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# In Vitro RNA Transcription by the New Jersey Serotype of Vesicular Stomatitis Virus

## I. Characterization of the mRNA Species

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The in vitro RNA synthesis by the virion-associated RNA polymerase of vesicular stomatitis virus (VSV), New Jersey serotype, was compared with that of the serologically distinct Indiana serotype of VSV. The New Jersey serotype of VSV synthesized five distinct mRNA species in vitro, three of which were smaller than the corresponding species synthesized by the Indiana serotype of VSV. These included the mRNA's coding for the G, M, and NS proteins. By hybridization experiments, virtually no sequence homology was detected between the mRNA's of the two serotypes. Despite this lack of overall homology, the 12 to 18S mRNA species of both serotypes contained a common 5'-terminal hexanucleotide sequence, $G_{(5')}ppp_{(5')}A\text{-}A\text{-}C\text{-}A\text{-}G$. The significance of this finding in light of specific interactions between the two serotypes of VSV in vivo is discussed.

Vesicular stomatitis virus (VSV) is a prototype of negative-strand rhabdoviruses and contains a linear single-stranded RNA of molecular weight $4 \times 10^6$ packaged within a characteristic bullet-shaped particle containing five structural proteins designated as L, G, M, NS, and N (26). The purified virions contain an RNA-dependent RNA polymerase (4) that transcribes the genome RNA in vitro and synthesizes monocistronic mRNA species (17) that code for five structural proteins of the virus (10). Recently it has been shown that the initiation of RNA transcription occurs in vitro at the 3' terminus of the genome RNA, and the mRNA's species are synthesized sequentially in the order 3'-N-NS-M-G-L 5' (2, 3). A characteristic feature of in vitro transcription by VSV is that a small leader RNA sequence (approximately 70 bases) is synthesized and released prior to the synthesis of any of the mRNA species (14). The 5'-terminal sequence of the leader RNA is unblocked and polyphosphorylated (14), whereas all the mRNA species contain a blocked 5'-terminal structure $G_{(5')}ppp_{(5')}A\ldots$ (3) and a common hexanucleotide sequence $G_{(5')}ppp_{(5')}A\text{-}A\text{-}C\text{-}A\text{-}G\ldots$ (25). Although the transcription process in vitro appears to involve cleavage of a precursor RNA molecule, the actual sequence of events leading to the biosynthesis of VSV mRNA species is still unclear (5).

The studies of in vitro RNA synthesis by VSV have been primarily with the Indiana serotype (designated as $VSV_{Ind}$). Another member of the VSV group is New Jersey isolate of VSV (designated as $VSV_{NJ}$), which has been shown to be serologically distinct from various subgroups of $VSV_{Ind}$ (11). $VSV_{NJ}$ contains a genome RNA larger than $VSV_{Ind}$, approximately $4.5 \times 10^6$ daltons, and five structural proteins (26). By complementation tests, the mutants of $VSV_{NJ}$ were assigned to six groups (21), indicating the probable existence of an additional structural gene in this virus. There are a number of interesting features of $VSV_{NJ}$ which clearly warrant studies on the structure and function of its genome RNA and mRNA's. Of considerable interest is the observation by Pringle et al. (21) of no genetic overlap of $VSV_{Ind}$ and $VSV_{NJ}$ viruses, despite evidence of relatedness based on cross-reacting group-specific antigen (ribonucleoprotein core) (11).

The recent studies also indicate that the genome RNAs of both the $VSV_{Ind}$ and $VSV_{NJ}$ share virtually no sequence homology (13), and similar conclusions were drawn from hybridization studies with $VSV_{NJ}$ genome RNA and the mRNA's isolated from $VSV_{Ind}$ infected cells (19, 23). Furthermore, using in vitro reconstitution experiments, it was shown that the L and NS proteins of $VSV_{NJ}$ failed to complement the corresponding proteins of $VSV_{Ind}$ to restore transcription of the N-RNA complex of $VSV_{Ind}$ (8). In contrast to these observations, it has been shown recently that the L protein of $VSV_{NJ}$ could be utilized in vivo in the transcription of a defective interfering

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particle obtained from a heat-resistant mutant of $VSV_{Ind}$ (HR-DI) which lacks the L protein gene (12). Moreover, Repik et al. (22) have shown that the wild-type $VSV_{NJ}$ is able to complement the transcription defect of a $VSV_{Ind}$ mutant of group I that is temperature sensitive in the L protein gene. Finally, it is also interesting to note that $VSV_{NJ}$ interferes with the replication of $VSV_{Ind}$ (16), and $HR\text{-}DI_{Ind}$ can cause heterotypic interference with $VSV_{NJ}$ in coinfected cells (20).

From the above observations, it seems quite possible that, despite apparent lack of gross homologies between the genome RNAs of the two serotypes of VSV, there may exist vital sequences in the genome RNA that are shared by both and recognized by the protein components of both viruses for transcription and possibly replication. With this general background information in mind, we initiated our studies on $VSV_{NJ}$. The present paper describes the in vitro transcription of mRNA species and their 5'-terminal sequence. The accompanying paper describes in detail the synthesis and characterization of the leader RNA of $VSV_{NJ}$.

## MATERIALS AND METHODS

**Viruses and cells.** The Ogden strain of the New Jersey serotype of VSV was clonally purified and used in this work. The seed stock of the virus was kindly provided by Robert Lazzarrini, National Institutes of Health, Bethesda, Md. Both $VSV_{NJ}$ and $VSV_{Ind}$ were grown in BHK-21/13 spinner cells and purified as described previously (6). [$^3$H]methionine-labeled viruses were purified as described (1).

**Synthesis and purification of in vitro mRNA's.** RNA was synthesized in vitro using Triton N101-disrupted VSV in standard incubation mixtures as detailed previously (25). [*Methyl*-$^3$H]*S*-adenosylmethionine was included in the reaction mixture to methylate the mRNA species (3). The specific radioactivities of the labeled substrates were: [$\alpha$-$^{32}$P]GTP (19.5 Ci/mmol); [$\alpha$-$^{32}$P]CTP (23.1 Ci/mmol); [$\alpha$-$^{32}$P]UTP (17.7 Ci/mmol); [$\alpha$-$^{32}$P]ATP (25.8 Ci/mmol). The synthesis was at 30°C for 2 h. The RNA was extracted with phenol and precipitated with ethanol at −20°C. The RNA was then selected for polyadenylic acid [poly(A)]-containing RNA by chromatography on an oligodeoxyribosylthymine-cellulose column and further purified by velocity sedimentation as described previously (17).

**Analysis of the 5'-terminal nuclease-treated fragments.** The RNase $T_1$-fragments of the labeled mRNA's were obtained as described previously (25). The purification of the fragment of *N*-[*N'*-(*m*-dihydroxyborylphenyl)succinamyl]-aminoethyl-cellulose (DBAE-cellulose) has been described (25). The conditions for the enzymatic digestions with RNase A, RNase $T_1$, nuclease $P_1$, nucleotide pyrophosphatase, and bacterial alkaline phosphatase have been described in detail elsewhere (1). High-voltage paper electrophoresis of various enzyme-treated products was at pH 3.5 in a pyridine-acetic acid-water (1:10:89) system as described previously (1). Descending paper chromatographic analysis was done using isobutyric acid–0.5 M ammonium hydroxide (10:6) as solvent on Whatman 3MM paper (25). DEAE-cellulose chromatography in 7 M urea has been described previously (7).

**Chemicals and enzymes.** The radiochemicals, chemicals, and enzymes used in these studies were obtained as detailed previously (25).

## RESULTS

**mRNA species synthesized in vitro by $VSV_{NJ}$.** The $VSV_{NJ}$ used in our studies is the Ogden strain, which was clonally purified and its serotype authenticated by neutralization with antisera prepared against $VSV_{NJ}$. The sizes of the structural proteins of $VSV_{NJ}$ are characteristically distinct (18) from those of $VSV_{Ind}$ when analyzed by polyacrylamide gel electrophoresis (Fig. 1). The G and M proteins of $VSV_{NJ}$ are smaller than the corresponding proteins of $VSV_{Ind}$. The N protein of $VSV_{NJ}$ migrates slightly slower than the N protein of $VSV_{Ind}$. The L proteins of both the viruses appear to have similar mobility, whereas the NS protein of $VSV_{NJ}$ (see reference 18) may be present at too low a level to be detected or may migrate at the same rate as the N protein under these electrophoretic conditions.

Highly purified $VSV_{NJ}$ was tested for RNA synthesis by the virion-associated transcriptase in vitro. Using [$^3$H]UTP as the labeled substrate, the specific activity of the transcriptase was found to be, on the average, 20 nmol of UMP incorporated per mg of protein per h. This specific activity is quite similar to that observed for the transcriptase activity of $VSV_{Ind}$ (17). To analyze the mRNA species synthesized in vitro, the poly(A)-containing RNA products labeled with [$\alpha$-$^{32}$P]UMP were analyzed by velocity sedimentation in sucrose gradients. mRNA species synthesized by $VSV_{Ind}$ were similarly synthesized, but labeled with [$^3$H]UMP and analyzed in the same gradient. A large mRNA species that sedimented around 31S was similar to the corresponding mRNA species from $VSV_{Ind}$ (Fig. 2A) (17). The smaller mRNA species, upon further fractionation, resolved into two distinguishable peaks of radioactivity sedimenting at 14.5S and 12S (Fig. 2B). On the other hand, the mRNA species synthesized by $VSV_{Ind}$ resolved into three peaks of radioactivity corresponding to four mRNA species (17); the mRNA coding for G protein sediments at 17S (fraction 13), mRNA coding for N protein sediments at 14.5S (fraction 15), and mRNA's coding for the M and NS proteins sediment at 12S (fraction 18) (17). Since the 17S region of the gradient did not contain

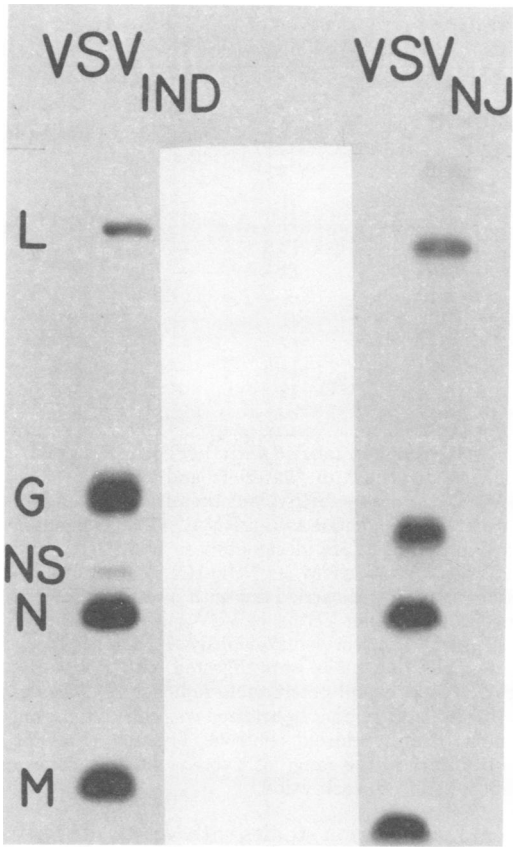


FIG. 1. *Polyacrylamide gel electrophoretic analysis of VSV proteins. VSV labeled with [$^3$H]methionine was solubilized and analyzed by sodium dodecyl sulfate-slab gel electrophoresis as described previously (1). Gels A and B represent the polypeptide pattern of VSV$_{Ind}$ and VSV$_{NJ}$, respectively. The $^3$H-labeled protein bands have been detected by the fluorographic procedure described by Bonner and Laskey (9). Approximately 80,000 cpm of $^3$H was present in each protein, and the fluorogram was exposed for 48 h. Electrophoresis was from top to bottom.*

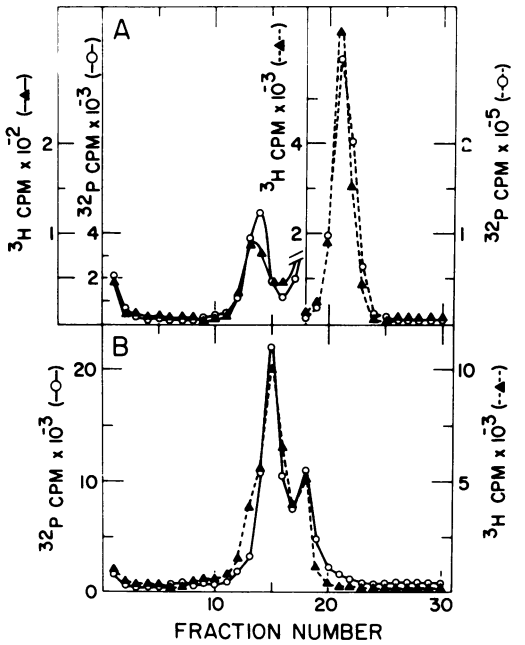


FIG. 2. *Velocity sedimentation analysis of the RNA products of the in vitro transcription of VSV$_{NJ}$. mRNA was synthesized in vitro in the presence of [α-$^{32}$P]UTP as the labeled precursor. The poly(A)-containing mRNA was purified as described in Materials and Methods and analyzed by velocity sedimentation in a sucrose gradient (17). VSV$_{Ind}$ mRNA synthesized in vitro and purified but labeled with [$^3$H]UMP was mixed with $^{32}$P-labeled VSV$_{NJ}$ mRNA before centrifugation. After centrifugation, fractions were collected by puncturing the tubes at the bottom, and trichloroacetic acid-precipitable radioactivity in each fraction was determined (17). (A) Sedimentation profile at low speed; 22,000 rpm for 17 h at 23°C in a Spinco SW-40 rotor. (B) Sedimentation profile at high speed; 33,000 rpm for 17 h at 23°C.*

radioactivity from the mRNA's synthesized by VSV$_{NJ}$, it seems that the mRNA species corresponding to 17S of VSV$_{NJ}$ may not be synthesized in vitro, or, more probably, it sediments with 14.5S mRNA species due to its smaller size than the corresponding mRNA species of VSV$_{Ind}$.

To resolve this question, the total 12 to 18S mRNA synthesized in vitro by VSV$_{NJ}$ was hybridized to VSV$_{NJ}$ genome RNA. The resulting hybrids were digested with RNase T$_2$, and the double-stranded RNA molecules, derived from each of the mRNA species, were resolved by electrophoresis in 5% polyacrylamide gels. This technique has been successfully used to separate and characterize each mRNA species synthesized by VSV$_{Ind}$ (15, 24). As shown in Fig. 3, four distinct double-stranded RNA molecules were resolved (1 to 4). When compared with the migration rates of the duplex RNAs of VSV$_{Ind}$ (1' to 4'), the RNA molecule migrating around fraction 44 is presumably the duplex RNA representing the 17S mRNA species and constitutes approximately 10% of the total RNA products. By analogy to the duplex RNA molecules of VSV$_{Ind}$ (10) and considering the relative mobilities of VSV$_{NJ}$ proteins during electrophoresis in sodium dodecyl sulfate-polyacrylamide gels (Fig. 1), we have tentatively assigned each duplex RNA in Fig. 3: the mRNA coding for G protein (RNA1), N protein (RNA2), NS protein (RNA3), and M protein (RNA4). The VSV$_{NJ}$ 31S mRNA species (Fig. 2A) presumably is the mRNA for the L protein. From the relative

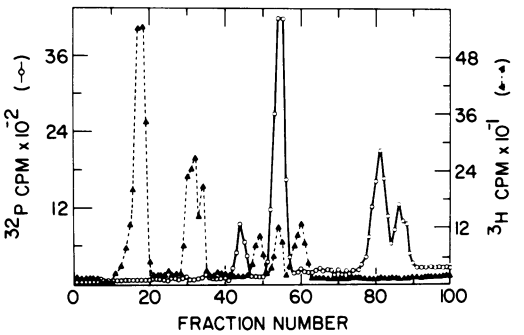


FIG. 3. *Separation of duplex RNAs derived from $VSV_{NJ}$ mRNA's by polyacrylamide gel electrophoresis. $VSV_{NJ}$ mRNA's were synthesized in vitro in the presence of [$\alpha$-$^{32}$P]CTP, and poly(A)-containing mRNA species were purified by sedimentation through sodium dodecyl sulfate-sucrose gradients as shown in Fig. 2B. The mRNA species sedimenting at 12 to 18S were pooled and precipitated with ethanol. Portions were annealed to $VSV_{NJ}$ genome RNA, digested with RNase $T_2$, and analyzed by electrophoresis on 5% polyacrylamide gels (25). $^3$H-labeled reovirus double-stranded RNA was mixed with the samples before electrophoresis. Migration is from left to right.*

mobilities of the RNA duplexes of $VSV_{NJ}$ compared with the corresponding RNA duplexes derived from $VSV_{Ind}$ (Fig. 3), it is apparent that some of the $VSV_{NJ}$ mRNA's are smaller than the corresponding counterparts in $VSV_{Ind}$ mRNA's. These are RNA1, -3, and -4. Thus, from the above results, it can be concluded that $VSV_{NJ}$ is capable of synthesizing in vitro five distinct mRNA species similar in size to those made by $VSV_{Ind}$, although some of the mRNA species are smaller than the corresponding species of $VSV_{Ind}$.

**Sequence homology of $VSV_{NJ}$ and $VSV_{Ind}$ mRNA's.** The synthesis of five mRNA species in vitro by purified $VSV_{NJ}$ offers an opportunity to study the presence of base homologies, if any, between $VSV_{NJ}$ and $VSV_{Ind}$ mRNA's. Each $VSV_{NJ}$ mRNA species coding for L, G, M, NS, and N (tentatively assigned; see above) was separated and individually hybridized with the $VSV_{Ind}$ genome RNA. As shown in Table 1, very little, if any, complementarity exists between $VSV_{NJ}$ mRNA's and $VSV_{Ind}$ genome RNA, which means no significant stretch of base sequence homology is present between the $VSV_{NJ}$ and $VSV_{Ind}$ mRNA species. It should be noted from Table 1 that the base level of hybridization ranges from 5 to 10% (for the smallest to the largest mRNA's, respectively), which could amount to sequence homologies of 40 to 600 bases which may be present in corresponding mRNA species. Similar results were obtained

TABLE 1. *Hybridization of $VSV_{NJ}$ mRNA species with $VSV_{Ind}$ genome RNA*[a]

| Genome RNA | | mRNA | | |
|---|---|---|---|---|
| New Jersey | Indiana | New Jersey ($s_{20,w}$) | Indiana ($s_{20,w}$) | % hybridized |
| + | − | 31 | — | 81 |
| + | − | 17 | — | 82 |
| + | − | 14.5 | — | 81 |
| + | − | 12 | — | 76 |
| + | − | — | 12-18 | 7 |
| − | + | 31 | — | 9.6 |
| − | + | 17 | — | 6.7 |
| − | + | 14.5 | — | 4.1 |
| − | + | 12 | — | 4.8 |
| − | + | — | 12-18 | 89 |

[a] $VSV_{NJ}$ mRNA labeled with [$\alpha$-$^{32}$P]UMP was purified as described in Materials and Methods. The poly(A)-containing mRNA was then fractionated into 31S RNA (designated as L-mRNA), 17 S (designated as G-mRNA), 14.5S (designated as N-mRNA), and 12S (designated as M + NS-mRNA) (see Fig. 1). Hybridization was carried out with individual mRNA species with either $VSV_{NJ}$ or $VSV_{Ind}$ genome RNA (1 to 2 $\mu$g/ml) as described previously (7). After hybridization, reaction tubes were digested with RNase $T_2$, trichloroacetic acid-precipitable radioactivity was determined, and percent hybridized was calculated from values of unhybridized controls. The self-annealing values were in the range of 4 to 6%, which has been subtracted from each value.

from hybridization studies with $VSV_{NJ}$ mRNA's isolated from infected cells and $VSV_{Ind}$ genome RNA (19, 23).

**5'-Terminal structure and sequence of $VSV_{NJ}$ mRNA species.** To determine the 5'-terminal structure of the in vitro synthesized mRNA's of $VSV_{NJ}$, the latter was synthesized in the presence of [$^3$H]*S*-adenosyl-L-methionine (AdoMet) and the poly(A)-containing mRNA's were purified by oligodeoxyribosylthymine-cellulose chromatography. A portion of the $^3$H-labeled mRNA's was then treated with nuclease $P_1$ and bacterial alkaline phosphatase, and the resulting mixture was analyzed by high-voltage paper electrophoresis. The corresponding labeled mRNA species synthesized by $VSV_{Ind}$ was treated with enzymes and analyzed in parallel during paper electrophoresis. As shown in Fig. 4A, the nuclease $P_1$ and phosphatase-resistant material migrated ahead of pA marker, indicating that the 5'-termini of the $VSV_{NJ}$ mRNA species were blocked and methylated. Another portion of the $^3$H-labeled mRNA's was treated with nuclease $P_1$ and nucleotide pyrophosphatase and analyzed by paper electrophoresis. As shown in Fig. 4B, two distinct peaks of equal radioactivity were seen, one staying at the origin and the other migrating with the pA marker. A

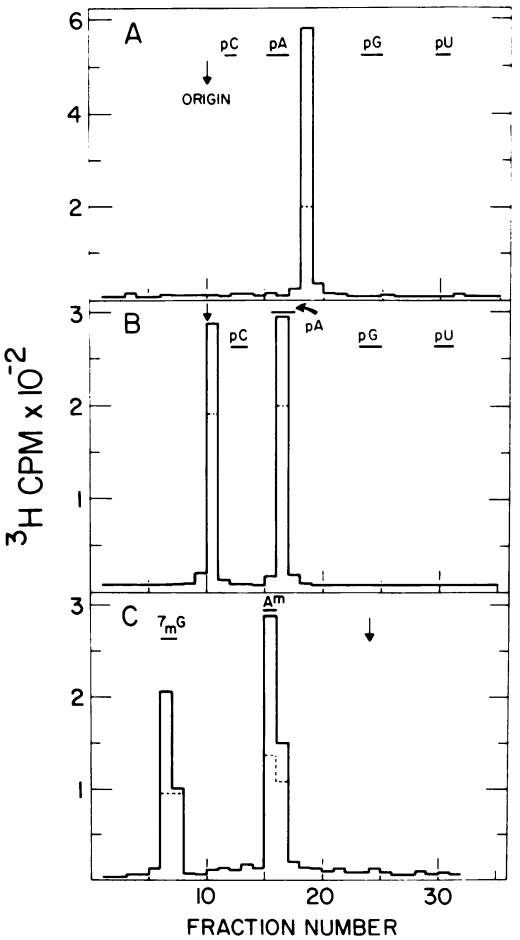


FIG. 4. *Paper electrophoretic analysis of enzymatic digestions of $^3$H-methylated RNA products. VSV$_{NJ}$ mRNA's were synthesized in vitro in the presence of [$^3$H]SAM to methylate the mRNA's (3). Poly(A)-containing mRNA's were purified and a portion was treated with nuclease P$_1$ prior to electrophoresis (A). A separate portion was digested with nuclease P$_1$ and nucleotide pyrophosphatase (B), and another was digested with nuclease P$_1$ and nucleotide pyrophosphatase followed by alkaline phosphatase (C) prior to analysis. The solid line denotes analyses of methylated VSV$_{NJ}$ mRNA's, and the dashed line denotes analyses of VSV$_{Ind}$ methylated mRNA's similarly treated and run in parallel. Marker nucleotides and nucleosides were included in each sample, and their positions after electrophoresis are shown.*

separate portion of $^3$H-labeled mRNA's was treated with nuclease P$_1$, nucleotide pyrophosphatase, and bacterial alkaline phosphatase and analyzed by paper electrophoresis (Fig. 4C). The radioactivities migrated towards the cathode with the markers m7G and A$^m$. These results were identical to those found for the methylated mRNA's from VSV$_{Ind}$ analyzed similarly. From the above results, it can be concluded that the 5'-terminal-blocked structure of methylated mRNA's from VSV$_{NJ}$ is m7G(5')ppp(5')A$^m$..., identical to the corresponding structure present in VSV$_{Ind}$ mRNA's.

To determine the 5'-terminal sequence of the VSV$_{NJ}$ mRNA species, the purified 12 to 18S mRNA's labeled with [$^3$H]methyl from [$^3$H] AdoMet was treated in separate portions with RNase T$_2$, RNase A, and RNase T$_1$, and the products from each digestion were analyzed by DEAE-cellulose chromatography in 7 M urea (25). As shown in Fig. 5A, digestion by RNase T$_2$ resulted in a single peak of radioactivity which eluted at a net charge of −5, consistent

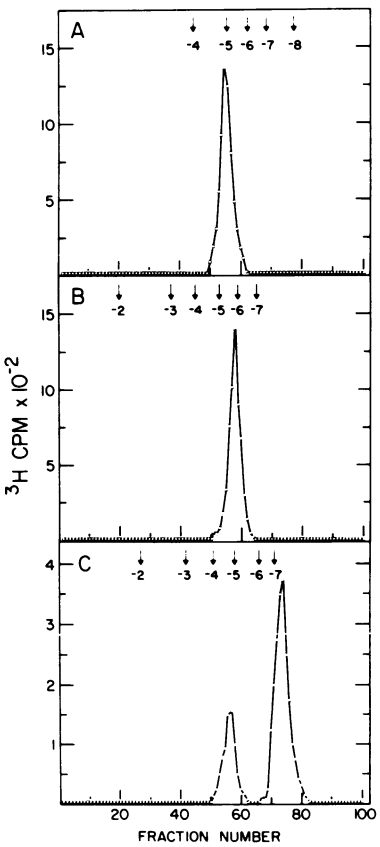


FIG. 5. *DEAE-cellulose chromatography of $^3$H-methylated RNA products following enzymatic digestions. VSV$_{NJ}$ mRNA was synthesized using [$^3$H]SAM as the labeled substrate; the 12–18S poly(A)-containing mRNA was purified, and a portion was digested with RNase T$_2$ and analyzed by DEAE-cellulose chromatography (A) as described (3). Aliquots treated with RNase A (B) or RNase T$_1$ (C) were analyzed in the same way. Arrows indicate the elution positions of marker oligonucleotides expressed as their net charges.*

with the 5′-terminal sequence m7G(5′)ppp-(5′)A$^m$-Np (where N = purine or pyrimidine). Treatment with RNase A released an oligomer which eluted at a charge of −6, a single nucleotide larger than the RNase $T_2$ product (Fig. 5B). The 5′-terminal oligomer can thus be depicted as m7G(5′)ppp(5′)A$^m$-N-Pyp. Treatment with RNase $T_1$ released two peaks of radioactivity (Fig. 5C); the predominant species (85%) eluted ahead of −7 charge and the minor one eluted at −5 charge. The sequence of the larger oligomer should be m7G(5′)ppp(5′)A$^m$-N-Py-N-Gp. The smaller one was also subsequently identified as m7G(5′)ppp(5′)A$^m$-Ap... (see below).

To identify the base N in Fig. 5A, mRNA's were synthesized in vitro in the presence of [α-$^{32}$P]ATP and [$^3$H]AdoMet and purified. The labeled RNA was then treated with RNase $T_2$, and the digestion product was analyzed by DEAE-cellulose chromatography as detailed in Fig. 5A. A radioactivity peak containing $^3$H and $^{32}$P eluted at −5 (data not shown), and the oligonucleotide was purified. It was then treated with nuclease $P_1$, and the products were analyzed by paper chromatography on Whatman 3MM paper using isobutyric acid and $NH_4OH$ (see Fig. 7). Two radioactivity peaks were obtained and identified as m7G(5′)pp$^*$p(5′)A$^m$ and $^*$pA (data not shown). From these results, it was concluded that the base N (Fig. 5A) is adenosine, and the overall 5′-terminal sequence can be depicted as m7G(5′)ppp(5′)A$^m$-A-Py-N-G.

The Py in the sequence was confirmed to be cytosine in the following experiments. $VSV_{NJ}$ mRNA's were synthesized in vitro in the presence of [$^3$H]AdoMet and [α-$^{32}$P]CTP or [α-$^{32}$P]UTP as the labeled substrates and purified as described in Materials and Methods. The labeled RNA's were then digested with RNase $T_2$ and chromatographed in DEAE-cellulose columns. As shown in Fig. 6A, a single peak of $^3$H radioactivity containing the methylated 5′-capped oligomer eluted at a charge of −5 (see also Fig. 5A). On the other hand, the $^{32}$P radioactivity, derived from [α-$^{32}$P]CTP, was distributed into two peaks; one eluted at −2 charge containing labeled 5′-monophosphates, and the second peak coeluted with the $^3$H radioactivity. This result indicates that the 5′-terminal sequence is m7G(5′)ppp(5′)A$^m$-A$^*$p-Cp where the RNase $T_2$ released m7G(5′)ppp(5′)A$^m$-A$^*$p with $^{32}$P transferred from the nearest neighbor cytosine. That the $^{32}$P was indeed at the 3′ side of the adenosine was shown by its total sensitivity to alkaline phosphatase (data not shown). In contrast to [α-$^{32}$P]CMP-labeled RNA, when [α-$^{32}$P]UMP-labeled (and also [*methyl*-$^3$H]-labeled) RNA was analyzed identically, only the $^3$H label

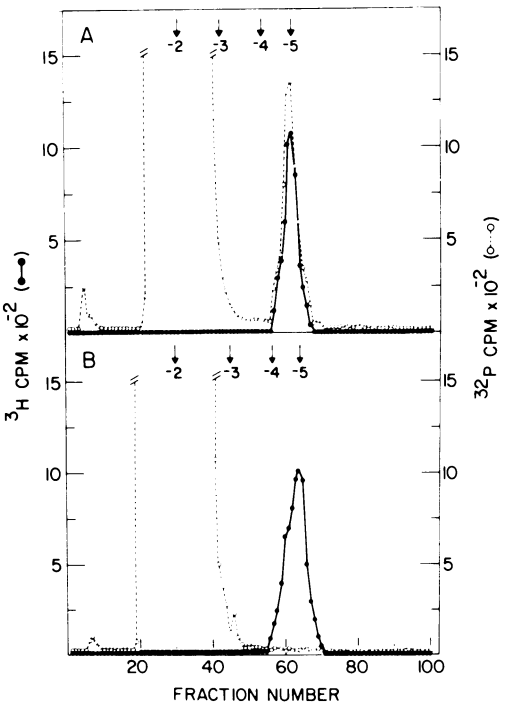


FIG. 6. *DEAE-cellulose chromatography of methylated RNA species after RNase $T_2$ digestion. $VSV_{NJ}$ mRNA was synthesized in vitro using [$^3$H]SAM and either (A) [α-$^{32}$P]CTP or (B) [α-$^{32}$P]UTP as the labeled substrates. The poly(A)-containing 12 to 18 S mRNA was isolated, digested with RNase $T_2$, and analyzed by DEAE-cellulose chromatography as described (3).*

eluted at a charge of −5, but no $^{32}$P was found coeluting at that position (Fig. 6B). This result eliminates the possibility that uridine may be present in the place of cytosine in some of the RNA molecules.

Thus, the 5′-terminal sequence of the $VSV_{NJ}$ mRNA's can now be depicted as m7G-(5′)ppp(5′)A$^m$-A-A-C-N-Gp.... To identify N, mRNA's were synthesized in the presence of [$^3$H]AdoMet and [α-$^{32}$P]GTP and purified. The labeled RNAs were then treated with RNase $T_1$, the products were chromatographed on DEAE-cellulose column, the radioactivity peak containing $^3$H and $^{32}$P eluting between −7 and −8 was pooled, and the oligonucleotide was purified by dialysis (data not shown). The labeled oligomer was then chromatographed on DBAE-cellulose column, which bound only the 5′-terminal-capped $T_1$ fragment (25). The $T_1$ fragment was eluted and purified (25) (data not shown). The fragment was then treated with RNase $T_2$ and analyzed by paper chromatography. There were two radioactivity spots: one containing both $^3$H

and $^{32}P$ migrating with pG and pU and the other containing only $^{32}P$ migrating with pA (Fig. 7). The former spot was eluted from the paper, and its structure was confirmed to be m7G(5')$\overset{*}{p}$pp(5')$A^m$-Ap; the latter spot was identified as $\overset{*}{A}p$ (data not shown). These results provide the complete 5'-terminal hexanucleotide sequence of $VSV_{NJ}$ 12 to 18S mRNA's as m7G(5')ppp(5')$A^m$-A-C-A-G. This sequence is identical to the corresponding sequence of the mRNA's synthesized in vitro by $VSV_{Ind}$.

It should be pointed out that the minor peak of radioactivity (Fig. 5C) eluting at −5 charge following RNase $T_1$ digestion was identified as m7G(5')ppp(5')$A^m$-Ap. This was achieved by labeling the in vitro mRNA's with [$^3$H]AdoMet and [$\alpha$-$^{32}$P]ATP. The purified mRNA was then treated with RNase $T_1$, and the material eluting at −5 charge (see above the processing of the radioactivity peak of 5A) was purified and treated with nuclease $P_1$ (data not shown). Analysis by paper chromatography revealed the presence of two radioactivity peaks, and both were identified as m7G(5')pp$\overset{*}{p}$(5')$A^m$ and $\overset{*}{p}$A (data not shown). Similar experiments using [$\alpha$-$^{32}$P] GTP and [$^3$H]AdoMet showed the presence of only one radioactive spot, m7G(5')$\overset{*}{p}$pp(5')$A^m$. These results indicate that the oligonucleotide present in the minor peak in Fig. 5C is indeed m7G(5')ppp(5')$A^m$-Ap. The reason for RNase $T_1$ cleaving at the adenosine site is unclear. The most probable explanation is that the commercial batch of RNase $T_1$ used may have contained trace amounts of RNase $T_2$-like activity. This was supported by the fact that the amount of the minor peak varied (5 to 20%) with the amount of RNase $T_1$ used for digestion.

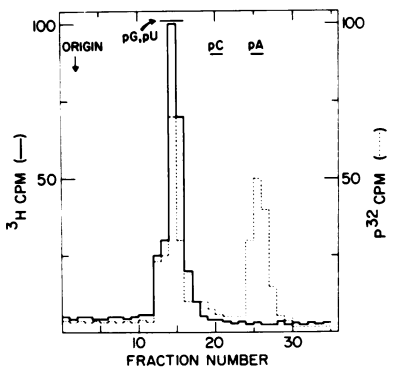


FIG. 7. *Analysis of methylated 5'-terminal $T_1$ fragment labeled with [$\alpha$-$^{32}$P]GTP. Poly(A)-containing $^3$H-methylated 12 to 18S RNA was synthesized with [$\alpha$-$^{32}$P]GTP and treated with RNase $T_1$, and the 5'-terminal RNase $T_1$ fragment was purified by DBAE-cellulose chromatography as described previously (25). The fragment was then treated with RNase $T_2$, and the digest was analyzed by descending paper chromatography (25).*

## DISCUSSION

In this communication, we have shown that the virion-associated RNA polymerase of the New Jersey serotype of VSV synthesizes in vitro five distinct RNA species (Fig. 2) similar to those made in the genetically distinct Indiana serotype of VSV (17). The sedimentation profile of the $VSV_{NJ}$ mRNA species is similar to the corresponding profile for the $VSV_{Ind}$ mRNA species (Fig. 2). It has been previously observed with $VSV_{Ind}$ that the relative amounts of the different mRNA species synthesized correspond to the gene order, i.e., N > NS > M > G > L (2). The similarity of the sedimentation profile of the mRNA's of both serotypes of VSV suggests that the gene order of $VSV_{NJ}$ may be the same as the gene order of $VSV_{Ind}$. Since six gene functions have been assigned to $VSV_{NJ}$ by complementation tests with various temperature-sensitive mutants (21), the presence of five in vitro mRNA species suggests that an additional mRNA species may be synthesized in vivo, but its corresponding gene is not transcribed in vitro. It is interesting to note that three mRNA species of $VSV_{NJ}$ tentatively assigned as G-, M-, and NS-mRNA's are smaller than the corresponding mRNA's of $VSV_{Ind}$ (Fig. 3). Since the genome RNA of $VSV_{NJ}$ is larger than the genome RNA of $VSV_{Ind}$ (26), the above results indicate that $VSV_{NJ}$ genome RNA may still have room for an additional gene, which may account for the sixth complementation group observed.

The synthesis of distinct mRNA species of $VSV_{NJ}$ gave us an opportunity to analyze the presence of sequence homology, if any, between the mRNA's of both the serotypes. When hybridization experiments were carried out with mRNA species of one serotype and the genome RNA of the other serotype, it was clear that virtually no sequence homology existed in the mRNA species (Table 1). These results confirm the results obtained previously using mRNA species from virus-infected cells (19, 23). It should be noted that a consistent and reproducible level of RNase-resistant values (5 to 10%) were always obtained, which may reflect the presence of small stretches of homology between the mRNA's and, conversely, between the genomes of two serotypes.

Despite lack of the overall sequence homologies detectable by RNA-RNA hybridization, it is apparent from experiments shown in this paper that some sequence homologies are present in the mRNA species of the two serotypes. The 5' termini of $VSV_{NJ}$ mRNA species are blocked,

methylated (Fig. 4), and polyadenylated, indicating that, like $VSV_{Ind}$, the purified virions of $VSV_{NJ}$ contain blocking, methyltransferase, and poly(A) polymerase activities. Moreover, the 5′-terminal hexanucleotide sequence of the methylated $VSV_{NJ}$ 12 to 18S mRNA species was found to be identical to m7G(5′)ppp(5′)$A^m$-A-C-A-G. This is identical to the corresponding sequence in the $VSV_{Ind}$ mRNA's (25). These results indicate that the mRNA species of the two serotypes do share some sequence homology, and, more importantly, they are shared at a specific site of the mRNA species. It should be pointed out that the relative amounts of 17S mRNA constitutes approximately 10% of the total 5′ termini present in the 12 to 18S mRNA (see Fig. 3). The 14.5S and 12S constitutes approximately 50 and 40%, respectively, of the 5′ terminus. Thus, if the 17S mRNA has a different 5′-terminal sequence, it might not be detected by the sensitivities of the method used. The 5′-terminal site of the mRNA species is the presumed "processing" site where individual mRNA species are cleaved off from a putative precursor (5). The common base sequence at that site may indicate that both the serotypes of VSV may use protein components that contain common active sites for recognition of the sequence for processing of mRNA's. Thus, the proteins of two serologically distinct viruses may contain similar amino acid sequences at specific regions of the polypeptide. This may explain the observed partial cross-reactivity of the antibodies prepared against the ribonucleoprotein cores of the two viruses (11). The nucleotide sequences adjacent to the poly(A) of the mRNA species of both the serotypes may also throw light on recognition sequences around the processing sites, and studies are underway to determine these sequences. As shown in the accompanying paper, the leader RNAs synthesized by the purified virions of both serotypes contain the identical 5′-trinucleotide ppA-C-G, and the genome RNAs both contain the 3′-terminal sequence, Py-G-$U_{OH}$. This result suggests that the genome RNA of both the serotypes may contain sufficiently similar 3′-terminal sequences for the transcriptase of either serotype to bind and initiate transcription. The overall importance of these common sequences are discussed in the accompanying paper.

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