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Poly(A) Polymerase Activity in Reovirus

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An enzymatic activity which synthesized oligo(A) in vitro was found in highly purified reovirus. The poly(A) polymerase activity was dependent on Mn^{2+} and utilized only ATP, whereas the virion-associated RNA polymerase required all four ribonucleoside triphosphates and Mg^{2+}. Oligo(A) synthesis was demonstrated with complete virions and infectious subviral particles derived from virus by limited chymotrypsin digestion but not with cores, a product of extensive chymotrypsin digestion of virus. The enzymatic product and the oligo(A) from purified virions were isolated by binding to oligo(dT)-cellulose columns. Most of the in vitro product was similar in size and structure to the oligo(A) from purified virions by the criteria of gel electrophoresis, DEAE-cellulose chromatography, end-group analysis, and sensitivity to RNase. The evidence suggests that oligo(A) synthesis is mediated by the poly(A) polymerase during a late step in viral morphogenesis and may result from an alternative activity of the virion-associated transcriptase.

Purified reoviruses contain about 1,200 molecules per virion of oligo(A) which is not covalently bonded to the 10 double-stranded genome RNA segments (28, 39). Although apparently virus specified (9, 13, 35), the origin and function of the oligo(A) in the viral replicative cycle have not been established. Many eukaryotic cell and viral mRNAs are known to contain poly(A) (15, 33). However, no long tracts of adenine were found in reovirus mRNA (40), indicating that the reovirus oligo(A) is probably not derived from viral mRNA. Since reovirus contains an RNA polymerase (10, 35), the oligo(A) could result by a transcription process, possibly by a slippage mechanism similar to that described for DNA-dependent RNA polymerases (14, 30, 31, 38). While testing this possibility, we observed that purified reovirus contained poly(A) polymerase activity. The properties of the enzyme activity and its in vitro products are described in this report.

MATERIALS AND METHODS

Cells and viruses. Reovirus type 3 (Dearing strain) was purified from infected suspension cultures of mouse L cells as described previously (34). For preparation of ^{32}P-labeled virus, suspension cultures of BHK cells (kindly supplied by D. Dubin, Rutgers Medical School) were infected in PO_4^{3-}-free media containing 0.25 μg of actinomycin D and 10 to 20 μCi of carrier-free [^{32}P]phosphoric acid per ml. Infectious subviral particles (SVPi) and cores were isolated by isopycnic sedimentation in CsCl after digesting virions with chymotrypsin in 0.01 M Tris buffer (pH 8.0) containing 0.15 M NaCl and no NaCl, respectively (34).

Standard incubation conditions. For oligo(A) synthesis, virions, or subviral particles or cores derived from an equivalent amount of virions (34), were incubated for 1 h at 43 C in 0.1 ml of 0.07 M Tris buffer (pH 7.5) containing 2.5 mM monocyclohexylammonium phosphoenolpyruvate, 1.4 μg of pyruvate kinase, 6 mM Mn^{2+}, and 0.1 mM ATP of the indicated specific activity. Particle protein concentrations and any alterations in the standard incubation procedure are indicated for each experiment. RNA was synthesized under the same conditions but with 7.5 mM Mg^{2+} rather than Mn^{2+} and in the presence of four ribonucleoside triphosphates, three unlabeled at a concentration of 2 mM and the labeled precursor at 1 mM (specific activity, 10 μCi/μmol). At the end of the incubation, samples were chilled, diluted with 20 volumes of 10% trichloroacetic acid and sodium pyrophosphate, collected on membrane filters (Millipore Corp.), dried, and counted in toluene-based scintillation fluid (Spectrafluor, Amersham/Searle, Arlington Heights, Ill.).

Product analysis. To isolate the oligo(A) synthesized in vitro, incubation mixtures were centrifuged to pellet the active particles (5 C, 10 min, 50,000 rpm, SW65 rotor). The pellets were washed in 0.07 M Tris buffer (pH 7.5) containing 0.1 mM ATP and resuspended in 0.5 ml of 0.01 M sodium acetate buffer (pH 4.5), 0.1 M NaCl, 0.001 M EDTA. An equal volume of phenol-chloroform (1:1) was added and, after shaking for 5 min, the sample was centrifuged briefly and the aqueous phase was removed (29). The phenol-chloroform phase was re-extracted with acetate buffer and the aqueous phases were combined, washed with

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ether to remove the phenol, and finally bubbled with N_2. To remove residual substrate and shorter oligo(A) molecules, the product was applied at 5 C in 0.01 M Tris buffer (pH 7.6) containing 0.5 M KCl and 0.001 M EDTA to a column (0.5 by 2 cm) of oligo(dT)-cellulose (2, 16), a kind gift of S. Kerwar, Roche Institute. After washing to remove the unbound material, the bound oligo(A) was eluted in the same buffer minus KCl. Oligo(A) was analyzed by electrophoresis in 20% polyacrylamide gels containing 8 M urea (26) and by chromatography on DEAE-cellulose in 7 M urea (39).

Materials. [^{3}H]ATP, [α-^{32}P]ATP, and carrier-free [^{32}P]phosphoric acid were purchased from New England Nuclear Corp., Boston, Mass. [^{3}H]UTP, [^{3}H]CTP, and [^{3}H]GTP were from Schwarz-Mann, Orangeburg, N.Y. DNase, chymotrypsin, and T1 and pancreatic RNases were purchased from Worthington Biochemical Corp., Freehold, N.J., and U2 RNase was kindly provided by E. Ehrenfeld, Albert Einstein College of Medicine. The deoxyribo- and ribopolymers were purchased from Miles Laboratories Inc., Kankakee, Ill. [β,γ-^{32}P]ATP was from ICN, Irvine, Calif.

RESULTS

Properties of virion-associated poly(A) polymerase. Purified reoviruses have firmly associated with the viral core an RNA polymerase activity that transcribes one strand of each of the 10 double-stranded genome RNA segments (5, 23, 37). The resulting single-stranded RNA functions as viral mRNA (17, 25) and also serves as a precursor of genome RNA (32). An additional polymerase activity could be detected in purified reovirus. It catalyzed the incorporation of ATP into an acid-insoluble product in vitro (Table 1). In contrast to the RNA polymerase which requires for optimal activity all four ribonucleoside triphosphates, Mg^{2+}, and activation by either proteolytic digestion (35) or brief exposure to elevated temperature (10), the homopolymer-synthesizing activity utilized only ATP as a substrate, required Mn^{2+}, and was active in the absence of chymotrypsin (Table 1). The poly(A) polymerase-specific activity expressed in picomoles of product per milligram of purified virions was less than one-tenth that of the RNA polymerase. Incorporation of [^{3}H]ATP into an acid-insoluble product in the presence of Mn^{2+} was reduced by the addition of the other three ribonucleoside triphosphates, but there was no RNA formation, as shown by the absence of [^{3}H]UTP utilization under these conditions (Table 1). In the presence of 1 mM ATP, the synthesis of oligo(A) by virions had a broad Mn^{2+} optimum of 4 to 8 mM. To obtain selective synthesis of poly(A) in the experiments shown in Table 1, a concentration of 12 mM Mn^{2+} was used. The reovirus RNA polymerase is inactive at this Mn^{2+} concentration, but in the presence of 6 mM Mn^{2+} and four ribonucleoside triphosphates, synthesis of viral mRNA occurs at 10% of the maximal rate (5). The optimal pH for poly(A) synthesis was 7.5 to 8.0, and maximal incorporation occurred at 45 C, values similar to those for reovirus RNA polymerase (19).

TABLE 1. *Synthesis of poly(A) and RNA by purified reovirus*[a]

Substrate	Incorporation (p mol)			
	Mn^{2+}		Mg^{2+}	
	−Chymotrypsin	+Chymotrypsin	−Chymotrypsin	+Chymotrypsin
[^{3}H]ATP	338	439	<1	<1
[^{3}H]CTP	<1	<1	<1	<1
[^{3}H]GTP	<1	<1	<1	<1
[^{3}H]UTP	<1	<1	5	8
[^{3}H]ATP plus CTP, GTP, UTP	129	191	<1	3,924
[^{3}H]UTP plus ATP, CTP, GTP	<1	<1	<1	4,838

[a] Purified reovirus (20 μg) was incubated for 1 h at 43 C plus or minus chymotrypsin (125 μg/ml) in 0.1 ml of 0.07 M Tris buffer (pH 7.5) containing 12 mM Mn^{2+} or 7.5 mM Mg^{2+} and 1 mM of the indicated ^{3}H-labeled ribonucleoside triphosphate (specific activity, 10 μCi/μmol). The unlabeled triphosphates were 2 mM each. Reactions were terminated by adding 2 ml of a solution of 10% trichloroacetic acid and sodium pyrophosphate at 0 C, and the samples were collected on membrane filters (Millipore Corp.), dried, and counted.

The quantity of poly(A) synthesized was proportional to the amount of virus added to the incubation mixture (Fig. 1). More extensive purification of the virus by additional velocity sedimentations in 20 to 40% glycerol density gradients or isopycnic sedimentation in CsCl solution (34), or both, did not reduce the specific activity of the poly(A) polymerase, indicating that it is firmly associated with, or a structural component of, virions. As further evidence that the poly(A) polymerase is an integral part of virions rather than adsorbed to the particle surface, SVPi were found to retain 50% of the poly(A) polymerase (Fig. 2). The SVPi were prepared by partial proteolytic digestion of purified virions and isolated by isopycnic sedimentation in CsCl as described previously (34). SVPi or virions incubated with or without chymotrypsin in the presence of 6 mM Mn^{2+} incorporated [^{3}H]ATP into an acid-insoluble product for 4 h or more (Fig. 2).

Digestion of reovirus type 3 with chymotryp-

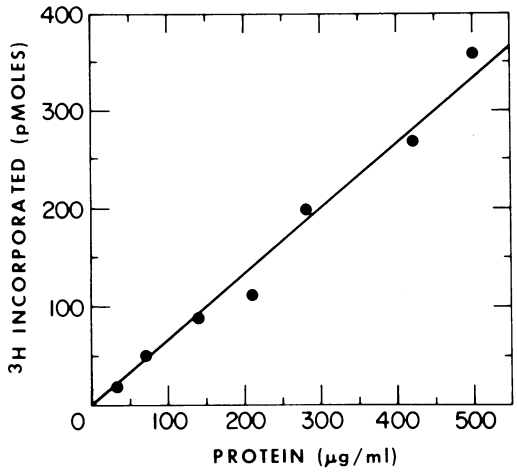

FIG. 1. *Dependence of oligo(A) synthesis on virion concentration. The indicated amount of purified reovirus expressed as micrograms of viral protein (24) was incubated for 1 h with [^{3}H]ATP (specific activity, 100 μCi/μmol) under standard conditions, chilled, acidified, filtered, and counted.*

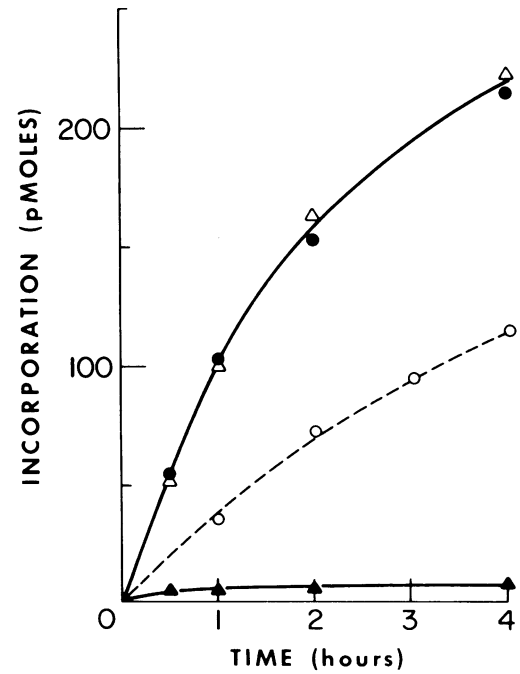

FIG. 2. *Oligo(A) synthesis by virions and subviral particles. Virions ± chymotrypsin (each 200 μg/ml) or cores (60 μg/ml) were incubated with [^{3}H]ATP (specific activity, 100 μCi/μmol) or, in the case of subviral particles (150 μg/ml), [α-^{32}P]ATP (specific activity, 200 μCi/μmol) under standard conditions. At the indicated times, samples were diluted with 2 ml of 10% trichloroacetic acid and sodium pyrophosphate at 0 C, collected on filters, and counted. Virions, ●; virions plus chymotrypsin, △; subviral particles, ○; cores, ▲.*

sin in solutions containing 0.15 M NaCl yield SVPi that retain the core polypeptides λ_1, λ_2, and σ_2 and a polypeptide derived by cleavage of μ_2, a constituent of the outer virion shell (34). The same polypeptide pattern was obtained when virions were digested with chymotrypsin in the presence of 12 mM Mn^{2+}, indicating that the divalent cation has a protective effect on μ_2 similar to that of 0.15 M NaCl. Chymotrypsin treatment of reovirus in buffers of low ionic strength and in the absence of Mn^{2+} degrades the μ_2 fragment completely and converts SVPi to core particles which lack the oligo(A) but contain polypeptides λ_1, λ_2, and σ_2 and the double-stranded RNA (18, 34). Core particles, in contrast to SVPi, did not synthesize poly(A) in vitro (Fig. 2). As reported previously (34), both SVPi and core particles synthesized reovirus mRNA in vitro. Thus, there is a correlation between reovirus particle structure and in vitro polymerase activity: virions contain latent RNA polymerase but functional poly(A) polymerase; the intermediate SVPi have both RNA and poly(A) polymerase activities; and core particles synthesize only RNA in vitro.

Properties of the in vitro product: (i) oligo-(dT)-cellulose binding. To test the possibility that the oligo(A) found in purified reovirus is synthesized by the virion-associated poly(A) polymerase activity, the properties of the in vitro product were compared with those of virion oligo(A). RNA was extracted with phenol from ^{32}P- labeled virions, and oligo(A) of chain length 10 to 20 residues was purified by gel filtration and DEAE-cellulose chromatography as described previously (6, 39). The oligo(A) was applied to oligo(dT)-cellulose at 5 C in 0.5 M KCl. Under these conditions, it binds to the extent of 82% and elutes in buffer devoid of KCl (Fig. 3). The in vitro oligo(A) product was similarly analyzed. Virions were incubated for 4 h under conditions of oligo(A) synthesis. At the end of the incubation, the virions were sedimented and washed to remove the residual substrate, [α-^{32}P]ATP. More than 90% of the total acid-precipitable radioactivity sedimented with the virions. The virions were then extracted with phenol-chloroform (1:1) (29), and the aqueous phase was applied to oligo(dT)-cellulose. Most of the radioactivity eluted in 0.5 M KCl (Fig. 3) and consisted of [α-^{32}P]ATP, [α-^{32}P]ADP, and lesser amounts of oligomers of chain length less than 10 adenine residues as identified by DEAE-cellulose chromatography. The remainder (11% of the labeled in vitro product that sedimented with virions) bound to the oligo(dT)-cellulose in 0.5 M KCl. The oligo(dT)-bound, in vitro product was eluted in buffer, and its composition and size were com-

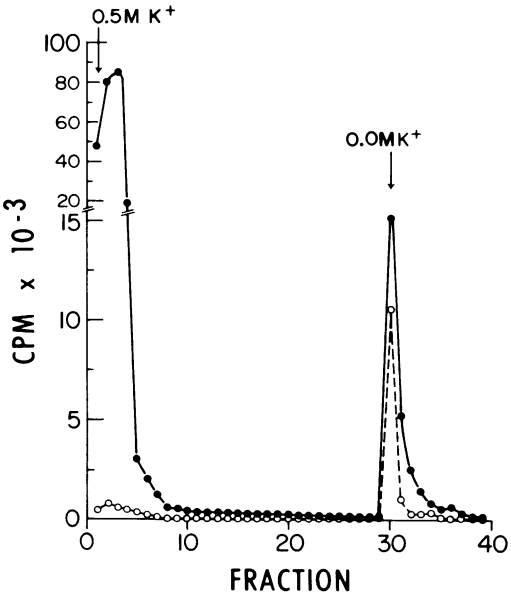

FIG. 3. *Binding of reovirus oligo(A) to oligo(dT)-cellulose. ^{32}P-labeled oligo(A) was isolated from purified virions by phenol extraction, gel filtration in Sephadex G-100, and chromatography on DEAE-cellulose as described previously (39). ^{32}P-labeled oligo(A) in 1 ml of 0.01 M Tris buffer (pH 7.6) containing 0.5 M KCl and 0.001 M EDTA was applied at 5 C to a column (0.5 by 2 cm) of oligo(dT)-cellulose. The column was washed with the same buffer, and fractions of 0.5 ml were collected until the radioactivity in the effluent was about twice the background level as measured by Cerenkov counting. The eluting buffer was then changed to Tris-EDTA without KCl. [^{32}P]oligo(A) synthesized in vitro by subviral particles (270 μg, 1.8 ml of standard incubation mixture, 4 h, [α-^{32}P]ATP of specific activity = 200 μCi/μmol) was extracted with phenol-chloroform and analyzed by the same procedure. Virion oligo(A), ○; in vitro product oligo(A), ●.*

pared with those of the virion oligo(A).

(ii) Sensitivity to alkali and nucleases. As expected for a ribopolymer, the in vitro product selected by oligo(dT)-cellulose binding was completely degraded to acid-soluble material by 0.3 N KOH but not by pancreatic DNase (Table 2). The product remained acid precipitable after treatment with a mixture of pancreatic and T1 RNases at a high salt concentration (0.3 M NaCl) but was partially degraded in the presence of 0.01 M NaCl. The salt-dependent resistance of poly(A) to pancreatic RNase digestion is well known (8). In contrast to pancreatic RNase, U2 RNase, which cleaves ribopolymers specifically at purine residues (1), digests the product almost completely to acid-soluble constituents (Table 2).

(iii) Size. ^{32}P-labeled oligo(A) of chain length 10 to 20 residues was isolated from purified virions as described above and mixed with ^{3}H-labeled in vitro product that had been bound and eluted from oligo(dT)-cellulose. The mixture was analyzed by electrophoresis in a 20% polyacrylamide gel in the presence of 8 M urea (26). The ^{32}P-labeled oligo(A) migrated as a single band as described previously (40) (Fig. 4A). The ^{3}H-labeled product was more heterogeneous, but the bulk of the ^{3}H- and ^{32}P-labeled oligo(A) migrated together, indicating that the products synthesized in vitro and in vivo were of similar size.

The mixture of ^{32}P- and ^{3}H-labeled oligo(A) was also analyzed by chromatography on DEAE-cellulose in 7 M urea, a procedure that separates oligomers predominantly on the basis of net negative charge (39). Again, the [^{3}H]- and [^{32}P]oligo(A) eluted together at an NaCl concentration of 0.22 to 0.32 M (Fig. 4B).

Initiation of oligo(A) synthesis in vitro. Oligo(A) isolated from purified reovirus contains 5'-terminal pAp, ppAp, and pppAp, implying that its synthesis, like that of reovirus mRNA (7), is initiated in a primer-independent reaction (28, 39). The similarity in size and charge of the in vitro and in vivo oligo(A) (Fig. 4) suggests that both are formed by a similar mechanism, and that the in vitro product is not formed by chain extension of the virion-associated oligo(A) acting as a primer for the

TABLE 2. *Properties of oligo(A) product*[a]

Treatment	Percent acid soluble
Pancreatic DNase	0
T1 + pancreatic RNase	
0.3 M NaCl	0
0.01 M NaCl	51
U2 RNase	97
KOH	>99

[a] Virions were incubated for 4 h under conditions of oligo(A) synthesis (6 mM Mn^{2+}, 0.1 mM ATP of specific activity = 100 μCi/μmol), sedimented, washed, and extracted with phenol-chloroform, and the [^{3}H]oligo(A) was isolated by oligo(dT)-cellulose adsorption chromatography (39). For each reaction, 4,000 counts/min of [^{3}H]oligo(A) was incubated for 30 min at 37 C in 0.5-ml samples as follows: pancreatic DNase, 5 μg per ml of 0.02 M Tris buffer (pH 7.3) and 0.005 M Mg^{2+}; pancreatic RNase, 5 μg/ml with T1 RNase, 80 U per ml of 0.02 M Tris buffer (pH 7.3) containing 0.001 M EDTA and 0.01 or 0.3 M NaCl; and U2 RNase, 3 U per ml of 0.01 M sodium acetate buffer (pH 4.5) with 0.1 M NaCl and 0.001 M EDTA. Digestion in 0.3 N KOH was for 18 h. All reactions were terminated by acidification at 0 C, and the products were collected by passage through membrane filters (Millipore Corp.), which were then dried and counted.

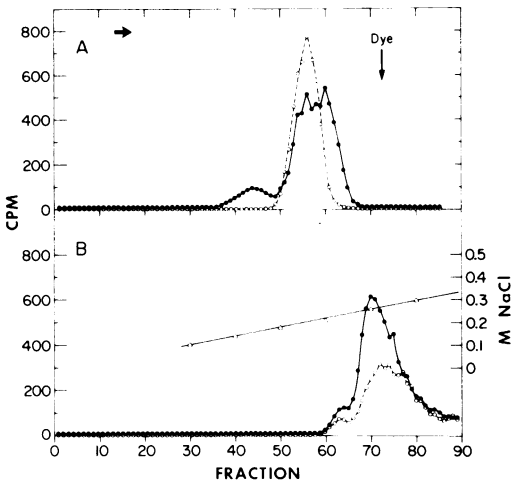

FIG. 4. *Analysis of oligo(A) by electrophoresis in 20% polyacrylamide gel and chromatography on DEAE-cellulose. (A) [^{32}P]oligo(A) from purified virions was mixed with [^{3}H]oligo(A) in vitro product that had been selected by adsorption to and elution from oligo(dT)-cellulose. The mixture was analyzed by electrophoresis for 17 h at 130 V in a 10-cm, 20% polyacrylamide gel containing 8 M urea as described previously (26, 40). The arrow indicates the position of the marker dye, bromophenol blue. (B) Another similar mixture of [^{32}P]- and [^{3}H]oligo(A) was applied to DEAE-cellulose (1 by 16 cm column) in 1 ml of 0.05 M Tris buffer (pH 8.0), 0.05 M NaCl, 7 M urea and eluted with a linear salt gradient of 0.05 to 0.5 M NaCl in Tris-urea. Fractions of 2 ml were collected, and 1 ml was counted in 8 ml of methyl cellosolve (2-methyl-oxy-ethanol) and 8 ml of toluene-based scintillation fluid (39). ^{32}P, ○; ^{3}H, ●.*

poly(A) polymerase. To test for the presence of initiated 5′ termini in the oligo(A) synthesized in vitro, virion-catalyzed product was prepared with [α-^{32}P]ATP as the radioactive precursor. The ^{32}P-labeled oligo(A) product was isolated by binding to and elution from oligo(dT)-cellulose. For comparison, ^{32}P-labeled oligo(A) of chain length 10 to 20 bases was obtained from purified virus (39). The oligo(A) preparations were digested with 0.3 N KOH, and the resulting nucleotides were separated by chromatography on DEAE-cellulose in 7 M urea. Digests of the oligo(A) from purified virions (Fig. 5A) or synthesized in vitro (Fig. 5B) yielded essentially identical elution profiles. Most of the radioactivity eluted at about 0.1 M NaCl, the position of AMP. A second peak eluted at 0.18 M NaCl, consistent with the structure ppAp. A third minor peak eluted between the two major peaks at 0.13 to 0.15 M NaCl, the position of pAp (39). In several experiments, the average amount of radioactivity in the position of ppAp was 9.0 and 8.5% for the in vivo and in vitro oligo(A), respectively, consistent with average chain lengths of about 30 and 25 nucleotides. Chain length measurements based on the proportion of 5′-terminal nucleoside polyphosphates may be overestimates because of a variable number of 5′-dephosphorylated molecules (28, 39) (see below). Chain length estimates, therefore, were also made on the basis of the amount of 3′-[^{3}H]adenosine and [^{3}H]AMP in alkaline digests of ^{3}H-labeled oligo(A) analyzed by paper chromatography (39). Average chain lengths of 11 were estimated for in vivo and in vitro oligo(A) (Table 3).

As a further test for primer-independent initiation of oligo(A) synthesis in vitro, incorporation of [β,γ-^{32}P]ATP and [^{3}H]ATP (specific activities, 1.83 and 0.35 Ci/mmol, respectively) were compared. Under standard conditions for oligo(A) synthesis, both precursors were incorporated by virions (350 μg/ml) at a linear rate for 2 h. [β,γ-^{32}P]ATP incorporation then declined, but [^{3}H]ATP utilization continued at the same rate for another 2 h. To determine the ratio of internal to 5′-terminal nucleotides in

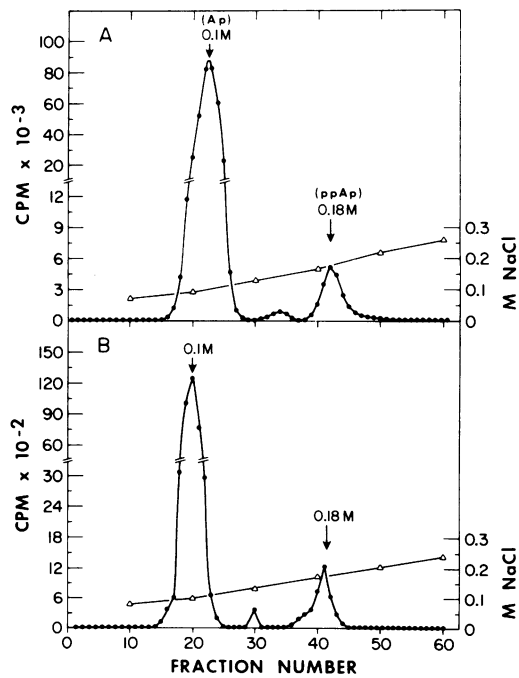

FIG. 5. *Chromatography on DEAE-cellulose of alkaline digests of [^{32}P]oligo(A). (A) Oligo(A) was isolated from ^{32}P-labeled purified virions as described in the legend to Fig. 4. (B) Oligo(A) was synthesized for 4 h with [α-^{32}P]ATP as the radioactive precursor, and the product was isolated by binding to oligo(dT)-cellulose. Each sample was digested in 0.3 N KOH for 18 h at 37 C, neutralized by passage through Dowex-50 resin (3), and analyzed by chromatography on DEAE-cellulose in 7 M urea as in Fig. 4B.*

TABLE 3. *Chain length estimates of oligo(A)*[a]

Source of Oligo(A)	^{3}H[AMP] (counts/min)	[^{3}H]adenosine (counts/min)	Chain length
Virion	2,318	227	11
In vitro product	7,988	775	11

[a] Virions were grown in the presence of [^{3}H]adenosine and purified. The ^{3}H-labeled RNA was extracted with phenol-chloroform, and the [^{3}H]oligo(A) was isolated by gel filtration and DEAE-cellulose chromatography (39). The in vitro product was synthesized with [^{3}H]ATP under standard conditions and purified by oligo(dT)-cellulose binding. The oligo(A) preparations were hydrolyzed with KOH as in Fig. 5 and analyzed by descending paper chromatography in *n*-butanol-isobutyric acid-water-NH_4OH (30:15:10:1). Authentic samples of AMP and adenosine were mixed with the hydrolysate before chromatography and located on the paper by absorbance of UV light. The paper was cut into 1-cm strips, and the samples were eluted and counted in aquasol.

the products, the oligo(A) made during 2 h, the period of linear synthesis, was extracted with chloroform-phenol and isolated by binding to oligo(dT)-cellulose. A total of 233 pmol of ^{3}H and 13.9 pmol of ^{32}P were present in the dT-cellulose-bound oligo(A) product. After KOH digestion (0.3 N, 18 h, 37 C) and chromatography on DEAE-cellulose as in Fig. 5, the ^{32}P-labeled nucleotides eluted in two peaks: 38% of the radioactivity at 0.18 M NaCl and 57% at 0.23 M NaCl, the positions of ppAp and pppAp, respectively. On the basis of the amount of [^{3}H]AMP versus 5′-^{32}ppAp and ^{32}p^{32}ppAp in the oligo(A) product, the average chain length was estimated to be 12 nucleotides. It should be noted that the 4-h oligo(A) product labeled with [β,γ-^{32}P]ATP liberated upon KOH hydrolysis a single peak of ^{32}P eluting from DEAE-cellulose in the position of ppAp. These results are consistent with the presence in the virion of phosphohydrolase (7, 11, 20), which removes the γ-^{32}P from the 5′ end of the product and the precursor [β,γ-^{32}P]ATP. The loss of terminal phosphates probably accounts for the decline in ^{32}P incorporation after 2 h, and may also explain the chain length overestimates of 25 to 30 nucleotides determined with [α-^{32}P]ATP (Fig. 5).

Since conversion of virions to core particles by chymotrypsin digestion, a treatment that results in the release of the virion oligo(A), also leads to the loss of poly(A) polymerase activity, it seemed important to test further whether oligo(A) or other polymers can serve as primers for 3′-terminal addition of AMP. Purified cores (60 μg of protein/ml) were incubated under conditions which support virion or SVPi-mediated oligo(A) synthesis (6 or 12 mM Mn^{2+}, 1 mM [^{3}H]ATP; specific activity, 20 μCi/μmol). Reactions were carried out in the presence or absence of oligo(A) that had been isolated from virions (30 to 300 μg/ml), or exogenous poly(A), poly(C), poly d(AT), or poly dA·dT, each at either 3 or 30 μg/ml. No stimulation of [^{3}H]AMP incorporation was observed, although under the appropriate conditions, i.e., Mg^{2+} and four ribonucleoside triphosphates, the RNA polymerase in these cores catalyzed the synthesis of 1.0 nmol of RNA during a 30-min in vitro reaction. The presence of the above exogenous polymers during the in vitro incubation also did not affect oligo(A) synthesis by virions. These results support the findings shown in Fig. 5, which indicate that oligo(A), like reovirus mRNA (7), is synthesized de novo.

Absence of poly(A) polymerase from reovirus empty virions. "Top component", i.e., noninfectious empty virions, has been shown to contain all of the reovirus polypeptides but no viral RNA or RNA polymerase activity (22). It was of interest to assay top component for poly(A) polymerase activity. Empty particles (ρ_{CsCl} = 1.30 g/ml) were isolated from infected cells by the usual purification procedures (5) and separated from infectious virions (ρ_{CsCl} = 1.37 g/ml) by isopycnic sedimentation in CsCl solutions. Analysis of solubilized empty particles by sodium dodecyl sulfate-polyacrylamide gel electrophoresis confirmed that their polypeptide composition was the same as that of infectious virions. As reported previously (22), the empty virions (210 μg/ml) with or without proteolytic digestion did not catalyze detectable RNA synthesis in vitro when incubated for 60 min under conditions which promoted the incorporation of 1.9 nmol of [^{3}H]UMP into RNA products by the same amount of chymotrypsin-digested infectious virions. Similarly, no poly(A) polymerase activity was observed when 210 μg of top component per ml was incubated under optimal conditions for homopolymer synthesis; in the control reaction, SVPi (150 μg/ml) incorporated 126 pmol of [^{3}H[AMP into acid-insoluble products in 1 h. Thus, poly(A) polymerase activity, like the virion transcriptase activity, is not detectable in top component and may also be template dependent.

DISCUSSION

Human reoviruses of all three serotypes (36) and avian reoviruses (21) contain oligo(A) which is not covalently associated with the genome RNA. Although its function, if any, in the viral replication cycle has not been eluci-

dated, we have detected a poly(A)-synthesizing activity in purified reovirus which presumably accounts for the origin of reovirus oligo(A). Poly(A) polymerase activities have been observed in a variety of eukaryotic cells (42) and in purified vaccinia virus (12, 27). In each case the enzyme is dependent upon, or stimulated by the addition of, exogenous oligoribonucleotide primers. These activities in vivo may catalyze the addition of multiple AMP residues to the 3′ termini of gene transcripts. A reaction of this type was recently observed in vitro with purified vesicular stomatitis virus, although no poly(A) polymerase activity was detected (4, 41). The reovirus-associated poly(A)-synthesizing activity differs from the other poly(A) polymerases in its ability to initiate synthesis of oligo(A) chains and its failure to utilize exogenous ribopolymers as primers. The in vitro oligo(A) product is similar in size and composition to the oligo(A) in reovirions, suggesting that the virion polymerase catalyzes synthesis of the oligo(A) during the reovirus infectious cycle.

It is not known whether the reovirus poly(A) polymerase activity is a modified form of the RNA transcriptase which is present in reoviruses. Both enzyme activities have similar pH and temperature optima. They are both absent from empty virions, suggesting that the poly(A) polymerase, like the transcriptase, is template dependent. Alternatively, the absence of polymerase activity from empty virions could result from an altered conformation of the viral polypeptides in RNA-deficient particles.

Reovirus cores prepared from virions by exhaustive chymotrypsin digestion of the outer-shell polypeptides synthesize RNA but not oligo(A) in vitro. Subviral particles obtained by partial proteolytic degradation of virions consist of cores and a fragment of one of the outer-shell proteins, polypeptide μ_2 (34). They mediate in vitro synthesis of mRNA in the presence of Mg^{2+} and four ribonucleoside triphosphates or oligo(A) if provided with Mn^{2+} and ATP. Virions contain an active oligo(A) polymerase but a latent transcriptase. These findings may indicate that the core transcriptase is converted to an oligo(A)-synthesizing activity by the addition of an outer-shell polypeptide(s) during a late step in viral morphogenesis. Perhaps the transcriptase in cores is prevented from traversing the double-stranded template RNA by the addition of one or more outer-shell polypeptides. As a consequence, mRNA formation is replaced by oligo(A) synthesis. Reovirus oligo(A) is readily released from particles when virions are converted to cores, suggesting that it is near or on the core surface. It may play a role in the assembly of outer-shell polypeptides on viral cores. It will be of interest to study the effect of oligo(A) or outer-shell polypeptides, or both, on the in vitro synthesis of mRNA by reovirus cores.

ADDENDUM IN PROOF

Silverstein et al. (J. Virol. **13**:740–752, 1974) recently described an oligo(A) polymerase activity in a particulate fraction from reovirus-infected cells and suggested that it is an alternative activity of the virion-bound transcriptase.

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