The SV40 transcription complex. I. Effect of viral chromatin proteins on endogenous RNA polymerase activity

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ABSTRACT.

SV40 chromatin obtained from infected monkey cells was used to study the effect of the viral chromatin proteins on endogenous RNA polymerase II. Ammonium sulfate activated the rate of transcription by endogenous RNA polymerase in two ways: 1) by direct action on the enzyme; and 2) by causing a reversible conformational change in the viral chromatin. Under optimal reaction conditions, the viral chromatin proteins did not limit the rate of RNA chain elongation, and high molecular weight RNA (1.6×10^6 d) was synthesized by the SV40 chromatin.

INTRODUCTION.

We have recently described a procedure for the isolation of a Triton soluble 55S viral transcription complex (VTC) from SV40 infected monkey cells (1). This complex contains predominantly closed circular viral DNA (1,2), approximately 60% protein by weight (3), and active RNA polymerase form II which initiated RNA chains in vivo (1). Electron microscopic (4-6) and biochemical (7-10) studies indicate that the viral complex closely resembles cellular chromatin and contains an average of 21 nucleosomes per DNA molecule (4). We plan to utilize the SV40 VTC as a model system in the study of the transcription process in eukaryotes. Certain properties of the SV40 VTC make it an excellent system for the study of the transcriptional process. The complex is relatively small and homogeneous (55S) and is soluble even at high salt concentrations (1). Furthermore, the size of the primary transcript in vivo is known (26S), as is that of the processed RNA products which are generated in the nucleus and cytoplasm (11,12).

The present work was aimed at obtaining reaction conditions in which the VTC could synthesize complete 26S transcripts of

the SV40 genome, such as those found in the nuclei of infected cells (11). We have previously shown that high molecular weight SV40 RNA can be synthesized in vitro by Sarkosyl treated VTC (1), but this procedure causes the dissociation of proteins from DNA (13). This report will describe reaction conditions which generate high molecular weight SV40 RNA at a maximum rate while retaining the association of protein with the bulk of the viral DNA in the preparation. In a second paper (14) we shall demonstrate that the SV40 DNA molecules which are actually utilized as templates for transcription also remain associated with the chromatin proteins during the process of synthesizing large RNA.

MATERIALS AND METHODS.

a. Virus and cell culture. The TC-7 subline of the CV-1 monkey cell line (15) was used for the propagation of small-plaque SV40 virus stocks (SV-S Takamoto strain) and all experiments. Conditions for cell growth and infections were as described previously (1).

b. Extraction and concentration of SV40 nucleoprotein complex. Cells were Triton extracted as described previously (1). All steps were performed at 0°C. The viral chromatin present in the Triton supernatant was concentrated by centrifugation into a 50% sucrose cushion (1). Sucrose was dissolved in a solution containing Triton (0.25%); 10 mM Tris, pH 7.9; 0.2 mM EDTA; 0.5 mM dithiothreitol; and either no NaCl (designated TTED), 0.4 M NaCl (TTEDS) or 0.3 M ammonium sulfate (TTED-0.3AS). When 55S radioactive complex was prepared for use as marker, it was labeled either from 41-43 hr post infection with ³H-thymidine (2-10 μ Ci/ml) or from 24-43 hr with ¹⁴C-thymidine (0.1-1 μ Ci/ml).

c. Preparation of SV40 DNA-RNA polymerase complex. Triton supernatant containing viral chromatin was treated with 0.4% Sarkosyl (w/v) for 30 min at 0°C. The resulting 21S DNA-RNA polymerase complex (1) was concentrated into a 50% sucrose cushion (dissolved in TTED) as previously described for nucleoprotein complex (1), except that centrifugation was for 5 hr at 45,000 rpm (4°C) in a SW 50.1 rotor.

d. Assays for endogenous RNA polymerase in viral complex. Assays for endogenous RNA polymerase activity were carried out

as described previously (16). Standard reaction mixtures (0.25 ml) contained a rate limiting concentration of ^3H -UTP ranging from 18-100 $\mu\text{Ci/ml}$ (4-7 μM). When the synthesis of high molecular weight RNA was required, a UTP concentration of 43 μM was utilized so as not to be rate limiting (our unpublished data). All reactions were carried out at 22°C. Backgrounds (zero time controls) ranged from 30-50 cpm, and were subtracted prior to presenting the data.

e. Sedimentation analysis of SV40 chromatin in sucrose gradients. Samples of 0.2 ml were layered onto 4.8 ml preformed 5-20% sucrose gradients. For the experiment shown in Fig. 3, sucrose was dissolved in TTED containing the appropriate ammonium sulfate concentration (0.05M or 0.3M). Sedimentation was for 90 min at 45,000 rpm (4°C) in the SW 50.1 Spinco rotor. Fractions were collected from the bottom of each tube directly onto Whatman 3MM paper squares and analyzed for acid insoluble radioactivity. Sedimentation in all sucrose gradients is depicted from right to left.

f. Sedimentation analysis of SV40 RNA in sucrose gradients. After the synthesis of ^3H -RNA, reaction mixtures were made 0.1% in sodium dodecyl sulfate (SDS) and then centrifuged through Sephadex G-25 to remove ^3H -UTP (see below). Samples were subsequently heated for 5 min at 95°C in order to release the RNA from its template DNA. SV40 ^{14}C -DNA form I was added as a marker (21S), and the samples were layered onto 4.8 ml 5-20% sucrose gradients (dissolved in TTEDS containing 0.1% SDS). Sedimentation was for 3 hr at 45,000 rpm (4°C) in a SW 50.1 rotor. Fractionation and analysis were as described above for SV40 chromatin, except that plots were produced from scintillation data stored on punch tape and used in conjunction with a Hewlett Packard tape reader, calculator, and calculator plotter.

g. Density analysis of SV40 chromatin in Conray gradients. Samples of 0.2 ml were layered onto 4.8 ml preformed 20-50% Conray gradients. Conray was diluted with TTED. Centrifugation was for 28 hr at 36,000 rpm (4°C) in a SW 50.1 rotor. Fractionation and analysis were as described above for sucrose gradients. Conray was used in preference to CsCl because the latter method requires fixation of the chromatin with formal-

dehyde and/or glutaraldehyde, which caused large losses of chromatin in the presence of 0.3 M $(\text{NH}_4)_2\text{SO}_4$.

h. Centrifugation through Sephadex G-25. This procedure was used to separate ^3H -RNA from ^3H -UTP rapidly and with little dilution. A Sephadex G-25 column (4 ml) was prepared in a 5 ml disposable syringe and equilibrated with 10 mM sodium acetate, pH 5.5, 1 mM EDTA, and 0.1% SDS. The column was spun dry in a clinical centrifuge at approximately 1,500 rpm for 4 min when ready for use. A small volume (≤ 0.25 ml) of RNA was then layered and similarly centrifuged through the column into a vial attached to the syringe inside a 15 ml Corex centrifuge tube. Virtually complete recovery of RNA was achieved with better than 99% removal of ^3H -UTP.

i. Isolation of *E. coli* ^{14}C -RNA. A culture of *E. coli* B^E was labeled for 5 generations with ^{14}C -uracil (2 $\mu\text{Ci/ml}$), washed, grown for another generation in the presence of uracil (50 $\mu\text{g/ml}$), then harvested, treated with lysozyme and SDS, and extracted twice with phenol at room temperature and once with chloroform. Details of this procedure have been published (17). The aqueous phase was passed over a Sephadex G-100 column and the excluded ^{14}C -RNA was stored frozen.

j. Preparation of SV40 DNA form I. SV40 DNA was extracted from infected TC-7 cells at approximately 40 hr by the method of Hirt (18). Supercoiled SV40 DNA (form I) was separated from open circular viral DNA and contaminating cell DNA by equilibrium centrifugation in CsCl containing ethidium bromide, and the dye was removed by extraction with isopropanol (19).

k. Chemicals and enzymes. Conray (Iothalamate meglumine, injection U.S.P. 60%) was purchased from Mallinckrodt, Inc. Other special reagents were obtained from sources indicated previously (13).

RESULTS.

a. Effect of salt on endogenous RNA polymerase activity. Partially purified SV40 transcription complex (VTC) was assayed for endogenous RNA polymerase activity as a function of ammonium sulfate concentration in the presence or absence of sarkosyl (Fig. 1A). As shown previously with cellular (13) and

viral (1) chromatin, this anionic detergent causes a marked stimulation in RNA polymerase activity by dissociating chromatin proteins from the DNA. Whereas sarkosyl caused a four-fold stimulation of transcription below 0.2 M $(\text{NH}_4)_2\text{SO}_4$, little or no stimulatory effect was produced by this detergent at concentrations above 0.2 M. The optimal KCl concentration was found to be in the range of 0.4-0.6 M (Fig. 1B), corresponding closely to the optimal concentration of the cation, ammonium (Fig. 1A). Since the VTC was extracted and purified in 0.4 M NaCl, it seems likely that the stimulatory effect of salt on endogenous RNA polymerase is not due to the release of a large fraction of the chromatin protein, unlike the case with sarkosyl.

We next asked whether the stimulatory effect of salt was due to a direct effect on RNA polymerase or on the chromatin proteins. SV40 DNA-RNA polymerase complex, devoid of other proteins, was prepared by treating VTC with sarkosyl, followed by purification of the complex by sedimentation. The stimulatory effect of $(\text{NH}_4)_2\text{SO}_4$ on this complex, as well as on intact VTC, is shown in Fig. 2. There was a direct effect of $(\text{NH}_4)_2\text{SO}_4$ on RNA polymerase, but only from 0-0.1 M salt. With intact SV40 chromatin, the salt stimulatory effect was considerably greater from 0-0.1 M than with the DNA-RNA polymerase complex, and the

Figure 1. Effect of salt and sarkosyl on RNA polymerase activity in SV40 chromatin (VTC). Concentrated VTC (in TTED, no NaCl) was used to synthesize ^3H -RNA over the designated range of $(\text{NH}_4)_2\text{SO}_4$ (part A) or KCl (part B) concentrations. Duplicate reactions were carried out at 22°C for 60 min. Sarkosyl (0.4%) was included in one set of reactions (●—●, Part A).

Figure 2. Ammonium sulfate activation of RNA polymerase in SV40 chromatin and SV40 DNA-RNA polymerase complex. RNA polymerase reactions were carried out for 60 min at 22°C with either SV40 chromatin (O—O) or SV40 DNA-RNA polymerase complex (■--■). The latter was prepared from chromatin by treatment with sarkosyl (see Methods). The "stimulation ratio" is the ratio of RNA polymerase activity at the designated concentration of $(\text{NH}_4)_2\text{SO}_4$ divided by the activity with no $(\text{NH}_4)_2\text{SO}_4$ in the reaction.

activity continued to increase in the range of 0.1 M to 0.2 M $(\text{NH}_4)_2\text{SO}_4$. It is thus evident that the chromatin proteins restrict the rate of transcription when less than 0.2 M $(\text{NH}_4)_2\text{SO}_4$ is present, but they apparently do not limit the activity above this salt concentration (see also Fig. 1A).

b. Relation of VTC structure to endogenous RNA polymerase activity. The following experiments were performed in an effort to determine whether the salt stimulatory effect on RNA polymerase was due to the release of an inhibitory protein present in the VTC, or perhaps to some structural change in the viral chromatin. As seen in Fig. 3, treatment of the viral chromatin with 0.3 M $(\text{NH}_4)_2\text{SO}_4$, followed by dilution to 0.05 M,

Figure 3. Effect of 0.3 M ammonium sulfate on the sedimentation rate of SV40 chromatin (VTC). SV40 chromatin containing ^3H -DNA was incubated with 0.3 M $(\text{NH}_4)_2\text{SO}_4$ for 30 min at 0°C before being concentrated into 50% sucrose at the same salt concentration. Aliquots of this chromatin were then diluted to 0.05 M $(\text{NH}_4)_2\text{SO}_4$ either in the presence (A) or absence (B) of salmon sperm DNA (100 $\mu\text{g}/\text{ml}$). A third aliquot was equally diluted into 0.3 M $(\text{NH}_4)_2\text{SO}_4$. ^{14}C -labeled SV40 chromatin was added as a 55S marker after dilution (parts A and B), or incubated with the ^3H -chromatin for 30 min at 0°C (part C) prior to dilution. The samples were then analyzed for sedimentation rate by sucrose gradient centrifugation as in Methods. ^3H -chromatin (●—●); ^{14}C -chromatin (■—■).

either in the presence (part A) or absence (part B) of a large excess of salmon sperm DNA, resulted in a decrease in sedimentation coefficient from 55S to 42S. Since the same S-value was obtained in the presence and absence of excess DNA, it is apparent that DNA binding proteins did not dissociate reversibly from the SV40 chromatin. In the control (Fig. 3C), both the ^3H -VTC and the marker ^{14}C -VTC were maintained at 0.3 M $(\text{NH}_4)_2\text{SO}_4$ throughout the experiment and were found to co-sediment. The decrease in S-value produced by 0.3 M $(\text{NH}_4)_2\text{SO}_4$ may have been due to the release of some protein or to an irreversible conformational change in the viral chromatin. An irreversible conformational change in the structure of polyoma chromatin has been reported to occur at 0.5 M NaCl (20).

Portions of the $(\text{NH}_4)_2\text{SO}_4$ treated VTC samples used in the above experiment (Fig. 3A,B) were also analyzed for density by centrifugation in Conray (Fig. 4A and B). This procedure has been shown to permit the banding of SV40 chromatin with no de-

Figure 4. Effect of 0.3 M ammonium sulfate on the density of SV40 chromatin (VTC). The samples from parts A and B of Fig. 3 were centrifuged to equilibrium in Conray gradients (parts A and B, respectively). As a control, SV40 ^{14}C -DNA form I and ^3H -labeled VTC (maintained in 0.4 M NaCl) were centrifuged in a third gradient (part C). ^3H -VTC (●—●); ^{14}C -VTC (■--■) or ^{14}C -DNA (part C, ■--■).

tectable change in structure as visualized in the electron microscope (J. Griffith, personal communication). It is evident that 0.3 M $(\text{NH}_4)_2\text{SO}_4$ produced no significant change in the density of the SV40 chromatin. As a control, the separation of SV40 chromatin from SV40 DNA in Conray is shown in Fig. 4C. Apparently little, if any, chromatin protein dissociated from the SV40 DNA as a result of treatment with 0.3 M $(\text{NH}_4)_2\text{SO}_4$. Thus, the decrease in sedimentation rate of the SV40 chromatin caused by this salt concentration was apparently due to a conformational change.

If the stimulation of RNA polymerase by salt was caused by the release of an inhibitory protein, it should be possible to separate VTC from this protein. The resulting VTC should then show relatively little salt activation of endogenous RNA polymerase. To examine this possibility, VTC was incubated in 0.3 M $(\text{NH}_4)_2\text{SO}_4$ and concentrated by centrifugation through sucrose containing this salt concentration. The VTC was then tested for its potential to be activated by salt or sarkosyl (Table 1). The control VTC, concentrated into a sucrose cushion containing no salt, showed the usual stimulation of RNA polymerase by sarkosyl at 0.05 M $(\text{NH}_4)_2\text{SO}_4$ (3.6-fold), and by 0.3 M $(\text{NH}_4)_2\text{SO}_4$ (3.4-fold). The VTC purified in the pre-

TABLE 1. Stimulation of RNA polymerase activity in VTC's prepared at high or low salt.

VTC PREPARATION	RNA POLYMERASE REACTION CONDITIONS			
	0.05M (NH ₄) ₂ SO ₄		0.3M (NH ₄) ₂ SO ₄	
	-SARKOSYL	+SARKOSYL	-SARKOSYL	+SARKOSYL
No salt	987	3527	3379	3829
0.3M (NH ₄) ₂ SO ₄	728	3338	2666	2816

Concentrated SV40 VTC was prepared either without salt or in 0.3 M (NH₄)₂SO₄, then assayed for the effect of (NH₄)₂SO₄ and sarkosyl (0.4%) on endogenous RNA polymerase activity. Duplicate reactions were carried out for 60 min at 22°C, and the average of the ³H-UTP incorporation minus a zero time background of 45 cpm is presented.

sence of 0.3 M (NH₄)₂SO₄, also showed a marked stimulation by both sarkosyl in low salt (4.5-fold) and by 0.3 M (NH₄)₂SO₄ (3.7-fold). Therefore, treatment of VTC with 0.3 M (NH₄)₂SO₄ did not cause the release of a protein which serves to inhibit endogenous RNA polymerase in reactions carried out at low salt. The activation of transcription by high salt is evidently due to some reversible conformational change produced in the VTC.

Figure 5. Effect of 0.3 M ammonium sulfate on the kinetics of RNA synthesis by SV40 chromatin. Concentrated viral chromatin (in TTED) was preincubated either with 0.3 M (O—O) or with 0.05 M (NH₄)₂SO₄ (■—■) for 30 min on ice prior to the addition of nucleoside triphosphates. Duplicate RNA polymerase reactions were carried out in the corresponding salt concentration for the designated times at 22°C.

c. Size of RNA and rate of chain elongation. The following experiments were aimed at determining the rate of RNA chain elongation and the ultimate size of the RNA product generated by the SV40 transcription complex under our optimal reaction conditions. The kinetics of the reaction carried out in 0.3 M ammonium sulfate ("high salt") as compared with the

Figure 6. Effect of ammonium sulfate on the size of RNA synthesized by SV40 chromatin. Concentrated VTC (in TTED) was pre-incubated either with 0.05 M (parts A-C) or 0.3 M (parts D-F) $(\text{NH}_4)_2\text{SO}_4$ for 30 min at 0°C prior to use in RNA polymerase reactions at the corresponding salt concentration. In parts A and D, a rate limiting concentration of ^3H -UTP ($7\ \mu\text{M}$) was utilized in short reactions (15 min). The other reactions were run for 120 min in excess ^3H -UTP ($43\ \mu\text{M}$). To test for the presence of RNase, the reactions in parts C and F were stopped by the addition of EDTA (20 mM) after 120 min, and incubation was continued for an additional 120 min. All samples were then treated with 0.1% SDS, centrifuged through Sephadex G-25, heated for 5 min at 95°C , and analyzed for sedimentation rate in sucrose gradients along with 21S ^{14}C -DNA. ^3H -RNA (—); ^{14}C -DNA form I (---).

previous "standard conditions", 0.05 M or "low salt", are shown in Fig. 5. The stimulatory effect of high salt is evident throughout the reaction, gradually increasing from 4.9-fold at 15 min to 8.5-fold at 240 min. This indicates that high salt exerts two effects on transcription by the VTC: 1) a stimulation of the initial rate; and 2) a prolongation of the reaction.

Figure 7. Size of RNA synthesized by SV40 chromatin as a function of reaction time. SV40 chromatin was used to synthesize ^3H -RNA in 0.3 M $(\text{NH}_4)_2\text{SO}_4$ as described in Fig. 6, and the RNA was analyzed in sucrose gradients. RNA polymerase reactions were carried out at 22°C in 43 μM UTP except for part A, where 7 μM UTP was used. The reaction times were 15 min (A and B), 30 min (C), 60 min (D), 120 min (E), and 240 min (F). ^3H -RNA (—); 21S ^{14}C -DNA form I (---).

The size of the RNA synthesized in low or high salt was analyzed by sucrose gradient centrifugation. The RNA synthesized in a 15 min reaction in low salt sedimented at ~10S, and no increase in size occurred when the reaction proceeded for 120 min (Fig. 6A,B). As seen in Fig. 6C, this may have been due to the presence of a small amount of RNase. Some decrease in RNA size occurred when the reaction was stopped with EDTA at 120 min and the mixture was incubated for an additional 2 hr. In a parallel series of reactions carried out in high salt (Fig. 6D-F), the RNA increased in S-value between 15 and 120 min with more than half the RNA becoming larger than the 21S marker (SV40 DNA). There was no evidence of active RNase in the reaction under these conditions. This conclusion is further substantiated by the observation that all of the newly synthesized RNA remains associated with the VTC during a 2 hr reaction (14).

To estimate the maximal rate of chain elongation, the S-value of the fastest major component synthesized in high salt and excess UTP was determined as a function of reaction time (Fig. 7). The S-values were converted to apparent molecular

Figure 8. Apparent molecular weight of ^3H -RNA synthesized by SV40 chromatin. The data of Fig. 7, parts B-F, was used to estimate the molecular weight of the major fast component of RNA (indicated by arrows) synthesized by the SV40 chromatin (O—O). Also shown is the total RNA synthesized at each time point (■ --- ■).

weights by Spirin's equation (21), and plotted together with the net RNA synthesized (Fig. 8). Between 60 and 240 min, the net RNA increased by 150% while the apparent molecular weight increased by only 36%. The largest RNA attained an apparent molecular weight of 1.6×10^6 d, equalling that of a complete transcript of one strand of the SV40 genome. From the increase in molecular weight between 15 and 60 min, which was nearly linear with time, we estimate that the largest RNA chains grew at a rate of 45 nucleotides per min.

DISCUSSION.

The isolation of viral transcription complexes (VTC's), which contain viral DNA in association with RNA polymerase, opens a new avenue to the approach of questions concerning the nature of the viral template. The Triton extraction procedure (1,22) offers an important advantage over the Sarkosyl method (23) in that it permits one to study the nature and role of proteins other than RNA polymerase which are associated with the viral DNA. The similarity in structure between the SV40 complex and cellular chromatin (4-10, 24,25) suggests the possibility that the VTC may serve as a useful model system for the study of the transcription process in eukaryotes.

Chromatin is generally thought to be a less efficient template for RNA synthesis than is free DNA (26). The inhibitory effect of chromatin proteins is exerted both on the initiation (27,28) and RNA chain elongation (29) stages of the transcription process, at least when catalyzed by exogenous DNA-dependent RNA polymerase. Endogenous RNA polymerase II in nuclei (30) and in chromatin (31) is markedly stimulated by high concentrations of salt, e.g., 0.4 M ammonium sulfate. It was proposed that the salt activation of transcription was due to the dissociation of protein from cellular DNA (31). The present report demonstrates that at least in the case of the SV40 chromatin, which contains RNA polymerase II (1), the salt stimulatory effect at 0.2-0.3 M $(\text{NH}_4)_2\text{SO}_4$ is not due to the release of an inhibitory protein, but rather to some reversible conformational change in the viral chromatin. Electron microscopic studies indicate that SV40 chromatin becomes less compact as the salt concentration is raised (32). High salt has also been shown to

cause a relaxation of the nucleosome structure, as seen by circular dichroism studies (33). It may be postulated from our present findings that such changes in chromatin structure permit endogenous RNA polymerase to transcribe chromatin as rapidly as free DNA.

Under optimal reaction conditions (0.3 M ammonium sulfate) the SV40 chromatin was able to synthesize large RNA transcripts with over half of the label incorporated into molecules which were greater than half the length of the viral genome. Several features of this reaction are worth noting:

(a) The RNA synthesized in a short reaction (15 min) with a rate limiting concentration of UTP was rather small and homogeneous, sedimenting as a single broad peak at ~10S. If nascent RNA chains had been associated with the VTC, one might expect that they would be very heterogeneous in length, ranging from 0-26S depending on the distance of the RNA polymerase from the promoter. A short reaction in vitro would radiolabel these chains without causing a marked alteration in the sedimentation profile. Two possible explanations for the appearance of 10S RNA are as follows: 1) either the nascent RNA chains were lost during the preparation of the VTC; or 2) most of the active RNA polymerases were localized near the promoter. The second alternative derives support from recent electron microscopic studies of Aloni and his co-workers (personal communication).

(b) Although starting out as a fairly homogeneous small component, the RNA synthesized by the VTC became very heterogeneous in size within the first 60 min of the reaction. During this time interval, net RNA increased linearly, and there was no evidence of RNA degradation. If termination of RNA chains had occurred, then new chains must also have been initiated at the same frequency in order that the net reaction continue linearly. Free RNA polymerase is thought not to be able to bind to DNA at 0.3 M $(\text{NH}_4)_2\text{SO}_4$ (34). Thus, reinitiation could only be due to enzymes which had already terminated chains but remained bound to DNA, or to those which for some reason had delayed functioning in vitro for various periods of time.

(c) Late in the reaction, the size of the largest RNA compo-

nent approached a limit of 1.6×10^6 daltons, which is close to that of a complete transcript of the SV40 genome. Between 60 and 240 min of the reaction, net RNA increased by 150% as compared to only a 36% increase in molecular weight of the fastest major component. While it is possible that this result was due to a trace of RNase, the absence of RNA degradation under these conditions offers a more interesting possibility, namely, that a termination signal on SV40 chromatin was recognized in vitro. Further experiments are necessary in order to resolve these alternatives.

(d) The rate of RNA chain elongation catalyzed by the endogenous RNA polymerase in the SV40 chromatin was only 0.8 nucleotides per second at 22°C under our optimal reaction conditions. As discussed above, this rate was not significantly limited by the presence of chromatin proteins. In the following paper, it will be shown that chromatin proteins do not dissociate from the SV40 DNA molecules which were utilized as templates for transcription (14). Although our observed elongation rate is considerably slower than the rate in vivo (11), it is 2- to 3-fold faster than that observed by Shani et al. (35) with the SV40 DNA-RNA polymerase complex obtained by the Sarkosyl extraction procedure (23). Using RNA polymerases purified from calf thymus, Mandel and Chambon (36) obtained chain elongation rates as high as 7-10 nucleotides per second at 37°C on purified SV40 DNA form I. The difference in the observed rates is apparently not due simply to the temperature at which the reactions were carried out, because we found no increase in rate by raising the temperature either to 30°C or 37°C (unpublished data). We also do not think the difference is due to the form of SV40 DNA used as a template, since the active viral chromatin contains form I DNA (Green and Brooks, manuscript in preparation). The system is evidently a complex one, and further studies will be necessary in order to understand the above features of the transcription process and how they relate to transcription in the intact cell.

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