MOLECULAR AND CELLULAR BIOLOGY, Aug. 1983, p. 1381–1388
0270-7306/83/081381-08$02.00/0

Vol. 3, No. 8

A Splice Junction Deletion Deficient in the Transport of RNA Does Not Polyadenylate Nuclear RNA

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Received 9 March 1983/Accepted 25 May 1983

A late region deletion mutant of simian virus 40 (*dl5*) was previously shown to be deficient in the transport of nuclear RNA. This is a splice junction deletion that has lost the 3′ end of an RNA leader, an intervening sequence, and the 5′ end of the splice acceptor site on the body of the mRNA. In this report, we analyzed the steady-state structure of the untransported nuclear RNA. The 5′ ends of this RNA are heterogeneous but contain a prominent 5′ end at the normal position (nucleotide 325) in addition to several other prominent 5′ ends not seen in wild-type RNA. The 3′ end of this RNA does not occur at the usual position (nucleotide 2674) of polyadenylation; instead, this RNA is non-polyadenylated, with the 3′ end occurring either downstream or upstream of the normal position.

Eucaryotic mRNA production occurs via a surprisingly complicated pathway. The sequence of events leading to stable mRNA includes promotion (which in itself may be multifunctional), 5′ capping, 3′ polyadenylation, splicing, and finally transport from the nucleus to the cytoplasm (for review, see references 9 and 14). Of these post-transcriptional events, the least investigated is the question of how nuclear transcripts are transported into the cytoplasm. Not all nuclear RNA sequences are destined to become cytoplasmic mRNA. It was reported that the sequence complexity of heterogeneous nuclear RNA is measurably greater than that of cytoplasmic RNA (31). It could be argued, however, that this may be because transcription runs well past the polyadenylation site and that subsequent cleavage and polyadenylation could result in the excision and degradation of downstream sequences, thereby accounting for the different complexities observed.

It seems unlikely, however, that such an explanation could account for the transport behavior of several constructed and naturally occurring deletion mutations in viral and cellular genes (7, 8, 12, 16, 18–22, 30; R. T. White, Ph.D. thesis, Stanford University, Stanford, Calif., 1980). There are now numerous examples of genes deleted downstream from promoters and upstream from polyadenylation sites that do not code for the production of stable mRNA. These deletions generally affect splice junctions. An early example of a transport deficiency mutation was constructed in the late region of simian virus 40 (SV40), in which an intervening sequence was precisely deleted (18, 19). The relation between splicing and transport, however, is complicated. There are, for example, deletion mutants which efficiently transport unspliced RNA (8,15,30; White, thesis), indicating that splicing per se need not occur to allow transport. Along these lines, a hypothesis that the intervening sequences were directly involved in the retention within the nucleus of nuclear RNA was proposed and tested (28). The results of those experiments indicated that retention of nuclear RNA is probably not simply the result of the presence of intervening sequences, as transcripts containing intronic sequences were efficiently transported.

What then can be the relation between splicing and transport of nuclear RNA? In an effort to address this question, we report here the detailed analysis of the nuclear RNA synthesized by a late region deletion mutant of SV40 that is deficient in the transport of nuclear RNA. It was observed that these RNAs were neither terminated nor polyadenylated at the normal polyadenylation site.

MATERIALS AND METHODS

Enzymes and reagents. Restriction endonucleases were purchased from Bethesda Research Laboratories, New England Biolabs, or Boehringer Mannheim Corp. T4 DNA ligase was a gift from Paul Hagerman, University of Colorado. The Klenow fragment of DNA polymerase I was purchased from Boehringer Mannheim. α-^{32}P-labeled deoxynucleotide triphosphate (7,800 Ci/mmol) was from New England Nuclear Corp. γ-^{32}p-labeled ATP (crude, carrier free) was from ICN. Oligodeoxythymidylate-cellulose was purchased from Collaborative Research Inc. Polynucleotide kinase and exonuclease VII were purchased

from Bethesda Research Laboratories. Cordycepin (3'-deoxyadenosine) was purchased from Calbiochem-Behring Corp.

Plasmids, bacteria, and plasmid DNA. The plasmid *dl5/322* was constructed by digesting SV40DNA with *Hha*I. The large fragment of this digest was recircularized after gel purification with T4 DNA ligase. This DNA was then digested with *Bam*HI and cloned into the *Bam*HI site of pBR322 (5). Transformation onto *Escherichia coli* HB101 cells was done by the method of Mandel and Higa (23). Tetracycline-sensitive colonies were screened for the presence of *dl5/322* DNA by restriction digestion. Plasmid DNA was purified as described previously (25). DNA from *dl5/322* was sequenced through the point of deletion, and a detailed restriction enzyme analysis was also performed. This analysis confirmed the DNA structure of *dl5* (White, thesis).

Cell culture and viruses. The history and protocols for growing virus in CV-1 monkey kidney cells were described previously (25). Cells were grown in Dulbecco modified essential medium with 5% fetal calf serum, penicillin (500 U/ml), and streptomycin (100 μg/ml).

DNA transfection. DNA transfections were performed as described by Graham and Van Der Eb (17) as modified by Parker and Stark (26). Viral DNA (10 μg per plate of 2×10^7 cells) was diluted into 1 ml of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-buffered saline containing (per liter); 8 g of NaCl, 0.36 g of KCl, 0.16 g of $Na_2HPO_4 \cdot 7H_2O$, 1 g of dextrose, and 5 g of HEPES (pH 7.05) and adjusted to 125 mM $CaCl_2$ from a 2 M stock solution. The solution was incubatd for 20 min at room temperature. The fine precipitate which formed was overlaid onto CV-1 cells without rinsing the monolayer and incubated for 30 min at room temperature. The cells were then overlaid with Dulbecco medium and incubated for 4 h at 37°C. The medium was then removed, and the cells were shocked by overlaying them with 2 ml of HEPES-buffered saline with 15% (wt/vol) glycerol for 4 min. The glycerol solution was then removed, and the cell monolayer was rinsed twice and then overlaid with Dulbecco medium with 10% calf serum. DNA for these transfections was prepared by excising the viral DNA from the plasmid DNA by *Bam*HI digestion. The gel-purified DNA was then recircularized with T4 DNA ligase.

Cell fractionation and extraction of RNA. The fractionation of cells into cytoplasmic and nuclear fractions and the extraction of RNA were described in detail previously (7).

Preparation of DNA hybridization probes. Recombinant plasmid DNA was digested with *Bam*HI, and the overhanging 5' termini were then end labeled with [γ-^{32}P]ATP by the exchange reaction (4). The labeled DNA was then purified by electrophoresis on a 1.0% agarose gel, and stained bands were cut out and electroeluted. The specific activity of the DNA was about 2×10^6 cpm/μg. This probe was used for 5'-end analysis of *dl5* RNA. A similar proble was prepared from SV40 DNA and used for the 5'-end analysis of wild-type (WT) SV40 RNA. Probes for 3'-end analysis of RNA were prepared by the fill-in reaction of the *Eco*RI site by using the Klenow fragment of DNA polymerase I and α-^{32}P-labeled dATP as described by others (11).

Analysis of RNA. Virus-specific RNA was analyzed by the nuclease S1 method of Berk and Sharp (3), modified as described previously (7). Nuclease-resistant DNA was electrophoresed on 1.5% alkaline agarose gels (24). Dried gels were exposed to Kodak AR5 film at −70°C for the times indicatd in the text in the presence of a Dupont Lightning-Plus intensifying screen. Viral RNA was also analyzed by the method of Alwine et al. (1). Nuclear RNA extracted from 2×10^8 cells transfected with either *dl5* or WT SV40 DNA was fractionated on oligodeoxythymidylate-cellulose. RNA was then electrophoresed on a 1.5% agarose gel containing methylmercury. Gels were then blotted onto diazotized paper and probed for the presence of late SV40 sequences with a nick-translated late region probe (0.758 to 0.175 map units [m.u.]).

RESULTS

Analysis of 5' ends of *dl5* nuclear RNA. Nuclear RNA from 4×10^7 cells was extracted 48 h after transfection with *dl5* DNA. As a control experiment, nuclear RNA was also extracted after transfection with WT SV40 DNA. RNA was analyzed with nuclease S1 and exonuclease VII for the determination of 5' ends. The DNA probes for these analyses were 5'-end labeled at the *Bam*HI site of the homologous DNAs. In addition, SV40 nuclear RNA was selected by two successive passages on oligodeoxythymidylate-cellulose for the presence of polyadenylated RNA (2) (Fig. 1). In the nuclease S1 analysis of *dl5* RNA, three relatively prominent bands were observed, 3.4, 1.7, and 0.93 kilobases (kb) long. The 5' ends of these bands corresponded to nucleotides (nt) 4300, 320, and 1600 of the SV40 genome (numbering is from Buchman et al. [6]). The 5' end at nt 320 corresponded to the major 5' end seen in WT SV40 nuclear RNA. The 5' end at nt 4300 has not been found in WT nuclear RNA, but 5' ends in the early region have been reported (21). The 5' end at nt 1600 was not detected in WT SV40 nuclear RNA. All of these determinations took into account the 500 nucleotides deleted in *dl5*. In addition to these three relatively prominent 5' ends, there was a substantial amount of 5'-end heterogeneity from nt 1600 to well into the early region, as well as some minor 5' ends between nt 830 to 1600. (A schematic summary of these results is presented in Fig. 6.)

As nuclease S1 analysis alone cannot distinguish 5' ends from splice junctions, we also digested DNA-RNA hybrids with exonuclease VII to confirm the location of authentic 5' ends. It was observed that the pattern of protected probe did not differ substantially from that seen with nuclease S1, indicating that most of this RNA was unspliced. Some relative loss in intensity of the 0.93-kb band was noted, however. It was therefore possible that some of 0.93-kb species was spliced to unknown 5' ends; nonetheless, it was clear that the great majority of *dl5*

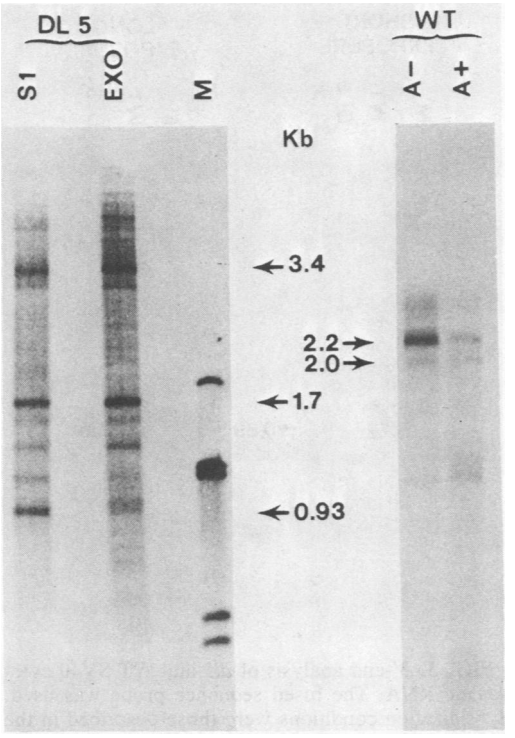

FIG. 1. 5'-end analysis of *dl5* and WT SV40 nuclear transcripts. The probe for *dl5* analysis was *Bam*HI-end-labeled cloned *dl5* DNA, and for WT analysis it was *Eco*RI-end-labeled SV40 DNA. The marker (lane M) is an *Hin*dIII digest of SV40 DNA. Nuclease S1 and exonuclease VII (exo) digests were preformed as described in the text with 50,000 cpm of probe and the nuclear RNA from 2×10^7 cells. Shown is a 14-h exposure of the autoradiograms from the dried alkaline gel. WT SV40 RNA was fractionated into polyadenylated (A+) and non-polyadenylated (A−) components as described in the text.

nuclear RNA was unspliced. A comparison of the 5'-end analyses of WT SV40 nuclear polyadenylated and non-polyadenylated RNAs showed only limited similarity. Here, the major 5' end was also at nt 320, but much less heterogeneity was observed. The WT heterogeneity present was mainly in the non-polyadenylated RNA and restricted to the region between nt 0 and 325.

3'-end analysis of nuclear RNA. Nuclear RNA from transfected cells was hybridized to 3'-end-labeled DNA probe that had been labeled at the *Eco*RI site by the fill-in reaction as described above. The probe for *dl5* RNA was a plasmid containing an *Hin*dIII-cut unit length of SV40 (cut at nt 3476) cloned into the *Hin*dIII site of pBR322. This DNA was linearized and labeled at the *Eco*RI site. The probe for WT SV40 RNA was the *Eco*RI (nt 1782)-*Hin*fI (nt 2824) fragment of SV40 DNA labeled at the *Eco*RI site. Hybrids were digested with S1 nuclease and analyzed as described above (Fig. 2). Analysis of WT SV40 nuclear polyadenylated RNA showed a major band at 0.89 kb. This corresponded to the 3' end of the RNA at nt 2674 (the normal polyadenylation site). In addition, a less abundant band at 1.04 kb was also seen. This band was the size of the probe (1,040 nucleotides) and may be partly the result of probe-probe rehybridization, representing a background of signal. It is also possible that some nuclear polyadenylated RNA was either contaminated with very long non-polyadenylated RNA or was itself very long and protecting the full length of the probe. The pattern observed with non-polyadenylated WT SV40 nuclear RNA is also shown (Fig. 2). Here, the 0.89-kb band was not apparent, indicating that the non-polyadenylated nuclear RNAs were not terminated at nt 2674; instead, a large amount of the 1.04-kb band was present. The intensity of this band was substantially above

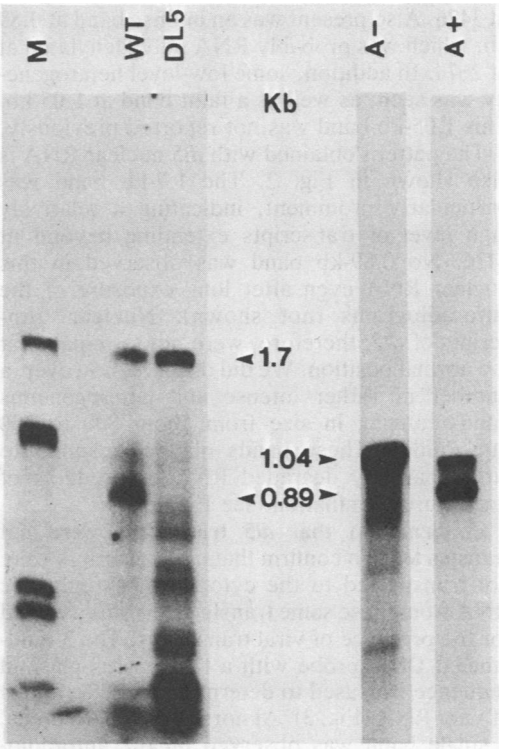

FIG. 2. 3'-end analysis of *dl5* and WT SV40 nuclear transcripts. The fused sequence probe was used for both the control WT SV40- and *dl5*-transfected total nuclear RNAs. The probe for 3' analysis of polyadenylated (A+) and non-polyadenylated (A−) WT nuclear RNAs was the *Eco*RI-*Hin*fI SV40 DNA fragment labeled by 3' fill-in at the *Eco*RI site. Shown is a 12-h exposure of the autoradiogram. Hybridization conditions were those described in the legend to Fig. 1.

the background protection of the probe, probably owing to hybridization with RNA having 3′ termini extending beyond the *Hin*fI site at nt 2824. In addition to this band, some heterogeneity of the 3′ ends was apparent, seen as a smearing down of the autoradiographic signal from the full length of the probe.

For the 3′-end mapping of *dl5* nuclear RNA, a different probe was used. A unit-length SV40 genome cloned into pBR322 at the *Hin*dIII site (SV40 nt 3476) was linearized and labeled at the *Eco*RI site (fused sequence probe). Because this probe has a fusion of virus and plasmid sequences at the *Hin*dIII site, any viral transcripts with 3′ termini beyond this point will yield nuclease S1 cleavage of the probe at this site. Therefore, all transcripts extending beyond nt 3476 will yield one band at 1.7 kb, allowing simple quantitation of this variable group of RNAs without a probe background. Using WT SV40-transfected total nuclear RNA, we observed the expected pattern. A prominent band at 1.7 kb was present, probably the result of non-polyadenylated nuclear RNA extending beyond nt 3476. Also present was an intense band at 0.89 kb, which was probably RNA polyadenylated at nt 2674. In addition, some low-level heterogeneity was seen, as well as a faint band at 1.05 kb. This 1.05-kb band was not reported previously.

The pattern obtained with *dl5* nuclear RNA is also shown in Fig. 2. The 1.7-kb band was particularly prominent, indicating a relatively high level of transcripts extending beyond nt 3476. No 0.89-kb band was observed in this nuclear RNA even after long exposure of the autoradiograms (not shown). Nuclear transcripts of *dl5*, therefore, were not terminated at the normal position. We did observe, however, a number of rather intense and heterogeneous bands ranging in size from about 800 to 200 nucleotides. These bands may correspond to either partially degraded RNA or products of premature termination (see Fig. 6).

Confirmation that *dl5* transcripts were not transported. To confirm that *dl5* transcripts were not transported to the cytoplasm, cytoplasmic RNA from these same transfections was assayed for the presence of viral transcripts. The 3′-end-labeled DNA probe with a fused virus-plasmid sequence was used to determine the 3′ terminus of viral RNA (Fig. 3). At normal exposure times, a single band was observed on the autoradiograms of WT-transfected cytoplasm. This 0.89-kb band derived from RNA polyadenylated at the normal position (nt 2674). Note that no detectable RNA with 3′ termini beyond nt 3476 was present in the WT-transfected cytoplasm. This was true even after long exposure of the autoradiograms. Because this RNA was not selected on oligodeoxythymidylate-cellulose,

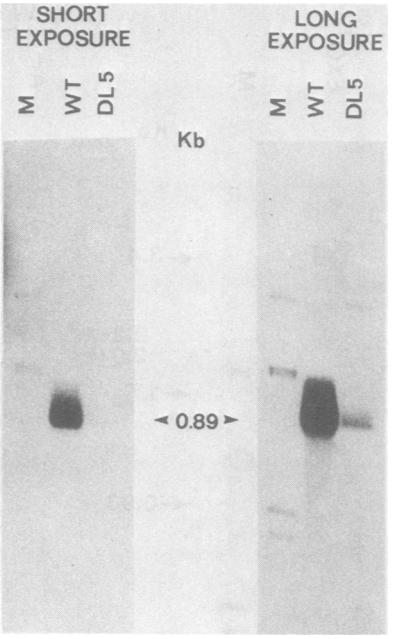

FIG. 3. 3′-end analysis of *dl5* and WT SV40 cytoplasmic RNA. The fused sequence probe was used. Hybridization conditions were those described in the legend to Fig. 1. RNAs are from matched transfected cytoplasms. Short exposure, 12 h; long exposure, 4 days.

both polyadenylated and non-polyadenylated populations of cytoplasmic RNA were represented. Both of these RNA populations were therefore terminated exclusively at nt 2674. The pattern of cytoplasmic transcripts from *dl5*-transfected cells is also shown in Fig. 3. At normal exposures, no *dl5* cytoplasmic RNA was detected, confirming the transport-deficient phenotype of *dl5*. At longer exposure times a curious observation was made: molecules with 3′ ends at nt 2674 became detectable. The level of this band corresponded to about 1% of the WT SV40 control levels. At this exposure sensitivity, no nuclear RNAs with 3′ ends at nt 2674 were detected (not shown). Quantitation of template DNA by Southern blot analysis established that *dl5* DNA replicated to within 50% of that of WT SV40 DNA (not shown).

Blot analysis of nuclear RNA. Because the nuclear RNA seen after *dl5* transfection did not terminate at the normal polyadenylation position (nt 2674), it was necessary to establish that *dl5* nuclear transcripts were not polyadenylated at unusual sites. A blot analysis was therefore performed with polyadenylated and non-polyadenylated nuclear RNAs present after *dl5* transfection, and these RNAs were analyzed

with a late region hybridization probe of SV40 DNA (Fig. 4). The control blot analysis of cytoplasmic RNA confirmed that *dl5* was a transport-deficient mutant, as little *dl5*-specific cytoplasmic RNA was present. As was observed with our 3'-end analysis, after long exposure times some *dl5* cytoplasmic RNA was eventually seen. This RNA appeared to be a 16S species and was present at about 1% of the level found in the control pattern. In the nucleus, no *dl5*-specific polyadenylated RNA was detected, even though *dl5*-specific non-polyadenylated nuclear RNA was. This result was consistent with our previous observation and established that *dl5* nuclear transcripts are not polyadenylated.

Inhibition of polyadenylation does not lead to accumulation of 3'-truncated nuclear RNA. Because much of the RNA present in *dl5*-transfected nuclei was 3' heterogeneous and truncated, it seemed possible that this pattern might be an indirect consequence of the inhibition of polyadenylation. To test this possibility, we treated infected cells with 50 μg of cordycepin per ml as described by others (10). At various times after drug addition, nuclear and cytoplasmic RNAs were extracted and analyzed by the nuclease S1 assay for the distribution of 3' ends (Fig. 5). The effect of cordycepin treatment on cytoplasmic RNA was not dramatic. The observed band at 0.89 kb did not change qualitatively during drug treatment; there was, however, a noticeable decrease in the intensity of the band after 4 h. During this treatment, nuclear RNA showed a clear accumulation of the 1.7-kb band and a corresponding decrease in the 0.89-kb band. This pattern was consistent with an accumulation within the nucleus of non-polyadenylated RNA extending beyond this *Hin*dIII site (nt 3476). This observation was also consistent with previous reports by others that cordycepin causes nuclear accumulation of non-polyadenylated RNA (27). Truncated or heterogeneous 3' ends of RNA did not accumulate during drug treatment; this pattern did not, therefore, resemble that observed with *dl5* nuclear RNA. This drug-induced accumulation of non-polyadenylated, nonterminated nuclear RNA did not continue with longer drug treatment. After 24 h of drug treatment, the nuclear RNA became barely detectable (not shown), and the cells showed significant cytopathology.

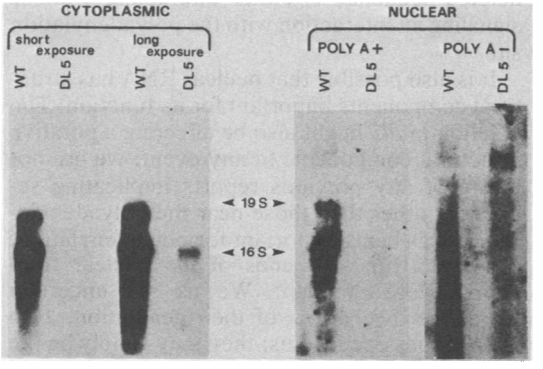

FIG. 4. Blot transfer analysis of nuclear and cytoplasmic RNAs. Polyadenylated cytoplasmic RNA was also analyzed. Cytoplasmic autoradiogram: short exposure, 24 h; long exposure, 7 days. Both the polyadenylated (polyA+) and non-polyadenylated (polyA−) nuclear RNA autoradiograms were exposed for 7 days.

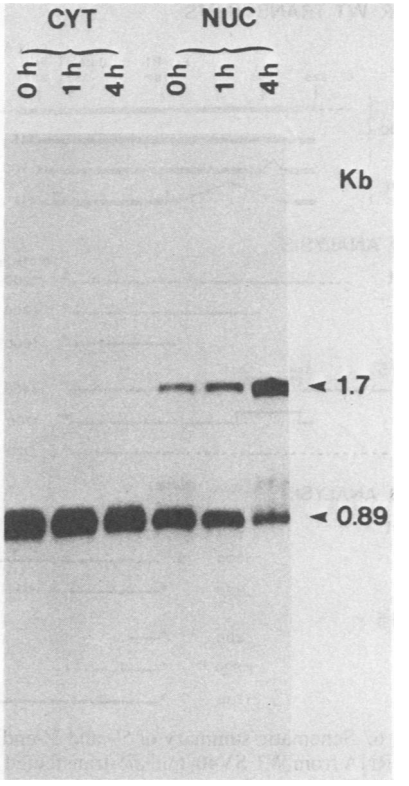

FIG. 5. 3'-end analysis of RNA from SV40-infected cells treated with cordycepin for the times indicated. Nuclear (nuc) and cytoplasmic (cyt) RNAs were analyzed with the fused sequence probe. Shown is a 6-h exposure of the autoradiograms.

DISCUSSION

The rationale of these experiments was to examine in detail the structure of nuclear RNA from a late region deletion mutant of SV40 (*dl5*) deficient in the transport of RNA. It was envisioned that this transport-deficient RNA might display some structural aberration that would correlate with its inability to transport. We analyzed the structure of this RNA, and a schematic summary of this analysis is shown in Fig. 6. We

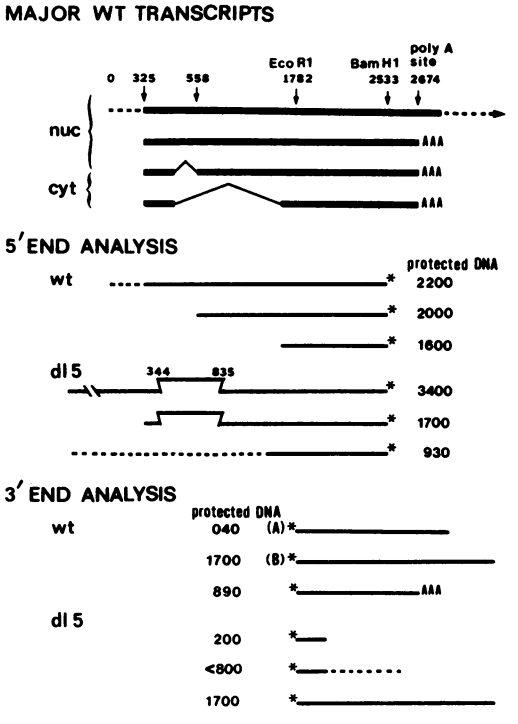

FIG. 6. Schematic summary of 5'- and 3'-end analyses of RNA from WT SV40- and *dl5*-transfected cells. Asterisks, Positions of the end-labeled probe. Dashed lines, Heterogeneous end distribution. The positions and sizes of the deletions in *dl5* are shown by the raised portions of the lines in the 5'-end analysis panel. *dl5* has a deletion of the two unique *HhaI* sites.

determined that nuclear RNA can be readily detected after *dl5* transfection. This RNA had a prominent 5' terminus at the usual position (nt 320). In addition, two other prominent 5' termini at nt 1600 and 4300 were also present. These ends are not normally abundant in WT SV40 nuclear RNA, indicating that some perturbation in 5'-end distribution had occurred. The end at nt 4300 was particularly puzzling. Because the promoter for the late region is at nt 0/5243, a 5' end at nt 4300 would be nearly 1,000 nucleotides upstream of the promoter. It is probable, therefore, that this 5' end was derived from transcripts greater than one unit length of SV40 and that degradation or 5' processing generated the nt 4200 end. The possibility that promotion occurred directly at nt 4300 has not been disproven, however.

In addition to these prominent 5' ends, there was also a substantial amount of 5' heterogeneity. This heterogeneity was not only quantitatively greater than that of the WT, but it also represented a greater diversity of SV40 sequences spanning the region from nt 1600 to well into the early region. In the WT SV40 nuclear RNA, 5' heterogeneity was confined to the area between nt 0 and 320. Because the DNA sequences for the viral promoter and major 5' end were intact and the observed 5' heterogeneity continued well upstream of the promoter, it seemed likely that much of this 5' heterogeneity also originated from a degradation process. This is especially likely in view of the non-polyadenylated nature of this RNA.

As *dl5* has a deletion of a splice junction, it was expected that the nuclear transcripts would not be spliced. Our analysis showed that little if any splicing occurred, confirming this expectation. The surprising observation was the result of the 3'-end analysis. It is generally thought that the polyadenylation event proceeds the splicing event, because unspliced but polyadenylated nuclear RNA is often observed in a number of different genes, including those of SV40 (21). We expected that the 3' ends of *dl5* transcripts would have normal levels of polyadenylation. What was observed, however, was that none of the *dl5* nuclear transcripts were terminated or polyadenylated at the normal position (nt 2674). Instead, the 3' ends of this RNA were either well beyond this terminus or truncated and located before it. Those 3' ends downstream of the normal terminus probably represent read-through by RNA polymerase. Kinetic studies of RNA synthesis imply that this read-through is a normal process for mRNA synthesis (14). It would therefore be expected that these molecules would function as substrate for subsequent cleavage and polyadenylation. That this did not occur implies that a more complicated process is occurring and that the context of the polyadenylation signal may be important. How the deletion of an intron junction region might perturb this context is not clear. The implication is that the deleted sequence is either interacting with or signaling an interaction with the polyadenylation site.

It is also possible that nuclear RNA has structural components important for its function. The deletion in *dl5* might also be affecting a putative structural component. In any event, we are not aware of any previous reports implicating sequences other than those near the polyadenylation site as being important for polyadenylation.

The 3' truncated ends of *dl5* nuclear transcripts were abundant. We are still uncertain about the mechanism of their generation. Two possibilities occur to us: they may simply be the product of degraded nuclear RNA, or they could derive from premature termination. Our steady-state analysis did not allow us to distinguish between these possibilities.

It seemed possible that the occurrence of these 3'-truncated ends was an indirect consequence of the inability to polyadenylate *dl5*

transcripts. Because non-polyadenylated nuclear RNA is thought to have a very short half-life (5 to 10 min [22]), prevention of polyadenylation would allow the accumulation of RNA degradation products. Cordycepin is known to inhibit polyadenylation without inhibiting RNA synthesis (10), thus allowing nuclear accumulation of non-polyadenylated RNA (27). If the 3'-truncated *dl5* transcripts were a consequence of non-polyadenylation, then the nuclear RNA of cordycepin-treated WT SV40-infected cells should also be 3' truncated. This was not observed, even though transient accumulation of non-polyadenylated nuclear RNA was seen.

dl5 is a splice junction deletion mutant that does not transport RNA. Deletions of splice junctions, however, are not uniformly transport deficient (7). The observation that *dl5* transcripts were not polyadenylated implies that the lack of transport is instead directly related to non-polyadenylation rather than to a splicing deficiency. Several observations were consistent with this idea. Even though unspliced SV40 RNA is predominantly nuclear, small amounts can be found in the cytoplasm (29). Our 3'-end analyses however, showed that only the ends at nt 2674 were detected in the cytoplasm; no read-through, truncated, or heterogeneous 3' ends were detected in the cytoplasm. There is, therefore, a better correlation between polyadenylation and transport than between splicing and transport. In addition, the transport phenotype of *dl5* was not absolute. Transcripts of *dl5* were transported at about 1% of the control levels. The mutant transcripts that were transported, however, all had 3' termini at nt 2674, even though this terminus could not be detected in the corresponding nuclear RNA. This implies that once polyadenylation has occurred, these transcripts are quickly transported into the cytoplasm. A rapid transport of polyadenylated nuclear RNA is consistent with kinetic measurements by others (22) of the half-life of polyadenylated and non-polyadenylated nuclear RNA.

ACKNOWLEDGMENTS

We thank Susan Carr for excellent technical assistance. We also thank Roberta Clugston for typing the manuscript.

This work was supported by Public Health Service grant CA-25819 from the National Cancer Institute. L.P.V. was also supported by a Rita Allen Scholarship.

LITERATURE CITED

1. **Alwine, J. C., D. C. Kemp, B. A. Parker, J. Riser, J. Renart, G. R. Stark, and G. M. Wahl.** 1979. Detection of specific RNAs or specific fragments of DNA by fractionation in gels and transfer to diazobenzyloxymethyl paper. Methods Enzymol. **68**:220–242.
2. **Aviv, H., and P. Leader.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidilic acid-cellulose. Proc. Natl. Acad. Sci. U.S.A. **69**:1408–1412.
3. **Berk, A. J., and P. A. Sharp.** 1978. Spliced early mRNAs of simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **75**:1274–1278.
4. **Berkner, K. L., and W. R. Folk.** 1977. Polynucleotide exchange reaction. J. Biol. Chem. **252**:3176–3184.
5. **Bolivan, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Hayneker, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
6. **Buchman, A. R., L. Burnett, and P. Berg.** 1980. The SV40 nucleotide sequence, p. 799–829. *In* J. Tooze (ed.), DNA tumor viruses; molecular biology of tumor viruses (2nd ed.), part 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Campos, R, and L. P. Villarreal.** 1982. An SV40 deletion mutant accumulates late transcripts in a paranuclear extract. Virology **119**:1–11.
8. **Carlock, L., and N. C. Jones.** 1981. Synthesis of an unspliced cytoplasmic message by an adenovirus 5 deletion mutant. Nature (London) **294**:572–574.
9. **Darnell, J. E.** 1979. Transcription units for mRNA production in eukaryotic cells and their DNA viruses. Prog. Nucleic Acid Res. Mol. Biol. **22**:327–353.
10. **Darnell, J. E., L. Philipson, and R. Wall.** 1971. Polyadenylic acid sequences: role in conversion of nuclear RNA into message RNA. Science **174**:507–510.
11. **Dorwin, J.** 1980. Cloning of human mitochondrial DNA in Escherichia coli. J. Mol. Biol. **140**:15–21.
12. **Dunnick, W., T. H. Rabbits, and C. Milstein.** 1980. An immunoglobulin deletion mutant with implications for the heavy-chain switch of RNA splicing. Nature (London) **286**:669–675.
13. **Esumi, H., Y. Takahashi, T. Sekiya, S. Sato, S. Nagase, and T. Sugimura.** 1982. Presence of albumin mRNA precursors in nuclei of analbumic rat livers lacking cytoplasmic mRNA. Proc. Natl. Acad. Sci. U.S.A. **79**:734–738.
14. **Ford, J., and M.-T. Hsu.** 1978. Transcription pattern of in vivo-labeled late simian virus 40 RNA: equimolar transcription beyond the mRNA 3' terminus. J. Virol. **28**:795–801.
15. **Ghosh, P. K., P. Roy, A. Barker, J. E. Mertz, S. M. Weissman, and P. Lebowitz.** 1981. Unspoiled functional late mRNAs containing intervening sequence are produced by a late leader mutant of simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **78**:1386–1390.
16. **Gilmore-Herbert, M., and R. Wall.** 1979. Nuclear RNA precursors in the processing pathway to MOPC 21K light chain messenger RNA. J. Mol. Biol. **139**:870–891.
17. **Graham, F. L., and A. J. Van Der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus DNA. Virology **52**:456–467.
18. **Gruss, P., C.-J. Lai, R. Dhar, and G. Khoury.** 1979. Splicing as a requirement for the biogenesis of functional 16S mRNA of simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **76**:4317–4321.
19. **Hamer, D. H., and P. Leder.** 1979. Splicing and the formation of stable 16S mRNA. Cell **18**:1299–1304.
20. **Kanton, J. A., P. H. Turner, and A. W. Neinhuis.** 1980. Beta thalassemia: mutations which affect processing of β-globin mRNA precursors. Cell **21**:149–257.
21. **Lai, C., R. Dhar, and G. Khoury.** 1978. Mapping of spliced and unspliced late lytic SV40 RNAs. Cell **14**:971–982.
22. **Levis, R., and S. Penman.** 1977. The metabolism of poly(A)$^+$ and poly(A)$^-$ hnRNA in cultured drosophila cells studied with a rapid uridine pulse-chase. Cell **11**:105–113.
23. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage-DNA interaction. J. Mol. Biol. **53**:159–162.
24. **McDonell, M. W., N. N. Simon, and F. W. Studier.** 1977. Analysis for restriction fragments of T7 DNA and determination of molecular weights by electrophoresis in neutral and alkaline gels. J. Mol. Biol. **110**:119–146.
25. **Mertz, J., and P. Berg.** 1974. Defective simian virus 40

genomes: isolation and growth of individual clones. Virology 62:112–129.
26. **Parker, B. A., and G. R. Stark.** 1979. Regulation of simian virus 40 transcription: sensitive analysis of the RNA species present early in infections by virus and viral DNA. J. Virol. 31:360–369.
27. **Rose, K. M., T. B. Leonard, and T. H. Carter.** 1982. Effects of adenine nucleosides on RNA synthesis in adenovirus infected cells. Mol. Pharm. 22:517–523.
28. **Villarreal, L. P., and S. Carr.** 1982. Genetic test for involvement of intervening sequences in transport of nuclear RNA. Mol. Cell. Biol. 2:1550–1557.
29. **Villarreal, L. P., and S. Jovanovich.** 1980. Leakage of nuclear transcripts late in simian virus 40-infected cells: quantitation of spliced and unspliced late 19S RNA. J. Virol. 36:595–600.
30. **White, R. T., P. Berg, and L. P. Villarreal.** 1982. Simian virus 40-rabbit β-globin recombinants lacking late mRNA splice sites express RNAs with altered structures. J. Virol. 42:262–274.
31. **Wold, B. J., W. H. Klein, B. R. Hough-Evans, R. J. Britten, and E. R. Davidson.** 1978. Sea urchin embryo mRNA sequences expressed in the nuclear RNA of adult tissue. Cell 14:941–950.
32. **Ziff, E. B.** 1980. Transcription and RNA processing by the DNA tumor viruses. Nature (London) 287:491–499.