

Separation and Properties of Two Kinds of Simian Virus 40 Late Transcription Complexes

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Simian virus 40 (SV40) transcription complexes were labeled in cells with 3-min pulses of [³H]uridine 48 h after infection and were extracted from nuclei in isotonic buffer or in a buffer containing Sarkosyl. In sucrose gradients, the labeled complexes sedimented faster than both free RNA and most SV40 nucleoproteins. Most of the pulse-labeled nascent RNA hybridized to the entire late region of SV40, remained bound to viral DNA in Cs₂SO₄ gradients, and ranged in size from a few nucleotides to about 5,000 nucleotides, with a peak at about 700. In contrast, the SV40-associated RNA polymerase activity in the same preparations sedimented near the major peak of SV40 nucleoproteins and was clearly separated from the transcription complexes bearing pulse-labeled nascent RNA. The two kinds of transcription complexes were released from isolated nuclei at different rates. Complexes bearing pulse-labeled RNA were released immediately when the nuclei were agitated in a Dounce homogenizer in isotonic buffer, whereas most of the complexes bearing RNA polymerase active *in vitro* were released more slowly, during subsequent incubation of the nuclei at 0°C. Since the complexes bearing pulse-labeled nascent RNA were virtually inactive *in vitro*, the blocked complexes described by Laub et al. (Proc. Natl. Acad. Sci. U.S.A. 77:3297-3301, 1980) probably account for almost all the SV40-associated RNA polymerase activity studied previously by many investigators. New procedures must be developed to preserve the activity of the pulse-labeled complexes if the many advantages of the SV40 system for studying transcription by nucleoprotein complexes *in vitro* are to be realized fully.

Girard et al. (10) prepared simian virus 40 (SV40) DNA-RNA complexes from infected cells that had been labeled with [³H]uridine for 8 to 40 min and showed that these complexes had the structure expected for transcriptional intermediates. Since the sodium dodecyl sulfate (SDS) used to isolate the complexes removed all the proteins, it was not possible to test for transcriptional activity *in vitro*. Gariglio and Mousset (9) and Green and Brooks (11) prepared active SV40 nucleoprotein complexes capable of elongating *in vitro* RNA chains that had been initiated in the infected cells. These complexes, representing about 1% of the total viral DNA present late in infection (18), contained SV40 DNA(I) and had a structure similar to that of chromatin (2, 8). These complexes were active when initiation by free RNA polymerase II did not occur *in vitro* (7-9), and although most of the RNA made *in vitro* was transcribed from the late strand (1, 6, 15), the early region was transcribed as well (16, 22). Since these properties were expected of SV40 transcription complexes active in the infected cells, it was assumed that the complexes isolated were also responsible for

making pre-mRNA in the cells. However, it was not shown that initiated RNA polymerase II could elongate *in vitro* the nascent RNA chains that had been pulse labeled in the cells.

More recent work has raised the possibility that most of the SV40 RNAs elongated *in vitro* might not be precursors to full-length mRNAs in infected cells after all. Laub et al. (17) showed that SV40 RNA pulse-labeled *in vitro* hybridizes almost exclusively to a short region near the origin of SV40 DNA (map position 0.67 to 0.76) and hybridizes much less to all other regions of the SV40 genome. The latter result would have been expected for RNA made by polymerase II transcribing many different regions of SV40 at the moment of isolation. These workers suggested that polymerase had halted preferentially a short distance from the initiation region on the late strand and had a low probability of continuing to transcribe late pre-mRNA. In a different type of study, Llopis and Stark (19) showed that transcription complexes isolated from cells infected with deletion mutant *dl-1261* or *dl-1262* have unusual properties. Complexes derived from wild-type SV40 are activated by high con-

centrations of salt or by Sarkosyl, whereas complexes derived from the mutants are activated only by Sarkosyl. Since the mutants carry deletions in the overlapping genes for virion proteins 2 and 3 (VP2, VP3) (4), we concluded that one or both of these structural proteins is bound to late transcription complexes in a way that inhibits the progress of initiated RNA polymerase. Our more recent work (unpublished) has shown that both the rate of transcription and the rate of accumulation of viral nucleoproteins are the same in cells infected with *dl*-1261 or with wild-type virus, i.e., the abnormality observed *in vitro* is not seen in infected cells. The results can be explained by postulating two different types of transcription complex. In one, VP2 or VP3 blocks RNA polymerase. This type would be essentially inactive in cells but could be activated *in vitro* with high concentrations of salt or with Sarkosyl. The other type of complex, free of VP2 and VP3, would be responsible for synthesizing late pre-mRNA in the cells. These ideas can be tested by isolating the two types of transcription complex. We now show that complexes bearing RNA chains pulse-labeled in the cells are readily separated and easily distinguished from complexes responsible for most of the SV40-associated RNA polymerase activity that can be measured *in vitro*. The two types of complex sediment at different rates in sucrose gradients, they are released at different rates from isolated nuclei, and they contain different nascent RNAs.

MATERIALS AND METHODS

Cells, virus, and pulse-labeling procedure. CV1 lines of green monkey kidney cells and wild-type SV40 were grown as described previously (19). After infection for 90 min with 10 PFU per cell, viral DNA was labeled with [¹⁴C]thymidine (50 mCi/mmol, 0.1 μ Ci per plate) from 24 to 48 h after infection to a specific activity of about 600 cpm/ μ g. At 48 h after infection, the cells were washed with 10 ml of warm TD buffer (137 mM NaCl, 5.1 mM KCl, 0.7 mM Na₂HPO₄, 50 mM Tris-hydrochloride, pH 7.4) and were pulse-labeled with 2 mCi of [³H]uridine (15 to 50 Ci/mmol) in 1 ml of medium per 10-cm plate of about 6×10^6 cells. At the end of the labeling period, the medium was removed, and the cells were chilled on ice. All subsequent operations were carried out at 2°C.

Preparation and analysis of SV40 nucleoproteins. Isotonic supernatant solutions were prepared after agitating isolated nuclei in a Dounce homogenizer at 0°C as described previously (19). To prepare supernatant solutions with Sarkosyl, nuclei prepared from infected cells with Nonidet P-40 (19) were suspended in chilled TD buffer containing 0.2 mM phenylmethanesulfonyl fluoride at a concentration of 2.4×10^7 nuclei per ml. Nuclei were transferred into an equal volume of TD buffer plus 0.4% Sarkosyl contained in a polyallomer tube for the SW56 rotor. The supernatant solution, recovered after 30 min of centrifugation at 35,000 rpm and 4°C, contained 10 to 15 μ g of SV40

DNA per 6×10^6 cells. Sedimentation of viral nucleoproteins was performed in sucrose gradients in buffer (137 mM NaCl, 5.1 mM KCl, 50 mM Tris-hydrochloride, pH 7.9) with or without 0.2% Sarkosyl. Further details are given in the individual figure legends. Equilibrium density gradient analyses in Cs₂SO₄ were performed as described previously (18). Fractions from sucrose gradients were assayed for RNA polymerase activity as described previously (8, 19). When gradients containing Sarkosyl were assayed, its final concentration was adjusted to 0.2%.

Mapping the viral RNAs. Purified SV40 DNA (10 μ g) was cleaved with restriction enzymes *Eco*RI, *Hpa*I, and *Bgl*II (New England Nuclear Corp., 10 U of each enzyme), and the fragments were separated in a 1.4% agarose gel and were transferred to nitrocellulose essentially as described by Southern (23). Nucleoproteins containing labeled nascent RNA were purified in sucrose gradients, extracted with phenol-chloroform-isoamyl alcohol as described previously (8), precipitated with ethanol, and treated with DNase (RNase-free, kindly provided by David Peabody, Department of Biochemistry, Stanford University). When the labeled DNA had been digested completely, the reaction mixture was extracted again with phenol-chloroform-isoamyl alcohol and was precipitated with ethanol. Hybridization of the labeled RNA to the filters was performed for 60 h at 42°C with a solution containing 50% formamide, 0.4 M NaCl, 5 mM EDTA, 0.2% SDS, 250 μ g of purified tRNA from *Escherichia coli* per ml, and 20 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) buffer, pH 6.8. One ml of this solution was used per 15 cm² of nitrocellulose. The filters were washed three times at 45°C for 5 min with a solution containing 0.36 M NaCl, 2 mM EDTA, 0.2% SDS, and 20 mM sodium phosphate, pH 7.0, and then were treated with En³Hance (New England Nuclear Corp.), dried, and autoradiographed at -70°C with an intensifying screen and preflashed Kodak X-Omat R film.

RESULTS

SV40 transcription complexes pulse-labeled with [³H]uridine in cells and extracted with isotonic buffer. Most of the SV40 DNA, pre-labeled with [¹⁴C]thymidine, was present in 70S nucleoprotein complexes, as observed previously (19). The complexes labeled by 3- or 4-min pulses of [³H]uridine sedimented ahead of these 70S complexes (Fig. 1). When a 2-min pulse was employed, we could not detect any incorporation of label into SV40 nucleoproteins. When the labeling period was prolonged to 3 h, most of the [³H]uridine was incorporated into free RNA that sedimented more slowly than the 70S complexes (Fig. 1). To obtain the patterns shown, it was essential to chill the cells immediately at the end of the labeling period. Without chilling, most of the pulse-labeled RNA sedimented more slowly than the 70S complexes, in the position of free RNA (Fig. 1c). However, chilling did disrupt most of the 200S previrions (19), adding nucleoproteins derived from these structures to the 70S region (data not shown). The material sedimenting in region A or B (Fig. 1a) was extracted with

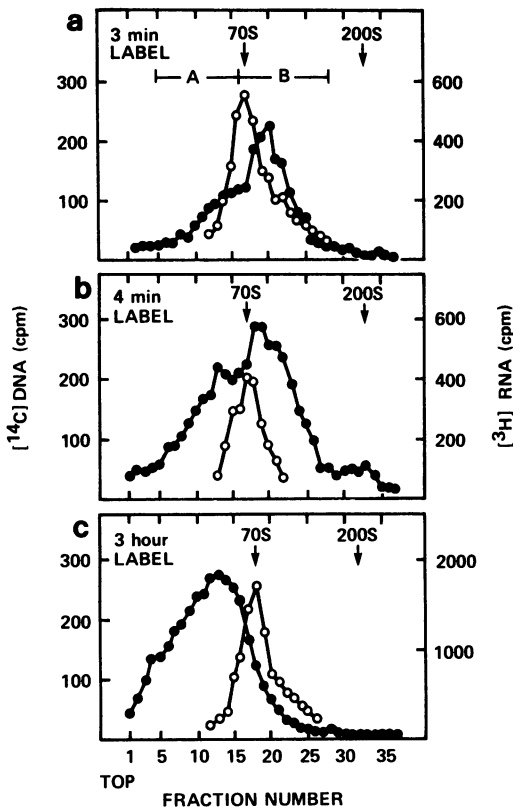


FIG. 1. Sedimentation of viral nucleoprotein complexes labeled in infected cells and extracted with isotonic buffer. Cells were labeled for 3 min (a), 4 min (b), or 3 h (c) with 2 mCi per plate of [^3H]uridine 48 h after infection or with 50 μCi per plate of [^3H]uridine 45 to 48 h after infection. Supernatant solutions obtained after incubating the nuclei for 1 h in isotonic buffer at 0°C (19) were sedimented through a 5 to 20% sucrose gradient in TD buffer for 40 min at 55,000 rpm in an SW56 rotor at 2°C . Fractions were collected from the bottom of the tube onto Whatman 3MM filters, which were washed five times with 5% trichloroacetic acid before counting. Symbols: \circ , [^{14}C]thymidine in DNA; \bullet , [^3H]uridine in RNA.

phenol, precipitated with ethanol, and banded to equilibrium in Cs_2SO_4 density gradients. Most of the labeled RNA near the top of the sucrose gradient (pool A) banded at the density of free RNA, whereas more than half of the labeled RNA lower in the gradient (pool B) banded near free DNA at the density expected for RNA-DNA complexes composed mostly of DNA (Fig. 2). The offset of the DNA and RNA peaks in Fig. 2b indicates that only a small part of the DNA is complexed with RNA, a conclusion consistent with previous estimates that only a small fraction of SV40 nucleoprotein is involved in transcription (18). The relative amounts of labeled RNA complexed to DNA in region B of sucrose

gradients after 3-min pulses with [^3H]uridine varied from experiment to experiment, probably owing to cross-contamination between different regions of the gradients and also to release of RNA chains during isolation and purification. When the time of preparation was minimized, as during a direct extraction of whole cells with SDS by the method of Hirt (14), up to 70% of the total label incorporated in a 3-min pulse was found in RNA-DNA complexes (data not shown). Also, better separation between free RNA and RNA in complexes was obtained in later experiments by varying the conditions of sedimentation (for example, see Fig. 6). The great majority of labeled RNA sedimenting ahead of the 70S peak in such an experiment is complexed with DNA.

SV40 transcription complexes pulse-labeled in cells and extracted with Sarkosyl. Isolated nuclei were extracted with the anionic detergent Sarkosyl after a 3-min pulse with [^3H]uridine. Sedimentation of the extract in a sucrose gradient (Fig. 3) revealed that a substantial fraction of the labeled RNA sedimented ahead of viral DNA that had been prelabeled with [^{14}C]thymidine. Much of the labeled RNA banded at the density of RNA-DNA complexes in Cs_2SO_4 gradients. Sarkosyl strips most of the proteins, including the core histones, from SV40 nucleoprotein complexes but leaves at least some initiated

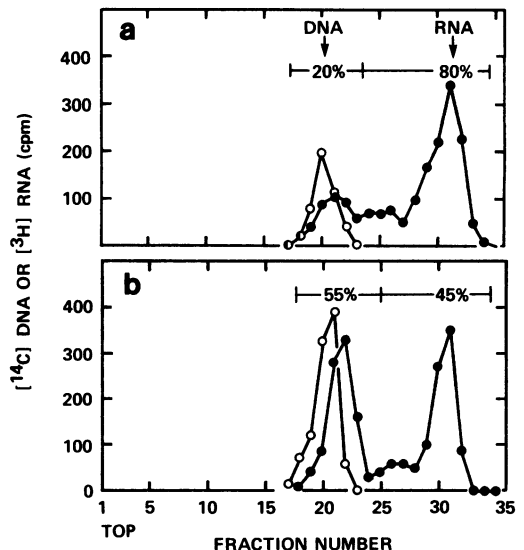


FIG. 2. Centrifugation in Cs_2SO_4 gradients of nucleic acids from the sucrose gradient of Fig. 1a (3-min label). Portions of pools A and B were extracted with phenol, precipitated with ethanol, and centrifuged to equilibrium. The radioactivity in each fraction was determined as described in the legend to Fig. 1. Symbols: \circ , [^{14}C]thymidine in DNA; \bullet , [^3H]uridine in RNA.

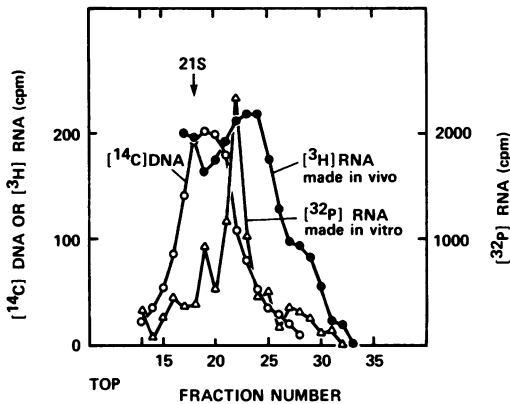


FIG. 3. Sedimentation of viral nucleoprotein complexes labeled in vivo and analysis of RNA polymerase activity in vitro. Cells were labeled for 3 min with 2 mCi per plate of [^3H]uridine 48 h after infection. The supernatant solution from nuclei treated with 0.4% Sarkosyl was sedimented through a 5 to 20% sucrose gradient in TD buffer containing 0.2% Sarkosyl for 3 h at 41,000 rpm in an SW41 rotor at 2°C. The RNA polymerase activity in a 90- μl portion of each fraction was determined by incubation in buffer containing 100 mM $(\text{NH}_4)_2\text{SO}_4$, 0.2% Sarkosyl, and 30 μM [^{32}P]UTP (2,000 cpm/pmol) for 1 h. Symbols: \circ , [^{14}C]thymidine in DNA; \bullet , [^3H]uridine in RNA made in cells; Δ , [^{32}P]UMP in RNA made in vitro.

RNA polymerase II bound in an active form (13). In extractions with Sarkosyl or with SDS, the yield of tritiated RNA-DNA complex was two to three times greater than in experiments in which we attempted to obtain transcription complexes in a more native state by agitating isolated nuclei in a Dounce homogenizer and then allowing the content of SV40 nucleoproteins to

leach into an isotonic buffer at 0°C. With the Sarkosyl procedure, significant incorporation of [^3H]uridine into RNA was detected with a pulse as short as 1 min.

Because Sarkosyl extraction was more efficient, it was used to obtain pulse-labeled RNA-DNA complexes for further characterization. The peak of tritiated material sedimenting ahead of the DNA was pooled, extracted with phenol, and precipitated with ethanol. About 90% of the uridine label could still be precipitated after treatment with DNase (RNase-free); all the label was soluble after treatment with RNase A. After denaturation with glyoxal (20), the size of the nascent labeled RNA was analyzed in a sucrose gradient (Fig. 4a) or in an agarose gel (data not shown). No specific bands were observed, but fragments from only a few nucleotides long to about 5,000 nucleotides were observed in a broad distribution, with a peak at about 700 nucleotides. Of the label, 25% was in fragments smaller than a 5S RNA marker, and 75% was in fragments larger than this marker (Fig. 4a). The size distribution was very similar to that obtained by Girard et al. (10), who isolated SV40 RNA-DNA complexes by using SDS.

About 70% of the labeled RNA from the pool hybridized to SV40 DNA covalently bound to diazotized paper (24). With correction for the hybridization of appropriate standards (24), we found that almost 100% of this RNA was complementary to SV40 DNA. The same labeled RNA hybridized mainly to restriction fragments of SV40 DNA from the late region (Fig. 4b, bands b and d); much less hybridized to fragments from the early region (Fig. 4B, bands a and c). This result is to be expected if the labeled RNA is transcribed in vivo predominantly from

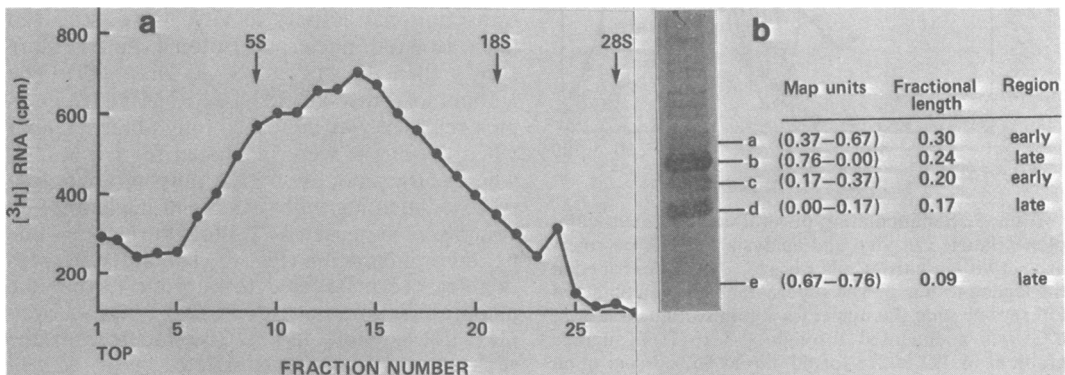


FIG. 4. Size distribution and complementarity of nascent SV40 pre-mRNA molecules pulse-labeled in cells for 3 min. (a) After sedimentation in the presence of Sarkosyl (Fig. 3), the labeled RNA was purified by phenol extraction, DNase treatment, and re-extraction. It was denatured with glyoxal (20) and sedimented in a 5 to 20% sucrose gradient in 0.4 M NaCl-1 mM EDTA-10 mM sodium phosphate buffer, pH 7.1, with an SW56 rotor for 4 h at 55,000 rpm. The markers were rRNAs treated with glyoxal and run in parallel. (b) The same preparation of RNA run in the sucrose gradient was hybridized to immobilized restriction fragments of SV40 DNA (see text and reference 17).

the whole of the late region during the period of the pulse. In contrast, Laub et al. (16, 17) have shown that RNA pulse-labeled *in vitro* hybridizes mainly to a fragment from the region immediately to the late side of the origin (Fig. 4b, band e). This result is to be expected if the RNA polymerase active *in vitro* is bound predominantly to this region.

Analysis of RNA polymerase activity in SV40 transcription complexes that have been pulse-labeled in cells. The complexes extracted from nuclei in isotonic buffer after a 3-min pulse of [³H]uridine were sedimented in a sucrose gradient, and the activity of RNA polymerase II in each fraction was determined (Fig. 5). The activity cosedimented with the peak of prelabeled viral DNA at 70S as expected, but we were surprised to find that the [³H]RNA in RNA-DNA complexes sedimented much faster. Tritium label was also found in the peak of free RNA that sedimented more slowly than the viral DNA. A similar experiment was performed with Sarkosyl rather than isotonic buffer used for extraction (Fig. 3). Again, the RNA polymerase activity sedimented as expected, on the forward edge of the peak of viral DNA, and again the [³H]RNA in RNA-DNA complexes sedimented much faster. We reported previous-

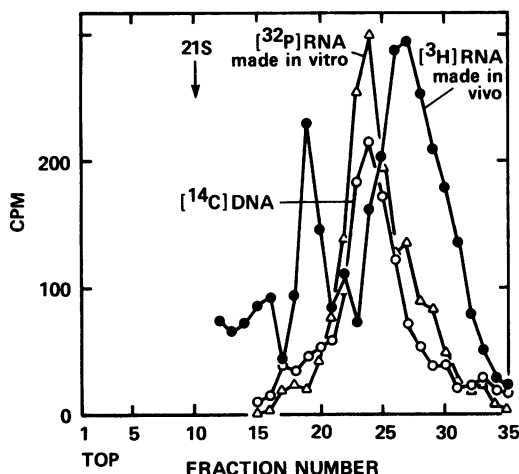


FIG. 5. Sedimentation of viral nucleoprotein complexes labeled *in vivo* and analysis of RNA polymerase activity *in vitro*. Cells were labeled as described in the legend to Fig. 3. The supernatant solution obtained after incubating the nuclei for 2 h in isotonic buffer at 0°C was sedimented through a 5 to 20% sucrose gradient in TD buffer for 60 min at 55,000 rpm in an SW56 rotor at 2°C. The radioactivity in each fraction was determined as described in the legend to Fig. 1. The RNA polymerase activity in a 100- μ l portion of each fraction was determined by incubation in buffer containing 300 mM (NH₄)₂SO₄ and 40 μ M [³²P]UTP (500 cpm/pmol) for 2 h. Symbols: \circ , [¹⁴C]thymidine in DNA; \bullet , [³H]uridine in RNA made in cells; Δ , [³²P]UMP in RNA made *in vitro*.

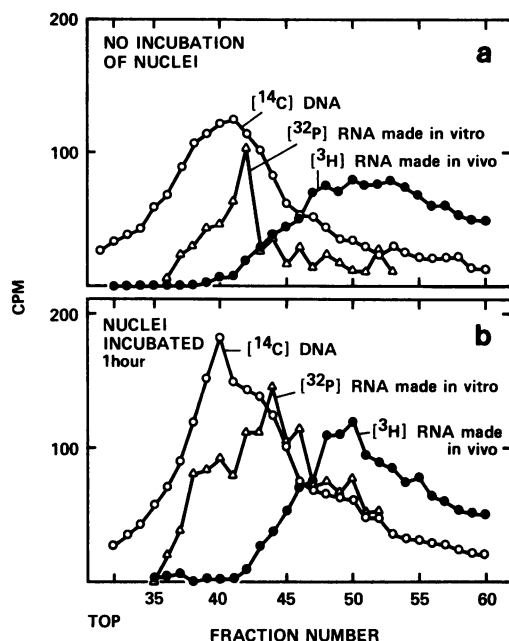


FIG. 6. Sedimentation analysis of viral transcription complexes released from isolated nuclei with or without incubation. Cells were labeled for 3 min with 2 mCi per plate of [³H]uridine 48 h after infection. Isotonic supernatant solutions were obtained from nuclei immediately after agitation with a Dounce homogenizer (a) or after subsequent incubation for 1 h at 0°C (b). The solutions were sedimented through a 5 to 20% sucrose gradient in TD buffer for 3 h at 41,000 rpm in an SW41 rotor at 2°C. Assays for RNA polymerase activity were carried out in each fraction. Symbols: \circ , [¹⁴C]thymidine in DNA; \bullet , [³H]uridine in RNA made in cells; Δ , [³²P]UMP in RNA made *in vitro*.

ly that the complexes responsible for SV40 transcriptional activity *in vitro* were extracted from isolated nuclei in isotonic buffer more slowly than the bulk of SV40 DNA (19). The amount of pulse-labeled [³H]RNA-DNA complex released was increased only about 1.2-fold when the nuclei were incubated for 1 h at 0°C, whereas the amount of RNA polymerase activity cosedimenting with 70S SV40 nucleoprotein complexes increased by 2.5-fold during the same period of incubation (Fig. 6). The different rates of release can be used to improve further the physical separation of these two types of complex. For example, in Fig. 6 (no incubation), the separation was almost complete.

We made several attempts to shift the position in sucrose gradients of transcription complexes pulse-labeled with [³H]uridine in cells or to shift the position in Cs₂SO₄ gradients of the corresponding [³H]RNA-DNA complexes by incubation *in vitro* in high salt, low salt, or Sarkosyl, for times as long as 90 min. We did not observe a

significant change in the positions of the ^3H label, indicating that substantial elongation of the pulse-labeled nascent RNA did not occur *in vitro*. In contrast, the unlabeled nascent RNA chains of the complexes bearing active RNA polymerase II were elongated readily in the same experiments, causing these complexes to sediment more rapidly, as has been demonstrated previously (8). We also made three unsuccessful attempts to detect RNA polymerase activity in 30 mM $(\text{NH}_4)_2\text{SO}_4$ (low salt) in gradients like the one shown in Fig. 6a (no incubation of the nuclei). In the same gradients, activity in the presence of 300 mM $(\text{NH}_4)_2\text{SO}_4$ (high salt) was observed reproducibly (Fig. 6).

DISCUSSION

***In vitro* activity of the complex that synthesizes SV40 pre-mRNA in infected cells.** The transcription complexes responsible for incorporating a pulse of [^3H]uridine into nascent RNA in the infected cells are inactivated almost completely during isolation by the procedures that have been used up to now, including extraction from nuclei with an isotonic buffer. However, extraction procedures with SDS (10), Sarkosyl, or isotonic buffer do preserve the association with the DNA templates of pulse-labeled nascent SV40 RNA and allow little or no degradation of the RNA. The small amount of RNA polymerase activity measured in low concentrations of salt is probably due to the transcription complexes bearing pulse-labeled nascent RNA, whereas the substantial activity measured in high salt is probably due to transcription complexes that are inactive in the cells. We have shown previously (19) that complexes active in low salt are extracted from nuclei immediately at 0°C , as are the nascent RNA chains pulse-labeled in cells (Fig. 6). The activity in low salt ceases after only 10 to 15 min of RNA synthesis *in vitro* (19) and is too labile to survive sedimentation in a sucrose gradient for 3 h as described above. Conversely, the activity measured in high salt is extracted relatively slowly upon incubation of the nuclei at 0°C and continues to make RNA at a linear rate *in vitro* even after 90 to 120 min (19). This activity sedimented near the bulk of the viral DNA, distinct from the complexes bearing nascent pulse-labeled RNA (Fig. 6). Since the activity measured in low salt *in vitro* is very small compared with the transcriptional activity in the infected cells, essential components must be lost during isolation. The potential advantages of studying SV40 transcription *in vitro* with isolated nucleoproteins have long been appreciated. To realize these advantages, procedures must be developed to preserve the RNA polymerase II activity associated with complexes bearing pulse-labeled nascent RNA. The ex-

periments done up to now (1-3, 6, 8, 9, 11, 12, 15, 16, 18, 22) have measured the properties of transcription complexes in which the initiated RNA polymerase, blocked in cells, has become active *in vitro* through the effect of high salt or Sarkosyl on the structure of the nucleoproteins.

The nature of the RNA polymerases in SV40 transcription complexes. Our data suggest that there may be two types of RNA polymerase II associated with the two types of transcription complex. The polymerase engaged in complexes actively making RNA in cells is labile during isolation by the procedures that have been used up to now and is active only in low salt. In contrast, the polymerase engaged in the transcriptional complexes that are blocked in cells is hardy enough to survive isolation and is activated not only by high salt but also by the anionic detergent Sarkosyl, which strips most other proteins, including the core histones, from SV40 nucleoprotein complexes. The possibility that there are two types of RNA polymerase II has been raised before by others on the basis of the differential sensitivity of transcription in whole cells, whole cell lysates, or isolated nuclei to changes in salt concentration and on the basis of differential inhibition of transcription by the nucleoside analog 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole, which causes premature chain termination (5, 7, 21, 25). Our present data are at least consistent with the possibility that two types of RNA polymerase II are responsible for the differential sensitivity of SV40 transcription complexes to 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole as observed by Laub et al. (17). This idea could be tested after isolation and separation of the two types of complex under conditions where both retain substantial activity.

Our previous results with transcription complexes from mutants carrying deletions in the genes for VP2 and VP3 indicate that one or both of these SV40 nucleocapsid proteins must be bound in a way that inhibits the ability of initiated polymerase II to continue RNA synthesis (19). We have argued that such blocked complexes may be at a dead end, unable to continue transcription and unable to enter virions because of the bound polymerase. The alternative possibility that the blocked complexes might have a functional role could be evaluated by determining the fate in infected cells of the small nascent RNAs bound to them.

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LITERATURE CITED

1. Birkenmeier, E. H., E. May, and N. P. Salzman. 1977. Characterization of simian virus 40 *tsA58* transcriptional intermediates at restrictive temperatures: relationship be-

- tween DNA replication and transcription. *J. Virol.* 22:702-710.
2. Birkenmeier, E. H., M. F. Radonovich, M. Shani, and N. P. Salzman. 1977. The SV40 DNA template for transcription of late mRNA in viral nucleoprotein complexes. *Cell* 11:495-504.
 3. Brooks, T. L., and M. H. Green. 1977. The SV40 transcription complex. I. Effect of viral chromatin proteins on endogenous RNA polymerase activity. *Nucleic Acids Res.* 4:4261-4277.
 4. Contreras, R., C. Cole, P. Berg, and W. Fiers. 1979. Nucleotide sequence analysis of two simian virus 40 mutants with deletions in the late region of the genome. *J. Virol.* 29:789-793.
 5. Dreyer, C., and P. Hausen. 1978. On the activity of RNA polymerase B in lysates from Ehrlich ascites cells. *Eur. J. Biochem.* 86:241-253.
 6. Ferdinand, F.-J., M. Brown, and G. Khoury. 1977. Synthesis and characterization of late lytic simian virus 40 RNA from transcriptional complexes. *Virology* 78:150-161.
 7. Fraser, N., P. B. Sehgal, and J. E. Darnell, Jr. 1979. Multiple discrete sites for premature RNA chain termination late in adenovirus-2 infection: enhancement by 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole. *Proc. Natl. Acad. Sci. U.S.A.* 76:2571-2575.
 8. Gariglio, P., R. Llopis, P. Oudet, and P. Chambon. 1979. The template of the isolated native simian virus 40 transcriptional complexes is a minichromosome. *J. Mol. Biol.* 131:75-105.
 9. Gariglio, P., and S. Mousset. 1975. Isolation and partial characterization of a nuclear RNA polymerase-SV40 DNA complex. *FEBS Lett.* 56:149-155.
 10. Girard, M., L. Marty, and S. Manteuil. 1974. Viral DNA-RNA hybrids in cells infected with simian virus: the simian virus 40 transcriptional intermediates. *Proc. Natl. Acad. Sci. U.S.A.* 71:1267-1271.
 11. Green, M. H., and T. L. Brooks. 1976. Isolation of two forms of SV40 nucleoprotein containing RNA polymerase from infected monkey cells. *Virology* 72:110-120.
 12. Green, M. H., and T. L. Brooks. 1977. The SV40 transcription complex. II. Non-dissociation of protein from SV40 chromatin during transcription. *Nucleic Acids Res.* 4:4279-4289.
 13. Green, M. H., J. Buss, and P. Gariglio. 1975. Activation of nuclear RNA polymerase by Sarkosyl. *Eur. J. Biochem.* 53:217-225.
 14. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. *J. Mol. Biol.* 26:365-369.
 15. Laub, O., and Y. Aloni. 1976. Transcription of simian virus 40. VI. SV40 DNA-RNA polymerase complex isolated from productively infected cells transcribed in vitro. *Virology* 75:346-354.
 16. Laub, O., S. Bratosin, M. Horowitz, and Y. Aloni. 1979. The initiation of transcription of SV40 DNA at late time after infection. *Virology* 92:310-323.
 17. Laub, O., E. B. Jakobovits, and Y. Aloni. 1980. 5,6-Dichloro-1- β -ribofuranosylbenzimidazole enhances premature termination of late transcription of simian virus 40 DNA. *Proc. Natl. Acad. Sci. U.S.A.* 77:3297-3301.
 18. Llopis, R., F. Perrin, F. Bellard, and P. Gariglio. 1981. Quantitation of transcribing native simian virus 40 minichromosomes extracted from CV1 cells late in infection. *J. Virol.* 38:82-90.
 19. Llopis, R., and G. R. Stark. 1981. Two deletions within genes for simian virus 40 structural proteins VP2 and VP3 lead to formation of abnormal transcriptional complexes. *J. Virol.* 38:91-103.
 20. McMaster, G. K., and G. G. Carmichael. 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. U.S.A.* 74:4835-4838.
 21. Sehgal, P. B., N. W. Fraser, and J. E. Darnell, Jr. 1979. Early Ad-2 transcription units: only promoter-proximal RNA continues to be made in the presence of DRB. *Virology* 94:185-191.
 22. Shani, M., E. Birkenmeier, E. May, and N. P. Salzman. 1977. Properties of simian virus 40 transcriptional intermediates isolated from nuclei of permissive cells. *J. Virol.* 23:20-28.
 23. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-517.
 24. Stark, G. R., and J. G. Williams. 1979. Quantitative analysis of specific labelled RNA's using DNA covalently linked to diazobenzylxymethyl-paper. *Nucleic Acids Res.* 6:195-203.
 25. Tamm, I., and T. Kikuchi. 1979. Early termination of heterogeneous nuclear RNA transcripts in mammalian cells: accentuation by 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole. *Proc. Natl. Acad. Sci. U.S.A.* 76:5750-5754.