Simian Virus 40 Maturation: Chromatin Modifications Increase the Accessibility of Viral DNA to Nuclease and RNA Polymerase

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The accessibility of extracellular and nuclear simian virus 40 (SV40-M and SV40-I, respectively) virion chromatin DNAs to micrococcal nuclease, DNase I, *Bgl*II, *Eco*RI, and RNA polymerase was examined. Our results support the following conclusions: (i) the intranucleosomal DNA of SV40-I chromatin, similar to the precursor 75S chromatin complex, is resistant to enzymatic activity; and (ii) SV40-M virion chromatin is modified in a manner which increases the accessibility of viral DNA to enzymes, and the distinction between nucleosomal DNA and linker DNA is absent. Micrococcal nuclease digestion of SV40-I virion chromatin gave a typical nucleosomal DNA ladder pattern with a repeat unit of 205 base pairs of DNA. SV40-I chromatin was sensitive to cleavage with endonuclease *Bgl*II, but not with *Eco*RI. When SV40-I virion chromatin was used as a template, the rate of incorporation of ribonucleoside triphosphates into RNA was 5% of that obtained with naked SV40 form I DNA. Micrococcal nuclease digestion of SV40-M virion chromatin resulted in submonomeric DNA fragments of approximately 55 base pairs, but no larger repeating unit of DNA was observed. SV40-M virion chromatin was sensitive to cleavage with either *Bgl*II or *Eco*RI and was approximately 20% more susceptible to digestion with DNase I than was SV40-I virion chromatin. The transcriptional efficiency of the extracellular virion chromatin was almost equivalent to that of naked SV40 form I DNA and was 16-fold higher than the rate observed with nuclear virion chromatin. The increased transcriptional activity was dependent upon the presence of nonhistone viral protein VP₁ or VP₂ or both.

The first stable simian virus 40 (SV40) chromatin complex which can be isolated after DNA replication is a compact, 30-nm particle which has a sedimentation value of 70 to 75S and is characterized by the presence of cellular histones H₁, H₃, H₂A, H₂B, and H₄, apparently in stoichiometric amounts (1, 3, 10, 11, 18, 22, 24, 27, 38). The products of micrococcal nuclease digestion of the 75S chromatin complex are similar to those of higher-order host cell chromatin, giving a nucleosomal DNA ladder pattern with a chromatin repeat unit of approximately 200 base pairs of DNA (3, 39, 41). The distribution of nucleosomes within the 75S complex is not completely random since a 400-base-pair length of DNA near the origin of replication and extending into the late coding region appears to be free of nucleosomes (30, 36, 37, 43). By use of procedures which allow the extraction of SV40 chromatin complexes without destroying their distinguishing structural composition, pulse-chase experiments demonstrate that radiola-

beled DNA and protein components of the 75S complex matures with time into a 200S previrion, a 240S nuclear (immature [SV40-I]) virion, and finally into an extracellular mature [SV40-M]) virion (1, 10, 11, 18, 22, 24, 38). As these transitions occur, three important changes in viral chromatin composition have been reported. First, the acetylation level of histones H₃ and H₄ in the previrion, nuclear virion, and extracellular virion are significantly higher than in either 75S virion chromatin or cellular chromatin, apparently due to a decreased rate of deacetylation (11, 24, 25). Second, during the final transition of nuclear to extracellular virions, histone H₁ is either specifically degraded or displaced from the virion since nuclear, but not extracellular, virion chromatin contains H₁ (11, 25, 28). Third, the specific association of nonhistone viral proteins with a chromatin complex isolated from extracellular virions after *in vitro* ethylene glycol-bis(β -aminoethyl ether)-*N,N'*-tetraacetic acid and dithiothreitol (EGTA-DTT) dissocia-

tion has been demonstrated (4). Since the final extracellular virion must ultimately reinitiate a cycle of virus replication, and the chromatin complex released in the nucleus must serve as a template for early viral transcription, it is important to understand what effect these chromatin modifications might have on the accessibility of viral DNA for genetic expression.

Nuclear and extracellular SV40 virions can be obtained with a high degree of purity and subsequently dissociated *in vitro* with EGTA-DTT under identical conditions. The virion dissociation conditions do not alter chromatin structure and therefore should minimize artifactual results due to the procedure of chromatin isolation. In addition, for the polyoma virion, the chromatin complex isolated *in vitro* accurately reflects chromatin complexes which can be isolated during the early stages of viral infection (46). By using enzymes to probe chromatin structure, our results demonstrate that the chromatin complexes of nuclear and extracellular SV40 virions are significantly different. The intranucleosomal DNA of SV40-I virion chromatin, similar to the precursor 75S complex, is resistant to enzymatic activity. In contrast, SV40-M virion chromatin is modified in such a manner that proteins do not inhibit access of the viral DNA to a variety of enzymes, and the distinction between nucleosomal DNA and linker DNA is absent.

MATERIALS AND METHODS

Viruses and cells. Wild-type SV40 (strain 776) was originally obtained from K. Takemoto, National Institutes of Health, Bethesda, Md. Virus was grown in BSC-1 cells.

Infection and purification of SV40 virions. Confluent monolayers of BSC-1 cells maintained in Eagles medium supplemented with 0.03% glutamine, 10% fetal calf serum, and a mixture of streptomycin, penicillin, ampicillin, and mycostatin were infected with SV40 at a multiplicity of 10 PFU per cell. After a 2-h adsorption at 37°C, Eagle minimal essential medium supplemented with 2% fetal calf serum was added. After 7 to 8 days, the infected cells and supernatant were collected, and the virus was purified as described previously (4).

Extraction and purification of nuclear (immature) SV40 virions. Confluent BSC-1 monolayers were infected as described above, and nuclear virions were extracted 48 to 72 h postinfection by the method of Garber et al. (22). Purified nuclear virions were adjusted to 10 mM Tris-hydrochloride (pH 7.4) by dialysis and stored at 4°C.

Preparation of virion chromatin complexes. Preparation of SV40 chromatin complexes was carried out by EGTA-DTT dissociation of SV40 virions as previously described (4, 5). Dissociated SV40 virions were layered onto isokinetic 10 to 30% sucrose gradients containing 5 mM EGTA, 3 mM DTT, and 150 mM NaCl in 10 mM Tris-hydrochloride (pH 7.8) and

0.05% Triton X-100. Centrifugation was in an SW41 rotor at 40,000 rpm (4°C) for 2 h. Isokinetic gradients were prepared by the method of McCarty et al. (28).

Micrococcal nuclease, DNase I, *Bgl*II, and *Eco*RI digestions of chromatin complexes. [³H]thymidine-labeled mature and immature SV40 virions were dissociated in EGTA-DTT buffer (pH 8.6). Micrococcal nuclease digestions were initiated by the addition of nuclease (0.25 U/μg of DNA) and 7 mM CaCl₂. Samples were removed at 2, 5, 10, and 20 min of incubation and stopped by the addition of sodium dodecyl sulfate (SDS) to 0.5%. DNA samples were extracted with phenol-chloroform-isoamyl alcohol (50:50:1) and then with chloroform, ethanol precipitated, and analyzed by agarose gel electrophoresis (see below).

*Bgl*II and *Eco*RI restriction digests were initiated by the addition of 7 mM MgCl₂ and the appropriate enzyme at a concentration of 8 to 10 enzyme units per μg of DNA. Samples were removed at 0, 2, 5, 10, and 20 min of incubation at 32°C, and the reactions were stopped by the addition of 5 mM EDTA-0.5% SDS. The SV40 DNA was analyzed by agarose gel electrophoresis to separate form 1, 2, and 3 (F_I, F_{II}, and F_{III}, respectively) DNAs. Individual DNA species were cut out of the agarose gel and solubilized by incubation in 0.1 N HCl for 1 h at 90°C. A 15-ml amount of Aqualos scintillation fluid was added, and the total counts per minute were determined.

For kinetic analyses of chromatin DNA digests, micrococcal nuclease was added with 7 mM CaCl₂, whereas DNase I digestions were performed in the presence of 7 mM MgCl₂. At indicated times, samples were removed and the reaction was stopped by the addition of SDS to 0.5%. DNA samples were spotted on 3MM filter pads (Whatman, Inc.), dried, and trichloroacetic acid precipitated, and radioactivity was determined in nonaqueous scintillation fluid.

Agarose gel electrophoresis of SV40 DNA. DNA samples were electrophoresed on a 1.5% agarose slab gel with 40 mM Tris-acetate (pH 7.2)-20 mM sodium acetate-1 mM sodium EDTA buffer. Electrophoresis of micrococcal nuclease digestion samples was at 150 V for 2 h. A *Hind*II-*Hind*III SV40 DNA digest was used for molecular weight standards. Electrophoresis of *Bgl*II- and *Eco*RI-restricted DNA samples was at 60 V for 16 h to fully separate F_I, F_{II}, and F_{III} SV40 DNAs. Gels were stained with ethidium bromide and photographed under a UV light source.

***In vitro* transcription of SV40 DNA and chromatin complexes.** Each reaction contained, in a total volume of 200 μl, 50 mM Tris (pH 7.8), 1 mM DTT, 6 mM MgCl₂, 150 mM KCl, 37.5 mM NaCl, 0.005% Triton X-100, 0.1 mM EDTA, 1.25 mM EGTA, 1 mM each ATP, GTP, and CTP, 0.1 mM UTP, and 50 μCi of [³H]UTP (35 Ci/mmol; New England Nuclear Corp.). *Escherichia coli* RNA polymerase (Holoenzyme; Worthington Diagnostics) was added at a molar enzyme/DNA ratio of 150. Enzyme and DNA were preincubated for 5 min at 37°C in the above buffer without ribonucleoside triphosphates. A 50-μl amount of buffer with ribonucleoside triphosphates was then added to initiate the assay. For kinetic assays, 10 to 20 μl of transcription assay mix was removed at the indicated times and added to 50 μl of polymerase stopping buffer containing 1% SDS, 0.15 M NaCl, and

5 mM EDTA in 10 mM Tris buffer (pH 7.4). A 50- μ l amount of each sample was then spotted on a 3MM filter disc (Whatman), dried, and trichloroacetic acid precipitated, and the total counts per minute were determined.

RESULTS

Sedimentation and buoyant density comparison of immature and mature SV40 virions. SV40-I virions were isolated 48 to 72 h postinfection by the PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)]-KCl-Brij 58 extraction procedure described by Garber et al. (22). [3 H]thymidine-labeled nuclear extracts were sedimented on sucrose gradients, and the 240S virions were collected. SV40-M virions were isolated from infected cell lysates 8 to 9 days postinfection, when the cytopathic effect was complete and greater than 95% of the cells were detached from the support surface. Mature SV40 virions were purified as described previously (4). Purified SV40-I and SV40-M virion preparations were dialyzed against 10 mM Tris (pH 7.4) and stored at 4°C.

The sedimentation patterns of [3 H]thymidine-labeled SV40-I and SV40-M virions were analyzed by velocity sedimentation on parallel isokinetic sucrose gradients. The two virion populations showed no significant differences in either sedimentation rate or purity since both sedimented as a single peak at 240S (Fig. 1A). The shift of immature SV40 virions by one fraction is not a reproducible result. Equilibrium

cesium chloride density gradient analysis of unfixed SV40-I and SV40-M virions (Fig. 1B) demonstrated that both virions species have a similar buoyant density of 1.33 g/cm³. The broad peak of radioactivity toward the bottom of the SV40-I gradient probably represented salt-labile populations of SV40-I virions (1) and constituted approximately 25 to 30% of the total radioactivity. These results support previous observations that SV40-I and SV40-M virions have similar biophysical properties (24).

Sedimentation analysis of chromatin extracted from immature SV40 virions. We recently demonstrated that *in vitro* EGTA-DTT dissociation of SV40-M virions released a homogeneous chromatin complex with a sedimentation value of 110S (4). The purity and sedimentation properties of chromatin complexes released from SV40-I virions under identical conditions were analyzed by sedimentation on isokinetic sucrose gradients (Fig. 2). 110S chromatin cores and 55S minichromosomes served as sedimentation markers on parallel gradients. The results of this experiment demonstrated that, although the chromatin derived from SV40-I virions is homogeneous, the complex has a significantly different sedimentation value of 75S. The difference in sedimentation coefficients of SV40-I and SV40-M chromatin cannot be attributed to the isolation procedure since both virion populations were dissociated under identical conditions. The 75S sedimentation coefficient of SV40-I chromatin is consistent with the

FIG. 1. Velocity sedimentation and buoyant density comparison of immature and mature SV40 virions. [3 H]thymidine ($^3\text{H-TdR}$)-labeled SV40-I virions were isolated 48 to 72 h postinfection as described by Garber et al. (22). [3 H]thymidine-labeled SV40-M virions were purified as described previously (4). (A) Virion samples were layered onto parallel isokinetic 10 to 30% sucrose gradients containing 150 mM NaCl in 10 mM Tris-hydrochloride (pH 7.4). Centrifugation was in an SW41 rotor at 40,000 rpm (4°C) for 50 min. (B) Virion samples were added to 5 ml of 1.33-g/cm³ CsCl and centrifuged in an SW50.1 rotor at 35,000 rpm (10°C) for 72 h. Gradients were fractionated, and radioactivity in each sample was determined. Density determinations were made on every fifth fraction. Symbols: ○, [3 H]thymidine SV40-I virions; ●, [3 H]-thymidine SV40-M virions.

FIG. 2. Velocity sedimentation of SV40-I and SV40-M virions dissociated *in vitro*. [^3H]thymidine (^3H -TdR)-labeled SV40-I and SV40-M virions were dissociated by treatment with 5 mM EGTA-3 mM DTT-0.15 M NaCl in 50 mM Tris (pH 8.7) for 30 min at 32°C. [^3H]thymidine-labeled SV40-M virions were also dissociated in 100 mM glycine (pH 9.85) for 5 min at 37°C as described by Christiansen *et al.* (9) for the preparation of marker 55S minichromosomes. After dissociation, 2.5% Triton X-100 was added to a final concentration of 0.05%, and reactions were transferred to an ice bucket. Samples were layered onto isokinetic 10 to 30% sucrose gradients and centrifuged in an SW41 rotor at 40,000 rpm for 2 h at 4°C. The sedimentation positions of 110S SV40-M cores and 55S SV40-M minichromosomes is indicated by arrows. Symbol: ■, [^3H]thymidine SV40-I chromatin.

original observations of Nedospasov *et al.*, which suggest that complete SV40 virions obtained from nuclear extracts of productively infected cells can be dissociated to give a chromatin complex which is similar in composition to intracellular SV40 chromatin (29).

Accessibility of SV40-I and SV40-M chromatin complexes to single-cut endonucleases. Endonuclease sensitivity studies have provided important information about the structural organization of SV40 chromatin complexes (14, 15, 30, 36, 37, 39, 41, 43). As pointed out above, one distinguishing characteristic of 75S cellular chromatin is a 400-base-pair region of DNA surrounding the origin of replication which is free of nucleosomes and accessible to restriction enzyme cleavage. The DNA in all other regions of the chromatin complex is resistant to cleavage due to chromatin structure. The susceptibility of SV40-I and SV40-M chromatin complexes to cleavage with single-cut restriction enzymes *Bgl*II, which cuts SV40 DNA at the

origin of replication, and *Eco*RI, which cuts SV40 DNA at 0.34 map unit on the *Bgl*II SV40 DNA physical map, was compared. [^3H]thymidine-labeled SV40-I and SV40-M virions were dissociated in EGTA-DTT buffer (pH 8.6), followed by addition of the appropriate restriction enzyme (8 to 10 U/ μg of DNA). Samples were removed at 0, 2, 5, 10, and 20 min of incubation, and the reactions were stopped by the addition of EDTA-SDS. The SV40 DNA was then analyzed by agarose gel electrophoresis to separate F_I , F_{II} , and F_{III} DNA species. Individual species were cut out of the agarose gel and solubilized with 0.1 N HCl, and the distribution of radiolabeled DNA was determined. Figure 3 shows the results of a typical restriction analysis of SV40-I and SV40-M chromatin complexes. After 20 min of incubation of SV40-I chromatin with restriction enzyme *Bgl*II, approximately 44% of the total DNA had been converted to linear F_{III} molecules (Fig. 3A). A similar analysis of SV40-M virion chromatin revealed that, at 20 min, 74% of the total DNA had been converted to F_{III} DNA (Fig. 3B). In the case of both SV40-I and SV40-M chromatin complexes, no increase in the level of F_{III} DNA was detected without the addition of enzyme, indicating that no endogenous nuclease was present (data not shown). A comparative analysis of the susceptibility of SV40-I and SV40-M chromatin to cleavage with *Eco*RI revealed quite different results. After 20 min of incubation with *Eco*RI, 20% of the SV40-I chromatin DNA had been converted to F_{III} DNA (Fig. 3C). At an equal enzyme/DNA ratio, the SV40-I virion chromatin was on the average 2.5 times less susceptible to cleavage with *Eco*RI than with *Bgl*II. In contrast, when SV40-M virion chromatin was incubated with *Eco*RI, we again found that 74% of the viral DNA was converted to F_{III} linear DNA after 20 min of incubation (Fig. 3D). Therefore, unlike SV40-I chromatin, in the SV40-M chromatin the origin of replication (*Bgl*II) as well as chromatin DNA that was distant from the origin was equally sensitive to cleavage with single-cut restriction enzymes. The possibility that a nucleosome-free region occurs at the *Eco*RI site in SV40-M chromatin cannot be excluded, although the micrococcal nuclease digestion pattern argues against this possibility (see below). Sedimentation coefficient and density determinations demonstrated that the 110S SV40-M virion chromatin was stable in the restriction enzyme buffer conditions, indicating that the increased sensitivity of the 110S chromatin was not due to loss of protein (data not shown).

Micrococcal nuclease digestion pattern of virion chromatin complexes. Micrococcal

FIG. 3. Restriction endonuclease accessibility of SV40-I and SV40-M chromatin complexes. [^3H]thymidine-labeled SV40-I and SV40-M virions were dissociated by treatment with 5 mM EGTA-3 mM DTT-0.15 M NaCl in 50 mM Tris (pH 8.7) for 30 min at 32°C, followed by the addition of Triton X-100 to 0.05%. *Bgl*II and *Eco*RI restriction digests were initiated by the addition of 7 mM MgCl_2 and the appropriate enzyme at a concentration of 8 to 10 enzyme units/ μg of DNA. At 0, 5, 10, and 20 min of incubation at 32°C, samples were removed and stopped by the addition of 5 mM EDTA-0.5% SDS. SV40 DNA was analyzed by agarose gel electrophoresis to separate F_I , F_{II} , and F_{III} DNAs as described previously (4). Individual DNA species were cut out of the agarose gel and solubilized by incubation in 1.0 ml of 0.1 N HCl for 1 h at 90°C. A 15-ml amount of Aquasol scintillation fluid (New England Nuclear Corp.) was added, the total counts per minute were determined, and the percentage of F_{III} DNA present was calculated. (A) *Bgl*II restriction, SV40-I chromatin; (B) *Bgl*II restriction, SV40-M chromatin; (C) *Eco*RI restriction, SV40-I chromatin; (D) *Eco*RI restriction, SV40-M chromatin.

nuclease preferentially digests sensitive chromatin DNA "spacer" or "linker" regions which separate the repeating nucleosomal DNA unit (32). Limited digestion releases nucleosome oligomers and monomers which reveal the fundamental chromatin repeat unit. The amount of DNA per histone octamer varies between about 140 and 240 base pairs, depending upon the presence of histone H_1 and the tissue from which the nucleosomes are isolated (17). In view of the differential susceptibility of SV40-I and SV40-M virion chromatin to single-cut restriction en-

zymes, micrococcal nuclease digestion patterns of the chromatin were analyzed. Virus samples were dissociated in EGTA-DTT buffer (pH 8.6), micrococcal nuclease was added, and digestion was initiated by the addition of 7 mM CaCl_2 . Samples were removed at 2, 5, 10, and 20 min of incubation, treated with SDS, extracted with phenol-chloroform-isoamyl alcohol, and analyzed by agarose gel electrophoresis. The digestion patterns of the two chromatin samples were quite different. After 10 min of micrococcal digestion of SV40-I chromatin, bands corresponding to monomer, dimer, and higher oligomeric nucleosomal DNA bands were detected (Fig. 4, insert, lane 4). At 20 min of incubation, the repeating chromatin DNA unit was even more evident, with up to pentameric lengths of DNA distinguishable (Fig. 4, insert, lane 5; spectrophotometric scan). DNA lengths of monomer

FIG. 4. Micrococcal nuclease digestion of SV40-I and SV40-M chromatin. SV40-I and SV40-M virions were dissociated as described in the legend to Fig. 3. Nuclease digestions were initiated by the addition of 7 mM CaCl_2 and micrococcal nuclease (0.25 U/ μg of DNA). Samples were removed at 2, 5, 10, and 20 min of incubation and stopped by the addition of SDS to 0.5%. DNA samples were then phenol-chloroform-isoamyl extracted twice, chloroform extracted twice, and ethanol precipitated. DNA samples were electrophoresed on a 1.5% agarose gel with 40 mM Tris-acetate (pH 7.2)-20 mM sodium acetate-1 mM sodium-EDTA buffer at 150 V for 2 h. A *Hind*II-*Hind*III digest of SV40 DNA was used as a molecular weight standard. Gels were stained with ethidium bromide and photographed under a UV light source. Lane: 1, *Hind*II-*Hind*III SV40 DNA standard; 2 through 5, SV40-I chromatin at 2, 5, 10, and 20 min of digestion, respectively; 6, *Hind*II-*Hind*III SV40 DNA standard; 7 through 10, SV40-M chromatin at 2, 5, 10, and 20 min of digestion, respectively; 11, *Hind*II-*Hind*III SV40 DNA standard. Densitometric scans of lane 5 (—) and lane 10 (----) were obtained on a Gilford 24002 scanning spectrophotometer.

through pentamer were determined by comparison with DNA markers and averaged to obtain the DNA repeat unit length, which was 205 base pairs. In contrast, the micrococcal digestion pattern of SV40-M virion chromatin did not reveal any distinguishable DNA repeat unit. After 10 min of incubation, the digested DNA appeared as a heterogeneous population of molecules from full-length linear DNA to DNA species of about 200 base pairs. As digestion continued, the heterogeneous species decreased in average molecular weight, and only when a large percentage of the DNA had been fully digested were any distinguishable bands observed (Fig. 4, insert, lane 10; spectrophotometric scan). The most apparent DNA species was a submonomeric fragment of 55 base pairs and a less reproducible DNA band corresponding to approximately 170 base pairs. The inability to resolve any distinguishable chromatin unit was not due to buffer conditions, nuclease/DNA ratio, or temperature since control digestions of nuclear chromatin or SV40-I virion chromatin under identical conditions repeatedly demonstrated chromatin DNA ladder patterns, whereas SV40-M virion chromatin did not. In fact, the 170- and 55-base-pair DNA fragments were detectable only under optimal conditions and were not always reproducible from experiment to experiment. These results suggest that the distinction between spacer and nucleosomal DNAs is lost in SV40-M virion chromatin.

Sensitivity of virion chromatin DNA to solubilization by micrococcal nuclease and DNase I. Another useful approach in probing chromatin structure is to compare the sensitivity of the DNA to acid solubilization by micrococcal nuclease and DNase I. Kinetic analysis of nuclease digestion of SV40-I and SV40-M virion chromatin is shown in Fig. 5. SV40-M virion chromatin was found to be approximately 20% more susceptible to digestion by either micrococcal nuclease or DNase I than was SV40-I virion chromatin (Fig. 5A and B). It is interesting to note that the initial kinetics of chromatin digestion with both enzymes were similar. Since both enzymes attacked linker DNA preferentially, at least in the case of the SV40-I chromatin complex, the initial digestion rate should have represented digestion of naked DNA. The slower digestion rate at later points was presumably due to an impediment of nuclease activity due to chromatin proteins. Since it was at these later points that SV40-M chromatin exhibited increased sensitivity, these results might suggest that nucleosomal structure is modified or "opened" in some manner which permits the micrococcal nuclease and DNase I to digest the DNA. This interpretation is consistent with the

FIG. 5. Solubilization of SV40-I and SV40-M chromatin DNAs by micrococcal nuclease and DNase I. SV40-I and SV40-M virions were dissociated as described in the legend to Fig. 3. Micrococcal nuclease was added with 7 mM CaCl_2 , whereas DNase I digestions were performed in the presence of 7 mM MgCl_2 . At the indicated times, samples were removed, and the reaction was stopped by the addition of SDS to 0.5%. DNA samples were then spotted on 3 MM filter pads, dried, and trichloroacetic acid (TCA) precipitated, and radioactivity was determined in nonaqueous scintillation fluid. (A) Micrococcal nuclease digestion; (B) DNase I digestion. Symbols: ○, SV40-I chromatin; ●, SV40-M chromatin.

results of the analysis of both single-cut restriction enzyme susceptibility and micrococcal digest patterns discussed above. It should be noted that the percent DNA digested at the final time points in no way reflected an endpoint since, with higher nuclease concentrations, 100% of the DNA in SV40-I and SV40-M chromatin can ultimately be solubilized (data not shown).

Transcription of virion chromatin complexes. In view of the differences in nuclease sensitivity exhibited by SV40-I and SV40-M virion chromatin, it was interesting to compare the transcriptional efficiency of the two chromatin complexes. Since nucleosomes inhibit both procaryotic and eucaryotic RNA polymerase chain initiation and elongation, these enzymes serve as useful probes of chromatin structure to determine what effect chromatin proteins of SV40-I and SV40-M virions have on the ability of RNA polymerase to recognize promoter sequences and elongate RNA chains through nucleosomal units. SV40-I and SV40-M virions were dissociated in EGTA-DTT buffer (pH 8.6), and 280 ng of DNA in the form of chromatin or naked SV40 F₁ DNA was added to the transcription assay mixture (Fig. 6). Consistent with previously published data, the SV40-M virion chromatin complex was found to be an efficient transcriptional template since the rate of incorporation of [^3H]UMP was 82% of that obtained

FIG. 6. Transcription kinetics of SV40 F₁ DNA, SV40-I chromatin, and SV40-M chromatin. SV40-I and SV40-M virions were dissociated as described in the legend to Fig. 3. SV40 F₁ DNA or chromatin (280 ng of DNA equivalent of each) was added to the polymerase preincubation mix and incubated for 5 min at 37°C; ribonucleoside triphosphate mix was then added to initiate transcription. Samples were taken at 5, 10, 15, and 20 min and processed as described in the text. Symbols: ○, SV40 F₁ DNA; △, SV40-M chromatin; ●, SV40-I chromatin.

with SV40 F₁ DNA. In contrast, the SV40-I viral chromatin was a poor transcriptional template, as it incorporated 20-fold less [³H]UMP than did SV40 F₁ DNA and 16-fold less [³H]UMP than did mature virion chromatin. The possibility that the high transcriptional efficiency of the SV40-M virion chromatin was due to synthesis of RNA on protein-free regions of the genome is unlikely since the average chain length of the RNA is approximately 2,300 nucleotides, 44% of the total SV40 genome length.

DISCUSSION

Two distinct SV40 virus particles can be isolated during a lytic infection cycle. Although the biophysical properties of the two virions are remarkably similar (Fig. 1), the chromatin complexes released by EGTA-DTT dissociation are significantly different. The accessibility of chromatin DNA to single-cut restriction enzymes *Bgl*I and *Eco*RI (Fig. 3), sedimentation coefficient (Fig. 2), micrococcal nuclease digestion pattern (Fig. 4), rate of DNA digestion with DNase I and micrococcal nuclease (Fig. 5), and

transcriptional efficiency (Fig. 6) all demonstrate that the chromatin complexes are distinct.

The nuclease analysis presented in this study strongly suggests that the basic nucleosome structure of SV40-M chromatin is modified. Intranucleosomal DNA, by virtue of histone arrangement, is quite resistant to enzymatic activity (7, 13, 45). When the number of nucleosomes and the length of nucleosomal DNA are taken into consideration and it is assumed that nucleosomes are fixed and randomly spaced on the SV40 DNA (with the exception of a 400-base-pair region near the origin of replication), only 20% of the total DNA molecules would be expected to have a given single-cut restriction enzyme site available for cleavage. The sensitivity of SV40-M chromatin DNA to *Eco*RI restriction (75%) suggests that this chromatin structure allows greater accessibility of the viral DNA to enzymatic activity.

The micrococcal nuclease digestion pattern of SV40-M chromatin provides further evidence that the nucleosomal structure is modified. It must be stressed that the inability to resolve any distinguishable chromatin structure does not appear to be an artifact generated by nuclease digestion conditions. Control digestions of cellular chromatin and nuclear viral chromatin under a wide range of buffer conditions and nuclease/DNA ratios repeatedly demonstrated the presence of defined chromatin DNA units, whereas no such pattern was discernible with SV40-M viral chromatin. Micrococcal nuclease digestions were also performed at 4°C. At this temperature, the ratio of the rate of DNA cleavage to the rate of DNA degradation is 20-fold greater than at 37°C (32). The rate of cleavage between repeating chromatin units is thereby increased, and the conversion of DNA to distinguishable chromatin subunits is enhanced. Even under these more stringent conditions of digestion, no chromatin DNA subunit was observed with SV40-M viral chromatin. Since nucleosomes inhibit both eucaryotic and procaryotic RNA polymerase initiation and elongation, the ability of SV40-M viral chromatin to serve as an efficient template for RNA transcription also suggests that the basic nucleosome structure is altered in SV40-M chromatin. Further investigation is required to determine whether actual nucleosomes (octamer of four histone pairs) are preserved in SV40-M chromatin with modified DNA-protein interaction or whether chromatin modifications lead to a redistribution of the protein along the DNA into a new type of structure which remains to be identified.

It is important to note at this point that the results presented in this paper are not necessarily inconsistent with those of Beard (2), Ponder

and Crawford (35), and Polisky and McCarthy (34) concerning the presence of typical nucleosome units in chromatin isolated from purified SV40 virions. Certainly, from our analysis of SV40-I virion chromatin, we agree that SV40 populations exist in which the nucleosomal DNA is resistant to cleavage with restriction enzymes and micrococcal nuclease. At the time that these previous studies were published, no distinction between different SV40 populations existed. The method of virus purification used in the first two studies was apparently a modification of polyoma virus purification described by Crawford (12). The low pH and temperature of the purification procedure allow most polyoma virions to adsorb to cells, presumably due to the hemagglutinin receptor of polyoma virions. Since SV40 virions do not have this receptor, extracellular SV40 virions might be lost when the supernatant fluid is discarded. The method of SV40 purification used by Polisky and McCarthy also involved the isolation of cell-associated virions by the method of Tan and Sokol (42). Our interpretation, therefore, is that these studies may have dealt with virions similar to the SV40-I nuclear virions described in this paper. Extracellular SV40-M virions may not have been present, or may have been present in low amounts, due to the method of virion purification.

It is not evident from present information which chromatin modification is responsible for the increased nuclease sensitivity of SV40-M chromatin. Previous studies suggest that viral protein VP₁ or VP₂ or both are important in maintaining the high transcriptional potential of SV40-M chromatin. The stable Sarkosyl-resistant association of VP₁ to SV40 DNA that we recently demonstrated in SV40 chromatin could be necessary for transcriptional efficiency (3a). However, it is not known whether viral proteins also dictate nuclease sensitivity or whether histone acetylation is the controlling factor. Recent studies in our laboratory suggest that the histone stoichiometry in SV40-M chromatin is modified, and the relationship of this observation to nuclease sensitivity must be considered in detail.

Virion chromatin modifications influence the accessibility of nucleosomal DNA to enzymes and so may affect the genetic expression of SV40 DNA. Transcriptionally active eucaryotic genes such as globin (44), ovalbumin (23), rat liver rRNA (40), integrated viral genomes (6, 8, 19, 20, 21, 33), and the *Drosophila* GP 70 heat shock gene (47, 48) demonstrate that active chromatin shows enhanced DNase I or micrococcal nuclease sensitivity or both. Though the structural basis of nuclease hypersensitivity remains to be established, circumstantial evidence that his-

tone acetylation (31) and nonhistone proteins (16, 26) may be important factors has been presented. The nuclease sensitivity, histone acetylation levels, association of nonhistone proteins, and high transcriptional efficiency of SV40-M chromatin suggest similarities to transcriptionally active chromatin. Since these chromatin modifications occur during the maturation of precursor nuclear SV40 to the final extracellular SV40 virion particle, one possible interpretation might be that these processes ultimately result in the encapsidation of a transcriptionally activated chromatin complex in preparation for the efficient expression of the viral genome in the subsequent infection cycle. In this context, it is of interest that the specific infectivity of extracellular SV40 virions has been reported to be 5- to 20-fold higher than that of the nuclear SV40 virion (24). Although the results presented in this manuscript certainly do not prove the above interpretation, the properties of SV40-M viral chromatin are similar to present concepts of transcriptionally active chromatin and merit further consideration.

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