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RNase III Cleaves Vesicular Stomatitis Virus Genome-Length RNAs but Fails to Cleave Viral mRNA's

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The procaryotic RNA processing enzyme RNase III (endoribonuclease III [EC 3.1.4.24]) was used to probe vesicular stomatitis virus (VSV) RNAs for specific sites that could be recognized and cleaved. The effect of the enzyme on the RNAs was monitored by measuring their subsequent migration in denaturing agarose-urea gels. VSV virion RNA (negative strand; M_r, 4×10^6) was cleaved by the enzyme to yield a set of discrete fragments which ranged in size from 3.5×10^6 to 0.2×10^6 daltons. The cleavage was a function of enzyme concentration, salt concentration, and time. A maximum of 20 to 22 fragments was generated under conditions of low enzyme concentration or short times of incubation. VSV genome-length intracellular RNA of both + and − polarity was also cleaved by RNase III. In contrast to the findings with virion-length RNA, however, the migration rates of VSV mRNA's purified by chromatography on polyuridylic acid-Sepharose were unaffected by treatment with RNase III. These results show that specific sites in the virion RNA and its full-length complement can be recognized by RNase III. Sites of this type are not present in the polyadenylic acid-containing mRNA, however.

Vesicular stomatitis virus (VSV), a rhabdovirus, contains a single strand of RNA having a molecular weight of 4×10^6 that is of antimessage (negative) polarity (19). The virus replicates in the cytoplasm of infected cells and uses a virus-coded RNA-dependent RNA polymerase to transcribe five mRNA's, each of which codes for one of the five virion proteins (10, 13). Transcription occurs in a sequential fashion beginning at the 3′ end of the genome, with the synthesis of a short leader RNA preceding the synthesis of the mRNA's (1, 2, 4). At present it is not known whether each of the five messages is initiated independently in a sequential fashion or whether one initiation event occurs followed by synthesis and processing, perhaps by cleavage, of a precursor molecule. Transcription can occur in vitro or in the cell in the presence of cycloheximide (22). Under either of these conditions, no larger precursor RNAs are detected. A full-length virion complementary (vc) RNA is found, however, in infected cells after viral protein synthesis occurs. This vc RNA has been implicated as the template for replication of the virion negative strand (21). The relationship between the synthesis of this vc RNA and the synthesis of the discrete mRNA's and the leader RNA is unknown.

Enzymes that function specifically to process RNA made from DNA templates have been identified in bacterial and animal cells (6). RNase III (endoribonuclease III [EC 3.1.4.24]) from *Escherichia coli* is one such enzyme. Site-specific cleavages by RNase III, for example, are responsible for generating the individual early mRNA's of bacteriophage T7 (8, 9). The cleavage takes place at specific processing signals that are present in the RNA. Available evidence from sequence data of RNase III cleavage sites indicates that processing sites may include one of several specific sequence elements (15, 16, 18). In addition to specific cleavage of single-stranded (ss) RNAs, it has also been shown that completely double-stranded (ds) RNA is a substrate for RNase III. The enzyme extensively digests ds RNA but digestion is not sequence specific. RNase III cleaves ds RNA to ss chain lengths of approximately 15 nucleotides, but the mere presence of short regions of double strandedness in a molecule is not sufficient to promote cleavage (5, 15, 17).

Based on the above findings and the sequence analysis of RNase III processing sites (16, 18), Robertson (15) has proposed that RNase III cleavage may involve a complex reaction in which a certain primary sequence may act in combination with particular RNA structural features of either hydrogen-bonded or non-hydrogen-bonded character to signal cleavage. We have examined VSV RNAs for the presence of

a cleavage site(s) that may be recognized by RNase III. In this communication we report that RNase III can recognize and cleave at discrete sites in VSV virion RNA and in full-length vc RNA. VSV mRNA's, however, contain no sites that are attacked by this enzyme.

MATERIALS AND METHODS

Cells and virus. Baby hamster kidney (BHK) cells, clone 21, were grown as described previously (21). Wild-type VSV, Indiana serotype, was propagated as described by Wertz and Levine (22).

Preparation of radiolabeled RNA. (i) ^{32}P-labeled 40S virion RNA. Confluent monolayers of BHK cells were infected with wild-type VSV at an input multiplicity of 0.02. At 1 h postinfection, phosphate-free minimal essential medium containing 2% calf serum and 67 μCi of $^{32}P_i$ per ml was added, and incubation was continued at 37°C for 15.5 h. The clarified culture supernatants were extracted once with trifluorotrichloroethane and centrifuged for 90 min at 54,450 × *g*. The virus pellet was dissolved in 1% sodium dodecyl sulfate (SDS) and extracted twice with phenol. Virion RNA, concentrated by ethanol precipitation, was further purified by sedimentation through a 15 to 30% (wt/wt) sucrose gradient (21). The virion RNA had a sedimentation coefficient of 40S relative to rRNA markers.

(ii) ^{32}P-labeled intracellular 40S RNA. Confluent monolayers of BHK cells, infected with VSV at an input multiplicity of 1, were incubated at 37°C in phosphate-free minimal essential medium containing 2% calf serum and 4 μg of actinomycin D per ml. At 3 h postinfection, the culture medium was replaced with phosphate-free minimal essential medium containing 2% calf serum and 600 μCi of $^{32}P_i$ per ml. After 2 h of incubation, a cytoplasmic extract was prepared, brought to 1% SDS, and centrifuged through a 15 to 30% (wt/wt) sucrose gradient (21). The 40S peak was collected, ethanol precipitated, and purified by sedimentation through a second 15 to 30% sucrose gradient.

(iii) ^{32}P-labeled VSV mRNA's. VSV RNA synthesized in actinomycin D-treated BHK cells was labeled with ^{32}P as described above. The cytoplasmic extract (21) was brought to 1% SDS and 0.1 M NaCl and extracted with phenol-chloroform. The ethanol-precipitated RNA was dissolved in buffer (10 mM Tris-hydrochloride [pH 7.4], 10 mM EDTA, 0.2% SDS) containing 0.4 M NaCl and adsorbed to a polyuridylic acid [poly(U)]-Sepharose column at 23°C. After washing with buffer containing no NaCl and with buffered 10% formamide, the polyadenylic acid [poly(A)]-containing RNA was eluted with buffered 90% formamide and collected by ethanol precipitation.

(iv) ^{14}C-labeled VSV intracellular RNA. VSV RNA synthesized in actinomycin D-treated BHK cells was labeled with [^{14}C]uridine (2 μCi/ml) between 3 and 5 h after infection. RNA was purified from cytoplasmic extracts as described previously (21).

Purification of *E. coli* RNase III. RNase III was purified from *E. coli* strain MRE-600 by the method of Dunn (7) as modified by Leis et al. (11). Enzyme assays were performed at 30°C by the method of Dunn (7), using ^{3}H-labeled poly(U) substrate alone or hybridized to unlabeled poly(A) to test for and differentiate between activity against ss or ds molecules, respectively. Briefly, the 100,000 × *g* supernatant from a DNase-treated lysate was passed through a DE52 column equilibrated with buffer A containing 0.1 M KCl. Those effluent fractions which contained at least 3.5-fold as much ds-specific RNase activity as ss-specific RNase activity were loaded onto a polyinosinic acid·polycytidylic acid-Sepharose column. The ds-specific RNase activity which was eluted from the column with 2 M NH_4Cl was found to contain no detectable ss-specific activity when incubated either with ^{3}H-labeled poly(U) or with ^{32}P-labeled VSV mRNA. In some cases, the RNase III was further purified by sedimentation through a 20 to 40% (vol/vol) glycerol gradient (11). Comparable results were obtained with and without the gradient step. Purified RNase III was stored in 1 to 1.48 M NH_4Cl–30% glycerol at −20°C.

Digestion of RNA by *E. coli* RNase III. RNase III reaction mixtures of 0.1 ml contained 10 mM Tris-hydrochloride (pH 7.5), 0.1 mM EDTA, 1 mM dithioerythritol, 10 mM $MgCl_2$, RNA, and NH_4Cl as indicated. Enzyme [2 to 3 μl containing about 3 to 5 U of RNase III or enough to digest 3 to 5 nmol of nucleotide in 1 h at 30°C, using poly(U)·poly(A) as substrate] was added immediately before incubation at 37°C. The reaction was stopped by dilution into 1 ml of buffer containing 0.5% SDS and 23 μg of yeast tRNA carrier and immediate precipitation with 2 volumes of ethanol at −20°C.

Gel electrophoresis. RNase III reaction products and RNA markers were analyzed by electrophoresis on 1.5% agarose horizontal slab gels containing 6 M urea and 0.025 M sodium citrate buffer, pH 3.5 (12). Samples were dissolved in 20 μl of 0.0025 M sodium citrate (pH 3.5)–6 M urea–20% sucrose–0.005% bromophenol blue and subjected to electrophoresis in the cold for 18.5 h at 150 V (5 V/cm). Urea was removed from the gel by three 20-min washes in cold gel buffer. The gel was then dried onto Whatman 3MM paper and used to expose du Pont Cronex 2DC X-ray film for 48 to 96 h. In some cases a du Pont Cronex Hi-plus intensifying screen was used at −70°C for 24 to 48 h.

RNA-RNA hybridization. Conditions for annealing of RNA, preparation of reagents, and assay of hybridization were exactly as described previously (21).

Materials. $^{32}P_i$, carrier-free, was obtained from New England Nuclear Corp. [^{14}C]uridine was purchased from Moravek Biochemical. ^{3}H-labeled poly(U) was obtained from Schwarz/Mann (5 cpm/pmol) or from Miles Laboratories, Inc. (33 cpm/pmol). Unlabeled poly(A) (162 optical density units per ml) was a gift of Jonathan Leis, Duke University Medical Center. Poly(U)-Sepharose was a gift of Steven Bachenheimer of this department. Formamide was purchased from MCB, SeaKem agarose was from Marine Colloids, Inc., and urea was from Mallinckrodt. Reovirus RNA was a gift of Gary Cobon, Duke University Medical Center.

RESULTS

Effect of incubation with RNase III on VSV virion RNA. *E. coli* RNase III is known

to recognize and cleave specific sites in ss RNA. The exact specificity of the cleavage of ss RNA is not known. Current evidence indicates that both secondary structure and specific sequence may be involved (5, 15–18). We have used RNase III to probe VSV RNAs for the presence of specific cleavage sites.

We have found that VSV virion RNA labeled with ^{32}P and incubated with RNase III is cleaved. The result of this cleavage is the generation of a characteristic and reproducible pattern of size classes of RNA. None of the ^{32}P-labeled RNA was rendered acid soluble by incubation with the enzyme at salt concentrations of 1.0 to 1.5 M. The extent of digestion was a function of enzyme concentration, salt concentration, and time of incubation.

Enzyme concentration. Purified ^{32}P-labeled VSV virion RNA was incubated with RNase III at various concentrations. The products of the enzyme digestion were analyzed by electrophoresis in 1.5% agarose gels containing 6 M urea. The gels were dried, and the relative migration of the ^{32}P-containing products was detected by autoradiography (Fig. 1). The unincubated 40S negative-strand virion RNA and virion RNA incubated in the standard reaction mixture with no enzyme migrated in the denaturing gels with an apparent molecular weight of 4×10^6. Exposure of the virion RNA to RNase III resulted in the cleavage of the virion-length molecule into a set of molecules all of which migrated more rapidly than did virion RNA. A maximum of 20 to 22 discrete fragments, which ranged in size from approximately 3.5×10^6 to 0.2×10^6 daltons, was discernible after digestion. As the concentration of enzyme was increased, all of the virion RNA substrate could be cleaved (Fig. 1e). The disappearance of all 40S substrate is referred to as complete cleavage in this case. This implies that all 40S molecules have at least one site that has been recognized and cleaved. Also, as the enzyme concentration increased, there was a disappearance of several of the high-molecular-weight bands and an increase in the amount of the smaller-molecular-weight fragments. This indicates that the higher-molecular-weight bands represent partial digestion products of the virion-length RNA.

Effects of monovalent salt concentration. ^{32}P-labeled virion RNA was digested with a constant amount of RNase III over a range of salt concentrations (Fig. 2). At a salt concentration of 0.1 M, a maximum of 20 to 22 discrete size classes of RNA was produced as a result of enzyme treatment. All full-length substrate was cleaved in a 5-min incubation. As the salt concentration was increased, a smaller number of discrete size fragments was generated, and these fragments were larger in size. To achieve digestion of all of the 40S substrate at higher salt concentrations, a longer incubation time was required. Digestion of all 40S substrate could be

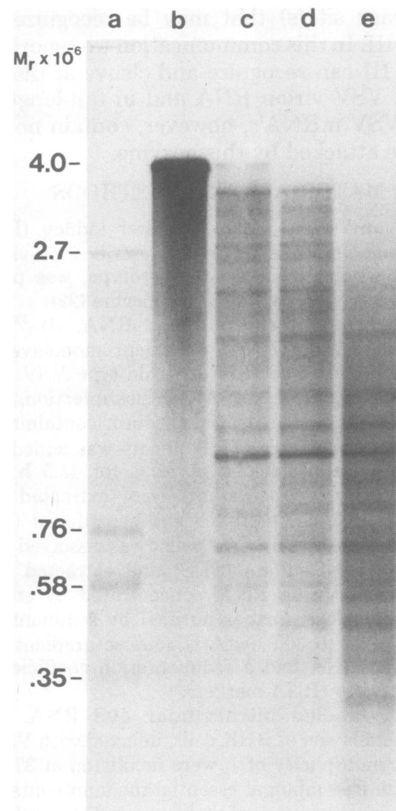

FIG. 1. *Gel electrophoresis of fragments produced by RNase III cleavage of VSV virion RNA. ^{32}P-labeled virion RNA was incubated with various amounts of RNase III in reaction buffer containing 0.14 M NH_4Cl at 37°C for 15 min. The reaction products were ethanol precipitated and analyzed as described in the text. (a) [^{14}C]uridine-labeled VSV intracellular RNAs (90,000 cpm) were subjected to electrophoresis for size references; (b) ^{32}P-labeled virion RNA (20,000 cpm) incubated without enzyme; (c) ^{32}P-labeled virion RNA plus 3 U of RNase III; (d) 8 U of RNase III; (e) 16 U of RNase III. Approximate molecular weight calculations in this gel system were made for the VSV mRNA's relative to the electrophoretic mobility of HeLa cell 28S and 18S rRNA markers that had been coelectrophoresed in adjacent wells in the same gel. When values of 1.7×10^6 and 0.67×10^6 daltons, respectively, were used for the rRNA's, as determined by Wellauer and Dawid by electron microscopy (20), the following molecular weight values were calculated for the VSV mRNA's: L, 2.7×10^6; G, 0.76×10^6; N, 0.58×10^6; NS and M, 0.35×10^6. These values are indicated in the left margin.*

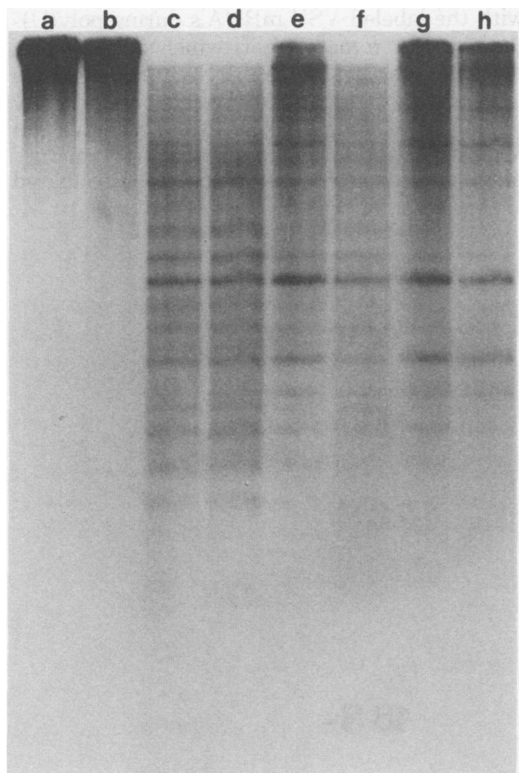

FIG. 2. *Effect of monovalent salt concentration on the cleavage of VSV virion RNA by RNase III. ^{32}P-labeled virion RNA was incubated with 5 U of RNase III at 37°C for the indicated times at various NH_4Cl concentrations. Reaction products were analyzed as described in the text. (a) ^{32}P-labeled virion RNA, unincubated control; (b) ^{32}P-labeled virion RNA, 20 min in 0.1 M NH_4Cl without RNase III; (c) 5 min in 0.1 M NH_4Cl plus RNase III; (d) 10 min in 0.1 M NH_4Cl plus RNase III; (e) 10 min in 0.15 M NH_4Cl plus RNase III; (f) 20 min in 0.15 M NH_4Cl plus RNase III; (g) 10 min in 0.2 M NH_4Cl plus RNase III; (h) 20 min in 0.2 M NH_4Cl plus RNase III.*

achieved in 5 min at 0.1 M NH_4Cl, whereas at 0.15 M NH_4Cl a 20-min incubation was required to achieve complete digestion. At 0.2 M NH_4Cl, complete digestion was not observed in a 20-min incubation. These results show that high salt concentrations are less favorable to digestion with RNase III and that under these conditions a greater proportion of high-molecular-weight partial digestion products are generated. At present it is not possible to determine whether this is due to an effect on the substrate or the enzyme. Dunn (7) has reported that the specificity of cleavage of T7 early mRNA's is influenced by ionic strength.

Digestion in the presence of ds RNA. In addition to specific processing of ss RNA, RNase III degrades ds RNA in a nonspecific manner (15, 17). It has been shown that the two enzyme activities copurify and that ds RNA can compete efficiently with the ss RNA processing activity (15, 17). The effect of the presence of excess ds RNA on the cleavage of VSV virion RNA by RNase III was examined. ^{32}P-labeled virion RNA was incubated with RNase III in the standard reaction mixture to which 6 μg of ds reovirus RNA had been added. In the presence of this concentration of reovirus RNA, cleavage of the VSV RNA was completely inhibited. The treated ^{32}P-labeled virion RNA migrated at the same rate as the untreated control (data not shown).

Digestion of VSV intracellular virion RNA and full-length vc RNA by RNase III. Infection of cells with VSV results in production of five small positive-polarity mRNA's which code for the five viral proteins. In addition to the mRNA's, a complete virion-length complementary strand of RNA (vc) is produced. This vc strand has been implicated as the template for replication of the negative strand (21). The preceding results have shown that the VSV virion RNA contains sites which are recognized and cleaved by RNase III. It is possible that the vc RNA has sites which, because of similarities in secondary structure and perhaps in sequence, will be recognized and cleaved by RNase III. The following experiments were done to determine whether 40S vc RNA contained sites for RNase III cleavage. Virion-length RNA was isolated from infected cells and purified by two cycles of sedimentation through sucrose velocity gradients. The polarity of this RNA was analyzed by hybridization to an excess of cold virion (negative strand) or messenger (positive strand) RNA. Approximately 13 to 16% of the intracellular 40S RNA was of positive polarity (Table 1). Attempts were made to purify the 40S vc RNA strand from a mixture of 40S intracellular RNAs. Morrison et al. (14) reported that 40S vc RNA contained poly(A) residues, in contrast to 40S virion RNA which did not. We attempted to purify 40S vc RNA by chromatography on poly(U)-Sepharose. Using the conditions described above for the preparation of VSV mRNA, we found that no 40S RNA bound after chromatography of infected-cell cytoplasmic extracts (see Fig. 4a). Since the pure 40S vc RNA could not be obtained by available techniques, we examined the effect of RNase III on 40S intracellular RNA containing both plus and minus strands.

The mixture of intracellular 40S RNA was digested with RNase III, and the products were examined on denaturing gels (Fig. 3). A pattern

TABLE 1. *Annealing of VSV 40S intracellular RNA*[a]

Sample	% RNase resistant
VSV 40S intracellular RNA, denatured, not annealed	3
VSV 40S intracellular RNA, denatured, annealed with:	
Self	25
mRNA[b] (5 μg)	86
mRNA (7.5 μg)	86
Virion RNA[c] (2 μg)	16

[a] VSV 40S intracellular RNA prepared as described in the text was isolated by two cycles of gradient centrifugation. The 40S RNA was collected by ethanol precipitation, suspended in buffer (0.01 M Tris [pH 7.6], 0.001 M EDTA), and denatured by heating to 100°C for 1.5 min, followed by immersion in an ice bath for 5 min. Samples containing 3,000 cpm each were adjusted to 0.3 M NaCl. Annealing conditions and RNase digestions were exactly as described previously (7).

[b] VSV mRNA was prepared, purified by chromatography on cellulose CF-11, and characterized exactly as described previously (7). The concentration used here was greater than fivefold in excess of that needed to achieve saturation.

[c] VSV virion RNA was extracted, purified, and characterized as described previously (7). The concentration used here was greater than fivefold in excess of that needed to achieve saturation.

of size fragments of RNA was generated after cleavage of the 40S virion and vc-strand RNAs which was similar to that generated by RNase III treatment of the 40S virion RNA. Complete cleavage of the material migrating as 40S RNA could be achieved. However, because of the small amount (13 to 16%) of 40S vc RNA present in the initial reaction mixture, it was not possible to determine whether the vc RNA alone generated the same pattern of cleavage products as did the virion strand; it was only possible to determine that all molecules in this mixture of negative and positive strands contained cleavage site(s).

RNase III does not cleave VSV mRNA. VSV mRNA was labeled with ^{32}P and purified by chromatography on poly(U)-Sepharose. This mRNA was 93% complementary to VSV genome RNA. The ^{32}P-labeled VSV mRNA was incubated with RNase III in the standard reaction mixture, and the products of the digestion were analyzed by gel electrophoresis (Fig. 4a and b). There was no alteration in the migration rate of the mRNA's after incubation with enzyme under conditions which digested purified virion RNA (Fig. 4c and d).

It is possible that an inhibitor and/or competitor of RNase III activity might have copurified with the labeled VSV mRNA's during poly(U)-Sepharose chromatography which subsequently blocked digestion of the mRNA. VSV mRNA's labeled with ^{32}P and isolated by chromatography on poly(U)-Sepharose were mixed with purified VSV virion RNA labeled with ^{32}P. The mixture of VSV virion RNA and mRNA was incubated

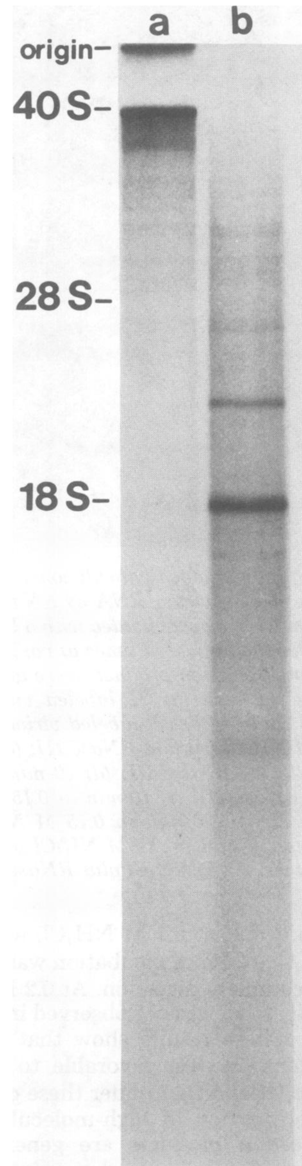

FIG. 3. *Gel electrophoresis of fragments produced by RNase III cleavage of VSV intracellular 40S RNA. Purified ^{32}P-labeled intracellular VSV 40S RNA was incubated at 37°C for 20 min in reaction buffer containing 0.14 M NH_4Cl without (a) or with (b) 8 U of RNase III. Reaction products were analyzed by gel electrophoresis as described in the text.*

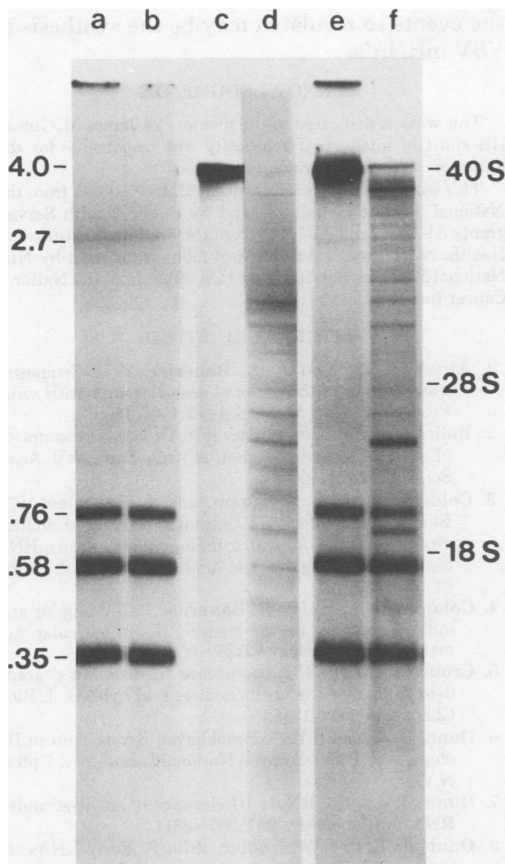

FIG. 4. *Effect of RNase III on electrophoretic mobility of VSV mRNA's. ^{32}P-labeled poly(A)-containing intracellular VSV mRNA's (17,300 cpm) were incubated at 37°C for 5 min in reaction buffer containing 0.13 M NH_4Cl with no RNase III (a) or with 8 U of RNase III (b). ^{32}P-labeled virion RNA (22,500 cpm) was incubated at 37°C for 5 min in reaction buffer containing 0.13 M NH_4Cl with no RNase III (c) or with 8 U of RNase III (d). ^{32}P-labeled poly(A)-containing VSV mRNA's (17,300 cpm) and ^{32}P-labeled VSV virion RNA (22,500 cpm) were mixed and incubated at 37°C for 5 min in reaction buffer containing 0.13 M NH_4Cl with no RNase III (e) or with 8 U of RNase III (f). Reaction products were analyzed by gel electrophoresis as described in the text. Approximate molecular weights for VSV mRNA's in this gel system were calculated as described in the legend to Fig. 1 and are indicated in the left margin; the positions of 28S and 18S rRNA markers coelectrophoresed in the same gel are indicated in the right margin.*

in the standard reaction mixture with (Fig. 4f) or without (Fig. 4e) added RNase III. The products of the incubation were analyzed by electrophoresis in agarose-urea gels.

The data presented in Fig. 4f show that in the mixture of virion and mRNA the virion RNA was digested by RNase III to yield a set of discrete size classes of RNA molecules. The pattern of fragments generated was essentially the same as that generated by RNase III cleavage of virion RNA alone (Fig. 4c and d). However, the migration rate of the VSV mRNA's present in the same reaction mixture was unaffected by incubation with RNase III. This experiment has been repeated with ^{14}C-labeled virion RNA and ^{32}P-labeled mRNA's so that the molecules can be discriminated before and after cleavage, and the results are essentially the same (data not shown). These results demonstrate that RNase III cleavage sites exist in VSV virion RNA; these sites are not present in VSV mRNA.

DISCUSSION

VSV virion RNA and full-length vc RNA can be cleaved in vitro by RNase III. VSV mRNA's are not attacked by this enzyme. These results will be considered from both a physical and a biological perspective.

First, from the physical point of view, these studies have allowed us to determine that an enzyme (RNase III) exists which can cleave VSV virion RNA into a series of discrete size fragments. Cleavage of VSV RNA by this enzyme is not random; VSV mRNA's are not cleaved by RNase III. These findings offer a powerful new tool for obtaining specific fragments of VSV genome RNA for use in mapping studies and for use in sequencing of VSV RNAs.

From a biological point of view, these studies have identified another measurable difference between VSV virus-specific RNA molecules. These findings show that the sequence(s) and/or structural features necessary to signal cleavage by RNase III are present in VSV genome-size RNAs but not in mRNA's. These findings raise the possibility that RNase III may be cleaving VSV RNA at intercistronic sites.

Cleavage of the 4×10^6-molecular-weight virion RNA generates a maximum of 20 to 22 fragments discernible on 1.5% agarose-urea denaturing gels under conditions of low enzyme concentration or short times of incubation. Longer times of incubation or a higher enzyme concentration results in the disappearance of some higher-molecular-weight bands and the appearance of only 14 to 16 fragments. Among the set of fragments generated, there are fragments which migrate with the apparent molecular weight one would predict for VSV mRNA's lacking their 3'-poly(A) residues. The majority of the remaining fragments generated are of an apparent size to represent partial digestion products of the full-length molecule.

Previous work using the technique of UV in-

activation of gene expression has shown that VSV transcription occurs in an ordered sequential fashion (1, 2). The observation that transcription occurs sequentially led to the postulation that syntheses of mRNA and 40S vc RNA might be related processes (1, 2). In particular, it was suggested that, in the case of mRNA synthesis, transcription is initiated at the 3′ end of the genome and that monocistronic mRNA's are generated by a processing mechanism that involves nucleolytic cleavage of the nascent positive strand. As a corollary, the genome-length vc RNA, which is required as a template for RNA replication, could be formed by lack of processing.

To test the hypothesis that RNase III cleavage occurs at intercistronic sites, it will be necessary to do two things. First, the VSV 40S vc RNA will have to be purified, and its RNase III cleavage products will have to be examined. Second, it will be necessary to map the fragments generated by RNase III cleavage of virion RNA by hybridization to purified VSV mRNA's and, correspondingly, to map the fragments generated by cleavage of 40S vc RNA on the viral genome. These studies are in progress.

At present, the relationship between transcription of discrete mRNA's and synthesis of the 40S vc RNA template for replication is unknown, as is the mechanism by which the two processes may be controlled. The active templates for transcription and replication are the RNA in a nucleocapsid structure. The control of these two processes then could involve not only recognition of specific RNA sequences and/or structural sites but also the specific interaction of viral proteins.

Obviously, the procaryotic enzyme RNase III employed here does not play a functional role in processing of VSV RNAs in vivo. However, using this enzyme as a probe, we have observed that recognition sites for this enzyme exist in the genome-size RNAs but not in the mRNA's. The information available at present indicates that RNase III recognition sites involve a certain primary sequence acting in combination with particular RNA secondary structural features (15, 16, 18). The findings reported here indicate that features required to signal RNase III processing are present in the virion RNAs and not in the mRNA's.

These findings lead us to postulate that primary-sequence-determined secondary structural features exist in VSV RNAs and that these features alone or in combination with the binding of specific viral proteins may play a role in the regulation of RNA synthetic events. One of the events so regulated may be the synthesis of VSV mRNA's.

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