

## Extensive Symmetrical Transcription of Simian Virus 40 DNA in Virus-Yielding Cells

(SV40/monkey cells/actinomycin D/RNase/hybridization)

YOSEF ALONI

Department of Genetics, Weizmann Institute of Science, Rehovot, Israel

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**ABSTRACT** Rapidly labeled RNA was extracted from monkey cells after infection with Simian Virus 40 (SV40) and exposure to short pulses of [ $^3\text{H}$ ]uridine late in infection. When this RNA was self-annealed, it became resistant to digestion with ribonuclease. The fraction of RNA that resisted the ribonuclease treatment decreased with increased labeling time, or when a short pulse of radioactivity was followed by incubation with unlabeled uridine and actinomycin D. The RNase-resistant RNA was isolated by chromatography on Sephadex G-100 and shown to be double-stranded by its susceptibility to ribonuclease as a function of salt concentration and temperature. This behavior was not due to RNA-DNA hybrid formation, since deoxyribonuclease had no effect upon the double-stranded molecules, even after their denaturation. The relation of the double-stranded RNA to SV40 was demonstrated by the hybridization of about 50% (corrected value, >90%) of the separated RNA strands with component I of SV40 DNA from plaque-purified virus. After self-annealing in formamide at low temperature, about 10% of the rapidly labeled, viral RNA sedimented at 13 S. This value corresponds in size to about 60% of the SV40 DNA.

These observations indicate that late in infection of monkey cells, SV40 DNA is transcribed symmetrically over a considerable portion of its length, and that subsequently some sequences from one or both of the RNA strands are degraded.

It has been generally accepted that transcription is asymmetric, in the sense that only one of the two DNA strands of a given gene serves as a template for RNA (1). However, the possibility that both DNA strands are transcribed, and that some sequences from one or both of the RNA strands are subsequently degraded, has not been excluded. Indeed, it has recently been found that transcription of mitochondrial DNA in HeLa cells operates according to the latter possibility (2, 3). SV40 DNA has circular configurations similar to those of mitochondrial DNA from HeLa cells (4). Early after infection, and in SV40-transformed cells, the viral genome is only partially transcribed, while at later stages in the infection process most or all of the genetic information contained in the SV40 genome is transcribed (5, 6). I show that late after infection of monkey cells with SV40, the viral DNA is transcribed symmetrically over a considerable portion of its length and, subsequently, some sequences from one or both of the RNA strands are degraded.

Abbreviations: SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl-0.015 M  $\text{Na}_3\text{citrate}$ .

### MATERIALS AND METHODS

Growth of SV40 (strain 777) not purified by plaque formation on monkey cells of the BS-C-1 line, as well as concentration and purification of the virus from the tissue culture lysates, were described (5). In all experiments, cultures ( $4 \times 10^6$  cells) were infected with 1.0 ml of the same stock of SV40 [ $4 \times 10^8$  plaque-forming units (PFU)/ml] except in the experiment described in Table 2, where both plaque-purified and nonpurified virus was used for infection of the cells. DNA was extracted from the purified virus with phenol (7), and

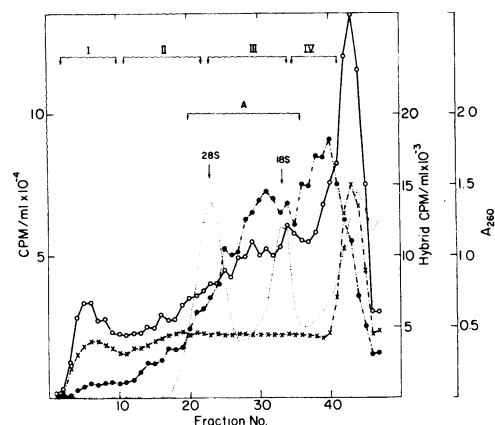


FIG. 1. Sedimentation patterns of [ $^3\text{H}$ ]RNA extracted from the Hirt supernatant fraction of SV40-infected or mock-infected cells. 48 hr after infection or mock-infection,  $4 \times 10^7$  cells were exposed for 20 min to [ $^3\text{H}$ ]uridine (0.25 mCi/ml, 29 Ci/mmol). In each case, RNA was extracted from the Hirt supernatant fraction and placed on a linear gradient of 15-30% (w/w) sucrose in SDS buffer (11) containing a 1.5 ml cushion of 60% sucrose. Centrifugation was for 16 hr at 23,000 rpm at  $20^\circ$  in a Spinco SW 41 rotor. Fractions were collected and 50- $\mu\text{l}$  portions were precipitated with  $\text{Cl}_3\text{CCOOH}$  and counted. The  $A_{260}$  profile shown is of the rRNA components from the  $^3\text{H}$ -labeled, infected cells. The cpm mock-infected curve was normalized to this absorbancy profile. Cpm hybrid represents the radioactivity bound to filters (7-mm diameter) containing SV40 DNA. The filters were incubated with 50  $\mu\text{l}$  from each fraction of the sucrose gradient in 1 ml of 4  $\times$  SSC for 18 hr at  $68^\circ$ , then treated with RNase (blank filters contained <5 cpm). The results are shown as cpm/ml. With RNA extracted from mock-infected cells, less than 7 cpm in any one fraction hybridized with SV40 DNA (pattern not shown).  $A_{260}$ ,  $\cdots\cdots$ ; cpm hybrid,  $\bullet\cdots\bullet$ ; cpm infected,  $\circ\cdots\circ$ ; cpm mock-infected  $\times\cdots\times$ .

fractionated by sedimentation through a solution of alkaline CsCl (pH 12.9). Under these conditions the supercoiled component I (8) sediments at 53 S, and is effectively separated from component II (16–18 S), other DNA of low molecular weight, and any contaminating proteins. Component I was used in all RNA–DNA hybridization experiments. DNA was extracted from BS-C-1 cells as described (9).

In all experiments, RNA was extracted from a sodium dodecyl sulfate (SDS)–high salt supernatant fraction (10). The Hirt supernatant was collected by ethanol precipitation and centrifugation, and the pellet was dissolved in buffer [0.01 M Tris·HCl (pH 7.0)–0.1 M NaCl–1 mM EDTA–0.5% SDS] and subjected to Pronase treatment (40 µg/ml, 2 hr, 37°) followed by phenol extraction. This procedure gave an almost complete recovery of pulse-labeled RNA, as with pulse-labeled mitochondrial RNA in HeLa cells (2). All RNA samples were digested with 25–100 µg/ml of DNase I (Worthington, RNase-free electrophoretically purified) in 0.05 M Tris·HCl (pH 6.7 at 25°)–25 mM KCl–2.5 mM MgCl<sub>2</sub> at 2° for 60 min. (The batch of DNase used was free of detectable RNase activity, as shown by sucrose gradient sedimentation of rRNA treated under identical conditions to those used in the actual experiments.) The digest was extracted with SDS–phenol, and the extract was passed through Sephadex G-100 (1.1 × 55-cm column) to give total RNA, or fractionated on sucrose gradients (11), as required. The virus-specific, pulse-labeled RNA in the Hirt supernatant fraction was 40–50% of the amount extracted from whole cells.

RNA–DNA hybridization with the DNA immobilized on Millipore filters (25-mm diameter if not otherwise specified, 0.45-µm pore size) was reported (5, 11). Self-annealing of the RNA at high temperature, and estimation of the RNase-resistant RNA was done in 2 × SSC (SSC is 0.15 M NaCl–0.015 M sodium citrate) (2).

## RESULTS

*Sedimentation Patterns of Pulse-Labeled RNA Extracted from SV40-Infected and Mock-Infected BS-C-1 Cells.* 48 hr after infection with SV40, BS-C-1 cells were exposed to [<sup>3</sup>H]-uridine for 20 min. Mock-infected cells were similarly treated. Fig. 1 shows the superimposed sedimentation profiles of labeled RNA extracted from the infected and mock-infected cells, with the rRNA components from the infected cells serving as sedimentation markers (*A*<sub>260</sub>). The radioactivity profile of the RNA from infected cells (cpm infected) shows a major peak in the 4S region, as well as an accumulation of RNA components from 4 to 28 S, and higher. However, in the case of the RNA from mock-infected cells (cpm mock-infected) only the 4S peak is clearly defined. The portion of RNA from infected cells that is SV40-specific represents, in various experiments, from 10 to 20% of the total radioactivity incorporated into RNA. This portion is the radioactivity remaining bound to filters after hybridization of radioactive RNA with an excess of SV40 DNA and treatment with RNase (cpm hybrid). Since the efficiency of the RNA–DNA hybridization reaction is about 50–60% (unpublished observations), these results suggest that a considerable portion of the pulse-labeled RNA extracted from the Hirt-supernatant fraction of SV40-infected cells late in infection is virus-specific. It also appears, as reported (12), that there is a general induction of cellular RNA synthesis in the infected cells, as compared to the mock-infected cells. This is seen most clearly in

the 4S region, where the infected sample shows about twice the specific radioactivity of the mock-infected one. This cannot be attributed to the synthesis of virus-specific RNA in infected cells, since the counts hybridizing with SV40 DNA fall off rapidly in this region of the gradient (Fig. 1). A similar pattern of radioactivity and hybridization was obtained upon centrifugation of the 4S region for a longer time through a second sucrose gradient (data not shown).

*RNase Resistance of Pulse-Labeled RNA from Infected and Mock-Infected Cells.* The double-helical content of the labeled RNA shown in Fig. 1, before and after self-annealing, was estimated by study of the degradation of RNA by pancreatic RNase A. Regions I–IV from the sucrose gradients of Fig. 1 were pooled as indicated, and the RNA was collected by ethanol precipitation and centrifugation and tested for RNase resistance (50 µg/ml of RNase in 2 × SSC for 90 min at 25°). Without annealing, 8–10% of the pulse-labeled RNA from infected cells was RNase resistant, as compared to 3–5% of the RNA from mock-infected cells. Prior incubation of the RNA in 4 × SSC for 18 hr at 68° resulted in a considerable increase in the RNase resistance of the RNA from infected cells, but not from mock-infected cells. In infected cells, the resistance increased to 23% in region II and to 30% in regions III and IV, while only a slight increase was registered in region I. Thus, double-helical RNA content is highest in those regions of the sucrose gradient that show an accumulation of virus-specific RNA.

*Relationship between Labeling Time and the Amount of RNase-Resistant RNA.* Table 1 shows that the percentage of the self-annealed, RNase-resistant RNA from infected cells decreased with increased labeling time: from 35% after 2 min to 20% after 20 min. In another experiment, infected cells were pulse-labeled with [<sup>3</sup>H]uridine for 5 min, followed by a 60-min incubation in unlabeled uridine with a high concentration of actinomycin D [The concentration of actinomycin D used (50 µg/ml) blocks further cellular and viral RNA synthesis (13, 14).] In this case, the percentage of RNase-resistant RNA was reduced to the background level of the mock-infected control, which did not show any change

TABLE 1. Ribonuclease resistance of self-annealed RNA from infected and mock-infected cells labeled for different periods of time

Labeling time (min)	Ribonuclease resistance (%)	
	Infected	Mock-infected
2	35	8
5	25	8
20	20	9
5, and 60-min chase*	9	8

Total RNA was prepared from 10<sup>7</sup> cells labeled with [<sup>3</sup>H]-uridine (0.25 mCi/ml, 29 Ci/mmol) for the indicated times. Self-annealing was for 18 hr at 70° in 0.2 ml of 4 × SSC. The RNase-resistance tests were performed with samples containing between 1000–2000 cpm with 50 µg/ml of RNase in 2 × SSC for 90 min at 25° (2).

\* Cells were labeled for 5 min, washed 3 times with Eagle's medium containing 0.1 mM uridine, then incubated for 60 min in the presence of 0.1 mM uridine and 50 µg/ml of actinomycin D.

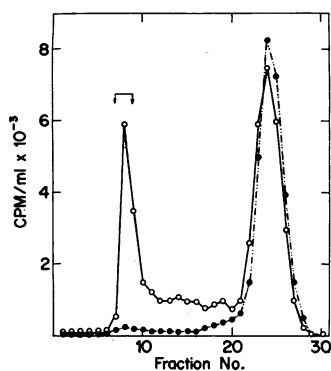


Fig. 2. Exclusion chromatography of self-annealed, RNase-treated RNA on a column of Sephadex G-100. RNA samples from infected and mock-infected cells were dissolved in 0.5 ml of 0.25 M NaCl and layered on separate  $1.1 \times 55$ -cm Sephadex G-100 columns equilibrated with 0.25 M NaCl. 2-ml Fractions were collected (0.3 ml/min), and 0.1-ml portions were counted in Triton X-100 (31). The first peak is excluded with the void volume, while the second peak contains the lower molecular weight products.  $\circ$ — $\circ$ , infected;  $\bullet$ — $\bullet$ , mock-infected.

in RNase resistance with labeling time. These results suggest that some sequences from the complementary RNA strands have a shorter half-life than others.

*Isolation of RNase-Resistant RNA by Chromatography on Columns of Sephadex G-100.* Region A from the sucrose gradient shown in Fig. 1 was collected by ethanol precipitation and centrifugation. The slower-sedimenting RNA components

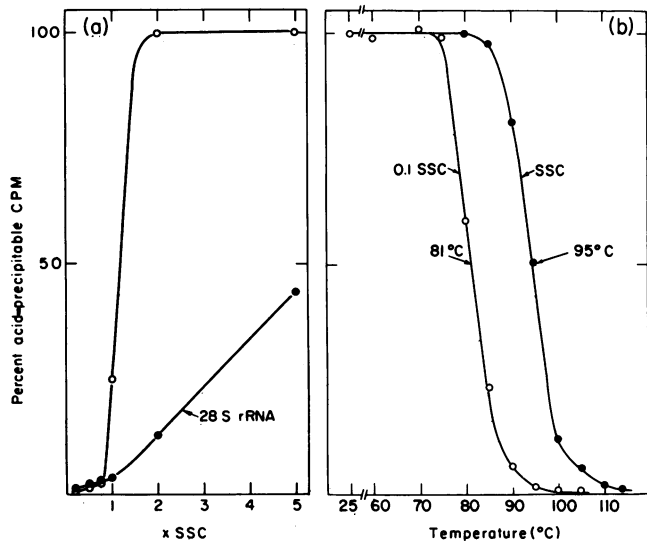


Fig. 3. Degradation of the material present in the excluded peak of the Sephadex column by RNase at different salt concentrations and at different temperatures. (a) Each sample in 2 ml contained the indicated concentrations of SSC,  $^{32}$ P-labeled 28S rRNA (800 cpm), and  $^3$ H-labeled RNA (800 cpm) from the excluded peak of the Sephadex column. RNase-resistance was tested with  $50 \mu\text{g/ml}$  of enzyme for 30 min at  $25^\circ$ . The samples were then precipitated with  $\text{Cl}_3\text{CCOOH}$  and counted. The amount of acid-precipitable material before treatment with RNase was assigned a value of 100%. (b) 0.6 ml Samples of  $^3$ H-labeled RNA (700 cpm) from the excluded peak of the Sephadex column were brought to SSC or  $0.1 \times \text{SSC}$ , heated for 4 min at the indicated temperatures, and rapidly cooled. The samples were then brought to  $2 \times \text{SSC}$ , and RNase-resistance tests were performed as in (a).

from this gradient were not collected, in order to exclude DNA degradation products. The material present in the pooled fractions was incubated in 0.1 ml of  $4 \times \text{SSC}$  at  $70^\circ$  for 18 hr, then treated with RNase ( $130 \mu\text{g/ml}$  in  $2 \times \text{SSC}$  at  $25^\circ$  for 90 min), extracted with SDS-phenol, collected by ethanol precipitation, dissolved in 0.25 M NaCl, and passed through columns of Sephadex G-100. Fig. 2 shows that only a small amount of the labeled RNA from mock-infected cells treated as above is in the excluded peak, as compared with the RNA from infected cells. The amount of labeled RNA present in these peaks is comparable with the amount of RNase-resistant, labeled RNA found in the RNase resistance tests described above. The RNA from infected cells found in the excluded peak of the Sephadex column was used in all further experiments.

*RNase Susceptibility as a Function of Salt Concentration and Temperature.* The material eluted in the excluded peak of the Sephadex column was tested for susceptibility to RNase as a function of salt concentration and temperature. The RNase susceptibility decreased with increasing ionic strength (Fig. 3a). This was also true of single-stranded 28S ribosomal RNA, which at a salt concentration greater than  $2 \times \text{SSC}$  (about 0.4 M) showed significant resistance to the enzyme. The most pronounced difference between the 28S rRNA and the RNA present in the excluded peak of the Sephadex column is in the steepness of the transition from RNase-resistance to sensitivity. In addition, at  $2 \times \text{SSC}$  the RNA from the excluded peak is almost completely resistant to the enzyme. A similar dependence on salt concentration has been shown for double-stranded MS2 RNA (15).

To determine the  $T_m$  of the transition from RNase-resistance to RNase-sensitivity, samples were heated to different temperatures and rapidly cooled; the RNase resistance in  $2 \times \text{SSC}$  was measured. Fig. 3b shows the results obtained when samples were heated at two different salt concentrations. Sharp transitions, with midpoints at  $95^\circ$  in  $1 \times \text{SSC}$  and at  $81^\circ$  in  $0.1 \times \text{SSC}$ , were observed. Such transitions and temperatures are indicative of double-stranded molecules. Indeed, with a base composition of 41% (G + C) for double-stranded SV40 RNA (16), these  $T_m$  values would fit the linear relationship between % (G + C) and  $T_m$  determined by hyperchromicity for double-stranded RNAs (17).

It is important to determine that the material eluted in the excluded peak of the Sephadex column in Fig. 2 is, indeed, self-complementary RNA and not a RNA-DNA hybrid:

(i) SV40 RNA-DNA hybrid has a considerably lower  $T_m$  value [about  $65^\circ$  at  $0.1 \times \text{SSC}$  (18) and about  $80^\circ$  at  $2 \times \text{SSC}$  (19, and personal observation)] than that found for the RNA-RNA duplex shown in Fig. 3 ( $81^\circ$  at  $0.1 \times \text{SSC}$  and  $95^\circ$  at  $1 \times \text{SSC}$ ). (ii) This RNA was never subjected to conditions, during its preparation, that would cause denaturation of DNA. Thus, single-stranded DNA would not be available for the formation of RNA-DNA hybrid. (iii) The self-complementary RNA was digested with DNase and collected in the void volume after passage through Sephadex G-100 (see *Methods*). This combined procedure excluded virtually all oligodeoxynucleotides from the RNA fraction. To further exclude the possibility of trace amounts of single-stranded oligodeoxynucleotides participating in the formation of double-stranded structures, the material from the excluded peak of the Sephadex column was heat-denatured and an

aliquot was again treated with DNase, extracted with SDS-phenol, and passed through Sephadex G-100. The treated and untreated samples were then separately reannealed and tested for their resistance to RNase. Fig. 4 shows that the second DNase treatment had no measurable effect upon the ability of the samples to reanneal. Within 24 hr, 40% renaturation was obtained in both the untreated and DNase-treated samples, indicating that the duplex is composed of double-stranded RNA. Failure to attain more complete renaturation is attributed to the RNA being fragmented more or less at random during the heating steps, and the improbability that complete renaturation of fragments would occur.

*Relation of the Annealed, Self-Complementary RNA to SV40 DNA.* RNA-DNA hybridization experiments were performed to determine the relationship between the double-stranded RNA from infected cells and SV40 DNA. "Native" and denatured RNA samples were incubated with filters containing excess amounts of component I SV40 DNA from plaque-purified virus or with filters containing BS-C-1 DNA. Almost 50% (>90% if corrected for efficiency of hybridization) of the denatured RNA hybridized with SV40 DNA, and about 8% hybridized with BS-C-1 DNA (Table 2). No hybridization was registered with either SV40 DNA or BS-C-1 DNA when "native" RNA was used. These results indicate again the duplex nature of the RNA, as well as its high specificity towards SV40 DNA.

*Molecular Weight of the Double-Stranded RNA.* The molecular weight of the virus-specific, double-stranded RNA was estimated from its position in sucrose gradients, as shown in Fig. 5. To minimize thermal and enzymatic degradation, self-annealing was at 37° in 60% formamide (20), and the RNase digestion was performed with a low concentration of enzyme (12). Fig. 5a shows a broad peak of RNase-resistant, <sup>3</sup>H-labeled RNA at about 10 S, as well as slower sedimenting RNA. The faster-sedimenting components from the 10S region (about 10% of the RNA) were pooled and recentrifuged through a second sucrose gradient. As shown in Fig. 5b, a symmetrical peak was obtained at 13 S, which corresponds to a molecular weight of about  $2 \times 10^6$  (15, 21).

The double-stranded nature of the RNA present in the 13S peak was verified by a test of its susceptibility to RNase as a function of temperature. A sharp melting profile, with a  $T_m$  value of 82° in  $0.1 \times$  SSC, was obtained, in good correspondence with the melting curve of the duplex RNA shown in Fig. 3b. The material showed essentially complete resistance to RNase, up to a temperature of 75°. The relationship of the 13S RNA component to SV40 DNA was also determined. RNA-DNA hybridization tests similar to those described in Table 2 showed that 60% (corrected value, 100%) of the denatured 13S RNA hybridized with SV40 DNA, indicating its viral origin. The 13S RNA component of Fig. 5b does not arise from the reannealing of small, single-stranded RNA components. This was shown by centrifugation of the 13S material through a sucrose gradient under denaturing conditions (22). As seen in Fig. 5c, 10-15% of the single-stranded, <sup>3</sup>H-labeled RNA sedimented at about 18 S (molecular weight =  $0.71 \times 10^6$ ) (23). Since hidden breaks in the RNA were probably introduced during the RNase treatment to which the molecules had been subjected during preparation, the size and amount of the high molecular weight RNA should be considered as minimum figures.

TABLE 2. Hybridization of SV40 DNA with "native" and denatured RNA

RNA	DNA on filter	μg/DNA filter	cpm bound to filter*
"Native"	SV40	10	15 (0.5)
	BS-C-1	50	12 (0.4)
	None	0	8 (0.3)
Denatured	SV40	10	1360 (47.5)
	BS-C-1	50	233 (8.2)
	None	0	14 (0.5)

Cells were infected with nonpurified virus. Labeled RNA used in the hybridization assay was obtained from the excluded peak of a Sephadex G-100 column (see Fig. 2). SV40 DNA was extracted from plaque-purified virus. Similar hybridization results were obtained when cells were infected with 100 PFU/cell of plaque-purified virus. Each hybridization mixture contained DNA immobilized on Millipore filters, as indicated, and 2850 cpm of "native" or denatured RNA (prepared as in Fig. 3b).

\* The numbers in parentheses give cpm bound as % of input, not corrected for efficiency of hybridization, which was 50-60% (unpublished observations).

## DISCUSSION

The most plausible interpretation of the observations reported here is that late in infection, SV40 DNA is transcribed to a major extent, if not completely, in a symmetrical fashion. Such a mode of transcription predicts the formation of RNA products capable of extensive self-pairing. Indeed, after

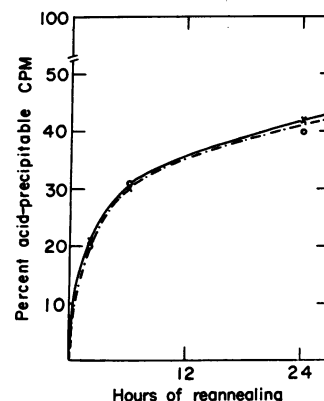


FIG. 4. The effect of DNase treatment on the ability of heat-denatured RNA from the excluded peak of the Sephadex column to reanneal. Total RNA from  $2 \times 10^7$  cells was self-annealed, treated with RNase, and passed through a column of Sephadex G-100 as in Fig. 2; the material at the void volume was collected. This was heated in 1.0 ml of  $0.1 \times$  SSC at 95° for 4 min, and rapidly cooled. DNase treatment was in 0.5 ml of TKM buffer with 25 μg/ml of enzyme at 2° for 60 min, followed by extraction with SDS-phenol. The extract was then passed through Sephadex G-100. Under these conditions, 60% of 1 μg of SV40 [<sup>14</sup>C]DNA was rendered acid-soluble, and was completely excluded by the Sephadex G-100 treatment, while single-stranded mitochondrial DNA, with a similar base composition to that of SV40 DNA, was rendered 40% acid-soluble and was also completely excluded by the Sephadex treatment. RNA was reannealed in 0.2 ml of  $4 \times$  SSC at 70°. After the indicated times, equal portions were removed and treated with 50 μg/ml of RNase in 1 ml of  $2 \times$  SSC at 25° for 60 min. They were then precipitated with  $\text{Cl}_3\text{CCOOH}$  and counted.  $\times$ — $\times$ , DNase treated;  $\circ$ — $\circ$ , untreated.

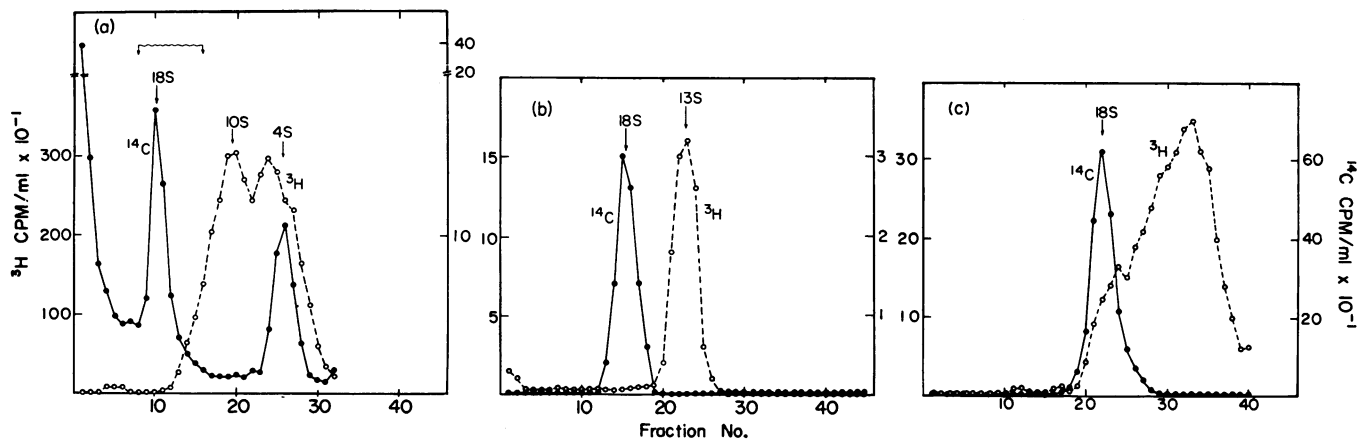


FIG. 5. Sedimentation of RNase-resistant RNA through sucrose gradients before and after denaturation. [ $^3\text{H}$ ]RNA from region A of Fig. 1 was collected and self-annealed at  $37^\circ$  for 18 hr in 60% (v/v) formamide–0.75 M NaCl–0.5% (w/v) SDS–0.01 M Tris (pH 7.9 at  $25^\circ$ ), digested with  $1\ \mu\text{g}/\text{ml}$  of RNase in  $2 \times \text{SSC}$  for 1 hr at  $25^\circ$ , and extracted with SDS–phenol. (a) RNA was sedimented through a linear gradient of 15–30% (w/w) sucrose in SDS buffer (11) in a Spinco SW41 rotor for 18 hr at 32,000 rpm at  $20^\circ$ . [ $^{14}\text{C}$ ]18S rRNA and 4S RNA served as sedimentation markers. At the end of the run,  $5\text{-}\mu\text{l}$  fractions were collected, precipitated with  $\text{Cl}_3\text{CCOOH}$ , and counted. (b) The region between the arrows in (a) was pooled, and a portion was rerun as in (a). (c) In a parallel experiment to that of (a) the region between the arrows was pooled, and the RNA was collected by ethanol precipitation. The RNA was then denatured with 18% formaldehyde at  $70^\circ$  for 9 min and run through a 5–20% (w/w) sucrose gradient in 0.02 M phosphate buffer (pH 7.4)–0.1 M NaCl–1% formaldehyde (22), in an SW41 rotor at 32,000 rpm for 16 hr at  $4^\circ$ . Fractions were collected and counted in Triton X-100 (31).

thermal annealing, RNase-resistant, double-stranded RNA was found in infected cells. The double-stranded nature of this RNA was shown by: (i) an almost complete RNase resistance at high ionic strengths, and a sharp transition to sensitivity with decreased salt concentrations; (ii) a sharp thermal transition from RNase resistance to sensitivity, with a characteristically high  $T_m$  value; and (iii) an inability to hybridize with complementary DNA unless first treated to denaturing conditions. In addition, the absence of DNA from the RNase-resistant, double-stranded material was shown by the lack of effect of DNase on the formation of the double-helical structure. The double-stranded RNA was further characterized; sedimentation through a sucrose gradient demonstrated that about 10% of the material has an  $s$  value of about 13. This corresponds to a molecular weight of about  $2 \times 10^6$  (15, 21), which is equivalent to about 60% of the SV40 genome ( $3.4$  to  $3.6 \times 10^6$  dalton) (24, 25). The relation of the double-stranded RNA to SV40 was suggested by its occurrence in infected cells and not in mock-infected cells, and was clearly established by the demonstration that after heat denaturation, about 50% (>90% if corrected for efficiency of hybridization) of the RNA hybridized with component I SV40 DNA from plaque-purified virus.

In order to determine whether the production of self-complementary RNA is in some way dependent upon the type of virus used, hybridization experiments were done with cells infected with either unpurified or plaque-purified virus. The formation of the self-complementary RNA was independent of the type of virus used for the infection (see Table 2). The self-complementary RNA represents a major fraction of the newly synthesized viral RNA. Thus, almost 20% (corrected value, 40%) of the total RNA from infected cells labeled for 2 min hybridized with excess SV40 DNA and 35% of this total RNA became RNase resistant after self-annealing (Table 1). These data, however, do not exclude the possibility of viral-induced, symmetrical transcription of

cellular DNA segments homologous to viral DNA, or synthesis of cellular RNA strands complementary to viral RNA strands. The about 8% hybridization registered with BS-C-1 DNA (Table 2), might arise from symmetrical transcription of cellular DNA, either unlinked or covalently linked to viral genes, or from transcription of a free or integrated viral DNA in which some segments have base sequences in common with cellular DNA (9, 26).

The percent of newly synthesized RNA that is virus-specific remains almost constant during the first 20 min after labeling (data not shown). Judged from the decrease in the amount of the self-complementary RNA with increased labeling time and from a pulse-chase experiment (Table 1), it appears that some sequences from the complementary RNA strands have a shorter half-life than others. This conclusion is supported by the findings that while the newly synthesized RNA that is self-complementary is transcribed from at least 60% of the SV40 DNA (see above), the viral RNA that accumulates during infection is transcribed from about 75% of the SV40 DNA (27) and it is not self-complementary (unpublished observations). It is, however, not known whether the newly synthesized sequences with a shorter half-life represent selective parts from one or both of the RNA strands. If the latter possibility is correct, this would suggest that both viral DNA strands contain mRNA genes. The kinetic labeling data thus imply the existence of a control mechanism, which acts by regulation of the *stability* of the primary gene product.

The first reported instance of symmetrical transcription that occurs *in vivo* over almost the entire genome is mitochondrial DNA in HeLa cells (2, 3). SV40 DNA is thus the second known case in which symmetrical transcription operates over a considerable portion of the DNA length. Symmetrical transcriptions over small portions of viral genomes was reported (28–30). More work is needed to identify the template for the synthesis of self-complementary viral RNA. The possibilities include both SV40 DNA strands, a free viral RNA strand, or a RNA–DNA hybrid.

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