The SV40 transcription complex. II. Non-dissociation of protein from SV40 chromatin during transcription

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ABSTRACT.

A small fraction of the SV40 chromatin isolated from infected monkey cell cultures by the Triton method contains active RNA polymerase which had initiated transcription in vivo. This viral transcription complex (VTC) was utilized to answer the question of whether proteins dissociate from chromatin during transcription in vitro. ^3H -RNA was synthesized by the VTC under conditions such that over half the label was in transcripts which were longer than half the length of the SV40 genome. Virtually all of the ^3H -RNA remained associated with the SV40 chromatin, causing an increase in sedimentation rate from 55S to 78S. The density of the VTC- ^3H -RNA complex indicated that less than 5% of the original protein dissociated from the SV40 DNA which served as a template for transcription. We conclude that SV40 chromatin can be transcribed while the proteins remain associated with the DNA.

INTRODUCTION.

In cells which have been lytically infected by polyoma or SV40 viruses, essentially all of the non-encapsidated viral DNA exists as a 55S nucleoprotein complex (1-3), which can be easily separated from cellular chromatin by our Triton extraction procedure (1). The viral complex has been shown by electron microscopic (4-6) and biochemical (5,7-10) analyses to possess a structure which closely resembles that of cellular chromatin. There is an average of 21 beads (nucleosomes) per SV40 genome, each having a diameter of ca. $110\text{\AA}$ (4-6). Nucleosomes are comprised of about 200 base pairs of DNA wound around a complex containing two each of the four major histones (11-17). Transcriptionally active and inactive regions of cellular chromatin are both organized in this basic manner (18-20), although there is some structural difference between them, as evidenced by a selective deoxyribonuclease I sensitivity of active genes (21,22).

One fundamental question regarding the process of transcription is whether RNA polymerase can transcribe DNA which is present in nucleosomes without causing the dissociation of the histones from DNA. The present report examines this question by utilizing the Triton soluble SV40 "viral transcription complex" (VTC) (23). The VTC comprises a small fraction (<1%) of the total Triton extractable SV40 chromatin, and contains RNA polymerase II which had initiated RNA chains in vivo (23). The VTC sediments at nearly the same rate as the bulk of the SV40 chromatin, and is thus presumed to contain a similar structure.

In this study, we have utilized RNA polymerase reaction conditions in which very large RNA transcripts are generated by the VTC. This requires the use of a rather high salt concentration, but not so high as to promote the dissociation of protein from the SV40 chromatin (24). It is the purpose of this report to demonstrate that protein does not dissociate from SV40 DNA molecules during the process of transcription.

MATERIALS AND METHODS.

Except for the following procedures, all materials and methods are as described in the preceding paper (24).

a. Density of SV40 DNA-RNA hybrid (see Fig. 2). After the synthesis of ^3H -RNA, samples were made 0.1% in sodium dodecyl sulfate (SDS), then centrifuged through Sephadex G-25 equilibrated with 0.1% SDS (24). SV40 ^{14}C -DNA form I was added as marker, and the samples were adjusted to a density of 1.75 g/cc by the addition of saturated CsCl and buffer (10 mM Tris, pH 7.4; 10 mM EDTA; 0.25% Triton X-100) in a final volume of 5 ml. Paraffin oil was layered over the CsCl, and the samples were centrifuged for 68 hr at 30,000 rpm (21°C) in a SW 50.1 rotor.

b. Density of SV40 chromatin containing ^3H -RNA (see Fig. 4). After the synthesis of ^3H -RNA, reactions were stopped by the addition of 17 mM EDTA and layered onto 4.7 ml 15-30% sucrose gradients (dissolved in TTED containing 0.01 M NaCl). Centrifugation was for 90 min at 45,000 rpm (4°C) in a SW 50.1 rotor. Fractions were collected preparatively, the ^3H -RNA peak was located, and the appropriate fractions were pooled.

Following the addition of E. coli ^{14}C -RNA as a free RNA control, the sample was made 10 mM in phosphate buffer, pH 7.5, and formaldehyde and glutaraldehyde was added consecutively according to the method of Christiansen and Griffith (25). The samples were then adjusted to a density of 1.55 g/cc by the addition of saturated CsCl and buffer in a final volume of 5 ml. Paraffin oil was layered over the CsCl, and the samples were centrifuged for 66 hr at 36,000 rpm (21°C) in a SW 50.1 rotor.

RESULTS.

(a) Size of RNA synthesized by SV40 transcription complex (VTC). Purified SV40 transcription complex (23) was used to synthesize ^3H -RNA in vitro under conditions which do not cause the dissociation of protein from the bulk of the viral DNA (24). Reactions were carried out for either 15 min with a rate-limiting concentration of ^3H -UTP (Fig. 1A), or for 120 min with excess ^3H -UTP (Fig. 1B), and the sedimentation properties of the ^3H -RNA was analyzed by sucrose gradient centrifugation. In part A, all of the label was found in short RNA chains sedimenting at 10S. The maximum size of unlabeled nascent RNA chains which can be further elongated in vitro is thus 560 bases (26). In part B, the ^3H -RNA sedimented much more rapidly, with 56% sedimenting faster than 22S. This is the sedimentation rate which corresponds to RNA containing half the length of the SV40 genome plus 560 bases. Thus, a majority of the ^3H -RNA was transcribed in vitro by RNA polymerase which traversed at least half the length of the SV40 genome.

The demonstration of extensive RNA chain elongation in vitro by the SV40 transcription complex was substantiated by a different means of analysis. RNA polymerase reactions were carried out for varying periods of time in rate-limiting (Fig. 2A) or excess UTP (Fig. 2B-D). After the reactions, protein was dissociated from the complex by treatment with sodium dodecyl sulfate (SDS), and the density of the newly synthesized ^3H -RNA was analyzed by CsCl equilibrium density gradient centrifugation.

Figure 1. Sedimentation rate of the RNA synthesized by the SV40 transcription complex. Concentrated viral transcription complex (VTC), prepared without salt, was incubated with 0.3 M $(\text{NH}_4)_2\text{SO}_4$ for 30 min at 0°C . RNA polymerase reactions were then carried out at 22°C in 0.3 M $(\text{NH}_4)_2\text{SO}_4$, either in the presence of a limiting amount of ^3H -UTP ($7\ \mu\text{M}$) for 15 min (part A) or in excess ^3H -UTP ($43\ \mu\text{M}$) for 120 min (part B). ^3H -RNA was prepared and sedimented through sucrose gradients as described previously (24). Fractions were analyzed for ^3H -RNA (—) and 21S SV40 ^{14}C -DNA form I (---).

A large portion (30-40%) of the ^3H -RNA synthesized during a 15 min period with limiting UTP banded coincidentally with SV40 DNA (Fig. 2A), again indicating that the unlabeled nascent RNA chains are very short in the VTC as prepared. The density of essentially all of the ^3H -RNA-DNA complex increased with time of reaction, but once again it is apparent that the RNA chains grew to very different extents. From the density of the DNA-RNA hybrid, we estimate that over 50% of the ^3H -RNA synthesized in a 120 min reaction had a length greater than half that of the SV40

Figure 2. Density of the SV40 DNA-RNA hybrid as a function of reaction time. Concentrated VTC (in TTED) was pretreated with 0.3 M $(\text{NH}_4)_2\text{SO}_4$ for 30 min at 0°C , then used in RNA polymerase reactions at 22°C containing 0.3 M $(\text{NH}_4)_2\text{SO}_4$ and native salmon sperm DNA (100 $\mu\text{g}/\text{ml}$). Reactions were as follows: (A) 15 min in 7 μM ^3H -UTP; (B,C,D) in 43 μM ^3H -UTP for 15, 30 and 120 min, respectively. After SDS treatment, the samples were centrifuged to equilibrium in CsCl . ^3H -RNA (—); SV40 ^{14}C -DNA form I ($\rho = 1.70$) (---).

genome. This assumes that only one RNA chain contributes significantly to the mass of each DNA-RNA hybrid. The correspondence of the RNA size estimated by density and by sedimentation analysis

(Fig. 1) helps to support this assumption.

(b) Protein in SV40 chromatin following transcription.

The protein in the SV40 chromatin condenses the 21S DNA (4-6) and causes it to sediment at 55S (2). In order to determine whether protein dissociated from the viral DNA during the process of transcription, we examined the sedimentation rate and the density of the complex containing newly synthesized ^3H -RNA. The ^3H -RNA was synthesized under conditions which ensured the formation of long transcripts (see Fig. 1B and 2D). In addition, the reaction mixtures contained a large excess of salmon sperm DNA. This was included as a "sponge" so as to prevent any his-

Figure 3. Sedimentation rate of the SV40 chromatin containing ^3H -RNA. ^3H -RNA was synthesized by the SV40 VTC as described in the legend to Fig. 2D. Aliquots of the reaction mixture were then spun through 10-30% sucrose gradients under the following conditions: (A) In the presence of ^{14}C -VTC marker (55S); (B) Aliquot was treated with 0.1% SDS, and the sucrose gradient contained 0.1% SDS; SV40 ^{14}C -DNA form I marker (21S). Sedimentation was for 90 min at 45,000 rpm (4°C) in a SW 50.1 rotor. ^3H -RNA (—); ^{14}C -VTC (---); ^{14}C -DNA (----).

tones which might have dissociated from the SV40 chromatin from reassociating with the viral transcription complex during subsequent transcription or in the ensuing steps of the experiment.

As seen in Fig. 3A, essentially all of the ^3H -RNA sedimented at 78S relative to the 55S marker complex. When the protein in the complex was released by treatment with SDS (Fig. 3B), the resultant ^3H -RNA-DNA complex sedimented at 38S relative to the 21S SV40 ^{14}C -DNA marker. It is thus evident that a large amount of protein remains associated with SV40 DNA during the synthesis of long RNA transcripts in vitro.

For a more precise estimate of the quantity of protein which remains associated with SV40 DNA following transcription, the density of the viral chromatin containing ^3H -RNA was measured (Fig. 4). Once again, an excess of salmon sperm DNA was included in the reaction in order to bind to any histones which

Figure 4. Density of SV40 chromatin containing ^3H -RNA. VTC labeled with ^3H -RNA was pooled from the 78S peak of a sucrose gradient (see Fig. 3A). *E. coli* ^{14}C -RNA was added and the mixture was "fixed" with formaldehyde and glutaraldehyde (25), then centrifuged to equilibrium in CsCl . Fractions including the pellet (P) were analyzed for acid insoluble ^3H -RNA (■—■), ^{14}C -RNA (O---O), and for density by refractive index (●—●). The arrow indicates the position of untranscribed VTC run in a separate bucket in the same rotor.

might dissociate from the viral DNA serving as template. After the polymerase reaction, the protein in the SV40 chromatin was cross-linked to the DNA by treatment with glutaraldehyde and formaldehyde under conditions which had been shown to be optimal for the retention of chromatin structure (25). SV40 chromatin containing ^{14}C -DNA was added prior to the fixation reaction and served as a density reference in one tube, and ^{14}C -labeled ribosomal RNA was added as a control to a separate reaction mixture. The chromatin containing ^3H -RNA had a density of 1.51 g/cc, while that of the ^{14}C -chromatin marker was 1.47 g/cc (Fig. 4). The ^{14}C -ribosomal RNA banded at the bottom of the CsCl gradient, indicating that little if any free protein became spuriously fixed on it.

As shown in Table 1, the difference in density between the DNA-protein- ^3H -RNA complex and the ^{14}C -DNA-protein complex (0.04 g/cc) is due primarily to the presence of RNA and not to the loss of protein during transcription. The density values (ρ_{obs}) for the various complexes analyzed in the preceding experiment (Fig. 4) are listed together with their corresponding partial specific volumes ($\bar{v}$). Even if the difference between the two $\bar{v}$'s was caused entirely by a loss of protein in the complex which served for transcription, and not by the presence of RNA, the molar ratio of protein would have decreased by only 5%. This value is within the experimental error of our density measurements.

TABLE 1. Change in partial specific volume of VTC resulting from transcription.

	ρ_{obs}	$\bar{v}$	$\Delta\bar{v}$
DNA	1.72	0.582	0.099
*DNA-PROTEIN	1.47	0.681	
DNA-RNA	1.76	0.569	0.094
*DNA-Protein-RNA	1.51	0.663	

*Fixed by treatment with glutaraldehyde and formaldehyde.

It is thus concluded that extensive transcription of SV40 chromatin in vitro by endogenous RNA polymerase II (23) does not result in the release of protein from the viral DNA.

DISCUSSION.

The present study demonstrates that the SV40 viral transcription complex (VTC) can synthesize long RNA transcripts under conditions in which protein does not dissociate from the transcriptionally active chromatin. Electron microscopic and biochemical studies have revealed a close similarity between the structure of SV40 and cellular chromatin (4-10). On the basis of sedimentation rate (23) and density in CsCl (Fig. 4), the VTC has a structure which closely resembles that of the bulk of the Triton soluble SV40 chromatin. However, it is important to note that only a small fraction (less than 1%) of the viral DNA present in our preparations of SV40 chromatin is associated with active RNA polymerase (our unpublished data). It is thus not certain that the template active viral chromatin (VTC) possesses nucleosomes like those observed in the bulk of the viral chromatin. The ability to synthesize long RNA transcripts without dissociating protein from the VTC provides a method for readily separating the active from the inactive viral chromatin (see Fig. 3), and should facilitate studies on the structure of these complexes.

Ammonium sulfate is known to stimulate endogenous RNA polymerase II in cellular chromatin (27), but the basis of this effect is not clear. In the previous paper, we have shown that ammonium sulfate stimulates RNA polymerase II in the SV40 VTC by a process which involves proteins other than the polymerase (24). The present work demonstrates that this effect is not due to the release of protein from the viral template before or during the process of transcription. It may thus be postulated that the stimulatory effect of high salt is due to some conformational change in the chromatin protein rather than to its dissociation from DNA. Biophysical and electron microscopic methods have indicated that high salt causes chromatin to become less compact (25,28). Assuming that the viral transcription complex contains nucleosomes, as appears to be the case for template active cellular chromatin (18-20), a salt-mediated

conformational change such as the conversion from an octamer to two tetramers may facilitate a maximal rate of RNA chain elongation in nucleosomal DNA, at least in vitro.

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