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Minichromosome of simian virus 40: presence of histone HI

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ABSTRACT

In contrast to conclusions of previous studies /I-3/ claiming the absence of histone HI from the SV40 and polyoma viral minichromosomes we have found that a preparation of purified SV40 minichromosomes does contain histone HI. The content of HI in relation to other four histones in the SV40 minichromosomes is close to that in the cellular chromatin. Histone HI in the isolated SV40 minichromosomes is bound apparently to internucleosomal DNA stretches as was shown already for HI in the cellular chromatin /4/.

In addition it was found that more than 90% of the purified SV40 minichromosomes migrated as a single discrete deoxyribonucleoprotein band upon agarose gel electrophoresis.

INTRODUCTION

Recent biochemical and electron microscopic evidence strongly suggests that the structure of eukaryotic chromatin fiber is based on repeating subunits (nucleosomes) /4-19/. In this respect Simian Virus 40 (SV40) appears to be a particularly attractive experimental object, since it has been found that SV40 DNA and cellular histones are associated in infected cells and in SV40 virions in a chromatin-like structure called a minichromosome /1-3, 20-22/. The SV40 and polyoma minichromosomes were visualized in the electron microscope as ring-shaped beaded fibers which consisted of 20-22 nucleosomes joined by short DNA-like threads /20-22/.

Analysis of histones in the SV40 and polyoma minichromosomes /1-3/ has led to the now widely accepted belief that the minichromosomes contain four kinds of histones (H2a, H2b, H3 and H4) but completely lack the lysine-rich histone HI /1-3, 21,22,25,26/. However, we have found that the purified SV40 minichromosome does contain histone HI, the content of

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HI in relation to other four histones being close to its relative content in the cellular chromatin.

MATERIALS AND METHODS

Virus and cells. Four times plaque-purified SV40 virus (strain 777) used throughout this study was grown on monolayers of monkey kidney cells (CV-I) in roller bottles. Cells were grown to confluence in Eagle medium which contained half amounts of amino acids and vitamins, 0.25% lactalbumin hydrolysate and I0% calf serum. Cultures were infected with SV40 24 to 36 hr after confluence at a multiplicity of about I0 PFU per cell and the virus was allowed to adsorb for 60 min at 37°C. After adsorbtion 70 ml of Eagle medium supplemented with I% calf serum were added to each roller bottle which contained 4-5xI0^7 cells and the incubation was continued for 36 hr at 37°C. Thereafter /Me-^{3}H/thymidine (I5 Ci/mmole) was added to a final concentration of I0 μCi/ml and the incubation was continued for additional 6-8 hr.

Isolation and purification of SV40 minichromosomes. The procedure used was a modification of the previously developed methods /I,2I-24/. Labelled cells at 42-44 hr after infection were washed thrice with 0.I4 M NaCl. Each roller bottle was then treated with 2-3 ml of 0.25% Triton X-I00, I0 mM Na-EDTA, I0 mM triethanolamine (TEA)-HCl, pH 7.8. The cells were allowed to swell for I0 min at room temperature, thereafter cooled to 3-4°C and I M NaCl was added to a final concentration of 0.I5 M. The lysate obtained was decanted from the bottles or gently scraped with a rubber policeman and thereafter centrifuged at 5,000 g for 5 min at 0-3°C. The cytoplasmic supernatant which contained in different experiments from I0 to 30% of the total ^{3}H-labelled SV40 DNA was not studied in this work. The nuclear pellet from 8-I2xI0^8 cells was resuspended in 30 ml of 0.25% Triton X-I00, 0.I5 M NaCl, I0 mM Na-EDTA, I0 mM TEA-HCl, pH 7.8 followed by extraction of the SV40 minichromosomes at 3-4°C for 2.5 hr with continuos gentle stirring by a glass rod. The suspension was then centrifuged at 5,000 g for I5 min and the supernatant (30 ml) which contained I-2 mg of the SV40 minichromosomes was layered onto 600 ml of a li-

near 10-30% sucrose gradient in 0.15 M NaCl, 1 mM Na-EDTA, 1 mM TEA-HCl, pH 7.8 which was prepared in the Ti 14 zonal rotor (Beckman). The rotor was centrifuged at 45,000 rpm for 3 hr at 3°C. More than 95% of the total ^{3}H in the extract were recovered in a sharp symmetrical peak of the SV40 minichromosomes which sedimented at about 55 S (Fig.1). The specific radioactivity of the SV40 ^{3}H-DNA ranged in different experiments from 20,000 to 40,000 cpm/µg and was equal to that of the cellular ^{3}H-DNA. Peak fractions (see Fig.1) were pooled, dialysed overnight against 1 mM Na-EDTA, 1 mM TEA-HCl, pH 7.8 and thereafter concentrated to about 500 µg of DNA per ml by ultrafiltration in the Amicon apparatus. No aggregation of the minichromosomes occured during ultrafiltration. The concentrated solution of minichromosomes was directly used for the next experimental step or alternatively, preliminarilly passed through a Sephadex G25 column (equilibrated with 1 mM TEA-HCl, pH 7.6) in order to remove EDTA . The yield of the minichromosomes after a second (overnight) extraction of the nuclear pellet constituted 20-40% of the yield in the first 2.5-hr extract. In small-scale experiments the minichromosomes were purified by sucrose gradient centrifugation in the SW25.2 rotor (Beckman).

Polyacrylamide gel electrophoresis of deoxyribonucleoproteins. Concentrated staphylococcal nuclease-treated deoxyribonucleoprotein samples were electrophoresed in 5 or 6% polyacrylamide slab gels as described previously /4/. The buffer in the electrode vessels and in the gels was 2 mM Na--EDTA, 10 mM TEA-HCl, pH 7.6. The gels were stained with ethidium bromide for DNA or alternatively, with Coomassie Brilliant Blue for proteins /4/.

SDS-gel electrophoresis of proteins. It was carried out in 15% polyacrylamide slab gels using a discontinuous system of Laemmli /27/ as modified by Weintraub et al. /17/.

Electron microscopy of minichromosomes. The samples were fixed overnight with 1% formaldehyde (pH 7.6) or alternatively, analysed in the absence of fixing. The samples were dialysed against 5 mM NaCl, 1 mM TEA-HCl, pH 7.6, thereafter diluted to 3-10 µg of DNA per ml with the same buffer followed

by application to carbon- or I% Parlodion-coated grids. After I5-60 sec, the excess liquid was removed from the grid followed by washing of the grid on the surface of H_2O drop for 3-5 min. Washed grids were dried on filter paper and thereafter rotatory shadowed with Pt-Pd (4:I) at an angle of 6°. The grids were examined in a JEM-I00B electron microscope (Jeol) at an instrumental magnification of I5,000.

Agarose gel electrophoresis of minichromosomes. From 0.5 to 3 µg of the minichromosomes (I0-20 µl) in I mM Na-EDTA, pH 7.6 were loaded onto I% agarose gel (0.6 cm diameter, 7 cm long). The buffer in the electrode vessels and in the gels was 2 mM Na-EDTA, I0 mM TEA-HCl, pH 7.6. Electrophoresis was carried out at 5 mA per gel until Bromphenol blue tracking dye reached the end of the gel. Minichromosomes were stained with ethidium bromide (0.5 µg/ml in H_2O).

RESULTS AND DISCUSSION

Minichromosomes of the SV40 virus were extracted from infected monkey kidney cells as described in Methods and thereafter centrifuged through a sucrose gradient in the Ti I4 zonal rotor (Fig.I). Minichromosomes sedimented as a sharp sym-

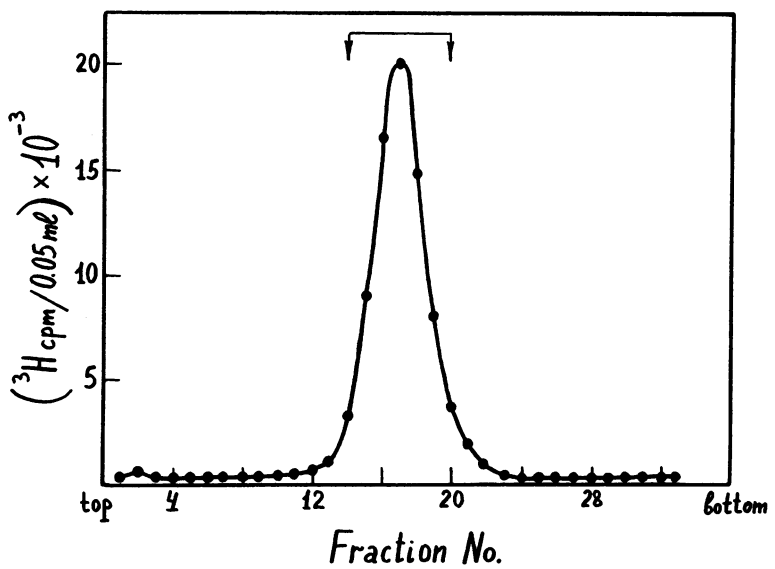

Fig.I. Sucrose gradient centrifugation of SV40 minichromosomes in the Ti I4 zonal rotor. See Methods for detail.

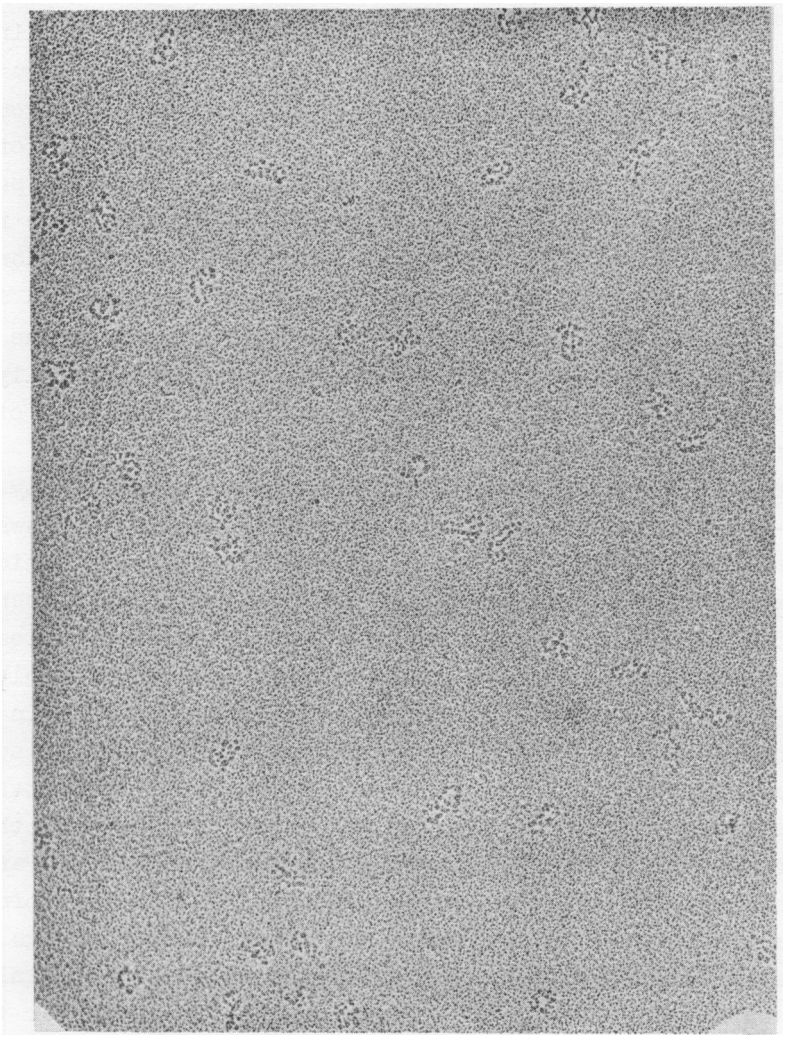

Fig.2. Low-magnification electron micrograph of purified SV40 minichromosomes. Magnification X 40,000.

metrical peak at 55-60 S, the peak contained essentially all the radioactivity of the initial extract.

Figure 2 illustrates an electron microscopic appearance of the purified minichromosomes. One can see numerous ring--shaped beaded fibers with a characteristic appearance of the SV40 or polyoma minichromosomes (cf. refs. 20-22). For the present study the most important feature of this typical micrograph is the absence of any significant amounts of contami-

nating material. Specifically, there were virtually no linear fibers, no large clumps etc., that is, almost all structures on the grid could be unambigiously identified as minichromosomes (Fig.2). Furthermore, agarose gel electrophoresis of DNA from the purified minichromosomes showed that the major part of DNA was a covalently closed supercoiled SV40 DNA, the remaining part being circular nicked SV40 DNA (data not shown). Thus both electron microscopy of the minichromosomes and analysis of their DNA suggest a high degree of purity of the minichromosome preparation. This conclusion was additionally strengthened by the results of electrophoretic analysis of the minichromosomes in agarose gels (see below).

Protein composition of the minichromosomes was analysed by SDS-polyacrylamide gel electrophoresis. Figure 3a shows a protein composition of the purified minichromosomes which were extracted from the nuclei of CV-I cells in the presence of 0.I5 M NaCl. One can clearly see that the minichromosome protein complement consists of five histones including histone HI (Fig.3a). Histone HI as well as other four histones in the minichromosomes were unambigiously identified by comparison with reference histone patterns from mouse Ehrlich ascites tumor chromatin /28/ and also by gel electrophoresis of the minichromosomal histones in acetic acid-urea system of Panyim and Chalkley /29/. In the acetic acid-urea system /29/ proteins are separated on the basis of either size or charge, whereas in the SDS system /I7/ the separation occurs mainly on the basis of sizes of protein molecules. Densitometer tracings of the histone patterns of the minichromosomes (Fig.3a) were compared with those of the nuclei isolated from uninfected (Fig.3c,d) and infected (Fig.3e) CV-I cells and also with densitometer tracings of histone patterns of mouse Ehrlich ascites tumor chromatin /I2,28/. Such a comparison has shown that the content of HI in the SV40 minichromosomes (Fig.3a) in relation to other four histones is practically identical to the relative content of HI in chromatin from either CV-I (Fig. 3d-e) or mouse Ehrlich tumor cells. However, when the SV40 minichromosomes were extracted from the nuclei of infected CV-I cells at a higher ionic strength (0.20 M NaCl instead of

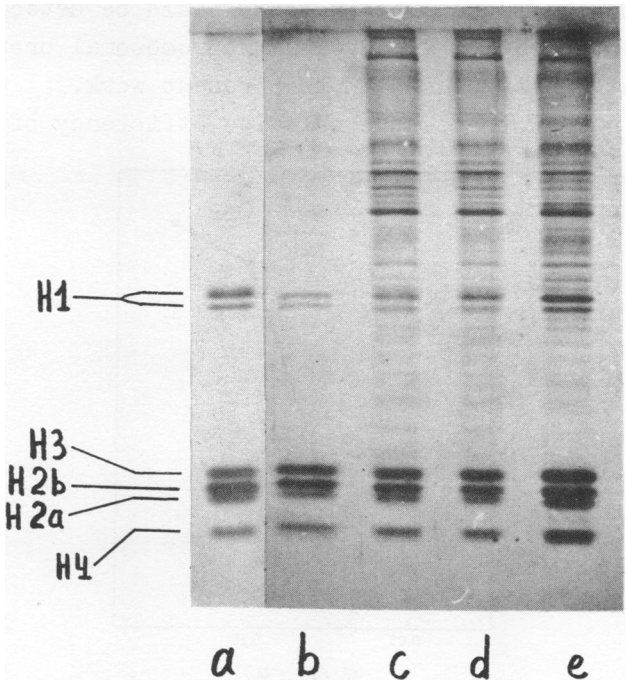

Fig.3. SDS-gel electrophoretic analysis of SV40 minichromosomal and cellular (CV-I) chromatin proteins. Proteins from:
(a) SV40 minichromosomes isolated in the presence of 0.15 M NaCl;
(b) The same as (a) but the minichromosomes were isolated in the presence of 0.20 M NaCl;
(c) Crude chromatin from uninfected CV-I cells which were incubated at 37°C for 30 min in 0.3 mM $CaCl_2$, I mM TEA-HCl, pH 7.6. The crude chromatin preparation was obtained by repeated extractions of the nuclei with 0.25% Triton X-100, 0.15 M NaCl, 10 mM Na-EDTA, 10 mM TEA-HCl, pH 7.6;
(d) The same as (c) but the crude chromatin was not incubated at 37°C;
(e) The crude chromatin from SV40-infected CV-I cells.

0.15 M NaCl in the extraction buffer; see Methods) the relative content of histone HI in the minichromosomes (Fig.3b) was found by densitometry to be 1.5–1.7 times lower than the relative content of HI in the 0.15 M NaCl-extracted minichromosomes (Fig.3a; cf. Fig.3b).

It should be noted that although the relative content of nonhistone proteins was very high in crude chromatin from either uninfected (Fig.3c,d) or infected (Fig.3e) CV-I cells,

virtually no nonhistone protein bands could be detected in the SDS-gel patterns of the SV40 minichromosomal proteins (Fig.3a,b) at loadings used in the present work.

Figure 4 illustrates the relative efficiency of extrac-

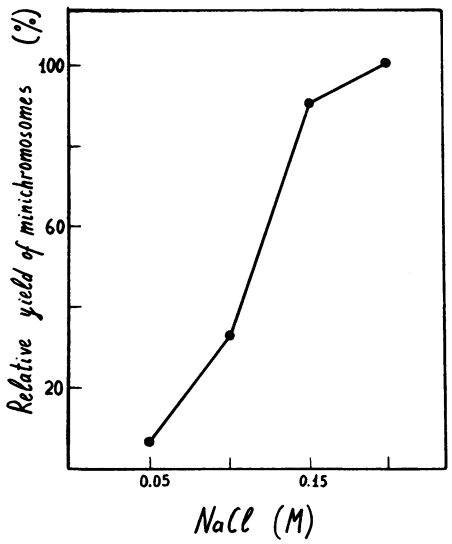

Fig.4. Dependence of relative efficiency of minichromosome extraction on the ionic strength of extraction buffer.

The yield of the SV40 minichromosomes in a 2.5-hr nuclear extract in the presence of 0.20 M NaCl (plus 0.25% Triton X-100, 10 mM Na-EDTA, 10 mM TEA-HCl, pH 7.8) was arbitrarily taken as 100%. See Methods for detail.

tion of the SV40 minichromosomes at different ionic strengths of extraction buffers. The efficiency of extraction was not changed significantly between 0.20 M NaCl and 0.I5 M NaCl but dropped to less than 40% in 0.I0 M NaCl and approached zero in 0.05 M NaCl (Fig.4).

Previous work with chromatin from mouse Ehrlich ascites tumor cells has shown that a mild digestion of the chromatin with staphylococcal nuclease produces chromatin subunits (mononucleosomes),their dimers (dinucleosomes) and also higher nucleosome oligomers which are heterogeneous with regard to the content of histone HI /4/. Specifically it has been found that a mild staphylococcal nuclease digest of the chromatin contains mononucleosomes of two discrete types which could be

separated from each other by polyacrylamide gel electrophoresis. Mononucleosome of the first type contained all five histones, whereas the mononucleosome of the second type lacked histone HI and in addition its DNA segment was approximately 30 base pairs shorter than the DNA segment of the mononucleosome of the first type /4/. Similar "HI-dependent" heterogeneity existed in nuclease-produced dinucleosomes and probably also in higher nucleosome oligomers. These and related findings strongly suggested the location of histone HI in the internucleosomal regions of chromatin fibers /4/.

We treated the purified SV40 minichromosoms with staphylococcal nuclease followed by separation of deoxyribonucleoprotein particles formed by electrophoresis in polyacrylamide gels. The patterns obtained were quite similar to the patterns which were given previously by nuclease-treated mouse Ehrlich tumor chromatin (Fig.5; cf. ref.4). At low times of digestion all the material was trapped at the origin of the gel (Fig.5 a,b). Longer digestion produced mono-, di- and oligonucleosomes which displayed a characteristic pattern in polyacrylamide gels (Fig.5c-i, k-n). Mononucleosomes were separated into two most rapidly migrating bands, the lower band being the histone HI-depleted mononucleosome, whereas the upper band contained a full complement of histones (see ref.4 for a detailed description of such patterns and of the methods used to study them). The relative amount of the HI-depleted mononucleosome in the total nuclease digest of the SV40 minichromosomes increased upon an increase of the time of digestion (Fig.5 c-i). Sufficiently long incubations of the minichromosomes with nuclease resulted in a digestion of all nucleosomes to limit-digest insoluble particles (Fig.5 h,i) as was already observed with various chromatins /4,9,10,12/. Figure 5j shows the electrophoretic pattern of the nuclease-digested crude chromatin from uninfected CV-I cells, which is similar to the patterns produced by either digested SV40 minichromosomes (Fig.5 a-i) or digested mouse Ehrlich tumor chromatin /4/. No significant degradation of histones (including HI) occured during incubation at 37°C in either the SV40 minichromosomes (data not shown) or the crude CV-I chromatin (Fig.3d; cf.

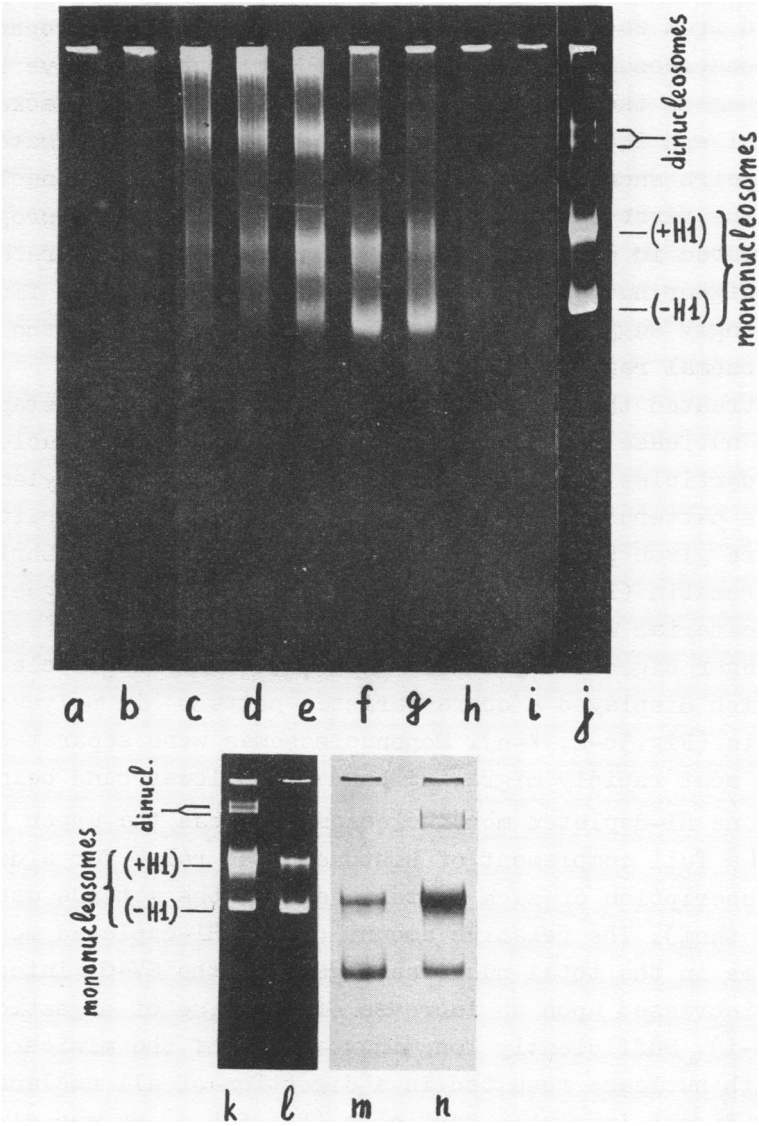

Fig.5. Polyacrylamide gel electrophoresis of nuclease-digested SV40 minichromosomes. Purified minichromosomes (500 µg of DNA per ml) were incubated with staphylococcal nuclease (10 µg/ml) in 0.3 mM $CaCl_2$, 1 mM TEA-HCl, pH 7.6 at 37°C as described previously for mouse Ehrlich tumor chromatin /4,12/. The reaction was stopped by addition of 50 mM Na-EDTA, pH 7.6 to a final concentration of 1 mM. Any insoluble material was removed by low-speed centrifugation and the samples were loaded onto a low-ionic-strength 5% polyacrylamide slab gel (see Methods for detail). (a) Undigested SV40 minichromosomes; (b)-(i) Minichromosomes digested with staphylococcal nuclease for 0.1, 0.2, 0.5, 1, 3, 7, 15 and 30 minutes, respectively; (j) Total 15-min nuclease digest of the crude chromatin from uninfected CV-1 cells; (k) and (l) The same as (e) and (g) but the samples were electrophoresed in 6% gels; (m) and (n) The same as (l) and (k) but deoxyribonucleo-protein bands were stained with Coomassie instead of ethidium bromide.

Fig.3c).

Finally, we have found that the purified SV40 minichromosomes migrated as a single discrete deoxyribonucleoprotein band upon a low-ionic-strength agarose gel electrophoresis

(Fig.6a). The electrophoretically purified minichromosomes also contained all five histones (data not shown). Higher loadings revealed a faint minor band which migrated slightly ahead of the major minichromosomal band (Fig.6b). Work is now in progress to determine the composition of this minor band. It should be emphasized that a low ionic strength (~ 0.01) during agarose gel electrophoresis was a necessary condition to obtain discrete minichromosomal bands. For example, electrophoresis at a five-fold higher ionic strength of solution resulted in a significant aggregation of the minichromosomes and in the loss of discrete patterns. Electrophoretic analysis of the purified SV40 minichromosomes in agarose gels additionally confirms a high degree of purity of the minichromosome preparation.

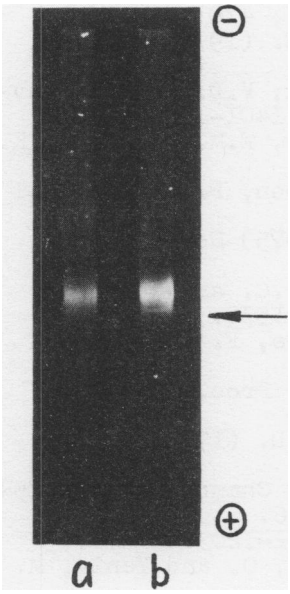

Fig. 6. Low-ionic-strength agarose gel electrophoresis of purified SV40 minichromosomes.
(a) 0.6-0.8 μg of minichromosomes;
(b) the same as (a) but a 3-fold higher loading.
Arrow indicates the minor band which is detected in overloaded gels.

The major result of this work is the finding of histone HI in the SV40 minichromosomes. Thus the SV40 minichromosome apparently contains a full complement of histones. This finding makes the minichromosome an even more promising experimental object for studies on chromatin structure, replication and transcription than was considered previously.

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