Properties of Adenoviral DNA Bound to the Nuclear Matrix†

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ABSTRACT: The association of adenoviral DNA with the high salt (2 M NaCl) resistant nuclear fraction, termed the nuclear matrix, has been investigated in HeLa cells at different times after infection with adenovirus type 5. When nuclear matrices were prepared in the absence of exogenously added nucleases, Ad5 DNA was quantitively associated with the matrix throughout the infection period (0–24 h). Moreover, early in infection (0–10 h) Ad5 DNA was severalfold enriched in DNase I digested nuclear matrices (8–15% of total nuclear DNA) compared to the high salt soluble chromatin fraction (85–90% of total nuclear DNA). At later times after infection, progressively more Ad5 DNA appeared in the chromatin fraction until, at 24 h, the nuclear matrix was strikingly depleted in Ad5 DNA. A large proportion of the Ad5 DNA in nuclear matrices prepared early in infection, e.g., 4 h, was full length in size. At later times (12–24 h) most of the viral DNA was fragmented to a size equivalent to total matrix DNA (100–1000 base pairs). The apparent switch of the matrix-associated viral DNA from a relatively DNase I resistant to sensitive state was initiated approximately at the time when viral DNA replication began (12 h). Since no discrete portion of the Ad5 genome was significantly enriched at the sites of attachment to the nuclear matrix throughout the infection period, the switch in DNase I sensitivity is not mediated by a change in DNA sequence attachment to the matrix. Appropriate 1-min pulse-label experiments with [^{3}H]thymidine revealed an enrichment of newly replicated Ad5 DNA on the nuclear matrix and suggested that the matrix is an important site for adenoviral DNA replication.

The nuclear matrix is a proteinaceous residual nuclear structure that has been isolated and characterized in a wide variety of eukaryotes (Berezney & Coffey, 1977; Shaper et al., 1979; Agutter & Richardson, 1980; Berezney, 1979, 1984). Matrix proteins and those of its metaphase counterpart, the chromosomal protein scaffold, have been proposed to organize DNA in the form of multiple supercoiled loops (Cook et al., 1976; Paulson & Laemmli, 1977; Georgiev et al., 1978; Okada & Comings, 1979; McCready et al., 1980; Vogelstein et al., 1980; Berezney, 1981; Lebkowski & Laemmli, 1982). While the precise relationships of the isolated nuclear matrix to in situ nuclear structure remain to be clarified, numerous functionally related components have been shown to be enriched in isolated nuclear matrix preparations (Berezney, 1984). Among these potential functional associations are those involving DNA and RNA containing viruses. For example, influenza viral RNA is associated with the matrix (Jackson et al., 1982), and primary transcripts and spliced RNA intermediates of adenoviral specific genes are bound to the matrix during the infectious cycle (Mariman et al., 1982). Adenoviral specific proteins (Hodge et al., 1977; Chin & Maizel, 1977; Sarnow et al., 1982), SV-40 (Jones & Su, 1982) and polyoma (Buckler-White et al., 1980) large T antigens and viral DNA sequences (Nelkin et al., 1980; Cook et al., 1982; Younghusband & Maundrell, 1982) are also enriched in the matrix. In this study we examined the association of adenoviral DNA with the nuclear matrix following infection of HeLa cells. The results suggest that the matrix is an important site of viral interaction.

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Materials and Methods

Cell Culturing and Infection. HeLa S3 cells were grown as suspension cultures in Joklik's medium (Gibco) containing 10% horse serum. Cells synchronized at the G_1/S border were obtained by the double thymidine block procedure (Stein & Borun, 1972). Log phase or synchronized cells released from the G_1/S block were infected with adenovirus type 5 (Ad5) at the indicated multiplicity of infection (moi) by concentrating the cells 20-fold and incubating them for 30 min at room temperature before diluting the cells to (2–4) × 10^5 cells/mL.

Nuclear and Matrix Preparation. Nuclei and nuclear matrix were prepared at different times after infection. Typically, 200 mL of culture at (2–4) × 10^5 cells/mL was collected and the cell pellet washed once with RSB [10 mM Tris [tris(hydroxymethyl)aminomethane], pH 7.4, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM phenylmethanesulfonyl fluoride]. Cells were incubated in one-third strength RSB for 30 min on ice and disrupted with 10 strokes of a tight-fitting Dounce homogenizer before and after the addition of 0.4% Triton X-100. Nuclear pellets were obtained by centrifugation at 1000*g* for 10 min.

DNA-rich nuclear matrices containing approximately 90% of the total nuclear DNA were prepared by the following procedure. Nuclei were resuspended to 2 mg of DNA/mL in 0.25 M sucrose, 10 mM Tris, pH 7.4, 5 mM MgCl, and 1 mM phenylmethanesulfonyl fluoride and incubated for 45 min at 37 °C. This resulted in nicking of the total nuclear DNA without extensive acid solubilization or release of DNA from the nuclei (recovery of total DNA in digested nuclei was greater than 95%). Ice-cold RSB was added to dilute the nuclei to 0.1 mg of DNA/mL, and the suspension was made 2 M in NaCl. Matrices were obtained by centrifugation at 10 000 rpm in an HS-4 rotor (16300*g*; Du Pont Instruments, Inc.) for 30 min. The supernatant was slowly drawn off, and the fluffy pellet was resuspended in 2 M NaCl in RSB with a wide-bore pipet and centrifuged at 10 000 rpm for 30 min.

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The supernatants were combined as the high salt soluble chromatin fraction, and the matrix pellet was resuspended in RSB.

DNA-depleted nuclear matrices containing approximately 5–10% of the total nuclear DNA were prepared by modifications of the procedures reported by Nelkin et al. (1980). Nuclei were diluted to 0.1 mg of DNA/mL in RSB and 4 M NaCl in RSB was gradually added with mixing to a final NaCl concentration of 2 M. DNase I (Sigma) was added to a final concentration of 50 units/mL (unless indicated otherwise), and the suspension was incubated for 20 min at 37 °C. At the end of the digestion period the suspension was chilled on ice, and matrices were isolated by centrifugation at 3000*g* for 20 min and resuspended in RSB. The 2 M NaCl supernatant containing the high salt soluble chromatin was dialyzed against RSB and used in the subsequent analyses.

In Vivo Labeling of Cells. Control and infected cells at (2–4) × 10^5 cells/mL were concentrated 10-fold and incubated at 37 °C for 1 min with [^{3}H]thymidine (83 Ci/mmol, New England Nuclear) at a final concentration of 10 μCi/mL. At 1 min, a 4-fold excess of iced medium was quickly added, and the cells were centrifuged at 2000*g* for 5 min,, resuspended in water, and precipitated with 5% trichloroacetic acid (TCA). Precipitates were washed onto GF-C (Whatman) filters, and the incorporation was determined by liquid scintillation counting. DNA concentration and specific labeling of DNA were determined as previously reported (Berezney & Buchholtz, 1981a).

DNA Blotting and Hybridization. Dot blots were prepared from 1 mL of culture at various times postinfection. Pelleted cells were resuspended in a small volume of RSB, lysed by the addition of 2 mL of 0.5 N NaOH, and incubated at 37 °C for 1 h. Lysates were brought to room temperature and an equal volume of 2 M ammonium acetate (pH 4.5) was added. By use of a 2.5-cm internal diameter filter assembly, the DNA was absorbed to nitrocellulose strips (BA-85, Schleicher & Schuell). A standard curve of Ad5 DNA concentrations was prepared by adding known amounts of viral DNA to cellular DNA. For Southern blots, 5–10 μg of DNA was resolved on 0.7% agarose gels by using 10 mM Tris, pH 8.3, 3 mM EDTA, and 90 mM boric acid in the gel and running buffer. Size-fractionated DNA was extracted from agarose gels by electroelution and transferred to nitrocellulose. Hybridization with ^{32}P-labeled nick-translated DNA probes and detection were by standard procedures (Southern, 1975; Rugby et al., 1977). For autoradiography, washed blots were air-dried and exposed to XAR-5 film (Kodak) at −70 °C with intensifying screens. Quantitation of the hybridized blots was by scintillation counting in Liquiscint cocktail solution (National Diagnostics).

Results

Kinetics of Adenovirus Infection. In order to accurately determine the time course of viral infection, the accumulation of viral DNA after infections at 10 or 50 pfu/cell was examined as a function of time in synchronized and log phase cells (see Materials and Methods). To do this, total infected cell DNA was immobilized on nitrocellulose and assayed for Ad5 DNA content by using nick-translated Ad5 DNA as the hybridization probe. Under all experimental conditions, Ad5 DNA replication was evident by 12–17 h as an increased hybridization signal (Table I).

Adenoviral DNA Is Bound to the Nuclear Matrix by High Salt Resistant Interactions. To determine if Ad5 DNA is associated with the nuclear matrix throughout the course of infection, nuclear matrices were prepared by a procedure in which most of the nuclear DNA remained associated by high salt resistant interactions (see Materials and Methods). Similar to previous results from rat liver tissue (Berezney & Buchholtz, 1981a), these DNA-rich nuclear matrices were virtually identical with DNA-depleted nuclear matrices in overall nuclear structure, in SDS–acrylamide gel polypeptide profile, and in their depletion of histones [<5% recovery of total nuclear histone based on densitometry of Coomassie blue stained gels (data not shown)]. As shown in Figure 1, by dot blot hybridization, 93–99% of the viral DNA was also associated with these DNA-rich matrices, which contained approximately 90% of the total nuclear DNA at all times throughout the infection.

Differential Sensitivity of Matrix-Attached Adenoviral DNA to DNase I. By appropriate digestion with exogenously added nuclease, it is possible to isolate nuclear matrices containing variable amounts of nuclear DNA. A direct relationship between the amount and average fragment size of DNA remaining associated with the matrix fraction has been demonstrated (Razin et al., 1979; Basler et al., 1981; Berezney et al., 1982). This has led several laboratories to propose that the small fragments of DNA that remain associated with the nuclear matrix after extensive digestion represent the basal

Table I: Ad5 DNA Content during HeLa Cell Infection[a]

hours postinfection	Ad5 DNA (pg)			
	10 moi		50 moi	
	log phase	synchronized	log phase	synchronized
0	64	68	140	190
4	57	60	82	160
8	65	65	170	220
12	170	77	220	150
17	750	300	1100	280
24	4600	3900	6300	3900
32	3400	3000	9200	8400

[a] Ad5 DNA was quantitated by dot blot hybridization (see Materials and Methods). Picograms of Ad5 DNA was calculated by using a constant of 100 cpm/pg of Ad5 DNA quantitated from a standard curve of dot blots. 13 μg of total cellular DNA was blotted for each sample.

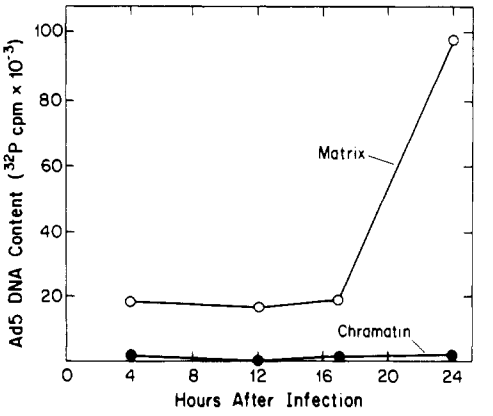

FIGURE 1: Association of Ad5 DNA with DNA-rich nuclear matrix and the high salt soluble chromatin. Nuclei from Ad5-infected cells were fractionated into a high salt soluble chromatin fraction containing 10% of the total nuclei DNA and a DNA-rich nuclear matrix containing ~90% of the total nuclear DNA (see Materials and Methods). The Ad5 DNA content of each nuclear fraction was then determined by dot blot hybridization with ^{32}P-labeled nick-translated Ad5 DNA as a probe. The amount of DNA placed on each dot blot was in direct proportion to the recovery of DNA in the nuclear subfractions for each infection time. The total DNA blotted for each infection time (nuclear matrix DNA plus high salt soluble chromatin DNA) was kept constant at 50 μg. This enabled a direct comparison of the Ad5 DNA distribution in the nuclear matrix and chromatin. The values reported represented the actual ^{32}P radioactivity counted for each dot blot after subtracting a background of 255 cpm.

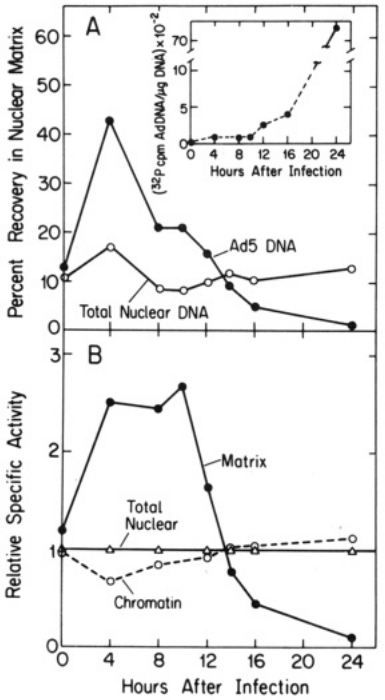

FIGURE 2: Association of Ad5 DNA with DNA-depleted nuclear matrix and the high salt soluble chromatin. Nuclei from Ad5-infected cells were fractionated following treatment with high concentrations of salt and DNase I into a high salt soluble chromatin fraction containing 83–92% of the total nuclear DNA (Ad5 plus cellular) and a DNA-depleted nuclear matrix containing 8–17% of the total nuclei DNA (see Materials and Methods). The Ad5 content of each nuclear fraction was determined by dot blot hybridization with 15 and 30 μg of chromatin and matrix DNA placed on duplicate filters. ^{32}P-Labeled nick-translated Ad5 DNA was used as the probe. Total nuclear DNA was measured as previously described (Berezney & Buchholtz, 1981a). (A) Percent recovery of Ad5 DNA and total nuclear DNA in DNA-depleted nuclei matrix. (●) Ad5 DNA; (○) total nuclei DNA. The insert shows the Ad5 DNA content (cpm/μg of DNA × 10^{-2}) for total nuclear DNA. (B) Specific activity of Ad5 DNA in the nuclear matrix and high salt soluble chromatin compared to total nuclear DNA (Ad5 plus cellular). Relative specific activity is calculated as the specific activity of the nuclear fractions (cpm/μg of DNA) divided by the specific activity of total nuclear DNA for the corresponding time point. By this calculation the relative specific activity of total nuclear DNA is always equal to 1.0. (●) Matrix DNA; (○) high salt soluble chromatin DNA; (△) total nuclear DNA.

attachment sites for nuclear DNA loops (Dijkwel et al., 1979; Razin et al., 1979; Nelkin et al., 1980; Basler et al., 1981; Berezney et al., 1982). It was therefore of interest to examine the association of adenoviral DNA with these DNA-depleted nuclear matrices.

At each time point after infection the amount of Ad5 DNA remaining attached to the nuclear matrix was determined together with the amount of total nuclear DNA. Figure 2A shows the percent recovery of both total and Ad5 nuclear DNA in the matrix fractions. Figure 2B shows the amount of Ad5 DNA in these fractions expressed as a relative specific activity (cpm/μg of DNA in each fraction divided by cpm/μg of DNA in total nuclei).

These data demonstrate an enrichment of Ad5 DNA in the nuclear matrix at 4 h after infection. Greater than 40% of total nuclear Ad5 DNA was recovered in this fraction compared to only about 15% of total nuclear DNA (Figure 2A). Figure 2B shows that this content of Ad5 DNA corresponds to a 2.5-fold enrichment. At 14 h, the recovery of Ad5 DNA was no greater than that of total nuclear DNA (approximately 10%), and by 24 h, there was a strikingly lower percentage of matrix-bound Ad5 DNA (approximately 1%) that corresponded to a 10-fold depletion. Since Figure 1 clearly demonstrates that Ad5 DNA remains at all times quantitatively bound to the nuclear matrix prepared in the absence of DNase I but in the presence of high salt, this observed depletion at later times after infection is likely due to an enhanced sensitivity of Ad5 DNA to DNase I following high-salt extraction.

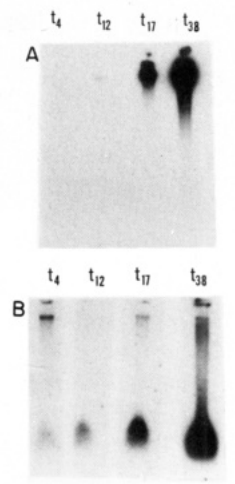

FIGURE 3: Southern blot analysis of nuclear and matrix-bound Ad5 DNA. DNA prepared from Ad5-infected nuclei (A) and DNA-depleted matrices (B) at 4, 12, 17, and 38 h after viral infection was resolved on 0.7% agarose gels, transferred to nitrocellulose, hybridized to ^{32}P-labeled nick-translated Ad5 DNA, and exposed for autoradiography.

Size of Matrix-Attached Ad5 DNA following DNase I Digestion. Next, we examined the size distribution of Ad5 DNA bound to DNA-depleted matrices. DNA was purified from matrices at different times after infection and subjected to electrophoresis on an agarose gel. The DNA was transferred to nitrocellulose by the method of Southern and hybridized to nick-translated ^{32}P-labeled Ad5 DNA. As a control, DNA isolated from nuclei before matrix preparation was also examined. Prior to blotting the DNA was visualized by ethidium bromide staining. As anticipated, total nuclear DNA migrated predominantly as a high molecular weight band at the top of the gel. In contrast, the DNA associated with matrices following DNase I treatment was not detected in this high molecular weight region but was observed as a diffuse band between 100 and 1000 base pairs (data not shown). The autoradiograms resulting from these blots are shown in Figure 3. At 4 h after infection the bulk of Ad5 DNA from the matrix is found in a high molecular weight band migrating at the position of intact Ad5 DNA (37 kilobase pairs; Figure 3B). Very little material is observed in regions of the blot corresponding to lower molecular weights. By 12 h, most of the Ad5 DNA is no longer in the high molecular weight band but is found in the lower molecular weight regions of the blot corresponding to a range of 100–1000 base pairs (Figure 3B). Matrix DNA analyzed from later times after infection showed a similar size distribution as the 12-h time point although a small percentage of the DNA is found in a high molecular weight band. In contrast, Ad5 DNA in nuclei isolated before treatment with high concentrations of salt and DNase I always was found predominantly in the 37-kilobase band (Figure 3A).

These results correlate well with the nuclease sensitivity data described above and suggest that the striking enrichment of Ad5 DNA on the matrix between 4 and 10 h is largely a result of a population of Ad5 DNA molecules that are refractile to DNase I digestion.

Sequence Analysis of Matrix-Bound Ad5 DNA. In order to determine the nature of the Ad5 DNA sequences remaining

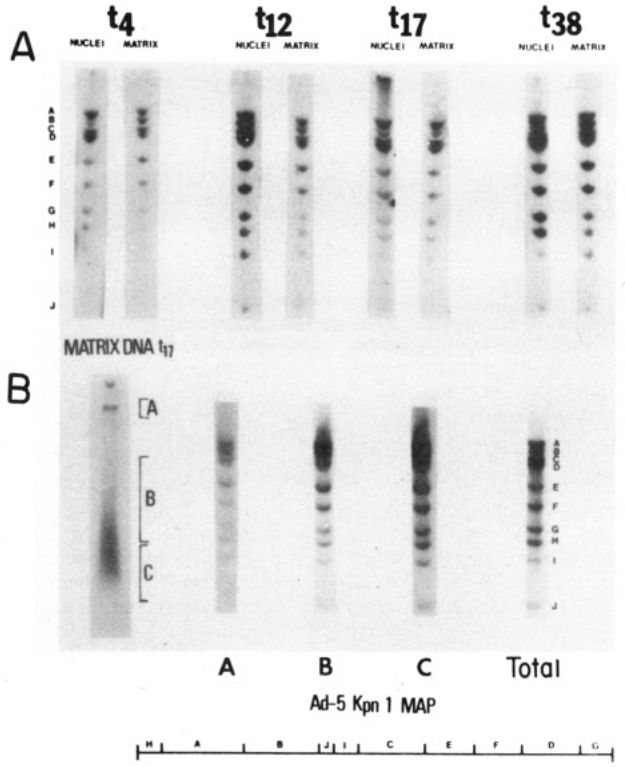

FIGURE 4: Southern blot analysis of Ad5 DNA sequences associated with the nuclear matrix. (A) Total nuclear and matrix DNA was prepared 4, 12, 17, and 38 h after infection with Ad5, nick-translated, hybridized to Southern blots of Kpn-1 restriction fragments of Ad5 DNA, and exposed for autoradiography. (B) Matrix DNA from 17-h postinfected cells was resolved on a preparative gel. DNA was eluted from the indicated regions of the gel (see the inset to the left), nick-translated, and hybridized to Southern blots of Kpn-1 restriction fragments of Ad5 DNA. The inset to the left is an autoradiograph of Ad5 DNA hybridized to a Southern blot of matrix DNA prepared 17 h after infection. A–C are autoradiographs of Southern blots of Kpn-1 digested Ad5 DNA hybridized with the corresponding matrix DNA from the regions indicated in the inset (A, B, and C, respectively).

attached to the DNA-depleted matrix, the following experiment was performed. Matrix DNA was prepared at various times after infection, nick-translated with [^{32}P]dCTP, and used as a hybridization probe against KpnI-digested Ad5 DNA that was run on agarose gels and blotted to nitrocellulose. As a control Ad5 DNA from whole nuclei was also prepared and used as a probe. Figure 4A demonstrates that at all times after infection there were no differences between the pattern of DNA fragments hybridizing to the total nuclear and matrix probes. In both cases all of the KpnI fragments from Ad5 DNA hybridized to the probes in approximate proportion to their molecular weights. Since the results of Figure 3 indicated that a small proportion of Ad5 DNA in the DNA-depleted matrix was refractile to DNase I treatment and migrated as intact Ad5 DNA on agarose gels, the possibility remained that the above hybridization pattern was influenced by this component. The results presented in Figure 4B demonstrate that this is not the case. Matrix DNA obtained 17 h after infection was separated into a fraction containing the intact Ad5 DNA component and two other fractions of lower molecular weight. These fractions were then nick-translated and used separately as hybridization probes. The results show that there is no difference in the hybridization pattern of the different fractions and the amount of DNA hybridized to each DNA fragment is again proportional to its molecular weight. It thus appears that all regions of the Ad5 DNA molecule are bound to the matrix in equal proportion.

Newly Replicated Ad5 DNA Is Associated with the Nuclear Matrix. We next examined the possible association of replicating Ad5 DNA with the nuclear matrix. Nascent viral DNA was pulse labeled by incubating G_1/S border synchronized cells at 24 h after infection with [^{3}H]thymidine for 1 min. As a control, uninfected HeLa cells were also pulse labeled for 1 min. Nuclei and DNA-depleted nuclear matrices were then isolated from the pulse-labeled cells. Previous studies have demonstrated that under these conditions label is incorporated predominantly into viral DNA (Hodge & Scharff, 1969). This is consistent with the large increase in Ad5 detected in cells 24 h after infection (Table I). To determine whether the nuclear matrix associated DNA was enriched with replicating viral DNA, we calculated the ratio of specific activity of matrix DNA (cpm/μg of DNA) to that of total nuclear DNA. DNA-depleted nuclear matrix from 24 h infected cells was enriched nearly 4-fold in nascent DNA (Table II). Significantly, the corresponding matrices from uninfected HeLa cells had a similar level of enrichment.

A number of previous studies with uninfected cells have demonstrated an inverse relationship between the enrichment of newly replicated DNA and the percent recovery of total nuclear DNA on the matrix (Dijwel et al., 1979; Pardoll et al., 1980; McCready et al., 1980). It was found that as the percent recovery of total nuclear DNA decreased, the specific activity of the matrix-bound DNA increased. Since the decreasing recovery of total DNA on the matrix results in a proportional decrease in the average fragment size of the matrix-attached DNA (Razin et al., 1979; Berezney et al., 1982), the corresponding enrichment in newly replicated DNA prompted investigators to propose that the sites of DNA replication are located at or close to the points of attachment of the replicating DNA to the matrix.

To investigate this relationship during Ad5 DNA replication, cells were pulse labeled for 1 min with [^{3}H]thymidine 24 h after infection and nuclear matrices were prepared with decreasing amounts of DNA ranging from approximately 80 to 3% of total nuclear DNA by DNase I digestion. The relative specific activity was then calculated as the cpm/μg of DNA on the matrix divided by the cpm/μg of total nuclear DNA. These calculations demonstrated an increase in the relative specific activity of newly replicated DNA as the percent recovery of total nuclear DNA on the matrix decreased (Figure 5). Particularly striking was the large increase in specific activity as the recovery of DNA on the matrix decreased from approximately 10 to 3%. These results are very similar to those previously described for eukaryotic replication (McCready et al., 1980; Dijwel et al., 1979; Pardoll et al., 1980) and strongly implicate the matrix as a major intranuclear site for adenoviral DNA replication.

Discussion

The results presented in this paper demonstrate that at all times after infection (0–24 h) both input and replicated Ad5 DNA are quantitatively associated with the HeLa cell nuclear matrix isolated before DNase I digestion (Figure 1). Furthermore, experiments involving 1-min pulse labeling with [^{3}H]thymidine are consistent with the nuclear matrix being the site of viral DNA replication (Figure 5 and Table II).

When we examined the nature of the DNA fragments remaining bound to the matrix following DNase I digestion, we observed that all regions of the viral genome were present in equal proportions (Figure 5). However, these results do not exclude the possibility that different populations of viral DNA, such as replicating or transcribing molecules, may have different sites of association with the matrix. Together these

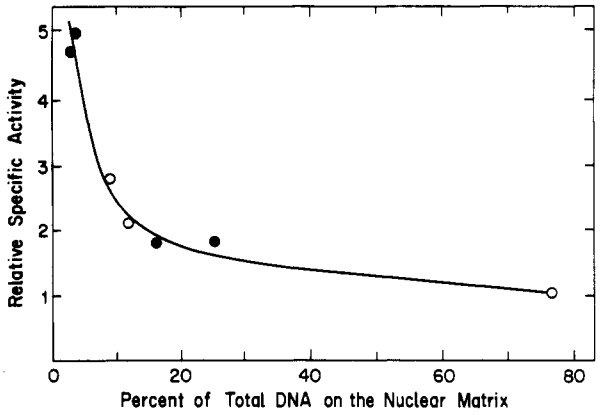

FIGURE 5: Relative specific activity of 1-min pulse-labeled Ad5 DNA on the nuclear matrix. One-minute in vivo pulse-labeled nuclear and matrix DNA from 24 h infected cells was extracted and the specific labeling of DNA determined (see Materials and Methods). Matrices with progressively decreasing recoveries of DNA (Ad5 plus cellular) were prepared by either digesting nuclei in high salt (2 M NaCl) with increasing concentrations of DNase I (10, 15, and 50 units/mL) at 37 °C for 20 min (○) or increasing the time of digestion (5, 40, and 60 min) with a fixed DNase I concentration of 10 units/mL at 37 °C (●). See Materials and Methods for more details of nuclear matrix isolation.

Table II: Association of Replicating DNA with DNA-Depleted Matrices

	% recovery		
nuclear matrix	total nuclear DNA	1 min labeled DNA	rel enrichment[a]
uninfected	8.6	29	3.4
infected	6.4	25	3.9

[a] Relative enrichment was calculated as the ratio of specific activity of matrix DNA (cpm/μg) to that of total nuclear DNA.

populations could generate an apparent random pattern of association.

While our results clearly demonstrate that Ad5 DNA is quantitatively bound to the nuclear matrix with no apparent sequence specificity, we cannot at present rule out the possibility that these associations are induced during the isolation protocol. In this regard only trace levels of high salt resistant binding were observed in appropriate reconstruction experiments utilizing matrix preparations and exogenously added adenoviral DNA (Younghusband & Maundrell, 1982) or cellular DNA (Pardoll et al., 1980; Berezney & Buchholtz, 1981a,b). The similar trace recovery of histones in both the DNA-rich and DNA-depleted nuclear matrices further suggests that the quantitative association of DNA with the matrix is not a result of precipitation by residual histones. It is apparent, however, that identification of the specific components involved in binding the Ad5 DNA to the nuclear matrix will be required before the issue is completely resolved.

During the course of these studies Younghusband & Maundrell (1982) also reported the association of Ad5 DNA with the nuclear matrix of infected cells. While our results are in basic agreement, we differ in our findings concerning the amount of Ad5 DNA bound to the matrix at various times after infection. We have found that, at all times examined, Ad5 DNA is quantitatively bound to the matrix whereas Younghusband and Maundrell reported a progressive increase in the association of viral DNA with the matrix, from 33% at 1 h to 87% at 24 h.

Matrix-attached Ad5 DNA at early times after viral infection was strikingly resistant to DNase I (Figure 2). The nuclease digestion was performed following high-salt extraction that removes histones and other soluble macromolecules. This suggests that the differences observed in DNase I sensitivity are due to high salt resistant factors. Remarkably, we observed that at 4 h postinfection a substantial proportion of the matrix-associated Ad5 DNA remained full length even though all detectable cellular DNA was degraded to small fragments (Figure 3). This high degree of resistance to nuclease attack at early times may be a reflection of the lack of uncoating of some of the input virions associated with the nuclear matrix or of the presence of uncoating intermediates that may also be high salt resistant. Alternatively, the association of viral DNA with some undefined, high salt resistant nuclear component could explain this phenomenon.

DNase I insensitivity of matrix associated viral DNA is maintained until about 12 h postinfection. This temporally coincides with the onset of viral DNA replication (Figure 2) and late gene expression. Presumably these new viral functions as well as virion assembly require a change in the structure of the viral chromatin. In support of this notion a difference in the digestion pattern of early and late viral chromatin with micrococcal nuclease has been reported (Daniell et al., 1981). Later times after infection, there is a progressive increase in DNase I sensitivity of the matrix-attached Ad5 DNA (Figure 2). At 24 h viral DNA is strikingly more sensitive than that of the host. This is not due to replicating viral DNA as discussed below but is apparently related to the postreplicative structure of Ad5 DNA and its association with the nuclear matrix. Further studies will be required to resolve this matter.

There are several features of adenovirus DNA replication that are distinct from that of the cell. These include a novel protein priming initiation mechanism, a strand displacement mode of elongation, and a unique viral DNA polymerase that carries out at least part of the replication process [for reviews, see Sussenbach & van der Vliet (1983) and Stillman (1983)]. A number of laboratories have demonstrated that newly replicated DNA in uninfected eukaryotic cells is initially bound to the nuclear matrix (Berezney & Coffey, 1975, 1976; Dijkwel et al., 1979; McCready, 1980; Pardoll et al., 1980; Berezney & Buchholtz, 1981a). This had led several groups to propose that DNA replication occurs in association with the matrix structure (Dijwel et al., 1979; McCready et al., 1980; Pardoll et al., 1980; Berezney & Buchholtz, 1981a). In this paper, we demonstrated that newly replicated Ad5 DNA is also associated with the nuclear matrix despite its unique properties of replication. There is a 4-fold enrichment of replicating viral DNA in the nuclear matrix following a 1-min pulse label with [^{3}H]thymidine. This is nearly identical with that of replicating DNA in uninfected cells (Table II). Furthermore, the specific activity of the matrix-bound labeled DNA progressively increases as the percent recovery of total nuclear DNA on the matrix (and hence average fragment size; Razin et al., 1979; Berezney et al., 1981) decreases. Thus, our results with adenoviral DNA replication are virtually identical with those previously described for cellular replication in uninfected cells.

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