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Psoralen-Cross-Linking Study of the Organization of Intracellular Adenovirus Nucleoprotein Complexes

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We used the psoralen-cross-linking technique to investigate the structures of adenovirus nucleoprotein complexes during infection. At late times after infection, three types of psoralen cross-linking patterns were observed. A high cross-linking pattern (type I), with about one cross-link in every 10 to 17 base pairs, was found for the newly synthesized and the bulk of the adenovirus late chromatin. Viral templates involved in replication, transcription, and recombination were all found to exhibit this cross-linking pattern. These results suggest that there is no nucleosome-like organization in the unpackaged late adenovirus nucleoprotein complexes. The second type of cross-linking pattern (type II) had a low cross-linking density of about one cross-link in every 700 to 1,000 base pairs. This cross-linking pattern was found to be associated with the viral DNA in the mature virus particles. The sequences at the termini of the virion DNAs, however, were found to have higher cross-linking densities, as shown by electron microscopy. The third type of cross-linking pattern (type III) was composed of a mixture of various proportions of type I and type II patterns in a single molecule. This mixed cross-linking pattern suggests that these molecules are virion assembly intermediates, with viral DNA being partially packaged in the virus particles. The organization of adenovirus nucleoprotein complexes at early times after infection was analyzed by the gel electrophoresis technique following digestion of the DNA with a restriction enzyme that was inhibited by cross-links. Our data suggest that the viral nucleoprotein complexes at early times after infection have accessibility to psoralen cross-linking between the virion DNA and the late viral nucleoprotein complexes. The observed cross-linking density of the early nucleoprotein complex DNA, however, was inconsistent with the nucleosomelike organization suggested by previous investigators.

Results of studies of simian virus 40 (13) and adenovirus (18) gene expression have shown that template maturation is a crucial step in the regulation of late RNA transcription. The mechanisms by which viral templates are altered at the early-late switch and how template alterations affect viral gene expression are still not understood. In several laboratories an attempt has been made to analyze the structure of adenovirus nucleoprotein complexes (NPCs) during lytic infection by the micrococcal nuclease digestion technique (3, 6, 7, 11, 14, 16). In these studies dynamic changes in viral NPCs during lytic infection were shown. At early times after infection, viral NPC is found to be changed from the virion core structure to a structure similar to that of cellular chromatin. Viral NPC is then switched to a core-like structure at late times after infection (3, 6, 7), although the newly synthesized and the parental viral templates are still found to be organized in a nucleosome-like structure (6, 7).

Contrary to the results of nuclease digestion studies, analyses of viral NPCs extracted from adenovirus type 2 (Ad2)-infected HeLa cell nuclei at late times after infection by electron microscopic and density gradient techniques have shown that the structures of the unpackaged late viral NPCs, including those that are actively engaged in viral DNA and RNA syntheses, are quite different from those of the virion core and the cellular chromatin (2, 9, 10; unpublished data). In these electron microscopic studies, the structures of adenovirus NPCs involved in transcription or replication were not distinguishable from the bulk of the unpackaged viral NPC, which was found to contain only a few nucleosome-like particles on an otherwise naked viral DNA. That the late adenovirus NPC is essentially naked is also indicated by the low DNA compaction ratio (1 to 1.5, as compared with 7 in cellular chromatin).

The reasons for the conflicting conclusions obtained by different techniques are not clear at present. In this study we used another approach, namely, the psoralen-cross-linking technique, to analyze the structure of adenovirus NPCs during lytic infection. Psoralen induces cross-links in double-stranded nucleic acids, and the cross-linking reaction is highly sensitive to the conformation of nucleic acids as well as protein-nucleic acid interactions (for a review, see reference 5). Furthermore, the psoralen molecule is capable of penetrating intact cells or virus particles, and therefore, it allows nucleic acids to be cross-linked in situ. These unique properties of the psoralen-cross-linking reaction have made psoralen a very useful reagent for probing the structures of NPCs in cells (5). In this study we used electron microscopic and gel electrophoresis techniques to investigate the cross-linking patterns of adenovirus NPCs during infection of HeLa cells. Our results indicated that the structure of the unpackaged adenovirus NPCs, including the putative replication, transcription, and recombination intermediates, is distinct from that of virion NPCs and cellular chromatin. Our data also suggest that the nucleoprotein organization of early and late viral NPCs is different.

MATERIALS AND METHODS

Cell and virus infection. HeLa cells were grown in a monolayer on Dulbecco modified Eagle medium supplemented with 5% fetal bovine serum and 50 μg of gentamicin per ml. The cells were infected with human Ad2 or adenovirus type 5 (Ad5) with a multiplicity of infection of 2,000 particles per cell.

Psoralen-cross-linking procedures. Adenovirus was cross-linked with 12 μg of aminomethyltrioxsalen (AMT; HRI

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Associates) per ml for 1 h; AMT was added at 20-min intervals during the cross-linking reaction. Cross-linking of DNA was induced by irradiation with a 365-nm UV lamp (model TL365; Spectroline) at a dose of 70 J/m^2 per s. For cross-linking of intracellular viral DNA, the monolayer cell culture was first incubated with 12 μg of AMT per ml for 1 min, and then the cells were irradiated with UV light as described above. After cross-linking, virus or cells were lysed with buffer containing 1% sodium dodecyl sulfate, 10 mM Tris hydrochloride, 100 mM NaCl, and 1 mM EDTA (pH 7.4) and digested with 100 μg of proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml for 3 h. Intracellular viral DNA was separated from high-molecular-weight cellular DNA by filtering the DNAs through a disposable, 0.45-μm-pore-size filter (Millex-HA; Millipore Corp., Bedford, Mass.). High-molecular-weight cellular DNA was trapped by the filter, while viral DNA was quantitatively recovered in the filtrate (the method will be described in a separate report). Viral DNA was purified by extraction with phenol-chloroform.

Electron microscopy. Conditions for denaturing cross-linked DNA and for the preparation of DNA samples for electron microscopy have been described previously (8).

Two-dimensional gel electrophoresis. For two-dimensional gel electrophoresis, DNA samples were run in the first dimension in a 1 to 1.4% agarose gel in 40 mM Tris hydrochloride–5 mM sodium acetate–1 mM EDTA (pH 7.4). The second dimension was run in a 1.2 to 1.6% agarose gel in 30 mM NaOH–1 mM EDTA. Alkaline buffer was changed every 8 to 12 h during electrophoresis. Alkaline gels were run at 200 mA in a cold room. After electrophoresis gels were neutralized with 50 mM Tris (pH 7.4) and stained with ethidium bromide. For fluorography, gels were first dried and then incubated with 0.7 M sodium salicylate (Fisher Scientific Co., Pittsburgh, Pa.) for 1 h at room temperature.

RESULTS

Three cross-linking patterns of intracellular Ad5 DNA at late times after infection. When Ad5 DNA was cross-linked with AMT at 24 h after infection and examined in the electron microscope, three types of cross-linking patterns were observed (Fig. 1). The first type of Ad5 DNA (type I DNA; 71% of 200 molecules counted) showed a high density of cross-links with few denaturation bubbles. The second type of DNA showed a low cross-linking density, with about one cross-link in every 700 base pairs (type II DNA; 21%). The third type of viral DNA was composed of various proportions of type I and type II DNAs linked together (type III DNA; 8%). At 12 h after infection, when few virus particles were produced, the majority (99%) of cross-linking patterns of the DNA were of type I. This result indicates that type I molecules represent unpackaged Ad5 NPCs. Results of studies done by the two-dimensional gel electrophoresis technique also support this interpretation (see below). The proportion of type II and type III molecules increased as the infection proceeded and as virus particles accumulated. At 46 h postinfection (p.i.), the proportion of type II and type III molecules increased to 31 and 13%, respectively. These DNA molecules therefore may be associated with the virion assembly pathway. Studies of the cross-linking pattern of DNA in the purified Ad5 virion showed that type II molecules are packaged viral DNA. Since packaging of adenovirus DNA is known to be initiated from the left end of the molecule, the mixed cross-linking pattern in type III molecules suggests that they are packaging intermediates, with various amounts of viral DNA being packaged. Results of the study of DNA cross-linking patterns of the 200 to 700s virion assembly intermediates support this interpretation (data not shown).

The high cross-linking pattern of type I molecules was quite distinct from the cross-linking pattern observed in cellular chromatin. The electron microscopic analysis therefore suggests that during late phases of the infection cycle, unpackaged adenovirus NPCs are not organized into nucleosomelike structures.

Adenovirus NPCs involved in DNA replication, transcription, and recombination are highly accessible to psoralen cross-links. The results described above indicate that the unpackaged adenovirus late NPC is highly accessible to psoralen cross-linking. To examine whether this type of nucleoprotein organization is also present in the Ad5 NPCs that are involved in biosynthetic processes, we analyzed the cross-linking patterns of Ad5 replication, transcription, and recombination intermediates with an electron microscope. The intermediates for replication (Fig. 2a), recombination (Fig. 2c), and the putative transcription complex (Fig. 2b) all exhibited the type I cross-linking pattern. The criterion for assigning the molecule in Fig. 2b as a transcription complex is based on the observation that the denaturation bubble was seen at the base of the nascent RNA, presumably due to the protection of DNA from cross-linking by RNA polymerase II. For replication intermediates denaturation at the replication fork would generate single-stranded tails instead of single-stranded bubbles. These results indicate that during the late phase of infection, the active viral templates are all organized in such a manner that they are highly accessible to the intercalation and cross-linking of psoralen molecules.

Two-dimensional gel electrophoresis analysis of AMT-induced cross-linking of intracellular Ad5 DNA. Gel electrophoresis analysis of *Xho*I restriction fragments of Ad5 DNA cross-linked with AMT at 24 h p.i. revealed two populations of viral DNA, as indicated by the two closely migrating bands for each of the restriction fragments (Fig. 3A, lane ×L). One population of DNA had the same electrophoretic mobility as that of the control uncross-linked DNA (Fig. 3A, lane C), whereas the other population had a retarded mobility relative to that of the control. The retarded mobility of psoralen-cross-linked DNA is due to a high cross-linking density, as shown previously by Sogo et al. (15) in nucleolar chromatin. Reconstruction experiments with purified Ad5 DNA fragments in the psoralen-cross-linking experiments confirmed this interpretation (data not shown). The higher cross-linking density of the slower-migrating bands in Fig. 3A was also shown by two-dimensional (neutral-alkaline) gel analysis. The slower-migrating bands in the neutral dimension became faster-moving bands in the second dimension (Fig. 3B). In the alkaline dimension (second dimension), DNA with a higher density of cross-linking had a higher mobility due to the smaller denaturation bubbles. These data therefore indicate that there are two types of intracellular Ad5 DNA, each of which has a different accessibility to psoralen cross-linking.

The nature of the two populations of intracellular viral DNA was investigated by pulse-labeling experiments. Only the DNA fragments with a high density of cross-linking become labeled during a 2-h pulse with [^{3}H]thymidine at 24 h p.i. (Fig. 3C). This result indicates that the highly cross-linked viral DNA is derived from newly synthesized Ad5 NPC. The observation that only the highly cross-linked DNA was found at 16 h p.i. supports this interpretation. The proportion of bands with a low cross-linking density in-

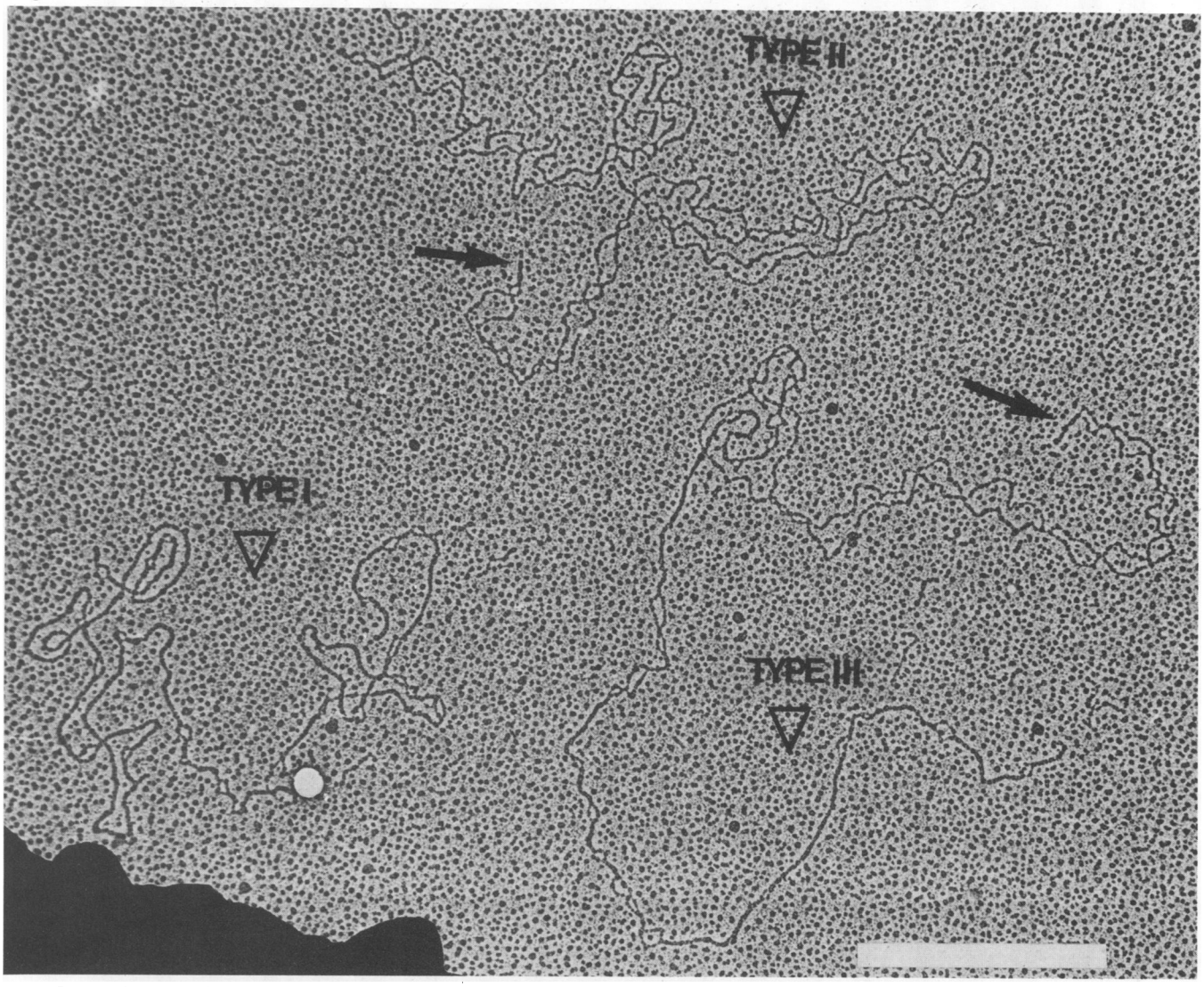

FIG. 1. Electron micrographs of intracellular Ad5 DNA cross-linked with AMT and denatured with glyoxal-formamide. Three types of cross-linking patterns are shown: high cross-linking density (type I), low cross-linking density (type II), and a mixture of high and low cross-linking densities (type III). The ends of type II molecules and the low cross-linking region of type III molecules are found to have a high cross-linking density (arrows). Bar, 1 μm.

creased as mature virions accumulated during infection, suggesting that they are derived from virions or virion assembly intermediates. Results of cross-linking studies of DNA in purified Ad5 virus particles showed that virion DNA indeed has a low accessibility to psoralen cross-linking, similar to that of the low-cross-linking bands seen in the intracellular viral DNA at late times of infection. These results are consistent with the electron microscopic data discussed above and indicate that DNA in the adenovirus NPC is highly accessible to psoralen cross-linking, while virion DNA is cross-linked at a much lower density.

The cross-linking density of the two DNA populations can be estimated by gel electrophoresis techniques. For the adenovirus NPC DNA with a high cross-linking density, the change in the apparent molecular weights of the cross-linked restriction fragments in a neutral gel was the consequence of the elongation of the DNA by the intercalation of AMT. Since each psoralen molecule has been shown to induce an approximate 28-degree unwinding of double-stranded DNA (19), the cross-linking density of a DNA fragment can be calculated from the percentage increase in the molecular weight of the cross-linked fragment by the following formula: $D = dWx\ [360/(28 \times 10.5)] = 1.22dW$, where D is the cross-linking density (number of cross-links per 100 base pairs) and dW is the percentage increase in molecular weight of the cross-linked DNA. Based on this formula, we estimated that adenovirus chromatin DNA has a cross-linking density of 6 to 10 cross-links per 100 base pairs, or about 10 to 17 base pairs per cross-link. This value is 1 order of magnitude higher than that for the nucleosomal DNA. This result indicates that the majority of unpackaged adenovirus NPC, as well as newly synthesized viral NPC, at late times after infection is not organized in a nucleosome-type structure.

For adenovirus virion DNA, which has a low cross-linking density, we used the two-dimensional gel technique to estimate the average cross-linking density. In the two-dimensional gels, uncross-linked and cross-linked DNA can

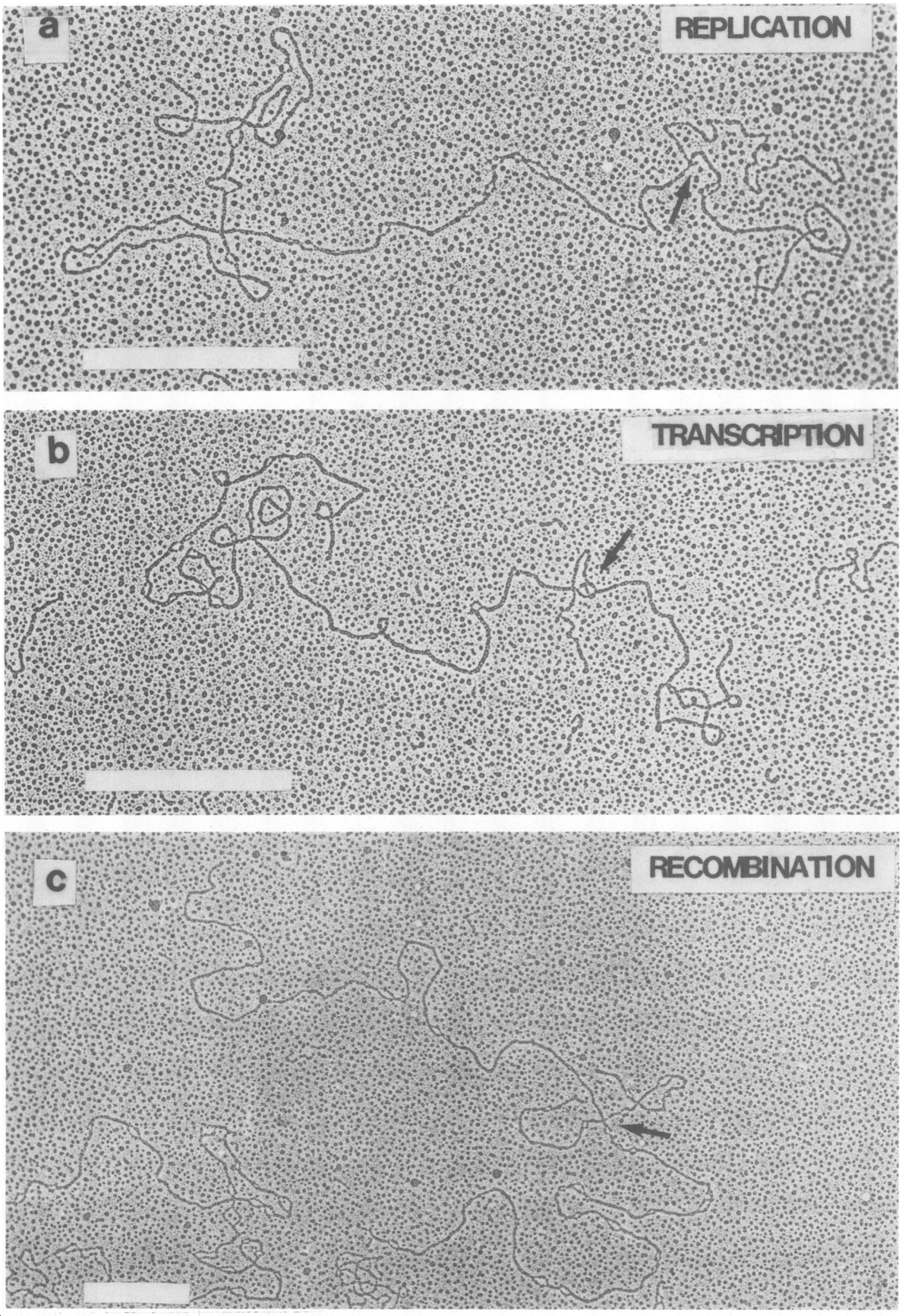

FIG. 2. Electron micrographs of replication, transcription, and recombination intermediates showing a high cross-linking density (type I). (a) Replication intermediate with a single-stranded branch (arrow). (b) Transcription intermediate with a nascent RNA transcript (arrow). (c) Recombination intermediate (chi structure; the Holliday junction is indicated by an arrow). Bar, 1 μm.

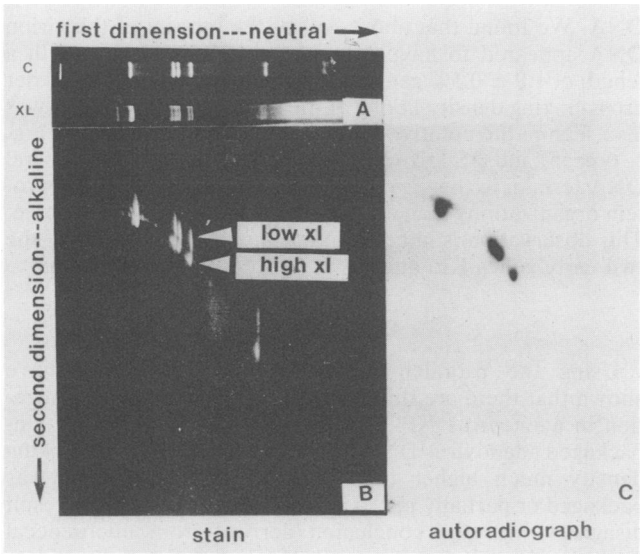

FIG. 3. Gel electrophoretic analysis of AMT-cross-linked, [^{3}H] thymidine pulse-labeled intracellular Ad5 DNA. Intracellular Ad5 DNA was pulse-labeled for 2 h at 24 h p.i. and cross-linked with AMT. The cross-linked viral DNA was cleaved with the restriction endonuclease *Xho*I and run in a 1% agarose gel in neutral buffer (A). Each gel band of the restriction fragments was found to be a doublet in the cross-linked (×L) sample. The first-dimensional gel was turned 90 degrees and run in a second dimension (alkaline buffer, 1.2% agarose gel) (B). The slower-migrating band of doublets in the first dimension migrated faster in the second dimension (high ×L). (C) Fluorograph of the gel shown in panel B. Only the faster-migrating bands in the second dimension became labeled.

be readily separated. By using the Poisson distribution, $P(0) = \exp(-n)$, where $P(0)$ is the probability of molecules without cross-links and n is the average number of cross-links, one can easily calculate n for a given DNA fragment from the proportion of uncross-linked DNA fragments determined from the two-dimensional gel data. By using this approach, the average cross-linking density of the virion DNA was found to be about 1 crosslink in every 700 to 1,000 base pairs.

Inhibition of restriction endonuclease cleavage of cross-linked Ad5 chromatin DNA. The major DNA sequence cross-linked by psoralen has been shown by Tessman et al. (17) to be TA. This result suggests that cleavage of highly cross-linked DNA by restriction endonucleases with the TA sequence in the recognition sites is strongly inhibited. We used this approach to demonstrate further the high cross-linking density in the intracellular Ad5 NPC DNA. We chose two restriction endonucleases, *Dra*I (recognition sequence, TTTAAA) and *Eco*RV (recognition sequence, GATATC) to cleave adenovirus DNA cross-linked by AMT. Partial digestion products resistant to further digestion by *Eco*RV can be seen in Fig. 4 in adenovirus DNA cross-linked at 24 h after infection. Results of pulse-labeling experiments indicated that the majority of the newly synthesized Ad5 DNA is enriched in the partial digestion products (Fig. 4A). Cross-linked virion DNA, on the other hand, was cut to completion by these two restriction enzymes (data not shown). Furthermore, cross-linked virion DNA fragments had the same gel mobilities as the uncross-linked control, whereas the newly synthesized DNA fragments had retarded mobilities. These results agree with the electron microscopic and two-dimensional gel data described above and reinforce our conclusion that adenovirus NPC DNA is highly accessible to psoralen cross-linking.

Psoralen cross-linking pattern of DNA in adenovirus early NPC is different from that of virus particles and the late viral NPC. We took advantage of the restriction endonuclease inhibition assay to examine the cross-linking density of adenovirus DNA at early times after infection. Adenovirus DNA was cross-linked at 3, 6, 12, and 24 h after infection; and the purified DNA was cleaved with the restriction endonuclease *Dra*I. After restriction fragments were separated in an agarose gel, the viral DNA was revealed by the Southern blotting technique. As expected, viral DNA at 24 h p.i. showed many partial digestion products and DNA fragments with retarded gel mobilities (Fig. 5, lane f). DNA cross-linked at 3, 6, and 12 h p.i. showed few *Dra*I partial digestion products, suggesting that at early times after infection Ad5 DNA is organized differently than unpackaged late viral NPC (Fig. 5, lanes b to d). However, DNA bands with retarded mobilities were observed along with bands with normal mobilities (compare lanes a and b in Fig. 5). The DNA bands with normal gel mobilities were probably derived from the virus particles that did not become uncoated during infection since at a low multiplicity of infection the intensities of these bands were greatly reduced (data not shown). These results suggest that at early times after infection the viral NPCs are organized differently than the virus particles and the late unpackaged viral NPCs.

The termini of adenovirus virion DNA are more accessible to psoralen cross-linking. During our analysis of the cross-linking pattern of adenovirus virion DNA, we observed an interesting distribution of cross-linking density in the virion

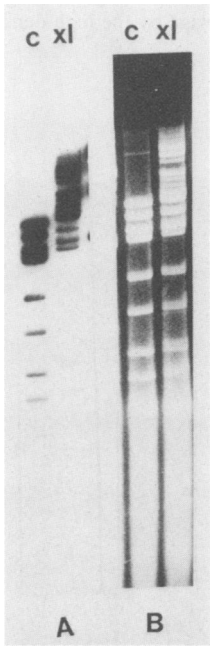

FIG. 4. Resistance of newly synthesized, AMT-cross-linked Ad5 DNA to cleavage by *Eco*RV. Ad5 DNA was pulse-labeled for 2 h beginning at 24 h p.i. and cross-linked with AMT. The viral DNA was then digested with *Eco*RV and analyzed in a 1% agarose gel (lanes ×l). Ad5 DNA without cross-links served as a control (lanes c). (A) Fluorograph of the gel showing the preferential accumulation of pulse-labeled DNA in the partially digested products. (B) Ethidium bromide staining of the same gel shown in panel A. Note that a doublet of restriction fragments can be seen in the lower-molecular-weight fragments.

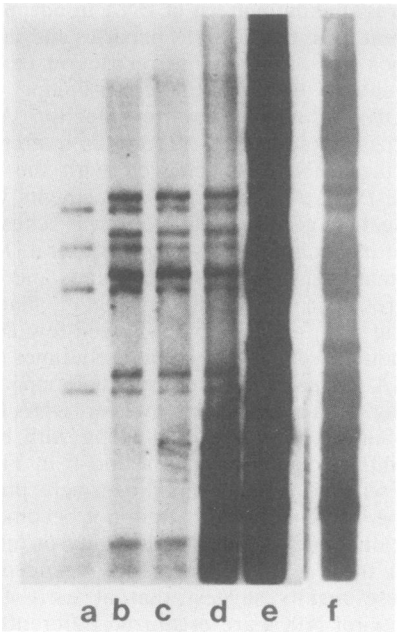

FIG. 5. Gel electrophoretic analysis of AMT-cross-linked Ad5 DNA during infection. Ad5 DNA was cross-linked with AMT at 3 h (lane b), 6 h (lane c), 12 h (lane d), and 24 h (lanes e and f) p.i. Viral DNA was extracted, digested with the restriction enzyme *Dra*I, and analyzed by blotting hybridization with total Ad5 DNA probe. Lane a, Control Ad5 virion DNA without cross-links; lane f, a lighter exposure of lane e showing the partial digestion products due to inhibition of *Dra*I cleavage by the high-density cross-linking in late Ad5 DNA.

DNA. We found that about half of the termini of the virion DNA appeared to have a high-cross-linking region with a length of 1.9 ± 0.5% genome lengths (see Fig. 6). The higher cross-linking density at one of the ends of the Ad5 DNA was also seen in the putative virion assembly intermediates (Fig. 1, types II and III, arrows). This result suggests that the viral DNA termini in the virus particles have different nucleoprotein organizations than do the other regions of viral genome. This observation is interesting because the promoters of the two early genes E1a and E4 are located in these regions.

DISCUSSION

Using the psoralen-cross-linking technique, we have shown that there are three types of nucleoprotein organization in adenovirus NPCs at late times after infection. Unpackaged adenovirus DNA was found to have a cross-linking density much higher than that of viral DNA that was packaged or partially packaged in virus particles. This result disagrees with the conclusion derived from micrococcal nuclease digestion studies, which is that the bulk of late adenovirus NPC is organized in a virus core-like structure (3, 6, 7), which we have shown has a much lower cross-linking density than that of unpackaged viral NPC. The conclusion that adenovirus late NPC is organized in a core-like structure is based on the similarities between micrococcal nuclease digestion patterns of viral DNA at late times after infection and those of virion core DNA. It is possible that the similarities of the two micrococcal nuclease digestion patterns are coincidental.

Results of psoralen-cross-linking studies reported here also indicated that the late adenovirus chromatin, including those engaged in replication, transcription, and recombination, are not organized in nucleosomes. This conclusion is derived from three types of evidence. First, electron microscopic examination of psoralen-cross-linked DNA showed that the intercross-link distances in the majority of adeno-

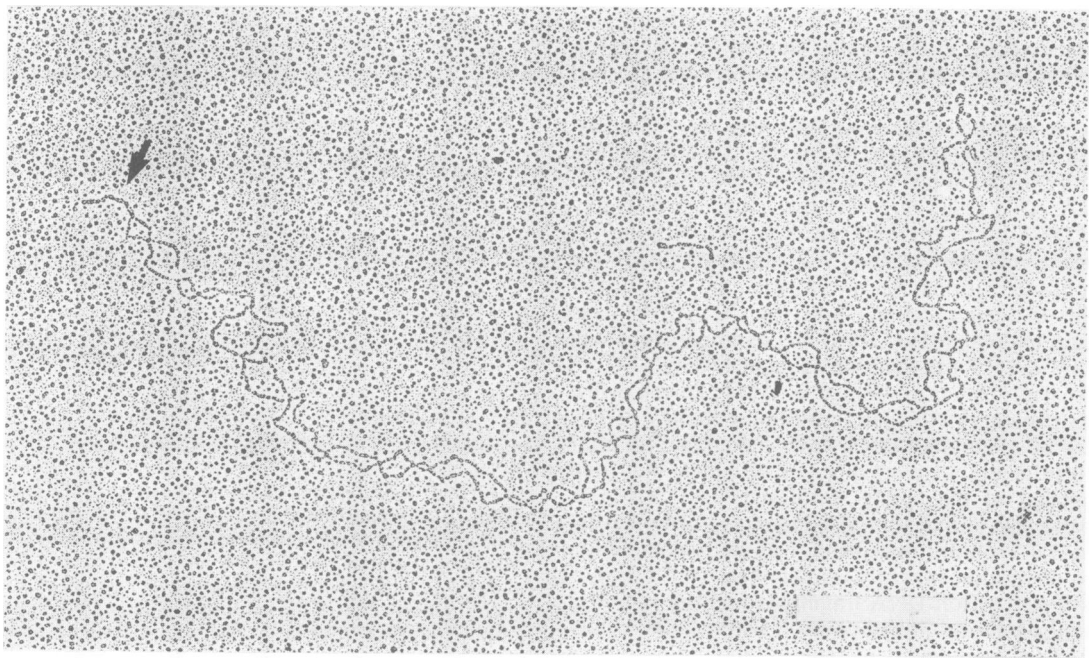

FIG. 6. Electron micrograph of Ad5 virion DNA cross-linked with AMT and denatured in glyoxal-formamide. The arrow points to the end of the Ad5 DNA with a high cross-linking density.

virus DNA, including active templates, are much shorter than those expected for the nucleosome structure. Second, newly synthesized adenovirus cross-linked with psoralen in vivo was found to have a retarded gel mobility in the neutral gel but an increased mobility in the denaturing gel. Nucleosomal DNA cross-linked with psoralen does not have an altered gel mobility in a neutral gel (5). On the other hand, psoralen-cross-linked DNA of transcriptionally active nucleolar chromatin which is not organized in nucleosomes exhibits gel retardation in a neutral gel (15). Estimation of the cross-linking density of highly cross-linked adenovirus DNA by using the known unwinding angle of psoralen intercalation showed that the cross-linking density is an order of magnitude higher than that of nucleosomal DNA. Third, psoralen-cross-linked, newly synthesized adenovirus was found to be resistant to digestion by restriction endonucleases that contain the cross-linked sequence TA in the recognition sites. These results indicate that adenovirus chromatin at late times after infection are not protected from psoralen cross-linking by proteins.

That adenovirus late NPC is essentially naked is also supported by other types of evidence. (i) Except for the presence of a few protein particles, the thickness and the contour length of adenovirus late NPC, including the replication and transcription intermediates examined under the electron microscope, are similar to those of naked adenovirus DNA (2, 9, 10); (ii) the buoyant density of the unpackaged late adenovirus NPC and replication templates is similar to that of naked adenovirus DNA in a metrizamide density gradient (9); (iii) sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis shows that there are few proteins associated with the bulk of the adenovirus late NPC or the replication intermediates (9); and (iv) adenovirus late NPC is fully accessible to restriction endonucleases digestion (unpublished data).

Our data and other evidence cited above therefore disagree with the results of Daniell et al. (6) and Dery et al. (7) that newly synthesized adenovirus NPC is arranged in nucleosomes. In the micrococcal nuclease digestion studies, the evidence for the nucleosomal organization of newly synthesized adenovirus NPC is the observation of nucleosome-like DNA ladders in DNA that was pulse-labeled at late times after infection. These investigators assumed that the pulse-labeled DNA fragments represent viral sequences because host DNA synthesis has been shown to be inhibited during the late phase of adenovirus infection. No evidence was presented, however, in their studies that host DNA synthesis is completely shut off and that the labeled DNA could hybridize to adenovirus DNA. In view of this uncertainty in the interpretation of micrococcal nuclease digestion data and the psoralen-cross-linking data presented here, we believe that the majority of adenovirus replication templates is not organized in nucleosomes. It remains possible, though, that there is a minor fraction of viral NPC organized in the nucleosome structure. This minor population would be manifested after the nuclease-sensitive, nucleosome-free adenovirus NPC has been removed by micrococcal nuclease digestion.

The observation of nucleosome-free adenovirus late NPC in the mammalian cell nucleus is intriguing. Nucleosome-free structures have been reported in the control region of papovavirus chromatin and in the transcriptionally active nucleolar chromatin (for a review, see reference 20). Because the adenovirus major late transcription unit covers most of the viral genome, we speculate that the nucleosome-free conformation of adenovirus late NPC may be a prerequisite for late gene expression. The requirement for template maturation in the expression of the major late transcription unit, as shown by Thomas and Matthews (18), is consistent with this interpretation.

Based on micrococcal nuclease digestion analysis, several investigators (6, 7, 16) have suggested that, during the early phase of adenovirus infection, the viral genome becomes associated with cellular histones. The nucleosome organization of the parental viral genome during virus infection suggests that the nucleoprotein structure plays a role in the regulation of early viral gene expression. This view was challenged recently by Chatterjee et al. (4). Using UV light to cross-link proteins associated with ^{32}P-labeled parental adenovirus DNA, Chatterjee et al. (4) showed that core protein VII, and not cellular histones, is associated with the parental adenovirus DNA at early times after infection. Our data obtained by the psoralen-cross-linking technique also do not support a nucleosome-type organization of early adenovirus NPC. Recently, we repeated the micrococcal nuclease digestion of early adenovirus NPC and found that the nucleosomal DNA ladder observed with the adenovirus DNA probe did not withstand stringent washing conditions, which do not affect the autoradiographic intensity of the genuine adenovirus DNA band. Therefore, the nucleosomal organization of early adenovirus NPC reported previously by several investigators (6, 7, 11, 14, 16) is probably the result of the cross-hybridization of adenovirus probes with cellular DNA. Nevertheless, our cross-linking data, as well as results of the micrococcal nuclease digestion studies, indicate that the nucleoprotein structure of late adenovirus NPC is different from that of the early viral NPC. The requirement for the switch of the nucleoprotein structure during early-late transition would presumably account for the template maturation needed for late gene expression (18).

Our observation that the sequences at the two ends of adenovirus DNA in virus particles are more accessible to psoralen cross-linking suggests that the nucleoprotein organization at the two ends is different from that of the rest of the viral genome in virus particles. The unique nucleoprotein organization at the termini of the viral NPC in the virus particles is interesting because the promoters of the two early viral genes E1a and E4 are located at the extreme termini of the adenovirus genome. Since E1a is the first viral gene expressed during lytic infection and since its gene products control the expression of other viral genes (for reviews, see references 1 and 12), the special nucleoprotein organization in the region near the E1a promoter in the virion NPC may play a role in the facilitation of transcription of E1a following virus uncoating in the cell nucleus.

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