Volume 8 Number 3 1980 Nucleic Acids Research

Extraction of transcribing SV40 chromosomes in very low salt

Howard J.Edenberg

Department of Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46223, USA

Received 14 December 1979

ABSTRACT

SV40 chromosomes capable of continued RNA synthesis *in vitro* have been extracted from infected cells in solutions of very low ionic strength (5 mM HEPES, 0.25 mM $MgCl_2$). The RNA made is of high molecular weight, and synthesis is sensitive to α-amanitin. More RNA is made than from previously described (high salt-extracted) transcription complexes. Transcribing SV40 chromosomes sediment at a rate intermediate between replicating and mature chromosomes, and have a higher ratio of protein to nucleic acid than either. They retain proteins that are lost during exposure to high salt, and might prove valuable in identifying proteins involved in SV40 transcription.

INTRODUCTION

Simian virus 40 has proven to be a useful model with which to study the chromosome structure, replication, and gene expression of mammalian cells. The use of *in vitro* transcription complexes has added to our knowledge of the template for SV40 transcription (2,3,14-16,19-23). SV40 exists within cells as a nucleoprotein complex (44), containing histones (7,8,25,26,30,33,34,43) and other proteins (e.g. 7,13,31,34,40,42). Two methods of extracting SV40 transcription complexes have previously been reported: one uses Sarkosyl to extract SV40 DNA with RNA polymerase attached but lacking most other proteins (2,3,14,15,19,20,29,41), and the other uses Triton X-100 in the presence of 0.4M NaCl (5,21-23). Both methods share the disadvantage of removing some of the proteins normally found associated with intracellular SV40. An *in vitro* transcription complex which retained more of these proteins could be useful in studying factors that modify transcription or proteins that process the primary transcript. We decided to examine whether conditions similar to those which extract replicating SV40 chromosomes (11-13)

allow the isolation of transcription complexes.

This report describes the extraction and characterization of transcribing SV40 chromosomes which retain proteins that are lost in high salt.

MATERIALS AND METHODS

Cells, virus, and labelling:

CV-1 cells obtained from Dr. J. Huberman and Dr. M. DePamphilis were grown at 37°C in 150 mm plastic culture dishes (Lux) in Minimum Essential Medium (autoclavable, GIBCO F-17) supplemented with 2mM glutamine and 10% fetal bovine serum (Flow). Stocks of SV40 were grown at low multiplicity (.01-.04 PFU/cell) from a stock originally from Dr. P. Tegtmeyer. Confluent cells were infected with SV40 at input multiplicity of approximately 2 PFU/cell, and kept in medium with 2% fetal bovine serum. Where specified, [methyl-^{14}C] thymidine was added at 0.05 μCi/ml, 57 mCi/mmol (Amersham) at 18-24 h after infection, to label mature SV40 DNA. To label replicating chromosomes, immediately before extraction the medium was removed from several dishes of cells and 2 ml per dish of TS buffer (20mM Tris-HCl (pH 7.4), 137mM NaCl, 5mM KCl, 1mM $CaCl_2$, 0.5mM $MgCl_2$) containing 2% fetal bovine serum and 100 μCi/ml of [methyl-^{3}H] thymidine at 47.5 Ci/mmol (Amersham) were added for 4 minutes at 37°C.

Preparation of SV40 chromosomes in low salt:

SV40 chromosomes were extracted from infected cells at 36-40 h post infection in solutions of extremely low ionic strength, as described previously (13). Briefly, "chromatin bodies" (washed, swollen nuclei lacking membranes (11)), were prepared in 0.1M sucrose, 0.2mM K_2HPO_4, pH 7.5. SV40 chromosomes were extracted by adding HEPES (pH 7.8) to 5mM, dithiothreitol to 0.5mM, and $MgCl_2$ to 0.25mM; cellular chromatin was pelleted. The supernate was then sedimented through 10 to 25% sucrose gradients in 0.2mM K_2HPO_4 (pH 7.5) over a 0.4 ml shelf of 70% sucrose in the Beckman SW41Ti rotor at 40000 rpm, 2°C for 135-150 min. Fractions were collected from the bottom of the gradient. Radioactivity was determined on aliquots either 1) dried on filters, 2) acid precipitated, filtered and washed, or 3) added directly to Aquasol II (New England Nuclear). For methods 1 and 2, filters were placed into toluene containing 5g/l PPO + 0.125 g/l

dimethyl-POPOP. Radioactivity was determined in a Beckman LS-8000 scintillation spectrometer.

Comparison with high-salt extract:

Infected cells were split into 2 groups: one extracted as above and the other essentially as described by Green & Brooks (22). Briefly, dishes were rinsed twice in PBS lacking Ca and Mg, drained, and 1.6 ml of 10mM Tris-HCl,pH 7.9, 10mM EDTA, 0.25% Triton X-100, 0.5mM dithiothreitol, was added to each dish on ice. After 10 min, 0.4 ml of 2 M NaCl was added and after a further 10 min cells were gently scraped into a tube and centrifuged at 4000xg, 2°C, for 10 min. The cloudy supernate was concentrated by sedimentation in a discontinuous gradient of 0.5 ml 70% sucrose, 1 ml 50% sucrose, and 2 ml 10% sucrose (all in 10mM Tris HCl pH 7.9, 0.2mM EDTA, 0.4M NaCl) in the SW41Ti rotor at 40000 rpm, 2°C, 150 min. Transcription complexes were recovered from the 50% sucrose.

Assays for RNA Synthesis in vitro:

Assays were designed to detect incorporation of [^{32}P]rUTP into polynucleotides under conditions optimized for cellular RNA polymerases. Assays were carried out in either 50 or 100 µl total volume containing 50mM Tris-HCl (pH 7.8), 2mM $MnCl_2$, 400 µM each of rATP, rGTP, rCTP; 15 µM rUTP + [^{32}P]rUTP (New England Nuclear or ICN, final specific activities 800 to 8000 cpm per picomole), 400 µg/ml bovine serum albumin. For different experiments, the assays also contained varying amounts of ammonium sulfate, purified salmon testes DNA, and α-amanitin as noted. Assays contained aliquots directly from the sucrose gradients constituting 40 to 60% of the total volume, and were carried out at 30°C unless otherwise noted. Assays were stopped by transferring the tubes to an ice-water bath; the assay mixtures were pipetted onto pieces of DE-81 ion exchange paper (Whatman) and washed at least 6 times with 0.4 M K_2HPO_4, twice each with water and 95% ethanol, dried, and radioactivity determined as above. Using DE-81 paper gave low and reproducible backgrounds; assays were done in triplicate. Specific activity was determined from aliquots of the assay mixture spotted on DE-81 pieces.

Size of RNA made in vitro:

RNA was synthesized in the presence of 0.4 M ammonium sulfate for 50 min at 37°C. The reaction was stopped with SDS to 0.5% and EDTA to 25 mM and the mixture sedimented through a Sephadex G-25 column to

remove most free [^{32}P]rUTP (5). RNA was precipitated with ethanol in the presence of 25 µg of yeast tRNA as carrier and resuspended in 10mM NaCl, 10mM Tris HCl, 50mM EDTA, 0.5% sarkosyl (all at pH 8.0). The mixture was placed at 95° for 7 min, quenched on ice, and sedimented through 5 to 20% sucrose gradients (50mM Tris, pH 8.0, 5mM EDTA, 0.95M NaCl) in the Beckman SW50.1 rotor for 3 h, 20°C. Fractions were collected from the bottom and acid precipitated in the presence of carrier DNA, filtered, washed, and radioactivity determined as above.

Density of the transcription complexes:

The relative density of the transcription complexes was determined by centrifugation in gradients of metrizamide (Nyegaard). SV40 chromosomes were extracted as described. One portion was allowed to synthesize RNA *in vitro* for 30 to 60 min at 30°C in the presence of 0.3M ammonium sulfate, under standard conditions. The reaction was terminated by chilling on ice and adding EDTA (pH 7.5) to 2.2mM; most free [^{32}P]rUTP was removed by sedimentation through Sephadex G-25 in M buffer (0.1M sucrose, 0.2mM K_2HPO_4, 10mM HEPES pH 7.8). The other portion (no reaction) was held on ice. Each sample was adjusted to 1.8 ml and 25% metrizamide, and layered over preformed discontinuous gradients of metrizamide in M buffer with 0.5mM dithiothreitol (1.6ml 40%, 1.6ml 33%). Centrifugation was for 18 h at 45000rpm, 4°C in the Beckman SW50.1 rotor. Gradients were fractionated from the bottom, and the refractive index determined. SV40 DNA was run in a parallel gradient as a marker. Aliquots were then either acid precipitated to determine radioactivity or used for *in vitro* synthesis of RNA, both as described. Similar experiments were performed and sedimented on discontinuous gradients of metrizamide at different average densities; the effect of brief treatment with 0.4M NaCl on the density of SV40 chromosomes was similarly examined (see legend of Figure 5).

RESULTS

Extraction and sedimentation of SV40 transcription complex.

Infected cells were lysed and "chromatin bodies" prepared at very low salt concentrations (0.2mM K_2HPO_4, 0.1M sucrose, pH 7.5); this washes away cytoplasmic and loosely associated constituents (11). SV40 chromosomes remain associated with chromatin bodies and can be

extracted either by gentle homogenization (11,12) or by adding magnesium to 0.25 mM and buffer to 5 mM (13). Sedimentation of the extract through neutral sucrose gradients separates the faster sedimenting replicating SV40 chromosomes from the mature SV40 chromosomes, as shown in Figure 1A,C. Aliquots of the gradient fractions were tested for the ability to synthesize RNA *in vitro*, and RNA polymerase activity was found to cosediment with a subset of these SV40 chromosomes (Figure 1B,C). The bulk of the RNA polymerase activity sediments faster than mature SV40 chromosomes but not as rapidly as replicating chromosomes. The *in vitro* RNA synthesizing activity which cosediments with SV40 chromosomes utilizes the

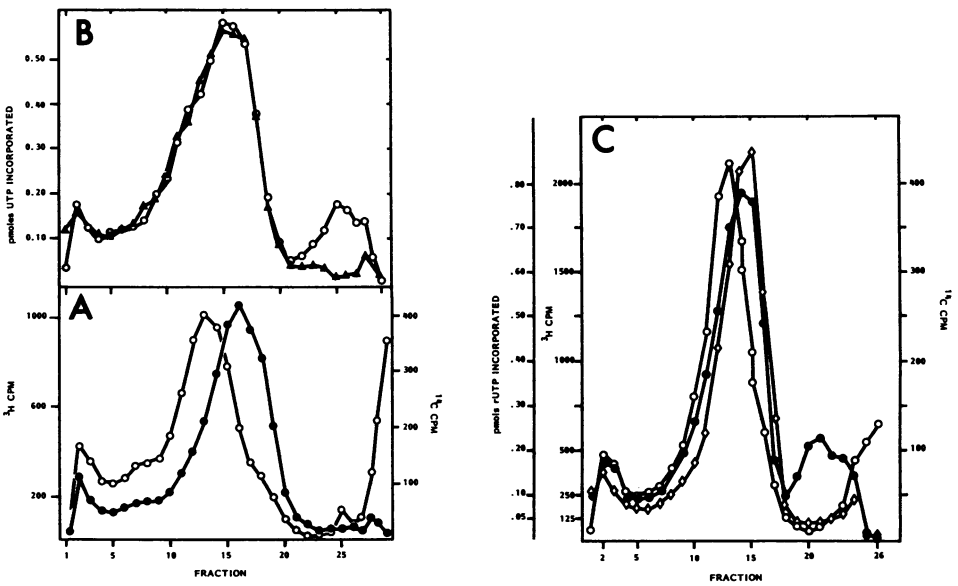

Figure 1: Sedimentation of SV40 chromosomes and detection of transcription complexes: A. Sedimentation: SV40 chromosomes were prepared and sedimented as described in Materials and Methods. 50 µl aliquots were added to Aquasol II to determine ^{3}H (O; replicating chromosomes) and ^{14}C (●; mature chromosomes). Sedimentation is from right to left. B. RNA synthesis *in vitro*: triplicate 40 µl aliquots of each fraction shown in A were assayed in 100 µl reaction mixtures containing 0.4 M ammonium sulfate. One set of triplicates contained 100 µg ml^{-1} exogenous DNA (O), the other had no added DNA (▲). C. SV40 chromosomes were prepared as above, ^{3}H (O) and ^{14}C (◇) were determined on 50 µl aliquots spotted on glass fiber filters. Triplicate 50 µl aliquots were assayed for RNA synthesis for 25 min at 37°C in 0.4 M ammonium sulfate, with 100 µg ml^{-1} added DNA (●).

endogenous template: exactly the same amount of RNA is synthesized whether or not exogenous DNA is added to the reaction (Figure 1B). The small amount of free RNA polymerase activity found at the top of the gradients is detected only when exogenous DNA is present (Fig. 1B,C), showing that no contaminant of the reaction mixture provides a template.

Effect of temperature and salt on in vitro RNA synthesis:

The in vitro synthesis of RNA occurs linearly with time at both 30°C and 22°C for at least one hour (Figure 2); at 37° synthesis is linear for only about 30 min. Synthesis increases about 50% as the temperature is raised from 22 to 30°C, but increases very little upon increase to 37°C. It thus appears as if some loss of activity begins soon at 37°. Figure 2 also shows, for comparison, the incorporation at 30°C of viral transcription complexes extracted in 0.4 M NaCl. RNA synthesis in the high-salt viral transcription complexes is less than in the low salt complexes when both are assayed at their optimum ammonium sulfate concentration, as shown. It should be noted that the number of cell-equivalents contained in the high salt extract was approximately 1.5 fold greater, so the difference in incorporation per cell-equivalent is even larger than shown. Our yield of RNA made in vitro in 30 minutes is routinely about 7 pmoles per 150mm dish (10^7 cells).

Salt greatly stimulates SV40 transcription in vitro (Figure 2B); twenty fold stimulation is common. The optimum concentration of ammonium sulfate is approximately 0.3 to 0.4M; there is some variability between preparations. However, there is no detectible difference in optima between chromosomes from the leading edge or from the trailing edge of the peak (cosedimenting with replicating and mature chromosomes, respectively): the results overlap and are both shown in Figure 2B as the solid circles. Addition of sarkosyl to the low salt transcription complexes does not stimulate RNA synthesis as much as addition of ammonium sulfate; optimal synthesis in the presence of sarkosyl is only about 60% of the optimum in its absence (data not shown).

Identity of the RNA polymerase :

To distinguish among the three RNA polymerase activities found in mammalian cells we used the drug α-amanitin: very low concentrations (<.5 μg/ml) inhibit RNA polymerase II, high

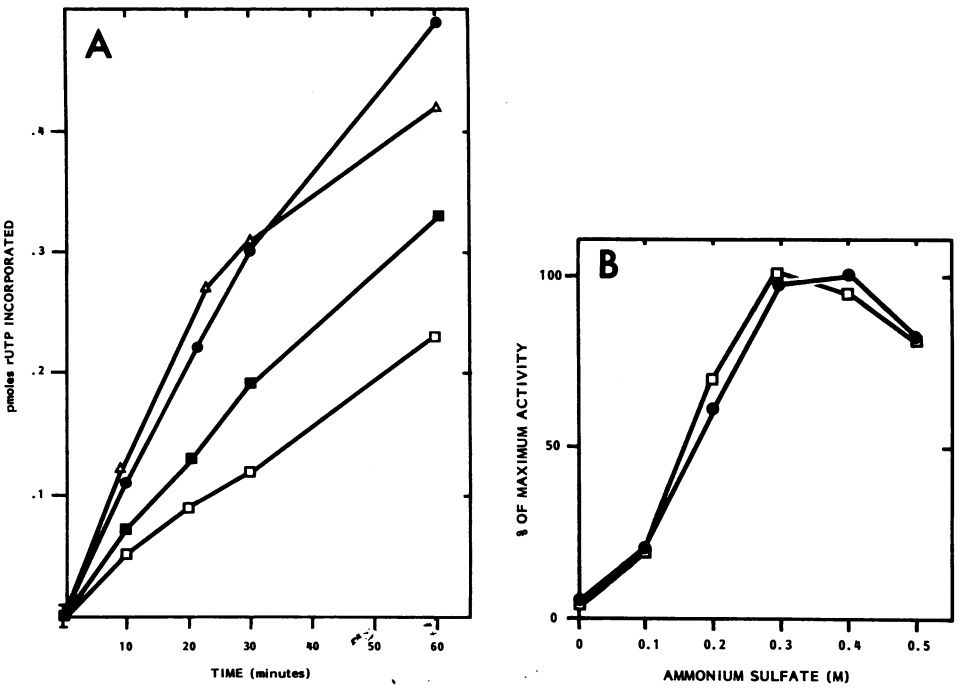

Figure 2: A. Kinetics of RNA synthesis in vitro: A pool of fractions containing replicating and mature chromosomes (from gradients similar to Figure 1) was assayed for RNA synthesis in a standard reaction mixture with 0.3 M ammonium sulfate and no added DNA. Duplicate aliquots of 50 µl each were taken at t=0 (on ice) and after the times noted: 22°C incubation (■); 30°C incubation(●); 37°C incubation (▲). A high salt extract was made in parallel as described. RNA synthesis was carried out as above; incubation was at 30°C (□).
B. Enhancement of RNA synthesis by ammonium sulfate: Pools enriched for either replicating or mature SV40 chromosomes were assayed without added DNA for 30 min at 30°C at each salt concentration. Incorporation was normalized to the maximum value obtained; both pools behaved identically and are represented as the solid circles. Results from a preparation made another day are shown as the squares.

concentrations (10-100 µg/ml) inhibit RNA polymerase III, and RNA polymerase I is unaffected (6,38). When assays are run across neutral sucrose gradients (such as those in Figure 1) or on pools from such gradients essentially all of the detectable RNA polymerase is abolished by 0.01 to 0.5 µg/ml of α-amanitin. This is true at all concentrations of ammonium sulfate tested, and in the presence of sarkosyl (data not shown). This argues that RNA polymerase II is

responsible for the synthesis seen in vitro.

Size of RNA made in vitro:

RNA synthesized in the presence of 0.4 M ammonium sulfate was sedimented through neutral sucrose gradients; endogenous mature SV40 DNA (Form I) serves as a marker. As seen in Figure 3, much of the RNA is as large as or larger than the SV40 Form I marker. The profile of RNA size is the same when the rUTP concentration is increased to 45 uM (3 fold); this indicates that the concentration of rUTP routinely used did not limit the size of the RNA made.

Density of the SV40 transcription complexes:

To compare the ratio of proteins to nucleic acids in SV40 chromosomes extracted at low and at high salt, isopycnic centrifugation in metrizamide was used. Metrizamide forms density gradients of low ionic strength in which unfixed protein-nucleic acid complexes can be analyzed and in which proteins are denser than nucleic acids (4). RNA polymerase activity of the chromosomes recovered from the gradients can be measured. When SV40 chromosomes are centrifuged in metrizamide as described, replicating and mature

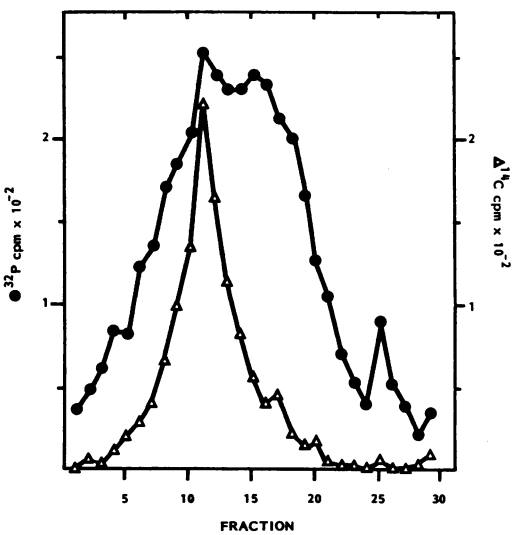

Figure 3: Size of the RNA made in vitro: SV40 chromosomes were isolated and RNA synthesized in vitro in 0.4M ammonium sulfate. The RNA was extracted and centrifuged as described. Triangles represent an internal ^{14}C-SV40 (Form I) marker; circles represent ^{32}P-RNA synthesized in vitro. Sedimentation is from right to left.

chromosomes band at nearly the same density (Figure 4A). Gradients of higher average metrizamide concentration reveal that replicating chromosomes are slightly denser than mature chromosomes (Figure 5A). Aliquots of the gradients were tested for the ability to synthesize RNA *in vitro*, and such activity was found at higher density than either replicating or mature chromosomes (Figure 4A,5A). This indicates that transcribing chromosomes have a higher average ratio of proteins to nucleic acids than other SV40 chromosomes.

If an assay for RNA polymerase is carried out in the presence of 0.3M ammonium sulfate before centrifugation in metrizamide, all three classes of SV40 chromosomes are found at lower densities, indicating loss of protein (Figure 4B). In this case also, the transcription complexes are denser than either replicating or mature chromosomes (Figure 4B). The presence of a small shoulder at lower density indicates that some chromosomes have lost even more protein than the majority. Under these conditions the RNA synthesized *in vitro*

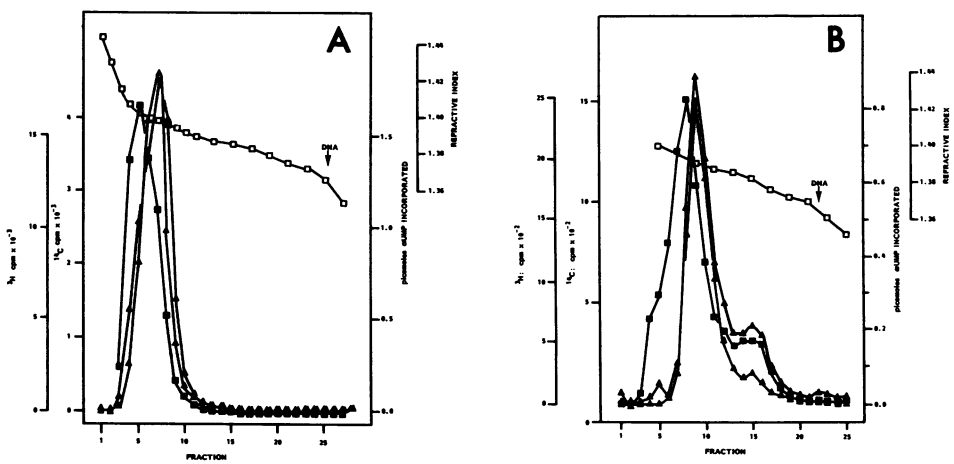

Figure 4: Density of the SV40 transcription complexes:
A. The density of transcription complexes that have never been exposed to high salt was compared to that of both replicating and mature SV40 chromosomes in a metrizamide gradient, as described.
B. A parallel gradient showing the density of transcription complexes and SV40 chromosomes after synthesis of RNA *in vitro* in 0.3M ammonium sulfate for 60 min. Density decreases from right to left. ^{3}H-replicating chromosomes (Δ); ^{14}C-mature chromosomes(▲); [^{32}P]rUMP incorporated *in vitro* (in aliquots of fractions from A; before sedimentation in B) (■); refractive index (□).

remains associated with the SV40 chromosomes, as shown by its density.

Treatment of the SV40 chromosomes with 0.4M NaCl on ice is sufficient to produce a similar decrease in the density of both replicating and mature chromosomes (Figure 5B). Transcribing chromosomes which have been exposed to 0.4 M NaCl retain their ability to synthesize RNA after centrifugation in metrizamide. They are denser than similarly treated replicating and mature chromosomes, but significantly less dense than transcribing chromosomes never exposed to high salt (compare 5A and B).

DISCUSSION

We have shown here that extraction of SV40-infected cells under conditions of very low ionic strength yields SV40 chromosomes capable of continuing RNA synthesis *in vitro* for over one hour at 30°C. This is consistent with one earlier report (34), but differs from others in

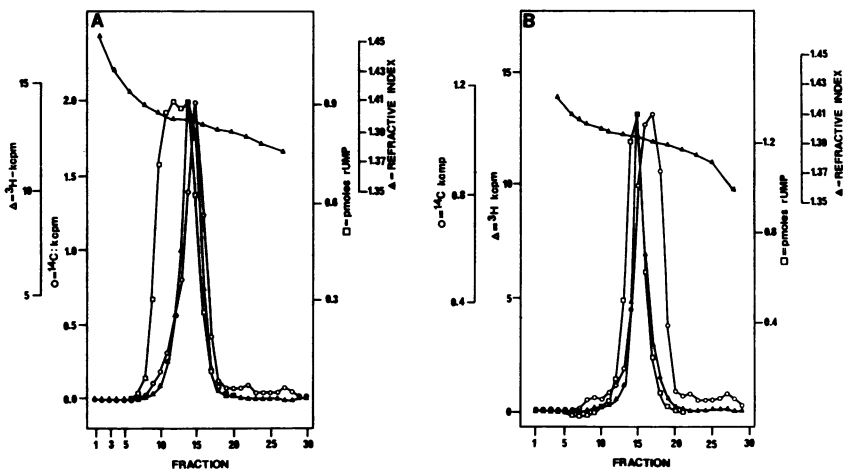

Figure 5: Exposure of SV40 chromosomes to 0.4M NaCl reduces their density in metrizamide. SV40 chromosomes were extracted and divided into two portions, one left untreated (A) and one adjusted to 0.4M NaCl and held on ice for 8 min (B). Both were then adjusted to 28% metrizamide (in M buffer without added salt) and 0.9 ml aliquots layered over gradients of 1.2 ml each 45%, 40%, and 35% metrizamide. After centrifugation for 20 h, fractions were collected and aliquots taken to determine refractive index (▲), ^{3}H (Δ), ^{14}C (O), and *in vitro* RNA synthesis in 0.3M ammonium sulfate (□).
A: Chromosomes never exposed to salt;
B: chromosomes exposed to 0.4M NaCl on ice.

which 0.4 M NaCl was required for extraction of transcription complexes (22). This difference probably reflects the critical dependence of the higher order structure of chromatin upon ionic conditions and upon the presence or absence of histone H1 (7,24,28,34,35). Chromosomes extracted in very low salt retain proteins that are lost upon exposure to high salt. RNA synthesis is due to RNA polymerase II, as determined by its sensitivity to low levels of α-amanitin. SV40 transcription in nuclei has been shown to be sensitive to these low levels of α-amanitin (27) as has transcription in previously reported in vitro preparations (19-22,29,41 and data not shown). Synthesis is greatly stimulated by the addition of ammonium sulfate, which also stimulates RNA synthesis in both Sarkosyl- (20,21) and high-salt-extracted (5,21) transcription complexes.

Transcribing SV40 chromosomes isolated by this low ionic strength method sediment just slightly ahead of the majority of mature SV40 chromosomes but not as rapidly as replicating chromosomes. Transcription complexes isolated in 0.4 M NaCl have also been shown to sediment very slightly ahead of mature SV40 chromosomes (5,21-23); they have not previously been compared to replicating chromosomes. SV40 chromosomes extracted by our method sediment slightly faster than those extracted in high salt (data not shown), and retain more proteins.

We have shown that transcribing chromosomes are denser in metrizamide than mature chromosomes, indicating a higher ratio of protein to nucleic acid. [The pre-existing RNA chains associated with SV40 chromosomes are short (21), thus density differences due to the presence of either free or protein-bound RNA should be small.] The increased density of transcribing chromosomes and their ability to synthesize RNA after centrifugation in metrizamide could enable one to purify them and determine which proteins (in addition to RNA polymerase II) are specifically associated with transcribing SV40 chromosomes.

SV40 chromosomes exposed to high salt (0.4 M NaCl or 0.3 M ammonium sulfate) remain associated with proteins, as seen in Figures 4B and 5B. However, the ratio of proteins to nucleic acids is significantly lower than in chromosomes kept in solutions of low ionic strength (compare 4A,5A). Thus exposure to high salt removes much of

the protein originally bound, although the RNA polymerase remains. The three classes of SV40 chromosomes all lose protein to a similar extent upon exposure to 0.4 M NaCl; transcribing chromosomes remain denser than replicating and mature chromosomes. A report published during the writing of this manuscript (21) has shown that SV40 transcription complexes isolated in 0.4 M NaCl have a nucleosome structure similar to the bulk of SV40 chromosomes. This is consistent with our finding that the density in metrizamide of transcribing chromosomes shifts in parallel with that of the bulk of mature chromosomes after treatment with different salt concentrations. Earlier work with SV40 chromosomes extracted in 0.2M NaCl showed that centrifugation in Metrizamide solutions containing 1 mM EDTA could result in loss of 1 or 2 nucleosomes (37). The density of our chromosomes in metrizamide, even after exposure to 0.4M NaCl, is higher than that previously reported; these differences may reflect loss of more protein when EDTA is present than in its absence, or differences in hydration of the nucleoprotein complexes in EDTA. Our demonstration that even brief exposure to 0.4 M NaCl results in a significant loss of protein could be important. From other work we know that histone H1 (25,28,34,35) and DNA polymerases (H. Edenberg, unpublished observations) are removed from SV40 chromosomes by high salt, although the basic nucleosome structure is retained (21). We do not know what other proteins may be lost, but it is possible that factors which modify transcription are among them.

Several laboratories have reported that extraction of SV40-infected cells under certain conditions can disrupt "salt labile virions" and "previrions," releasing SV40 chromosomes (1,16,17,39). It is theoretically possible for soluble RNA polymerase II to associate with the chromosomes so released to create an artifactual complex. There is strong evidence that the transcribing chromosomes isolated here are not artifactual. We have shown that the transcribing chromosomes we isolate can synthesize RNA _in vitro_ even after treatment with Sarkosyl. Sarkosyl inactivates RNA polymerase molecules that have not already initiated transcription (18,19) while only slightly inhibiting those which have initiated (18). Thus the ability of the transcribing chromosomes to synthesize RNA in the presence of Sarkosyl demonstrates that the RNA synthesis observed is a continuation of RNA synthesis initiated _in vivo_. [The possibility

that adventitious binding is followed by initiation of transcription during the extraction at 0^{o} is an unlikely prospect, since initiation of transcription by RNA polymerase II requires a time- and temperature- dependent step (32).]

It has been reported that replicating and mature chromosomes extracted in 0.2 M NaCl have the same density in metrizamide (10). Under our extraction and centrifugation conditions, replicating chromosomes are denser than mature chromosomes (Figure 5). The density of replicating chromosomes shifts in parallel with that of mature chromosomes after treatment with salt; as noted above this is consistent with their having similar nucleosome structures. [After fixation in glutaraldehyde and formaldehyde, replicating chromosomes are less dense than mature chromosomes in CsCl gradients (data not shown). This confirms a higher protein to nucleic acid ratio.]

Transcribing SV40 chromosomes isolated as described here could be useful as substrates in searches for RNA processing enzymes. They are soluble, purified away from most cytoplasmic and nuclear components; the yield of RNA synthesized in vitro is severalfold greater than from high-salt-extracted complexes; and the RNA remains associated with the chromosomes (Figure 4).

The discovery that transcribing chromosomes are extracted under the same conditions as replicating and mature chromosomes points out an additional complexity in studying the association of enzymes and other proteins with SV40 chromosomes (e.g. 13,30,31,34,36,40). All three classes of chromosomes must be considered in analyses of the specificity of association of such proteins.

ACKNOWLEDGEMENTS

This research was supported by grants from the Public Health Service (GM-25681, National Institute of General Medical Sciences), a Biomedical Research Support Grant, an institutional grant from the American Cancer Society, and the Grace M. Showalter Trust.

REFERENCES

1 Baumgartner,I., Kuhn,C. and Fanning,E. (1979) Virol. 96, 54-63
2 Birkenmeier,E.H., May,E. and Salzman,N.P. (1977) J. Virol.22,702-710
3 Birkenmeier,E.H., Radonovich,M.F., Shani,M., Salzman,N.P. (1977) Cell 11, 495-504
4 Birnie,G.D., Rickwood,D. and Hell,A. (1973) Biochim. Biophys. Acta 331, 283-294
5 Brooks,T.L. and Green,M.H. (1977) Nuc. Acids Res. 4, 4261-4277
6 Chambon,P. (1975) Annu. Rev. Biochem. 44, 613-638
7 Chen,Y.H., MacGregor,J.P., Goldstein,D.A. and Hall,M.R. (1979)

J. Virol. 30, 218-224
8 Chestier,A., and Yaniv,M. (1979) Proc.Natl.Acad.Sci.USA 76, 46-50
9 Christiansen,G. and Griffith,J.D. (1977) Nuc.Acids Res. 4,1837-1851
10 Cremisi,C., Pignatti,P.F., Croissant,O. and Yaniv,M. (1976) J.Virol. 17, 204-211
11 Edenberg,H.J., Waqar,M.A. and Huberman,J.A. (1976) Proc.Natl.Acad. Sci.USA, 73, 4392-4396
12 Edenberg,H.J., Waqar,M.A. and Huberman,J.A. (1977) Nuc.Acids Res.4, 3083-3095
13 Edenberg,H.J., Anderson,S. and DePamphilis,M.L. (1978) J. Biol. Chem. 253, 3272-3280
14 Ferdinand,F.J., Brown,M. and Khoury,G. (1977) Virol. 78, 150-161
15 Ferdinand,F.J., Brown,M. and Khoury,G. (1977) Proc.Natl.Acad.Sci. USA 74, 5443-5447
16 Fernandez-Munoz,R., Coca-Prados,M. and Hsu,M.-T. (1979) J. Virol. 29, 612-623
17 Garber,E.A., Seidman,M.M. and Levine,A.J. (1978) Virol. 90, 305-316
18 Gariglio,P., Buss,J. and Green,M.H. (1974) FEBS Lett. 44, 330-333
19 Gariglio,P. and Mousset,S. (1975) FEBS Letters 56, 149-155
20 Gariglio,P. and Mousset,S. (1977) Eur. J. Biochem. 76, 583-590
21 Gariglio,P., Llopis,R., Oudet,P. and Chambon,P. (1979) J. Mol.Biol. 131, 75-105
22 Green,M.H. and Brooks,T.L. (1976) Virol. 72, 110-120
23 Green,M.H. and Brooks,T.L. (1977) Nuc. Acids Res. 4, 4279-4289
24 Griffith,J.D. (1975) Science 187, 1202-1203
25 Griffith,J.D. and Christiansen,G. Cold Spring Harbor Symp. Quant. Biol. 42, 215-226
26 Howe,C.C. and Tan,K.B. (1977) Virology 78, 45-56
27 Jackson,A.H. and Sugden,B. (1972) J. Virol. 10, 1086-1089
28 Keller,W., Muller,U., Eicken,I., Wendel,I. and Zentgraf,H. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 227-244
29 Laub,O. and Aloni,Y. (1976) Virol. 75, 346-354
30 MacGregor,J.P., Chen,Y.H., Goldstein,D.A. and Hall,M.R. (1978) Biochem. Biophys. Res. Comm. 83, 814-821
31 Mann,K. and Hunter,T. (1979) J. Virol. 29, 232-241
32 Meilhac,M., Tysper,Z. and Chambon,P. (1972) Eur.J.Biochem. 28, 291-300
33 Meinke,W., Hall,M.R. and Goldstein,D.A. (1975) J.Virol.15, 439-448
34 Muller,U., Zentgraf,H., Eicken,I. and Keller,W. (1978) Science 201, 406-415
35 Oudet,P., Spadafora,C. and Chambon,P. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 301-312
36 Otto,B. and Fanning,E. (1978) Nuc. Acids Res. 5, 1715-1728
37 Pignatti,P.F., Cremisi,C., Croissant,O. and Yaniv,M. (1975) FEBS Lett. 60, 369-373
38 Roeder,R.G. (1976) in RNA Polymerase, R.Losick, M.Chamberlin, Eds., pp 285-329. Cold Spring Harbor Laboratory, N.Y
39 Seidman,M., Garber,E. and Levine,A.J. (1979) Virol. 95, 256-259
40 Sen,A., Hancock,R. and Levine,A.J. (1974) Virol. 61, 11-21
41 Shani,M., Birkenmeier,E., May,E., and Salzman,N.P. (1977) J.Virol. 23, 20-28
42 Tsubota,Y., Waqar,A., Burke,J., Milavetz,J., Evans,M., Kowalski,D. and Huberman,J. (1979) Cold Spring Harbor Symp. Quant.Biol. 43, 693-704
43 Varshavsky,A.J., Bakayev,V.V., Chumackov,P.M. and Georgiev,G.P. (1976) Nuc. Acids Res. 3, 2101-2113
44 White,M. and Eason,R. (1971) J. Virol. 8, 363-371