Proc. Nat. Acad. Sci. USA
Vol. 70, No. 12, Part I, pp. 3566-3570, December 1973

In Vitro Synthesis of RNA That Contains Polyadenylate by Virion-Associated RNA Polymerase of Vesicular Stomatitis Virus

(poly(U) filters/oligo(dT)-cellulose/RNA gel electrophoresis/transcriptase/viral replication)

AMIYA K. BANERJEE AND DENNIS P. RHODES

Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Communicated by B. L. Horecker, August 9, 1973

ABSTRACT **The RNA synthesized *in vitro* by the virion-associated RNA-instructed RNA polymerase of purified vesicular stomatitis virus contains polyadenylate sequences. These have been demonstrated by their partial resistance to pancreatic and T_1 ribonucleases and their capacity to bind to poly(U) filters and oligo(dT)-cellulose. The polyadenylate sequences range in apparent size from 50 to 200 bases, similar to the size of the poly(A) in mRNA from vesicular stomatitis virus-infected cells. Possible mechanisms of polyadenylylation of the *in vitro* RNA product are discussed.**

Vesicular stomatitis virus (VSV) is a bullet-shaped, membrane-maturing virus belonging to the rhabdovirus group (1). The genome of VSV consists of a single-stranded RNA with a molecular weight of approximately 4×10^6 (2, 3). Upon treatment of purified virions with nonionic detergents, an RNA polymerase is activated that, in the presence of four ribonucleoside triphosphates, synthesizes RNA species complementary to the genome RNA (4). These RNAs (referred to as product RNA) are smaller than the VSV genome RNA and include several RNA species, ranging in molecular weights from 2 to 10×10^5, that are partially resolved by electrophoresis in polyacrylamide gels (5). However, the product RNA contains sequences that are representative of the entire genome (6). The RNA isolated from polysomes of infected cells also corresponds in size to the RNA synthesized *in vitro* (7-11), except that an additional 28S virus-specific single-stranded RNA is present (8, 11). The RNA species made *in vitro* and *in vivo* hybridize to the genome RNA, indicating that they arise by a transcription process and presumably function as mRNA. It has been shown that mRNA isolated from VSV-infected cells contains polyadenylate sequences ranging in size from 70 to 250 bases (12). We demonstrate here that the RNA product synthesized *in vitro* by the virion-associated polymerase also contains polyadenylate sequences of similar length.

METHODS

Purification of VSV and [^3H]Adenosine-Labeled VSV. Baby hamster kidney (BHK 21, clone 13) cells adapted to suspension cultures were kindly provided by Dr. D. T. Dubin, Rutgers University. Cells were grown in Eagle's minimum medium, supplemented with 5% fetal-calf serum, glutamine (4 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml), and twice the normal amounts of nonessential amino acids and vitamins. Cells were concentrated to a density of 10^7 cells per ml in the same medium and infected with VSV (Indiana serotype; kindly provided by Dr. D. Summers, Albert Einstein College of Medicine, New York) at a multiplicity of infection of 1. After adsorption for 30 min at 37°, the cells were diluted to 10^6 cells per ml; actinomycin D and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.2) (HEPES, Nutritional Biochemical Corp.) (7), were added to concentrations of 1 μg/ml and 14 mM, respectively. After 16-24 hr at 37°, the cells were pelleted by centrifugation at 600 $\times$ *g* for 15 min. The virus from the supernatant was recovered by centrifugation at 27,000 rpm in a Spinco rotor no. 30 for 1 hr at 4°, suspended in buffer containing 1 mM Tris·HCl (pH 7.4) and 1 mM EDTA. After sonication in an MSE-60 sonicator at full power for 10 sec, the virus suspension was layered onto a 7-ml linear sucrose graident [20-70% (w/v) in 1 mM Tris·HCl (pH 7.4) and 1 mM EDTA] and centrifuged to equilibrium at 35,000 rpm for 16 hr at 4°. The virus band was collected, dialyzed against reticulocyte standard buffer [10 mM NaCl, 3.0 mM $MgCl_2$, and 10 mM Tris·HCl (pH 7.4)], layered onto an 8.5-ml linear sucrose gradient [15-30% (w/v) in reticulocyte standard buffer] over an 0.5-ml cushion of 70% sucrose, and centrifuged at 35,000 rpm in an SW41 Spinco rotor for 35 min at 4°. The virus band was collected, dialyzed against 1 mM Tris·HCl (pH 7.4) and 1 mM EDTA in 2.5% (v/v) $(CH_3)_2SO$, and stored in a liquid nitrogen freezer after adjusting the $(CH_3)_2SO$ concentration to 10%. VSV was also purified from BHK 21/13 monolayers. The cells were grown to confluence (2×10^7 cells per bottle) and infected with VSV at a multiplicity of infection of 1. After 16 hr, the medium was collected and virus was purified as described above. The virus preparations from both suspension and monolayer cells contained predominantly infectious B particles (2), and very little, if any, contaminating interfering T particles (13) (see below in Fig. 1). [2,8-^{3}H]Adenosine-labeled VSV was isolated from BHK 21/13 suspension cultures in a similar manner, except that [^{3}H]adenosine (5 μCi/mmol) was added 3 hr post-infection.

Isolation of Labeled RNA from VSV and VSV-Infected Cells. RNA from [^{3}H]adenosine-labeled VSV was extracted with phenol containing 0.5% sodium dodecyl sulfate (phenol-SDS) at 60°. RNA from the aqueous phase was precipitated with ethanol at −20°. For isolation of ^{3}H-labeled VSV mRNA from infected cells, [^{3}H]adenosine was added at 3 hr post-infection as described above, and after 8 hr the cells were harvested. Total RNA was extracted from the cells by the method of Scherrer, *et al.* (14), except that phenol-chloroform (1:1, v/v) was used as the organic phase. Single-stranded ^{3}H-labeled VSV RNA was isolated from the total RNA by LiCl precipitation as described (15).

Poly(U) Filter Binding and Oligo(dT)-Cellulose Binding. Preparation of poly(U) filters and assays for poly(A)-con-

Abbreviations: VSV, vesicular stomatitis virus; SDS, sodium dodecyl sulfate.

taining RNA were according to the method of Sheldon *et al.* (16). Oligo(dT)-cellulose (17), was a kind gift from Dr. S. S. Kerwar, Roche Institute of Molecular Biology. Oligo(dT)-cellulose was packed in a column (0.5 × 2 cm) and equilibrated with high-salt buffer [0.5 M KCl, 10 mM Tris·HCl (pH 7.5) and 1 mM EDTA]. Radioactive RNA samples in high-salt buffer were loaded onto the column and eluted with the same buffer. Ten 1-ml fractions were collected. The buffer was changed to low-salt buffer (as above, without KCl) and another ten 1-ml factions were collected. The radioactive content of all fractions (referred to as acid-precipitable radioactivity in the text) was determined by adding 5 ml of ice-cold 5% trichloroacetic acid, filtering through Millipore filters, type HA, and counting in a toluene-based scintillation fluid in the Beckman LS-250 scintillation counter.

RESULTS

Proteins in Purified VSV. To determine the purity of the virus preparation, we subjected VSV polypeptides to electrophoresis in a 10% SDS–polyacrylamide gel. Five distinct polypeptide bands with molecular weights of 190,000, 69,000, 50,000, 45,500, and 29,000, were observed. Other workers have observed and named these bands (18). There was no significant amount of material in other bands, suggesting little, if any, contamination by cellular proteins.

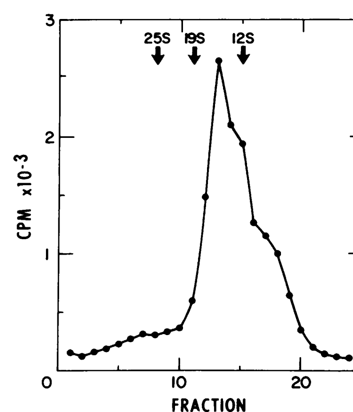

FIG. 2. Glycerol gradient centrifugation of [^{3}H]AMP-labeled product RNA. RNA was synthesized *in vitro* as described in Fig. 1 except that the concentrations of ATP, GTP, and CTP were each 0.5 mM, [α-^{32}P]UTP was replaced by [^{3}H]ATP (1.2 Ci/mmol, final specific activity = 35 cpm/pmol), and 13 μg of purified VSV was used. The ^{3}H-labeled RNA product was purified as described in Fig. 1. A portion of the RNA was layered onto a glycerol gradient as in Fig. 1 and centrifuged at 26,000 rpm for 18 hr at 5°. *Arrows* indicate the positions of the three size classes of [^{3}H]A-labeled reovirus mRNAs, 25 S, 19 S, and 12 S, synthesized *in vitro* by the virion-associated transcriptase (19) and centrifuged under the same conditions. Acid-precipitable radioactivity in each fraction was determined.

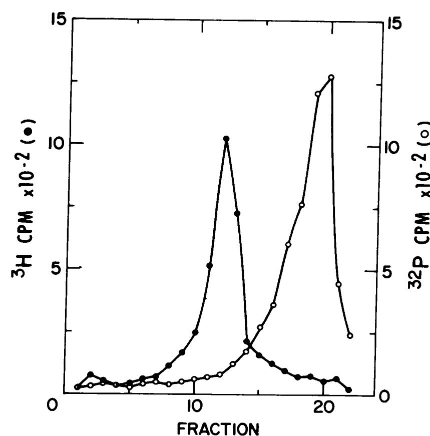

FIG. 1. Glycerol gradient sedimentation of VSV genome RNA and product RNA. RNA was synthesized *in vitro* in an incubation mixture (0.2 ml) which contained 0.1 M NaCl, 0.05 M Tris·HCl (pH 8.0), 5 mM $MgCl_2$, 4 mM dithiothreitol, 1 mM ATP, GTP, and CTP, 0.2 mM UTP and [α-^{32}P]UTP (40 Ci/mmol, final specific activity = 230 cpm/pmol), 0.05% Triton N101, and 85 μg of purified VSV. Incubation was carried out at 30° (20) for 2 hr. The reaction was terminated by addition of SDS (0.5%) and the labeled RNA was extracted with phenol. The RNA was precipitated from the aqueous phase with ethanol at −20°. Unreacted [α-^{32}P]UTP was removed by Sephadex G-100 chromatography and the RNA present in the void volume was precipitated with ethanol. The purified ^{32}P-labeled product RNA was denatured with $(CH_3)_2SO$ (19), and precipitated with ethanol. A portion of the labeled product RNA was mixed with [^{3}H]A-labeled VSV genome RNA and layered onto a 5–30% glycerol gradient containing 20 mM Tris·HCl (pH 8.0), 0.1 M NaCl, and 5 mM EDTA, and centrifuged at 35,000 rpm at 4° in an SW41 Spinco rotor for 5 hr. The fractions were collected from a hole pierced at the bottom of the tube and acid-precipitable radioactivity in each fraction was determined (19). In this and the following figures, the quantities indicated on the ordinate labels are the product of the experimental values and the given scale factor.

Transcription of VSV RNA In Vitro by the Virion-Associated Transcriptase. Purified VSV was incubated at 30° in an incubation mixture containing four ribonucleoside triphosphates, including [α-^{32}P]UTP as the labeled precursor (see legend of Fig. 1). Incorporation of radioactivity into RNA was linear for at least 2 hr, resulting in a 3- to 4-fold net RNA synthesis. After incubation for 2 hr, the reaction mixture was extracted with phenol–SDS, and the product RNA was separated from the radioactive precursor by Sephadex G-100 column chromatography. Labeled product RNA was denatured with $(CH_3)_2SO$, mixed with [^{3}H]A-labeled VSV genome RNA, and layered onto a 5–30% glycerol gradient. As shown in Fig. 1, ^{3}H-labeled VSV RNA sedimented in the position expected of 42 S; the *in vitro* ^{32}P-labeled RNA sedimented more slowly. The results show that the virus contains only 42S RNA and that the *in vitro* products are smaller than the genome RNA. In order to separate further the product RNA species, [^{3}H] AMP-labeled product RNA synthesized with [^{3}H]ATP as the labeled substrate was centrifuged in a gradient for a longer time (Fig. 2). The *in vitro* RNA products sedimented in a broad peak ranging predominantly from 10 to 19 S. However, 5–10% of the total acid-precipitable radioactivity applied to the gradient sedimented more rapidly than 19 S (Fig. 2).

Polyadenylate Sequences in the RNA Product Synthesized In Vitro. The first indication that product RNA synthesized by purified VSV contains poly(A) sequences came from the observation that [^{3}H]AMP- and [^{32}P]UMP-labeled product RNAs were differentially sensitive to pancreatic RNase digestion. Product RNAs were synthesized *in vitro* by purified VSV in separate reaction mixtures containing [^{3}H]ATP

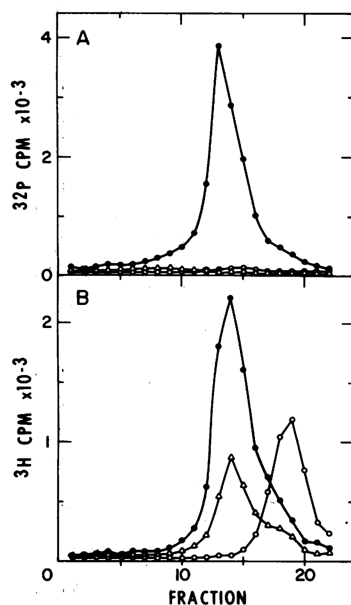

FIG. 3. Glycerol gradient analysis of the nuclease-resistant fragments in product RNAs. [^{3}H]AMP- and [^{32}P]UMP-labeled product RNAs were separately synthesized *in vitro*, and denatured as detailed in Figs. 1 and 2. Portions of the [^{32}P]UMP-labeled product (*A*) and [^{3}H]AMP-labeled product (*B*) were directly layered onto 5–30% glycerol gradients and centrifuged under conditions described in Fig. 2. Acid-precipitable radioactivity in each fraction was assayed (●). A second aliquot of ^{32}P- or ^{3}H-labeled RNA product was also centrifuged in a separate gradient. The salt concentration of each of the collected fractions was adjusted to 0.3 M NaCl, and pancreatic RNase (5 μg/ml) was added. The fractions were incubated for 30 min at 37° and acid-precipitable radioactivity was determined (△). Other aliquots of ^{32}P- and ^{3}H-labeled product RNAs were incubated at 37° for 30 min with pancreatic RNase (5 μg/ml) in buffer containing 0.01 M Tris·HCl (pH 7) and 0.4 M NaCl, and the digest was layered onto the gradient and centrifuged. Acid-precipitable radioactivity in each fraction was determined (○). In each case the samples were adjusted to 0.2% SDS before layering onto gradients.

or [α-^{32}P]UTP as the labeled precursor. After the reaction, the two mixtures were extracted separately with phenol–SDS and the product RNAs were isolated by Sephadex G-100 column chromatography. ^{3}H- and ^{32}P-labeled product RNAs were then denatured with $(CH_3)_2SO$ to eliminate secondary structure before digestion with RNase. As shown in Table 1, line 2, the pancreatic RNase resistances of [^{3}H]AMP- and [^{32}P]UMP-labeled products were markedly different. Only 3% of the [^{32}P]UMP-labeled product RNA was RNase-resistant, whereas 35% of the [^{3}H]AMP-labeled product RNA was resistant. The same results were obtained when the labeled product RNAs were treated with both pancreatic and T_1 RNases. Both of the labeled products hybridized to the VSV genome RNA to 90–100% at a genome RNA concentration of 5 μg/ml (Table 1, line 8). The results indicate that the product RNAs are copied from the genome RNA and the RNase-resistant radioactivity in the [^{3}H]AMP-labeled product RNA is apparently not due to the presence of double-stranded regions, but instead to the presence of either free poly(A) or poly(A) sequences in the product RNA.

To ascertain if the poly(A) sequences are covalently linked to VSV-specific product RNA and if all of the RNA species contain these sequences, we performed the following experiment. Product RNA labeled with [^{3}H]AMP was denatured and layered onto a 5–30% glycerol gradient and centrifuged. Acid-precipitable radioactivity was determined in each of the collected fractions. In a similar gradient individual fractions were collected and digested with pancreatic RNase before measuring the acid-precipitable radioactivity. In additional samples, the labeled product RNA was treated with RNase before gradient centrifugation. Fig. 3 shows the results of this experiment. When individual gradient fractions of the [^{3}H]AMP-labeled product RNA were treated with RNase, the profile of radioactivity resistant to RNase and precipitable by acid coincided with that of the undigested RNA (Fig. 3*B*). This suggests that if there are free molecules of poly(A) present, they are of the same size as the *in vitro* VSV-specific product RNA. However, this was found not to be the case, since when [^{3}H]AMP-labeled product RNA was treated with pancreatic RNase (or both pancreatic and T_1 RNases) before centrifugation, the RNase-resistant radioactivity sedimented in the 4S region of the gradient (Fig. 3*B*), showing that these sequences arise from the RNA species sedimenting from 10 to 19 S. In a similar experiment, the radioactivity in [^{32}P]UMP-labeled product RNA was completely sensitive to RNase digestion when treated before or after centrifugation (Fig. 3*A*). The results suggest that most, if not all, RNA species synthesized *in vitro* by the VSV-associated RNA polymerase contain covalently linked poly(A) sequences.

TABLE 1. *Hybridization of [^{3}H]AMP- and [^{32}P]UMP-labeled product RNAs with VSV genome RNA*

Virion RNA (μg/ml)	Treatment	Acid-insoluble (cpm) [^{3}H]-RNA	Acid-insoluble (cpm) [^{32}P]-RNA	RNase resistance (%) [^{3}H]-RNA	RNase resistance (%) [^{32}P]-RNA
None	None	4306	3453	—	—
None	RNase	1500	102	35	3
None	Hybridized, RNase	1920	357	44	10
0.1	Hybridized, RNase	1982	450	46	13
0.2	Hybridized, RNase	2224	655	52	19
0.5	Hybridized, RNase	2329	1447	54	42
1.0	Hybridized, RNase	2766	1898	64	55
5.0	Hybridized RNase	3959	3506	92	101
10.0	Hybridized, RNase	4244	3762	99	109

[^{3}H]AMP- and [^{32}P]UMP-labeled RNAs were synthesized *in vitro* and purified as described in the legends of Figs. 1 and 2. Both products were denatured with $(CH_3)_2SO$ and precipitated with ethanol. Aliquots of each product were hybridized for 2 hr at 60° with unlabeled VSV genome RNA in 0.01 M Tris·HCl (pH 7.4) containing 0.4 M NaCl in 0.2 ml (6). RNA samples were digested with 1 μg of pancreatic RNase (Worthington Biochemical Co.) for 30 min at 37° in 0.4 M NaCl buffer as indicated. Acid-precipitable radioactivity in each reaction mixture was determined.

Poly(U) Filter and Oligo(dT)-Cellulose Binding of Product RNA. Poly(U) immobilized on glass filters and oligo(dT) covalently attached to cellulose have been used to detect poly(A) sequences in RNA molecules (16, 17). Under appropriate salt conditions, both poly(U) and oligo(dT) form duplexes with poly(A). Product RNA was tested for the presence of covalently linked poly(A) by both of these binding tests. Both [³H]AMP- and [³²P]UMP-labeled product RNAs, which were differentially RNase-resistant (35 and 3%, respectively), were similarly and efficiently bound to oligo(dT)-cellulose and poly(U) filters (Table 2). In control experiments, VSV mRNA isolated from infected cells, which was previously shown to contain poly(A) sequences (12), was bound to poly(U) filters and oligo(dT)-cellulose to a similar extent (60%). [³H]Adenosine-labeled VSV genome RNA, which was 3% RNase-resistant, bound only 0.4% to poly(U) filters and 6% to oligo(dT)-cellulose. The results indicate that the product RNA contains poly(A) sequences, and confirm that the genome RNA (12) contains insufficient adenine-rich sequences to allow binding to poly(U) filters or to oligo(dT)-cellulose. It can be seen from Table 2 that about 30% of the [³H]AMP-labeled product RNA and 36% of the [³²P]UMP-labeled product RNA do not bind to oligo(dT)-cellulose. This could be due to the absence of poly(A) sequences from some RNA species or to partial degradation of some RNA by nucleases during the transcriptase reaction.

Size of the Poly(A) Sequences. To determine the approximate length of the poly(A) sequences in the product RNA, *in vitro* [³H]AMP-labeled product RNA and *in vivo* [³H]-adenosine-labeled mRNA were digested with pancreatic and T_1 ribonucleases. The resistant fragments were subjected to electrophoresis in 20% polyacrylamide gels containing 8 M urea. Radioactive poly(A) fragments derived from both *in vitro* and *in vivo* RNAs showed a heterogenous distribution (Fig. 4). From the positions of RNA markers it was estimated that the poly(A) from RNase-treated *in vivo* mRNAs is approximately 50–200 bases (12). In view of the similarity of the mobilities of the RNase-resistant fragments, we conclude that the poly(A) in RNA products synthesized *in vitro* is also 50–200 bases long.

TABLE 2. *VSV RNA binding to poly(U) filters and oligo(dT)-cellulose*

Sample	RNase resistance (%)	percent binding by	
		Poly(U) filter	Oligo(dT)-cellulose
In vitro RNA			
[³H]AMP-labeled product	35	68	70
[³²P]UMP-labeled product	3	52	64
In vivo RNA			
[³H]A-labeled mRNA	44	63	66
[³H]A-labeled virion RNA	3	0.4	6
[³H]poly(A)	99	79	100

[³H]AMP- and [³²P]UMP-labeled product RNAs were synthesized *in vitro* and purified as described in the legends of Figs. 1 and 2. Aliquots of each RNA product were denatured with $(CH_3)_2SO$ and tested for RNase resistance. Poly(U) filter binding was carried out as described (15), while oligo(dT)-cellulose binding was as described in *Methods*. [³H]Poly(A) (11.6 Ci/mol; $S_{20,w}$ = 8.8) was from Miles Laboratories, and [³H]A-labeled VSV mRNA was isolated from infected cells as described in *Methods*.

TABLE 3. *Ribonucleoside triphosphate requirements for in vitro RNA synthesis*

Reaction	ATP (mM)	[³H]AMP incorporation (nmol/2 hr)
Complete	1.0	1.1
− UTP	1.0	<0.05
− CTP	1.0	<0.05
− GTP	1.0	<0.05
− UTP, CTP, GTP	1.0	<0.05
− UTP, CTP, GTP	0.1	<0.05

Transcriptase assay conditions were the same as described in Fig. 2, except that [³H]ATP (40 Ci/mmol) was used as the labeled substrate and the concentration of unlabeled ATP was changed and UTP, GTP, and CTP were omitted as indicated in the table. 32 μg of purified VSV was used in each reaction.

Test for Poly(A) Polymerase Activity in Purified VSV. The possibility remained that purified VSV possesses a poly(A) polymerase activity similar to that isolated from calf thymus (22). This enzyme covalently attaches AMP residues to either endogenous or exogenous RNA primers at their 3′-termini. To find out if endogenous viral RNA can act as primer for AMP incorporation, or if poly(A) can be synthesized in an unprimed reaction, the transcriptase assay was

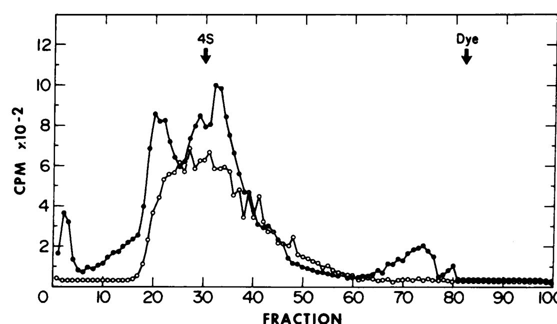

FIG. 4. Polyacrylamide-gel analysis of polyadenylate sequences in RNA product synthesized *in vitro* and in infected cells. [³H]AMP-labeled product RNA was synthesized *in vitro* and purified as described in Fig. 2. A portion of the labeled RNA was incubated with pancreatic RNase (10 μg) and T_1 RNase (1 μg) in 0.15 M Tris·HCl (pH 7.4) containing 0.005 M EDTA and yeast tRNA (100 μg). The incubation was carried out at 37° for 60 min. The RNase-resistant fragments were precipitated with two volumes of ethanol at −20° for 18 hr (15). The precipitate was collected and electrophoresed in a 20% polyacrylamide gel containing 8 M urea (32) for 16 hr at 130 V at room temperature. [³H]Adenosine-labeled VSV mRNA isolated from infected cells was similarly processed and electrophoresis was carried out in a separate gel. The gels were fractionated in a Gilson automatic gel crusher. Each gel fraction (1 mm) was dissolved in H_2O_2 and counted in Aquasol (New England Nuclear Corp). Migrations of labeled tRNA from *Bombyx mori* (kindly provided by Dr. L. P. Gage, Roche Institute of Molecular Biology) and bromphenol blue dye marker are shown by the *arrows*. *In vitro* product RNA (O); *in vivo* mRNA (●).

carried out in the presence or absence of various ribonucleoside triphosphates with [^{3}H]ATP as the only labeled precursor. As shown in Table 3, the *in vitro* transcriptase reaction was dependent on the presence of four ribonucleoside triphosphates and there was virtually no polymerization of [^{3}H]AMP when ATP was the only substrate present. Thus purified VSV, under these conditions, does not synthesize free poly(A) or add AMP residues to endogenous viral RNA.

DISCUSSION

The virion-associated transcriptase in purified VSV synthesizes, *in vitro*, RNA complementary to genome RNA. When labeled with [^{3}H]AMP, the *in vitro* product RNA was 35% resistant to pancreatic and T_1 RNases, whereas [^{32}P]UMP-labeled product RNA was only 3% resistant. RNA products labeled *in vitro* with either precursor bind efficiently to poly(U) filters and oligo(dT)-cellulose, as does *in vivo* mRNA. On the other hand, [^{3}H]adenosine-labeled VSV genome RNA is only 3% resistant to RNase and binds poorly to poly(U) filters or oligo(dT)-cellulose. These results show that *in vitro* synthesized RNA species contain covalently linked poly(A) sequences and confirm that the genome RNA contains very little, if any, poly(A) (12). The size of the poly(A) sequences is similar to that of mRNA from VSV-infected cells, 50–200 bases. Purified VSV does not possess detectable poly(A)-polymerase activity and does not add AMP residues to endogenous genome RNA.

There are several possible mechanisms by which adenylylation of product RNA might occur *in vitro*. First, virion-associated polymerase may transcribe poly(U) sequences located in the viral genome, resulting in the synthesis of poly(A). A similar mechanism was suggested for adenylylation of vaccinia RNA synthesized *in vitro* by transcription of thymidine-rich regions in vaccinia DNA by the core-associated transcriptase (21). Second, AMP residues may be added sequentially to the 3′ ends of RNA chains in the absence of a poly(U) template, presumably by an enzyme similar to that reported by Edmonds and Abrams (22). Such post-transcriptional modification has been suggested for adenylylation of mRNAs isolated from eukaryotic cells as well as from cells infected with DNA- or RNA-containing viruses (23–26). Third, virion-associated transcriptase may synthesize poly(A) by repeated transcription of short tracts of uridine residues in the RNA template by a mechanism similar to that reported in the *E. coli* DNA-dependent RNA polymerase system (27), where the enzyme synthesizes poly(A) from ATP by slipping on oligo(dT) sequences in the denatured DNA template *in vitro*. Similar results were also obtained with phage T3 RNA polymerase and denatured T3 DNA (28). In the VSV system, poly(A) is found covalently associated with newly synthesized RNA chains and no free poly(A) is synthesized or is added to the endogenous viral RNA in the presence of ATP alone. Recently, it has been shown that VSV genome RNA does not contain U-rich sequences (29), although uridylic acid is the predominant base in VSV genome RNA, comprising 31 mole percent (7). From these observations, it appears that adenylylation of VSV product RNA *in vitro* is not mediated by transcription of U-rich regions in the genome RNA template, but possibly by a slippage mechanism from short uridine tracts. It is also possible that the poly(A) sequences in product RNA may be synthesized by an enzyme similar to that reported by Edmonds and Abrams (22) with newly synthesized RNA acting as a primer for AMP incorporation. The synthesis of poly(A) could be mediated by the viral transcriptase alone or in combination with any of the other viral structural proteins.

While this work was in progress, we learned that cell-free extracts from VSV-infected L-cells also synthesize RNA containing poly(A) sequences (30). Similar observations have also been made with purified Newcastle disease virus (31).

We thank Drs. Aaron J. Shatkin and C. Martin Stoltzfus for reviewing the manuscript.

1. Howatson, A. F. (1970) *Advances in Virus Research*, eds. Smith, K. M., Lauffer, M. A. & Bang, F. B. (Academic Press Inc., New York), Vol. 16, pp. 195–256.
2. Huang, A. S. & Wagner, R. R. (1966) *J. Mol. Biol.* **22**, 381–384.
3. Schincariol, A. L. & Howatson, A. F. (1970) *Virology* **42**, 732–743.
4. Baltimore, D., Huang, A. S. & Stampfer, M. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 572–576.
5. Bishop, D. H. L. & Roy, P. (1971) *J. Mol. Biol.* **57**, 513–527.
6. Bishop, D. H. L. (1971) *J. Virol.* **7**, 486–490.
7. Mudd, J. A. & Summers, D. F. (1970) *Virology* **42**, 958–968.
8. Huang, A. S., Baltimore, D. & Stampfer, M. (1970) *Virology* **42**, 946–957.
9. Schincariol, A. L. & Howatson, A. F. (1972) *Virology* **49**, 766–783.
10. Wild, T. F. (1971) *J. Gen. Virol.* **13**, 295–310.
11. Huang, A. S. & Manders, E. K. (1972) *J. Virol.* **9**, 909–916.
12. Ehrenfeld, E. & Summers, D. F. (1972) *J. Virol.* **10**, 683–688.
13. Huang, A. S., Greenawalt, J. W. & Wagner, R. R. (1966) *Virology* **30**, 161–172.
14. Scherrer, K., Marcaud, L., Zajdela, F., Breckenridge, B. & Gros, F. (1966) *Bull. Soc. Chim. Biol.* **48**, 1037–1075.
15. Stoltzfus, C. M., Shatkin, A. J. & Banerjee, A. K. (1973) *J. Biol. Chem.*, in press.
16. Sheldon, R., Jurale, C. & Kates, J. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 417–421.
17. Gilham, P. T. (1964) *J. Amer. Chem. Soc.* **86**, 4982–4985.
18. Wagner, R. R., Prevec, L., Summers, D. F., Sokol, F. & MacLeod, R. (1972) *J. Virol.* **10**, 1228–1230.
19. Banerjee, A. K. & Shatkin, A. J. (1971) *J. Virol.* **6**, 1–11.
20. Bishop, D. H. L. & Roy, P. (1971) *J. Mol. Biol.* **58**, 799–812.
21. Kates, J. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 743–752.
22. Edmonds, M. & Abrams, R. (1960) *J. Biol. Chem.* **235**, 1142–1149.
23. Darnell, J. E., Philipson, L., Wall, R. & Adesnik, M. (1971) *Science* **174**, 507–510.
24. Philipson, L., Wall, R., Glickman, G. & Darnell, J. E. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2806–2809.
25. Weinberg, R. A., Ben-Ishai, Z. & Newbold, J. E. (1972) *Nature New Biol.* **238**, 111–113.
26. Shatkin, A. J. (1974) *Annu. Rev. Biochem.*, in press.
27. Chamberlin, M. & Berg, P. (1964) *J. Mol. Biol.* **8**, 708–726.
28. Salvo, R. A., Chakraborty, P. R. & Maitra, U. (1973) *Fed. Proc.* **32**, 645.
29. Marshall, S. & Gillespie, D. (1972) *Nature New Biol.* **240**, 43–45.
30. Galet, H. & Prevec, L. (1973) *Nature New Biol.* **243**, 200–203.
31. Weiss, S. R. & Bratt, M. A. (1973) *Amer. Soc. Microbiol. Meeting*, p. 202.
32. Malloy, G. R., Thomas, W. L. & Darnell, J. E. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3684–3688.