Transcription of the SV40 Genome in Transformed Cells and during Lytic Infection

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Cultured cells respond largely in two different ways to infection by Simian vacuolating virus (SV40), a small DNA-containing oncogenic virus. For example, the BSC-1 line of African green monkey cells are *permissive* to SV40, i.e., the virus can multiply and produce infectious progeny, eventually killing the host (lytic infection). On the other hand, the 3T3 line (Todaro and Green, 1963) of mouse cells is *restrictive* to SV40, i.e., the virus cannot multiply and cannot produce infectious progenies, although it can enter the cell and express some of its gene functions (abortive infection). As much as 50% (Todaro and Green, 1966) of the abortively infected mouse cells acquire the heritable changes of growth properties known as *transformation*, an equivalent process of carcinogenesis.

The transformed cells (SV3T3) do not contain or produce infectious virus or infectious viral DNA; they do, however, harbor the complete information of the viral genome, for synthesis of infectious virus is induced by fusion with permissive cells (Koprowski et al., 1967; Watkins and Dulbecco, 1967). The viral genes are expressed only partially in the transformed cells; a virus-specific nuclear antigen (T antigen) (Black et al., 1963) and surface antigen are observed, whereas no viral capsid antigen is detectable. The molecular basis of the regulation of viral genomes in transformed cells is unknown. Its elucidation will contribute to the understanding of the mechanism of transformation, and also may throw light on the mechanism of gene control in mammalian cells.

By the use of the RNA-DNA hybridization competition technique, both Oda and Dulbecco (Oda and Dulbecco, 1968) and Aloni and collaborators (Aloni et al., 1968) showed that SV40 mRNA in SV3T3 cells lacks 60% to 70% of the nucleotide base sequences of the viral mRNA synthesized at a late time after lytic infection of BSC-1 cells. Since the whole viral genome persists in the SV3T3 cells (Watkins and Dulbecco, 1967), their results suggested that the expression of the viral genes is controlled on the transcriptional level. Their results, however, did not exclude the possibility that the whole viral genome is transcribed and viral mRNA with certain base sequences (such as those in the capsid protein genes) is selectively degraded later. Recent findings of Lindberg and Darnell on large viral mRNAs in the nuclei of SV3T3 cells is consistent with this notion (Lindberg and Darnell, 1970).

In order to obtain basic information on the regulation of viral gene expression accompanying transformation and lytic infection, we have been characterizing viral mRNA in SV3T3 cells (3T3 cells transformed by SV40) as well as BSC-1 cells lytically infected by SV40. The results indicate that in the SV3T3 cells, viral RNA is synthesized as several molecules with sizes corresponding to three-fourths to two or three times the length of a single strand of SV40 DNA. These RNA molecules lack "late" viral RNA base sequences and are most probably composed of partially cellular and partially viral RNA. They are converted during the nucleo-cytoplasmic transfer to a molecule corresponding in size to one-half the length of a single strand of SV40 DNA.

In lytic infected cells characteristic patterns of virus-specific RNA can be detected, both "early" and "late" after infection. Early RNA contains a major component of 8×10^5 daltons mol wt, corresponding in size to one-half of the SV40 genome. Late RNA contains two main components with mol wt of 7.9×10^5 and 5.6×10^5 daltons; in addition, late RNA is heterogeneous in the nucleus and the majority of it is larger than cytoplasmic RNA.

Synthesis of Viral mRNA in SV3T3 Cells

Figure 1 shows the sedimentation profiles of SV3T3 RNA which was labeled with ^{3}H-uridine for (*a*) 15 min or (*b*) 6 hr. When labeling is brief, RNA hybridizable with SV40 DNA is distributed in two rather broad bands. These viral RNA components are hereafter called "large" viral RNA. A shoulder is also observed under 18 S rRNA marker. This viral RNA component is hereafter called "small" viral RNA.

Strauss et al. (1968) showed that the secondary structure of bacteriophage MS2 RNA is completely destroyed in 99% dimethylsulfoxide (DMSO) at room temperature, thus under the sedimentation conditions used in the present work, all species

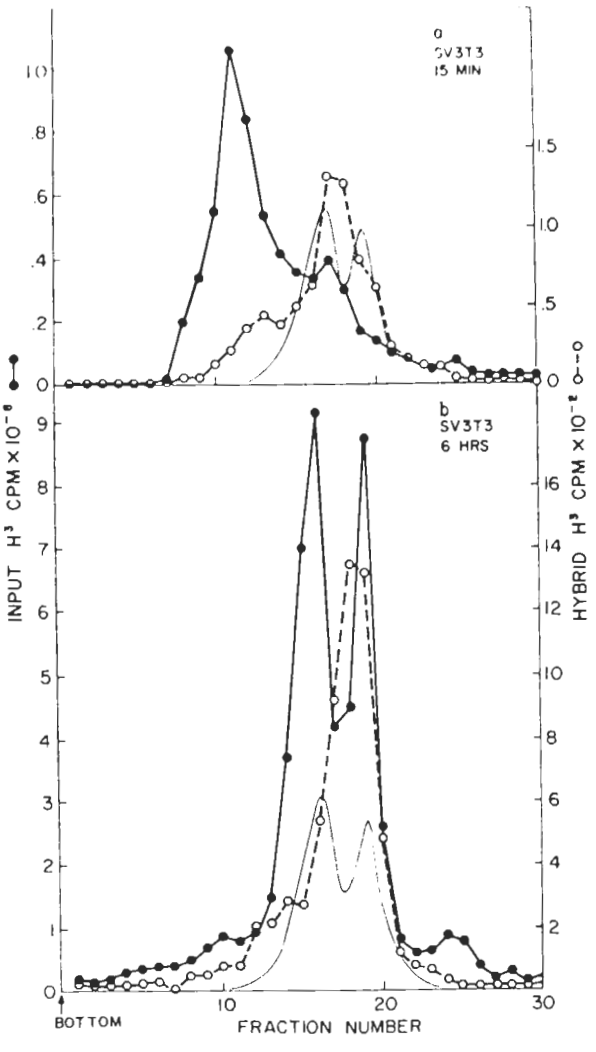

FIGURE 1. Sedimentation of SV3T3 RNA in 99% DMSO. SV3T3 cells were grown at 37°C on 10 cm Nunc plastic petri dishes in reinforced Eagle's medium containing 10% calf serum. When the culture became nearly confluent, RNA was labeled by changing the medium with 2.5 ml of reinforced Eagle's containing 2% calf serum and 500 μc of ^{3}H-uridine (~20 c/mmole). At 15 min or 6 hr later, the cells were washed three times with cold saline and lysed by adding directly to the petri dishes as follows: 6% p-aminosalicylate, 1% tri-iso-propylnaphthalene sulfonate, 6% n-butanol and 1% NaCl. Extraction of RNA was carried out at 0–4°C by a modification of Kirby's method (Kirby, 1966). 50 μg of the purified RNA were layered over 99% DMSO, stabilized by linear gradients of 0–10% sucrose and 0–99% ^{2}H-DMSO (Strauss et al., 1968). The sedimentation was for 18–3/4 hr at 29°C at 40,000 rpm in a Spinco SW-41 rotor. Fractions were collected from the bottom of the tube. RNA was precipitated by adding two volumes of ethanol and was annealed with 3 μg of denatured SV40 DNA fixed on Millipore filters for 20 hr at 65°C. The amount of DNA used was well in excess. Under the conditions used (Westphal and Dulbecco, 1968), the blank filter retained less than 0.002% of the input ^{3}H counts. In this figure and Fig. 2, 5, 9, 10 and 11, the thin continuous lines represent ^{14}C-labeled SV3T3 rRNAs used as sedimentation markers.

of RNA are expected to sediment as a function of the chain length. Ring-shaped SV40 DNA with a single nick in one of the two strands sedimented closely together with the 28 S rRNA marker under the condition used (data not shown). Thus in Fig. 1*a* approximately 50% of the RNA hybridizable with SV40 DNA are not smaller than a single strand of SV40 DNA. In contrast, when RNA is labeled for 6 hr (Fig. 1*b*), counts hybridizable with SV40 DNA are predominant in the small viral RNA component.

The specificity of the hybridization was checked by use of RNA from 3T3 cells, an untransformed mouse cell line. This RNA, when sedimented in DMSO, cross-hybridized with SV40 DNA at a level no more than 0.001% of the input all the way through the gradient (data not shown). The counts in the hybrids at the two peak positions in Fig. 1*a* were 0.009% and 0.033% of the respective inputs.

Precursor-Product Relationship of Viral mRNAs in SV3T3

In order to study the precursor-product relationship between the large viral RNAs and the small viral RNA, pulse-chase experiments were carried out. Figure 2 shows the sedimentation profiles of SV3T3 RNAs pulsed or pulse-chased with uridine. The results are replotted in Fig. 3. The ^{3}H-uridine-pulsed RNA (Fig. 2*a*) again shows the characteristic distribution of viral RNA components. The counts in the large viral RNAs decrease upon 2 hr chase (Fig. 2*b*) or 6 hr chase (Fig. 2*c*). The counts in the small viral RNA increase during the first 2 hr chase and then start decreasing on further chase until they become almost undetectable by 16 hr chase (Fig. 2*d*). The residual incorporation of ^{3}H-uridine during the chase periods and possible reutilization of nucleotides make the quantitative analysis of this experiment rather difficult. Nevertheless, the fact that the count increase in the small viral RNA during the first 2 hr chase period is more than the increase of the total viral RNA is consistent with the notion that the large viral RNA is the precursor of the small viral RNA.

In order to circumvent the difficulties described above, a similar pulse-chase experiment was carried out with 10 μg/ml of actinomycin D during the chase periods (Fig. 4). Since shutoff of RNA synthesis is nearly complete under this condition, the increase of the counts in the small viral RNA during the chase periods must be attributed to conversion from the large viral RNAs.

From these experiments, we conclude that the large viral RNAs are precursors of the small viral RNA.

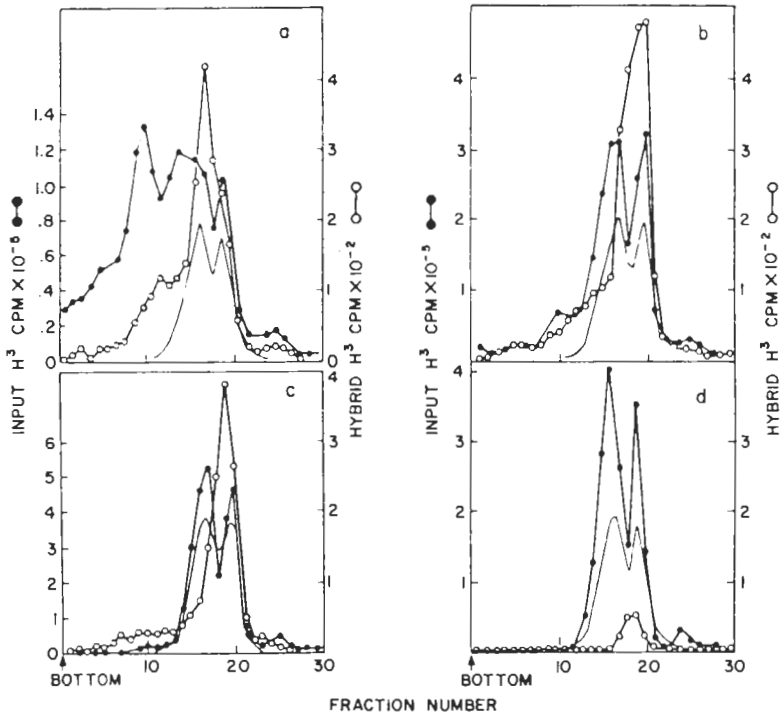

FIGURE 2. Pulse-chase experiment with SV3T3 RNA. Growth and labeling conditions of SV3T3 cells are as described in the legend to Fig. 1 except that the cells were labeled for 1 hr. RNA was extracted immediately after the labeling period from one 10 cm petri dish. The remaining cultures were washed four times with Eagle's medium containing 10% calf serum and 100 μg/ml of uridine and were allowed to grow at 37°C in the same medium for 2, 6, or 16 hr before RNA was extracted. The procedures of sedimentation in DMSO and RNA-DNA hybridization are as described in the legend to Fig. 1.

(*a*) = 1 hr pulse; (*b*) = 1 hr pulse and 2 hr chase; (*c*) = 1 hr pulse and 6 hr chase; (*d*) = 1 hr pulse and 16 hr chase.

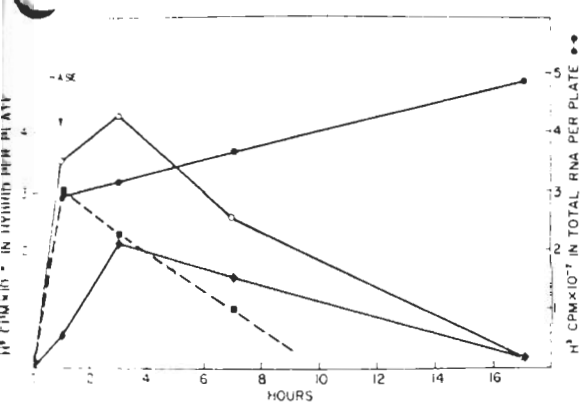

FIGURE 3. Replotting of the pulse-chase experiment. In order to construct this picture, the counts hybridized to SV40 DNA in Fig. 2 were divided into two groups: "large viral RNA" and "small viral RNA." For 1 hr pulse (Fig. 2*a*) and for the 2 hr chase (Fig. 2*b*), the viral RNA in fractions 9 to 18 and that in fractions 19 to 22 were classified as large RNA and small RNA, respectively. For the 6 hr chase sample (Fig. 2*c*) and the 16 hr chase sample (Fig. 2*d*) the viral RNA in fractions 9 to 17 plus one-half *the counts in fraction 18 were classified as large RNA* and the viral RNA in fractions 19 to 21 plus one-half in fraction 18 were classified as the small RNA. The total RNA counts were calculated from the counts in the 5% cold TCA-precipitable fraction of an aliquot of each of the pooled RNA samples. ●——● = total RNA; ○——○ = total RNA hybridizable to SV40 DNA; ■- - -■ = large viral RNA; ◆——◆ = small viral RNA. The values are expressed as count/min/petri dish.

Half-life of the large viral RNAs is approximately 1.8 hr and that of the total viral RNA is 3.9 hr.

SITE OF THE CONVERSION OF LARGE VIRAL RNAS TO SMALL VIRAL RNA

The intracellular distribution of the viral RNA components was examined by analyzing the RNAs prepared from isolated nuclei and cytoplasm of SV3T3 cells. The results (Fig. 5) show that the large viral RNAs and the small viral RNA are richer in the nuclei (Fig. 5*b*) and cytoplasm (Fig. 5*c*) respectively. The small viral RNA in the nuclei preparation may be at least partially explained by contamination from the cytoplasm because the relative amounts of the 45 S ribosomal precursor RNA and 18 S rRNA indicate that the nuclei preparation is only 75% to 80% pure. On the other hand, the near complete absence of the 45 S and 32 S rRNA precursors indicates that the cytoplasmic fraction has a high degree of purity. In spite of this, there seems to be also a small amount of large viral RNA in the cytoplasm. These results suggest that at least a part of the conversion process takes place in the cytoplasm. It is noteworthy that no large viral RNA sedimenting faster than 28 S rRNA is found in the cytoplasm.

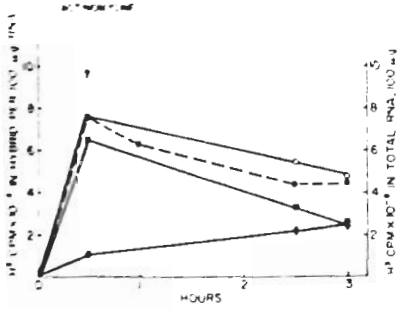

FIGURE 4. Short-term pulse-chase experiment with actinomycin D. The procedures of the experiment are as described in the legend to Fig. 2 and 3 except that the pulse was for 30 min and the chase was carried out for shorter periods in the presence of 10 μg/ml of actinomycin D. ●– – –● = total RNA; ○——○ = total viral RNA; ■——■ = large viral RNA; ◆——◆ = small viral RNA. Results are expressed as count/min/100 μg of total RNA.

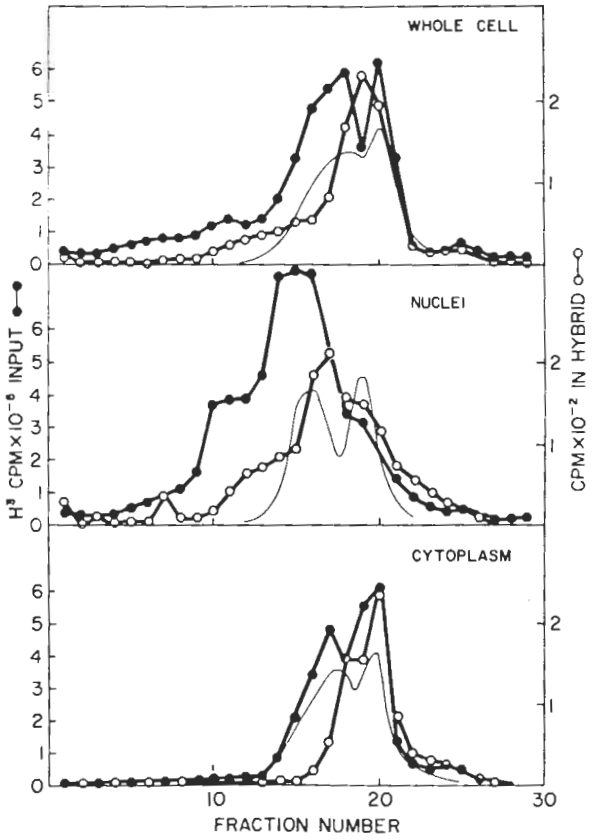

FIGURE 5. Distribution of viral RNA in the nucleus and cytoplasm of SV3T3 cells. SV3T3 cells were labeled for 90 min with 500 μc of ^{3}H-uridine per petri dish. Cells from four 10 cm petri dishes were swollen in 4 ml of RSB (0.01 M Tris-HCl pH 7.4, 0.003 M $MgCl_2$, 0.01 M NaCl) for 10 min at 0°C and disrupted by a glass homogenizer in the presence of 0.4% Triton-X 100. The cell breakage was monitored by phase microscopy. The nuclei and cytoplasm were separated by centrifuging the broken cells at 1,000 rpm for 2.5 min in a refrigerated International centrifuge. The nuclear pellet was suspended by vigorous agitation in 2 ml of TES (0.02 M Tris-HCl pH 7.5, 0.001 M sodium salt of EDTA, 0.1 M NaCl). Nucleic acids were extracted from the suspended nuclei and cytoplasm by the procedures described in the legend to Fig. 1. Under the conditions used, the purified RNA, as calculated from the OD at 260 mμ, was distributed between the nuclear and cytoplasmic fractions at a ratio of 1:4:18. The amounts of the RNA layered on each DMSO gradient were: 59 μg of whole cell RNA, 24 μg of nuclear RNA and 66 μg of cytoplasmic RNA.

Further studies are necessary in order to clarify whether the subcomponents of the large viral RNAs behave differently during the post-transcriptional processing.

NATURE OF THE LARGE VIRAL RNA

As discussed in the introduction, previous studies by use of RNA-DNA hybridization suggested that the regulation of viral gene expression in SV3T3 cells is on the transcriptional level. The finding of the large precursor viral RNAs with a shorter half-life, however, opened the possibility that the whole viral genome is transcribed but specific sections of the RNA molecules are degraded later. In this case regulation is on a post-transcriptional level.

In order to differentiate between these models, the large precursor viral RNAs were examined to see whether they contain late sequences. In one experiment, either pulsed or pulse-chased total SV3T3 RNA was competed with unlabeled RNA isolated from SV3T3 for sites on the SV40 DNA. The pulsed RNA sample consisted of 80% of large viral RNAs and 20% of small viral RNAs, whereas the chased RNA sample consisted of 15% of large viral RNAs and 85% of small viral RNAs. Figure 6 shows the results of the competition experiment. Both pulsed and pulse-chased RNA were completely competed by unlabeled SV3T3 RNA. Furthermore, there were essentially no differences between the two cases in the competing efficiency of unlabeled SV3T3 RNA. Unlabeled RNA isolated from the secondary culture of mouse embryo cells competes only 15% indicating that the observed total competition with SV3T3 RNA is due to viral RNA.

In another experiment, the gradient fractions containing the large viral RNA were pooled and used in the competition with unlabeled SV3T3 RNA (Fig. 7). The results again show that at least 90% of the base sequences in the large viral RNAs are competed by unlabeled SV3T3 RNA. The outcome of the competition experiment between late lytic RNA and SV3T3 RNA (Fig. 7) indicates that the unlabeled SV3T3 RNA used in these experiments lacks late base sequences, conforming with previous results by others (Oda and Dulbecco, 1968; Aloni et al., 1968). From these experiments, we conclude that the

large viral RNAs contain no appreciable amount of late RNA base sequences. The results within the limit of the experimental technique exclude the post-transcriptional regulation model. The large viral RNAs then may be best explained as composed of hybrid molecules containing both viral and cellular sequences. The generation of this type of RNA molecules in SV3T3 cells seems to be well justified since Sambrook et al. (1968) showed that in these cells SV40 DNA is integrated into the chromosome by bonds resistant to alkali as well as detergent. It is quite possible that the viral DNA duplex is inserted into the cellular DNA duplex by a phosphodiester bond resulting from a recombinational event in the manner similar to bacterial lysogeny (Campbell, 1961). RNA polymerase may then start transcription at the nearest cellular initiation site and proceed into the viral DNA until

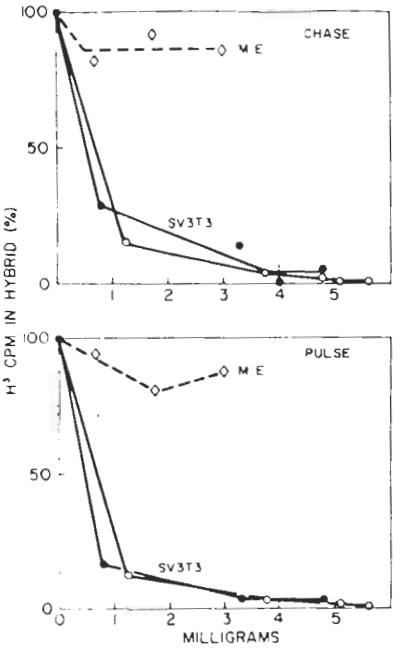

FIGURE 6. Competition of pulsed or pulse-chased RNA with total SV3T3 RNA. SV3T3 cultures were pulsed or pulse-chased as described in the legend to Fig. 2. The pulse was for 1 hr and the chase was for 6.5 hr. The indicated amount of unlabeled SV3T3 RNA was first hybridized with 0.1 μg of ^{14}C-labeled SV40 DNA for 16–18 hr; then the labeled RNA was added and the mixture was incubated for an additional 16 hr at 65°C. The partial detachment of the DNA during annealing was checked by the ^{14}C counts and the results are adjusted for the loss of DNA. The amount of the ^{3}H-RNA used was 87 μg of pulsed RNA or 115 μg of chased RNA. The filled circles and the open circles represent two different experiments. The experiment represented by the filled circles was carried out in a final total volume of 0.7 ml. The experiment represented by the open circles was in 0.3 ml. Count/min hybridized without competing RNA were: 183 (●——●, chase), 420 (○- - -○, chase), 130 (● ——●, pulse) and 377 (○ ——○, pulse). ×——× represents the experiment in which unlabeled RNA isolated from secondary mouse embryo (M.E.) cells was used as the competitor.

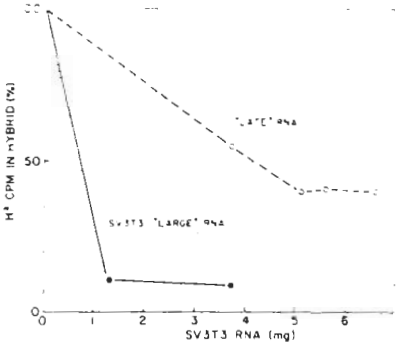

FIGURE 7. Competition of the large viral RNA with total SV3T3 RNA. An SV3T3 culture was labeled for 1 hr with ^{3}H-uridine. Purified RNA was sedimented through 99% DMSO. Those fractions which correspond to fractions 9 to 17 of Fig. 2*a* were pooled and RNA was precipitated with 2 vol of ethanol. The precipitated RNA was dissolved in 2 × SSC and was used for the hybridization competition as described in the legend to Fig. 6. Late RNA was prepared from BSC-1 cells (an established line of African green monkey cells) infected by SV40, at a multiplicity of infection of 100. The infected cells were labeled with 750 μc per petri dish of ^{3}H-uridine from 49 to 50 hr after infection.

it hits a termination signal within the viral DNA or vice versa. This would result in a nucleotide chain consisting of partially cellular and partially viral RNA.

The possibility that the large viral RNAs are tandems of the small viral RNA devoid of late base sequences has not been excluded, but it may be unlikely in the light of the observation (see next section) that the molecular weights of the large viral RNA molecules seem not to be simple multiples of that of the small viral RNA.

Resolution of the Large Viral RNA into Subcomponents

In order to resolve the multicomponents of the large precursor RNA, SV3T3 RNA was subjected to polyacrylamide gel electrophoresis. In comparing the molecular weights of in vitro transcribed SV40 RNA determined by DMSO gradient and polyacrylamide gel electrophoresis, it was found that the apparent molecular weight in polyacrylamide gel was 1.4 times larger than that in DMSO gradient, using rRNAs as markers (Fig. 8). Based on the assumption that in DMSO all species of RNA molecules assume a random configuration, we can conclude that under the conditions of the gel electrophoresis, rRNA and SV40 RNA differ in their secondary structures. Therefore, molecular weights of viral RNA determined by acrylamide gel electrophoresis using rRNA markers were corrected accordingly.

Figure 9 shows gel electrophoregrams of RNA prepared from SV3T3 cells pulse-labeled for 1 hr with ^{3}H-uridine (Fig. 9*a*) and chased for 4 hr after a 1 hr pulse (Fig. 9*b*). The molecular weight

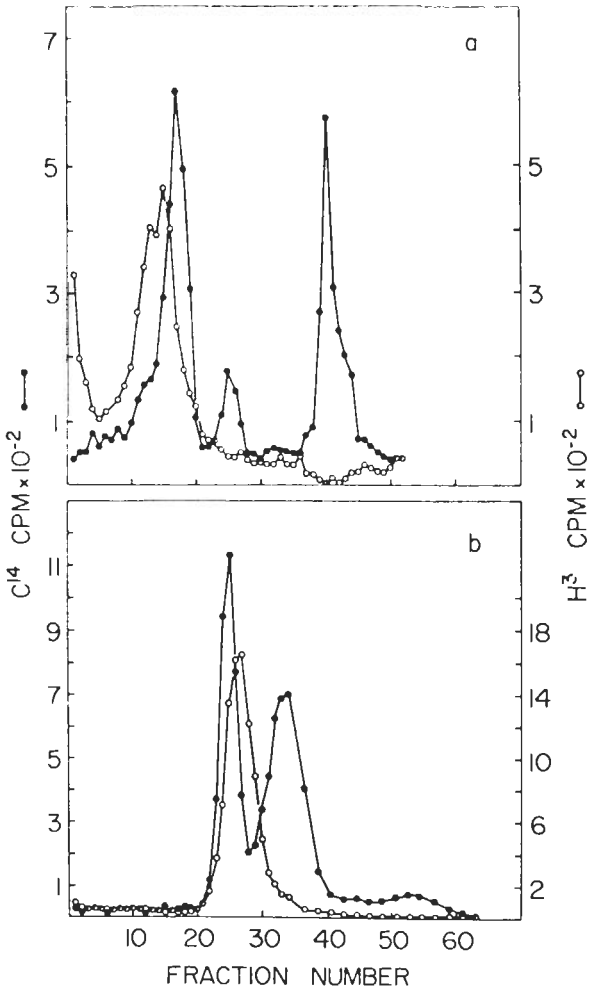

FIGURE 8. Comparison of the molecular weight of in vitro SV40 RNA determined by polyacrylamide gel electrophoresis and sedimentation in DMSO gradients. In vitro RNA is synthesized with *E. coli* RNA polymerase on superhelical SV40 DNA (Form I) as template. The conditions of synthesis are described by H. Westphal and D. Kiehn (this volume) 112 μg enzyme, 104 μg DNA, 0.5 mc of both ^{3}H-UTP (specific activity 20 c/mmole) and ^{3}H-CTP (specific activity 10 c/mmole) in 0.5 ml final volume were used. The time of incubation is 5 min at 37°C.

The RNA, extracted as described by Summers (1970), contained 2.3×10^8 count/min, specific activity 2 to 5×10^5 count/min per μg. 2.3×10^7 count/min were sedimented on a DMSO gradient for 25 hr, 39,000 rpm in an SW-41 Spinco rotor at 29°C. Sixty-two fractions were collected. The RNA from fractions 27, 31 and 35 (Fig. not shown) was precipitated with ethanol and analyzed both on DMSO gradients (conditions as above) and on polyacrylamide gels, together with rRNA from BSC-1 cells, using the method described by Summers (1969) and Loening (1967). The gel 0.6 × 12 cm was composed of 2.2% acrylamide and 0.5% agarose. Electrophoresis was carried out at room temperature for 6 hr at 7 ma per gel. The gel was fractionated by use of a Savant gel divider. The samples were frozen, warmed to 60°C for 5 min and counted. The molecular weights on DMSO gradients calculated according to Strauss (Strauss et al., 1968) were 1.45×10^6 (fraction 27), 8.4×10^5 (fraction 31), 5.4×10^5 (fraction 35); on gels according to Bishop (Bishop et al., 1967), 1.88×10^6 (fraction 27), 1.30×10^6 (fraction 31) and 7.9×10^5 (fraction 35).

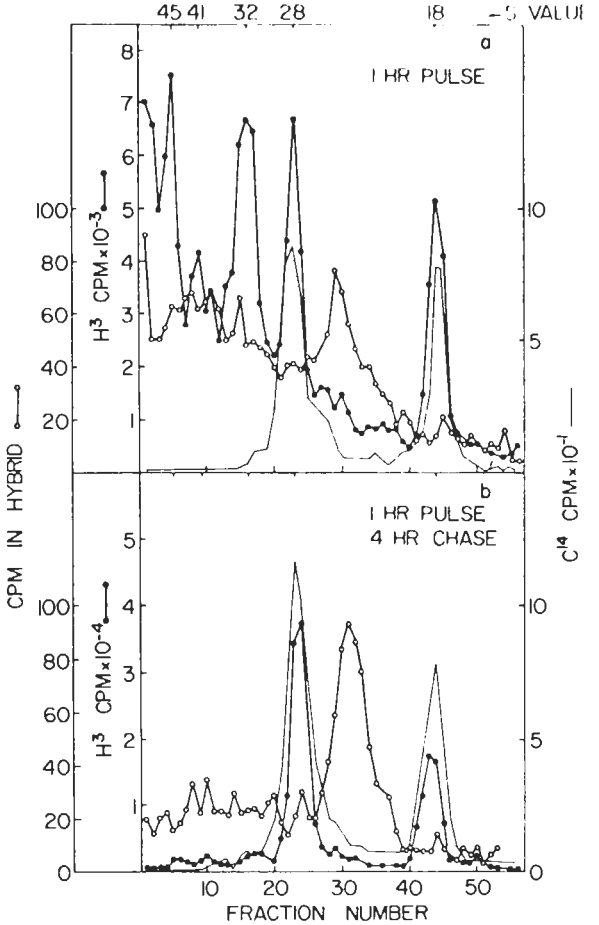

FIGURE 9. Polyacrylamide gel electrophoregrams of pulsed or pulse-chased SV3T3 RNA. Pulse-chase conditions are as described in the legend to Fig. 2. Polyacrylamide gel electrophoresis was carried out as described in the legend to Fig. 8. RNA was eluted from the fine gel beads by freezing and thawing, and then hybridized with SV40 DNA under the conditions described in the legend to Fig. 1.
(*a*) 1 hr pulse; (*b*) 4 hr chase after 1 hr pulse.

of the fastest migrating peak (fraction 29 of Fig. 9*a* or fraction 31 of Fig. 9*b*) was estimated as 8.5×10^5 daltons which is approximately 50% of the molecular weight of a single strand of SV40 DNA. The large viral RNAs are now distributed among five or six peaks (Fig. 9*a*) whose mol wt were $1.25–1.4 \times 10^6$, $1.6–1.65 \times 10^6$, $1.8–1.95 \times 10^6$, $2.2–2.3 \times 10^6$, $2.5–2.6 \times 10^6$, and $2.9–3.0 \times 10^6$ daltons. There also is an even larger component(s) which did not migrate into the gel. Consistent with the pulse-chase experiments in the DMSO gradient (Fig. 2), the counts were reduced in these large viral RNAs upon chase and accumulated

The molecular weights are, as determined on gels, 1.43 ± 0.02 times higher than on DMSO gradients.
(*a*) gel of fraction 27; (*b*) DMSO gradient of fraction 27; ○——○ = in vitro RNA, ^{3}H, ●- - -● = ribosomal marker, ^{14}C.

the 9×10^5 dalton species. Westphal and Dulbecco (1968) found that the SV3T3 cells contain 20 genome equivalents of SV40 DNA per cell. The multiple precursor RNA species may arise from different integration sites, each with a different contribution of cellular RNA.

Viral RNA Synthesis in SV40-infected BSC-1 Cells

During lytic infection of monkey cells (BSC-1) with SV40, two classes of viral mRNA are transcribed. Early RNA is transcribed prior to the onset of viral DNA replication, while late RNA is that made after DNA replication begins. Results from hybridization competition experiments have indicated that the sequences transcribed before DNA replication are about 40% of those found in late RNA. This implies that during lytic infection the expression of the SV40 genome is regulated on the level of transcription (Aloni et al., 1968; Oda and Dulbecco, 1968).

The RNA in SV40 infected BSC-1 cells was labeled with ^{3}H-uridine at different times after infection and was fractionated by sedimentation in 99% DMSO. The result is shown in Fig. 10*b*, *c*, and *d*. For comparison, a sedimentation profile of SV3T3 RNA labeled under similar conditions is presented in Fig. 10*a*. The main component of the viral RNA synthesized between 9 hr and 10 hr after infection sediments with a mol wt of about 8×10^5 daltons (fraction 19 of Fig. 10*b*) whereas that synthesized between 15 and 16 hr or 49 and 50 hr after infection has an approximate mol wt of 6.5×10^5 daltons (Fig. 10*c* and *d*). In Fig. 10*b*, approximately one-third of the viral RNA sediments much faster than the main component. The mol wt of this fraction of RNA range between 2×10^6 and 6×10^6 daltons, corresponding in size to more than one genome length.

The sedimentation profile of the viral RNA from BSC-1 cells infected by SV40 in the presence of 5-fluorodeoxyuridine (FUdR) and labeled between 15 and 16 hr after infection was similar to that in Fig. 10*b* (data not shown). Furthermore, the proportion of viral RNA was similar in the two RNA samples: 0.02% of the total RNA as compared to 0.5% and 4.8% in the samples shown in Fig. 10*c* and *d*, respectively. These observations indicate that the viral RNA shown in Fig. 10*b* is synthesized before the onset of viral DNA replication (early RNA), whereas that shown in Fig. 10*c* and *d* is late RNA. The nature of the larger early RNAs is not known. Experiments are in progress to determine the stability of these RNAs and to see if they contain late RNA sequences.

The late viral RNA shown in Fig. 10*d* is separated on polyacrylamide gels into two peaks with mol wt of 7.9×10^5 and 5.6×10^5 daltons (Fig. 11). In addition, there is a fraction of hybridizable RNA which is heterogeneous in size, ranging from 2×10^5 up to 4×10^6 daltons.

Virus-specific RNA in Nuclei and Cytoplasm of SV40-Infected BSC-1 Cells

In the following experiment, SV40-infected BSC-1 cells were labeled with ^{3}H-uridine for 3 hr

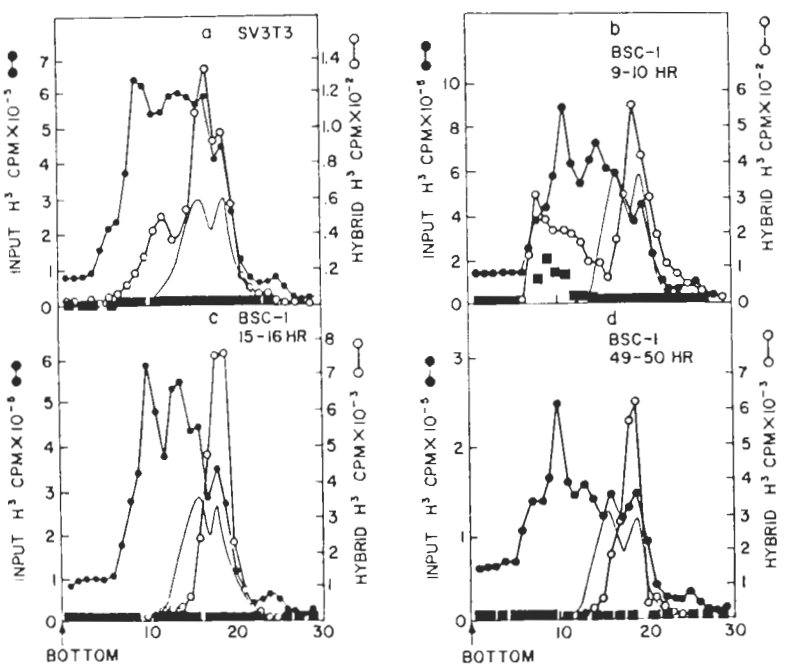

FIGURE 10. Sedimentation of SV3T3 RNA and SV40-infected whole cell RNA in 99% DMSO. SV3T3 and BSC-1 cells were grown as described in the legend to Fig. 1. The multiplicity of infection for BSC-1 cells was 100 PFU per cell. 500 μc of ^{3}H-uridine per petri dish was used. RNA was purified as described in the legend to Fig. 1. 50 μg of purified RNA was layered on the DMSO gradient as described in the legend to Fig. 1. The sedimentation time was 19 hr at 29°C at 40,000 rpm in a Spinco SW-41 rotor.

(*a*) SV3T3 cells, time of labeling 1 hr; (*b*) BSC-1 cells, labeling time from 9 to 10 hr after infection; (*c*) BSC-1 cells, labeling time from 15 to 16 hr after infection; (*d*) BSC-1 cells, labeling time from 49 to 50 hr after infection. ●——● = ^{3}H counts; ○——○ = ^{3}H counts hybridizable to SV40 DNA; ▲——▲ = ^{3}H counts retained on a filter without DNA.

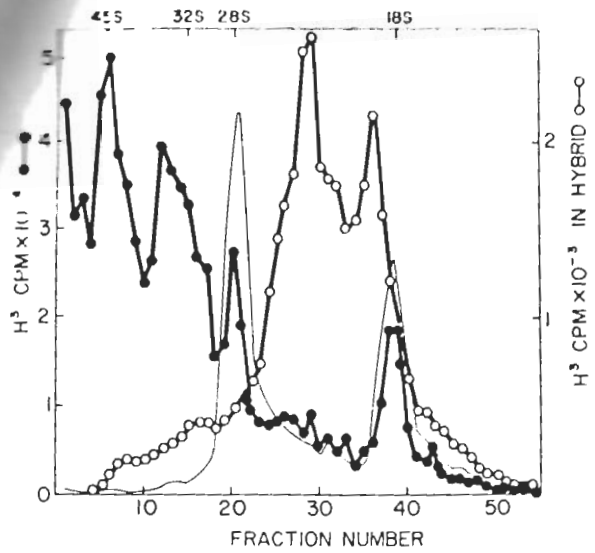

FIGURE 11. Fractionation of SV40-infected whole cell RNA in polyacrylamide gel. BSC-1 cells were grown and infected as described in the legend to Fig. 10. The time of labeling was from 49 hr to 50 hr after infection. 20 μg of whole cell RNA was analyzed by polyacrylamide gel electrophoresis, as described in the legend to Fig. 8.
●——● = ^{3}H counts; ○——○ = total ^{3}H counts hybridizable to SV40 DNA.

starting 50 hr after infection. Nuclei were isolated, using the procedure of Penman (Penman, 1966). The RNA was extracted and analyzed by sedimentation through a DMSO gradient and by gel electrophoresis. The results are shown in Fig. 12. The virus-specific RNA is heterogeneous in size with a broad peak of around 8×10^5 daltons. Nuclear RNA from infected cells labeled for 15 min has a similar pattern on polyacrylamide gels or in DMSO gradients (data not shown). The heterogeneity in the large molecular weight region of the gel (Fig. 12*a*) is probably not due to aggregation of smaller molecules since a similar size distribution is found in the DMSO gradient (Fig. 12*b*).

SV40 RNA in the cytoplasm of infected BSC-1 cells labeled with ^{3}H-uridine from 48 hr to 51 hr after infection has a mol wt of 5.7×10^5 daltons (Fig. 13). This suggests that in whole cells the viral RNA with a mol wt of 5.6×10^5 daltons is identical to cytoplasmic RNA and the larger component is nuclear RNA. The difference in size between nuclear and cytoplasmic RNA at late time of infection may be due to processing of the larger nuclear RNA or to selective transport of the smaller component from the nucleus to the cytoplasm.

SUMMARY

In SV3T3 cells, viral RNA is synthesized as several molecules with sizes corresponding to three-fourths to two or three times the length of a single strand of SV40 DNA. These RNA molecules lack late viral RNA base sequences and are most probably composed of partially cellular and partially viral RNA. They are converted during the nucleo-cytoplasmic transfer to a molecule corresponding in size to one-half the length of a single strand of SV40 DNA.

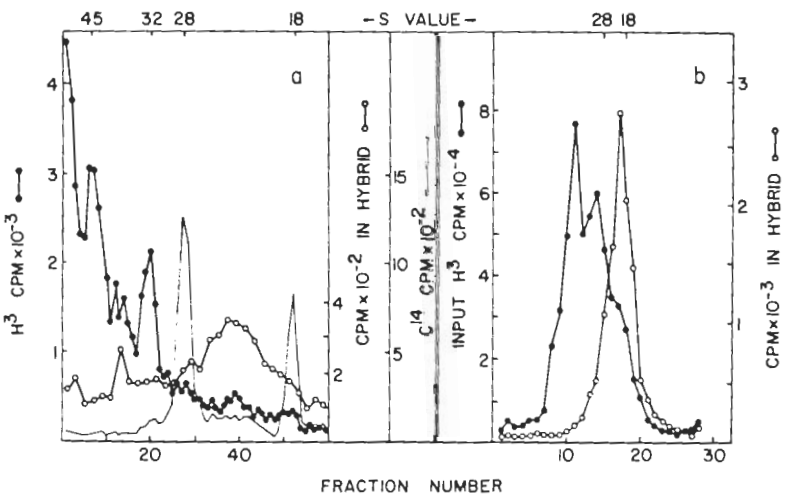

FIGURE 12. Fractionation of SV40 infected BSC-1 nuclear RNA by polyacrylamide gel electrophoresis and sedimentation in 99% DMSO. BSC-1 cells were grown as described in the legend to Fig. 1. The cells, near to confluence, were infected at a multiplicity of infection of 35 PFU per cell. ^{3}H-uridine was added, 500 μc per plate, from 50 hr to 53 hr after infection. Isolation of nuclei was carried out according to Penman (1966). RNA was extracted from isolated nuclei by the procedure described in the legend to Fig. 5.

(*a*) Fractionation of pulse-labeled nuclear RNA in polyacrylamide gels. 3 μg nuclear RNA from BSC-1 cells labeled from 50 hr to 53 hr post infection with 500 μc per 10^7 cells of ^{3}H-uridine was analyzed by polyacrylamide gel electrophoresis. The procedures are described in the legend to Fig. 8, except that the gel length was 20 cm, and the electrophoresis was carried out for 10 hr at a constant current of 6.5 ma per gel. 0.1 ml aliquots of each 1 ml fraction were counted and the remaining hybridized to SV40 DNA filters as described in the legend to Fig. 1.
●——● = ^{3}H counts of 0.1 ml aliquots; ○——○ = total ^{3}H counts hybridizable to SV40 DNA; —— = ^{14}C rRNA markers.

(*b*) Sedimentation of pulse-labeled nuclear RNA in 99% DMSO. 7 μg of the purified nuclear RNA, described above, were layered on a DMSO gradient, fractionated and hybridized as described in the legend to Fig. 1. The sedimentation time was 6 hr at 64,000 rpm at 29°C in a Spinco SW 65 rotor.
●——● = total ^{3}H counts; ○——○ = total ^{3}H counts hybridizable to SV40 DNA.

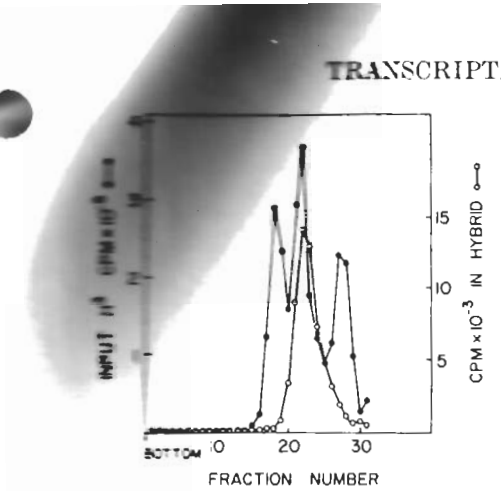

FIG. 13. Sedimentation of cytoplasmic RNA in 99% DMSO. BSC-1 cells were grown, infected and labeled as described in the legend to Fig. 1. The time of labeling was from 48 hr to 51 hr after infection. Cells were washed in RSB at pH 8.5 (Penman et al., 1969) for 5 min at 0°C and disrupted by means of a glass homogenizer. Nuclei and cytoplasm were separated by centrifugation. RNA was extracted at 0°C from the cytoplasm by Kirby's method.

19 μg of purified RNA was layered on the DMSO gradient as described in the legend to Fig. 1. The sedimentation time was 20 hr at 29°C at 40,000 rpm in a Spinco SW-41 rotor.

●——● = total ^{3}H RNA; ○——○ = total ^{3}H counts hybridizable with SV40 DNA.

In lytic infected cells, characteristic patterns of virus-specific RNA can be detected, both early and late after infection. Early RNA contains a major component of 8×10^5 dalton mol wt, corresponding in size to one-half of the SV40 genome. Late RNA contains two main components with mol wt of 7.9×10^5 and 5.6×10^5 daltons; in addition, late RNA is heterogeneous in the nucleus and the majority of it is larger than cytoplasmic RNA.

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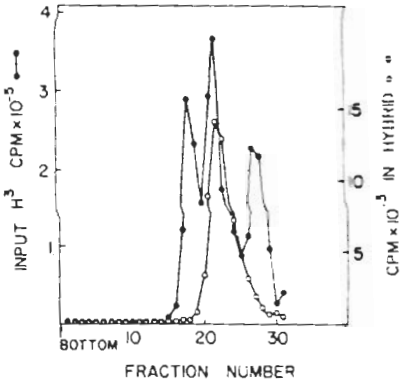

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