Characteristics of the Polyadenylic Acid Segment Associated with Messenger Ribonucleic Acid in Mouse Sarcoma 180 Ascites Cells†

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ABSTRACT: It has been shown recently that rapidly labeled polysomal RNA and nuclear RNA molecules contain a large poly(A) segment. The poly(A)-containing polysomal RNA of mouse sarcoma 180 cells, which consists of a heterogeneous population of molecules with sedimentation values of 10–30 S, shows a DNA-like base composition. The poly(A) segments could be isolated by adsorption on Millipore filters after RNase treatment of the RNA preparations. Nucleotide analysis of the isolated nuclear and polysomal poly(A) indicates an adenylate content of 97–99 mole %. About 0.5 mole % adenosine is found in alkaline hydrolysates of this material, suggesting an average chain length close to 200 nucleotides for the poly(A) segment, as well as the presence of a free 3′-OH terminus. The time course of poly(A) labeling in polysomes indicates that this sequence is assembled after the rest of the RNA molecule has been completed. Poly(A) synthesis is sensitive to actinomycin D to an extent somewhat smaller than that of the rest of the RNA. Cordycepin, an analog of adenosine, inhibits preferentially the labeling of the poly(A)-containing polysomal RNA. The poly(A) segments in the cordycepin-treated cells are shorter, as indicated both by increased adenosine yields after alkaline hydrolysis and by increased electrophoretic mobilities in polyacrylamide. The same techniques indicated that in untreated cells the polysomal poly(A) is somewhat shorter than the nuclear material. The results indicate that the poly(A) segment may be localized at the 3′ end of the polysomal and nRNA molecules, and that it is synthesized by sequential addition of adenylate residues to the newly formed RNA molecules. Cordycepin appears to affect primarily the enzymic process concerned with poly(A) synthesis. This would lead to poly(A)-deficient mRNA molecules unable to migrate to the polysomes.

There is recent evidence for the occurrence of a poly(A) segment in mammalian mRNA. The rapidly labeled non-rRNA components of both mouse sarcoma 180 and HeLa cell polysomes have been shown to contain adenylate-rich RNase-resistant material of relatively large size (Lee *et al.*, 1971a; Edmonds *et al.*, 1971; Darnell *et al.*, 1971). Evidence has also been presented for the occurrence of an adenylate-rich sequence in the 10S rabbit reticulocyte RNA (Lim and Canellakis, 1970). The occurrence of poly(A) in rat liver cytoplasm had been reported previously, but its relation to mRNA was not then evident (Hadjivassiliou and Brawerman, 1966, 1967). RNA transcribed from vaccinia virus DNA has also been shown to contain a large poly(A) segment, apparently localized at the 3′ end of the RNA molecules (Kates, 1970). It was observed that the occurrence of poly(A) in RNA causes it to bind to Millipore filters at high ionic strength, thus providing a convenient method for the assay and isolation of this type of RNA (Lee *et al.*, 1971a; Brawerman *et al.*, 1972). The fractionation of cytoplasmic RNA (cRNA) by sequential phenol extractions with neutral and alkaline Tris-HCl buffers (Hadjivassiliou and Brawerman, 1967) was also found to be due to a property of the poly(A) segment (Brawerman *et al.*, 1972).

There is little information available concerning the significance of poly(A) in mRNA. Its occurrence in nuclear RNA (nRNA) as well (Edmonds *et al.*, 1971; Darnell *et al.*, 1971) suggests that it is synthesized in the nucleus and transported to the cytoplasm together with the mRNA. It has been suggested that it may serve as a special site for the binding of mRNA to some cell structure concerned with mRNA transport (Lee *et al.*, 1971a). The findings presented in this report indicate that the poly(A) segment contains about 200 nucleotides and appears to be localized at the 3′ terminus of the RNA molecules. We also present evidence for its formation apparently at the last stage of mRNA synthesis. Experiments with cordycepin, which appears to inhibit preferentially poly(A) synthesis, suggest that the poly(A) segment may be required for the transport of mRNA from nucleus to polysomes.

Experimental Section

Cell Incubations, Polysome Preparation, and RNA Extraction. The detailed procedures for the handling, incubation, and lysis of the mouse sarcoma 180 ascites cells, as well as for the preparation of polysomes and the phenol extraction of the polysomal RNA, have been described previously (Lee *et al.*, 1971a,b). In the present study, the first incubation in Krebs bicarbonate medium lacking nutrients was omitted. The cells were incubated in the medium supplemented with amino acids, bovine serum, and glucose in the presence of 0.04 μg/ml of actinomycin D to prevent rRNA synthesis Penman *et al.*, 1968), and 1 μg/ml of ethidium bromide to inhibit mitochondrial RNA (mtRNA) labeling (Zylber *et al.*, 1969). The cells were always incubated in the presence of the inhibitors for 30 min prior to addition of labeled precursors.

Polysomes were isolated either by sedimentation at 100,000*g* as described previously (Lee *et al.*, 1971b), or by a procedure involving precipitation with Mg^{2+}. The latter method, which

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was used when quantitative recovery of the polysomes was required, consisted of lysing the cells in a low ionic strength solution (10 mM KCl, 10 mM Tris-HCl (pH 7.6), 1.5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 12% sucrose, and 0.12% Triton X-100), removing the nuclei by centrifugation at 1000*g* for 5 min, and supplementing the supernatant with 0.1 volume of 0.3 M $MgCl_2$. This caused precipitation of the polysomes and ribosomes. After 30 min in ice, these particles were recovered by centrifugation at 2000*g* for 10 min. KCl concentrations greater than 10 mM prevented the precipitation of polysomes.

For isolation of the nRNA, the 1000*g* pellet was first washed by suspension in 10% sucrose, 50 mM Tris (pH 7.6), and 3 mM $CaCl_2$, recentrifugation, and resuspension in H_2O. One-tenth volumes of 1 M sodium acetate (pH 5.2) and of 5% sodium dodecyl sulfate were added to the nuclear suspension, followed by an equal volume of water-saturated phenol. After mixing in the cold, the suspension was kept at 65° for 5 min, cooled, and centrifuged to separate the phases. The nonaqueous residue (interphase plus phenol phase) was reextracted with 0.1 M sodium acetate in 0.5% sodium dodecyl sulfate at room temperature, and the aqueous phases pooled.

Polysomal RNA was prepared either by phenol extraction in the presence of 0.1 M Tris (pH 9.0) and 0.5% sodium dodecyl sulfate, or by sequential extractions at pH 7.6 and 9.0 as described previously (Lee *et al.*, 1971a). The aqueous phases were reextracted three times with fresh phenol, to remove both residual protein and the sodium dodecyl sulfate, the phenol was removed from the aqueous phases by three extractions with ether, and the ether removed with a stream of air.

For the preparation of ^{32}P-labeled RNA, the cells were incubated in complete medium with a reduced phosphate content (0.01 mM KH_2PO_4). Carrier-free [^{32}P]phosphate was added and the cells were harvested after a 1-hr labeling period. Nuclear and polysomal RNA fractions were prepared as usual, but the aqueous phases were dialyzed overnight against repeated changes of 0.1 M phosphate buffer (pH 7.0), and finally against H_2O.

Isolation of Poly(A) Segments. The RNA preparations were incubated for 40 min at 30° in 50 mM Tris (pH 7.6), 50 mM KCl, and 1 mM $MgCl_2$ in the presence of 1 μg/ml of pancreatic RNase and, in the case of nRNA, 5 μg/ml of DNase. The reaction mixtures were next diluted with 20 volumes of ice-cold 500 mM KCl, 10 mM Tris (pH 7.6), and 1 mM $MgCl_2$, and filtered slowly through Millipore filters previously soaked in the same solution for about 30 min. For radioactivity assays, the filters were heat dried and placed in toluene scintillation mix. When the poly(A) had to be recovered, the filters were placed in 0.5–1 ml of 0.5% sodium dodecyl sulfate in 0.1 M Tris (pH 9.0) immediately after filtration, and kept at 0° for about 30 min with occasional shaking. The heavy precipitate of potassium dodecyl sulfate did not interfere with the elution and subsequent operations.

Analysis of Nucleotides and Adenosine. RNA samples were precipitated with cold 10% trichloroacetic acid, after addition of rRNA as carrier when required. The precipitates were washed with a 3-l. mixture of ether and ethanol, dissolved in a small volume of 0.3 N KOH, and incubated at 37° for 18 hr. After neutralization with 1 N acetic acid, samples were spotted on Whatman No. 1 paper, together with 5 μg of adenosine/spot when required. After chromatography, the appropriate zones were cut out. In the case of tritium-labeled material, they were eluted with 1 ml of water and the eluates mixed with 10 ml of Triton X-100 scintillation mixture. Zones with ^{32}P-labeled nucleotides were placed directly in vials with toluene scintillation mix. The counting procedures have been described previously (Lee *et al.*, 1971a,b).

Polyacrylamide Gel Electrophoresis. Poly(A) preparations were subjected to electrophoresis in 7.5% polyacrylamide gels (10 cm long) as described by Loening (1967). The electrophoreses were run at room temperature at 10 mA/gel until the bromophenol blue indicator had started to run out of the tubes, plus an additional 15 min. The gels were divided in 1-mm slices, which were placed in pairs in scintillation vials together with 0.5 ml of 0.5% sodium dodecyl sulfate in 0.1 M Tris (pH 9.0). The vials were incubated for 24 hr at 37°, and 5 ml of Triton X-100 scintillation mix was added.

Results

Poly(A) Content of Polysomes and Nuclei. Polysomal RNA, extracted with phenol in the presence of Tris-HCl (pH 9.0) in order to obtain the totality of the RNA, contains nonribosomal species with poly(A) segments as well as others free of substantial poly(A) sequences, as judged by the criterion of capacity to bind to Millipore filters (Lee *et al.*, 1971a). The extent of adenosine label in the poly(A) segments of the RNA could be conveniently determined by pancreatic RNase digestion followed by adsorption on Millipore. It was found to range from 30 to 70% of the total radioactivity, depending on the labeling period (see below).

The analysis of poly(A) in the nRNA proved more difficult. The phenol extraction procedure used for the polysomes was unsatisfactory, since it led to highly viscous aqueous phases due to the presence of DNA. This DNA, which contained a substantial amount of adenosine label, had to be eliminated by extensive DNase treatment. The hot sodium dodecyl sulfate–phenol extraction procedure used by Edmonds *et al.* (1971) proved as effective as the Tris (pH 9) procedure for obtaining the poly(A)-containing RNA species, and little DNA contamination was observed. The procedure for the assay of poly(A) in polysomal RNA was effective for the nuclear extracts, but DNase had to be included in the enzyme treatment. A large portion of the labeled nuclease-resistant material did not represent poly(A), and adsorption on Millipore was essential for the assay of nuclear poly(A). This is in contrast to the behavior of the polysomal extracts, where nearly all the RNase-resistant adenosine-labeled material was retained on Millipore as poly(A) (Lee *et al.*, 1971a). As in the case of the polysomal RNA, inclusion of RNase T_1 in the enzyme treatment of the nuclear extracts did not significantly affect the amount of radioactivity on the filters.

The proportion of poly(A) detected in the nRNA was far lower than in the case of polysomal RNA. The total amount of radioactive poly(A) in the nuclear extracts, however, was about equal to that of the polysomes (see Tables III and IV, and Figure 3). The proportion of the nRNA molecules that contain poly(A) could not be estimated with any certainty. A large portion of the RNA was capable of binding to Millipore, but much of this binding was eliminated by pretreatment with DNase presumably free of contaminating RNase (purchased from Sigma Chemical Co., Saint Louis, Mo.). It is not clear whether the DNase-sensitive binding was caused by single-stranded DNA fragments somehow associated with the nRNA, or whether the enzyme, through possible contamination by traces of RNase, caused fragmentation of the RNA with resulting loss of Millipore-binding capacity for some of the fragments.

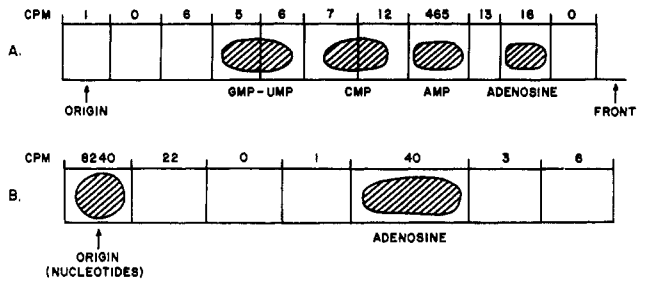

FIGURE 1: Distribution of radioactivity in alkaline hydrolysates of adenosine-labeled poly(A) preparations. Poly(A) prepared from cells labeled for 2 hr with 5 μCi/ml of [2,8-^{3}H]adenosine (15 Ci/mmole; New England Nuclear, Boston, Mass). (A) Chromatography with isobutyric acid–0.5 N NH_4OH (10:6, v/v) for 6 hr; (B) chromatography with 1-butanol–0.1 N NH_4OH (6:1, v/v) for 24 hr. Paper strips divided as indicated. Different preparations used for parts A and B.

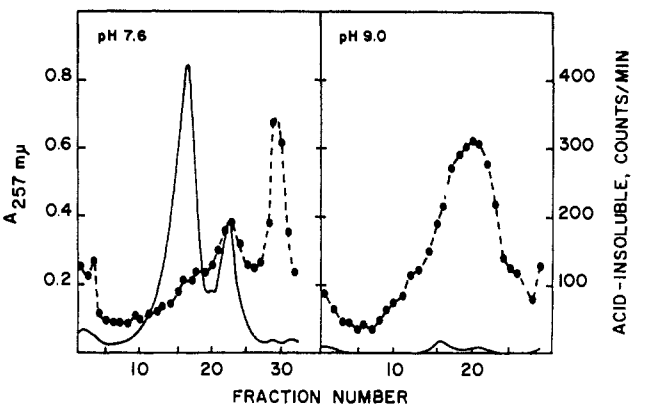

FIGURE 2: Zone sedimentation patterns of ^{32}P-labeled polysomal RNA fractions. RNA prepared by sequential phenol extraction from cells incubated as described in Table I. Samples subjected to zone centrifugation in SW50 Spinco rotor through 5-ml 5–20% linear sucrose gradients in 20 mM Tris-HCl (pH 7.6) and 10 mM KCl at 45,000 rpm for 225 min at 8°. Fractions analyzed as described previously (Lee *et al.*, 1971a).

Characteristics of the Nuclear and Polysomal Poly(A). In order to determine whether the nuclease-resistant radioactivity retained on Millipore represents poly(A), and not a polynucleotide fragment with a peculiar structure, or perhaps even nonnucleic acid material, the distribution of labeled nucleotides in the Millipore-bound material was examined. All the radioactivity from adenosine-labeled polysomal preparations was found in adenylic acid after alkaline hydrolysis (Figure 1A). When preparations from ^{32}P-labeled cells were examined, about 99% of the radioactivity was in adenylic acid in the case of the polysomal material, and only slightly less in the nuclear counterpart (Table I). Thus the Millipore-bound material after nuclease digestion clearly represents poly(A).

Labeled adenosine was detected in the alkaline hydrolysates of the poly(A) preparations (Figure 1B). The yields of adenosine were the same whether the poly(A) was prepared as described in the Experimental Section, or more extensively purified by an additional trichloroacetic acid precipitation step. The conditions used for the alkaline hydrolysis have been shown by Sugiyama and Fraenkel-Conrat (1961) not to cause any significant dephosphorylation of nucleotides. Thus the nucleoside could have originated only from a free 3′-OH terminus on the polynucleotide chains. If it is assumed that all the poly(A) molecules are terminated in this fashion, the ratio of labeled nucleoside to nucleotide provides an average value of about 200 nucleotides for the chain length of the isolated poly(A). The finding of adenosine also indicates that at least part of the poly(A) segments are localized at the 3′ end of the polysomal RNA molecules, since the enzyme treatments used to eliminate the rest of the RNA could not have generated free 3′-OH ends on the poly(A).

Characteristics of the Two Classes of Labeled Polysomal RNA. It has been shown previously that the polysomal RNA molecules containing poly(A) can be separated from those lacking this segment by sequential phenol extractions in the presence of Tris buffers of pH 7.6 and 9.0 (Lee *et al.*, 1971a). The RNA of the pH 9 fraction, which contains the poly(A), sediments as heterogeneous material with a broad peak between the 28S and 18S ribosomal components (Figure 2; see also Lee *et al.*, 1971a). Nucleotide analysis of a ^{32}P-labeled preparation shows that it has a base composition close to that of DNA (Table II). The RNA lacking poly(A) (pH 7.6 fraction) shows a greater G + C content, intermediate between that of DNA (43% G + C) and rRNA (64% G + C). It is more heterogeneous than the pH 9 RNA, with additional light material and a component that cosediments with the 18S ribosomal. It also appears to possess a greater proportion of rapidly sedimenting material.

Time Course of Labeling of Polysomal RNA Components. Since it appeared from the above results that the poly(A)

TABLE I: Distribution of ^{32}P-Labeled Nucleotides in Nuclear and Polysomal Poly(A) Preparations.[a]

	Nuclear	Polysomal
Adenylic acid	96.7	98.8
Guanylic acid	1.9	0.6
Cytidylic acid	0.7	0.3
Uridylic acid	0.7	0.3

[a] Poly(A) prepared from cells incubated for 1 hr in presence of 0.2 mCi/ml of ^{32}P-labeled phosphate. Nucleotides separated by two-dimensional paper chromatography, in isobutyric acid–0.5 N NH_4OH (10:6, v/v) for 7 hr and in saturated $(NH_4)_2SO_4$–1 N sodium acetate–isopropyl alcohol (80:18:2, v/v) for 7 hr. Values expressed as per cent of total radioactivity in nucleotides. Single determination done on each preparation.

TABLE II: Distribution of ^{32}P-Labeled Nucleotides in Polysomal RNA Fractions.[a]

	pH 7.6 RNA	pH 9.0 RNA
Adenylic acid	21.0 (1.1)	26.4 (0.9)
Guanylic acid	29.2 (1.2)	23.3 (0.8)
Cytidylic acid	27.1 (1.4)	22.1 (1.7)
Uridylic acid	22.8 (0.4)	28.2 (0.6)

[a] RNA fractions prepared as in Figure 2. Values in parentheses represent maximum deviations from mean. Three determinations were done on single preparation of each fraction. For other details, see Table I.

segments are localized at the 3′ end of the RNA molecules, experiments were designed to determine whether they are synthesized at the final stage of the transcription process. In this event, the first radioactive RNA molecules to appear in the polysomes after addition of radioactive adenosine should be labeled primarily in the poly(A) segment, and the proportion of the radioactivity in the poly(A) portion of the molecules should decrease gradually. This is the case, as shown in Figure 3 and Table III. The time course of labeling of the polysomal RNA bearing poly(A) (capable of binding to Millipore) shows a lag period. This is particularly evident when the radioactivity values for the non-poly(A) portion of the molecules are plotted. The labeling of the poly(A) segments shows little, if any, lag period. The rate of labeling of poly(A) in the nucleus is greater than that in the polysomes (Figure 3, Table III).

The RNA devoid of poly(A) that is present in the polysomal preparations shows no noticeable lag in the time course of labeling. It can be seen in Figure 3 that it represents the major labeled species at early times, and that its rate of labeling rapidly declines while that of the Millipore-bound RNA remains constant for an extended period of time.

Effect of Actinomycin D on the Labeling of the Polysomal RNA Components. The poly(A) segments could be synthesized as distinct entities and then joined to completed RNA molecules, or by sequential addition of adenylate residues to the 3′ end of the RNA chain. This latter process could be accomplished as part of transcription if the terminal portion of the DNA being transcribed were a poly(T) sequence. It could also be performed by a separate enzyme after transcription is completed. Synthesis of poly(A) as a separate entity should be relatively insensitive to actinomycin, since this drug acts by binding to guanine residues of DNA. Addition of adenylate residues to RNA chains *via* transcription should be effectively blocked by actinomycin. In the case of posttranscriptional addition, poly(A) formation could possibly continue, but should cease rapidly because of exhaustion of primer RNA molecules.

As can be seen in Table III, the synthesis of poly(A) is quite strongly affected by actinomycin. The effect on poly(A), however, is smaller than that on total RNA, as judged by the increased ratios of labeled poly(A) to RNA. The relatively small magnitude of this differential effect, as well as the lack of precise kinetic data, make it difficult to distinguish between poly(A) synthesis as part of transcription or by a separate addition process. The above results, however, tend to rule out separate poly(A) synthesis followed by joining to completed RNA chains.

Effect of Cordycepin on Polysomal RNA Synthesis. It has been reported by Penman *et al.* (1970) that cordycepin (3′-deoxyadenosine) can strongly inhibit the labeling of the rapidly synthesized polysomal RNA in HeLa cells while hardly affecting the nuclear heterodisperse RNA. As can be seen in Table IV, the polysomal RNA component bearing poly(A) is particularly sensitive to the drug. The RNA lacking poly(A) is not significantly affected after a labeling period of 30 min. Poly(A) itself is inhibited to about the same extent as the Millipore-bound RNA. As with HeLa cells, the nRNA as a whole is much less affected.

Size of the Poly(A) Components. As shown above, labeled adenosine was detected in alkaline hydrolysates of polysomal poly(A) preparations to the extent of 1 residue/about 200 nucleotides. The nucleoside was also present in hydrolysates of the nuclear material. A comparison of the adenosine yields from the two types of preparations indicated that the poly-

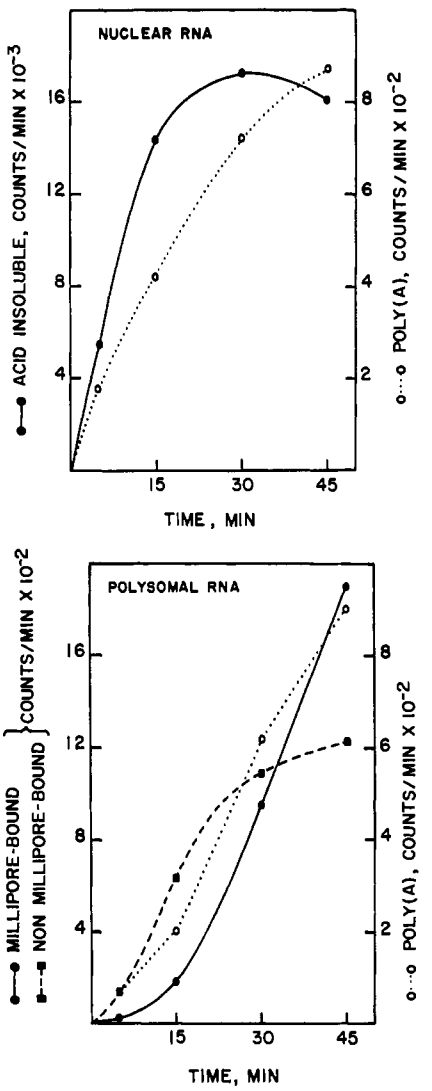

FIGURE 3: Time course of appearance of labeled RNA components in nuclei and polysomes. Cells incubated in the presence of 5 μCi/ml of tritiated adenosine. Samples taken at indicated times, chilled rapidly, washed, and lyzed in low ionic strength solution as described in the Experimental Section; polysomes obtained by Mg precipitation. Aliquots of RNA solutions used for analysis of cold acid-insoluble RNA, Millipore-bound RNA, and poly(A). Millipore-bound RNA values represent radioactivity associated with non-poly(A) portion of Millipore RNA (difference between Millipore-bound radioactivity and that due to poly(A) component); non-Millipore-bound RNA values represent differences between acid-insoluble radioactivity and Millipore-bound radioactivity. Values are expressed as cpm per constant volume of cell suspension.

somal poly(A) might be somewhat smaller than its nuclear counterpart (Table V). This was verified by comparing the mobilities of the poly(A) preparations during polyacrylamide gel electrophoresis. In order to provide reliable comparisons, poly(A) from cells labeled for 2 hr with [^{14}C]adenosine was run together with tritium-labeled material. The poly(A) preparations migrated as relatively homogeneous components, as has already been shown by Edmonds *et al.* (1971). The polysomal poly(A) showed a rate of migration substantially greater than that of the nuclear material (Figure 4). Thus the conclusion concerning the sizes of nuclear and polysomal poly(A) based on the data on adenosine yields was confirmed by the measurements of electrophoretic mobility. The relative mobilities determined from the experiment described in

TABLE III: Effect of Actinomycin D on Labeling of Polysomal and nRNA.[a]

		Control			Actinomycin	
		5 min	15 min	30 min	20 min	35 min
Polysomes	Non-Millipore RNA	134	630	1,090	151	133
	Millipore RNA	90	384	1,560	173	206
	Poly(A)	69	202	620	131	149
	Per cent poly(A) in Millipore RNA	77	73	40	76	72
Nuclei	Total RNA	5360	14,400	17,300	2290	2310
	Poly(A)	178	423	725	115	110
	Per cent Poly(A) in total RNA	3.3	2.9	4.2	5.0	4.8

[a] Cell suspensions incubated as in Figure 3. Five minutes after addition of [^{3}H]adenosine, a portion of the suspension was supplemented with 10 μg/ml of actinomycin D. Millipore RNA values represent total Millipore-bound radioactivity. For other details, see Figure 3.

TABLE IV: Effect of Cordycepin on the Labeling of Polysomal and nRNA.[a]

		Control	Cordycepin Treated	% Inhibn by Cordycepin
Polysomes	Non-Millipore RNA	1,830	2,230	−20
	Millipore RNA	4,160	1,130	73
	Poly(A)	1,180	270	77
	Per cent poly(A) in Millipore RNA	28	24	
Nuclei	Total RNA	40,500	29,000	28
	Poly(A)	1,010	210	79
	Per cent poly(A) in total RNA	2.5	0.7	

[a] Cell suspensions incubated in complete medium supplemented with 1 μg/ml of ethidium bromide and 0.04 μg/ml of actinomycin D, in the presence or absence of 25 μg/ml of cordycepin. After 30 min, labeled adenosine was added, the incubations were continued for 30 min, and the cells harvested. For other details, see Table III.

Figure 4, and from a second experiment using different poly(A) preparations, are summarized in Table VI. The polysomal poly(A) from cells labeled for 2 hr shows a greater mobility than that from cells labeled for 30 min. It is not clear whether the same relation applies to the nuclear components, since the results from the two experiments were not consistent in this respect (Table VI).

Poly(A) preparations from cordycepin-treated cells yielded amounts of labeled adenosine considerably greater than those obtained from untreated cells (Table V). This finding was rather unexpected, since the adenosine analog is believed to exert its inhibitory effect by substituting for AMP in the growing polynucleotide chain, and thereby terminate chain elongation (see Guarino, 1967). Chains terminated with a cordycepin residue would not yield adenosine upon alkaline hydrolysis. It is possible, therefore, that the drug functioned in these experiments by inhibiting the activity of an enzyme concerned with poly(A) synthesis, without becoming incorporated into the polymer. Polyacrylamide gel electrophoresis showed a considerably greater mobility for the poly(A) preparations from cordycepin-treated cells (Figure 5, Table VI). Thus, both the data on adenosine yields and electrophoretic mobilities indicate that cordycepin causes the appearance of shorter poly(A) chains. The chains also seem to be more heterogeneous in size (Figure 5). An effect of cordycepin on poly(A) has also been found by J. Darnell (personal communication).

Discussion

The findings in this report indicate the occurrence of poly(A) in polysomal and nuclear rapidly labeled RNA as a segment about 200 nucleotides long probably localized at the 3′ end of the RNA molecules. This conclusion, based primarily on the detection of about 0.5 mole % adenosine in the alkaline hydrolysates of the poly(A) preparations, requires the assumption that all the poly(A) molecules in the preparations are terminated with a free 3′-OH. Since chain-length values of about 200 can also be deduced from the 4S sedimentation value (Fresco and Doty, 1957) and from the electrophoretic mobility in acrylamide gel (Edmonds *et al.*, 1971), the above assumption is probably valid. The possibility that the adenosine is derived from small poly(A) fragments, and not from the main component evident in the acrylamide gel patterns, is rather remote, since the yields of nucleoside in different preparations correlate quite well with the electrophoretic mobilities of the corresponding main components. The present findings are similar to those of Kates (1970), who

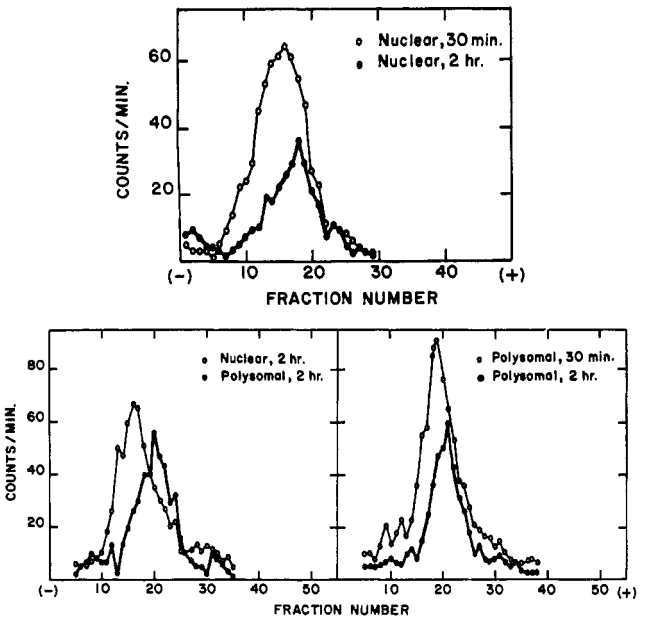

FIGURE 4: Polyacrylamide gel electrophoresis of nuclear and polysomal poly(A) preparations. Poly(A) prepared from cells labeled either with 5 μCi/ml of tritiated adenosine for 30 min or 2 hr, or with 0.2 μCi/ml of [8-^{14}C]adenosine (40 mCi/mmole) for 2 hr. Preparations labeled with tritium (open circles with thin lines) were mixed with ^{14}C-labeled poly(A) (closed circles with thick lines) for the electrophoretic runs.

showed the occurrence of a poly(A) segment of about 100 nucleotides apparently localized at the 3′ terminus of the RNA synthesized by vaccinia virus.

The conclusion concerning the localization of the poly(A) in the polysomal RNA molecules is supported by the kinetic data. These indicate that the poly(A) segment is synthesized subsequently to completion of the rest of the RNA molecule.

TABLE V: Terminal Adenosine Content of Poly(A) Preparations from Normal and Cordycepin-Treated Cells.[a]

	Control		Cordycepin Treated	
	Mole % Adenosine	Chain Length[b]	Mole % Adenosine	Chain Length[b]
Nuclear	0.47	212	0.60	167
	0.50	200	0.58	172
	0.53	190		
Polysomal	0.48	208	0.74	135
	0.53	190	1.04	96
	0.59	170		
	0.59	170		
	0.59	170		

[a] Cells incubated for 2 hr with labeled adenosine in the presence or absence of 25 μg/ml of cordycepin. Poly(A) preparations subjected to alkaline hydrolysis and paper chromatography as in Figure 1B. Different values in each set represent results obtained with different preparations. [b] Average size of poly(A) estimated by ratio of adenosine to total nucleotide, assuming that all poly(A) molecules carry a free 3′-OH terminus.

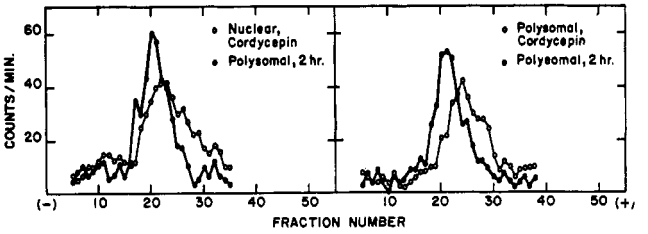

FIGURE 5: Polyacrylamide gel electrophoresis of poly(A) from cordycepin-treated cells. Preparations from cells incubated with cordycepin as in Table IV were run together with poly(A) from cells labeled with ^{14}C as in Figure 4.

Poly(A) synthesis appears to proceed *via* stepwise additions of adenylate residues to the RNA chains. The present results cannot clearly distinguish between addition as part of the transcription process, or by a separate enzymic reaction after transcription is completed. The latter process seems more likely, since enzymes capable of poly(A) synthesis using as primer RNA molecules with a free 3′-OH have been shown to occur in animal cell nuclei (Edmonds and Abrams, 1960; Hyatt, 1967).

The observed effect of cordycepin on the labeling of various RNA components suggests that poly(A) synthesis may be the major site of action of the drug. As reported first by Penman *et al.* (1970), polysomal RNA labeling is strongly inhibited while nRNA synthesis is much less affected. As shown here, it is the poly(A)-containing polysomal RNA that is primarily affected. Moreover, the poly(A) recovered from cordycepin-treated cells is considerably reduced in size. These effects could be explained by assuming that gene transcription is little affected by the drug levels used in our experiments, but that the enzyme concerned with subsequent poly(A) formation is particularly sensitive to cordycepin. mRNA molecules deficient in poly(A) would be unable to migrate from nucleus to polysomes, thus leading to the observed inhibition of polysome labeling. This interpretation of the cordycepin effect implies a role for poly(A) in mRNA transport.

The significance of the observed differences in poly(A) sizes is obscure. The smaller size of the material from polysomes suggests that adenylate residues are removed after the RNA molecules leave the nucleus. This could occur in the course of mRNA transport or after the mRNA has become incorporated into polysomes. As for the differences between cells

TABLE VI: Relative Electrophoretic Mobilities of Poly(A) Components from Normal and Cordycepin-Treated Cells.[a]

Time of Incubn (min)	Experiment I		Experiment II	
	Nuclear	Polysomal	Nuclear	Polysomal
30	1.00	1.26	1.00	1.05
120	1.12	1.40	1.00	1.19
30, cordycepin	1.53	1.60	1.30	1.37

[a] Mobilities computed from comparison of positions of poly(A) peaks in polyacrylamide gel electrophoretic patterns as in Figures 4 and 5. Different poly(A) preparations were used in the two experiments. Values are relative to those of the nuclear poly(A) labeled for 30 min.

labeled for 30 min and 2 hr these could be caused by a decrease with time in the size of the poly(A) segment in the RNA molecules. They could also be due to synthesis in the nucleus of smaller poly(A) segments after the cells have been incubated for an extended period of time *in vitro*, probably under less than optimal conditions.

It remains to be determined whether all species of mammalian mRNA carry the poly(A) segment. The polysomes also contain rapidly labeled non-rRNA that lacks poly(A). This RNA appears to reach the polysomes immediately after its synthesis is completed, and it does not seem to be affected by cordycepin. Part of this RNA shows sedimentation characteristics expected of mRNA. The exact relation of this RNA to the polysomes, however, remains to be determined.

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Hybridization of Ribonucleic Acid with Unique Sequences of Mouse Deoxyribonucleic Acid†

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ABSTRACT: The proportion of the genome transcribed in various mouse organs was estimated by hybridizing purified unique sequences of DNA with excess RNA. Saturation hybridization values of 4–5% were obtained for liver, kidney, and spleen, but as much as 11% for mouse brain. Recycling and additivity tests demonstrated a partial overlap in base sequences transcribed in various organs. The saturation value increased with age for brain RNA but little effect of age was apparent with liver RNA. Parallel experiments with *Bacillus subtilis* nucleic acids revealed that a relatively larger per cent of the bacterial genome is transcribed in exponentially growing bacteria.

For all the rapid increase in factual knowledge which has accumulated during recent years, the details of mechanisms controlling selective gene expression in mammalian cells remain obscure. For example, although the existence of a rapid turnover of nuclear RNA in mammalian cells has been established since the early work of Harris (1963), the relationship of this phenomenon to regulation of gene function is not yet understood. Since the size of the mammalian genome is so large compared to that of bacteria, it might be supposed that the fraction of the genome being transcribed into mRNA molecules at a given instant is quite small. Certainly a few per cent of the mammalian genome would suffice to specify essentially all known enzymes. Notwithstanding the appeal of such an argument, little quantitative data exist concerning the fraction of active genes in any mammalian cell or tissue.

An obvious approach to this question involves the use of DNA–RNA hybridization. In principle, the DNA of a cell may be titrated by total RNA to provide an estimate of the fractional transcriptional activity. Only those DNA segments which produced RNA in that cell will react. In practice, however, the assay is a difficult one for many reasons. In the first place, saturation of the DNA is all but impossible to achieve in cases where the frequency of various RNA molecules in a

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