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Deletion mutants of simian virus 40 defective in biosynthesis of late viral mRNA

(mRNA synthesis/intervening sequences/splice junctions)

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ABSTRACT Deletion mutants of simian virus 40 defective in late gene functions have been examined for genetic elements that control the biosynthesis of the viral mRNAs. Mutant-specific RNA from infected cells was identified by $CH_3Hg(OH)$/agarose gel electrophoresis and mapped by the nuclease S1 technique. Altered RNA species, shortened by a size equivalent to the deletion region, can be detected in cells infected with these mutants lacking sequences within the body RNA segments. On the other hand, mutants that lack the 5′-end of the body sequences (a splice junction) fail to accumulate the respective shortened RNA species. In particular, mutant dl-2301 whose deletion includes both the leader and body splice junctions plus the intervening sequences, exhibits a polar effect on a distal gene. Whereas the late region of dl-2301 can be transcribed normally, the mutant defect appears to be associated with little or no accumulation of the mutant-specific late RNA in the infected cells. These results suggest that splice junctions or the intervening sequences, or both, in the viral genome are control signals for post-transcriptional processing of the viral RNA.

Studies from a number of laboratories have demonstrated that simian virus 40 (SV40) mRNA molecules, like many eukaryotic mRNAs, undergo significant post-transcriptional modifications (1-6). These modifications include formation of a 5′-"cap" structure, 3′-polyadenylylation, and internal splicing in the transcripts. Recent results of mapping of SV40 RNA from virus-infected cells showed that each species of late cytoplasmic RNA molecules contains a leader structure spliced to a main body segment. These cytoplasmic RNAs presumably are generated by a splicing process to remove the intervening sequences from continuous viral transcripts that can be detected in the nuclei of the infected cells (7). Such findings suggest the involvement of specific nucleotide sequences (signals) in controlling the steps of viral mRNA processing. In an attempt to delineate these specific viral sequences, we have examined viral mRNA biosynthesis from SV40 deletion mutants lacking sequences in the late genomic region. The results show that stable altered SV40 mRNA can be detected in cells infected with deletion mutants lacking sequences within the coding regions. However, no stable RNA was detected from deletion mutants lacking either one splice junction located at or near the 5′-end of the body segment or two splice junctions plus the intervening sequences. We further demonstrate that the defect in viral mRNA synthesis in these deletion mutants occurs at a post-transcriptional level.

MATERIALS AND METHODS

Cell Culture and Virus Strains. Primary African green monkey kidney cells were cultivated in minimal essential medium plus 10% fetal calf serum for two or three passages before infection with SV40. In this study we examined several SV40 deletion mutants including dl-1003 (deletion between 0.86 and 0.94 map unit), dl-1007 (0.83–0.07 unit), and dl-1010 (0.99–0.11 unit) constructed and characterized earlier (8, 9). Mutant dl-2301 (0.73–0.82 unit) was similarly constructed by complementation cloning of the 91% wild-type DNA fragment (0.73–0.82 map unit) cleaved with *Hpa* II and *Hae* II (8, 10). All of these mutants were derived from the wild-type strain 776 and grown in the presence of a helper virus (tsA28) derived from strain VA-45-54 (11).

Preparation of Viral RNA. Confluent monolayers of AGMK cells were infected with viral stocks containing deletion mutants and helper SV40 tsA28 at a multiplicity of 1–10 plaque-forming units per cell at 37°C. At a late stage, generally 48–60 hr after infection, cytoplasmic and nuclear RNAs were prepared as described (7). Poly(A)-containing RNA was selected by chromatography on an oligo(dT)-cellulose column (12). For $CH_3Hg(OH)$/agarose gel analysis, SV40-specific RNA was obtained by selection of the viral RNA on SV40 DNA-containing nitrocellulose filters and subsequently released in 90% formamide buffer (13). When DNA infection was performed, AGMK cells were treated with SV40 deletion mutant or wild-type virus DNA (10 $\mu g/150\ cm^2$) in the presence of DEAE-dextran (250 $\mu g/ml$) for 2 hr at room temperature. After incubation at 37°C for 3–4 days, the infected cells were harvested and the viral transcriptional complexes were prepared (14). The *in vitro* labeling of the transcriptional complexes with [α-^{32}P]ATP (350 Ci/mmol) has been described (15).

Analysis of Viral RNA. (*i*) *Nuclease S1/alkaline agarose gel electrophoresis.* The technique of Berk and Sharp (16) was used to map the size and positions of mutant specific transcripts. The poly(A)-containing viral RNA was mixed with a 5- to 10-fold molar excess of *Bam*HI-cleaved [^{32}P]DNA probe (specific activity, $1-2 \times 10^6$ cpm/μg) in 80% formamide, denatured at 68°C for 10 min, and annealed for 3–4 hr at 49°C. The hybridization mixture was treated with nuclease S1 at 45°C for 30 min and analyzed by alkaline agarose gel electrophoresis as detailed (7).

(*ii*) *Analysis by blots. In vitro* labeled RNA from the viral transcriptional complexes was analyzed by hybridization to nitrocellulose filters containing immobilized SV40 DNA fragments (15). One blot contained fragments of SV40 DNA resulting from cleavages with *Hae* II, *Hpa* II, and *Bam*HI. For analysis of strandedness, the labeled RNA was similarly hybridized to filters containing separated strands of SV40 DNA segments from an *Hpa* II/*Bam*HI digest (15).

Complementation Analysis. Plaquing defective mutants by complementation with ts mutants as originally described by Brockman and Nathans (10) was applied to construct deletion mutants used in this study. Similar plaquing analysis was also

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Abbreviations: SV40, simian virus 40; VTC, viral transcriptional complex.

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used to characterize the biological defects of these deletion mutants.

RESULTS

Studies on Biogenesis of Late SV40 RNA from Deletion Mutants. Defective mutants of SV40 containing a deletion in the late gene region were examined for signals or nucleotide sequences that may be involved in the biosynthesis of the late SV40 mRNAs. Two major categories of deletion mutants were studied. The mutants in the first category contained deletions within the structural genes for viral capsid proteins VP1, VP2, and VP3. The positions of these deletions therefore reside within the coding sequences of the late 16S and 19S mRNAs. The construction and biological characterization of these mutants have been described (8). Mutants in the second category had deletions that included a portion of the leader sequence and the intervening sequences as well as a portion of bodies of the late viral RNAs. These mutants were similarly constructed by endonucleolytic excision of wild-type SV40 DNA segments followed by complementation plaquing (10).

We examined mRNA biosynthesis of deletion mutants that lacked sequences within the coding regions of the late viral structural proteins (Fig. 1). Three mutants were used: (*i*) mutant dl-1010 with a deletion region mapping between 0.99 and 0.11 unit, within the coding region of VP1; (*ii*) dl-1003 which is missing the *Hin* E fragment (0.86–0.94 unit), within the coding region of VP2 and VP3; and (*iii*) dl-1007 containing a deletion (0.83–0.07 unit) within the coding region for VP1, VP2, and VP3. Because these mutants are defective, they were grown and propagated in the presence of a helper virus (tsA28); the late viral mRNA isolated from the infected cells therefore contains the RNA species from the helper virus. We present here two different RNA mapping approaches to define the RNA species specifically transcribed from the deletion mutants.

Mapping Late SV40 RNA of Deletion Mutants Lacking Sequences in the Coding Regions. Initially, experiments were performed to detect the synthesis of mutant-specific RNA by using agarose gel electrophoresis in the presence of $CH_3Hg(OH)$ which denatures RNA and therefore separates RNA species according to size (17). An RNA band equivalent to 0.20 SV40 unit length was present in dl-1007-infected cells in addition to the helper virus-derived RNA molecules. The size estimation indicated that the additional RNA represented an altered 19S RNA. Mutant dl-1010-infected cells contained two altered RNA species, 0.32 and 0.16 unit length, corresponding to the shortened 19S and 16S RNAs, respectively (data not presented).

Further analysis of deletion mutant-derived RNAs was carried out by using the technique of Berk and Sharp (16) in which

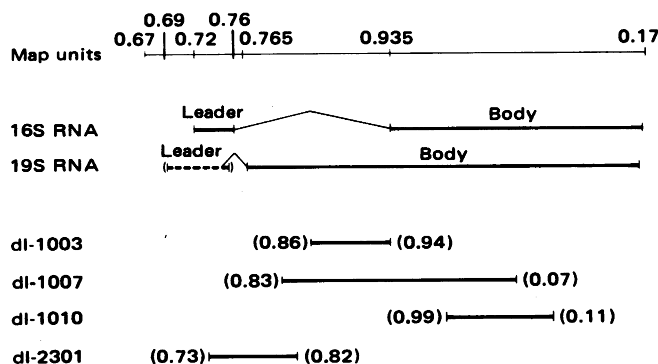

FIG. 1. Map of deletion mutants of SV40 and the late viral RNAs, showing the map positions of two major late cytoplasmic SV40 RNAs. The map positions of several deletion mutants of SV40 used in this study, as determined by electron microscopic heteroduplex mapping, are also indicated.

nuclease S1 is used to map the RNA-protected DNA segments. This mapping technique allowed us to determine the size and the genomic positions of the viral transcripts. Poly(A)-containing late SV40 RNAs were prepared from cytoplasm of cells 48 hr after infection with viral stocks of dl-1003, dl-1007, or dl-1010 (all contain SV40 tsA28 helper virus). ^{32}P-Labeled wild-type SV40 DNA of strain 776, from which these deletion mutants were derived, was cleaved with *Bam*HI and used for hybridization with the cytoplasmic RNAs. The hybrid molecules were digested with nuclease S1 and the digest was analyzed by alkaline agarose gel electrophoresis. Because DNA of the wild-type strain differs slightly from that of tsA28 helper in the late SV40 gene region (18), a preliminary experiment of S1 nuclease mapping was performed with hybrids between the late tsA28 RNA and [^{32}P]DNA from the wild-type strain. Two major DNA segments corresponding to coding sequences of the late 16S and 19S RNAs were observed, indicating that strain differences did not significantly affect the nuclease S1 analysis under the conditions described (data not shown).

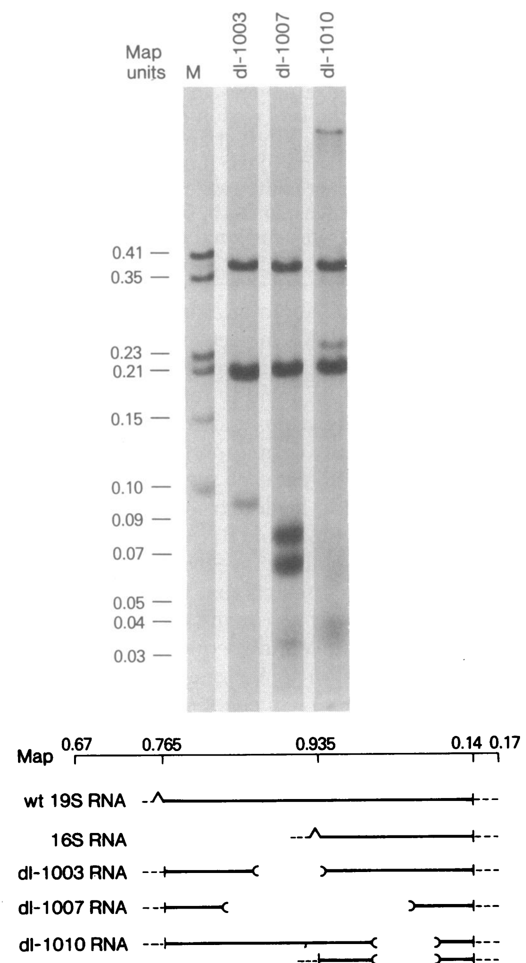

FIG. 2. Nuclease S1 analysis of RNA from deletion mutants of SV40. Late cytoplasmic RNA from cells infected with SV40 deletion stocks containing dl-1003, dl-1007, or dl-1010 was annealed with ^{32}P-labeled SV40 DNA cleaved by *Bam*HI, and the hybrids were analyzed by the nuclease S1 technique of Berk and Sharp (16). The body sequences of late 16S and 19S RNAs (0.21 and 0.38 unit, respectively) from the helper virus tsA28 were also detected. Additional radioactively labeled bands indicate stable altered RNA molecules transcribed from each mutant. M, SV40 DNA fragments obtained from a restriction enzyme digest. Diagrammed below the figure are the deletion locations in each mutant and the corresponding altered RNA segments seen. wt, Wild type.

The results of mapping cytoplasmic RNAs from infections with mutants dl-1003, dl-1007, and dl-1010 are presented in Fig. 2. Because the mutant transcripts lacking sequences in the coding region would hybridize with the wild-type DNA probe in the region transcribed, alkaline agarose gel electrophoretic analysis of the hybrids after nuclease S1 digestion would yield two DNA segments corresponding to the ends of the remaining sequences. In the analysis of dl-1003 RNA (deletion of 0.86–0.94 unit), two new segments were detected in addition to the 0.38 and 0.21 unit bands (which correspond to helper 19S and 16S RNAs, respectively). One new fragment, migrating at a position approximately 0.095 unit length, corresponds to the RNA transcribed from the region between 0.765 and 0.86 map unit; the other new segment (0.20 unit), slightly below the 16S RNA-derived 0.21 unit band, represents the 3′-end portion of the altered RNA (0.94–0.14 unit). The two additional segments were present in approximately equimolar quantities, suggesting that they are derived from a single altered 19S RNA species.

Analysis of the RNA from dl-1007 also showed two additional fragments—a 0.06-unit (0.77–0.83 unit) and a 0.08-unit (from 0.07 ± 0.01 to 0.14) segment—both present in approximately equal molar quantities, suggesting that they are also derived from an altered 19S RNA. An altered 19S RNA from dl-1010 was similarly represented by the presence of a 0.23-unit segment (0.765–0.99 unit) and a 0.03-unit fragment (0.11–0.14 unit). In addition, an altered 16S RNA was also detected from the transcripts of mutant dl-1010-infected cells as characterized by the presence of a 0.06-unit segment (0.935–0.99 unit) and a 0.03-unit segment (0.11–0.14 unit). The results from the nucleases S1 mapping of RNA confirmed the findings of the presence or the absence of altered RNA transcribed from the deletion mutants in our earlier analysis using $CH_3Hg(OH)$. Taken together, these experiments demonstrate that stable altered late 19S RNA molecules are synthesized from all three deletion mutants and that an altered 16S RNA can be found only in cells infected by dl-1010. Because only dl-1010 contains the 5′-terminus of the 16S RNA body sequences, the defect in synthesizing altered 16S RNA molecules in dl-1003 and dl-1007 appears to be associated with the missing region which corresponds to sequences at or near the 5′-end of the 16S RNA body segment.

Complementation Analysis of dl-2301. Mutant dl-2301 (0.73–0.82 unit), in another category of late defective mutants, lacks a DNA segment corresponding to a portion of the leader RNA, the intervening sequences, and the 5′-terminal portion of the 19S RNA coding sequences. Mutant dl-2301 was isolated by complementation cloning of the shortened SV40 DNA genome obtained from enzymatic excision using *Hpa* II (cleavage at 0.73 unit) and *Hae* II (0.82 unit). The mutant stock contains a helper virus, tsA28. *Hind* II+III analysis of dl-2301 showed that *Hind* fragments C and D are missing and a new fused fragment of 0.12 unit long is present (data not presented). Mutant dl-2301 appears to be a simple deletion with no detectable sequence rearrangement.

An additional experiment to demonstrate the functional defect of dl-2301 was conducted by complementation analysis. An infectious center assay was performed by coinfection of BSC-1 cells with DNA from dl-2301 and mutant virus from tsA28 or tsB4. As controls, DNAs of dl-1007 and tsD202 were also used for coinfection with tsA28 or tsB4. Mutant dl-1007 (previously characterized) lacks all late functions whereas tsD202 is defective in D function (VP2 or VP3) at 41°C (19, 9). dl-1007 complemented mutant tsA but failed to form plaques with tsB (Table 1). On the other hand, tsD202 complemented both tsA and tsB with equal efficiency. Mutant dl-2301, like dl-1007, was able to complement mutant tsA but not mutant tsB. Because the *B* gene product, VP1, is encoded by 16S mRNA, these findings suggest that dl-2301 is defective in the 16S RNA function. Although the structural gene for VP1 and the entire body sequences (0.935–0.17 unit) of 16S mRNA are intact in dl-2301, the mutant failed to express the VP1 gene function. This analysis indicates that dl-2301 is a polar mutant in which a deletion in the region between 0.73 and 0.82 unit affects the expression of a distal gene. A similar polar effect has been observed recently in a mutant of SV40 containing a deletion between 0.73 and 0.80 unit (20).

Table 1. Complementation between defective mutants and ts mutants of SV40

Defective mutant DNA	ts mutant A28	ts mutant B4	ts mutant None
tsD202	18; 28	24; 30	0; 0
	3; 2	4; 5	0; 0
dl-1007	25; 36	3; 2	1; 1
	14; 12	0; 0	0; 0
dl-2301	53; 51	0; 0	0; 0
	20; 16	0; 0	0; 0
None	0; 0	0; 0	—

BSC-1 cells (5×10^4) were coinfected with DNA from a defective mutant and ts mutants (1–5 plaque-forming units per cell). An infectious center assay was performed; duplicate infections were carried out for each analysis. Results are expressed as plaque-forming units per dish after infection with 20 ng of DNA (upper rows) or 4 ng DNA (lower rows).

Mapping RNA of Defective Mutant dl-2301. Infection of primary AGMK cells with a stock of dl-2301 (containing tsA28 helper) was performed. Late RNA was prepared from the infected cells and separated into cytoplasmic and nuclear fractions. The poly(A)-containing RNA from dl-2301 was analyzed by the nuclease S1 technique using the *Bam*HI-cleaved [^{32}P]-DNA probe from the isogenic SV40 strain 776. Similar nuclease S1 sizing was also performed with hybrid DNA molecules between *Bam*HI-cleaved [^{32}P]DNA and *Bam*HI-cleaved dl-2301 DNA. The results of these experiments are presented in Fig. 3. From the DNA·DNA sizing (results shown at the left side), two segments corresponding to 0.32 and 0.59 unit in size were observed. The 0.32-unit segment mapped from 0.82 to 0.14 unit, or from one end of the deletion to the *Bam*HI site in the late region. The cytoplasmic RNA prepared from infection with the mutant showed two prominent bands, at 0.38 and 0.21 unit, representing the 19S and 16S late SV40 RNAs, respectively, derived from the tsA helper. No segment corresponding in size to the 0.32 unit was detectable from cytoplasmic RNA of the defective mutant dl-2301. Poly(A)-containing nuclear RNA from a similar infection was analyzed by the same technique and compared with the results with cytoplasmic RNA. In addition to the cytoplasmic RNA species, we observed a number of nuclease S1-resistant bands (0.42 unit and larger) corresponding to uninterrupted RNA molecules transcribed from the helper virus as described previously (7). Again, the predicted 0.32-unit segment was not present in detectable quantities in the nuclear RNA fraction.

These results indicate that altered 19S RNA from dl-2301 is not synthesized in sufficient quantity to be detected by the methods used. The results of complementation analysis further imply that dl-2301 fails to synthesize a functional 16S RNA. In summary, these findings suggest that the nucleotide sequences in the region deleted play an important role in the biosynthesis of the late SV40 RNA species.

Transcription of dl-2301 Late RNA *In Vitro*. The absence of stable late RNA from dl-2301-infected cells suggests that the

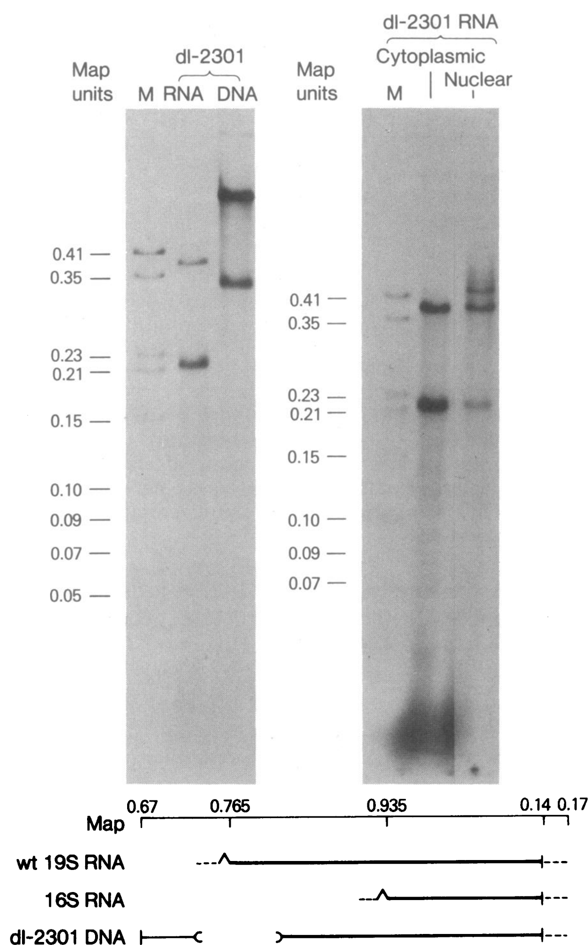

FIG. 3. Nuclease S1 analysis of late dl-2301 RNA. The late cytoplasmic and nuclear RNA, prepared from cells infected with dl-2301 and helper virus tsA28, was hybridized to SV40[^{32}P]DNA cleaved by *Bam*HI (0.14 unit) and the hybrids were digested with nuclease S1. Alkaline agarose gel electrophoresis was performed to determine the size of nuclease-resistant DNA segments. Map units refer to fractional length of the SV40 genome as determined from the migration of ^{32}P-labeled restriction DNA fragments (M). At the left, mapping of the cytoplasmic RNA from cells infected with dl-2301 containing tsA28 helper virus is shown in the track marked RNA. Mutant dl-2301 DNA was similarly mapped, in the track designated DNA, as a marker for detection of any dl-2301 late RNA sequences. At the right, results of a comparative analysis between the cytoplasmic RNA and the nuclear RNA from dl-2301/tsA28 infected cells are shown. The map positions of DNA segments corresponding to 16S and 19S RNAs and dl-2301 DNA are diagrammed below. wt, Wild type.

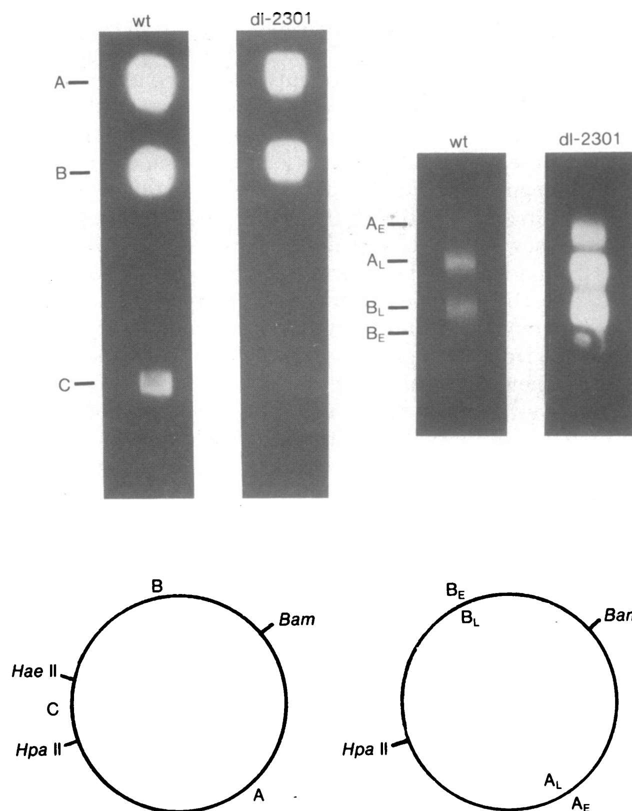

FIG. 4. Mapping *in vitro* labeled RNA from infection with wild-type (wt) SV40 and dl-2301 DNAs. Late after infection the VTCs were prepared from AGMK cells infected with either wild-type SV40 DNA or dl-2301 DNA. The viral RNA chains were elongated with [α-^{32}P]ribonucleoside triphosphates by an *in vitro* reaction (14, 15). The labeled virus-specific RNA sequences were detected by hybridization to SV40 DNA fragments immobilized on nitrocellulose filters (21). (*Left*) Hybridizations of the RNA to nitrocellulose blots of SV40 DNA segments cleaved by *Bam*HI, *Hpa* II, and *Hae* II. (*Right*) Hybridizations of the same RNA preparation to the separated strands of *Bam*HI/*Hpa* II-cleaved SV40 DNA. The DNA fragments and the separated strands used in this analysis are presented in the SV40 map.

deleted sequences are involved in the processing of the RNA transcripts. Alternatively, it is possible that this mutant is defective in initiation of late transcription. To differentiate between these possibilities, we examined the SV40 RNA from viral transcriptional complexes (VTCs) obtained from cells infected with dl-2301 DNA in the absence of a helper virus. RNA chains from the isolated VTCs, extended *in vitro* with radiolabeled nucleoside triphosphates, were purified and analyzed by hybridization with two sets of blots (Fig. 4). One set contained all three SV40 DNA fragments from cleavages with *Hpa* II, *Hae* II, and *Bam*HI; the other set contained the separated strands of the two viral DNA fragments from *Hpa*II/*Bam*HI cleavages. Fig. 4 *left* shows that, whereas VTC RNA obtained from cells infected with wild-type SV40 DNA annealed with all three fragments, the labeled VTC RNA similarly prepared from cells infected with dl-2301 DNA hybridized with only fragments A and B, confirming that the VTC RNA is mutant specific. The results of annealing these RNA preparations to the separated SV40 DNA strands (Fig. 4 *right*) showed that greater than 90% of both wild-type and dl-2301 VTC RNAs is late viral sequence specific. Because no initiation of transcription and processing of the RNA transcripts occurs in VTCs, these experiments demonstrate that the late RNA synthesis is equally active in cells infected with dl-2301 or wild-type DNA. Thus, the apparent instability of the late dl-2301 RNA in the infected cells appears to result from a defect at a post-transcriptional level.

DISCUSSION

In this study we attempted to examine the biogenesis of the late SV40 mRNAs in mammalian cells by using a series of defective deletion mutants lacking sequences in the late region of the SV40 genome. Results of mapping RNA from the deletion mutants can be summarized on the basis of the mutant classes. Mutants belonging to the deletion class that lacks sequences within the body region synthesized altered 16S or 19S RNA or both, depending on the location of the deletion mutant. On the other hand, mutants of the deletion class that is missing se-

quences at or near the 5′-end of a coding segment, such as deletions in dl-1003 (0.86–0.94 unit) and dl-2301 (0.73–0.82 unit), do not produce altered 16S or 16S and 19S cytoplasmic RNA species, respectively. The deletion in dl-2301 also includes the 3′-end of the leader segment (0.76 unit).

The defect in mRNA synthesis in these mutants is further demonstrated by the results of a complementation analysis. For example, an earlier study (22) demonstrated that dl-1003, although containing an entire intact VP1 structural gene, fails to complement tsB4, a temperature-sensitive mutant defective in VP1. The distal gene defect resulting from the deletion in dl-1003 appears to be associated with the physical absence of 16S RNA. The failure of dl-2301 to form plaques with tsB4 at a nonpermissive temperature indicates that dl-2301 is also defective in the biosynthesis of 16S RNA molecules. The failure of dl-2301 to express any late functions, however, does not occur at the level of transcription because the late transcripts of the mutant can be demonstrated by *in vitro* labeling RNA on VTCs that had initiated transcription *in vivo*. We suspect that the RNA transcripts from dl-2301 lack certain processing signals and, as a result, are not stably accumulated in nuclei and in cytoplasm. Similar observations have recently been made in analysis of hybrid RNA from SV40–rabbit β-globin gene recombinant molecules in which the late splice junctions and the intervening sequences of the SV40 genome have been deleted (D. Hamer and P. Leder, personal communication). Results from a different approach using translation analysis of early SV40 mRNA also show that the prespliced RNA transcripts from viable deletion mutants lacking a splice junction and a portion of the intervening sequences can be detected in nuclei but not in cytoplasm of the infected cells (23).

Although the exact nature of RNA processing signals remains unclear, their locations can be suggested from the deletion region of dl-2301 which includes at least one splice junction for 16S RNA (0.760 unit) and one for 19S RNA (0.765 unit), the intervening sequences (0.760–0.765 unit), and a portion of the leader and coding sequences (7, 24). Results presented in this study show that deletions in a significant portion of the coding sequences do not seem to affect mRNA biosynthesis. Similar results have also been observed in mutants that lack a significant portion of the leader sequences (unpublished data). We conclude from this analysis that splice junctions or intervening sequences are signals for regulatory steps in viral mRNA biosynthesis that are important for generating stable mRNA molecules.

In the late gene region of SV40, sets of intervening sequences with splice junctions precede the main body of structural genes. One may speculate that removal of these sequences brings the coding region closer to the RNA 5′-end and therefore more accessible for translation. It is reasonable to further speculate that splicing out the intervening sequences may also play a role in gene regulation, perhaps by stabilizing mRNA or facilitating the transport of transcripts from the cell nucleus to the cytoplasm. Whether the putative splicing function is associated with the transport of a special class of mRNA or represents a general mechanism remains to be critically tested.

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1. Kelly, T. J. & Nathans, D. (1977) in *Advances in Virus Research*, eds. Lauffer, M. A., Bang, F. B., Maramorosch, K. & Smith, K. M. (Academic, New York), Vol. 21, pp. 86–173.
2. Gilbert, W. (1978) *Nature (London)* **271**, 501.
3. Aloni, Y., Dhar, R., Laub, O., Horowitz, M. & Khoury, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3686–3690.
4. Celma, M. L., Dhar, R., Pan, J. & Weissman, S. M. (1977) *Nucleic Acids Res.* **4**, 2549–2560.
5. Hsu, M.-T. & Ford, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4982–4985.
6. Lavi, S. & Groner, Y. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5323–5327.
7. Lai, C.-J., Dhar, R. & Khoury, G. (1978) *Cell* **14**, 971–982.
8. Lai, C.-J. & Nathans, D. (1974) *J. Mol. Biol.* **83**, 179–193.
9. Lai, C.-J. & Nathans, D. (1976) *Virology* **75**, 335–345.
10. Brockman, W. W. & Nathans, D. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 942–946.
11. Tegtmeyer, P. & Ozer, H. L. (1971) *J. Virol.* **8**, 516–524.
12. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
13. Weinberg, R., Warnaar, S. O. & Winocour, E. (1972) *J. Virol.* **10**, 193–201.
14. Gariglio, P. & Mousset, S. (1975) *FEBS Lett.* **56**, 149–155.
15. Ferdinand, F.-J., Brown, M. & Khoury, G. (1977) *Virology* **78**, 150–161.
16. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12**, 721–732.
17. Bailey, J. M. & Davidson, N. (1976) *Anal. Biochem.* **70**, 75–85.
18. Lai, C.-J. & Nathans, D. (1974) *Virology* **60**, 466–475.
19. Chou, J. Y. & Martin, R. G. (1974) *J. Virol.* **13**, 1101.
20. Cole, C. N., Landers, T., Goff, S. P., Manteuil-Bruttag, S. & Berg, P. (1977) *J. Virol.* **24**, 277–294.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
22. Lai, C.-J. & Nathans, D. (1975) *Cold Spring Harbor Symp. Quant. Biol.* **32**, 54–60.
23. May, E., Kress, M. & May, P. (1978) *Nucleic Acids Res.* **5**, 3083–3099.
24. Reddy, V. B., Thimmappaya, B., Dhar, R., Subramanian, K. N., Zain, B. S., Pan, P. K., Ghosh, P. K., Celma, M. L. & Weissman, S. M. (1978) *Science* **200**, 494–502.