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Adenovirus DNA Is Associated with the Nuclear Matrix of Infected Cells

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Viral DNA was found to be tightly associated with the nuclear matrix from HeLa cells lytically infected with human adenovirus type 5. The bound viral DNA, like cell DNA, was resistant to nonionic detergent and to extraction with high-salt (2 M NaCl) solution. However, whereas over 95% of the cell DNA was recovered in the matrix fraction, the amount of associated viral DNA varied during infection. Throughout the lytic cycle, the amount of matrix-associated adenovirus type 5 DNA increased until it reached a plateau level at 20 to 24 h after infection. At this stage, the matrix-bound DNA represented 87% of the total viral DNA; after this stage, additional newly synthesized viral DNA accumulated as non-matrix-associated DNA. DNase digestion studies revealed that all viral DNA sequences were equally represented in the matrix-bound DNA both early and late in infection; thus, unlike cell DNA, there seem to be no preferred attachment sites on the viral genome. An enrichment of viral DNA relative to cell DNA was found in the matrix-associated DNA after extensive DNase I digestion. This finding, together with an in situ hybridization study, suggests that the viral DNA is more intimately associated with the nuclear matrix than is cell DNA and probably does not exist in extended loops.

The DNA of eucaryotic cell nuclei is organized into supercoiled loops (3, 9) which are structurally maintained by attachment to a proteinaceous substructure, the nuclear matrix (5). The nuclear matrix consists of a peripheral lamina, nuclear pore structures, a residual nucleolus, and an intranuclear matrix (for review, see reference 4). It is resistant to high-salt solution, nonionic detergents, and nuclease digestion and is composed of three major polypeptides and a large number of less abundant proteins. In addition to its role as an important component in the structural organization of the nuclear DNA, the nuclear matrix is the site of DNA replication (6, 17, 20, 30) and possibly also the site of heterogeneous nuclear RNA processing (12, 16, 29). Furthermore, Barrack and Coffey (1) have demonstrated specific binding of hormones to the nuclear matrix.

The DNA loops of interphase nuclei are apparently anchored to the nuclear matrix at specific sites along the chromosome. When the loops are digested with a nuclease, the DNA which remains associated with the matrix is enriched for particular sequences. For example, Cook and Brazell (10) have demonstrated an enrichment of α-globin, but not β- or γ-globin, sequences in the matrix-associated DNA of HeLa cells. The virus-specific sequences of a simian virus 40-transformed cell line are enriched in the DNase-digested nuclear matrix (18), and rDNA has also been found closely associated with the nuclear matrix (19).

The human adenoviruses are DNA viruses which replicate in the nuclei of infected cells. Hodge et al. (13) have suggested that the nuclear matrix of HeLa cells infected by human adenovirus type 5 (Ad5) undergoes a change in protein composition. More recently, it has been demonstrated that the DNA of polyoma virus, a papovavirus, is associated with the nuclear matrix of productively infected cells (7).

In this communication we examine the association between Ad5 DNA and the nuclear matrix of lytically infected HeLa cells. We found that a proportion of the viral DNA became tightly bound to the nuclear matrix during the early phase of infection and that this proportion continued to increase until it reached a plateau level late in infection. This association was not mediated through specific viral sequences, and the viral DNA was more resistant to nuclease digestion than was the cell DNA.

MATERIALS AND METHODS

Virus and cells. Ad5 and HeLa cells were kindly provided by F. L. Graham, McMaster University, Hamilton, Ontario. HeLa cells were grown in Dulbecco modified Eagle medium (Flow Laboratories,

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Inc., Rockville, Md.) supplemented with 10% bovine serum. Virus was propagated in monolayer cultures of HeLa cells and titrated by the fluorescent cell counting method (23).

Nuclear matrix. The nuclear matrix was prepared from HeLa cells by one of the following methods.

(i) The cells were washed with phosphate-buffered saline and scraped from the dish with a rubber policeman. The pelleted cells were then suspended in nuclear wash buffer (0.25 M sucrose, 10 mM triethanolamine [pH 7.4], 10 mM NaCl, 5 mM $MgCl_2$) containing 0.5% Nonidet P-40 (NP-40) and were incubated at 0°C for 15 min. The cells were disrupted in a Dounce homogenizer, and lysis was monitored by phase-contrast microscopy. Nuclei were pelleted at 1,000 × *g* for 10 min, washed in nuclear wash buffer, and resuspended in nuclear wash buffer. NaCl was increased to 2.0 M by the addition of 0.67 volume of 5 M NaCl, and DNase I (Boehringer Mannheim Corp., New York, N.Y.) was added to 50 μg/ml. The mixture was incubated at 37°C for 30 min. EDTA was added to 10 mM, and the nuclei were layered over a solution of 15% glycerol–2 M NaCl–10 mM triethanolamine–10 mM EDTA–0.1% NP-40 (pH 7.4) and centrifuged in an SW41 rotor at 15,000 rpm for 45 min. This pellet was termed the nuclear matrix. To extract DNA, the pellets were suspended in 3% sodium dodecyl sulfate–2% β-mercaptoethanol and extracted twice with an equal volume of double-distilled phenol saturated with 100 mM Tris base. Nucleic acids were precipitated with 2 volumes of ethanol and suspended in 10 mM NaCl–10 mM Tris (pH 7.5)–1 mM EDTA. RNA was digested by treatment for 30 min at 37°C with 50 μg of RNase A per ml and 20 U of RNase T1 per ml, and the mixture was again phenol extracted. DNA recoveries were normally between 60 and 80% of the initial preparation and, when appropriate, could be normalized for losses.

(ii) This method is based on the preparation of a nuclear "cage" which is composed of cytoskeletal and nuclear components (11). Briefly, cells were washed with TBS (10 mM Tris [pH 7.6], 0.14 M NaCl), scraped off the dishes, washed again, and resuspended in TBS. The cells were then lysed by adding 3 volumes of 1.33× lysis buffer (1× lysis buffer is 1.95 M NaCl, 0.10 M EDTA, 2 mM Tris [pH 8.0], and 0.5% Triton X-100) and incubating at 0°C for 15 min. By using a wide-bore pipette, the nuclear cage was then layered over a step gradient consisting of 2.5% sucrose in lysis buffer, 15% sucrose in 1.5 M NaCl–10 mM $MgCl_2$–1 mM Tris (pH 7.6), and 45% sucrose in 10 mM Tris (pH 7.6)–10 mM $MgCl_2$–10 mM $NaCl_2$ and centrifuged in an SW27 or SW41 rotor at 7,500 rpm for 1 h. The nuclear matrix was collected on the interface between the 15 and 45% sucrose layers.

For DNase digestion experiments, the interphase fraction was recovered, diluted severalfold in the appropriate enzyme buffer, and digested at 37°C as indicated below. Digested preparations were layered over 15% glycerol in 2 M NaCl–10 mM Tris (pH 7.6)–10 mM EDTA–0.1% NP-40 and centrifuged at 15,000 rpm for 45 min. The pellets were resuspended in 3.0% sodium dodecyl sulfate–2% β-mercaptoethanol and processed as described for method i. For preparations that were not digested with DNase, the lysate was centrifuged as above, and DNA was extracted from the pellet and from the supernatant.

Nick translation. DNA was labeled in vitro by the method of Rigby et al. (24).

In situ hybridization. HeLa cells were grown in culture wells attached to microscope slides (Lab Tek Products, Div. Miles Laboratories Inc., Westmont, Ill.); one-half of the wells on each slide were infected with Ad5. Some slides were labled for 3 h with [^{3}H]thymidine ([^{3}H]dThd) before infection. At 24 h postinfection, the cells were washed in phosphate-buffered saline and lysed at 0°C by immersion in 0.5% NP-40–0.22 M sucrose–0.5 mM $CaCl_2$–50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.8). Nuclear matrix was made by sequential treatment with 0.2 mM $MgCl_2$–10 mM Tris (pH 7.4) containing increasing concentrations of NaCl (30). Ethidium bromide (100 μg/ml) was added to the final buffer. DNA was nicked by exposure to a UV light source for 1 min. Preparations were fixed for 2 min in 3 parts ethanol–1 part acetic acid, dehydrated in 95% ethanol for 10 min, and air dried. In situ hybridization was essentially as described by Pardue and Gall (21). RNA was removed by digestion for 1 h at 37°C with 100 μg of RNase per ml in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and the preparation was washed three times in 2× SSC, dehydrated through 70 and 95% ethanol, and air dried. DNA was denatured with 0.07 N NaOH for 3 min, and the preparation was again dehydrated with three changes in 70% ethanol and two changes in 95% ethanol and air dried. The hybridization mixture contained ^{3}H-nick-translated Ad5 DNA (10^7 cpm/μg), 200 μg of sonicated herring sperm DNA per ml, and 0.3 M NaCl. A portion (60 μl) of the mixture was added to each slide under a cover slip (22 by 60 mm) and allowed to hybridize for 15 h at 65°C. The slides were washed in three changes of SSC at 60°C, dehydrated, and air dried. Autoradiographs were made by using Kodak NTB-2 dipping emulsion. Preparations were stained with Giemsa after photographic development.

Reassociation kinetics. The concentration of viral DNA in the preparations was determined by solution hybridization with ^{32}P-nick-translated Ad5 DNA by the method of Pettersson and Sambrook (22) as described previously (27).

RESULTS

Nuclear matrix. The nuclear matrix is a residual three-dimensional structure which remains after extraction of nuclei with nonionic detergent–2 M NaCl followed by digestion with DNase and RNase (see above). The protein composition of our nuclear matrix preparations from HeLa cells (Fig. 1) was similar to that reported for a variety of cell types (4). The predominant proteins were of 40,000 to 65,000 daltons, and no histones remained. The nuclear matrix of infected cells was indistinguishable from that of mock-infected cells (Fig. 1). The DNA content of our nuclear matrix preparations varied from more than 90% of the cell DNA before nuclease digestion to approximately 0.4% of the cell DNA after DNase I digestion. The nuclear matrix preparations used for this study were made by one of two methods (see above).

The method used for each experiment is indicated below and in the figure legends.

Association of viral DNA with the nuclear matrix. The Ad5 lytic cycle can be divided into two phases, an early phase during which a few viral proteins are synthesized in relatively small amounts and a late stage, which begins about 8 h postinfection, during which viral DNA synthesis occurs and virus-specific proteins are synthesized in large amounts. The amount of Ad5 DNA associated with the nuclear matrix was determined at various times throughout the lytic cycle. HeLa cells were grown for 24 h in [^{3}H]dThd to label the cell DNA uniformly. The cells were then washed and infected with Ad5; nuclear matrix was prepared by method ii at 1, 4, 8, 24, and 36 h post infection, and the amounts of cell and viral DNA in the matrix-bound and free fractions were determined. The cell DNA concentration was determined by ^{3}H radioactivity; viral DNA levels were measured by reassociation kinetics. The proportions of cell and viral DNA associated with the nuclear matrix are shown in Table 1. More than 90% of the cell DNA remained associated with the matrix at all times, whereas the viral DNA increased from 33% attached to the nuclear matrix at 1 h postinfection to a peak of 87% associated late in infection. The absolute amount of viral DNA associated with the nuclear matrix late in infection remained constant (Table 1); however, from 24 h onward, the amount of free viral DNA increased, and the matrix-bound viral DNA represented a smaller proportion of the total Ad5 DNA.

TABLE 1. Distribution of DNA in Ad5-infected cells[a]

Time (h) postinfection	Amt (ng/10^6 cells) of Ad5 DNA		Matrix-associated cell DNA (% of total cell DNA)
	Free	Matrix associated	
1	0.98	0.48 (33)[b]	95
4	0.54	0.48 (47)	93
8	0.74	0.99 (57)	90
24	20.6	133 (87)	94
35	153	132 (46)	93

[a] Nuclear matrix was prepared by method ii at the indicated times after Ad5 infection of HeLa cells at a multiplicity of infection of 50 IU per cell. DNA was prepared from the matrix fraction, and free DNA was prepared from the supernatants. The amounts of cell and viral DNA were determined by radioactive counting and reassociation kinetics, respectively.

[b] Numbers within parentheses indicate percentages.

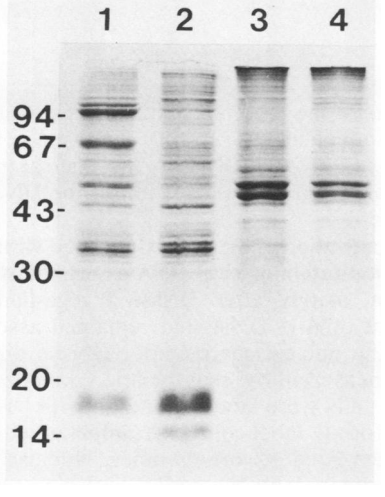

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the nuclear proteins from Ad5-infected and uninfected HeLa cells. Nuclear matrix was prepared by method i (see text). Lanes: 1, 2 M NaCl extract of nuclei from Ad5-infected cells; 2, 2 M NaCl extract from uninfected cells; 3, nuclear matrix from uninfected HeLa cells; 4, nuclear matrix from Ad5-infected cells. The proteins were separated on a 10% linear gel and stained with Coomassie blue. The numbers to the left of lane 1 indicate the positions of molecular weight standards ($\times 10^{-3}$).

DNA sequences involved in the attachment of the viral genome to the nuclear matrix. To determine whether Ad5 DNA is associated with the nuclear matrix through specific sequences, we used progressive restriction endonuclease digestion of the DNA bound to the nuclear matrix (10). As the nuclease digestion proceeds, the DNA remaining attached to the matrix becomes enriched for sequences close to the attachment sites. The DNA which remained attached to the matrix was isolated and digested to completion with the same restriction enzyme. Equal amounts of completely restricted DNA were separated by agarose gel electrophoresis, blotted to diazobenzyloxymethyl-paper, and tested for Ad5 sequences by hybridization with nick-translated Ad5 DNA. Figure 2 shows an autoradiograph of ^{32}P-labeled Ad5 DNA hybridized to matrix DNA isolated at 6 and 24 h postinfection which is digested to increasing extents while associated with the matrix.

All regions of the Ad5 genome were present in the nuclear matrix-associated DNA both early and late in infection at all levels of digestion tested (Fig. 2). Furthermore, there was no enrichment for any fragment, which suggests that all sequences in the viral genome are equally involved in the attachment of Ad5 DNA to the matrix. There was a trend to an increased amount of Ad5 DNA relative to cell DNA with increasing digestion in the early sample (Fig.

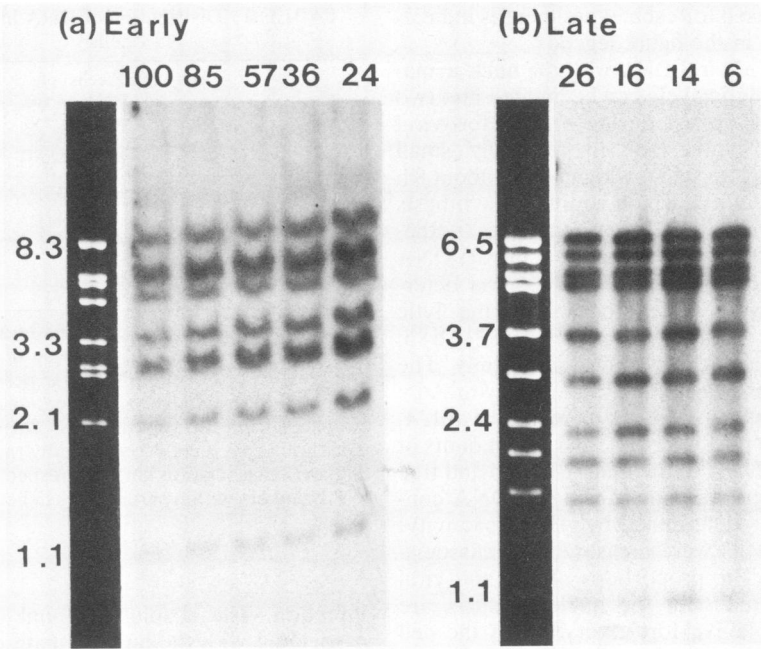

FIG. 2. Autoradiographic detection of Ad5 DNA sequences associated with the nuclear matrix. Nuclear matrix was prepared by method ii (see text) at (a) 6 h and (b) 24 h postinfection. Matrix preparations were digested to increasing amounts with *Hin*dIII or *Kpn*I, respectively. DNA that remained matrix associated was purified, digested to completion with the same restriction enzyme, and separated on a 1% agarose gel. Equal amounts of cell DNA were applied to each lane. The gel was blotted to diazobenzyloxymethyl-paper, probed with ^{32}P-nick-translated Ad5 DNA, and autoradiographed. The numbers above each lane indicate the percentage of cell DNA that remained matrix associated. Lanes 1 and 7 are stained gels showing complete *Hin*dIII and *Kpn*I digests of Ad5; sizes are in kilobase pairs.

2a). This suggests that the Ad5 DNA is attached in such a way that it is less easily removed by restriction enzyme digestion than is the cell DNA.

These experiments, which demonstrated an equal representation of all parts of the Ad5 genome in the matrix-associated DNA, used digestions with restriction endonucleases to a level at which up to 94% of the cell DNA had been removed. It was possible that more extensive digestion would demonstrate an enrichment for specific regions of the Ad5 genome. To this end a second experimental approach was used. HeLa cells were infected with Ad5, and nuclear matrix was prepared both early (8 h in the presence of 25 μg of cytosine arabinoside per ml) and late (25 h) in infection. The early and late nuclear matrix preparations were digested with DNase I to remove 99 and 96%, respectively, of the DNA. The DNA which remained associated with the matrix was ^{32}P labeled in vitro by nick translation and used to probe a blot of a *Hin*dIII digest of Ad5 DNA. The matrix-associated DNA hybridized to all bands in the same proportions as did the total infected-cell DNA (Fig. 3). This confirmed the previous result suggesting that all viral sequences are equally involved in the attachment of Ad5 DNA to the nuclear matrix, even after extensive digestion of the DNA with DNase I.

Organization of the matrix-attached viral DNA. (i) Quantitation of viral DNA associated with the nuclear matrix after DNase I digestion. The amount of Ad5 DNA that remained associated with the nuclear matrix after DNase digestion was measured by reassociation kinetics (22). HeLa cells were labeled for 24 h with [^{3}H]dThd to uniformly label cell DNA, infected with Ad5, and harvested at various times. Nuclear matrix was prepared by method ii and digested with DNase I to more than 99% digestion of ^{3}H-labeled cell DNA. The residual matrix-associated DNA was purified and analyzed for cell and viral sequences. The amounts of viral DNA in the total cell DNA and the DNase I-digested matrix preparations were determined by reassociation kinetics with ^{32}P-nick-translated Ad5 DNA (Fig. 4). The amount of cell DNA was determined by ^{3}H radioactivity. The proportion of viral DNA relative to cell DNA was greater in

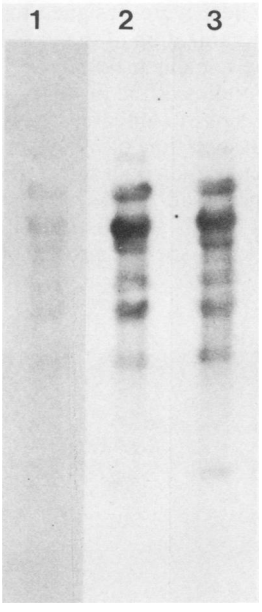

FIG. 3. Ad5 DNA sequences in DNase I-digested nuclear matrix. Nuclear matrix was made from Ad5-infected cells by method ii both early and late in infection and was digested with DNase I to remove 99 and 96%, respectively, of the cell DNA. The residual matrix-associated DNA was purified, ^{32}P labeled by nick translation, and used to probe blots of *Hin*dIII-digested Ad5 DNA. Lanes: 1, [^{32}P]DNA from HeLa cell nuclear matrix early in infection with Ad5 used as a probe; 2, ^{32}P-labeled total late infected-cell DNA as a probe; 3, [^{32}P]DNA from HeLa cell nuclear matrix late in infection.

the DNase I-digested matrix sample than in the total cell DNA preparation at all times postinfection. This result indicates that the viral DNA is less accessible to DNase I digestion than is cell DNA.

(ii) In situ hybridization of Ad5 DNA to infected-cell nuclear matrix. When cells growing on cover slips are permeabilized with nonionic detergent, extracted with high-salt solution, and treated with ethidium bromide and UV light to nick the supercoiled loops of DNA, the DNA extends to form fluorescent halos around the residual nuclei (Fig. 5A and reference 30). Autoradiographs of cells which have been prelabeled with [^3H]dThd, treated in this way, and prepared for in situ hybridization have a halo of silver grains surrounding the nuclear matrix (Fig. 5B and reference 30). This confirms that the fluorescent halo corresponds to the relaxed nuclear DNA and that the DNA halo remains intact during in situ hybridization. To similar unlabeled preparations from Ad5-infected and noninfected cells, we hybridized, in situ, ^3H-nick-translated Ad5 DNA. All of the silver grains in the autoradiographs are confined to the residual nucleus (Fig. 5C) and do not extend into the halo region. Hybridization of ^3H-labeled Ad5 DNA to uninfected cells showed only background labeling (Fig. 5D). This result suggests that the Ad5 DNA, which is approximately 12 μm long, is intimately associated with the nuclear matrix and does not extend from it as long strands.

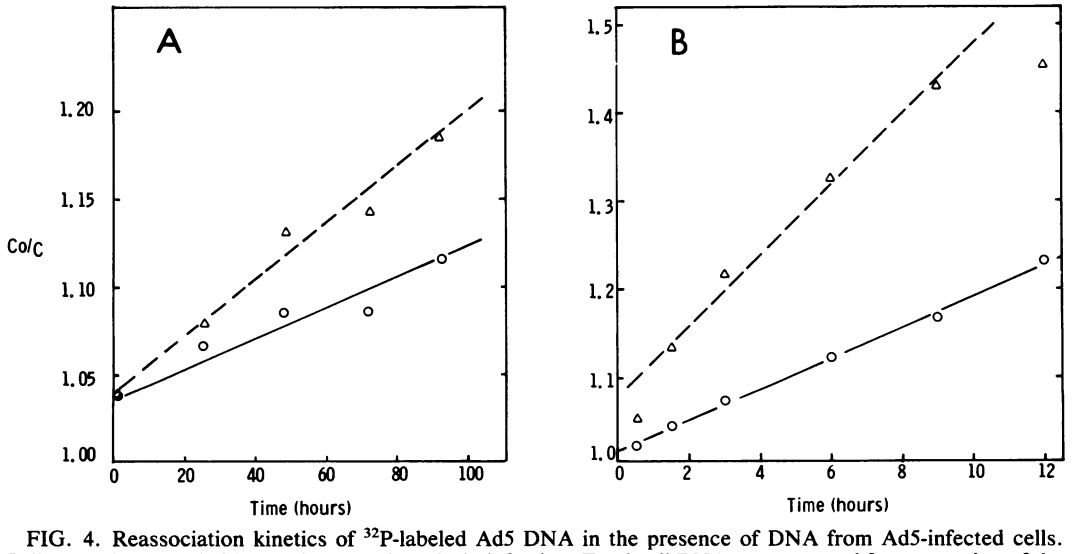

FIG. 4. Reassociation kinetics of ^{32}P-labeled Ad5 DNA in the presence of DNA from Ad5-infected cells. Cells were harvested either early (A) or late (B) in infection. Total cell DNA was prepared from a portion of the cells, and nuclear matrix was prepared from the remainder. The nuclear matrix was digested with DNase I to remove 99% of the cell DNA, and the residual matrix-associated DNA was purified. Equal amounts of total or matrix-associated DNA were used in reassociation experiments (27). The slope of the line is proportional to the amount of Ad5 DNA in the sample. Symbols: ○, total cell DNA; △, matrix-associated DNA.

DISCUSSION

Eucaryotic cell DNA is tightly associated with the nuclear matrix (4, 5, 9–11, 18, 19), and this association is involved in DNA replication (4, 6, 17, 20, 30) and possibly in transcription regulation (12, 16, 29). Human adenoviruses replicate in the cell nucleus and have a strictly regulated sequence of gene transcription, DNA replication, and assembly of mature virions. The studies reported here were designed to examine the extent and organization of the viral DNA associated with the nuclear matrix.

Previous studies of the proteins of the nuclear matrix of adenovirus-infected cells have demonstrated an association of virus-specific protein with the nuclear matrix of infected cells. Hodge et al. (13) prepared nuclear matrix from Ad2-infected HeLa cells at 22 h after infection. They found three virus-specific proteins (the 100,000-

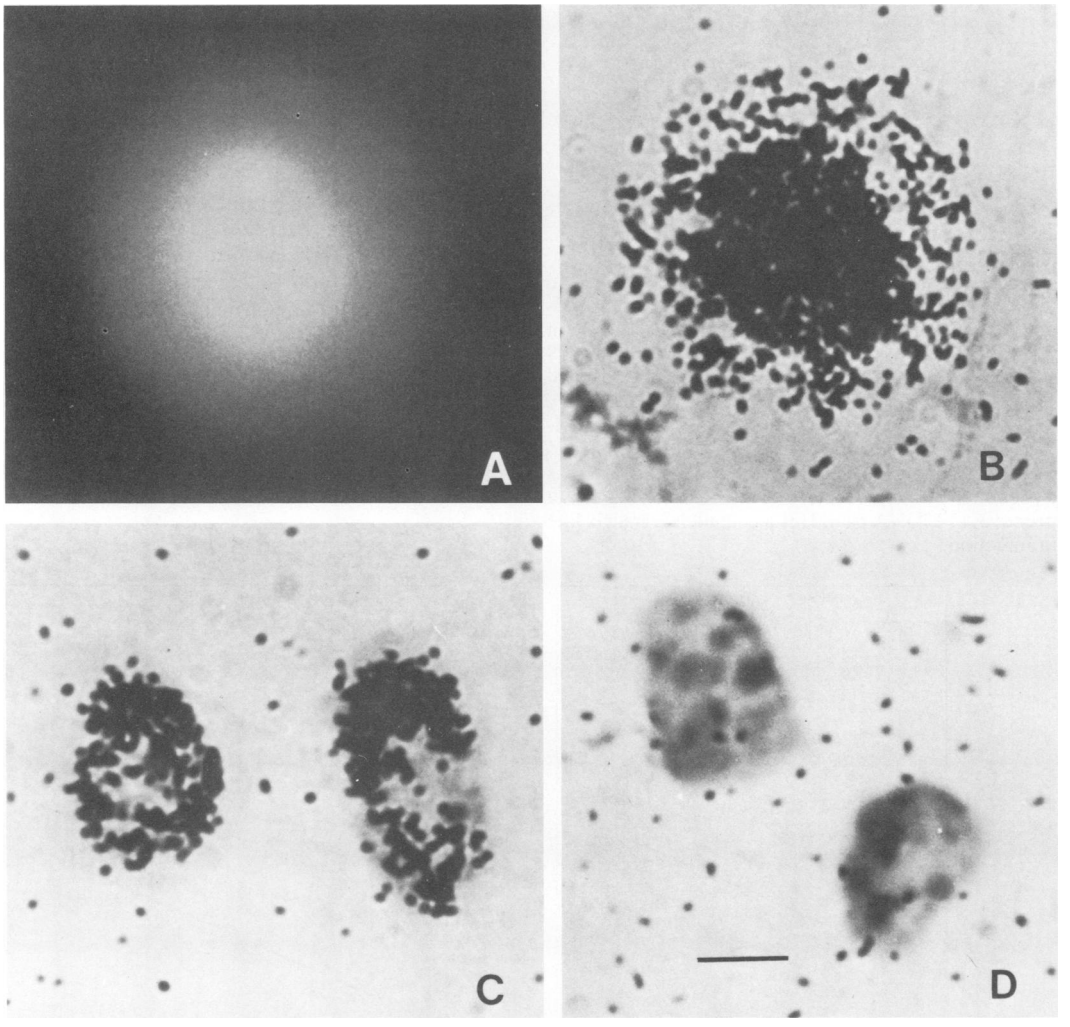

FIG. 5. In situ hybridization of Ad5 DNA to the nuclear matrix of infected HeLa cells. Nuclear matrix was prepared from cells growing on slides by permeabilizing with NP-40 and extracting with high-salt solutions. The DNA was then relaxed by treatment with ethidium bromide and UV light (30). (A) Fluorescence photomicrograph showing ethidium bromide-stained DNA as a relaxed halo around the residual nucleus. (B) Autoradiograph of an Ad5-infected HeLa cell which had been labeled for 3 h with [^{3}H]dThd before matrix preparation. Nuclear matrix was prepared as for (A), and the preparation was subjected to mock in situ hybridization (see text). (C) Autoradiograph of in situ hybridized Ad5-infected nuclear matrix. Cells were processed as in (A) at 24 h postinfection and hybridized in situ with ^{3}H-nick-translated Ad5 DNA. (D) In situ hybridization of ^{3}H-labeled Ad5 DNA to uninfected HeLa cell matrix. Bar, 10 μm.

dalton protein, pVII, and VII) associated with the nuclear matrix fraction and a significant reduction in the amount of the major cellular matrix polypeptides in the range of 45,000 to 60,000 daltons. Chin and Maizel (8) found the Ad2 early protein E3 (11,000 daltons) enriched in the nuclear matrix fraction of infected cells. Both of these studies used 0.5 M NaCl extractions; we used 2.0 M NaCl. The higher-concentration salt extraction which we used probably accounts for the absence of these virus-specific proteins in our nuclear matrix preparations from infected cells. Similarly, both of the previous studies show a considerable amount of histones in the matrix fractions; the higher salt concentrations which we used also eliminated these nonmatrix proteins from our preparations. This result indicates that although some adenovirus-specific proteins are associated with the nuclear matrix at intermediate salt concentrations, the nuclear matrix proteins of Ad5-infected and uninfected HeLa cells that are resistant to high salt concentrations are indistinguishable by Coomassie blue staining (Fig. 1).

Viral DNA was associated with the nuclear matrix throughout the lytic cycle. The association was similar to the attachment of cell DNA to the nuclear matrix in that it was resistant to extraction by nonionic detergent (1% Triton X-100 or 1% NP-40) and 2.0 M NaCl. Infecting viral DNA became associated with the matrix within 1 h after infection and accumulated on the matrix until a plateau level was reached during the late phase of infection (Table 1). The associated viral DNA represented as much as 87% of the intracellular viral DNA at the time of maximal DNA synthesis. This differs from polyoma virus, the only other DNA virus which has been examined for nuclear matrix association. Polyoma virus is transported to the nucleus shortly after infection, and uncoating intermediates have been found in association with a nuclear matrix-like component shortly after infection (31). Polyoma virus DNA is associated with the nuclear matrix throughout the lytic cycle (7); however, only a small proportion of it is matrix associated. As the total viral DNA increases in polyoma virus-infected nuclei, the amount of matrix-bound viral DNA increases in parallel, resulting in a constant proportion (about 10% of the total) associated with the matrix throughout the lytic cycle (7).

The plateau of matrix-associated Ad5 DNA late in infection, in contrast to the continual increase of free viral DNA (Table 1), could be due to a saturation of matrix binding sites or an equilibrium between synthesis of viral DNA and incapsidation of viral DNA into mature virions. Mature virions did not remain matrix associated after extraction with high-salt solution (Fig. 1). The association of viral DNA with the matrix did not displace cell DNA from the matrix. More than 90% of the cell DNA sedimented with the nuclear matrix from cells at all stages of the lytic cycle.

Cell DNA seems to have fixed attachment sites. For example, Cook and Brazell (10) found that the cellular globin gene, but not the β- or the γ-globin gene, is involved in matrix association. Similarly, rDNA has been found to be preferentially associated with the matrix (19). In contrast, we found no evidence that any region of the viral genome is preferentially involved in matrix attachment (Fig. 2 and 3). The lack of sequence specificity, together with the enrichment of viral DNA in the nuclease-digested matrix preparation (Fig. 2), suggests that the physical organization of the matrix-associated Ad5 DNA may be different from the loop structure which has been proposed for cell DNA (3, 9). The size of the Ad5 genome, 36.5 kilobase pairs, and the fact that adenovirus DNA does not become concatemerized into larger structures (2, 14, 25) requires that if it were in a loop conformation, the loops would be much smaller than the cell DNA loops, which have been estimated to be 80 to 220 kilobases (3, 9, 10). Thus, even a single attachment site on each Ad5 molecule would represent two- to sixfold more attachment points per unit length of viral DNA compared with cell DNA. This difference could account for the enrichment of viral DNA in the matrix fraction after DNase I digestion (Fig. 4).

The in situ hybridization results suggest that the viral DNA is confined to the original nuclear dimensions and after relaxation of supercoiling does not extend out into the halo created by the cell DNA (Fig. 5). The Ad5 DNA molecule is approximately 12 μm long, and the nuclear structures are 15 μm in diameter. Thus, if the Ad5 molecules are attached at two points, they could extend a considerable distance into the halo region. Since no accumulation of silver grains has been detected in the halo regions of in situ hybridization, even in overexposed autoradiograms, it appears that the viral DNA must be in a rather intimate association with the matrix rather than in extended loops.

The data presented here could also be interpreted as indicating a random, artifactual association of viral DNA with the nuclear matrix during isolation. Although we cannot completely rule out this possibility, we performed reconstruction experiments and found that when ^{32}P-labeled Ad5 DNA was added to HeLa cell nuclei, less than 1% was associated with the nuclear matrix after the 2 M NaCl extraction. Furthermore, when a nuclear matrix preparation was digested with restriction endonucleases, the high-molecular-weight DNA fragments which

were generated did not reassociate with the matrix but rather were released into the supernatant (Fig. 2). These observations, together with the results of others (20), strongly imply an in vivo relationship between the nuclear matrix and DNA.

Adenovirus DNA replicates by a strand displacement mechanism (15) which is quite different from the bidirectional mode of cell DNA replication. Also, adenovirus DNA synthesis may require the cell DNA polymerase γ, which is not utilized for cellular replicative DNA synthesis (28). This raises a question as to whether the replication of viral DNA occurs in a type of association with the matrix similar to that of cell DNA. The data presented here concern the total intracellular viral DNA and do not look specifically at replicating DNA. Thus, the significance of this association with respect to viral DNA replication cannot be assessed from this study. Experiments to examine a possible association between replicating Ad5 DNA and the nuclear matrix are in progress.

In the late phase of the lytic cycle, some viral DNA does not reenter the replicative pool but rather is incapsidated to form mature virions. The presence of the viral DNA and some viral proteins (8, 13) on the nuclear matrix suggests a possible role for the nuclear matrix as a site for the assembly of mature virions in the infected-cell nuclei. It has also been hypothesized that the early-to-late transition in adenovirus might involve sequestration of template viral molecules in the infected nucleus (26). The association of the viral DNA with the nuclear matrix could provide the compartmentalization suggested for these regulatory events.

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