Simian Virus 40 Encapsidation: Characterization of Early Intermediates

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Simian virus 40 chromosomes were separated into various species by a two-step purification consisting of low-ionic-strength glycerol gradient sedimentation followed by low-ionic-strength agarose gel electrophoresis. For each species of simian virus 40 chromosome purified, the comigrating DNA and proteins were identified by agarose or polyacrylamide gel electrophoresis, respectively. Two species of chromosomes were identified which contained form I and form II DNA and large amounts of viral protein; they migrated more slowly than most of the free simian virus 40 chromosomes, which contained very little viral protein. The nuclease susceptibility of these chromosomes suggests to us that they are intermediates in encapsidation, and we describe an encapsidation model.

The mechanism by which the simian virus 40 (SV40) chromosome is encapsidated is of interest because (i) the initiation of encapsidation may act as a rate-limiting step regulating the pool size of replicating and transcribing SV40 chromosomes, and (ii) the conformational changes resulting from the binding of viral proteins to SV40 chromosomes may mimic similar conformational changes resulting from protein-DNA interactions in cellular chromatin.

The product of encapsidation, the virion, is composed of SV40 DNA and cellular histones surrounded by a capsid consisting of the viral proteins VP1, VP2, and VP3 (1, 9, 10, 14, 20, 22, 27) in the proportion of 420/36/96, respectively, per capsid (18) arranged into a structure containing 72 subunits, of which 60 are hexons and 12 are pentons (1). The intracellular SV40 chromosome that is incorporated into the virion consists of SV40 DNA and cellular histones in a structure similar to eukaryotic chromatin (4, 9, 11, 15, 19, 21-23, 25, 27, 31, 33-36, 39, 41, 49, 50, 52-54).

The simplest encapsidation mechanism requires self-assembly of viral proteins to form capsids and sites of interaction between the viral proteins and SV40 chromosomes. Although self-assembly of the viral proteins of SV40 has not been observed, VP1 has been shown to exist as a monomer with a molecular weight of 45,000 and as a polymer which sediments at 8S (44). This size is comparable to that of the hexon of polyoma (a similar virus) obtained either from virus or from self-assembly of purified polyoma VP1 (6). If self-assembly is responsible for the formation of the capsid structure, two models of encapsidation are possible depending on whether self-assembly precedes or follows the interac-

tions between the viral proteins and the SV40 chromosomes. The former model predicts the existence of preformed empty capsids as intermediates in encapsidation, which, after insertion of the SV40 chromosome, are sealed to yield virions. With the use of cesium chloride density gradient analysis, empty capsids have been identified that, by pulse-chase experiments, possess the kinetics of formation consistent with their role as intermediates in encapsidation; the presence of these empty capsids is the basis for the encapsidation model (44, 48, 50).

Recently, evidence has been obtained which casts doubt on this interpretation. Empty capsids have been shown to be derived from a salt-labile form of previrion which was identified kinetically as an intermediate in encapsidation (3, 11, 13, 17, 18, 26). The presence of this intermediate and the absence of empty capsids suggested an encapsidation model in which the capsid formation follows binding of viral proteins to the SV40 chromosome (3, 11, 17, 18, 26, 28). By a careful analysis of the proteins associated with the 75S nonreplicating chromosome, 200S previrion, 240S previrion, and 250S virion, a model was proposed in which VP1 and VP3 first interact with the SV40 chromosome to yield a 200S previrion which, by the addition of VP1, VP2, and VP3, becomes a 240S previrion. After a conformational change, the 240S previrion becomes a 250S virion (18). This conformational change may be due to sulfhydryl cross-linking (42, 55), cation-mediated cross-linking (7), or an as yet unknown mechanism.

The encapsidation models described do not, however, address themselves to the critical first steps of encapsidation leading from the commit-

ted SV40 chromosome to the previrion. We have previously reported that a portion of the 75S chromosomes contain bound VP1 and that SV40 chromosomes of increasing S value contain proportionately greater amounts of VP1. These chromosomes were considered to be early encapsidation intermediates by the above-mentioned model (40). To analyze the structures of these early encapsidation intermediates, they were purified by low-ionic-strength agarose gel electrophoresis. After characterization of their associated DNA and proteins, the structure of the chromatin was analyzed by nuclease digestions. The results suggest that the structures of the intermediates consist of SV40 chromosomes surrounded by different numbers of capsomeres, each of which contains five hexons of VP1 around an empty penton binding site.

MATERIALS AND METHODS

Viruses and cells. BSC-1 cells (a gift from M. Gutai) were used for chromosome preparations, virus production, and plaque assays. Cells were grown in a 5% CO₂ atmosphere in Eagle modified minimal essential medium (GIBCO Laboratories) supplemented with 5% fetal calf serum (GIBCO Laboratories) and 100 µg of gentamicin (Schering Corp.) per ml. Strain VA45-54 SV40 (a gift from P. Tegtmeier) was propagated at low multiplicity of infection. The propagated virus used for SV40 chromosome preparations had a titer of 5×10^8 PFU/ml.

Infection of cells. Logarithmically growing BSC-1 cells were infected with SV40 virus (50 PFU per cell) as previously described (40) or were mock infected with medium lacking virus.

Radiolabeling of SV40 chromosomes. At 42 to 44 h postinfection, cells were labeled with either 0.25 to 1.0 mCi of [³H]thymidine (specific activity, 40 Ci/mmol; New England Nuclear Corp.) per plate for 3 h or a mixture of 0.5 mCi of [³H]thymidine and 1.5 mCi of [³⁵S]methionine (specific activity, 600 to 800 Ci/mmol; New England Nuclear Corp.) per plate for 3 h in 2 ml of methionine-free minimal essential media.

Isolation and purification of SV40 chromosomes. SV40 chromosomes were isolated and partially purified by glycerol gradient sedimentation as previously described (40). Cells washed with phosphate-buffered saline were suspended in buffer A (5% glycerol, 2 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; pH 7.5], 0.5 mM MgCl₂, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride) and scraped from the plate. After the nuclei were isolated by a Triton X-100 wash, the SV40 chromosomes were leached from the nuclei in buffer B (10 mM HEPES [pH 7.5], 1 mM EDTA, 0.5 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride) and were partially purified by sedimentation on a 10 to 30% linear glycerol gradient in buffer C (10 mM HEPES [pH 7.5], 5 mM KCl, 1 mM EDTA, 0.2 mM MgCl₂, 0.5 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride) in an SW41 rotor at 40,000 rpm for 135 min at 4°C.

Nuclease digestion. Solutions of DNase (1 mg/ml; Worthington Diagnostics; 2,839 U/mg) were stored in

50% glycerol–0.005 M sodium acetate (pH 5.0) at –20°C (46). Solutions of micrococcal nuclease (1 mg/ml; Boehringer-Mannheim Corp.; 15,000 U/mg) in 50% glycerol–1.0 mM Tris-hydrochloride (pH 8.0)–0.1 mM CaCl₂ at –20°C (46). Glycerol gradient fractions containing SV40 chromosomes were incubated in Eppendorf tubes (Bio-Rad Laboratories) and washed with bovine serum albumin (10 mg/ml; Sigma Chemical Co.), with a final concentration of either 80.0 U of DNase per ml (20 U/µg of DNA) and 2 mM MgCl₂, or 600 U/ml of micrococcal nuclease (120 U/µg of DNA) and 6 mM CaCl₂, for times and temperatures to be specified. Samples for chromosome analysis were mixed with EDTA to a final concentration of 10 mM. Samples for DNA analysis were mixed with sodium dodecyl sulfate and EDTA to a final concentration 0.2% and 10 mM, respectively.

Agarose gel electrophoresis. Glycerol gradient fractions containing SV40 chromosomes were prepared for agarose gel electrophoresis by the addition of bromophenol blue and glycerol to final concentrations of 0.08 and 15%, respectively, to the fractions. Electrophoresis was in a submerged 0.5% agarose (Sigma type II) gel buffered with 0.036 M Tris, 0.03 M NaH₂PO₄, and 0.001 M EDTA (pH 7.5) for 20 h at 30 V. After electrophoresis, the gels were stained with ethidium bromide (1 µg/ml) in electrophoresis buffer. Radiolabeled gels were prepared for fluorography by two equilibrations in 1 M sodium salicylate for 30 min each (8). Gels were dried on a Bio-Rad gel dryer and fluorographed at –70°C with Kodak XAR-5 film. Samples of SV40 DNA for agarose gel electrophoresis were prepared by incubation with 0.2% sodium dodecyl sulfate, 1 mM EDTA, and 0.008% bromophenol blue at 37°C for 15 min. SV40 form I and form II DNA were resolved on submerged 1% agarose gels as above (51).

Two-dimensional agarose-agarose and agarose-polyacrylamide gel electrophoresis. Agarose-agarose gel electrophoresis was performed by removing the lane containing SV40 chromosomes electrophoresed in a 0.5% agarose gel obtained as above, soaking the agarose in buffer containing 0.6% sodium dodecyl sulfate and 2 mM EDTA for 30 min, and finally incubating it in fresh buffer for 30 min. The agarose was then attached to a 1.0% agarose slab gel with 0.5% agarose, electrophoresed, stained, and fluorographed as described above. These gels resolved SV40 form I and form II DNA. Agarose-polyacrylamide gel electrophoresis was performed by removing the lane containing [³⁵S]methionine-radiolabeled SV40 chromosomes as described above, attaching the gel to a 10% polyacrylamide gel (32) with 0.5% agarose in running buffer, and electrophoresing at 25 mA until the bromophenol blue marker migrated to the end of the gel. Nuclease-digested SV40 DNA was electrophoresed on 2.5% agarose gels as described above. SV40 DNA, obtained by the procedure of Hirt (24), was used for markers. Marker DNA for the nuclease digestion gels was obtained by *Hind*III (Boehringer-Mannheim Corp.) digestion of purified SV40 DNA according to the supplier's directions.

RESULTS

Purification of SV40 chromosomes by glycerol gradient sedimentation and agarose gel electro-

FIG. 1. Purification of SV40 chromosomes by glycerol gradient sedimentation. Nuclear extracts were obtained from infected cells (●) and mock-infected cells (○) labeled for 3 h with [3 H]thymidine and purified as described. Samples (25 μ l) from each fraction were counted. V, RC, and NRC indicate the position of virions, replicating chromosomes, and non-replicating chromosomes, respectively (40). Sedimentation is from right to left.

phoresis. When [3 H]thymidine-labeled nuclear extracts from infected cells are fractionated by sedimentation in a 10 to 30% glycerol gradient, the pattern shown in Fig. 1 is obtained showing nonreplicating chromosomes (NRC), replicating chromosomes (RC), and virions (V) (40). These peaks are absent from mock-infected preparations when treated similarly. Highly condensed chromosomes and large amounts of VP1 are present in the RC region (40). One interpretation of these observations is that the RC region contains, in addition to replicating chromosomes, SV40 chromosomes in the process of forming into virus particles (40) with the VP1 contributing to the condensation of the chromosomes.

To characterize further the structures of the chromosomes found in the RC region, SV40 chromosomes were analyzed by low-ionic-strength agarose gel electrophoresis. Agarose gel electrophoresis was chosen because unfixed SV40 chromosomes migrate in agarose gels (2, 54) and fixed SV40 chromosomes are partially resolved into various subfractions depending upon their migration (2). Furthermore, the resulting agarose gel can be easily manipulated for

further analysis. Individual fractions from glycerol gradients of [3 H]thymidine-labeled SV40 chromosomes (Fig. 1) were electrophoresed on 0.5% agarose gels for 22 h, and the label was visualized by fluorography. The fractions in the V region contained radiolabeled material that migrated to the positions labeled β and γ (Fig. 2, lane 1). Radiolabeled material from the RC region of gradients (Fig. 2, lane 2) migrated as a band of label at β and a smear of label at γ . The radiolabeled material in the NRC region (Fig. 2, lanes 3 and 4) migrated heterogeneously as a principal band at α and a smear at β . Figure 2, lane 5, shows SV40 form I and form II DNA.

When comparable fractions from mock-infected cells were analyzed, the results shown in Fig. 2, lanes 6 to 9, were obtained. Because of the much smaller amount of radiolabel incorporated, the bands in the fluorograms are fainter and have been outlined to show their position. (A 1-month exposure was necessary to detect the labeled chromatin.) Each gradient fraction contained radiolabeled chromatin which migrated as a single band whose mobility varied inversely with its sedimentation value. Unfortunately, the

FIG. 2. Agarose gel electrophoresis of [3 H]thymidine-labeled SV40 chromosomes in fractions from the glycerol gradient shown in Fig. 1. Portions were electrophoresed on 0.5% agarose gels for 22 h and fluorographed as described. Infected extracts: V region, lane 1; RC, lane 2; NRC fraction 14, lane 3; NRC fraction 16, lane 4; SV40 DNA marker, lane 5; mock-infected extracts corresponding to NRC, lane 6; RC, lane 7; fraction 5, lane 8; and V region, lane 9.

cellular chromatin in the gradient fractions corresponding to those containing replicating and nonreplicating SV40 chromosome migrated to the same general position on the gel as the SV40 chromosomes.

Because of the possibility of the presence of cellular chromatin migrating similarly to the α , β , or γ species of SV40 chromosomes, it was necessary to show that these species actually contained SV40 DNA. After electrophoresis in the 0.5% agarose gel to separate the different species of SV40 chromosomes (labeled with [3 H]thymidine) in the various gradient fractions, the individual lanes of interest were excised. They were incubated in a buffer containing sodium dodecyl sulfate and EDTA to disrupt protein-DNA interactions, attached to a 1.0% agarose gel with molten 0.5% agarose, and electrophoresed. Fractions corresponding to glycerol gradient regions NRC, RC, and V were analyzed, and fluorograms of the gels are shown in Fig. 3. The α , β , and γ chromosomal species (Fig. 2) in fractions containing NRC (Fig. 3A) and RC (Fig. 3B) clearly contained SV40 form I and form II DNA. The γ species present in fractions from the V region also contained SV40 DNA. There was very little labeled chromatin contamination apparent in the NRC, RC, or V gradient fractions. The labeled DNA present at the interface of the first and second agarose gels may represent trapped DNA, since one-dimensional agarose gel electrophoresis of the identical fractions did not reveal high-molecular-weight DNA (data not shown). The trapping could have resulted from the immobilization of some of the circular SV40 DNA by the molten agar used to join the two agarose gels.

Proteins migrating with the different species of SV40 chromosomes. Because the SV40 chromosomes in the glycerol gradient fractions which migrated as the β and γ forms are more compact than the bulk of the SV40 chromosomes (the α forms) by electron microscopic analysis, a likely explanation for the reduced mobilities of the β and γ species relative to the α species of SV40 chromosome is that the charge of the nucleic acid is shielded or neutralized by associated proteins. To determine whether major differences in associated proteins, especially viral proteins, existed, the material fractionated on the 0.5% agarose gel was analyzed by sodium dodecyl sulfate-acrylamide gel electrophoresis. In this experiment, [35 S]methionine was used to label the proteins comigrating in the agarose gel with the α , β , and γ species. Autoradiographs of the sodium dodecyl sulfate gels are shown in Fig. 4. VP1 was identified by protease digestion and comparison to an authentic sample of VP1 (40; data not shown). As is apparent, VP1 was associated primarily with the β and γ species. In

FIG. 3. Two-dimensional agarose-agarose gel electrophoresis of [3 H]thymidine-labeled SV40 chromosome DNA. Fractions of the glycerol gradient shown in Fig. 1 were first analyzed on 0.5% agarose gels (migration, left to right) and, after excision of the lane and deproteinization, were electrophoresed on 1.0% agarose gels (migration, top to bottom). The gels were dried and fluorographed as described. SV40 form I and form II DNA were used as markers. (A) NRC region, fraction 17; (B) RC region, fraction 12; and (C) V region, fraction 1. The positions of the α , β , and γ species were identified by comparison to standards included on the 0.5% agarose gels.

addition to VP1, the α , β , and γ species each contained other comigrating proteins whose identities are presently unknown. The 25,000-dalton protein comigrating with γ species is probably VP3. Many other proteins found to remain at the origin or to migrate to various positions in the gel represent cellular proteins, since they were also seen in mock-infected cells.

Nuclease digestion of the different SV40 chromosome species. Since the β and γ species contain large amounts of form I and form II DNA and viral structural protein, they probably represent encapsidation intermediates. The reduced mobilities of these forms relative to the α species must then be caused by fundamental differences in their structures, possibly resulting from the

FIG. 4. Two-dimensional agarose-polyacrylamide gel electrophoresis of SV40 chromosomal proteins. Fractions of glycerol gradients containing [35 S]methionine-labeled protein were electrophoresed in 0.5% agarose gels (migration, left to right). After excision, the lane containing the fraction of interest was transferred to a 10% polyacrylamide gel, and proteins were separated by gel electrophoresis. Radiolabeled proteins were visualized by autoradiography. (A) Mixed NRC and RC region, (B) V region. O is the sample origin. The positions of the α , β , and γ species were identified by comparison to standards electrophoresed on the 0.5% agarose gel.

association of the viral structural proteins. The most likely structural modification that could cause the reduction in mobility observed is shielding of the nucleic acid present in each form by viral structural proteins, effectively reducing the negative charge of the chromosome.

To test whether such structural modifications exist, the various species were digested with nucleases. The digestion products were analyzed by agarose gel electrophoresis. Nuclease digestion has been used extensively to study the structure of chromatin (38). DNase I and micrococcal nuclease were chosen for this analysis because of the ability of DNase I to digest along nucleosomes and the specificity of micrococcal nuclease for sites between nucleosomes. Since the nucleases differ in size, it is possible that modifications in structure may affect the accessibility to digestion by either of the enzymes.

The results of digestion of fractions from NRC and RC regions of gradients are shown in Fig. 5.

Digestion of NRC fractions containing principally α species with DNase I for 1 min (Fig. 5A, lane 5) caused substantial digestion of the DNA present, and extensive digestion (Fig. 5, lane 4) reduced the size of the fragments to less than 70 base pairs. A similar effect was observed when the β and γ species found in RC glycerol gradient fractions were used as substrates (Fig. 5, lanes 9 and 10, respectively).

Comparable results were obtained when micrococcal nuclease was used for digestions, with one exception. Although all species were rapidly digested (Fig. 5B, lanes 4, 5, 9, and 10), the final product of digestion of the α species was a fragment of DNA 150 base pairs long (Fig. 5C, lane 4). The typical pattern of DNA fragments observed with moderate digestion of the α species (Fig. 5A and B, lane 5), which represent DNA from mono-, di-, and tri-nucleosomes, is also present in digestion of the mixed β and γ species, but is very faint. The bands are more prominent when digestion is extremely brief, although the β and γ species are always more extensively digested regardless of the time of digestion (data not shown).

In contrast to the extensive digestion which occurred in the presence of nuclease and divalent cation, only slight nicking of form I DNA in the SV40 chromosomes occurred with either nuclease or cation alone (Fig. 5A and B, lanes 2, 3, 7, and 8). Perhaps not surprisingly, extensive digestion of any of the three chromosome species with either nuclease destroyed the integrity of the species (Fig. 5C and D). A small amount of the α species remained after mild digestion with micrococcal nuclease (Fig. 4D, lane 5). Neither nuclease nor cation alone had an appreciable effect on the structure of the species. The greater streakiness of the species on these gels is an artifact due to the presence of bovine serum albumin, which was used to stabilize the nuclease and chromosomes during digestion (data not shown).

The accessibility of the DNA in the β and γ species of SV40 chromosomes to nuclease digestion could be explained either by the chromosomes being encapsidation intermediates lacking a portion of the capsid or being degradation products derived from virions. If the latter were the case, it might be expected that the virions found in the V region of the glycerol gradient would be partially degraded and therefore susceptible to digestion. Although the radiolabeled DNA in this region (γ species) was extensively digested by DNase I (Fig. 6A, lanes 1 and 2), the stained DNA (in virus particles) was not appreciably digested (Fig. 6B, lanes 1 and 2). Most of the radiolabeled DNA present in the native virion species was degraded and no longer migrated to its original position (Fig. 6A, lanes 3

FIG. 5. Nuclease digestion of α and β plus γ species of SV40 chromosomes. Lanes 1 to 5 and 6 to 10 contain glycerol gradient fractions containing either α species (NRC; lanes 1 to 5) or β plus γ species (RC; lanes 6 to 10) SV40 chromosomes, respectively, were digested as described with either DNase I (A and C) or micrococcal nuclease (B and D). Products of digestion were analyzed for DNA (A and B) on 2.5% agarose gels or chromosome species (C and D) on 0.5% agarose gels. Lanes 1 and 6, chromosomes; lanes 2 and 7, chromosomes and nuclease; lanes 3 and 8, chromosomes and cation; lanes 4 and 9, chromosomes, nuclease, and cation plus incubation at 37°C for 15 min; lanes 5 and 10, chromosomes, nuclease, and cation plus incubation at room temperature for 1 min; lane 11, *Hind*III digest of SV40 DNA; and lane 12, SV40 DNA. The sizes of the *Hind*III fragments of SV40 DNA are shown to the right.

and 4). The digestion was very rapid, and similar results were obtained with micrococcal nuclease (data not shown).

DISCUSSION

Agarose gel electrophoresis. The mobility of nucleoprotein in low-ionic-strength agarose gel electrophoresis depends upon the size and charge of the nucleoprotein. An increase in size with little effect upon charge will reduce the mobility of the nucleoprotein, whereas an in-

crease in negative charge without appreciable modification of its size will increase its mobility. The mobility of an SV40 chromosome will therefore vary with its degree of compaction and with the amount of shielding of its nucleic acid by associated proteins. Considering the known heterogeneity in compaction of SV40 chromosomes, as determined by sedimentation analysis and electron microscopy (4, 11, 27, 40, 41, 54), it is not surprising that in this study, agarose gel electrophoresis resolved radiolabeled SV40

FIG. 6. Nuclease digestion of virions. Glycerol gradient fractions from the V region were obtained as described and digested with DNase I (at 37°C for 15 min) and analyzed on a 0.5% agarose gel with or without deproteinization. (A) Fluorography: lane 1, undigested deproteinized DNA; lane 2, digested deproteinized DNA; lane 3, undigested, no deproteinization; lane 4, digested, no deproteinization; and lane 5, SV40 DNA marker. (B) Ethidium bromide staining: lane 1, undigested deproteinized DNA; lane 2, digested deproteinized DNA; and lane 3, SV40 DNA marker.

chromosomes into a major species at position α and a group of species found between positions β and γ , with SV40 virions migrating to position γ , in order of decreasing mobility. The sharpness of the radiolabeled bands in the α , β , and γ positions is somewhat variable depending upon the particular chromosome preparation and the freshness of the sample.

The SV40 chromosome species were analyzed by agarose-agarose or agarose-polyacrylamide gel electrophoresis to determine whether the differences in their mobilities were due to differences in the DNA or proteins present, respectively. The fact that the bulk of the radiolabeled DNA present in each chromosome species consists of SV40 form I and form II DNA is not surprising. Since SV40 form I and form II DNA are present in each type of SV40 chromosome in exactly the same position, nicking of the DNA does not produce a sufficient difference in chromatin structure to be observed in this electro-

phoresis system. The nicking of the radiolabeled DNA may be due to its high specific activity. By using these radiolabeled samples, no replicating SV40 chromosomes could be identified. Since the pulse time was long, this result was expected. The position of migration of replicating SV40 chromosomes is presently being determined.

The agarose-polyacrylamide two-dimensional analysis of proteins present in each SV40 chromosome form was also useful, although the analysis has a major limitation. A one-dimensional polyacrylamide gel analysis of radiolabeled proteins based only upon molecular weight is inadequate for their identification (40). VP1 was identified for this analysis by its absence in mock-infected extracts and its protease fingerprint.

Only trace amounts of viral proteins were present in the α species of SV40 chromosomes. The β species contained some VP1, whereas the γ species and virions contained the largest quantities relative to the amount of labeled DNA present. It is likely that the VP1 present is actually associated with the chromosomes, since it has copurified with the chromosomes after glycerol gradient sedimentation and agarose gel electrophoresis. In addition, the VP1 no longer migrated to the β or γ positions if the chromosomes were first digested with DNase I (data not shown.) In addition to the nuclease-sensitive VP1 that migrated with the γ species SV40 chromosomes, VP1 was also found that migrated more slowly than the γ species and was not nuclease sensitive. This VP1 was probably present in aggregation with other proteins. That the viral protein is the cause of the change in mobility observed for the chromosomes is consistent with its potential to shield the nucleic acid present within the structure. The presence of other as yet unidentified proteins may also affect the migration of the SV40 chromosomes. Other proteins migrating with the various forms are presently being identified.

The migration of SV40 chromosomes subjected to agarose gel electrophoresis has been previously reported (2, 54), although resolution of the various chromosome forms was not observed if the nucleoprotein was not cross-linked (2). The difference in results between that report (2) and this study may in part be due to differences in sample preparation, electrophoresis conditions, and chromosome visualization. In the earlier work, the chromosomes were concentrated by dialysis, resolved on a 1% agarose gel, and visualized by ethidium bromide staining for nucleic acid (2). As described here, untreated chromosomes are resolved on 0.5% agarose gels and visualized by fluorography of the radiolabeled SV40 DNA. Better resolution of chromosomes was obtained when 0.5% agarose gels

were used rather than with 1% agarose gels. The radiolabeled DNA represented only the more recently synthesized portion of the stained material present on the gel.

Nuclease digestion. All species of SV40 radiolabeled chromosomes and the virions were susceptible to digestion by both DNase I and micrococcal nuclease. The α , β , and γ species of SV40 chromosomes did not migrate to their original positions after extensive digestion with either nuclease, suggesting that the proteins associated with each chromosomal species were not capable of retaining the structural integrity of the species after digestion. The DNA produced by very limited digestion with either nuclease consisted of fragments of approximately 200, 400, and 600 base pairs. This result is in agreement with the nucleosome model for chromatin (15, 29) and with previous experiments with micrococcal nuclease digestion of pooled SV40 chromosomes (4, 14). Extensive digestion with DNase I of susceptible species yields very small DNA fragments. In contrast, extensive digestion of the α species of SV40 chromosome by micrococcal nuclease yielded a DNA fragment of approximately 150 base pairs. This fragment is comparable to the nucleosome core size previously reported for SV40 chromatin (4). Interestingly, no core-sized DNA was observed after extensive digestion of the other radiolabeled chromosome species or virions. Whether this apparent increased susceptibility to digestion represents a fundamental difference in nucleosome structure is presently under investigation. Such a difference could result from conformational modifications resulting from the association of viral proteins or from modifications in the histones present in the nucleosomes (30, 31). Histone acetylation is known to increase the susceptibility of chromatin to nuclease digestion (37).

Proposed encapsidation model. Since the β and γ species of SV40 chromosomes contain SV40 DNA I and II and VP1, it is quite probable that they are encapsidating intermediates. One explanation of their nuclease sensitivity is that the capsid structure of these intermediates contains regularly spaced openings (Fig. 7) which allow the passage of the nuclease into the capsid. Because of the absence of VP2 in early intermediates (18), the proposed openings have been shown at the penton sites. An encapsidation model which includes these observations and previous evidence on the protein complement (18) and sulfhydryl sensitivity (42) of virions is briefly described.

Although nothing is known about the process which commits an SV40 chromosome to encapsidation, the distinction between the α species (the typical SV40 minichromosome) and β spe-

FIG. 7. Proposed partial capsid structure of early encapsidation intermediates. The potential penton binding sites are the shaded areas.

cies suggests that the process may involve a fundamental change in the structure of the nucleosomes of the SV40 chromosome such as modification of histones. Such a change could make the chromosome available to binding of the viral protein derived hexons. The hexons are likely formed by self-assembly from monomer VP1 (6). VP1 contains cysteine located as two domains of three cysteines each and one cysteine at the highly basic amino terminus (45). Each VP1 could then form two sulfhydryl bridges to each of its neighbors within a hexon, two bridges to adjacent hexons, and one bridge to pentons or to the encapsidated chromosome. The β species of SV40 chromosome might consist of a limited number of hexons in an uncross-linked structure. Addition of hexons to available sites on the β species eventually would yield the γ species. When all the hexons in the γ species of SV40 chromosome had cross-linked, the conformational change would result in formation of the nuclease-sensitive virion with a structure as shown in Fig. 7. Although there is as yet no direct evidence for this cross-linking, the fact that the chromosomes which contain VP1 undergo a substantial increase in sedimentation when they become virions is suggestive that cross-linking is occurring. Addition of the penton proteins and their cross-linking to the hexons would result in a stable virion.

This model differs from other self-assembly models (11, 13, 18, 26, 40) principally in the suggestion that the structure of SV40 nucleosomes is modified prior to hexon assembly to chromosomes and the hexon-hexon cross-linking precedes penton assembly. The absence of pentons until late in encapsidation may be necessary for the movement of encapsidation proteins or cofactors in and out of the previrion.

Although mature virions can be degraded by

Triton X-100, high EDTA concentrations, mechanical disruption (9, 26, 47), or β -mercaptoethanol (42), the majority of the virions observed in this study are not degraded, since they are resistant to nuclease digestion. Only the pulse-labeled virions were accessible to digestion. It is possible, however, that the binding of penton proteins to the immature encapsidation intermediates is easily disrupted. Such a selective loss of the pentons could also result in the structures observed. A portion of the encapsidation intermediates may be contaminated by such specific degradation products. The extent of specific penton loss is under investigation.

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