, [6,5-^{3}H]UTP) per ml (35 Ci/mmol). After 15 min of incubation at 37°C, the mixture was diluted with an equal volume of 0.05 M Tris-hydrochloride (pH 7.9), 0.2 M NaCl, 0.01 M EDTA, 2% *N*-lauryl sarcosine, 200 μg of dextran sulfate per ml, 0.002 M UTP, and 300 μg of proteinase K (E. Merck; fungal, chromatographically purified and lyophilized) per ml. The suspension was pipetted gently, using a large-bore pipette, and incubated overnight at 37°C. RNAs were purified by phenol extraction (39). RNA-DNA hybridization experiments were performed with adenovirus 2 DNA immobilized on 6.5-mm cellulose nitrate membranes (type B6, Schleicher & Schuell Co.) (10).

RESULTS

Preparation of nuclei containing parental viral DNA. Three methods of nuclear preparation were compared (Table 1) before analyzing the physical state of parental viral DNA early in infection. For all three methods, nuclei were treated with nonionic detergents to remove cytoplasmic material. Nuclei prepared in isotonic (i) or hypotonic (ii) buffers, used routinely for preparation of nuclei for in vitro RNA synthesis (9, 38, 40), contained the same amount of parental DNA as did nuclei prepared in sucrose–calcium buffer for micrococcal nuclease digestion (iii). Comparable amounts of viral RNA were synthesized in vitro with the three types of nuclear preparations (Table 1).

Since nuclei prepared for micrococcal nuclease digestion exhibited typical amounts of viral transcriptional activity, we used this method to study the fate and structure of parental viral DNA. The subcellular distribution of parental ^{32}P-labeled adenovirus 2 DNA 3 h after infection is shown in Table 2. The percentage of parental DNA found in nuclei ranged from 14 to 21%. To determine if the ^{32}P-labeled DNA in nuclei 3 h after infection represented viral DNA, nuclear DNA was analyzed by CsCl equilibrium density gradient centrifugation. The results of such an analysis are shown in Fig. 1. Greater than 95% of the ^{32}P-labeled DNA had the buoyant density of viral DNA (1.714 g/cm^3). A similar result was obtained when parental viral DNA was examined 18 h after infection (data not shown). Thus, 5% is the upper limit of viral DNA molecules that might be covalently attached to cellular DNA (16) or trapped with cellular DNA, or, alternatively, which were degraded and reincorporated into cellular DNA.

Kinetics of digestion of intranuclear adenovirus 2 DNA by micrococcal nuclease. The rate at which DNA is rendered acid soluble when eucaryotic cell nuclei are digested by micrococcal nuclease is linear early in the course of digestion and gradually reaches a plateau when 50 to 75% of the DNA is digested (1, 6, 30). Digestion of the intranuclear parental adenovirus 2 DNA by micrococcal nuclease in a digest buffer containing 1.0 mM $CaCl_2$ yielded kinetics of degradation similar to those described for eucaryotic chromatin; a plateau was reached when about 60% of the viral DNA was rendered acid soluble (Fig. 2). The digestion kinetics of purified adenovirus 2 DNA mixed with mock-

TABLE 1. *Recovery of ^{32}P-labeled adenovirus 2 DNA and viral transcriptional activity in nuclei prepared for micrococcal nuclease digestion (iii), as compared to crude nuclear preparations (i, ii)*[a]

Nuclear prepn	Intranuclear adenovirus 2 DNA		Total [^{3}H]-UTP incorporated (cpm/10^8 cells)	Viral transcriptional activity (% hybridization of [^{3}H]RNA to adenovirus 2 DNA)
	Genomes per nucleus	% of input		
Isotonic–Triton (i)	500	15	423,000	1.90
RSB–Triton (ii)[b]	700	22	490,000	2.60
Sucrose–calcium–Nonidet P-40 (iii)	600	19	327,000	3.00

[a] KB cells were infected with 3 × 10^3 particles per cell using ^{32}P-labeled adenovirus 2. After 3 h of infection, nuclei were isolated by the indicated methods, the amount of intranuclear ^{32}P-labeled adenovirus 2 DNA was determined, and the nuclei were incubated in vitro for RNA synthesis as described in the text. [^{3}H]RNA was extracted from nuclei and hybridized to 10 μg of adenovirus 2 DNA.

[b] RSB, Reticulocyte standard buffer.

TABLE 2. *Subcellular distribution of parental adenovirus 2 DNA in cultures harvested early in infection*[a]

Fraction	% of infective ^{32}P-labeled adenovirus 2[b]
Cells[c]	37–58 (47.5)
Postnuclear supernatant	16–33 (24.5)
Nuclear washes[d]	1.6–4 (2.8)
Nuclei	14–21 (17.5)

[a] KB cells were infected with ^{32}P-labeled adenovirus 2 at an MOI of 3,000 particles per cell. Cultures were harvested 3 h after infection, and nuclei were fractionated by the sucrose–calcium method.

[b] The numbers give the range of values obtained from six experiments. Numbers in parentheses represent average values.

[c] KB cells after two washes with cold phosphate-buffered saline.

[d] Nuclear washes include two washes with Nonidet P-40 buffer and two washes with the micrococcal

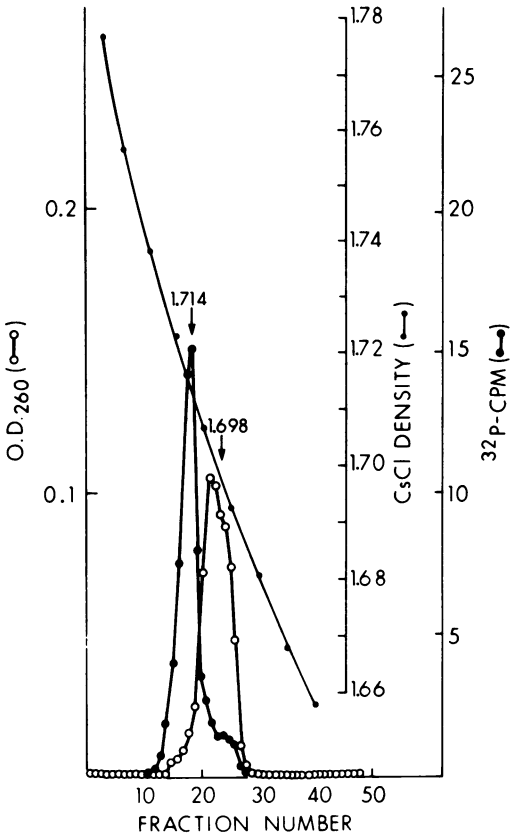

FIG. 1. *CsCl density gradient analysis of intranuclear ^{32}P-labeled parental adenovirus 2 DNA. Nuclei containing 30 μg of DNA were prepared 3 h after infection. The nuclear DNA was centrifuged to equilibrium in 10-ml CsCl gradients (32). Centrifugation was at 100,000 × g for 69 h at 15°C in a Spinco 50 Ti rotor. Gradient fractions (0.2 ml) were collected, and density was determined by refractive index. Symbols: ●, ^{32}P-labeled adenovirus 2 DNA; O, optical density at 260 nm (OD_{260}) of cellular DNA; ——, CsCl density. Under these conditions, the desities of adenovirus 2 DNA and KB nuclear DNA are, respectively, 1.714 and 1.698 g/cm^3.*

infected KB cell nuclei were significantly different. After 30 s of digestion, as much as 55% of the ^{32}P-labeled viral DNA was digested, as compared to 4% of the intranuclear parental viral DNA (Fig. 2). The digestion of purified viral DNA reached a plateau when about 90% had been rendered acid soluble. As a further control purified ^{32}P-labeled viral DNA was mixed with ^{3}H-labeled KB cell chromatin and subjected to digestion. Under these conditions viral DNA was degraded at least as fast as in the absence of chromatin. For example, after 1 min of digestion (micrococcal nuclease, 1 U/μg of DNA), 18% of the [^{3}H]chromatin counts per minute were solubilized as compared to 70% of the ^{32}P-labeled viral DNA.

Relationship between MOI and the intranuclear concentration of nuclease-resistant viral DNA. To determine the effect of MOI on intranuclear viral DNA, three parameters were measured at various multiplicities (Fig. 3). The amount of intranuclear viral DNA increased linearly up to 4,000 particles per cell. As measured in isolated nuclei, the amount of viral transcriptional activity increased up to MOIs of 3,000 to 4,000 particles per cell (Fig. 3A). However, the increase was not directly proportional to MOI.

Nuclei purified from cultures infected at various multiplicities were digested with micrococcal nuclease (Fig. 3B). The kinetics of digestion were the same irrespective of the intranuclear concentration of parental viral DNA. We conclude that a similar percentage of the viral DNA is in a nuclease-resistant structure irrespective of the MOI.

Nuclease resistance of parental viral DNA at various times during the course of infection. The fate of parental viral DNA during the course of infection was analyzed by harvesting cultures and purifying nuclei at various times in infection (Fig. 4). Parental viral DNA was detectable in nuclei by 1 h after infection,

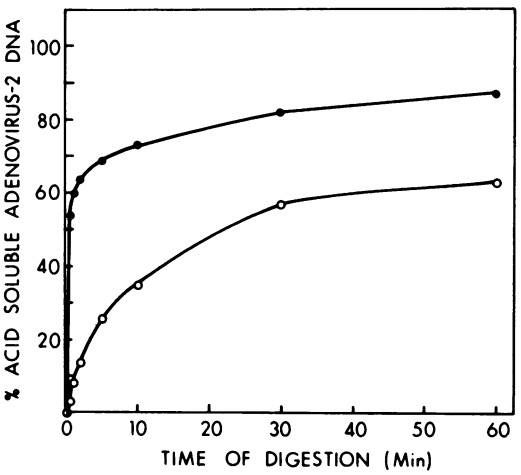

FIG. 2. *Kinetics of micrococcal nuclease digestion of ^{32}P-labeled parental viral DNA in purified KB cell nuclei. Nuclei were digested with micrococcal nuclease as described in the text. Aliquots were removed from the digest at the indicated times, and the amount of ^{32}P-labeled adenovirus 2 DNA rendered acid soluble in 10% trichloroacetic acid was determined. Symbols: O, infectious intranuclear ^{32}P-labeled parental viral DNA; ●, deproteinized ^{32}P-labeled adenovirus 2 DNA mixed with mock-infected KB cell nuclei.*

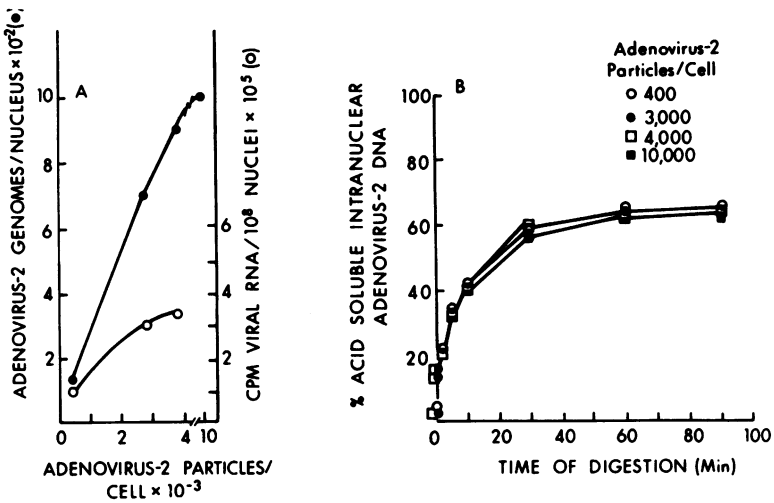

FIG. 3. *Effect of increasing MOI on the amount of intranuclear viral DNA, virus-specific RNA synthesis in vitro, and nuclease-resistant viral DNA. Nuclei were purified from ^{32}P-labeled adenovirus 2-infected cells by method iii. The MOI was either 400, 3,000, 4,000, or 10,000 adenovirus 2 virions per cell. (A) ●, Recovery of intranuclear ^{32}P-labeled adenovirus 2 DNA; ○, synthesis of virus-specific RNAs in nuclei incubated in vitro. (B) Kinetics of micrococcal nuclease digestion of intranuclear adenovirus 2 DNA at different MOIs.*

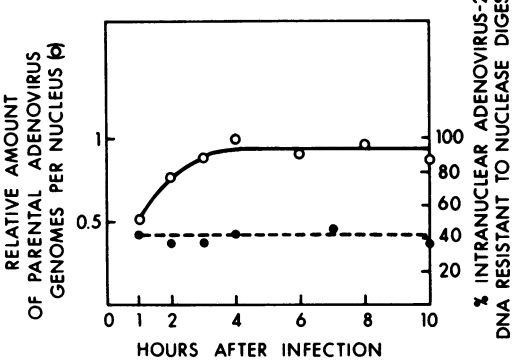

FIG. 4. *Micrococcal nuclease digestion of intranuclear parental adenovirus 2 DNA at different times during productive infection. Nuclei were isolated by the sucrose-calcium method from KB cells infected with ^{32}P-labeled adenovirus 2 particles. Cultures were harvested at the indicated times during the productive infection. Symbols: ○, Recovery of intranuclear ^{32}P-labeled parental adenovirus 2 DNA; ●, limit digestion of intranuclear ^{32}P-labeled parental adenovirus 2 DNA by micrococcal nuclease.*

although maximal transfer to nuclei was not achieved until 4 h. The amount of intranuclear viral DNA remained constant until the latest times examined, 10 h after infection. For each time sample the percentage of parental ^{32}P-labeled DNA resistant to micrococcal nuclease digestion (after limit digestion) was determined. For all samples tested the percent was constant (Fig. 4), and the kinetics of digestion were the same (not shown).

Electrophoresis of DNA fragments generated by micrococcal nuclease digestion. When nuclei from human cells are digested with micrococcal nuclease, the DNA fragments are integral multiples of a monomeric unit of about 185 base pairs, consisting of a core of nuclease-resistant material about 140 base pairs in length and a heterogeneous nuclease-sensitive linker with a mean length of about 45 base pairs (19, 21, 23). The sizes of cellular and viral DNAs resistant to digestion were compared. KB cells were labeled with [^{3}H]thymidine for 72 h before infection with ^{32}P-labeled adenovirus 2. At 3 h after infection nuclei were purified and prepared for micrococcal nuclease digestion. Samples were taken from the digestion mixture at various times, and the DNA was extracted and then fractionated on 2% agarose cylindrical gels (Fig. 5).

As the digestion proceeded, the sizes of both cellular and viral oligomers decreased. The high-molecular-weight fragments diminished, and DNA shifted to the mobilities of monomers and material smaller than 140 base pairs. The viral oligomers were somewhat smaller than corresponding cellular units. Also, the viral nucleosomes shifted to smaller sizes at a more rapid rate than the cellular chromatin. The most prominent difference between cellular and viral patterns was the rate of accumulation of submonomer fragments at the expense of monomer. These submonomers migrated faster than the dye front (slice 80). Higher-resolution gels (not

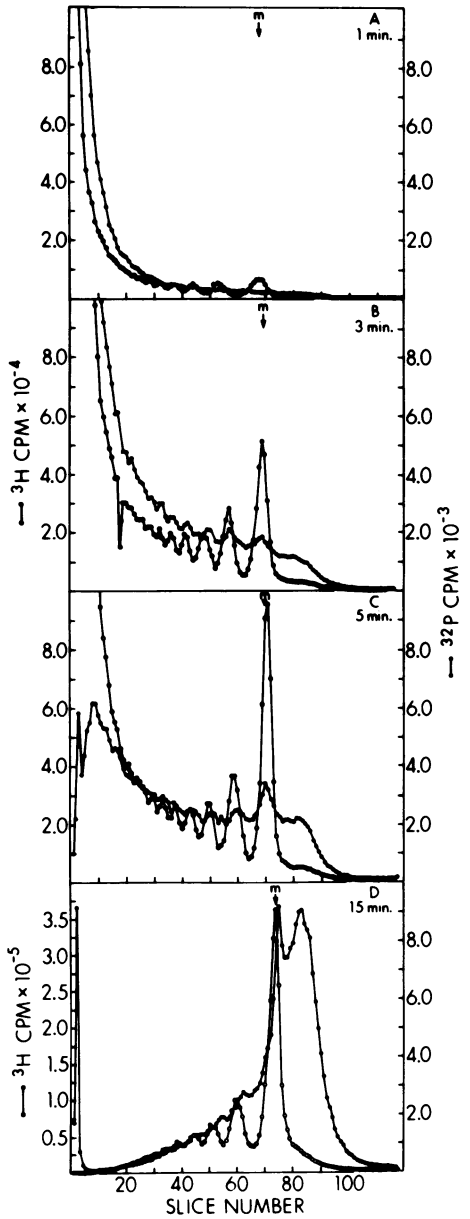

FIG. 5. *Micrococcal nuclease digestion patterns of DNA from ^{3}H-labeled KB cells 3 h after infection with ^{32}P-labeled adenovirus 2. Nuclei from KB cells infected with 3,000 virus particles per cell were prepared and digested with micrococcal nuclease as described. At the indicated times, samples were removed for phenol extraction and electrophoresis in 2% agarose cylindrical gels. (A) 1-min digest, 1% acid soluble; (B) 3-min digest, 10% acid soluble; (C) 5-min digest, 15% acid soluble; (D) 15-min digest, 20% acid soluble.*

shown) showed that these fragments ranged in size from 20 to 80 base pairs.

The parental viral DNA fragments resistant to nuclease digestion were also displayed as DNP. Nuclei were prepared from unlabeled KB cells infected for 3 h with [^{3}H]thymidine labeled adenovirus 2. A sample was prepared after 10 min of digestion; electrophoresis was performed before deproteinization (Fig. 6). As was the case with viral DNA from infected nuclei, viral DNP dimers and trimers migrated in the gel more rapidly than cellular forms, reflecting the reduced DNA content of these structures (34).

Viral "nucleosomes" are not formed by association of nuclear proteins with DNA during digestion. To evaluate the possibility that cellular nuclear proteins had exchanged with viral genomes during the course of digestion, ^{3}H-labeled cellular chromatin was mixed

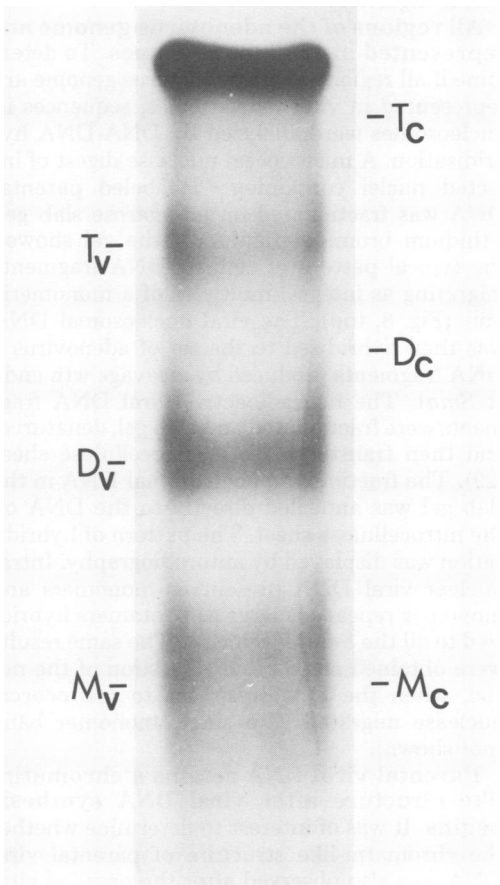

FIG. 6. *Electrophoretic analysis of DNP released from adenovirus 2-infected nuclei digested with micrococcal nuclease. KB cells were infected with [^{3}H]thymidine-labeled adenovirus 2 (3,000 particles per cell). Nuclei were isolated and digested as described in the text. Electrophoresis was in a composite 3.5% acrylamide–0.5% agarose gel. M_v, M_c: Nucleosome monomers, viral and cellular; D, T: nucleosome dimers and trimers.*

with phenol-extracted, ^{32}P-labeled adenovirus 2 DNA and then digested with micrococcal nuclease. The DNA was extracted and subjected to electrophoresis. To maximize the possibility of detecting resistant DNA, the ratio of ^{32}P-labeled DNA to ^{3}H-labeled DNA was 20 times greater than that present in isolated nuclei. The results are shown in Fig. 7A through C. At no time during the course of digestion did there appear ^{32}P-labeled DNA fragments of discrete sizes in the gel regions corresponding to nucleosomal DNA. In a separate experiment (Fig. 7D), purified ^{3}H-labeled cellular DNA was mixed with purified ^{32}P-labeled adenovirus 2 DNA. A similar result was obtained. Thus, it is clear that the nucleosome monomers and oligomers of viral DNA in the nucleus are not formed by exchange during digestion.

All regions of the adenovirus genome are represented in viral nucleosomes. To determine if all regions of the adenovirus genome are represented in viral nucleosomes, sequences in nucleosomes were analyzed by DNA-DNA hybridization. A micrococcal nuclease digest of infected nuclei containing ^{32}P-labeled parental DNA was fractionated on an agarose slab gel. Ethidium bromide staining of the gel showed the typical pattern of cellular DNA fragments migrating as integral multiples of a monomeric unit (Fig. 8, top). The viral nucleosomal DNA was then hybridized to the set of adenovirus 2 DNA fragments produced by cleavage wth endo R·*Sma*I. The nonradioactive viral DNA fragments were fractionated on a slab gel, denatured, and then transferred to a nitrocellulose sheet (29). The fractionated nucleosomal DNA in the slab gel was annealed directly to the DNA on the nitrocellulose sheet. The pattern of hybridization was displayed by autoradiography. Intranuclear viral DNA present as monomers and monomer repeats as large as pentamers hybridized to all the *Sma*I fragments. The same results were obtained after a limit digestion of the nuclei, when the DNA resistant to micrococcal nuclease migrated as a single monomer band (not shown).

Parental viral DNA retains a chromatin-like structure after viral DNA synthesis begins. It was of interest to determine whether the chromatin-like structure of parental viral DNA was also observed after the onset of viral DNA replication. To examine this possibility, ^{3}H-labeled KB cells infected with ^{32}P-labeled adenovirus 2 were continued in culture until 18 h after infection; the cells were then harvested, and nuclei were prepared and digested. The DNA was extracted and subjected to electrophoresis (Fig. 9). As with nuclei 3 h after infection (Fig. 5), a chromatin-like structure of viral DNA

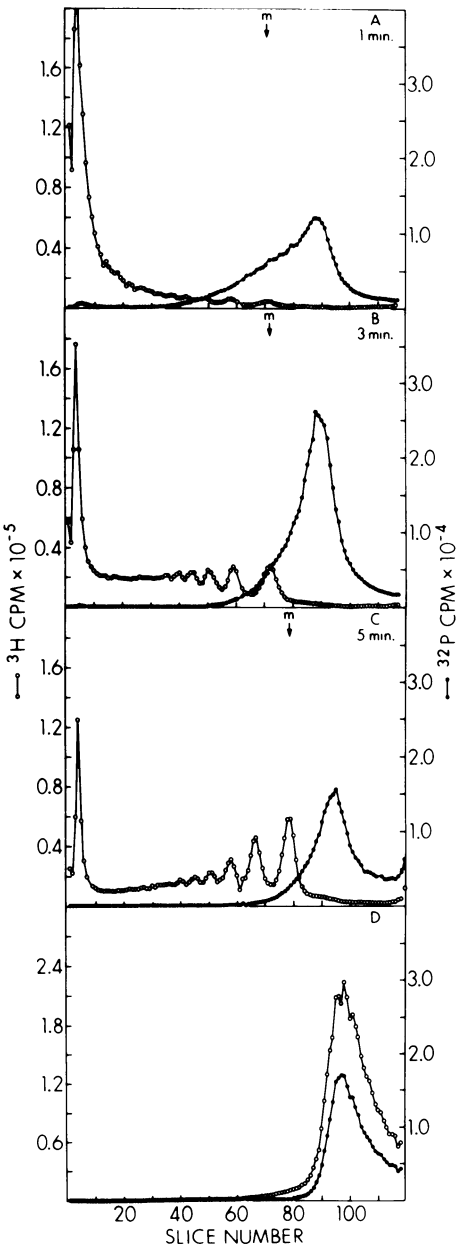

FIG. 7. *Micrococcal nuclease digest patterns of DNA from ^{3}H-labeled KB cell chromatin mixed with phenol-extracted, ^{32}P-labeled adenovirus 2 DNA. The isolation of chromatin and digestion by micrococcal nuclease are described in the text. Electrophoresis was in 2% agarose cylindrical gels. (A) 1-min digest, ^{3}H cpm, 0.1% acid soluble; ^{32}P cpm, 22% acid soluble. (B) 3-min digest, ^{3}H cpm, 3% acid soluble; ^{32}P cpm, 37% acid soluble. (C) 5-min digest, ^{3}H cpm, 8% acid soluble; ^{32}P cpm, 47% acid soluble. (D) Phenol extracted, ^{3}H-labeled KB cell DNA mixed with ^{32}P-labeled adenovirus 2 and digested to 25% acid-soluble ^{3}H cpm and 65% ^{32}P cpm.*

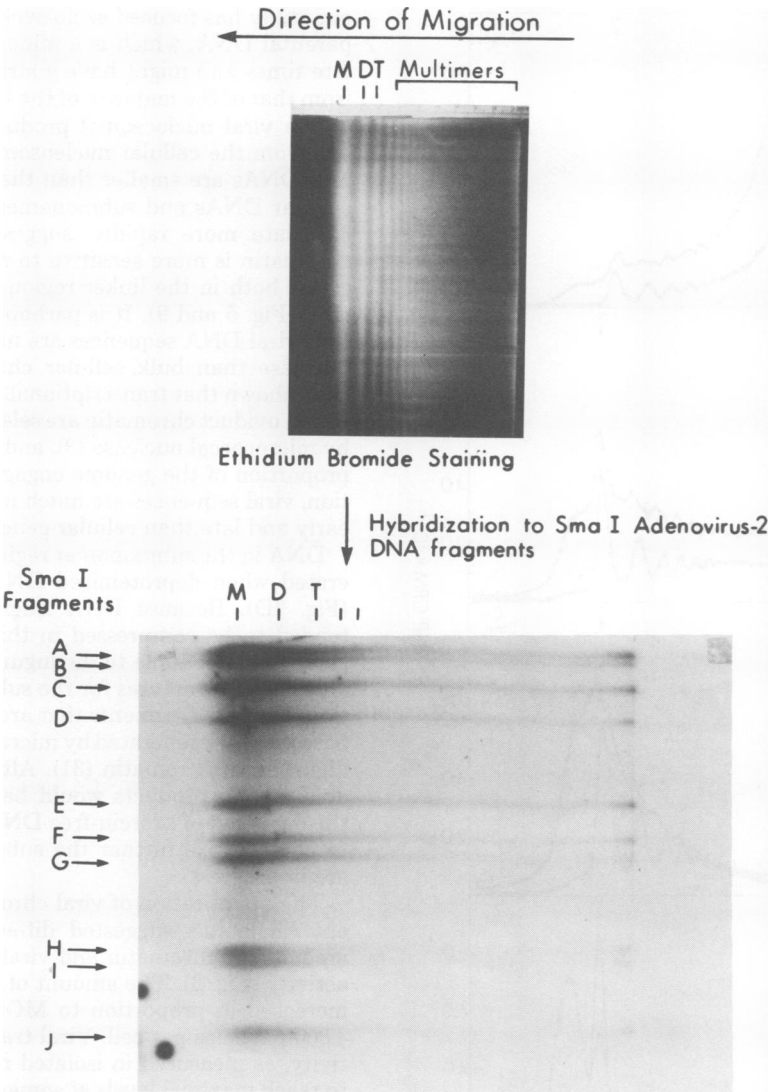

FIG. 8. *Hybridization transfer of DNA in nucleosomes to nitrocellulose-bound endo R·SmaI fragments of adenovirus 2 DNA. Nuclei isolated from ^{32}P-labeled adenovirus 2-infected KB cells (3 h after infection, 2,000 particles per cell) were digested for 5 min with micrococcal nuclease (500 μg of DNA per ml; 30 U of enzyme per ml). DNA fragments purified from the digest were run on a 1.4% agarose slab gel (top). ^{32}P-labeled DNA in the slab gel was then hybridized to nonradioactive adenovirus 2 SmaI fragments (A–J) which previously had been separated on a 1% agarose slab gel and transferred to nitrocellulose (20). For the hybridization step the radioactive gel was oriented such that the direction of migration was at right angles to the direction of migration of the DNA which had been transferred to nitrocellulose. (Bottom) Pattern of the autoradiogram after the hybridization step. M, D, and T designate nucleosome monomers, dimers, and trimers, respectively.*

was observed. In these preparations also, a submonomer fraction was observed.

DISCUSSION

Based on the studies presented here, we conclude that parental adenovirus DNA molecules entering the nucleus early in infection (3 h) are organized in a pattern similar to the nucleosomal structure of cellular chromatin (Fig. 5); parental DNA found in the nucleus at late times also has a chromatin-like structure (Fig. 9). Digestion of nuclei from infected cells with micrococcal nuclease releases viral and cellular DNP containing DNA sequences which are multiples of a monomeric unit. It is clear that at least 40% of the viral DNA exists in this organization; after 15 min of digestion 40% of the ^{32}P-labeled viral counts per minute are smaller than full-length

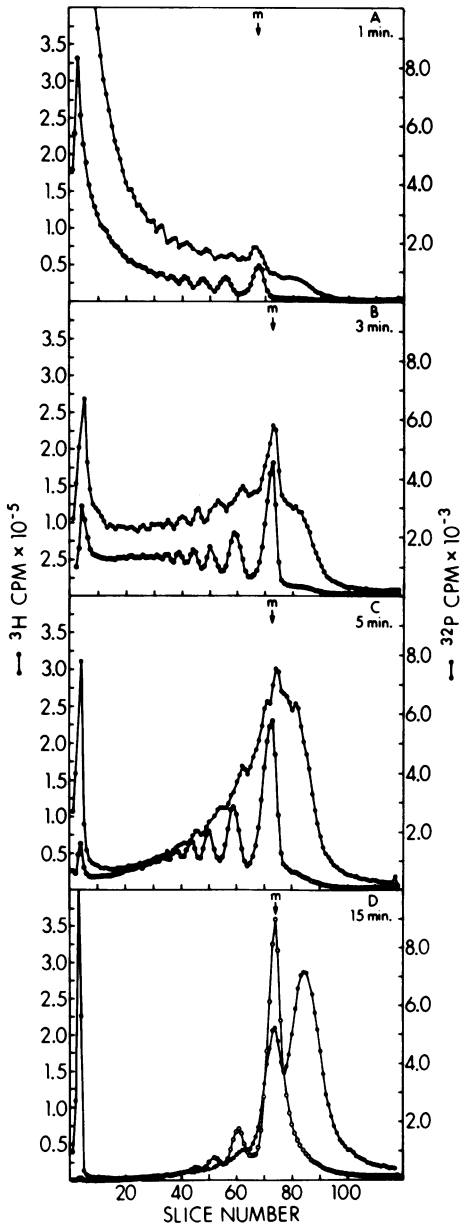

FIG. 9. *Micrococcal nuclease digestion patterns of DNA from ^{3}H-labeled KB cells 18 h after infection with ^{32}P-labeled adenovirus 2. The infection, digestion, and electrophoresis conditions are described in the legend to Fig. 5. (A) 1-min digest, 4% acid soluble; (B) 3-min digest, 15% acid soluble; (C) 5-min digest, 17% acid soluble; (D) 15-min digest, 20% acid soluble.*

adenovirus 2 DNA but equal to or larger than cellular monomers. An intracellular adenovirus chromatin structure was not observed in electron microscopy studies of nuclear replicative DNAs extracted late in infection (18). However, our study has focused exclusively on the fate of parental DNA, which is a minor component at late times and might have a structure different from that of the majority of the DNA molecules.

The viral nucleosomal products do differ in size from the cellular nucleosomes. Viral oligomer DNAs are smaller than the corresponding cellular DNAs and submonomer fragments accumulate more rapidly, suggesting that viral chromatin is more sensitive to micrococcal nuclease both in the linker region and within the core (Fig. 5 and 9). It is perhaps not surprising that viral DNA sequences are more sensitive to nuclease than bulk cellular chromatin. It has been shown that transcriptionally active regions of hen oviduct chromatin are selectively released by micrococcal nuclease (3), and, in terms of the proportion of the genome engaged in transcription, viral sequences are much more active both early and late than cellular genes.

DNA in the submonomer region was also generated when deproteinized DNA was digested (Fig. 7D). Because DNA fragment mobilities tended to be compressed in this region of the gels, it is impossible to distinguish between two alternative structures for the submonomer fraction. Discrete fragments that are multiples of 10 base pairs are generated by micrococcal nuclease digestion of chromatin (31). Alternatively, randomly sized products would be expected from the digestion of protein-free DNA. Experiments to characterize further the submonomer DNA are in progress.

The quantitation of viral chromatin at different MOIs has suggested differences between amounts of chromatin and viral transcriptional activity (Fig. 3). The amount of viral chromatin increased in proportion to MOI up to at least 4,000 particles per cell. Viral transcriptional activity, as measured in isolated nuclei, appeared to reach maximal levels at somewhat lower multiplicities. Thus, quantitation of chromatin structure is not necessarily a direct reflection of viral transcription. Viral templates may exist in chromatin structures but not be utilized for viral transcription. In fact, it may be that only a small fraction of the viral chromatin is actively transcribed. Although limitations in cellular RNA polymerase concentrations might be responsible for limited template utilization, previous studies suggest that other factors may influence template-enzyme interaction and thereby determine the templates actively transcribed (37).

Analysis of viral chromatin has provided additional evidence for the utilization of parental genomes in virus replication. Maximum transfer of parental DNA to the nucleus required 3 h from the beginning of virus adsorption, in agree-

ment with previous studies of viral penetration (24). The viral chromatin was shown to be present in nuclei as late as 18 h after infection; at all times examined, a constant proportion of parental DNA was resistant to nuclease digestion. Thus, the chromatin-like structure of parental DNA was preserved at least 11 to 12 h after viral DNA replication had begun. An earlier study had demonstrated the preservation of a substantial amount of parental DNA until late in infection (36). Since the late phase of viral transcription is initiated at the time DNA replication begins (14), our findings demonstrate that this late transcriptional pattern does not require or coincide with the elimination of chromatin structures containing parental DNA strands.

Although all regions of the viral genome are represented in chromatin before the beginning of viral DNA replication (Fig. 8), it is certainly possible that regions containing early genes have a structure different from those genes expressed only at late times (11, 12, 27). The polypeptides responsible for the chromatin-like organization of parental viral DNA in the nucleus have not been identified. Structural analysis of virions suggests a role for the internal proteins VII and V (7). However, the ability to infect cells with naked DNA (13, 25) indicates that virion proteins are not obligatory for early expression of the viral genome. Thus, a role for cellular histones in the early viral chromatin cannot be excluded.

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