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Synthesis of Complementary RNA Containing Polyadenylic Acid by Sendai Virions In Vitro

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Sendai virus synthesized, in vitro, [^{32}P]AMP- and [^{3}H]AMP-labeled RNA that ranged in size from 3 to 25*S* with major peaks at 7*S* and 13*S*. Both labeled products were predominantly single-stranded RNA and were complementary in base sequence to 50*S* virion RNA. Passage of the 3 to 25*S* in vitro RNA transcripts through a polyuridylic acid-cellulose column revealed that only the larger (predominantly 18*S*) RNA transcripts contained polyadenylic acid [poly(A)] segments capable of binding to the column. After treatment with a combination of RNase A and T_1, the majority of the in vitro poly(A) sedimented at 6*S* although the product ranged in size from 3 to 9*S*. Proof that the RNase-resistant material was indeed poly(A) was obtained by nearest-neighbor analysis when 95% of the radioactivity was recovered in AMP.

The universal presence of polyadenylic acid [poly(A)] sequences on the 3′ terminus of eukaryotic mRNA has been firmly established in a wide variety of systems (9) with only two known exceptions, histone mRNA (1, 9) and a fraction of HeLa cell mRNA (22). In mammalian cells, poly(A) is added post-transcriptionally to heterogenous nuclear RNA by a poly(A) polymerase present in the nucleus. Polyadenylation appears to play a role in the processing of heterogeneous nuclear RNA into cellular mRNA (9), in the subsequent transport of cellular mRNA from the nucleus into the cytoplasm (9), and in the functional stability of mRNA (13, 27).

Messenger RNAs from several cytoplasmic viruses have also been shown to contain poly(A) segments after extraction from infected cells (11, 12, 24, 30, 34). The observation that three of these cytoplasmic viruses, vaccinia virus (14), vesicular stomatitis virus (3, 33), and Newcastle disease virus (34), synthesize mRNA containing poly(A) segments in vitro as well as in vivo indicates that polyadenylation of viral mRNA is not just a consequence of cellular infection, but rather may be a viral function. The fact that cytoplasmic RNA viruses, which do not transport mRNA from the nucleus to the cytoplasm, synthesize mRNA containing poly(A) may offer a uniquely different scheme for the synthesis and function of poly(A) than that found in mammalian cells.

We now report that Sendai virus, another cytoplasmic virus that has previously been shown to synthesize poly(A)-containing complementary RNA in vivo (19, 24), is also capable of synthesizing complementary RNA containing poly(A) segments in vitro. Size determination of the poly(A) segments synthesized by Sendai virus in vitro indicate that they are almost twice as large as those synthesized by Sendai virus in vivo.

MATERIALS AND METHODS

Virus purification. Virus used in this experiment was plaque purified from the Enders strain of Sendai virus and was free of incomplete virions (15). Sendai virus was grown in embryonated hen eggs as previously described (32). The virus was concentrated from allantoic fluid by differential centrifugation. First, cells and debris were removed in a Sorvall GSA rotor (4 C, 5 min, 8,000 rpm), and then the virus was pelleted in a Beckman 19 rotor (4 C, 1 h, 19,000 rpm). The virus was resuspended in 5 mM Tris-hydrochloride (pH 7.4)-1 mM EDTA (TE buffer) with the aid of a Dounce homogenizer (Kontes, small clearance) and then centrifuged in a Sorvall GSA rotor (4 C, 10 min, 5,000 rpm) to remove virus clumps. Concentrated virus was purified by rate-zonal sedimentation through a linear 15 to 30% (wt/wt) 33-ml sucrose gradient (TE buffered) containing a 3-ml 60% (wt/wt) sucrose cushion, using a Beckman SW27 rotor (4 C, 30 min, 27,000 rpm). The virus band was collected, diluted with TE buffer, and isopycnically banded in a 25 to 60% (wt/wt) 30-ml TE-buffered sucrose gradient, using a Beckman SW27 rotor (4 C, 16 h, 20,000 rpm). The virus band was collected and pelleted in a Beckman 30 rotor (4 C, 45 min, 30,000 rpm). The pellet was resuspended in 0.01 M Tris-hydrochloride (pH 7.3)-0.03 M NaCl, the protein concentration was determined according to the procedure of Lowry (18), using bovine serum albumin as a standard, and the virus was then stored at −70 C. Typical preparations contained 10 mg of protein per ml and incorporated

410 pmol of UTP per mg of protein during a 30-h incubation.

To obtain labeled Sendai virus genomes, 10-day-old embryonated eggs were infected with Sendai virus as previously described (31). After a 24-h incubation at 37 C, the infected eggs were inoculated with 0.2 ml (200 μCi) of [^{3}H]uridine (20 Ci/mmol) and incubated an additional 24 h at 37 C. Labeled virions were then harvested and purified as above.

Synthesis and purification of in vitro product RNA. Reaction mixtures contained 50 mM Tris-hydrochloride (pH 8.0), 90 mM NaCl, 4 mM magnesium acetate, 3 mM dithiotreitol, 0.08% (vol/vol) Triton N-101, 0.7 mM CTP, 0.7 mM GTP, 0.7 mM UTP, 0.09 mM ATP, and 15 μCi of [^{3}H]ATP per ml (26 Ci/mmol) or 9 μCi of [α-^{32}P]ATP per ml (21 Ci/mmol). Purified Sendai virus was added to the reaction at 1 mg of protein per ml. Reactions ranging in volume from 2 to 12 ml were incubated for 30 h at 24 C and then terminated by the addition of 0.5% sodium dodecyl sulfate (SDS). Product RNA was purified by sequential phenol extraction at pH 7 and 9 as described by Lee et al. (17) and isolated free of mononucleotides as previously described (8).

Gradient sedimentation of in vitro RNA. Labeled RNA samples were layered over a 10-ml 15 to 30% (wt/wt) linear sucrose gradient in 5 mM Tris-hydrochloride (pH 7.4), 1 mM EDTA, 0.5% (wt/vol) lithium dodecyl sulfate, and 0.1 M lithium chloride, which had been formed over a 0.6-ml 60% (wt/wt) sucrose cushion. Gradients were centrifuged in a Beckman SW41 rotor (20 C, 11 h, 32,000 rpm), and [^{14}C]uridine-labeled rRNA from chicken embryo fibroblasts was centrifuged in a parallel gradient as markers. The gradients were fractionated (0.4 ml) from the top by using an ISCO (Instrumental Specialties Co.) model 640 density gradient fractionator, and samples of each fraction were assayed for radioactivity, using 0.5 ml of water and 9.0 ml of PCS (Amersham/Searle).

Hybridization of in vitro RNA to Sendai virus 50S genomes. Unlabeled 50S genome RNA was obtained from purified Sendai virions by phenol-SDS extraction and purified by sucrose gradient centrifugation (Beckman SW41, 20 C, 3 h, 41,000 rpm). Labeled in vitro product RNA and unlabeled genome RNA were resuspended in 0.03 M sodium citrate (pH 7.0) and heat denatured. Sodium chloride was added to 0.3 M, and duplicate samples (20 μl) of the [^{32}P]AMP- and [^{3}H]AMP-labeled product RNA were each placed into Pasteur pipettes, which had been previously sealed at the tip with a flame. Unlabeled 50S genome RNA (2 μg in 10 μl) was added, the pipettes were flame sealed, and the RNA was annealed by incubation in an 80 C oil bath for 1 h. Similar aliquots of labeled in vitro product RNA were annealed without the addition of genomes to serve as self-annealing controls. The hybrids were taken up in 1 ml of 0.3 M NaCl-0.03 M sodium citrate, half of which was treated with 5 μg of RNase A per ml for 30 min at 20 C before trichloroacetic acid precipitation.

Binding to poly(U)-cellulose column. A polyuridylic acid [poly(U)]-cellulose column was prepared by a procedure similar to that of Sheldon et al. (28). The in vitro RNA products were denatured in 90% dimethyl sulfoxide (Me_2SO) and ethanol precipitated. RNA samples {1 μg of ^{14}C-labeled synthetic poly(A) (Miles), [^{14}C]uridine-labeled 28S chicken embryo fibroblast rRNA, and Sendai virus [^{3}H]AMP- or [^{32}P]AMP-labeled RNA} were dissolved in 2 ml of 0.01 M Tris-hydrochloride (pH 7.5)-0.1 M NaCl and loaded on the poly(U)-cellulose column equilibrated at 5 C with 10 ml of the same buffer. The column was first washed at 5 C with 15 ml of 0.01 M Tris-hydrochloride-0.1 M NaCl and then at 45 C with 15 to 25 ml of 0.01 M Tris-hydrochloride (pH 7.5). Fractions of 1 ml were collected at both temperatures at a flow rate of 0.25 ml/min, and aliquots (0.1 ml) of each fraction were assayed for radioactivity, using 10 ml of PCS (Amersham/Searle). RNA that bound poly(A) - and RNA that did not bind poly(A) - were ethanol precipitated for use in subsequent experiments.

Test for RNase resistance. Poly(A)+ and poly(A) - RNAs were resuspended in 0.2 ml of distilled water, denatured in a boiling water bath for 3 min, and immediately plunged into an ice bath. Tris and NaCl were added to the denatured RNA to bring the concentration to 0.5 M Tris-hydrochloride (pH 7.5-0.3 M NaCl in a final volume of 0.5 ml. Samples (50 μl) of each tube were assayed for trichloroacetic acid-precipitable RNA, and these values were used to determine the total radioactive RNA in each fraction. As a presumptive test for the presence of poly(A) sequences, the remainder of the RNA was incubated with 50 μg of RNase A and 2 μg (678 U) of RNase T_1 per ml at 37 C for 30 min and assayed for acid-precipitable radioactivity.

Size determination of in vitro poly(A). Sendai virus [^{32}P]AMP-labeled RNA that bound to the poly(U) column was resuspended in 0.8 ml of distilled water and treated with RNase A and RNase T_1 as described above. The digestion was terminated by the addition of 0.5% SDS. The RNase digest was phenol extracted, and mononucleotides were removed by chromatography as previously described (8). RNase-resistant RNA was resuspended in 0.6 ml of TE and layered over a 15 to 30% (wt/wt) sucrose gradient in TE buffer with 0.1 M NaCl and 0.5% (wt/vol) lithium dodecyl sulfate. The gradient was centrifuged in a Beckman SW41 rotor (20 C, 24 h, 41,000 rpm). As 4S markers, [^{14}C]uridine-labeled chicken embryo fibroblast tRNA and unlabeled f-met tRNA from *Escherichia coli* were centrifuged in a parallel gradient. After fractionation, 0.1-ml aliquots from each fraction (0.4 ml) were assayed for radioactivity, using 0.5 ml of water and 9.0 ml of PCS (Amersham/Searle).

Nearest-neighbor analysis of RNase-resistant RNA. The [^{32}P]AMP-labeled RNase-resistant RNA, sedimenting in the 5 to 9S region (see Fig. 3), was pooled and precipitated with ethanol. The RNase-resistant RNA was resuspended in 0.5 ml of 0.3 N KOH and hydrolyzed in an 80 C oil bath for 2 h. The base composition was determined by the method of East (10), using Dowex 50W-X4 (Bio-Rad) cation exchange resin and ascending chromatography on a thin-layer cellulose plate (Brinkmann CEL-PLATE-F-22). Using the four ribonucleotide monophosphates as markers, the positions of individual nucleotides were located under UV light. The plate

was divided into a number of fractions, the cellulose powder was scraped directly into counting vials containing 0.1 ml of water, and the vials were assayed for radioactivity by the addition of 1.25 ml of NCS (Amersham/Searle) and Liquifluor (New England Nuclear Corp.).

RESULTS

Sedimentation properties of AMP-labeled RNA. To detect sequences of poly(A) in the RNA synthesized in vitro by Sendai virions, the RNA products were labeled in reaction mixtures containing [^{3}H]ATP or [α-^{32}P]ATP. The RNA product was purified by phenol extraction chromatography on Sephadex to remove mononucleotides, and then sedimentation on sucrose gradients (Fig. 1). The [^{32}P]AMP- and [^{3}H]AMP-labeled RNA products showed similar sedimentation patterns, and both products of the Sendai virion polymerase reaction sedimented in three major peaks. The two peaks at the top of the gradient correspond to approximately 7S and 13S in relation to the 18S and 28S rRNA markers, though the product ranged in size from 3 to 25S. A third major peak, found on the cushion of the gradient, has previously been shown to be the transcriptive intermediate composed of newly synthesized complementary RNA hydrogen bonded to unlabeled 50S genome templates (32).

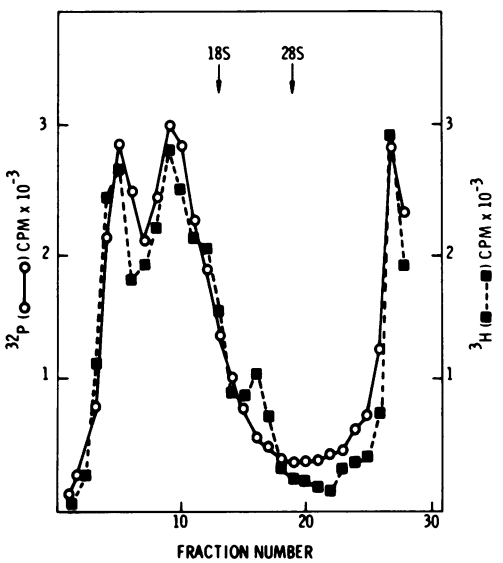

FIG. 1. *Sedimentation patterns of Sendai virus in vitro product RNA. Sendai virus [^{32}P]AMP- and [^{3}H]AMP-labeled RNAs synthesized in vitro were phenol-SDS extracted, chromatographed on a Sephadex G-50 column, and sedimented on a 15 to 30% linear sucrose gradient as described in the text. After collection of 0.4-ml fractions, 2.5-μl samples of each [^{32}P]AMP-labeled RNA fraction (○) and 15-μl samples of each [^{3}H]AMP-labeled RNA fraction (■) were assayed for radioactivity. The positions of [^{14}C]uridine chicken embryo fibroblast 18 and 28S rRNA are indicated by arrows.*

Both RNA products were virus specific. The [^{32}P]AMPand [^{3}H]AMP-labeled RNA that sedimented at 8S to 25S (Fig. 1, fractions 6–17) was pooled and ethanol precipitated. To demonstrate that the RNA products were virus specific, RNA from each gradient was either digested directly with RNase A or hybridized to purified Sendai virus 50S genome RNA before RNase digestion. The results (Table 1) illustrate that both [^{32}P]AMP- and [^{3}H]AMP-labeled products were predominantly single stranded as demonstrated by their sensitivity to RNase digestion. They were shown to be virus specific because approximately 90% of the [^{32}P]AMP-labeled RNA and of the [^{3}H]AMP-labeled RNA formed RNase-resistant hybrids with Sendai virus genomes.

Anomalous size of in vitro product. Although some 18S RNA species did occur on the shoulder of the 14S peak (Fig. 1), the size of the in vitro product (7S and 13S) was considerably smaller than the size (18S) of the major species of virus-specific RNA extracted from infected cells (4, 7). Predominantly 18S RNA has never been reported to be synthesized in vitro by Sendai virions (25, 31, 32). The smaller size of the RNA synthesized in vitro by Sendai virions could result from nuclease contamination, degraded genomes, differences in RNA configuration, or aberrant function of the virion-

TABLE 1. *Hybridization of labeled Sendai virus 8 to 25S in vitro product RNA with unlabeled Sendai virion 50S RNA*

Labeled RNA	Treatment	RNase resistance (%)
[^{32}P]AMP-labeled product RNA	Not annealed[a]	8.7
	Self-annealed[b]	10.4
	Annealed with 50S RNA genome[c]	99.8
[^{3}H]AMP-labeled product RNA	Not annealed[a]	9.4
	Self-annealed[b]	11.3
	Annealed with 50S RNA genome[c]	96.2

[a] Product RNA was treated directly with RNase A and T_1.

[b] Product RNA was first incubated under annealing conditions and then treated with RNase A and T_1.

[c] Product RNA was first annealed with 66 μg of 50S genome RNA per ml and then treated with RNase A and T_1.

associated RNA polymerase. Nuclease contamination could cause an accumulation of small RNA, although a nuclease does not appear to be responsible for the small product size obtained with Sendai virions for several reasons. Ribosomal 18S and 28S RNA remain intact when incubated in an RNA polymerase reaction (Fig. 2), and addition of the exogenous RNA failed to increase the product size. Addition of bentonite to the reaction mix or shortening the time of incubation did not increase the product size (data not shown).

Degraded genomes would also produce small RNA products. To test this possibility, Sendai virions were labeled with [^{3}H]uridine, purified as described in Materials and Methods, and then incubated in a [α-^{32}P]ATP reaction mixture. No degradation of the [^{3}H]uridine-labeled 50S genome was apparent (Fig. 3), yet the [^{32}P]AMP product was the same size as shown in Fig. 1. The 4 to 5S RNA at the top of the gradient has only been detected in radioactive virions when labeling is performed in the absence of actinomycin D and is presumed to be cellular in origin, probably the tRNA described by Kolakofsky (16).

An apparent small size could also result from conformational differences between RNA synthesized in vitro and in vivo, but denaturation of RNA with 90% (vol/vol) Me_2SO before centrifugation (Fig. 4) did not significantly alter the sedimentation coefficient (compare Me_2SO-

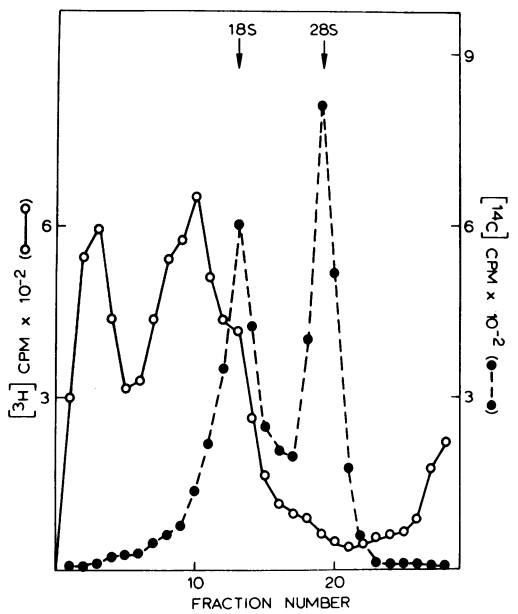

FIG. 2. *Incubation of 18 and 28S rRNA in a Sendai virus transcriptase reaction. A Sendai virus transcriptase reaction (0.2 ml) containing [^{14}C]uridine-labeled 18 and 28S rRNA from chicken embryo fibroblasts was incubated for 30 h at 24 C. The reaction contained [^{3}H]ATP to label the RNA product. The reaction mixture was phenol-SDS extracted and the RNA was sedimented on a linear 15 to 30% (wt/wt) sucrose gradient as described in the text. After fractionation, the RNA in each fraction was ethanol precipitated and assayed for [^{3}H]AMP-labeled product RNA (O) and ^{14}C-labeled 18 and 28S rRNA (●) by trichloracetic acid precipitation. Positions of standard unlabeled 18 and 28S ribosomal markers sedimented in a parallel gradient are indicated by the arrows.*

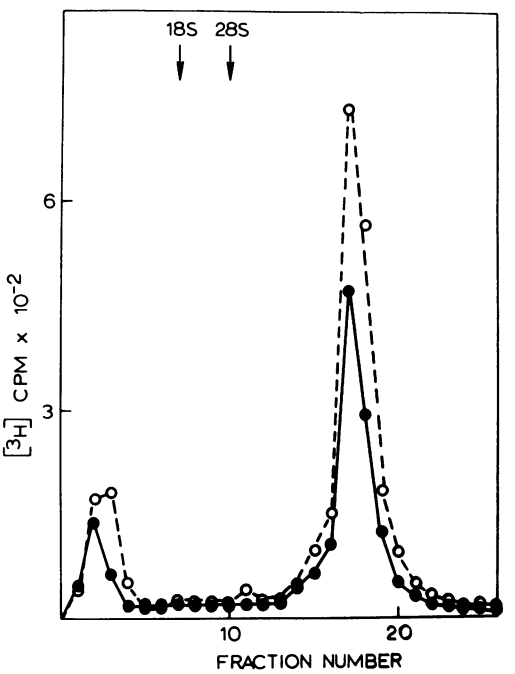

FIG. 3. *Sedimentation pattern of Sendai virus 50S genomes before and after incubation in a transcriptase reaction. Sendai virus was grown in eggs in the presence of [^{3}H]uridine, and the resulting labeled virus was purified as described in the text. A portion of the ^{3}H-labeled virions were either phenol-SDS extracted directly or incubated in a standard transcriptase reaction containing [^{32}P]ATP for 30 h at 24 C before extraction. Extracted RNA was then sedimented on a linear 15 to 30% (wt/wt) linear sucrose gradient in a Beckman SW41 rotor (20 C, 3.25 h, 41,000 rpm). Gradients were fractionated, ethanol precipitated, and assayed for trichloroacetic acid precipitable RNA. The sedimentation pattern of the RNA in Sendai virions is shown before (O) and after (●) a 30-h incubation in a transcriptase reaction. Positions of 18 and 28S rRNA sedimented in a parallel gradient are indicated by the arrows. The ^{32}P-labeled product had a sedimentation pattern identical to that shown in Fig. 1.*

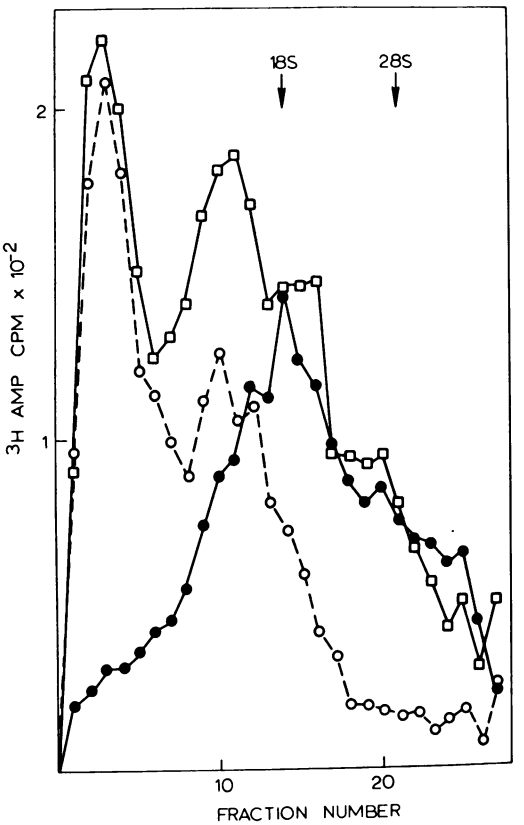

FIG. 4. *Sedimentation of Sendai virus product RNA after poly(U) chromatography. Purified [^{3}H]AMP-labeled product RNA was denatured in Me_2SO and fractionated on a poly(U)-cellulose column (1.6 by 2.0 cm). The poly(A)+ and poly(A)- RNA were sedimented along with unfractionated [^{3}H]AMP-labeled RNA on linear 15 to 30% (wt/wt) sucrose gradients as described in the text. Gradient fractions containing poly(A)+ (●), poly(A)- (○), and unfractionated Sendai virus product RNA (□) were assayed directly for [^{3}H]AMP radioactivity in PCS solubilizer (Amersham/Searle).*

treated RNA [Fig. 4] with untreated RNA [Fig. 1]).

Thus, the Sendai virion-associated enzymes appear to work aberrantly in vitro. In the data to be presented, we will provide evidence that the transcriptase does not appear to be synthesizing complete RNA molecules.

Poly(A) attached to some virus-specific RNA. To demonstrate a covalent linkage of poly(A) to in vitro product RNA and to determine approximately what percentage of the product RNA species contained poly(A), [^{3}H]AMP-labeled product RNA was denatured with Me_2SO and chromatographed on a poly(U)-cellulose column. As controls, passage of synthetic poly(A) or 28S rRNA through the column resulted in greater than 99% retention of the poly(A) and 0.5% retention of the 28S rRNA at 5 C (Table 2). Passage of [^{3}H]AMP-labeled product yielded 29.6% retention of radioactivity by the poly(U)-cellulose. The percentage of radioactivity bound by the poly(U)-cellulose column cannot be converted into the number of RNA molecules containing poly(A) segments because the molecular weight distribution and AMP composition of the molecules in the fractions are not known. But the results suggest that less than one-third of the [^{3}H]AMP-labeled transcripts had poly(A) attached.

Proof that the presence of poly(A) was the basis for separation on the poly(U)-cellulose column was obtained by nuclease digestion of the bound and unbound RNA. The [^{3}H]AMP-labeled RNA that passed through the poly(U) column was completely digested by a combination of RNase A and RNase T_1 (Table 3). Approximately 24% of the [^{3}H]AMP label in the RNA that was retained by the poly(U) column was resistant to the combined nucleases. These results suggest that the RNA retained by the column [poly(A)+] contained poly(A) segments, whereas the RNA which passed through the column [poly(A)-] did not.

TABLE 2. *Poly(U)-cellulose column chromatography*

RNA sample	Counts/min (%) eluted at 5 C[a]	Counts/min (%) eluted at 45 C[a]
^{14}C-labeled CEF[b] 28S RNA	2,862 (99.5)	17 (0.5)
^{14}C-labeled synthetic poly(A) (Miles)	1 (0.0)	2,981 (100.0)
[^{3}H]AMP-labeled Sendai virus in vitro product RNA	2,020 (70.4)	850 (29.6)

[a] In each experiment greater than 95% of the radioactivity loaded on the poly(U)-cellulose column was recovered.
[b] CEF, Chicken embryo fibroblast cells.

TABLE 3. *Nuclease digestion of fractions from poly(U) column*

RNA	Counts/min treated	Counts/min % RNase resistant[a]
[^{3}H]AMP labeled, eluted at 5 C	845	5 (0.6)
[^{3}H]AMP labeled, eluted at 45 C	1,080	256 (23.7)

[a] RNA samples resuspended in 0.5 M Tris-hydrochloride (pH 7.5)-0.3 M NaCl were incubated with 50 μg RNase A and 2 μg RNase T_1 per ml for 30 min at 37 C.

Only larger RNAs contain poly(A). To determine whether the size of the RNA molecules had any bearing on poly(A) content, the poly(A)+ and poly(A)− fractions from the poly(U) column were analyzed by sedimentation on rate-zonal sucrose gradients (Fig. 4). The poly(A)− RNA was composed of the smaller species of RNA. The RNA that was retained by the column [poly(A)+] sedimented predominantly at 18*S* and was composed of larger RNA species.

Sedimentation properties of the poly(A) segment. To characterize the poly(A) segment present on the in vitro RNA, [^{32}P]AMP-labeled product RNA was fractionated on a poly(U)-cellulose column. The poly(A)+ RNA was digested with a combination of RNase A and RNase T_1 and sedimented on a sucrose gradient. Sedimentation patterns of the RNase-resistant regions of the AMP-labeled RNA (Fig. 5) indicated that the poly(A) segments were very heterogeneous and that the majority of the poly(A) segments were larger than the 4*S* tRNA marker. The peak of radioactivity occurred at 6*S*, and the RNA in the 3 to 9*S* region (fractions 8–22) was pooled and used in the subsequent nearest-neighbor analysis.

Nearest-neighbor analysis of nuclease-resistant segment. Conclusive proof that these RNase-resistant segments are poly(A) was obtained by a nearest-neighbor analysis (Table 4). The RNase-resistant segments that sedimented in the 3 to 9*S* region were found to be composed of greater than 95% AMP, with UMP and CMP accounting for the remainder, possibly serving as the attachment points for some of the poly(A) segments.

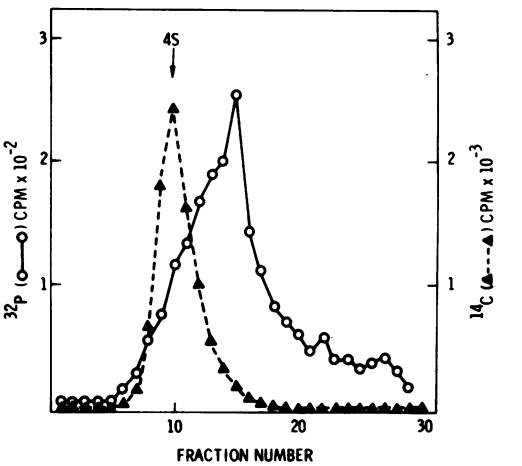

FIG. 5. *Size determination of Sendai virus in vitro poly(A). Sendai virus [^{32}P]AMP-labeled product RNA that bound to the poly(U) column was pooled and treated with a combination of RNase A and RNase T_1. The RNase digest was phenol-SDS extracted, chromatographed on a Sephadex G-50 column, and sedimented on a 15 to 30% linear sucrose gradient as described in the text. Samples (100 μl) of each [^{32}P]AMP labeled fraction (O) were assayed for radioactivity. As 4S markers, [^{14}C]uridine-labeled chicken embryo fibroblast tRNA (▲) and unlabeled f-met tRNA from E. coli were sedimented in a parallel gradient.*

TABLE 4. *Nearest-neighbor analysis by thin-layer chromatography of Sendai virus in vitro poly(A)*

Migration	Counts/min recovered[a]	Percent of total counts/min recovered
AMP	939	95.4
UMP	28	2.9
CMP	17	1.7
GMP	0	0.0

[a] In each experiment, greater than 98% of the radioactivity loaded on the thin-layer plate was recovered.

DISCUSSION

The [^{32}P]AMP- and [^{3}H]AMP-labeled in vitro product RNA, which ranged in size from 3 to 25*S* (Fig. 1), was considerably smaller than the 18, 22, and 35*S* complementary RNA species found when viral RNA is extracted from Sendai virus-infected chicken embryo lung cells (4, 7). The smaller size of the in vitro RNA appears to result from aberrant function of the virion-associated RNA polymerase. Evidence suggests that contaminating nucleases are not involved (Fig. 2) and that intact genomes are present in the reaction mixture (Fig. 3). Small product RNA could not have been transcribed from incomplete virions (15) because no 19*S* and 25*S* subgenomic RNAs were detected (Fig. 3), indicating that the virus preparation was free of incomplete virions. The reasons for the aberrant function of the Sendai virion RNA polymerase are not obvious. When Newcastle disease virus was grown and purified by the same procedures described for Sendai virus, it synthesized predominantly 18*S* RNA in vitro (8).

Only the larger RNA transcripts contained poly(A) (Table 3 and Fig. 4). The fact that the smaller in vitro RNAs do not contain poly(A) suggests that the Sendai virion transcriptase frequently terminates prematurely in vitro and that poly(A) segments are added only to completed transcripts. It is not possible that the 13*S*

product RNA molecules could be complete but are lacking poly(A), because loss of a 6S poly(A) segment is not sufficient to reduce an 18S RNA molecule to a 13S RNA molecule. The theory that the poly(A)+ RNAs were complete transcripts and the poly(A)- RNAs were prematurely terminated could be tested by experiments such as those performed with vesicular stomatitis virus (5) and influenza virus (6) to determine the amount of the genome transcribed in vitro. However, these experiments would be difficult to perform with Sendai virus because of the extremely low activity of the virion polymerase (32).

The in vitro poly(A) was very heterogenous and ranged in size from 3 to 9S, with a peak at 6S (Fig. 5). Thus, the size of the poly(A) synthesized in vitro was considerably larger than the 3.8S poly(A) segments found on Sendai virus mRNA in vivo (20, 24). This phenomenon was also seen with another paramyxovirus, Newcastle disease virus (34), and may be accounted for by the fact that poly(A) segments in a cellular environment become shortened with time (13, 21, 26, 27). Alternatively, the results obtained in this study seem to support the theory proposed by Sheldon and Kates (29), that poly(A) is added post-transcriptionally, with the size of the poly(A) being dependent on the rate at which the RNA polymerase can transcribe an mRNA molecule. The larger size of the poly(A) segments found in vitro could result from the slow rate of RNA synthesis by Sendai virus in vitro (only 10 pmol/mg of protein per h [32]).

Since Sendai virus replicates entirely within the cytoplasm of infected cells, the method by which the virus is able to synthesize and attach poly(A) segments is of considerable interest. The fact that the smaller (presumably incomplete) in vitro RNA transcripts do not contain poly(A) segments (Table 3 and Fig. 4) suggests that the synthesis of poly(A) may be restricted to the mRNA termination site. A transcriptional process was presumably eliminated by the finding that Sendai virus 50S RNA genomes do not contain large tracts of poly(U) (19). The in vitro synthesis of mRNA containing poly(A) has also been shown with Newcastle disease virus (34), vesicular stomatitis virus (3, 33), and vaccinia virus (14). Banerjee et al. (2) have obtained evidence that vesicular stomatitis virus does not contain a poly(A) polymerase and suggest that the transcriptase adds poly(A) onto completed transcripts by a template-slippage or template-independent mechanism. Whether a poly(A) polymerase exists in Sendai virions as a separate enzyme, as is the case with vaccinia virus (23), or whether the virion RNA polymerase serves a dual function is yet to be determined.

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