JOURNAL OF VIROLOGY, Apr. 1981, p. 256-262
0022-538X/81/040256-07$02.00/0

Vol. 38, No. 1

In Vivo Transcription of the 5'-Terminal Extracistronic Region of Vesicular Stomatitis Virus RNA

MANFRED SCHUBERT* AND ROBERT A. LAZZARINI

Laboratory of Molecular Genetics, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20205

Received 23 October 1980/Accepted 12 January 1981

In vivo transcription and polyadenylation at the junction of the L cistron and the 5'-terminal extracistronic region of vesicular stomatitis virus RNA was investigated. Annealing of 5' ^{32}P-labeled RNA representing the 5'-terminal noncoding 77 nucleotides of vesicular stomatitis virus genomic RNA to L gene mRNA resulted in specific duplex formation. Two specific RNase T_1- and RNase A-resistant duplexes, 66 and 77 nucleotides long, bound to oligodeoxythymidylic acid cellulose. The specific sizes of the duplexes and their selection by oligodeoxythymidylic acid cellulose chromatography demonstrated that they were covalently linked to the polyadenylic acid tail of L gene mRNA. These data strongly suggest that the viral polymerase polyadenylates L gene mRNA in vivo by using the stretch of seven uridine residues at the end of the L cistron and that the polymerase can resume transcribing the 5'-terminal extracistronic region, resulting in a covalent linkage of the transcript to the polyadenylic acid tail of L gene mRNA.

The antimessage-sense genomic RNA of vesicular stomatitis virus (VSV) is sequentially transcribed into five monocistronic mRNA's. The molar amount of each mRNA synthesized decreases with its distance from the 3' terminus of the genome in the order of 3' N-NS-M-G-L 5' (1, 4). The messages are modified and carry methylated cap structures (2, 3, 22) and polyadenylic acid [poly(A)] tails (6, 35).

The search for potential intermediates of transcription, such as triphosphate-bearing messages (13, 24, 27, 34; R. A. Lazzarini, I. Chien, and J. D. Keene, submitted for publication) or covalently linked polycistronic messages (14, 15), and the study of the capping reaction (2, 12) have led to two apparently mutually exclusive models for the mechanism of transcription.

The proposed models suggest either that messages are initiated and terminated at the beginning and end of each cistron (stop-start model) or that they are cleaved from a polycistronic transcript (processing model) (5). Both models can accommodate the single entry site for the viral polymerase at the 3' terminus of the genome suggested by the UV inactivation studies (1, 4). Thus, in both models, transcription begins with synthesis of leader RNA (7-9) which is encoded by the 3'-terminal extracistronic region of the VSV genome (16, 21, 26).

In this communication, we report about in vivo transcription at the junction of the L cistron and the 5'-terminal extracistronic region. We have identified a unique 59-nucleotide-long transcript which is covalently linked to the poly(A) tail of L gene mRNA. This transcript represents an exact copy of the 5'-terminal extracistronic region of VSV genomic RNA. These data indicate that transcription can resume after polyadenylation of L gene mRNA by the viral polymerase, using seven uridine residues as the template for polyadenylation at the end of the L protein cistron.

MATERIALS AND METHODS

Virus growth and RNA purification. The two defective interfering (DI) particles DI-LT_2 and DI-011 were derived from the heat-resistant strain and the Mudd-Summers strain, respectively, of VSV (Indiana). For the isolation of in vivo mRNA's, VSV Mudd-Summers strain was used. Propagation of the particles and the extraction of the RNAs have been described earlier (17, 19, 23).

Isolation of 5'-terminally labeled 77'mer RNA. After removal of the terminal phosphates with calf intestinal phosphatase, the 5' terminus of DI-LT_2 RNA was labeled with [γ-^{32}P]ATP and polynucleotide kinase as described earlier (29). [γ-^{32}P]ATP was removed by gel filtration through Sephadex G-150. The RNA was then self-annealed, and the single-stranded portion was digested with RNase T_1 followed by the inactivation of the RNase with proteinase K (31). The 77'mer/73'mer RNA duplex (J. D. Keene, R. A. Lazzarini, and I. Chien, Proc. Natl. Acad. Sci. U.S.A., in press) which survived the RNase digestion was heated for 1 min at 100°C, and the 5'-terminally labeled 77'mer RNA was separated from the unlabeled 73'mer

RNA by electrophoresis on a 12% polyacrylamide gel.

Isolation of in vivo mRNA's. BHK-21 cells were grown in suspension, and infection of 3×10^8 cells was carried out at a multiplicity of 10, using VSV Mudd-Summers strain as previously described (6). At 4 h postinfection, the cells were lysed, and the nuclei were removed. The cell sap was layered onto two parfait gradients consisting of an 8-ml linear 20 to 40% (wt/wt) CsCl gradient and a 2.5-ml hyperphase of 5% sucrose in 10 mM Tris-hydrochloride (pH 7.5)-1 mM EDTA (ET buffer) (18). After centrifugation in a Beckman SW41 rotor for 17 h at 33,000 rpm and 4°C, the RNA pellet was dissolved in ET buffer containing 0.5% sodium dodecyl sulfate and applied to two 10 to 30% sucrose gradients in the same buffer. Centrifugation was in an SW27 rotor for 16 h at 22,000 rpm and room temperature. Fractions (1 ml) of the gradients were collected, portions were trichloroacetic acid precipitated to measure the [^{3}H]uridine label, and the absorbance at 260 nm of each fraction was determined. Appropriate fractions were pooled, ethanol precipitated, and extracted with phenol and chloroform. After ethanol precipitation, the RNAs were stored in water.

Annealing of the 77'mer RNA. RNAs of the pooled sucrose gradient fractions corresponding to the amount from 10^7 infected BHK-21 cells were annealed for 4 h at 60°C to approximately 5×10^{-3} pmol of 5'-terminally labeled 77'mer RNA in 20 μl of 10 mM Na_2HPO_4-NaH_2PO_4 (pH 6.8)-0.4 M NaCl in the presence of 20 to 40 μg of rRNA or tRNA or both. Single-stranded RNA was completely digested in 20 min at 37°C with 60 U of RNase T_1 per mg of RNA. The RNase was inactivated by the addition of proteinase K (100 μg/ml) and incubation for 15 min at 37°C. With proteinase K as a carrier, the RNase-resistant duplexes were precipitated with ethanol. The pellets were washed with 80% ethanol, dried in vacuo, dissolved in 4 M urea, heated for 1 min at 100°C, and directly applied to a 12% polyacrylamide gel.

Selection of the RNA duplexes. VSV L mRNA was selected after denaturation in 80% dimethyl sulfoxide by oligodeoxythymidylic acid [oligo(dT)] cellulose as described previously (28). The poly(A)-containing fraction was annealed to 5'-terminally labeled 77'mer RNA as outlined above. Single-stranded portions other than poly(A) were digested for 20 min at 37°C with 10 μg of RNase A per mg of RNA and 2 U of RNase T_1 per mg of RNA. The RNases were inactivated with proteinase K, and the reaction mixture was passaged three times over oligo(dT) cellulose. Bound and unbound materials were pooled and precipitated.

Partial hydrolysis conditions. The 5'-terminally labeled RNAs were hydrolyzed either in formamide for 60 min at 100°C (33) or in a Na_2CO_3-$NaHCO_3$ buffer, pH 9, for 30 min at 90°C (10).

Separation of the RNAs. The RNAs were separated on 20 or 12% polyacrylamide gels as described previously (10).

RESULTS

It was first shown by Herman and associates (14), using electron microscopy, that polycistronic VSV mRNA's containing intervening poly(A) sequences are produced in VSV-infected cells and during in vitro transcription. We have recently demonstrated (15) by direct RNA sequencing that these intervening poly(A) sequences are extended at their 3' end by a faithful transcript of the two intercistronic nucleotides (20, 25) followed by sequences which correspond to the 5' terminus of the next mRNA.

In this communication, we report on transcription of the 5'-terminal region of the VSV genome, a region that includes both the end of the L gene and the terminal extracistronic sequences. The L protein cistron ends at position 60 from the 5' terminus of VSV genomic RNA with a stretch of seven uridine residues and is followed by an extracistronic region of 59 nucleotides (28). If transcription is resumed after polyadenylation of L gene mRNA, some of the L messages should bear a copy of this extracistronic region at their poly(A) tails. To test for this possibility, we have isolated a specific 77'mer RNA probe which is identical to the last 77 5'-terminal nucleotides of VSV genomic RNA and should be complementary to the postulated transcript. The probe was isolated from a DI particle RNA, DI-LT_2 RNA, which recently was shown to have termini that were complementary for 70 nucleotides (Keene et al., in press). Sequencing of the terminal regions revealed that the first non-base-paired guanosine residues after the complementary region are in position 77 from the 5' end and in position 73 from the 3' end (Keene et al., in press). Consequently, panhandles isolated by RNase T_1 digestion of the DI-LT_2 RNA would contain a 77-nucleotide chain containing the original 5' terminus and spanning the junction of the end of the L protein cistron with the extracistronic region.

After removal of the 5'-terminal phosphates DI-LT_2 RNA was labeled with [γ-^{32}P]ATP and polynucleotide kinase. The RNA was then self-annealed, and the single-stranded portion was completely digested with RNase T_1. RNase T_1 was inactivated by the addition of proteinase K. The RNase-resistant duplex was heat denatured, and the unlabeled 73'mer was separated from the labeled 77'mer by polyacrylamide gel electrophoresis (Fig. 1, lane 1). The marked RNA band running as an approximately 80-nucleotide-long single-stranded RNA was excised and eluted from the gel slice.

Figure 1, lanes 3 and 5, shows partial formamide hydrolysates of the same RNA separated next to those of DI-011 RNA (lanes 2 and 4). DI-011 RNA was also labeled at its 5' terminus, and we have shown earlier that its 5' end is conserved from VSV RNA for approximately 900 nucleotides (29). The ladder-like patterns of the hydrolysates demonstrate that the RNA in lane 1

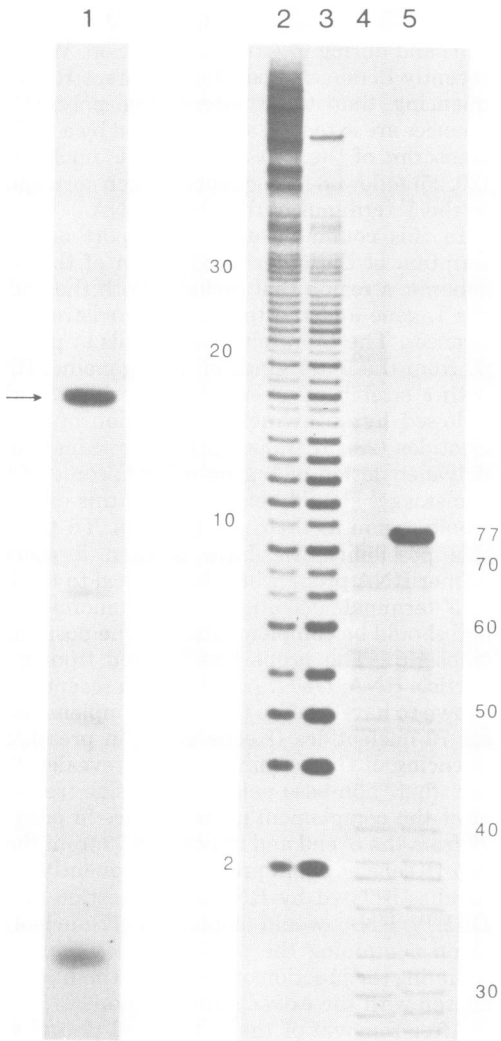

FIG. 1. *Isolation and characterization of 5'-terminally labeled 77'mer RNA. DI-LT$_2$ RNA (lane 1) was labeled at its 5' terminus with [γ-^{32}P]ATP and polynucleotide kinase and self-annealed. After digestion with RNase T$_1$ and inactivation of the RNase, the resistant duplex formed by the complementary terminal regions of the RNA was denatured and separated on a 12% polyacrylamide gel. The marked RNA was eluted from the gel slice. The 5'-terminally labeled DI-011 RNA (lanes 2 and 4) and the RNA from lane 1 (lanes 3 and 5) were partially hydrolyzed in formamide (33) and separated on a 20% polyacrylamide gel by using two different times of application (first, lanes 4 and 5; second, lanes 2 and 3). The lengths (nucleotides) of the RNA fragments are as indicated.*

was, as expected, exactly 77 nucleotides long and that all of its RNA fragments comigrated with those of DI-011 RNA. Positions of preferred hydrolysis at XpA bonds are also identical in both RNAs. These patterns are characteristic for each individual RNA species, and the correspondence between the 77'mer and DI-011 RNA patterns identifies the 77'mer as the 5'-terminal region of VSV genomic RNA. The sequence was also confirmed by direct RNA sequencing (data not shown; J. D. Keene, personal communication).

Annealing of the 77'mer RNA to VSV mRNA's. VSV in vivo mRNA's were isolated by pelleting through a CsCl gradient as described earlier (18), a process which separates them from the nucleocapsid template. The RNAs in the pellet were dissolved and applied to a 10 to 30% sucrose gradient (Fig. 2A). Indicated fractions were pooled and phenol extracted, and equal portions corresponding to RNA from approximately 10^7 VSV-infected BHK cells were annealed to the 77'mer RNA. Since 28S and 18S cellular rRNA's were present in some fractions, the RNA concentration in each annealing reaction was almost equalized by the addition of tRNA. After complete digestion with RNase T$_1$ followed by the inactivation of the RNase with proteinase K, the resistant duplexes were heat denatured and separated on a polyacrylamide gel next to partial hydrolysates of 5'-terminally labeled DI-011 RNA and 77'mer RNA (Fig. 2B). As can be seen from the autoradiogram, annealings of the 77'mer RNA occurred preferentially with RNAs from pools c and d (fractions 7 to 12), which sedimented at 28S and were the size of L gene mRNA. In addition, the size of the RNA probe remained unchanged after complete RNase T$_1$ digestion, indicating that all guanine residues, including position 59 of the 77'mer (see Fig. 4), were base paired, possibly with sequences present in L gene mRNA or associated with the L message. Because of the removal of the complementary plus strand of the DI-LT$_2$ panhandle during the isolation of the 77'mer RNA, self-annealing of the 77'mer RNA was not observed (data not shown).

To determine whether duplexes were formed with L message and not with RNAs associated with L message, pools c and d were combined, and the RNAs were selected after denaturation in 80% dimethyl sulfoxide for poly(A) sequences with oligo(dT) cellulose (28). The poly(A)-containing fraction was heated and annealed to the 77'mer RNA. Single-stranded RNA was digested with RNase T$_1$ and RNase A, the RNases inactivated by proteinase K, and the resistant duplexes were applied to oligo(dT) cellulose. The bound material (E) and the flow through fraction (FT) were heat denatured and separated on

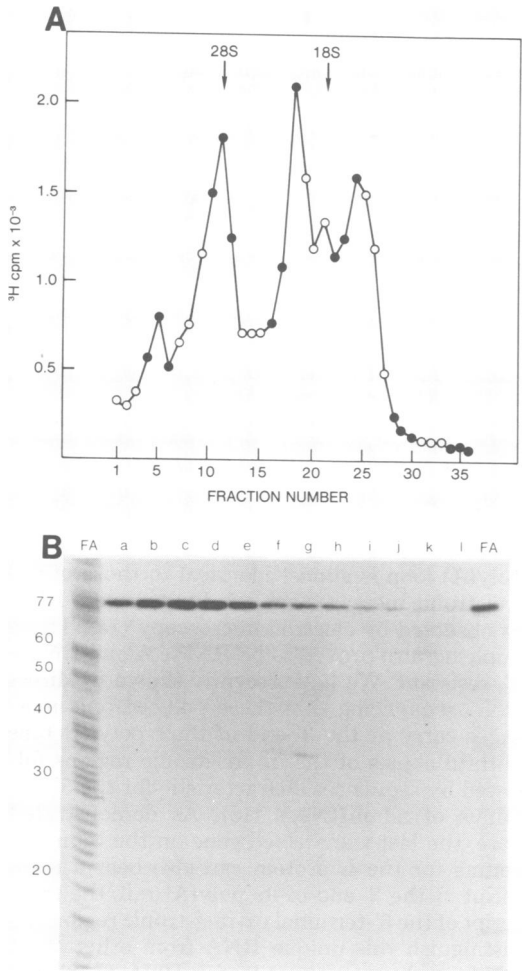

FIG. 2. *Annealing of 77'mer RNA to in vivo RNA's. Nucleocapsid-free in vivo mRNA's were isolated as described in the text and separated on a 10 to 30% sucrose gradient (A). The counts per minute for trichloroacetic acid-precipitable [^{3}H]uridine was determined for portions of each fraction. The arrows mark the positions of the host rRNA as determined by spectrophotometry at 260 nm. Adjacent fractions (○ and ●) were pooled and used in the annealing reaction with 5'-terminally labeled 77'mer RNA (B). RNase T_1-resistant duplexes formed with the RNAs of the pooled fractions (a to l) were denatured and separated on a 12% polyacrylamide gel next to partial hydrolysates of 5'-terminally labeled DI-011 (FA, left) and 77'mer RNA (FA, right). The sizes (nucleotides) of the RNA fragments are as indicated.*

a polyacrylamide gel next to a partial hydrolysate of the 77'mer RNA (Fig. 3). Virtually all of the duplexed RNA bound to oligo(dT) cellulose and migrated after heat denaturation as 77'mer and 66'mer RNAs with decreasing amounts of minor RNA bands in the order of 65, 64, 63, 62,

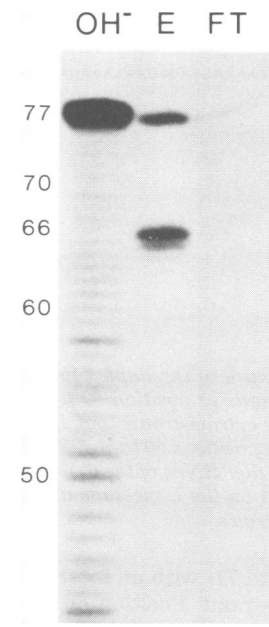

FIG. 3. *Oligo(dT) selection of the duplexes formed with L mRNA. The 5'-terminally labeled 77'mer RNA was annealed to poly(A)-selected in vivo L mRNA. After RNase T_1 and RNase A digestion and degradation of the RNases with proteinase K, the reaction mixture was passaged through an oligo(dT) cellulose column. The bound (E) and the unbound (FT) materials were heat denatured and separated next to a partial alkaline hydrolysate of 77'mer RNA on a 12% polyacrylamide gel. Sizes (nucleotides) are as indicated.*

and 61.

Since a mixture of RNase T_1 and RNase A was employed after annealing, all duplexes were covalently linked to poly(A) without any intervening residues (28). Any intervening nucleotides (other than adenine) would have led to a cleavage between the poly(A) and the duplex, and the specific selection of the duplex by oligo(dT) cellulose would not have been possible. In addition, the amount of 66'mer RNA could be favored over the amount of 77'mer RNA by increasing the RNase A concentration during the digestion (data not shown). This indicates that the 66'mer RNA duplex was generated at the expense of the 77'mer RNA duplex.

Since positions 67 to 77 of the 77'mer RNA are part of the L protein cistron (28) (Fig. 4), the duplex must have been formed with polyadenylated L mRNA. In addition, the conversion of 77'mer into 66'mer by increasing the RNase A concentration identifies the position of the poly (A) (Fig. 4). The RNA duplex appears to be a combination of a 66'mer duplex (positions 1 to 66 of the 77'mer RNA) and an 11'mer duplex

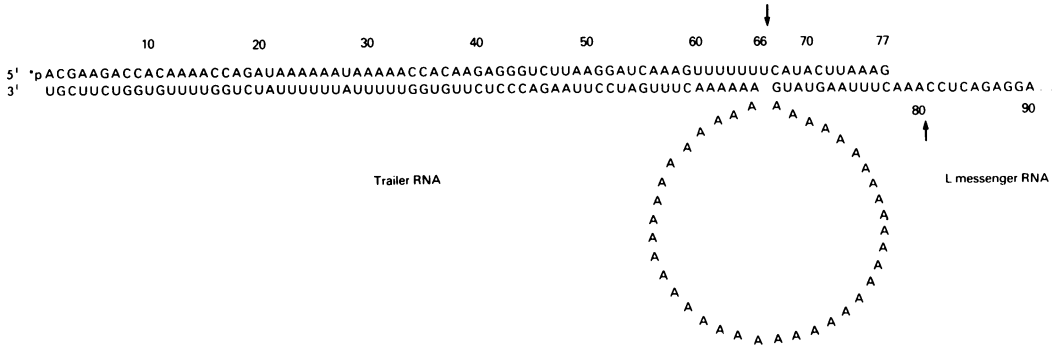

FIG. 4. *Structure of the duplex formed between 5'-terminally labeled 77'mer RNA and in vivo L mRNA. The L cistron ends at position 60 from the 5' terminus of VSV RNA, represented here by the 77'mer RNA, which spans 59 extracistronic and 18 intracistronic nucleotides. It also contains the polyadenylation site of the L gene from positions 60 to 65 (28). This short 77'mer RNA annealed specifically to L mRNA which carries a transcript (trailer RNA) of the 5'-terminal extracistronic region at the 3' end of its poly(A) tail. The cleavage site of RNase A on the L message and the more resistant cleavage site on the 77'mer RNA (position 66) are indicated by arrows.*

(positions 67 to 77) with an intervening poly(A) loop on one strand. Position 66 was held open due to steric constraints of the poly(A) loop (Fig. 4) and therefore was accessible to RNase A, although less sensitive to RNase A than single-stranded RNA.

The minor bands corresponding to the 65'mer to 61'mer duplexed RNAs suggest that the RNase A-sensitive site can be at various positions within the stretch of seven uridine residues, probably because the poly(A) loop can wander across the track of uridines; the longer 66'mer duplex, however, seems to be favored (Fig. 3). The data taken together demonstrate that L mRNA can carry a transcript of the 5'-terminal extracistronic region at its poly(A) tail.

DISCUSSION

In this communication, we have demonstrated that a specific 5'-terminally labeled RNA representing 77 nucleotides from the 5' end of VSV RNA anneals specifically to VSV in vivo L mRNA. The formed duplexes are RNase T_1 and RNase A resistant and bind to oligo(dT) cellulose. Therefore, no intervening nucleotides (other than adenine) link the duplexed RNA to poly(A). We have previously shown, using a similar method, that the L cistron ends at position 60 with a stretch of seven uridine residues (positions 60 to 66; Fig. 4), the site which specifies polyadenylation of L gene mRNA (28). The sizes of the recovered duplexes strongly suggest a duplex formation (Fig. 4). The duplex involves full-length 77'mer RNA with an RNase A-sensitive position at 66 due to the intervening poly(A) loop.

The structure of this duplex including the poly(A) loop is almost identical to those of polycistronic messages annealed to template RNA as observed by electron microscopy (14). These duplexes also proved to be RNase A and RNase T_1 resistant. We have recently shown by direct RNA sequencing that these polycistronic messages carry at the 3' end of their poly(A) tails faithful copies of the intercistronic regions followed by sequences characteristic for the 5' terminus of all mRNA's (15). As demonstrated here, the last transcribed gene on the template coding for the L protein can also bear a transcript at the 3' end of its poly(A) tail, the transcript of the 5'-terminal extracistronic region. To distinguish this unique RNA from other short RNA products such as leader RNA (7, 8) and the DI particle product (11, 30, 32), we refer to it as a "trailer" sequence since it appears to trail transcription of L gene mRNA.

The existence of the trailer sequence is important with respect to polyadenylation of L gene mRNA in vivo. The trailer sequence is specified by the viral genome and, therefore, like the L protein mRNA, is synthesized by the viral transcriptase. Consequently, the sequences that flank the polyadenylate at both ends are products of the transcriptase, an arrangement that strongly suggests that polyadenylation of L message results from chattering of the viral transcriptase at the end of the L gene, with the uridine stretch as a template.

To estimate the amount of linked trailer sequence, we annealed two minus-sense T_1 oligonucleotides from the L gene region to L message, the 18'mer positions 60 to 77 from the 5' terminus of VSV genomic RNA (28), and a 33'mer which is derived from a region approximately

800 nucleotides upstream from the L gene polyadenylation site (31) (data not shown). We compared the amounts of 18'mer and 33'mer RNAs which could be annealed to various amounts of L message to those of the 77'mer RNA. The frequency at which full-length trailer RNA linked to L message was found to be 1 to 3%. This corresponds to the frequency of poly(A)-linked polycistronic messages in in vitro transcripts (14, 15).

The significance of trailer RNA in respect to the mechanism of transcription (stop-start model versus processing model) remains open. One possibility is that trailer RNA is simply the product of an infrequent inaccuracy of the transcriptase, a failure to terminate transcription after polyadenylation of L message. If linked trailer RNA is a normal intermediate of transcription, it would have to be released from L message by a cleavage event since the vast majority of L messages do not carry a full-length trailer RNA. Further search for small RNAs which might represent processing products of the trailer RNA and their abundance relative to L message may give insight into whether or not trailer RNA is a normal intermediate of transcription.

LITERATURE CITED

1. **Abraham, G., and A. K. Banerjee.** 1976. Sequential transcription of the genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **73**:1504–1508.
2. **Abraham, G., D. P. Rhodes, and A. K. Banerjee.** 1975. The 5' terminal structure of the methylated mRNA synthesized *in vitro* by vesicular stomatitis virus. Cell **5**:51–58.
3. **Abraham, G., D. P. Rhodes, and A. K. Banerjee.** 1975. Novel initiation of RNA synthesis *in vitro* by vesicular stomatitis virus. Nature (London) **255**:37–40.
4. **Ball, L. A., and C. N. White.** 1976. Order of transcription of genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **73**:442–446.
5. **Banerjee, A. K., G. Abraham, and R. J. Colonno.** 1977. Vesicular stomatitis virus: mode of transcription. J. Gen. Virol. **34**:1–8.
6. **Banerjee, A. K., and D. P. Rhodes.** 1973. *In vitro* synthesis of RNA that contains polyadenylate by virion associated RNA polymerase of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **70**:3566–3570.
7. **Colonno, R. J., and A. K. Banerjee.** 1976. A unique RNA species involved in initiation of vesicular stomatitis virus RNA transcription *in vitro*. Cell **8**:197–204.
8. **Colonno, R. J., and A. K. Banerjee.** 1978. Complete nucleotide sequence of the leader RNA synthesized *in vitro* by vesicular stomatitis virus. Cell **15**:93–101.
9. **Colonno, R. J., R. A. Lazzarini, J. D. Keene, and A. K. Banerjee.** 1977. *In vitro* synthesis of messenger RNA by a defective interfering particle of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **74**:1884–1888.
10. **Donis-Keller, H., A. M. Maxam, and W. Gilbert.** 1977. Mapping adenines, guanines and pyrimidines in RNA. Nucleic Acids Res. **4**:2527–2538.
11. **Emerson, S. U., P. M. Dierks, and J. T. Parsons.** 1977. In vitro synthesis of a unique RNA species by a T particle of vesicular stomatitis virus. J. Virol. **23**:708–716.
12. **Gupta, K. C., and P. Roy.** 1980. Alternate capping mechanisms for transcription of spring viremia of carp virus: evidence for independent mRNA initiation. J. Virol. **33**: 292–303.
13. **Hefti, E., and D. H. L. Bishop.** 1976. The sequences of VSV *in vitro* transcription product RNA (± SAM). Biochem. Biophys. Res. Commun. **68**:393–400.
14. **Herman, R. C., S. Adler, R. A. Lazzarini, R. J. Colonno, A. K. Banerjee, and H. Westphal.** 1978. Intervening polyadenylate sequences in RNA transcripts of vesicular stomatitis virus. Cell **15**:587–596.
15. **Herman, R. C., M. Schubert, J. D. Keene, and R. A. Lazzarini.** 1980. Polycistronic vesicular stomatitis virus RNA transcripts. Proc. Natl. Acad. Sci. U.S.A. **77**: 4662–4665.
16. **Keene, J. D., M. Schubert, and R. A. Lazzarini.** 1980. Intervening sequence between the leader region and the nucleocapsid gene of vesicular stomatitis virus RNA. J. Virol. **33**:789–794.
17. **Keene, J. D., M. Schubert, R. A. Lazzarini, and M. Rosenberg.** 1978. Nucleotide sequence homology at the 3' termini of RNA from vesicular stomatitis virus and its defective interfering particles. Proc. Natl. Acad. Sci. U.S.A. **75**:3225–3229.
18. **Kolakofsky, D.** 1976. Isolation and characterization of Sendai virus DI RNAs. Cell **8**:547–555.
19. **Lazzarini, R. A., G. H. Weber, L. D. Johnson, and G. M. Stamminger.** 1975. Covalently linked message and anti-message (genomic) RNA from a defective vesicular stomatitis virus particle. J. Mol. Biol. **97**:289–307.
20. **McGeoch, D. J.** 1979. Structure of the gene N; gene NS intercistronic junction in the genome of VSV. Cell **17**: 673–681.
21. **McGeoch, D. J., and A. Dolan.** 1979. Sequence of 200 nucleotides at the 3'-terminus of the genome RNA of vesicular stomatitis virus. Nucleic Acids Res. **6**:3199–3211.
22. **Moyer, S. A., G. Abraham, R. Adler, and A. K. Banerjee.** 1975. Methylated and blocked 5' termini in vesicular stomatitis virus *in vivo* mRNAs. Cell **5**:59–67.
23. **Murphy, M. F., and R. A. Lazzarini.** 1974. Synthesis of viral mRNA and polyadenylate by a ribonucleoprotein complex from extracts of VSV-infected cells. Cell **3**:77–84.
24. **Rose, J. K.** 1975. Heterogeneous 5'-terminal structures occur on vesicular stomatitis virus mRNAs. J. Biol. Chem. **250**:8098–8104.
25. **Rose, J. K.** 1980. Complete intergenic and flanking gene sequences from the genome of vesicular stomatitis virus. Cell **19**:415–421.
26. **Rowlands, D. J.** 1979. Sequences of vesicular stomatitis virus RNA in the region coding for leader RNA, N protein mRNA, and their junction. Proc. Natl. Acad. Sci. U.S.A. **76**:4793–4797.
27. **Roy, P., and D. H. L. Bishop.** 1973. Initiation and direction of RNA transcription by vesicular stomatitis virus virion transcriptase. J. Virol. **11**:487–501.
28. **Schubert, M., J. D. Keene, R. C. Herman, and R. A. Lazzarini.** 1980. Site on the vesicular stomatitis virus genome specifying polyadenylation and the end of the L gene mRNA. J. Virol. **34**:550–559.
29. **Schubert, M., J. D. Keene, and R. A. Lazzarini.** 1979. A specific internal RNA polymerase recognition site of VSV RNA is involved in the generation of DI particles. Cell **18**:749–757.
30. **Schubert, M., J. D. Keene, R. A. Lazzarini, and S. U. Emerson.** 1978. The complete sequence of a unique RNA species synthesized by a DI particle of VSV. Cell **15**:103–112.
31. **Schubert, M., and R. A. Lazzarini.** 1981. Structure and

origin of a snapback Defective Interfering particle RNA of vesicular stomatitis virus. J. Virol. **37**:661–672.

32. **Semler, B. L., J. Perrault, J. Abelson, and J. J. Holland.** 1978. Sequence of a RNA templated by the 3'-OH RNA terminus of defective interfering particles of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **75**:4704–4708.
33. **Simoncsits, A., G. G. Brownlee, R. S. Brown, J. R. Rubin, and H. Guilley.** 1977. New rapid gel sequencing method for RNA. Nature (London) **269**:833–836.
34. **Testa, D., P. K. Chanda, and A. K. Banerjee.** 1980. Unique mode of transcription *in vitro* by vesicular stomatitis virus. Cell **21**:267–275.
35. **Villarreal, L. P., and J. J. Holland.** 1973. Synthesis of poly(A) *in vitro* purified virions of vesicular stomatitis virus. Nature (London) New Biol. **246**:17–19.