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Genome Localization of Simian Virus 40 RNA Species

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The topographical locations on the simian virus 40 (SV40) genome of the templates for virus-specific RNA species present late in the lytic infection were determined by RNA-DNA hybridization experiments with the *Hin*d restriction enzyme fragments. Two classes of late virus-specific cytoplasmic mRNA's can be separated on the basis of either sedimentation properties in neutral sucrose or electrophoretic mobility in polyacrylamide gels. In the 19*S* class, two species of RNA were identified by hybridization experiments. One of these species was complementary to sequences of the early template on the minus (E) strand (0.175 to 0.655 map units), and the other more abundant species was complementary to sequences present in the late template on the plus (L) strand (0.655 to 0.175 map units). In addition two species were detected in the 16*S* class of late cytoplasmic virus-specific mRNA. One of these species was the major late RNA detected and consisted of a polyadenylated transcript complementary to the plus (L) DNA strands of *Hin*d fragments K, F, J, and G (0.945 to 0.175 map units). This species appears to specify the major capsid protein (VP1). A less abundant non-polyadenylated 16*S* RNA species complementary to the plus (L) strands of *Hin*d fragments C, D, and E (0.655 to 0.945 map units) may result from post-transcriptional processing or nonspecific degradation of the 19*S* viral RNA complementary to the plus (L) strand.

The transcription of simian virus 40 (SV40) during productive infection of monkey kidney cells has been studied in considerable detail (20, 24). Prior to viral DNA replication, an early RNA species with an apparent sedimentation coefficient in neutral sucrose of 19*S* (25, 26) is found in the cytoplasm and anneals with the minus (E) strand of SV40 DNA (10, 16, 21). Late in the lytic cycle, the transcription of SV40 DNA is extensive and symmetrical on both DNA strands (2, 3, 12, 22). In the cytoplasm, however, two late virus-specific RNAs, a 19*S* and a 16*S* species, are found (25). These late SV40 RNAs are presumably the transcriptional or modified post-transcriptional products from the plus (L) strand of SV40 DNA. In DNA-RNA hybridization experiments with separated strands of restriction enzyme fragments of SV40 DNA and late lytic cytoplasmic RNA, the early and late SV40 templates were localized to specific segments of the viral genome (12, 13, 22). The early region occupies a portion of the minus SV40 strand from 0.17 to 0.65 units, whereas the late template extends from 0.65 to 0.17 units on the plus DNA strand (12). These values are in good agreement with those obtained from nucleotide sequence analysis of SV40-specific RNA (7, 8). In a previous study, Weinberg and his colleagues localized the 19*S* early RNA and the 19*S* and 16*S* late viral RNAs to their respective regions of the SV40 genome (25). Nevertheless, the precise position of the late 16*S* RNA species could not be ascertained. This assignment has assumed greater significance since it was recently demonstrated that late SV40-specific RNA (17, 19) and, more specifically, the late 16*S* species (18) can be translated in a cell-free system into VP1, the major virus capsid protein. Thus, a careful mapping study of late SV40-specific RNAs might be expected to provide information relating not only to the pattern and regulation of late transcription of the viral genome but also to the localization on the genome of late virus-coded proteins.

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MATERIALS AND METHODS

Virus and viral DNA. Confluent monolayers of primary African green monkey kidney (AGMK) cells were inoculated with plaque-purified SV40 (small

plaque, from strain 776) at a multiplicity of 0.01 PFU/cell. Carrier-free [^{32}P]orthophosphate was added 24 h after infection in phosphate-free medium and, after 7 days of incubation, the cells were harvested. ^{32}P-labeled SV40 DNA I was prepared from purified virus by equilibrium density centrifugation in CsCl-ethidium bromide, as previously described (10). The specific activity of the DNA preparations ranged from 2×10^5 to 8×10^5 counts/min per μg. Endonuclease R *Hin*d fragments of SV40 DNA were prepared as previously described (6), and the individual strands were separated by hydroxyapatite (HA) chromatography after reaction with complementary RNA (11). For certain experiments, two or more physically adjacent *Hin*d fragments were combined prior to strand separation. SV40 cRNA was made utilizing supercoiled SV40 DNA and *Escherichia coli* DNA-dependent RNA polymerase (kindly provided by T. Shih) as previously described (10).

Viral RNA. RNA was isolated from primary AGMK cells 48 h after infection (multiplicity of infection, 20 to 50 PFU/cell) with SV40 (strain 776). Most cultures were pulse labeled from 45 to 48 h in medium containing 75 to 100 μCi of [5,6-^{3}H]uridine per ml (Amersham/Searle, 45 Ci/mmol). Cells were washed with ice-cold phosphate-buffered saline and scraped from monolayers into cold HeLa buffer (0.14 M NaCl, 0.01 M Tris-hydrochloride [pH 8.0], 0.0015 M $MgCl_2$). After the addition of 1/20 volume of 10% Nonidet P-40, the cells were blended in a Vortex mixer for 30 s, and the nuclei were pelleted at 5,000 rpm in a Sorvall RC2-B centrifuge for 10 min at 4 C. The supernatant cytoplasmic RNA was concentrated by precipitation with 2 volumes of ethanol and resuspended in 0.01 M Tris-hydrochloride (pH 7.4), 0.01 M NaCl. In some experiments, the poly(A)-containing cytoplasmic RNA was further selected on oligo(dT)-cellulose (P-L Biochemicals) as follows. The RNA was bound to the oligo(dT)-cellulose in 0.5 M NaCl, 0.01 M Tris-hydrochloride (pH 7.5), 0.1% SDS, washed with 0.1 M NaCl, 0.01 M Tris, and then eluted in 0.01 M Tris. Prior to use, appropriate fractions were concentrated by precipitation with 2 volumes of ethanol as described above.

Sucrose gradient analysis. RNA samples in 0.2 to 0.6 ml were layered on 12 ml of 5 to 30% neutral sucrose gradients and sedimented in an SW41 rotor at 33,000 rpm for 9 h at 6 C. Fractions were collected dropwise from the bottom of the centrifuge tube. Portions of each fraction were analyzed for total radioactivity and for SV40-specific RNA, as determined by DNA-RNA filter hybridization.

Acrylamide gel electrophoresis. Cylindrical polyacrylamide gels (12 cm by 6 mm) in glass tubes consisted of 3% acrylamide and 0.15% *N,N'*-methylene bisacrylamide polymerized in E buffer (40 mM Tris, 5 mM sodium acetate, 1 mM EDTA, 0.1% SDS, adjusted to pH 7.8 with acetic acid) with *N,N,N',N'*-tetramethylenediamine (0.03%) and ammonium persulfate (0.05%). Gels were subjected to pre-electrophoresis for 2 h at 9 V/cm prior to the addition of RNA. The RNA sample, in less than 50 μl of sterile water, was mixed with 0.1 volume of 10× E buffer, 0.1 volume of 60% sucrose containing bromophenol blue dye, and 0.1 volume of 10% SDS, and then layered onto the gel beneath the buffer. Electrophoresis was carried out in E buffer at 9 V/cm (5 mA/gel) for 400 min at room temperature. After electrophoresis, the gels were frozen at −70 C and cut into 1.1-mm slices with stacked razor blades. For hybridization analysis, RNA was eluted from each gel slice by shaking at 37 C for 24 h in 0.3% SDS (0.3 ml).

Filter hybridization studies. RNA obtained from sucrose gradient fractions or eluted from acrylamide gel slices was adjusted to a final concentration of 6× SSC (SSC is standard saline citrate, 0.15 M NaCl, 0.015 M sodium citrate) in 0.1 to 0.3 ml and incubated with small (2 mm by 2 mm) filters which contained 0.2 μg of SV40 DNA. After 20 h of incubation at 68 C, filters were removed and washed extensively in 1× SSC. After treating with 20 μg of pancreatic RNase per ml for 1 h at 37 C in 2× SSC, the filters were washed, dried, and counted in a toluene-based scintillation fluid (1).

Solution hybridization studies. RNAs from polyacrylamide gel slices or sucrose gradient fractions were incubated with ^{32}P-labeled plus (L) strands of specific *Hin*d SV40 DNA fragments (generally 150 to 300 counts/min or about 0.2 to 1 ng of DNA) in 1.0 M NaCl, 0.03 M phosphate buffer, 0.1% SDS for 20 h at 68 C. In these reactions, the DNA concentrations of various fragments (or grouped fragments) was adjusted proportionately to its genetic complexity (genomic representation) so that equimolar RNA concentrations incubated for the same time would result in equivalent hybridization levels. Reaction mixtures were analyzed by HA chromatography as previously described to determine the percentage of ^{32}P-labeled DNA in duplex molecules (10).

RESULTS

Size of late lytic SV40-specific RNA. It was previously reported by others that late SV40-specific cytoplasmic RNAs have sedimentation values in neutral sucrose of 19*S* and 16*S* (18, 25, 26), and, furthermore, that these molecules have a slower electrophoretic mobility relative to an 18*S* rRNA marker when analyzed in polyacrylamide gels (5, 25). The data shown in Fig. 1 confirm both of these findings. Hybridization of neutral sucrose fractions of late lytic SV40 cytoplasmic RNA with SV40 DNA-containing filters shows the characteristic smaller 19*S* and larger 16*S* virus-specific RNA peaks (Fig. 1, upper panel). Fractions from parallel neutral sucrose gradients were incubated with small amounts of either the plus (L) or minus (E) strand SV40 DNA fragments, and the extent of hybridization was analyzed by HA chromatography. These experiments (unpublished) indicated that the 19*S* RNA peak contains both late and early SV40 RNA sequences whereas the 16*S* RNA species consists primarily if not entirely of late RNA. In 3% acrylamide gels, two peaks of SV40-specific RNA are observed in slices 24 and 37 with electrophoretic mobilities intermediate to those of ribosomal

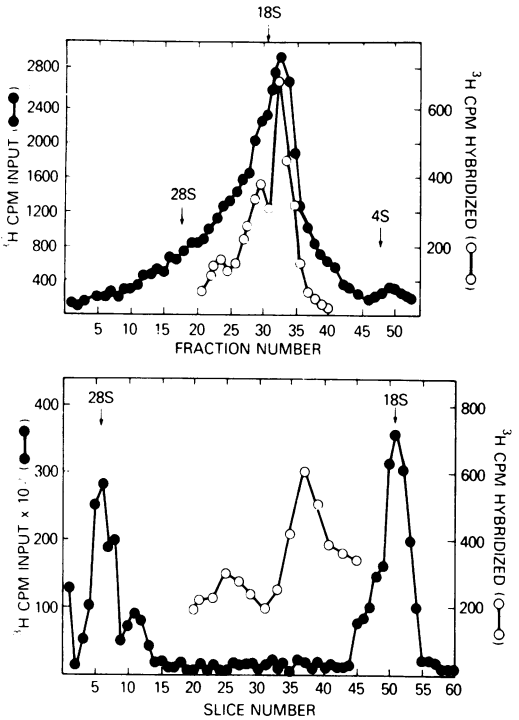

FIG. 1. *Fractionation and analysis of SV40 RNA. Upper panel, Late lytic oligo(dT)-cellulose selected cytoplasmic RNA from SV40-infected AGMK cells was sedimented through neutral sucrose (5 to 30% [wt/vol]) at 33,000 rpm for 9 h at 6 C in a Beckman SW41 rotor. Fractions of 0.25 ml were obtained from the bottom of the tube and 5-μl volumes were counted directly to determine the counts per minute per fraction (●). Approximately 5 μl from each fraction was used in hybridization reactions with SV40 DNA-containing filters in a total volume of 125 μl containing a final concentration of 6× SSC and 0.1% SDS (see Materials and Methods). After washing (2× SSC) and RNase treatment (20 μg/ml at 37 C for 1 h), the filters were counted in a toluene-based scintillation fluid to determine SV40-specific counts per minute* (O). *Lower panel, Total cytoplasmic SV40 late lytic RNA was subjected to electrophoresis in 3% polyacrylamide gels as described in Materials and Methods. After elution from gel slices, volumes of 30 μl were counted to determine counts per minute per slice (●). Approximately 30 μl of the eluent from each slice was used in hybridization reactions analogous to those described above to determine SV40-specific counts per minute* (O).

28S RNA and ribosomal 18S RNA (Fig. 1, lower panel). In separate experiments 19S and 16S RNAs were selected from sucrose gradients and subjected to electrophoresis in acrylamide gels (data not shown). These 19S and 16S RNA species correspond respectively to the slice 24 and slice 37 peaks in polyacrylamide gels, in agreement with the results of others (5, 25), and will subsequently be referred to as late SV40 19S and late SV40 16S RNAs. When virus-specific RNA was analyzed under denaturing conditions (Me_2SO-sucrose gradients and formamide gels), the mobilities of the 19S and 16S species were shifted relative to the rRNA markers; however, the resolution of these two species was not significantly enhanced (data not shown). Consequently, we employed aqueous polyacrylamide gels and neutral sucrose gradients in subsequent experiments.

Mapping of late SV40 16S and 19S RNAs on the viral genome. In previous studies we have shown that approximately half of the SV40 plus (L) DNA strand (from 0.645 to 0.175 units), corresponding to *Hin*d restriction enzyme fragments C, D, E, K, F, J, and G (see Fig. 6), functions as the template for late cytoplasmic viral RNA. In the mapping experiments described below, separated, ^{32}P-labeled plus (L) strands of these restriction enzyme fragments were incubated individually, or in groups, with varying concentrations of late lytic SV40 RNA which had first been selected on the basis of size.

Hybridization properties of RNA selected on oligo(dT)-cellulose following neutral sucrose sedimention. In the first set of hybridization experiments (Fig. 2), poly(A)-containing RNA was selected by passage of the crude cytoplasmic RNA preparation through an oligo(dT)-cellulose column as described in Materials and Methods. Such RNA was then sedimented through neutral sucrose as previously described and portions of various gradient fractions were annealed in solution to small quantities of the plus strands of *Hin*d fragments C, EK, and FJG as described in the legend to Fig. 2. The results of this experiment suggested that RNA homologous to each of the fragment groups employed is present in approximately equal amounts in the 19S region of the gradient. The RNA sedimenting at 16S, on the other hand, is complementary mainly to sequences contained in *Hin*d fragments GJF. Although RNA from this region reacts with *Hin*d fragments C and EK, it is clearly present in significantly lower concentrations than the more abundant species which reacts with DNA present in *Hin*d fragments GJF. In a parallel experiment, ^{32}P-labeled minus strand DNA from *Hin*d fragments A + B (representing the majority of the early region of the SV40 genome) was annealed with larger amounts of RNA from the same sucrose gradient fractions (Fig. 2). The broad peak to the left of the rRNA 18S marker confirms that the primary early viral RNA species is approximately 19S.

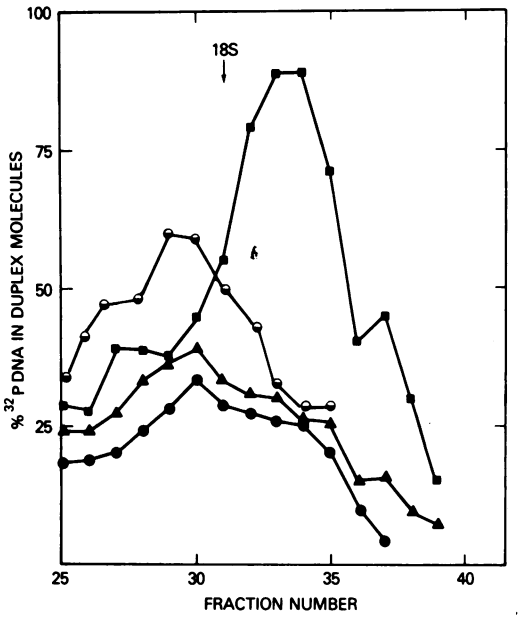

FIG. 2. *Analysis of sucrose gradient-fractionated late lytic SV40 RNA. Oligo(dT)-cellulose selected late lytic RNA was sedimented in 5 to 30% neutral sucrose as described in Fig. 1 (upper panel) and Materials and Methods. From fractions 25 through 39, volumes of 5 μl were added to hybridization mixtures containing 125 μl with a final concentration of 1.0 M NaCl, 0.03 M phosphate buffer, 0.1% SDS, and from 150 to 300 counts/min of ^{32}P-labeled plus (L) strands of the following SV40 Hind DNA fragments: C (●); EK (▲); or GJF (■). In a parallel experiment, 40 μl of RNA was added to a similar reaction mixture containing 150 counts/min of ^{32}P-labeled minus (E) strand fragments of Hind A+B (◒). These fragments represent the majority of the early region of the genome (see Fig. 6 for map positions of Hind fragments). After incubation at 68 C for 20 h, the percentage of [^{32}P]DNA fragments in duplex molecules was analyzed by HA chromatography.*

Hybridization properties of unfractionated late lytic SV40 cytoplasmic RNA after gel electrophoresis. In a parallel set of experiments, the plus DNA strands of *Hind* fragments CD, E, KF, and JG were annealed with late lytic cytoplasmic SV40 RNA that had been subjected to electrophoresis through 3% acrylamide gels (Fig. 3). In contrast to the experiment described in Fig. 2, this RNA preparation had not been preselected on oligo(dT)-cellulose, permitting the evaluation of virus-specific RNA species which did not contain poly(A) regions. The results shown in Fig. 3 are in good agreement with those previously presented (Fig. 2). In this analysis, *Hind* fragment K, as well as *Hind* fragments F, J, and G, were found to be complementary to the abundant species of 16S RNA. *Hind* fragment E, which had been pooled with *Hind* fragment K in the experiment shown in Fig. 2, did not react as well with 16S virus-specific RNA. RNA homologous to *Hind* fragments C and D was also present in this region of the gel, but at significantly lower concentrations. The better separation of 19S and 16S RNAs effected in gels suggests that the reaction of *Hind* fragments C, D, and E with RNA from the 16S region of the gel is not simply the result of cross-contamination from the 19S region. Nevertheless, the absence of clear-cut peaks in the hybridization profiles (lower panel of Fig. 3) with RNA present in lower concentrations as well as the lower efficiency of elution of higher-molecular-weight species was characteristic of RNA eluted from acrylamide gels. Thus, sucrose gradient analyses were used in subsequent experiments.

Hybridization properties of late lytic SV40

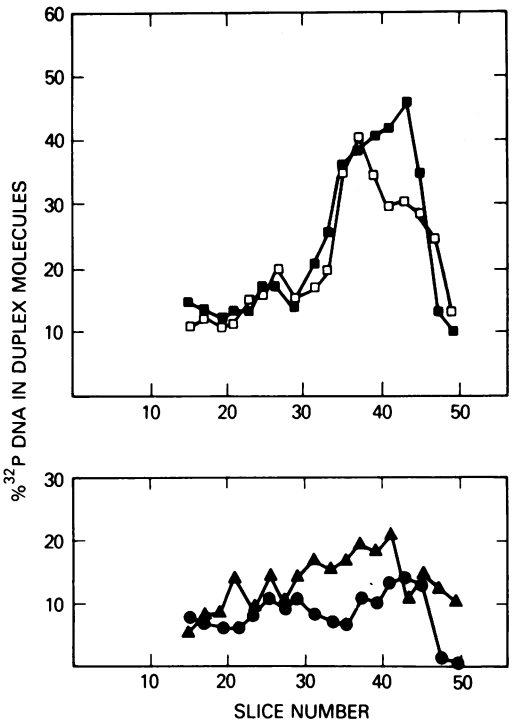

FIG. 3. *Analysis of polyacrylamide gel-fractionated late lytic SV40 RNA. Total cytoplasmic late lytic RNA was subjected to electrophoresis in polyacrylamide gels as described in Materials and Methods. From each gel slice, approximately 100 μl of eluent was added to a 150-μl hybridization mixture containing a final concentration of 1.0 M NaCl, 0.03 M phosphate buffer, and 0.1% SDS, and 150 to 300 counts/min of ^{32}P-labeled plus (L) strands of the following SV40 DNA Hind fragments: CD (●); E (▲); K (□); and FJG (■) (see Fig. 6 for map positions of Hind fragments).*

RNA purified by sedimentation in neutral sucrose, followed by oligo(dT)-cellulose selection. Unfractionated late lytic SV40 cytoplasmic RNA was first sedimented in neutral sucrose under conditions described in Fig. 1 (upper panel). Eight fractions on each side of the 18S rRNA peak were separately pooled, precipitated in 2 volumes of ethanol, resuspended, and resedimented through neutral sucrose, yielding twice-purified 19S and 16S RNA pools (Fig. 4). From the 19S preparation, two regions (a and b) were selected to the left side of the 18S marker, and from the 16S pool two regions (c and d) were selected from the right side of the 18S marker. In each case, the fractions closest to the 18S rRNA marker (b and c) contained the majority of RNA, but were more likely to be cross-contaminated than regions a and d. The four RNA pools (a through d) were diluted to 10 ml in 0.5 M NaCl, 0.01 M Tris-hydrochloride (pH 7.4) and passed over oligo(dT)-cellulose (see Materials and Methods). The flow-through from each region [without poly(A): poly(A)$^-$] as well as eluted poly(A)-containing [poly(A)$^+$] RNAs were precipitated in ethanol and resuspended in a small volume.

The results of hybridization experiments between each of the eight samples and the plus DNA strands of SV40 *Hin*d restriction fragments GJ, FK, C, D, and E are shown in Fig. 5. All of the late DNA fragments have similar reaction kinetics with poly(A)$^+$ RNA from region a, suggesting that 19S RNA is complementary to most if not all of the late region of SV40 DNA. On the other hand, poly(A)$^+$ RNA from region d reacted preferentially if not exclusively with *Hin*d G, J, F, and K, which represent the 3′ terminal half of the SV40 late region (13, 21). The annealing of poly(A)$^+$ RNA from regions b and c with the various *Hin*d fragments was similar to that seen with RNA from regions a and d, respectively. The increased rate of reaction between RNA from regions b and c and the DNA fragments probably reflects the higher concentrations of virus-specific RNA in these fractions. There was also some suggestion of cross-contamination between the 19S and 16S RNA in regions b and c. These data suggest that, whereas the 19S poly(A)$^+$ RNA is complementary to the entire late region of the plus (L) strand of SV40 DNA, the 16S poly(A)$^+$ RNA is complementary only to the 3′ half of this region.

The lower panels of Fig. 5 present the results of hybridization experiments between poly(A)$^-$ RNA from each region of the sucrose gradient and the late *Hin*d SV40 DNA fragments. Whereas most of the virus-specific poly(A)$^+$ RNA is found in regions b and c, the majority of poly(A)$^-$ SV40 RNA is present in region d, suggesting in vivo processing or breakage of large poly(A)-containing RNA during the purification procedures. It should be noted that all of the poly(A)$^-$ regions except region d contain 10 to 100 times lower concentrations of virus-

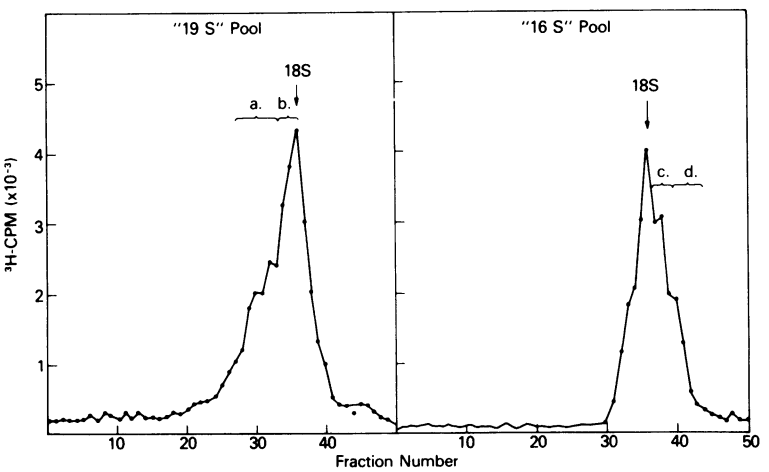

FIG. 4. *Selection of 19S and 16S RNA "pools" by sucrose gradient sedimentation. Total cytoplasmic late lytic RNA was sedimented in 5 to 30% (wt/vol) sucrose gradients as described in Fig. 1. Eight fractions on each side of the internal 18S rRNA marker were separately pooled, concentrated by precipitation in 2 volumes of ethanol, and resuspended in 0.4 ml of 0.01 M Tris-hydrochloride (pH 7.5), 0.01 M NaCl, 0.0025 M EDTA. The heavier and lighter RNA pools (19S and 16S pools) were resedimented under identical conditions and volumes of 2 μl from each fraction were directly counted. Two regions (a and b) were selected from the "19S pool" and two regions (c and d) were selected from the "16S pool." Fractions from these regions were combined and used in the experiments described in Fig. 5.*

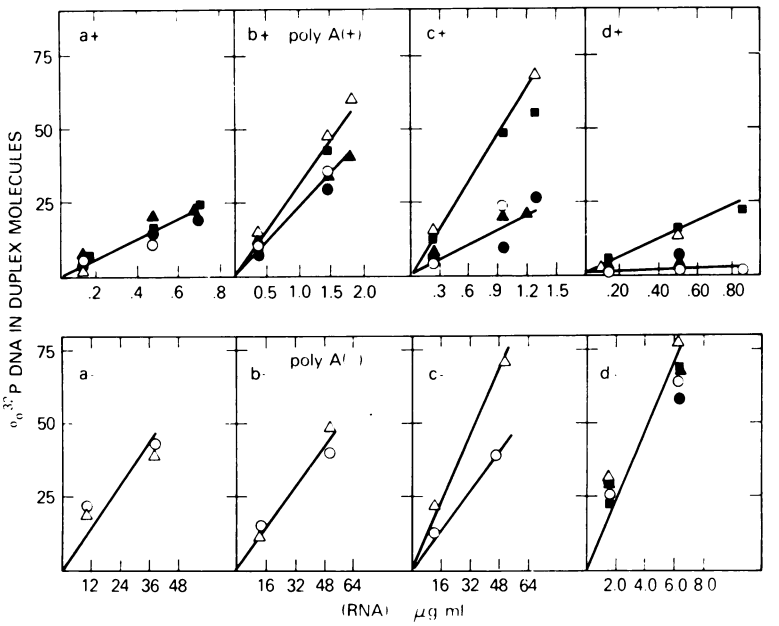

FIG. 5. *Mapping of RNA from regions of the 19S and 16S "pools." Twice-sedimented RNA from both regions of the 19S (a and b) and 16S (c and d) "pools" shown in Fig. 4 were passed through oligo(dT)-cellulose columns as described in the text. From this procedure, eight samples, poly(A)$^+$ and poly(A)$^-$ fractions from regions a, b, c, and d, were concentrated by ethanol precipitation and employed in hybridization experiments. Increasing concentrations of a particular RNA sample were incubated with 150 to 250 counts/min of the ^{32}P-labeled plus strands of various Hind SV40 DNA fragments under conditions described in Fig. 2 (C, ●; D, ○; E, ▲; KF, △; JG, ■). See Fig. 6 for map positions of Hind DNA fragments and text for discussion of results. The percentage of [^{32}P]DNA in duplex molecules was determined by HA chromatography.*

specific RNA than the corresponding poly(A)$^+$ RNA pools. This most likely reflects the higher concentrations of SV40-specific RNA in the poly(A)$^+$ fractions and the presence of ribosomal RNAs in the poly(A)$^-$ pools.

In each case (with the possible exception of region c) poly(A)$^-$ RNA is equally complementary to all segments of the late SV40 template. This differs from the results observed in the 16S poly(A)$^+$ regions c and d where little if any hybridization with DNA fragments located in the 5′ half of the late region was noted. These findings suggest that the majority of the reaction between RNA from the 16S region of sucrose gradients, and DNA fragments from the 5′ half of the late region of SV40 DNA, involves hybridization with virus-specific RNA containing no 3′ terminal poly(A) tracts.

DISCUSSION

The experiments included in this study analyze the pattern of transcription late in the lytic cycle of SV40. The sedimentation and electrophoretic properties of virus-specific RNAs are clearly dependent on the method of analysis employed; the 19S and 16S SV40 RNAs migrate more slowly relative to the 18S ribosomal marker in acrylamide gels (Fig. 3) and faster in denaturing gels (data not presented). These findings of variability in the migration of SV40 RNAs, as a function of the method of analysis, emphasize the difficulties in assigning molecular weights to RNAs on the basis of their electrophoretic and sedimentation properties relative to ribosomal RNA markers.

Hybridization experiments with the minus (E) strands of *Hind* fragments A and B (Fig. 2) confirmed that the major early SV40 RNA is a 19S species. Since the early viral RNA is present in relatively low concentration, it is possible that there are other less abundant early RNAs which are not detected by these methods of analysis. We have localized the late 19S SV40 RNA to the entire late region of the genome (Fig. 2, 3, and 5). Since the virus-specific RNA in the 19S region anneals to fragment *Hind* C with slower kinetics than it does to the rest of the late region (Fig. 2 and 3), it is possible that the 19S category of late RNA consists of more than one species in which RNA with sequences complementary to *Hind* C is less abundant. This result may be related to the finding by

Dhar et al. that a major late virus-specific RNA has its 5′ terminus near the *Hind* C-D junction (7). It should also be noted that Shenk et al. have produced a number of viable deletion mutants of SV40 which map in fragment *Hind* C, suggesting that a part of this segment of the genome may be nonessential (T. Shenk, J. Carbon, and P. Berg, personal communication).

The 16*S* category of late SV40-specific RNA appears to consist of at least two components. The major or abundant RNA is polyadenylated and represents the transcription product from the 3′ half of the late SV40 region (fragments *Hind* K, F, J, and G). Since our analysis was based on HA chromatography, in which partial hybrids are scored as duplex molecules, we cannot determine at what point in *Hind* K (which represents about 4% of the SV40 genome) this abundant transcript begins. These data support the model first suggested by Weinberg et al. (25) for the localization of SV40-specific RNAs and are in agreement with the recent findings of others for SV40 (E. May, H. Kopecka, and P. May, Nucleic Acids Res., in press) and polyoma virus transcription (Kamen et al., Cell, in press; N. Acheson and P. Beard, personal communication).

The finding that the major poly(A)-containing, late lytic 16*S* SV40 RNA maps in the region of 0.945 to 0.175 (*Hind* KFJG) suggests that it codes for the major late viral protein, VP1. Prives et al. have already shown that poly(A)-containing 16*S* SV40 RNA can be translated in a cell-free system into a polypeptide which co-migrates with VP1 in SDS-polyacrylamide gel electrophoresis. Similar results have been obtained by us for the translation of SV40 16*S* RNA into VP1 (F.-J. Ferdinand et al., manuscript in preparation) and by others for the translation of late polyoma virus 16*S* RNA into the major polyoma virus structural protein (23).

These data as well as the finding of an abnormal VP1 produced by SV40 deletion mutants in this segment of the SV40 genome (C-J. Lai and D. Nathans, personal communication) confirm the location of the gene coding for the late viral capsid protein between 0.945 and 0.175 SV40 map units. It should be noted this segment is equivalent to 23% of the viral genome (6) or about 450,000 daltons of RNA. It is therefore sufficient to code for a protein the size of VP1 (43,000 to 48,000 daltons; see 20, 24).

Since the late complementation groups of SV40 temperature-sensitive (ts) mutants (B, C,

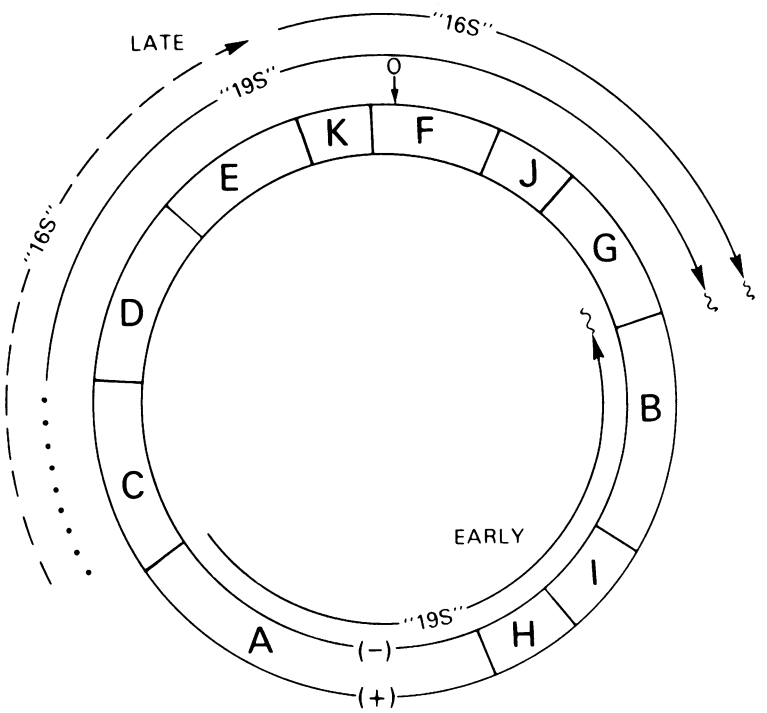

FIG. 6. *A map of the SV40 RNA species present in the cytoplasm of AGMK cells late after lytic infection (∿, 3′ poly A tracts; -----, 16S late poly(A)⁻ species; ·····, 5′ end of late 19S RNA present in lower abundance). See text for discussion.*

and BC groups) have been localized to the same segment of the genome as the 16S poly(A)-containing RNA (14, 15), the above data indicate that they contain ts defects in VP1.

We have also described a minor 16S RNA species which maps at the 5' end of the late RNA region from 0.655 to 0.945 map units (*Hind* fragments CDE). The absence of a 3'-poly(A) tail from essentially all of this RNA suggests that it may represent a degradation product of the late 19S RNA. Whether this degradation involves processing within the cell, physical disruption of the RNA during extraction and purification, or both, is unclear. Aloni and his colleagues have presented evidence based on pulse-chase experiments in enucleated cells suggesting that the late 19S RNA serves as a precursor to the late 16S RNA (4). This form of processing would preserve the 3'-terminal poly(A) sequence at the end of the 16S RNA species located to the 3' half of the precursor 19S RNA. The segment of the late region to the 5' side of *Hind* K may code for VP3 (and its related polypeptide, VP2; see 9). Cell-free translation of late SV40 19S RNA (which contains that segment in the 5' half of the molecule) results in the production of a peptide (X) which may be related to VP3 (18). In addition, the translation of the non-poly(A)-containing polyoma virus RNA (presumably enriched for the 5' end of the late region) leads to the synthesis of polypeptides which co-migrate with polyoma virus proteins VP2 and VP3 (T. Hunter and W. Gibson, personal communication).

The above data are summarized in Fig. 6. Early 19S and late 19S RNAs are found in the cytoplasm, perhaps after processing of the initial transcript (2-4, 11, 12, 22, 25). These RNA species are complementary to most if not all of the early and late genome regions, respectively. Abundant, polyadenylated late 16S RNA is also found in the cytoplasm; it is complementary to sequences between 0.945 and 0.175 map units and almost certainly codes for the major capsid protein, VP1. In addition, we detect smaller amounts of a non-polyadenylated 16S late RNA corresponding to the 5' half of the late region, which may result from processing or breakage of the 19S late RNA and which may code for the smaller SV40 structural proteins VP2 and VP3.

ADDENDUM IN PROOF

We recently learned that W. Fiers and his coworkers (personal communication) have found a nucleotide sequence in *Hind* K, close to the *Hind* E boundary, which corresponds to the N-terminal amino acid sequence of VP1. These data are in good agreement with the results presented in this manuscript and would appear to firmly establish the genomic location of the major capsid protein.

LITERATURE CITED

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