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Compact form of SV40 viral minichromosome is resistant to nuclease: possible implications for chromatin structure

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ABSTRACT

We report two new findings bearing on the "supranucleosomal" level of the structure of the Simian Virus 40 minichromosome. I) Isolated SV40 minichromosome which contains all five histones including HI /I/ exists in solution under approximately physiological ionic conditions as a compact roughly spherical particle ~300 Å in diameter which is capable of fitting within the virus capsid. In spite of such a compact conformation of the minichromosome individual nucleosomes can be readily visualized within the particle. Compact state of SV40 minichromosome depends on both the presence of histone HI and maintenance of approximately physiological ionic strength of solution ($\mu \approx 0.15$). Removal of HI results in a conversion of the compact minichromosome into an extended (circular beaded) structure. 2) The compact form of the SV40 minichromosome in contrast to its circular beaded form is virtually completely resistant to staphylococcal nuclease, strongly suggesting that in particular nuclease-sensitive parts of the internucleosomal DNA regions are not exposed on the outside of the compact SV40 minichromosome. On the other hand, DNase I which is known to attack both inter- and intranucleosomal DNA in the chromatin /2,3/ readily digests the compact form of the SV40 minichromosome. Possible models of the compact minichromosome and implications for higher order structures of the cellular chromatin are discussed.

INTRODUCTION

Simian Virus 40 appears to be a particularly attractive experimental object for studies on the structure and functioning of nucleosomes /2-17/ since SV40 DNA and cellular histones are associated in infected cells and in SV40 virions in a chromatin-like structure called a minichromosome /18/. The SV40 and polyoma minichromosomes were vizualized in the electron microscope as circular beaded fibers which consisted of 20-22 nucleosomes joined by short DNA-like threads /I,18-21/.

We have previously found that the SV40 minichromosomes extracted with 0.I5 M NaCl from nuclei of lytically infected cells contain not only four "nucleosomal" histones (H2a,H2b, H3 and H4) but histone HI as well /I/. This finding was independently confirmed by other authors (refs.22,23; A.Worcel, H.Weintraub and W.Keller, personal communications).

On the other hand, the SV40 minichromosomes extracted from the purified SV40 virions by a high pH treatment apparently do not contain histone HI /22/. In this paper we shall consider only histone HI-containing SV40 minichromosomes extracted from nuclei of lytically infected cells /I/.

MATERIALS AND METHODS

Isolation and purification of SV40 minichromosomes. Four times plaque-purified SV40 virus (strain 777) was grown on monolayers of green monkey kidney cells (CV-I) as described previously /I/. The cells were labelled with /Me-^{3}H/thymidine 30-35 hr after infection /I,I9-2I/. The procedure described below is a modification of the previously developed methods /I8-2I,24/. At 40-42 hr after infection the cells were washed twice with 0.I4 M NaCl. Each flask was then treated with 4-5 ml of 0.25% Triton X-I00, I0 mM Na-EDTA, I0 mM triethanolamine (TEA)-HCl, pH 6.8 (pH of all buffers was measured at 20°C). The cells were allowed to swell for I5 min at ~4°C followed by addition of I M NaCl to a final concentration of 0.I3 M. The lysate obtained was decanted from flasks or gently scraped with a rubber policeman and thereafter centrifuged at 5,000 g for 5 min at 3°C. The cytoplasmic supernatant was discarded, the nuclear pellet from 2-3x$I0^{8}$ cells was briefly resuspended in 50 ml of 0.25% Triton X-I00, 0.I3 M NaCl, I0 mM Na--EDTA, I0 mM TEA-HCl, pH 6.8 and then centrifuged at 5,000 g for 5 min. The pellet was resuspended in 5-6 ml of 0.25% Triton X-I00, 0.I3 M NaCl, I0 mM Na-EDTA, I0 mM TEA-HCl, pH 8.0. Proteinase inhibitor phenylmethylsulphonyl fluoride (PMSF; Sigma) was then added to a final concentration of I mM followed by extraction of the SV40 minichromosomes at 3-4°C for 3 hr with continuous gentle stirring of the suspension on a magnetic stirrer. The suspension was then centrifuged at 5000 g for

I5 min and the supernatant (5-6 ml) which contained I50-300 μg of the SV40 minichromosomes was layered onto three 50-ml linear sucrose gradients (5-25%) in 0.I3 M NaCl, I mM Na-EDTA, I mM TEA-HCl, pH 7.5. The samples were centrifuged in the SW25.2 rotor (Beckman) at I8,000 rpm for II hr at 3°C. Peak fractions (see Fig.I) were pooled and immediately used for the next experimental stage. In some experiments minichromosome solutions were concentrated by ultrafiltration after lowering of the ionic strength of solution to about 0.005 /I/ but not under the initial ionic conditions. We used 0.I3 M NaCl in the extraction buffer in contrast to 0.I5 M NaCl in our previous work /I/ or 0.20 M NaCl in works of other authors /I9-2I/ to prevent a partial loss of histone HI from minichromosomes. A partial loss of HI was already detectable in 0.20 M NaCl /I/.

The amount of the SV40 minichromosomes in the pooled supernatants obtained before extraction of the CV-I nuclei at pH 8.0 (see above) constituted I5-25% of the amount of the minichromosomes in the pH 8.0-extract.

Nuclease digestion of SV40 minichromosomes. Pooled fractions of the isolated minichromosomes (see Fig.I) in I0-I5% sucrose, 0.I3 M NaCl, I mM Na-EDTA, I mM TEA-HCl, pH 7.5 contained from 5 to I0 μg of the SV40 DNA per ml. Nuclease digestion under these ionic conditions (in which a compact form of the minichromosome is stable; see below) was carried out without a preliminary removal of sucrose and EDTA. At first 50 mM $CaCl_2$ was added to the minichromosome solution to a final total $CaCl_2$ concentration of 5 mM and to a final "effective" $CaCl_2$ concentration (taking into account the presence of I mM Na-EDTA) of approximately 3 mM. Thereafter staphylococcal nuclease (Worthington; I mg/ml in I mM NaCl) was added to a final concentration of 2 μg/ml. The digestion was carried out at first at ~4°C and thereafter at 37°C. Aliquots of the digestion mixture were mixed with ¼ volume of 50 mM Na-EDTA, pH 7.5 to stop the reaction. Purified total yeast tRNA was then added as a carrier to a final concentration of 30 μg/ml followed by precipitation of nucleic acids in the sample with 75% ethanol. The samples were incubated for 2 hr at -20°C and then centrifuged in polyallomer tubes at I5,000 rpm for I5 min in the

SW50.I rotor (Beckman). This procedure permits one to quantitatively pellet both DNA of the intact SV40 deoxyribonucleoprotein (DNP) particles and DNA of various DNP products of digestion down to at least the size of tRNA as was checked by appropriate controls. The pellets were dissolved in an SDS--containing buffer and subjected to agarose gel electrophoresis (see below).

Digestion of the compact minichromosomes with pancreatic DNase (DNase I) was carried out as described above for staphylococcal nuclease except that I mM $MgCl_2$ was added to the complete digestion buffer.

In parallel control experiments the enzymatic activity of the sample of nuclease which had been used for digestion of the compact SV40 minichromosomes (see above) was checked in several ways. In particular, this nuclease sample was used to digest purified SV40 DNA I and isolated mouse Ehrlich tumor chromatin under ionic conditions exactly identical to those used for digestion of the compact minichromosome (see above). In fact, one and the same sample of sucrose-NaCl-EDTA--TEA solution has been used for nuclease digestion of both the compact SV40 minichromosomes, SV40 DNA I and mouse chromatin. Furthermore, the above-mentioned nuclease sample was used to digest the minichromosomes which were converted to a partially or fully extended (circular beaded) form by a decrease of the ionic strength of solution.

Agarose gel electrophoresis of DNA. The ethanol-precipitated DNP particles (see above) were deproteinized by dissolving in I% SDS, 0.005% Bromphenol Blue, I mM Na-EDTA, pH 7.5 followed by heating of the samples at 50°C for 20 min and electrophoresis of DNA in SDS-containing I% agarose slab gels. The buffer in the electrode vessels and in the gel was 0.I% SDS, 2 mM Na-EDTA, 5 mM CH_3COONa, 40 mM Tris-HCl, pH 7.5. Free proteins in SDS-containing samples did not interfere with the electrophoresis of DNA. After electrophoresis at 3 V/cm for I0-I5 hr the gel was soaked in several changes of H_2O to remove SDS and then stained for DNA with ethidium bromide.

Low-ionic strength agarose gel electrophoresis of SV40 minichromosomes. It was carried out as previously described

/I/ except that slab gels were used.

SDS-gel electrophoresis of proteins. It was carried out as described previously /I/.

Analytical sucrose gradient centrifugation of SV40 minichromosomes. A minichromosome sample in I mM Na-EDTA, I mM TEA-HCl, pH 7.5 or in 0.I3 M NaCl, I mM Na-EDTA, I mM TEA-HCl, pH 7.5 (0.25 ml) was layered onto II.7 ml of a linear 5-25% sucrose gradient. The buffer in the gradient was identical to that in the sample. Centrifugation was carried out in the SW40 rotor at 37,000 rpm for 2.5 hr at 3°C. Fractions were collected from the bottom of a centrifuge tube directly onto glass fiber filters and thereafter processed and counted as described previously /25/.

Electron microscopy. A drop of a minichromosome solution (5-IO µg of DNA per ml) either in IO-I5% sucrose, 0.I3 M NaCl, I mM Na-EDTA, I mM TEA-HCl, pH 7.5 (see Fig.I) or in a low-ionic-strength buffer (3 mM TEA-HCl, I mM Na-EDTA, pH 7.5) was applied onto a carbon-coated electron microscopic grid. After IO-60 seconds the drop was removed by a filter paper touched to the edge of the grid followed by successive washings of the grid on surfaces of a few H_2O drops for a total time of 2-5 minutes. Alternatively, the grid was allowed to swim on the H_2O drop without preliminary removal of excessive sample liquid. The SV40 minichromosomes were applied onto grids either unfixed or preliminary fixed with formaldehyde (pH 7.5) for IO-60 min at ~4°C. These variations did not lead to qualitative changes of the results. However, it should be noted that the percentage of partially unfolded forms of minichromosomes on the grids was significantly higher in the absence of fixation. Washed grids were dried on a filter paper followed by rotatory shadowing with Pt-Pd (4:I) at the angle of 7° or, alternatively, by staining with uranyl salts /26/ or sodium phosphotungstate /I2/. Supporting carbon films were prepared by evaporation of carbon onto freshly cleaved mica plates followed by a transfer of the film from the mica onto 400-mesh copper grids. The carbon film was made hydrophillic by a glow discharge. The samples were examined in a JEM-IOOB electron microscope (Jeol) at instrumental magnifications of

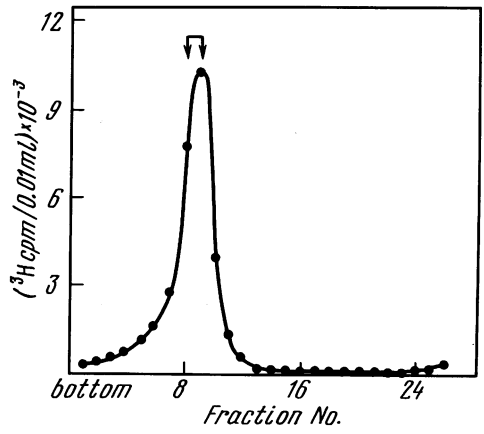

Fig. 1. Purification of the compact SV40 minichromosomes by sucrose gradient centrifugation (see Methods). Arrows indicate fractions which were pooled for further analysis.

I5,000 and 50,000 for metal-shadowed and stained samples, respectively.

RESULTS

Sedimentational and electrophoretic analysis of SV40 minichromosomes. The apparent sedimentational coefficient of the SV40 minichromosomes under "physiological" salt conditions ($\mu \approx 0.I5$) was 80-85S if measured with the use of the SV40 DNA I (2IS) as a sedimentational marker (Fig.2a). On the other hand, when the mouse 60S ribosomal subunit /2I/ was used as a sedimentational marker, the apparent sedimentational coefficient of the SV40 minichromosomes was found to be 70-75S (data not shown). This difference is probably due in particular to different buoyant densities of the SV40 DNA I and RNP particles used as sedimentational markers /2I/. Sedimentational data show that under "physiological" salt conditions ($\mu \approx 0.I5$) minichromosomes exist in a much more compact conformation than at a low ionic strength ($\mu \approx 0.005$) (Fig.2). As can be seen from Figure 2a the SV40 minichromosomes sediment at 80--85S (in relation to the SV40 DNA I marker) in a high ionic strength buffer ($\mu \approx 0.I5$; see also refs. I9-2I/, whereas at a low ionic strength ($\mu \approx 0.005$) they sediment at approximately

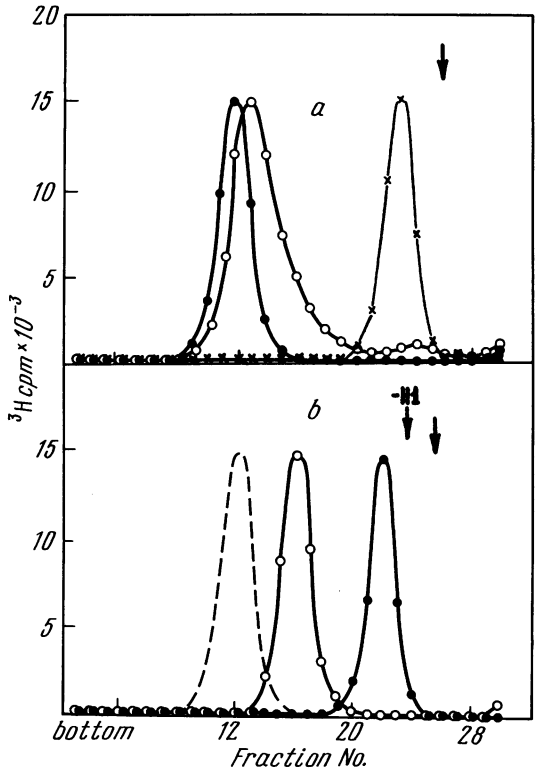

Fig. 2. Sedimentational patterns of compact and extended SV40 minichromosomes.

(a) —●—, Isolated minichromosomes (see Fig. 1) were centrifuged in the SW40 rotor through a 0.13 M NaCl-containing sucrose gradient (see Methods). —o— , A superimposed curve from the same experiment except that before layering onto the gradient the minichromosome preparation was dialysed overnight against 1 mM Na-EDTA, 1 mM TEA-HCl, pH 7.5 followed by addition of an equal volume of 0.26 M NaCl, 1 mM Na-EDTA, 1 mM TEA-HCl, pH 7.5. -x-,A superimposed curve of H1-depleted SV40 minichromosomes (see the legend for Fig. 3) which were centrifuged in a parallel tube under the same ionic conditions.

(b) —●— , Isolated minichromosomes were dialysed against 1 mM Na-EDTA, 1 mM TEA-HCl, pH 7.5 followed by centrifugation through a 5-25% sucrose gradient in the same buffer (see Methods). —o— , Isolated minichromosomes were treated overnight with 1% HCHO in 0.13 M NaCl, 1 mM Na-EDTA, pH 7.5 followed by dialysis against 1 mM EDTA, pH 7.5 and centrifugation through a low-ionic-strength sucrose gradient (a superimposed curve). The dashed line shows a superimposed sedimentational pattern of the SV40 minichromosomes in 0.13 M NaCl. Arrows in a and b indicate the positions of the SV40 DNA I used as a sedimentational marker (in parallel tubes). Additional arrow in b indicates the position of the peak of the H1-depleted minichromosomes (see the legend to Fig. 3) in 1 mM Na-EDTA, pH 7.5.

37S in relation to the same marker (Fig.2b). The "extended" histone HI-containing SV40 minichromosome undergoes compactization upon an increase of the ionic strength of solution to ~0.I5 (Fig.2a). Thus a transition from the compact to the extended minichromosome may be at least partially reversible. However, sedimentational criteria of compactization is clearly a rough one and therefore cannot be considered as a direct evidence for the restoration of a true initial compact minichromosomal structure from the extended minichromosome. Notice also that the peak of "reconstructed" compact minichromosomes has a prominent "light" shoulder which is not present in a symmetrical sharp peak of the initial compact minichromosomes (Fig.2a). Moreover, the whole peak of the "reconstructed" compact minichromosomes is slightly shifted (Fig.2a), additionally indicating that the restoration of the initial compact state of the SV40 minichromosome may be not complete under these conditions. Electron microscopic study of the "reconstructed" compact minichromosomes is currently under way. It should be noted that the salt-induced compactization of the extended histone HI-containing minichromosome apparently does not require the integrity of the covalently closed state of the SV40 DNA since the minichromosomes which contained nicked DNA were indistinguishable from the intact minichromosomes upon such a transition (data not shown).

The compact state of the initial SV40 minichromosome could be at least partially fixed with formaldehyde (Fig.2b). Minichromosomes which were treated with formaldehyde before lowering of the ionic strength of solution sediment significantly faster than the extended minichromosomes (Fig.2b). Finally it should be noted that the above-mentioned structural transitions critically depend on the presence of histone HI in the minichromosome. The histone HI-depleted minichromosomes (Fig.3a; cf. Fig.3b) sedimented in 0.I3 M NaCl at approximately 34S (in relation to the SV40 DNA I marker) as compared with 80-85S for the initial HI-containing compact minichromosomes under the same ionic conditions (Fig.2a). At low ionic strength ($\mu \approx 0.005$) the HI-depleted minichromosomes sedimented at approximately 30S (in relation to the SV40 DNAI

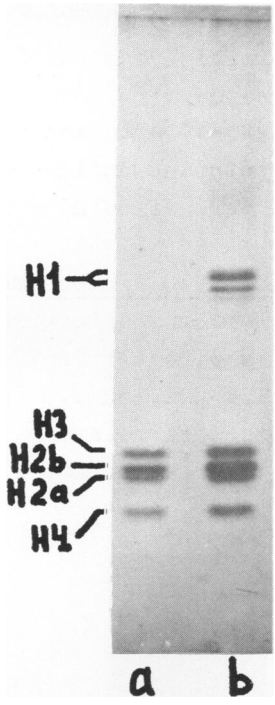

Fig. 3. Electrophoretic analysis of proteins in the SV40 minichromosomes.
(a)Purified SV40 minichromosomes (see Fig. 1) were treated with 0.60 M NaCl, 5 mM TEA-HCl, pH 7.5 to remove histone H1 followed by Sephadex G200 gel chromatography to separate minichromosomes from free proteins. The H1-depleted minichromosomes were analysed by SDS-gel electrophoresis (see Methods).
(b) Proteins of the initial preparation of the purified SV40 minichromosomes (see Fig. 1 and ref. 1).

marker) (Fig.2b). Electron microscopy of the HI-depleted minichromosomes revealed typical circular beaded structures both in a high (~0.I5) and low (~0.005) ionic strength buffers (data not shown; see e.g., ref. 22). Thus the HI-depleted minichromosomes exist in an extended state at any ionic strength of solution, whereas the HI-containing minichromosomes in a high ionic strength buffer ($\mu \approx 0.I5$) are in a much more compact conformation than at a low ionic strength ($\mu \approx$ ≈ 0.005) (see also below).

Similar results were obtained with the use of a low-

-ionic-strength agarose gel electrophoresis of the minichromosomes (Fig.4; see also ref.I). One can see that the minichromosomes which were treated with formaldehyde before a decrease of the ionic strength of solution migrate in the gel considerably faster than both the unfixed minichromosomes and the minichromosomes which were fixed at a low ionic strength ($\mu \approx 0.005$) (Fig.4).

Electron microscopy of SV40 minichromosomes. The samples were applied onto electron microscopic grids after purification of the minichromosomes directly in I0-I5% sucrose, 0.I3 M NaCl, I mM Na-EDTA, I mM TEA-HCl, pH 7.5 (see Methods and Fig.I). The minichromosomes were rotatory shadowed with platinum-palladium alloy (Fig.5) or alternatively, stained with

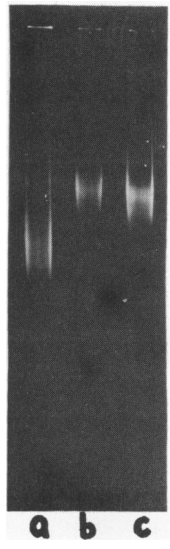

Fig. 4. Low-ionic-strength agarose gel electrophoresis of SV40 minichromosomes.
(a) Isolated minichromosomes were treated overnight with 1% HCHO in 0.13 M NaCl, 1 mM Na-EDTA, 1 mM TEA-HCl, pH 7.5 at ~4°C followed by dialysis against 1 mM Na-EDTA, 1 mM TEA-HCl, pH 7.5, concentration of the minichromosomes by ultrafiltration and a low-ionic-strength electrophoresis of the minichromosomes in a slab 1% agarose gel (see ref.1 for technical details).
(b) The same as a but the HCHO treatment was carried out in 1 mM Na-EDTA, 1 mM TEA, pH 7.5.
(c) Unfixed minichromosomes.

uranyl salts or sodium phosphotungstate (Fig.6). Most of the material on the metal-shadowed grids were roughly spherical particles 350-400 Å in diameter (Fig.5). The same samples but stained with uranyl acetate (Fig.6a), with sodium phosphotungstate (Fig.6c) or with uranyl formate (Fig.6b) contained a somewhat higher percentage of the partially unfolded SV40 minichromosomes but a major proportion of the material on the grid were still roughly spherical particles with a diameter of 250-300 Å (Fig.6). Thus the diameter of the metal-shadowed compact SV40 minichromosomes is significantly higher than that

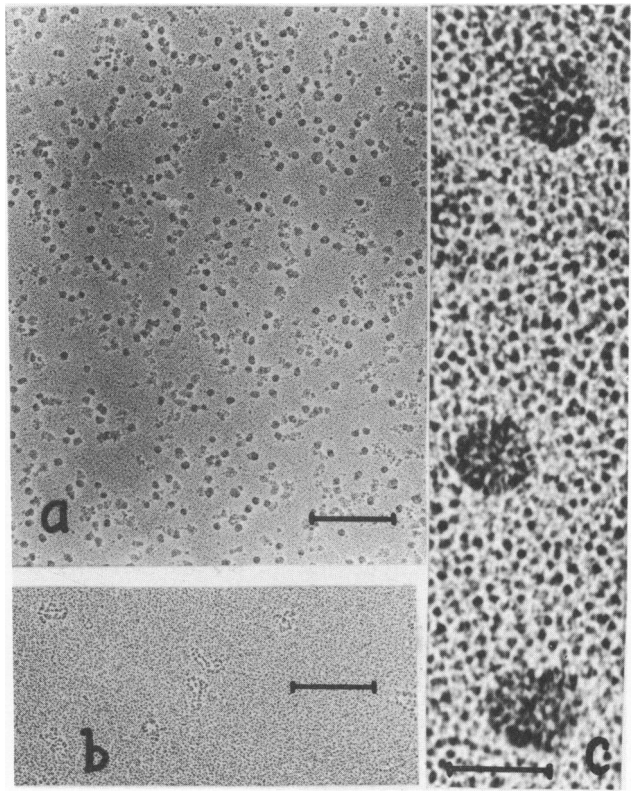

Fig.5. Electron microscopy of metal-shadowed compact SV40 minichromosomes.
(a) Minichromosomes were prepared for electron microscopy in the presence of 0.13 M NaCl (see Methods). (b) Preparation of the SV40 minichromosomes in a low-ionic-strength buffer (see Methods). (c) The same as a but a higher magnification. The bars correspond to 0.5 μ in a and b and to 500 Å in c.

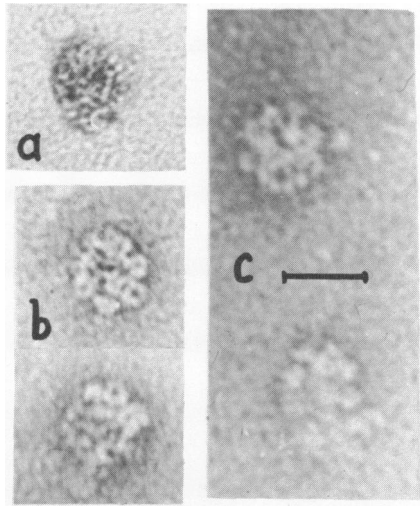

Fig. 6. Electron microscopy of stained compact SV40 minichromosomes.
(a) A compact SV40 minichromosome stained with uranyl acetate (see Methods). (b) The same but stained with uranyl formate. (c) The same but stained with sodium phosphotungstate. The bar in a-c corresponds to 300 Å.

of the stained particles (Fig.5; cf. Fig.6), apparently due to a relatively thick metal layer around the compact minichromosomes in the former case. It should be noted that the compact state of the formaldehyde-fixed minichromosomes is apparently quite stable upon storage since the electron microscopic appearance of the fixed samples was not significantly changed during a two-week interval at ~4°C. However, although the majority of fields of a given electron microscopic grid contained mainly compact minichromosomes, some of the fields (particularly in stained preparations) contained a much higher than average proportion of partially unfolded minichromosomes. Thus the compact SV40 minichromosomes, being quite stable in solution both in a fixed and unfixed state (see below), are relatively unstable upon electron microscopic procedures. A striking difference between the previously observed extended form of the histone HI-containing minichromosome at a low ionic strength of solution ($\mu \approx 0.005$) and its compact configuration at a high ionic strength ($\mu \approx 0.15$) is il-

lustrated by Fig.5a,b. It should be emphasized that the SV40 minichromosomes which were extracted and purified from nuclei of lytically infected CV-I cells at 40-42 hr after infection do not contain any significant amounts of virus-specific capsid proteins (Fig.3b; see also ref. I). Thus the compact configuration of the minichromosome is apparently entirely due to interactions within the complex between the covalently closed circular 5,000-bp DNA and five eukaryotic histones.

In contrast to the metal-shadowed compact minichromosomes (Fig.5) the particles stained with sodium phosphotungstate (Fig.6c) or with uranyl formate (Fig.6b) show a distinct internal structure. On the basis of staining properties of the mononucleosomes /I2,26/ we interpret the particles in Figure 6 as consisting of a tightly packed circular oligonucleosomal filament in which individual mononucleosomes with their characteristically stained central area are clearly seen. Some views of the stained compact minichromosome suggest (but do not proove) a solenoidal coiling of the nucleosomal filament with about three turns of the nucleosomal helix per the minichromosome particle (Fig.6c). Lowering of the ionic strength of solution and/or removal of histone HI leads to a conversion of the compact globular minichromosome into the extended (circular beaded) particle (Figs. 2 and 5).

Compact SV40 minichromosome is resistant to staphylococcal nuclease. Figure 7 shows that the treatment of the compact SV40 minichromosomes in I0% sucrose, 0.I3 M NaCl, 3 mM $CaCl_2$, I mM TEA-HCl, pH 7.5 with staphylococcal nuclease does not lead to any significant degradation of the covalently closed supercoiled SV40 DNA I in the minichromosomes even in the presence of relatively high concentrations of the nuclease both at a low (~4°C) and high (37°C) temperature. Only traces of the nicked SV40 DNA II and no linear DNA III could be detected after I hr of the incubation of the compact minichromosomes (7 µg of DNA per ml) with 2 µg/ml of the nuclease at 37°C (Fig.7h; cf. Fig.7a). Staphylococcal nuclease was enzymatically active under the above-stated solvent conditions since the same sample of the nuclease which did not attack the compact SV40 minichromosomes (Fig.7) rapidly digested un-

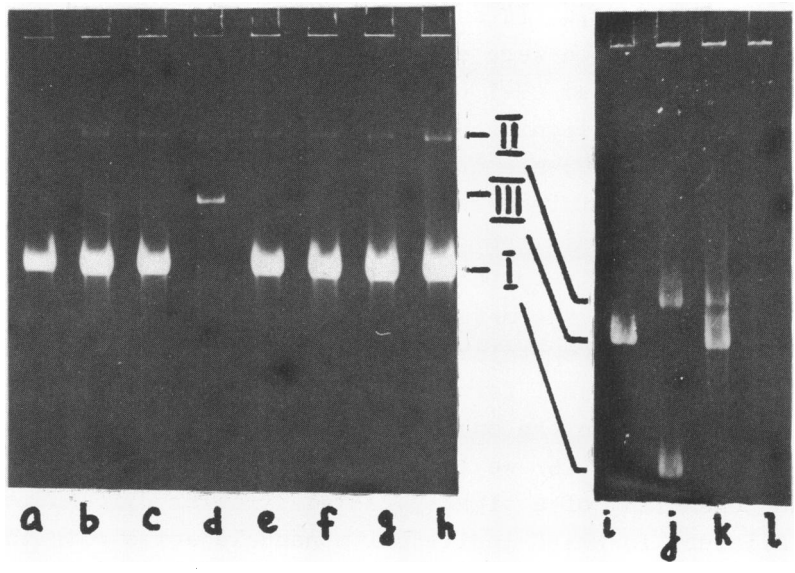

Fig. 7. Compact SV40 minichromosome is resistant to staphylococcal nuclease.

Isolated SV40 minichromosomes were treated with nuclease in the presence of 0.13 M NaCl at ~4°C for 0 (no enzyme), 3, 10, 30 and 60 min, respectively (slots a-c, e-f) followed by incubation at 37°C for 10 and 60 min, respectively (slots g and h) and agarose gel electrophoresis of DNA. Slot d contains the linear SV40 DNA III marker obtained by treatment of the SV40 DNA I with EcoRI restriction endonuclease.

Slab i-l are from another electrophoretic run and contain the linear SV40 DNA III marker (slot i) and DNA from DNase I-digested compact minichromosomes. The digestion was carried out as described in Methods (5 µg of DNase I per ml) at ~4°C for 1 min (slot g) followed by digestion at 37°C for 30 sec (slot k) and 5 min (slot l). Most of DNA fragments in l were less than 500 bp long (data not shown) and therefore were electrophoresed out of the gel under the conditions used.

der identical solvent conditions both the naked SV40 DNA I and the isolated mouse Ehrlich tumor chromatin to acid-soluble oligonucleotides and to the mono- and dinucleosomes, respectively (data not shown; see Methods for technical details). In addition, the same sample of the nuclease readily digested the partially unfolded minichromosomes in I mM $CaCl_2$, I mM TEA-HCl, pH 7.5 as was already shown previously /I,20/ (see ref. I for DNP electrophoretic patterns of the digests). To check a possibility of existence of nuclease-sensitive minichromosomes in a largerly nuclease-resistant population of the

compact minichromosomes we analysed not only the ethanol-precipitated DNP particles after nuclease digestion of the SV40 minichromosomes (see Methods) but also the corresponding supernatants. It was found that virtually all ^{3}H-labelled SV40 DNA I in the preparations of the compact minichromosomes remains intact after treatment with staphylococcal nuclease. Furthermore, the nuclease-resistant state of the compact minichromosome is apparently quite stable upon storage at ~4°C, since the result of nuclease treatment of the minichromosomes on the fourth day after their isolation was indistinguishable from that obtained with freshly prepared compact minichromosomes.

Since staphylococcal nuclease apparently preferentially attacks internucleosomal DNA stretches (linkers) in the chromatin /I3,I6,I7/, the observed resistance of the compact SV40 minichromosome to the nuclease strongly suggests that in particular nuclease-sensitive parts of the linker regions are not exposed on the outside of the compact minichromosome.

On the other hand, we have found that in contrast to staphylococcal nuclease pancreatic DNase (DNase I) readily attacks the compact SV40 minichromosome producing at first SV40 DNA II with multiple nicks and upon a longer digestion a linear SV40 DNA III, and its degradation products (Fig.7, 1). This result correlates with the previously established mode of action of DNase I on the chromatin according to which DNase I attacks both inter- and intranucleosomal DNA stretches with comparable efficiences /2,3/.

DISCUSSION

Presence of histones /I,2I,27,28/, nucleosomal organization /I,I8-20/, and the existence of a compact form of the SV40 minichromosome (see above) make it a very useful model of larger and probably more complex higher order structures of the cellular chromatin. Possible limitations of the SV40 system as a model one will be mentioned below, and now we shall briefly enumerate those facts about the SV40 minichromosome which we believe to be of the major importance for understanding of structural aspects of the problem.

I) The SV40 minichromosome isolated from lytically infected cells /I8-20/ or assembled in a partially purified in vitro system /29,30/ is a complex of the circular covalently closed SV40 DNA 5,000±200 bp in length with five eukaryotic histones including HI /I/.

2) There are on average 25±2 negative superhelical turns in the isolated SV40 DNA in 0.2 M NaCl at 37°C /3I-33/.

3) There are on average 2I±2 nucleosomes in the extended (circular beaded) form of the SV40 minichromosome /I8-20/.

4) Replication of DNA in the SV40 minichromosome proceeds bidirectionally from a fixed origin /34,35/. There is apparently a tightly DNA-bound protein(s) at or near the origin of replication in the SV40 minichromosome /36,37/.

5) The isolated SV40 minichromosome exists in solution under physiological ionic conditions apparently as a roughly spherical particle approximately 300 Å in diameter (see Figures 5 and 6).

6) In spite of such a compact conformation of the SV40 minichromosome which in this state is capable of fitting within the virus capsid, the individual nucleosomes can be readily visualized within the minichromosomal particle (see Fig.6).

7) Some views of the stained compact minichromosome suggest (but do not proove) a solenoidal coiling of the nucleosomal filament with about three turns of the nucleosomal helix per minichromosome (see Fig.6).

8) The compact state of the minichromosome depends on the presence of histone HI and on maintenance of appropriate ionic strength of solution ($\mu \approx 0.I5$) (see above). On the other hand, the compact state of the minichromosome apparently does not depend on the existence of a nick in the SV40 DNA, probably because the relaxation of the structure is prevented by histone--histone and/or DNA-histone interactions in the compact particle (unpublished data).

9) The compact form of the SV40 minichromosome in contrast to its partially or fully extended form is virtually completely resistant to staphylococcal nuclease (see Fig.7). This finding strongly suggests that nuclease-sensitive parts of the internucleosomal DNA stretches (linkers) are not ex-

posed on the outside of the compact minichromosomal particle.

A model for the structure of the compact minichromosome should account for its approximately spherical shape (see above). Although icosahedral symmetry of the small spherical viruses and in particular, of the SV40 virus /38,39/ suggests that the isolated compact minichromosome may also have approximately icosahedral or dodecahedral symmetry, some other possibilities are also not excluded. We shall briefly consider below some of the possible models for the compact minichromosome which are formally compatible with the presently available experimental data.

Although we start discussion from a solenoidal coiling of a circular oligonucleosomal fiber ~100 Å in diameter which consists of 20-22 nucleosomes it should be emphasized that other ("nonsolenoidal") models are by no means excluded and one of such models is briefly considered at the end of the paper (see **below**). A solenoidal coiling of a 100 Å fiber in cellular chromatin is generally assumed to be responsible for generation of the experimentally observed 200-300 Å chromatin fiber /40-42/. Since the compact form of the minichromosome is apparently completely resistant to staphylococcal nuclease (see above) we suggest that <u>all</u> internucleosomal (linker) DNA regions in the compact minichromosome face the inside surface of the 300 Å minichromosomal solenoid. Some views of the stained compact minichromosome suggest that a solenoidal coiling of the nucleosomal filament consists of approximately three turns of the nucleosomal helix (see above). If this is indeed the case we are faced with the problem of a "return" because of the circularity of the coiled nucleosomal filament. The "return" fiber a priori may vary in length from a very short one (300-400 Å) (Fig.8a) to a relatively long coiled fiber which forms several turns within the solenoidal structure of the oligonucleosomal filament (Fig.8b). As concerning the chemical nature of the "return" fiber it may be either naked DNA or a DNA-histone fiber consisting of fully or partially unfolded nucleosomes or finally, a typical nucleosomal fiber. Since there are on the average 20-22 nucleosomes per 5,000±200 bp of DNA in isolated SV40 minichromosome (see

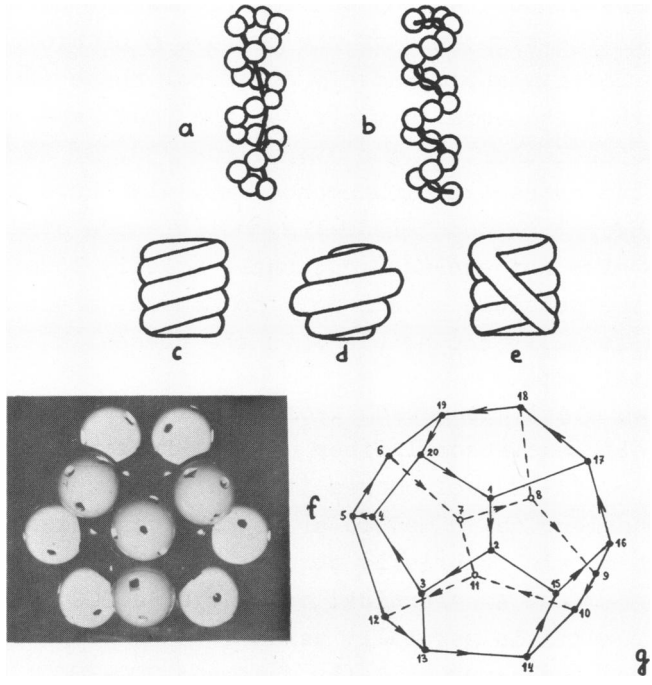

Fig. 8. Some of possible models of the compact SV40 minichromosome.
(a) and (b) are highly schematic drawings of a partially relaxed solenoidally coiled SV40 nucleosomal filament with a relatively short (a) and long (b) "return" fiber (see Discussion for details). Both a and b types of structure may correspond to general side views depicted roughly to-scale in (c) and (d). The model (c) is based on a constant number of nucleosomes per solenoidal turn (approximately six /39/). In the dodecahedral model (d) the number of nucleosomes per turn varies from approximately ten in the central turn to approximately five in the two other turns of the nucleosomal helix. The model (e) is an unlikely but formally not excluded minichromosome structure in which the two ends of the nucleosomal filament are joined together outside the helix. (f) A photograph of the model of the dodecahedral arrangement of nucleosomes in the compact SV40 minichromosome. The spheres correspond to histone "cores" of the nucleosomes. The model was photographed along a three-fold axis of symmetry of the dodecahedron (see Discussion). (g) A possible "nonsolenoidal" arrangement of nucleosomes within the framework of the dodecahedral model (see f). Nucleosomes of the circular oligonucleosomal filament are situated at the apexes of the dodecahedron and are connected to each other as indicated by arrows. Notice that this arrangement of nucleosomes does not require any "return" fiber (see Discussion). The dashed lines correspond to the back face of the dodecahedron.

above) one can calculate that there are from about 200 to about 800 bp of DNA which are apparently not used for nucleosome formation. Although an attractive "function" of such "excessive" DNA is to be a "return" fiber (Fig.8a,b) the question is insufficiently experimentally defined as to permit a more detailed pithy discussion.

Independently of the length of a "return" fiber (Fig.8a, b) a general side view of the above-considered structures is determined in particular, by the requirement of an approximately spherical shape of the compact minichromosome. The corresponding roughly to-scale drawings are shown in Figure 8c,d. The first model is based on a constant number of nucleosomes per a solenoidal turn (approximately six; see refs.4I,42) (Fig.8c). In the second model (Fig.8d) the number of nucleosomes per turn varies from approximately ten in the central turn to approximately five in the two other turns of the nucleosomal helix which gives the structure a more spherical shape.

Consider now a "superhelical" aspect of the problem. There are on average 25 ± 2 negative superhelical turns in the isolated SV40 DNA I under physiological ionic conditions and temperature /3I-33/. A priori these superhelical turns may be entirely due to the existence of nucleosomes in the minichromosome /32/ or, alternatively, some proportion of superhelical turns in the SV40 DNA I may be due to a higher order coiling of the nucleosomal filament in the compact minichromosome. If a higher order coiling in the minichromosome indeed plays a role in formation of superhelical turns in the SV40 DNA I (see below) the following important point should be emphasized. The final sign of those superhelical turns in the SV40 DNA which are introduced due to a higher order minichromosome structure depends not only on the sign of the higher order "solenoidal" turns (Fig.8) but also on the way of joining of the two opposite ends of the I00 Å fiber (Fig.8a-d; cf.Fig. 8e). It can be shown that a positively coiled helix which has its ends joined together by passing the "return" fiber along the axis of the helix (Fig.8a) will add negative supercoils to the SV40 DNA I, the exact number of supercoils being de-

pendent in particular, on the conformation of the "return" fiber (see ref. 43 for a proof). On the other hand, the same positively coiled helix which has its ends joined together outside the helix (Fig.8e) will add positive supercoils to the SV40 DNA I /43/. Although we consider this latter model (Fig.8e) unlikely, it is formally not excluded by the presently available data. It should be noted that in any case a maximum input of the higher order minichromosomal structure into the final superhelical state of the SV40 DNA I apparently does not significantly exceed three superhelical turns and may be even zero. As concerning experimental data on this point, both the relaxed appearance of the circular beaded SV40 minichromosomes in a low-salt buffer (Fig.5b; see also refs. I8-20,22,23) and results of direct determinations of a superhelix density of DNA from the compact and extended SV40 minichromosomes (unpublished data) suggest that the input of the higher order minichromosomal structure into the final superhelical state of the SV40 DNA I is close to zero. Thus the above topological considerations may be not essential for the particular case of the compact viral minichromosome.

It should be emphasized that the solenoidal packing of the type shown in Figure 8a-e is not the only possible way of the mutual arrangement of nucleosomes in the compact minichromosome. For example, 20 nucleosomes of the SV40 minichromosome may be situated at the 20 apexes of the dodecahedron (Fig.8f). This model also permits a solenoidal coiling of the circular nucleosomal filament providing that its opposite ends are joined together by a "return" fiber (see Fig. 8d). At the same time, it can be readily shown on the basis of properties of the dodecahedron that in contrast to the above-mentioned models (Fig.8a-e), the dodecahedral arrangement of nucleosomes (Fig.8f) provides another way of folding of the circular nucleosomal filament which does not require any "return" fiber (Fig.8g). Within the framework of this model "excessive" nucleosomes of those SV40 minichromosomes which contain more than 20 nucleosomes /I8-20/ occupy the cavity of dodecahedron (Fig.8f,g). Finally it should be noted that since both the icosahedron and dodecahedron have a five-fold axis of symmet-

ry, the dodecahedral or approximately dodecahedral symmetry of the compact SV40 minichromosome provides a natural basis for the experimentally observed icosahedral symmetry of the SV40 capsid /38,39/.

What is a role of histone HI in maintaining the compact structure of the minichromosome? Since histone HI-depleted minichromosomes exist in an extended (circular beaded) state (see Results and refs.22,23) it appears that HI plays a "cross-linking" role in the compact minichromosomal structure. Although several concrete arrangements of HI molecules within frameworks of the above-considered models can be suggested, the question seems to be insufficiently experimentally defined as to permit a detailed pithy discussion. As concerning the role of the ionic environment in stabilization of the compact state of the minichromosome (see above) it is probably similar to a role of counterions in stabilization of e.g., a compact Ψ-state of DNA in the presence of some neutral polymers /44, 45/. However some specific effects of particular inorganic ions on the compact minichromosomal structure cannot be excluded at the present time.

There are at least three obvious and significant differences between structural parameters of the SV40 minichromosome and cellular chromatin. Firstly, the 5,000-bp SV40 minichromosome is several orders of magnitude smaller than an average eukaryotic chromosome and about ten times smaller than an average chromomere /40/. Secondly, although the SV40 minichromosome contains a full complement of histones /I/, it apparently does not contain many other protein components of the cellular chromatin, in particular so-called HMG (high mobility group /46/) chromosomal nonhistone basic proteins (unpublished data). Thirdly, although DNA in the cellular chromatin appears to be constrained into independent supercoiled loops /47/, this constraint is apparently due to noncovalent protein-DNA and RNA-DNA interactions /47/ in contrast to a covalently closed state of DNA in the SV40 minichromosome.

However, in spite of these and possibly other limitations of the SV40 minichromosome as a model system, the already established striking similarity of the "nucleosomal" structure

of the minichromosome to that of the cellular chromatin /I8-20/ suggests that a characteristic "supranucleosomal" structure of the minichromosome described in this paper also has a "counterpart" in the cellular chromatin structures.

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