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Characterization of early simian virus 40 transcriptional complexes: Late transcription in the absence of detectable DNA replication

(early transcriptional complexes/*in vitro* RNA/early-late "switch")

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ABSTRACT **Isolation of early viral transcriptional complexes and incorporation *in vitro* of radiolabeled precursors into nascent RNA has permitted an analysis of early simian virus 40 (SV40) transcription. Under conditions such that viral DNA replication was undetectable, both early and late SV40 RNA were synthesized. This finding provides evidence that viral DNA replication is not an absolute requirement for late transcription and supports earlier observations that late viral RNA is synthesized in SV40-infected nonpermissive mouse cells. The majority of the early viral transcriptional activity can be solubilized, indicating that a substantial portion of this RNA is transcribed from free rather than integrated templates. Sedimentation analysis of the transcriptional complexes resulted in the detection of two separate peaks of activity, suggesting the possibility of two distinct types of early SV40 templates.**

It has been known for some time that simian virus 40 (SV40) RNA is synthesized in two phases (1–3). Prior to viral DNA replication, most of the viral mRNA appears to be transcribed from the early DNA strand (4–6). Subsequently, the bulk of viral transcription occurs from the late DNA strand and is processed into late 16S and 19S viral mRNAs (7, 8). It was generally assumed that this late phase of viral transcription was inextricably linked to viral DNA replication (see refs. 9 and 10). The absence of the late SV40 transcripts in most SV40-transformed cell lines supported this concept (11). Nevertheless, we consistently found late viral RNA sequences in abortively infected mouse cells, in which SV40 DNA replication could not be detected (5, 12). Furthermore, recent studies with early temperature-sensitive mutants of SV40 grown at 41° (the nonpermissive temperature) indicated the presence of a small but reproducible fraction of late virus-specific cytoplasmic RNA (13). This led us to speculate that viral DNA replication was not an absolute requirement for the transcription of sequences on the late SV40 DNA strand. It was difficult to test this hypothesis directly, because of the low levels of late virus-specific RNA found prior to viral DNA replication (1–6, 14) and the potential rapidity and efficiency of the RNA processing system (15).

We recently described the adaptation of an *in vitro* transcriptional system (16) established by Gariglio and Mousset (17). The supernatant fraction of SV40-infected cell nuclei, lysed in the presence of Sarkosyl, actively incorporates radiolabeled ribonucleotide triphosphates, and the newly synthesized RNA can be easily analyzed. Although it is unclear whether putative termination signals are recognized under such incorporation conditions, the absence of reinitiation allows one to assess accurately the frequency of *in vivo* transcription of the two viral DNA strands (16). Further advantages of this *in vitro* transcriptional system include: (*i*) the enrichment of virus-specific RNA sequences in the nuclear supernatant fraction; (*ii*) an increase in the specific activity of the viral RNA through *in vitro* incorporation of highly radiolabeled precursors; and (*iii*) elimination of the cellular processing of primary viral RNA transcripts (E. Kuff, F. Ferdinand, and G. Khoury, *J. Virol.*, in press). All of these features together provided a method that we have used to study the early phase of SV40 transcription.

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MATERIALS AND METHODS

Viruses and Cells. Plaque-purified wild-type SV40 (WT, strain 776) was originally obtained from D. Nathans and the early SV40 temperature-sensitive mutant, tsA58, from P. Tegtmeyer. Viruses were grown and titered in primary African green monkey kidney cells.

Infection of Cells. Confluent monolayers of primary or secondary cells grown in minimal essential medium (Grand Island Biological Co., Grand Island, NY) supplemented with 2 mM glutamine, 10% fetal calf serum, and penicillin and streptomycin were infected with SV40-WT at a multiplicity of 30 plaque-forming units per cell or with SV40-tsA58 at a multiplicity of 5–10 plaque-forming units per cell. After a 1-hr adsorption at room temperature, cells were re-fed with minimal essential medium containing 2% fetal calf serum.

Preparation of Viral Transcriptional Complexes (VTCs) and Transcription *In Vitro*. At 8.5 hr after infection, cells were harvested, and the nuclei were isolated and lysed with either 0.25% Sarkosyl in 0.1 M NaCl (17) or 0.25% Triton X-100 in 0.4 M NaCl (18). Transcription in Sarkosyl extracts has been described (16). The conditions for incorporation from VTCs isolated in Triton extracts were identical except NaCl concentration which was 425 mM. [^{3}H]UTP (1.6 μM, 25 Ci/mmol) or [α^{32}P]ATP (0.4–1.6 μM, 150–250 Ci/mmol) (Amersham/Searle Corp., Arlington Heights, IL) was labeled precursor.

Nucleic Acid Hybridizations. DNA·RNA filter hybridizations and DNA transfer by the Southern technique (19) as well as autoradiography of the nitrocellulose strips have been described in detail (16). In some experiments, we found it convenient to use DNA fragments produced with restriction endonucleases *Bam*HI and *Hpa* II, which cleave SV40 DNA at map positions 0.15 and 0.73, respectively. These enzymes divide the genome into an essentially early portion (fragment A) and a late portion (fragment B) (see Fig. 2). The early and late viral templates map from 0.17 to 0.67 and from 0.67 to 0.17 units, respectively, in a clockwise direction. Because the DNA from 0.67 to 0.72 is complementary to a minority of the stable late RNA sequences, fragment B contains essentially all of the late gene region except for the terminal 2.5% (0.15–0.175 map unit). Fragment A contains all of the early region (plus the terminal portion of the late region). The separated strands of both DNA fragments can be easily separated on 1.4% agarose gels (16).

Abbreviations: SV40, simian virus 40; WT, wild type; VTC, viral transcriptional complex; E, early DNA strand; L, late DNA strand; A, early DNA fragment; B, late DNA fragment.

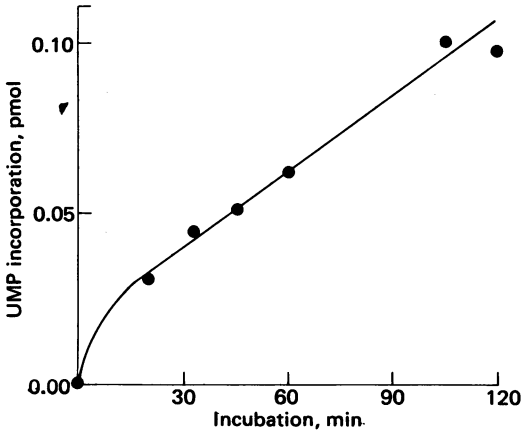

FIG. 1. Kinetics of *in vitro* RNA synthesis from the nuclear supernatant fraction. Cells (3.2×10^8) were infected with SV40 tsA58 at 5 plaque-forming units per cell and grown at the nonpermissive temperature (41°) for 8.5 hr. The RNA synthesizing activity was assayed in a final volume of 1.3 ml with [^{3}H]UTP (21 Ci/mmol) as a radiolabeled precursor. Aliquots (0.25 ml) were removed at the times indicated, the RNA was precipitated with trichloroacetic acid onto nitrocellulose filters, and the radioactivity was assayed in a toluene-based scintillation fluid.

RESULTS

Template Activity of Early VTCs. In previous experiments, we described the properties of late SV40 VTCs, harvested at 40 hr after infection. To investigate the properties of early VTCs, it was essential that cell cultures be harvested prior to the onset of viral DNA replication. The precise time of initiation of SV40 DNA replication is unclear, although most reports suggest that it occurs approximately 12 hr after infection (see refs. 9 and 10). In the experiments described below, we chose to extract VTCs as early as possible (8.5 hr after infection). As an additional safeguard against viral DNA replication, most experiments were performed at the nonpermissive temperature (41°) with a "nonleaky" early SV40 temperature-sensitive mutant, tsA58 (20). This mutant fails to produce any detectable progeny at temperatures above 39° (21).

In the first set of experiments, cells infected with tsA58 at a multiplicity of infection of 5–10 plaque forming units per cell were harvested at 8.5 hr after infection as described (16). Nuclear extracts from these cultures were separated into a supernatant fraction containing solubilized VTCs and a pelleted chromatin fraction. The RNA polymerizing activity on the endogenous early SV40 templates was determined for the supernatant fraction as described in Fig. 1; the incorporation of [^{3}H]UMP was approximately linear for the first 2 hr of sampling. We also investigated the transcriptional activity in the chromatin pellet fraction. Because it is difficult to obtain uniform aliquots from the viscous pellet, at the end of the 2-hr incorporation period the entire reaction mixture was extracted with phenol/chloroform/sodium dodecyl sulfate (16) and further purified by Sephadex chromatography. We tried to decrease loss of RNA by reextracting several times with phenol/chloroform. Nevertheless, because of the viscosity of the interphase, the amount of RNA may be slightly underestimated. In these experiments, the majority of the transcriptional activity is present in the chromatin pellet (about 40 times the total incorporation of UMP found in the supernatant fraction). When analyzed by SV40 DNA-filter hybridization (Table 1), however, only a small percentage of the chromatin-synthesized RNA was found to be SV40-specific (0.05–0.07%). On the other hand, 4–11% of the supernatant RNA was virus-specific. Thus, the majority of viral RNA (57% and 85% found in two separate experiments) is transcribed from the supernatant fraction of early SV40 VTCs.

In previous experiments (see also Table 1) we found that the pellet fraction from late SV40 VTCs contained only about 3 times as much transcriptional activity (in terms of incorporation of radiolabeled precursor) as the supernatant fraction. The higher ratio of supernatant to pellet activity in late VTCs compared to early VTCs (1:3 versus 1:40) reflects the significant increase of viral transcriptional activity in the late lytic Sarkosyl supernatant (16, 17). This observation is supported by the fact that essentially all of the late lytic Sarkosyl supernatant RNA (87%) is SV40-specific (Table 1). As was the case with the early VTCs, the majority of the virus-specific activity in the late VTCs (84%) was found in the supernatant fraction.

Analysis of Early SV40 RNA by DNA·Filter Hybridization. The templates for early and late SV40 mRNAs are localized within separate regions of the viral genome and are located on the opposite DNA strands (23, 24). Separation of the SV40 DNA

Table 1. Analysis of SV40 RNA synthesized in supernatant and pellet fractions by viral transcriptional complexes

Incorporation fraction	Total incorporation		SV40-specific RNA		Fraction of SV40-specific RNA
	cpm	%	cpm	%	
Early VTC 1*					
Supernatant	63,000	2.3	541/13,100	4.1	0.57
Pellet	2,670,000	97.7	243/330,000	0.07	0.43
Early VTC 2*					
Supernatant	40,000	2.5	512/4,800	10.7	0.85
Pellet	1,540,000	97.5	203/420,000	0.05	0.15
Late VTC†					
Supernatant	730,000	27.0	8700/10,000	87	0.84
Pellet	1,970,000	73.0	6300/105,000	6	0.16

* In two separate experiments (early VTC 1 and 2), 2×10^9 SV40-tsA58-infected cells (multiplicity of infection = 5) were incubated at the nonpermissive temperature (41°) for 8.5 hr. The nuclei were prepared and lysed with Sarkosyl and the extract was separated by centrifugation into supernatant and pelleted chromatin fractions. The pellet was taken up in 1.7 ml (the same volume as the supernatant) of 0.025 M Tris·HCl, pH 7.9/0.1 M NaCl and homogenized by vortexing and swelling for 1 hr at 23° prior to the reaction. The *in vitro* RNA synthesis on endogenous templates was examined with [α-^{32}P]ATP (0.5 mCi, 200–250 Ci/mmol) as radiolabeled precursor. After 90 min of incubation at 23°, both supernatant and pellet fractions were extracted twice with sodium dodecyl sulfate/phenol/chloroform and once with chloroform alone. After ethanol precipitation of the aqueous phase, the samples were digested with DNase (25 μg/ml), phenol extracted, and chromatographed on Sephadex (G-50, fine). The [^{32}P]RNA in the void volumes of supernatant and pellet fractions were concentrated by ethanol precipitation and compared. Exhaustive hybridization with SV40 DNA filters was performed as described (22).

† Late VTCs from 6×10^7 WT-SV40-infected cells were incubated in a final volume of 1 ml for 90 min with [α-^{32}P]ATP as radiolabeled precursor (0.8 μM, 180 Ci/mmol). The RNA was purified and the SV40-specific portion was determined by hybridization to SV40 DNA filters.

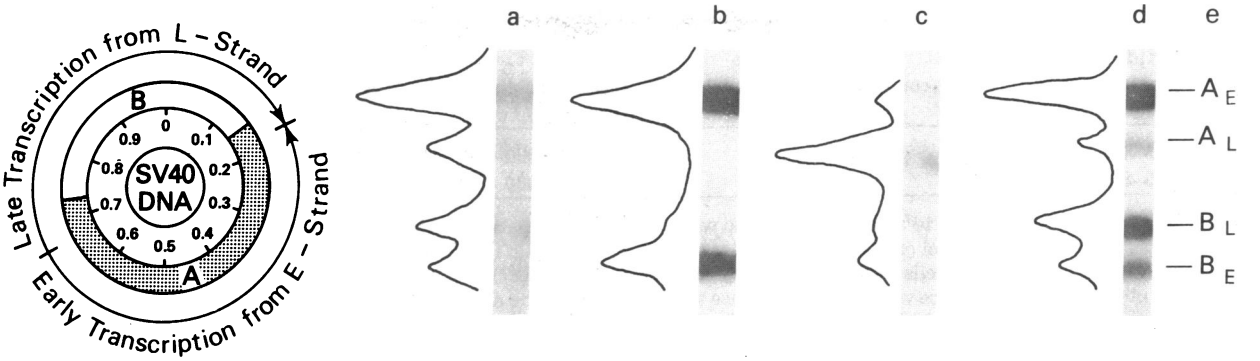

FIG. 2. Hybridization of *in vitro* [^{32}P]RNA to fragments of the SV40 genome. The map of the SV40 genome shows the position of fragments generated by cleavage with the restriction endonucleases *Bam*HI and *Hpa* II: fragment A, 0.15–0.72; fragment B, 0.72–0.15. The outermost line shows the direction of transcription and the positions of the early and late gene regions. At 8.5 hr after infection with SV40-WT or SV-40-tsA58, nuclear extracts were prepared either by the Sarkosyl or the Triton extraction procedure. ^{32}P-Labeled RNA was annealed with blots containing the separated strands of fragments A and B. The absence of crosscontamination of these DNA fragments has been demonstrated (16). Results of hybridization with RNA obtained from: (*a*) WT-early Sarkosyl VTC; (*b*) tsA58-early Sarkosyl VTC; (*c*) tsA58-early Sarkosyl VTC (hybridization of the self-annealing RNA fraction after pancreatic RNAse treatment and heat denaturation); (*d*) tsA58-Triton X-100 VTC. The fraction of ^{32}P-labeled RNA that annealed with the bands A_E, A_L, B_L, and B_E in each blot are presented in Table 2.

strands and restriction enzyme fragments of the viral genome thus provide probes to assay newly synthesized viral RNA.

The cleavage products of SV40 DNA generated with endonucleases *Bam*HI and *Hpa* II were denatured in alkali and electrophoresed in 1.4% agarose gels, separating the early (E) and late (L) strands of both the early (A) and the late (B) DNA fragments into four DNA bands. The DNA was transferred to nitrocellulose paper for hybridization studies (19).

In the first experiment, RNA was synthesized *in vitro* from the Sarkosyl extract of cells infected with SV40-WT. Cells were harvested 8.5 hr later and early VTCs were prepared. After a 2-hr incorporation *in vitro* with α-^{32}P-labeled ATP, RNA was purified and annealed with the *Bam*HI/*Hpa* II-separated DNA strands (Fig. 2*a*). RNA from both the supernatant and the pellet fraction (latter not shown) contained early (0.43) and anti-late sequences (0.18) that annealed with the E-strand DNA fragments (A_E and B_E, respectively) (Table 2). In addition, a significant but lesser amount of RNA annealed with the L strand DNA fragments, complementary to late (B_L = 0.20) and anti-early (A_L = 0.19) RNA sequences. In a previous study (16) we demonstrated that SV40 RNA synthesis is not initiated *in vitro* under the Sarkosyl-incorporation conditions. Thus, the tentative conclusion from this experiment is that viral transcription is initiated on both the E and L SV40 DNA strands prior to the onset of viral DNA synthesis.

Because we were concerned about the possibility of low levels of viral DNA replication in studies performed with SV40-WT, this experiment was repeated with the early SV40 mutant, tsA58, at the nonpermissive temperature (41°). Although the majority of RNA transcribed from the early tsA58 Sarkosyl supernatant annealed with the E-strand (A_E = 0.52; B_E = 0.38), a definite and reproducible fraction annealed with the late region (B_L = 0.10) (Fig. 2*b*). Although in this experiment no anti-early RNA could be detected (complementary to A_L), it seemed likely that these RNA sequences had been transcribed but were preferentially removed from the filter-binding reaction by hybridization in solution with an excess of true early RNA (A_E). To investigate this possibility, the *in vitro*-labeled early Sarkosyl supernatant RNA was self-annealed in 5× standard saline–citrate (0.75 M NaCl/0.075 M Na citrate, pH 7) for 36 hr at 68°. Remaining single-stranded RNA was digested with pancreatic RNase (50 μg/ml, 1 hr, 37°), and the resistant RNA duplexes were examined in subsequent hybridization experiments. After brief heat denaturation, the RNA was annealed with the separated strand blots which in turn were evaluated by autoradiography. As predicted (Fig. 2*c*) the labeled anti-early RNA (A_L) was clearly present in the RNA·RNA duplexes. The fact that it was the most prominent labeled species found in RNA·RNA hybrids most likely reflects the relative abundance of unlabeled early RNA (A_E) in the nuclear preparations. The presence of RNA complementary to the remaining bands (A_L, B_E, B_L) further demonstrates that transcription occurs from symmetrical regions of both DNA strands in the tsA58 Sarkosyl supernatant. The detection of labeled RNA complementary to the L strand in this experiment with tsA58 confirms the previous observation with WT virus—namely, that late SV40 transcripts are presumably initiated prior to viral DNA replication. Thus, the major difference between the early and late VTCs is quantitative rather than qualitative; transcription from early VTCs is predominantly (60–90%), but not exclusively, from the E strand (Fig. 2) whereas transcription from late VTCs is predominantly (85–90%) from the L strand (16, 25).

Table 2. Radiolabel distribution in bands shown in Fig. 2

Band	Fraction of radiolabel			
	Panel *a*	Panel *b*	Panel *c*	Panel *d*
A_E	0.43	0.52	0.07	0.39
A_L	0.19	—	0.62	0.10
B_L	0.20	0.10	0.13	0.32
B_E	0.18	0.38	0.18	0.19

Earlier studies (26) showed, by cesium chloride/propidium diiodide equilibrium centrifugation and by alkaline sucrose sedimentation, that essentially no tsA58 DNA replication occurs at ≥40°. A more sensitive method of detection of DNA replication is DNA·DNA filter hybridization of pulse-labeled DNA, in which as few as 200–250 virus-specific input counts could be detected with an efficiency of about 10% (26).

To document the absence of viral DNA replication in these experiments within the limits of the sensitivity of filter hybridization, two sets of cultures infected with tsA58 (multiplicity of infection = 10) were incubated for either 5.5 or 7.5 hr at 40.5°. At these times, cells were pulse-labeled for 1 hr with [^{3}H]dThd at 40.5°, and the Hirt supernatant DNA fractions were purified (27). These DNA preparations were denatured and annealed exhaustively with SV40 DNA-containing filters (22) (Table 3). Even after two consecutive incubations with SV40 filters, essentially no newly synthesized DNA hybridized in the 7.5-hr sample (the time was chosen so that the end of the

Table 3. DNA synthesis in tsA58 infected cells at the nonpermissive temperature

Time of [^{3}H]dThd, pulse, hr after infection	Total precipitable counts in supernatant,* cpm	Input hybridization reaction,† cpm	cpm hybridized to: SV40 filter†	Blank filter†	SV40 filter‡	Blank filter‡
5.5–6.5	136,000	88,000	36	33	38	23
7.5–8.5	150,000	63,000	26	21	26	33

Six flasks, each containing about 2×10^7 cells, were infected with tsA58 and incubated at 41°. At 5.5 hr and 7.5 hr after infection, the medium of three flasks was withdrawn and 10 ml of fresh medium containing 1 mCi of [^{3}H]dThd (20 Ci/mmol) was added to each flask; incubation at 41° was continued for 1 hr more. The cells were then lysed by the Hirt procedure (27).

* The supernatants were extracted twice with phenol and once with chloroform; the nucleic acids in the aqueous phase were precipitated with ethanol and resuspended in 2 ml of 0.15 M NaCl/0.015 M Na citrate.

† One milliliter of each sample was treated with pancreatic RNase (25 μg/ml) for 2 hr at 37°, followed by phenol extraction and ethanol precipitation of the aqueous phase. The precipitate was redissolved in 200 μl of H_2O, boiled for 45 min, and quenched. To each sample, 50 μl of 3 M NaCl/0.3 M Na citrate and one SV40-DNA-containing plus one blank filter were added for incubation at 68° for 24 hr.

‡ After the 24-hr incubation, the filters were removed and fresh filters were added for another 24 hr of hybridization. The total acid-precipitable radioactivity at the start of the hybridization was 58,000 cpm for the 5.5- to 6.5-hr pulse and 63,000 cpm for the 7.5- to 8.5-hr pulse. Under the same conditions, 36% of SV40 cRNA (i.e., RNA transcribed asymmetrically *in vitro* from the early DNA strand) hybridized to the SV40 DNA filter in the first 24 hr, as did 15–20% of denatured ^{3}H-labeled SV40 DNA I (data not shown).

pulse would correspond to the time of harvest of most early VTCs, 8.5 hr). The control sample, labeled even earlier (5.5–6.5 hr), also contained no detectable SV40 DNA.

The extent of transcription of each DNA strand beyond the early and late coding regions (i.e., the presence of anti-early RNA, A_L, and anti-late RNA, B_E) may reflect the symmetrical transcription of the SV40 genome as described (15). This finding is of questionable significance, however, because it can be argued that symmetrical transcription occurs on a minority of molecules and that factors required for recognition of a putative termination of viral transcription may have been removed by Sarkosyl extraction. Although it is difficult to circumvent this problem completely, we decided to prepare VTCs by using a milder procedure. Triton X-100 extraction apparently removes fewer viral DNA-bound proteins (28). Thus, for example, a late Triton X-100 VTC sediments at 55 S whereas a late Sarkosyl VTC sediments at 22–24-S (16, 17, 18, 28). The early Triton X-100 VTC (Fig. 2*d*) incorporated radiolabeled RNA complementary to both regions of both DNA strands. Although this finding again confirms the presence of L-strand transcription the the absence of detectable DNA replication, it does not provide any further evidence for the presence or absence of a localized termination site for SV40 transcription.

Sedimentation Analysis of the Early VTCs. For additional purification of the early SV40 VTCs and comparison of their sedimentation properties to those of the late VTCs (16, 17), the supernatants of Sarkosyl-treated nuclear extracts harvested 8.5 hr after infection with SV40 tsA58 were analyzed by sedimentation in 10–30% neutral sucrose gradients (3.6 ml) containing a 50% sucrose cushion (0.4 ml) at the bottom of the tube. Of the 25 fractions collected, the RNA polymerizing activity in endogenous templates was assayed in the first 20 fractions with [α-^{32}P]ATP as radiolabeled precursor. The fractions were extracted with phenol/chloroform/sodium dodecyl sulfate and the RNA was precipitated with ethanol. In experiments with late VTCs (16), essentially all of the newly synthesized RNA was SV40-specific; thus the virus-specific RNA incorporation curve was assumed to be the same as that of the total RNA incorporation curve. This assumption obviously cannot be made for early VTCs because less than 11% of the radiolabeled supernatant RNA was SV40 specific (Table 1). For this reason the sedimentation pattern for the viral transcriptional activity was analyzed as follows.

Precipitates of each fraction were resuspended in 0.75 M NaCl/0.075 M Na citrate, adjacent pairs of fractions were combined, and the RNA was annealed with filters containing SV40 DNA as described (16). Two major peaks of early SV40 VTC activity were found (Fig. 3). Compared with markers run in a parallel tube, these activities sedimented at 16–25 S and >45 S. In contrast, we previously found only one major peak of late SV40 transcriptional activity at 22–24S (16). It should be reemphasized, however, that the overall viral RNA incorporation of the late VTCs is substantially greater than that of the early VTCs. Thus, peaks of activity equivalent to those seen in the early transcriptional intermediate preparations would not have been easily detected in the late VTC profile. The RNA obtained from the two peaks of early VTC activity were analyzed by hybridization to SV40 strand-separated DNA blots (Fig. 3) as described in Fig. 2. Of particular interest was the observation that the two peaks of early VTC activity seemed to transcribe qualitatively different types of RNA. The slower-sedimenting activity appeared to synthesize both early and late SV40 RNA. On the other hand, the >45S early VTC

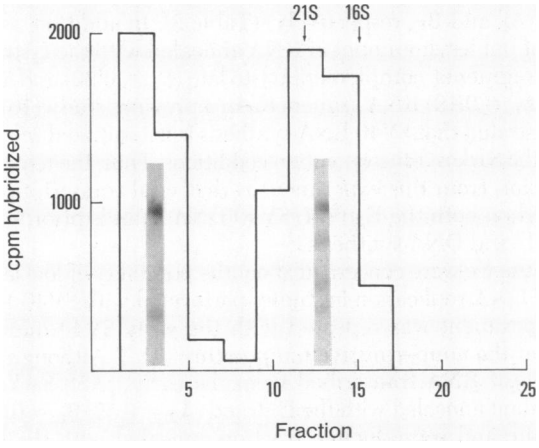

FIG. 3. Sedimentation through neutral sucrose of early VTCs. The nuclear supernatant in 0.6 ml, prepared from SV40-tsA58-infected cells (8½ hr after infection), was sedimented in a 10–30% sucrose gradient (3.6 ml) in 0.025 M Tris-HCl, pH 7.9/0.1 M NaCl/0.025 M KCl/1 mM dithiothreitol containing a 0.4-ml cushion of 50% sucrose (SW 60 rotor, 1.75 hr, 60,000 rpm at 4°). Twenty-five fractions were collected. The RNA polymerizing activity in the first 20 fractions was assayed in the presence of α^{32}P-labeled ATP. Each two adjacent fractions were combined as indicated and the RNA synthesized in these pools was extracted and hybridized to SV40 DNA immobilized on nitrocellulose filters, to establish the sedimentation profile of the VTCs as demonstrated by the bar graph. RNA from the two peaks of transcriptional activity (fractions 2–5 and 12–15) were subsequently hybridized to the *Hpa* II/*Bam*HI separated strand blots (see legend to Fig. 2). The pattern of hybridization is indicated in the *insets*.

activity appeared to transcribe only early SV40 RNA. Although the reasons for this observation are unclear, the finding was reproducible and raises the possibility that more than one type of VTC exists prior to viral DNA replication.

DISCUSSION

We have used the Sarkosyl extraction procedure to analyze early SV40 transcriptional complexes and early virus-specific RNA. This extraction procedure provides unprocessed RNA that, in the supernatant fraction, has been greatly enriched for virus-specific sequences (4–11% in the supernatant compared to 0.06% in the pellet).

The most remarkable result of these studies is that some late viral transcription is initiated before the onset of viral DNA replication. In the absence of detectable SV40 DNA synthesis and in the absence of reinitiation of SV40 transcription (16), as much as 10–20% of the viral RNA synthesized *in vitro* by either Sarkosyl or Triton X-100 nuclear extracts is late RNA (i.e., anneals with the late SV40 DNA strand). This observation is contrary to earlier notions that initiation of late SV40 transcription might somehow be directly coupled to and dependent upon viral DNA synthesis. As a result of these experiments, we suggest that the transition from early to late SV40 transcription reflects a quantitative change rather than the initiation of a new process. Thus, at later times there is an increase in the frequency of late transcription relative to early transcription rather than a *de novo* initiation of late transcription. Some of the factors that control this transition from early to late transcription are known. For example, it has recently been shown that SV40 T antigen regulates the level of early RNA synthesis (13, 29); this same antigen might also regulate the level of late RNA synthesis, either directly or indirectly.

Based on these observations and the findings obtained in the present study, we present the following speculative model to explain the early-late SV40 transcriptional "switch." In the absence of a functional T antigen, initiation of early transcription is more efficient than the initiation of late transcription. With the limited number of viral templates early in the infectious cycle, a small amount of SV40 RNA is transcribed (13); the majority of this RNA is early. As a functional T antigen is synthesized, (*i*) viral DNA replication is initiated and (*ii*) the amount of early transcription is limited or late transcription is augmented, or both. A portion of the newly synthesized SV40 DNA molecules presumably enters the transcriptional pool, and the majority of the transcriptional templates are involved in late transcription at this time. Whether initiation of DNA replication, as well as the presence of a functional T antigen, is necessary for the *predominance* of late SV40 transcription is not known. Nor is it clear whether the same molecule can serve as templates for transcription of early and late viral mRNAs, either simultaneously or at separate times.

The apparent independence of initiation of late transcription from viral DNA replication as demonstrated in this study supports the earlier finding of late SV40 RNA in abortively infected (nonpermissive) mouse cells (5, 12). It also provides a potential explanation for the detection of V antigen (the product of late SV40 RNA) in mouse cells injected with very high titers of SV40 virions or SV40 DNA I (30). Nevertheless, we should reemphasize that we have detected late strand specific RNA in these experiments. Whether this RNA is transcribed by the same mechanism as that for true late SV40 RNA and whether it is functional are still unknown.

A preliminary observation of particular interest is the detection of two active peaks of viral transcriptional activity in the SV40-tsA58 Sarkosyl VTCs. The lighter peak, sedimenting at 16–25 S, appears to synthesize RNA homologous to both viral DNA strands whereas the heavier peak (>45 S) is associated with an activity that appears to synthesize only early SV40 RNA. Although this finding is reproducible, its significance is not yet clear. For example, the 45S activity might reflect transcription from a distinct template that differs in its configuration or associated proteins. On the other hand, a trivial explanation would be that some RNA homologous to the L strand is synthesized by the VTCs sedimenting at 45 S, but it preferentially anneals with an excess of cold E-strand RNA that might be present in these fractions. Additional studies are required to determine the significance of these two separable early VTC activities.

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