Messenger Ribonucleic Acid Metabolism in Mammalian Mitochondria. Discrete Poly(adenylic acid) Lacking Messenger Ribonucleic Acid Species Associated with Mitochondrial Polysomes†

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ABSTRACT: The mRNA species released from mitochondrial polysomes prepared by the Mg^{2+} precipitation technique have been further characterized using various analytical techniques. Mitochondrial polysomes were dissociated by treatment with puromycin and chemically labeled with [^{3}H]dimethyl sulfate. About 51% of steady-state mitochondrial mRNA bind to oligo(dT)-cellulose indicating the presence of poly(adenylic acid) (poly(A)) in this fraction. The poly(A)-containing mRNAs resolve into discrete bands of 9–16 Sϵ, while the RNA fraction unable to bind to oligo(dT)-cellulose representing poly(A)-lacking mRNA contains 8–12 Sϵ species. About 90% of poly(A) lacking RNA hybridizes with mitochondrial DNA and less than 7% hybridizes with nuclear DNA. The extent of hybridization of poly(A)-lacking RNA with mitochondrial DNA was not significantly affected by the presence of excess mitochondrial rRNA, cytoplasmic rRNA, or a tenfold concentration of poly(A)-containing RNA isolated from total mitochondrial RNA. Possible differences in sequence properties between poly(A)-containing and -lacking mitochondrial mRNAs were further verified using a solid phase-bound cDNA procedure. Poly(A)-containing mRNA released from mitochondrial polysomes shows over 85% sequence homology with oligo(dT)-cellulose-bound cDNA prepared against total mitochondrial poly(A)-containing RNA. In contrast only about 1% of poly(A)-lacking mitochondrial mRNA hybridizes with the cDNA providing direct evidence for the distinct sequence properties of the two mRNA species.

Since the discovery of poly(A)[1] sequences in HeLa and Ehrlich ascites mitochondria (Perlman et al., 1973; Avadhani et al., 1973), there have been several attempts at isolation and characterization of poly(A)-containing RNAs in various mitochondrial (mt) systems (Gaitskhoki et al., 1973; Hirsch and Penman, 1974; Hirsch et al., 1974; Avadhani et al., 1974; Ojala and Attardi, 1974). It has been shown that mt poly(A) containing RNA is associated with polysome-like structures and is preferentially released by treatment with puromycin and EDTA (Perlman et al., 1973; Avadhani et al., 1974). In a previous report, we presented evidence that some poly(A)-lacking RNA, presumed to be mRNA, is also found associated with Ehrlich ascites mt polysomes (Avadhani et al., 1974). In the present paper we confirm and extend our previous observations using mt mRNA isolated from high Mg^{2+} polysomes (Lewis et al., 1976). A portion of the results included in this paper were reported in abstract form (Lewis et al., 1975).

Experimental Procedures

Materials. In all of the experiments reported in this paper, Ehrlich ascites hypotetraploid cells were used. Details of cell growth, fractionation of cells, and isolation of mitochondria were as described in Lewis et al. (1976).

Isolation of Mitochondrial Polysomes and Mitochondrial mRNA. Polysomes were prepared from purified mitochondria by the Mg^{2+} precipitation procedure described in Lewis et al. (1976). Both heparin and DEP were present during the entire isolation procedure. (See Lewis et al., 1976.)

Dissociation of polyribosomes by treatment with puromycin was similar to a procedure described by Blobel (1971). Mitochondrial polysomes were suspended in buffer B (30 mM Tris-HCl (pH 7.5), 100 mM KCl, 20 mM $Mg(CH_3COO)_2$, 5 mM 2-mercaptoethanol) containing 200 μg/ml of heparin and treated with puromycin at a concentration of 200 μg/ml. The suspension was incubated at 30 °C for 15 min and centrifuged at 250 000g for 3 h in an SW 65-L rotor using a Sorvall OTD-2 ultracentrifuge at 0–4 °C. The ribosome-free supernatant fraction was aspirated, extracted with sodium dodecyl sulfate–phenol–chloroform (LaTorre and Perry, 1973) and RNA was recovered from the resultant aqueous phase by precipitation with absolute ethanol in presence of 3% potassium acetate.

Methylation of RNA. High specific activity ^{3}H-labeled dimethyl sulfate (3–5 Ci/mmol) was purchased from New England Nuclear. Chemical labeling of RNA was performed according to Stull (1975). This procedure involves methylation of purified RNA in 0.05 M potassium phosphate buffer (pH 6.8) and 50% dimethyl sulfoxide. RNA (10–50 μg) was dissolved in 0.1 M potassium phosphate buffer (pH 6.8) at a concentration of 200 μg/ml and mixed with an equal volume of dimethyl sulfoxide. [^{3}H]Dimethyl sulfate in ether (about 1 mCi/10 μg of RNA) was added to the RNA suspension and ether was removed by evaporation with nitrogen gas. The methylation reaction was carried out at room temperature for 90 min. The reaction mixture was mixed with the help of a Vortex Genie every 10 min. Unbound [^{3}H]dimethyl sulfate was removed by chromatography on a Sephadex G-25 column.

Estimation of Poly(A). Poly(A) was estimated by the

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[1] Abbreviations used are: mt, mitochondrial; DEP, diethyl pyrocarbonate; poly(A), poly(adenylic acid); Sϵ, sedimentation value based on electrophoretic migration on gels; cDNA, complementary DNA; nDNA, nuclear DNA; EDTA, (ethylenedinitrilo)tetraacetic acid; SSC, standard saline citrate; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

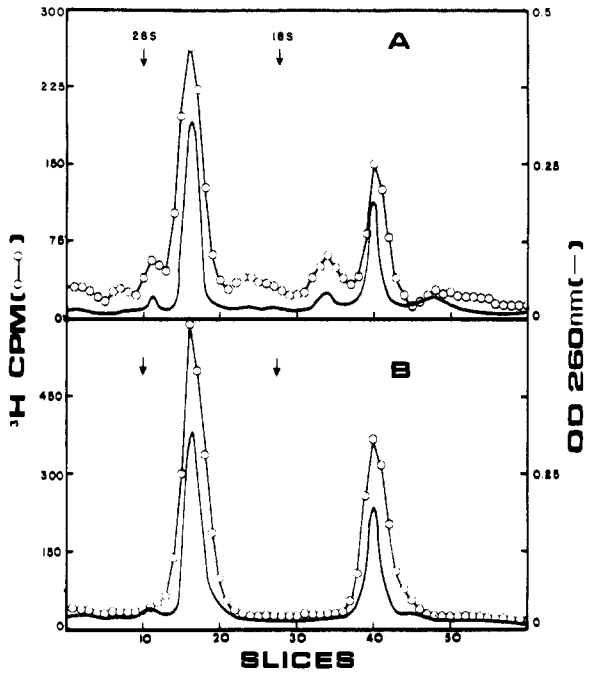

FIGURE 1: Electrophoretic patterns of ^{3}H methylated RNA. Total mtRNA (A) and mt rRNA isolated from puromycin treated particles (B) were methylated with [^{3}H]dimethyl sulfate as described under the Experimental Procedures. Methylated RNA samples (0.2–0.3 μg and 4000–5000 cpm) were mixed with the respective unlabeled RNA (25 μg) and electrophoresed on 3.8% polyacrylamide gels of 9.0 cm long. Gels were scanned in a Gilford 2400A gel scanner and then sliced and counted as described under the Experimental Procedures.

method of Gillespie et al. (1972) as micro-modified by Stull (1975). This modified technique can detect as little as 1 × 10^{-10} g of poly(A).

Oligo(dT)-Cellulose Chromatography. Mitochondrial poly(A) containing RNA was purified on oligo(dT)-cellulose columns essentially as described by Aviv and Leder (1972). A 0.6 × 6.0 cm column was prepared in water-jacketed glass tubes using 1.0 g of oligo(dT)-cellulose (Collaborative Research, Inc.). The column was equilibrated with 0.5 M KCl, 10 mM Tris-HCl (pH 7.5). RNA dissolved in 1–3 ml of the above buffer was applied to the column at 18 °C and was allowed to pass through the column at the rate of 0.1 ml/min. The column was then washed with 19 ml of 0.1 M KCl and 10 mM Tris-HCl (pH 7.5) at an elution rate of about 0.2 ml/min at 18 °C. The RNA retained on the oligo(dT)-cellulose column, hereafter referred to as "bound" RNA, was eluted with 10 mM Tris-HCl (pH 7.5) at 35 °C. Fractions of 0.5 ml each were collected and 0.025 ml aliquots were counted with 4 ml of Cab-O-Sil scintillation mixture in a Packard Tricarb spectrometer. When unlabeled RNA was analyzed, the fractions were taken in 0.3-ml microcuvettes for spectrophotometric reading at 260 nm. The fractions corresponding to the unbound and bound RNA were pooled separately and mixed with 3–4 volumes of absolute ethanol to precipitate the RNA. In cases where the RNA content was too low for precipitation, 50 μg of *Escherichia coli* tRNA was used as the carrier.

DNA–RNA Hybridization. DNA was isolated from purified nuclei and mitochondria as described before (Avadhani et al., 1974). Alkali denatured nuclear (50 μg) and mitochondrial (10 μg) DNA were fixed on Schleicher and Schuell membrane filter disks (22 mm diameter) as described by Gillespie and Spiegelman (1965). The reaction mixture contained 4× SSC and 50% formamide in a final volume of 0.25 ml. The reaction was carried out in tightly closed scintillation vials at 55 °C for 24 h. Each vial contained the appropriate DNA filter and also a blank filter without DNA. Treatment with RNase, and washing of the filters at 40 °C were essentially as described earlier (Avadhani et al., 1974). The radioactivity detected on the blank filters was subtracted from the total radioactivity bound to the DNA filters.

Synthesis of Solid Phase Bound cDNA. Preparation of oligo(dT)-cellulose-bound cDNA was according to the method of Venetianer and Leder (1974). The reaction mixture contained 0.1 M Tris-HCl (pH 7.5), 40 mM KCl, 10 mM $Mg(CH_3COO)_2$, 20 mM NaCl, 2 mM dithiothreitol, 0.4 mM each of dATP, dGTP, dCTP, and dTTP, 6 μg/ml of avian myeloblastosis virus DNA polymerase (RNA dependent DNA polymerase), 10 μg/ml of poly(A) containing RNA, 50 μg/ml of actinomycin D, and 30 mg/ml of oligo(dT)-cellulose. The reaction volume ranged from 0.2 to 8.0 ml. The reaction mixture was vigorously stirred at 37 °C for 150 min. At the end of reaction, the cellulose-bound cDNA was either poured into a water-jacketed column (0.5 cm diameter) or filtered through glass-fiber filters for radioactivity determination. In both cases, the cellulose-bound cDNA was washed thoroughly with 0.1 N NaOH and then with distilled H_2O.

Hybridization of mRNA to Solid Phase Bound cDNA. The mRNA sample to be tested for complementarity with cDNA was dissolved in 2 ml of 0.6 M NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 50% formamide, and applied to the preequilibrated cDNA column at 45 °C. The sample was allowed to pass through the column at a rate of 2 ml/h. The column was then washed with 13 ml of the same buffer at 45 °C. Subsequent washings were carried out with 7.5–15 ml of 10 mM Tris-HCl (pH 7.5) at temperatures ranging from 25 to 75 °C as required. Fractions of 0.5 ml each were collected and counted with 10 ml of Cab-O-Sil scintillation mixture.

Electrophoretic Analysis of RNA. RNA samples were electrophoresed on 4% polyacrylamide gels as described by Bishop et al. (1967). The amount of radioactivity in the gel slices was determined as described earlier (Avadhani et al., 1974).

Results

Chemical Labeling of mt RNA. Although chemical labeling with dimethyl sulfate presents a useful method of obtaining radioactive RNA for various analysis (Smith et al., 1968; Dawid, 1972; Gaubatz and Cutler, 1975), this method is known to produce nicks in the RNA chains (see Gaubatz and Cutler, 1975). In order to determine the extent of degradation caused during methylation, mt total RNA and mt rRNA were labeled and electrophoresed along with excess unlabeled RNAs. As shown in Figure 1A, mt total RNA resolves into two major peaks corresponding to large and small mt rRNAs with about 15% of the radioactivity showing heterogeneous migration. In case of mt RNA from ribosomal particles, over 95% of the radioactivity is detected in peaks corresponding to mt rRNAs (Figure 1B). Further, in both the cases, the radioactivity distribution matches with the optical density profile indicating very little degradation of mt RNAs during the chemical labeling with dimethyl sulfate. This may be due to the protective effects of dimethyl sulfoxide against RNase activity during labeling (Stull, 1975). The procedure used has been shown to yield intact 18 and 28S rRNAs and specific activities in the range of 18–24 000 cpm/μg of RNA (see Stull, 1975).

Release of mRNA by Puromycin. Treatment with puromycin dissociates polysomes releasing mRNA (Blobel, 1971; Perlman et al., 1973; Avadhani et al., 1974; Hirsch and Pen-

TABLE I: Distribution of Poly(A) in Various RNA Fractions.[a]

RNA Fractions	μg of Poly(A)/ 100 μg of RNA	Distribution of Poly(A) (%)
Total mt RNA	0.17	-
Total polysomal RNA	0.25	100
Post puromycin pellet (ribosomes)	0.002	<4
Puromycin supernatant (mRNA)	5.3	96

[a] Preparation of RNA and estimation of poly(A) were as described under the Experimental Procedures.

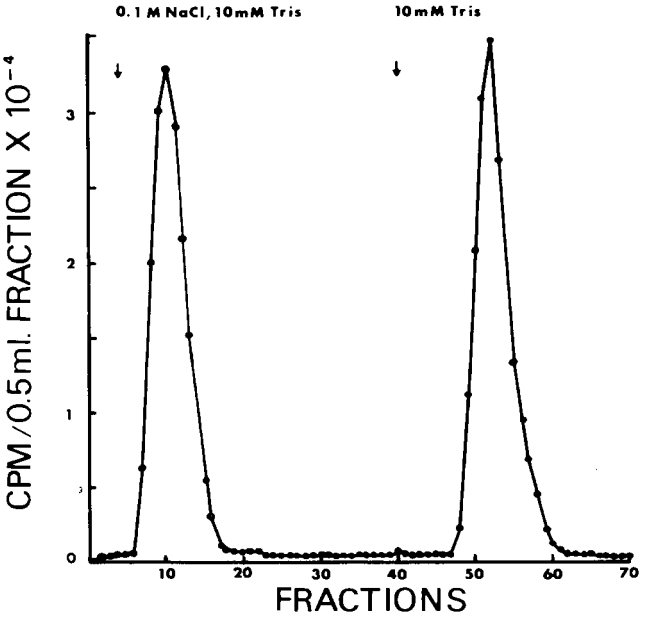

FIGURE 2: Oligo(dT)-cellulose chromatography of puromycin released mt mRNA. Polysomes were treated with puromycin to release the mRNA as described under the Experimental Procedures. The mRNA was methylated with [^{3}H]dimethyl sulfate and roughly 9.5 μg of RNA (21 000 cpm/μg) in 1 ml of 10 mM Tris-HCl (pH 7.5), 0.5 M KCl was layered on the oligo(dT)-cellulose column. Details of chromatography were as described under the Experimental Procedures. Fractions of 0.5 ml were collected and 0.025-ml aliquots counted with 4 ml of Cab-O-Sil mixture.

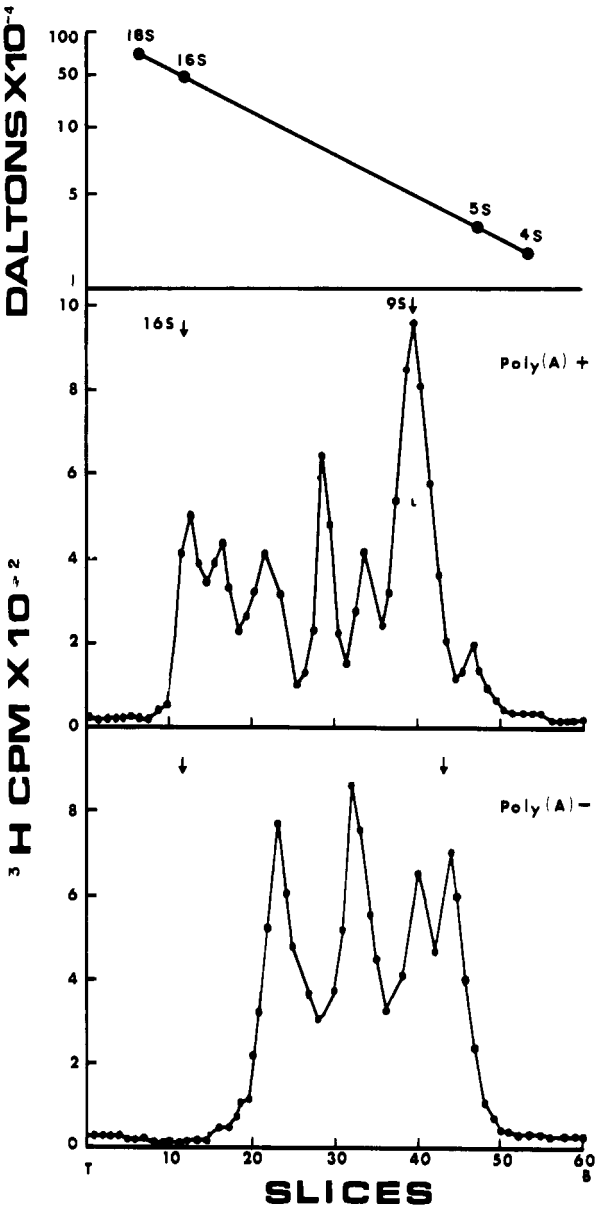

FIGURE 3: Electrophoretic analysis of steady-state mRNA from mt polysomes. The oligo(dT)-cellulose-bound (poly(A) containing) and -unbound (poly(A) lacking) RNA fractions from Figure 2 were further purified by three additional passages through the oligo(dT)-cellulose column. RNA samples containing 16 000–18 000 cpm were electrophoresed on 4% polyacrylamide gels (9.0 cm long) as described under the Experimental Procedures. *E. coli* 16S and Ehrlich ascites cytoplasmic 18S, 5S, and 4S RNAs were used for determining the relative Sϵ of mt mRNA peaks.

man, 1974). Since very little is known about the quantitative aspect of mRNA release by puromycin, we examined the poly(A) distribution in various fractions. As shown in Table I, total mt RNA and RNA extracted from mt polysomes contain 0.17 and 0.25% poly(A), respectively. The puromycin released fraction, presumably mRNA, contains 6.3% poly(A), while the ribosomal particles (165 000*g* pellet) contain less than 0.002% poly(A). The poly(A) detectable in puromycin released fraction represent >96% of poly(A) detectable in the mt polysomes. These results suggest that puromycin method used in these experiments yields a total release of mRNA from the polysomes.

Isolation and Analysis of mt Poly(A)-Lacking mRNA. In a previous report from this laboratory (Avadhani et al., 1974) it was shown that only 60–65% of pulse-labeled RNA released from mt polysomes selectively binds to oligo(dT)-cellulose, indicating possible existence of some poly(A) lacking mRNA in mitochondria. In order to further verify this possibility, mt polysomal RNA released by puromycin was labeled with [^{3}H]dimethyl sulfate and analyzed on a oligo(dT)-cellulose column. As shown in Figure 2, 51% of chemically labeled mt mRNA binds to oligo(dT)-cellulose. The oligo(dT)-cellulose-"bound" and -"unbound" RNAs were further purified by three additional passages through the oligo(dT)-cellulose column and referred to as poly(A)-containing and -lacking mRNAs, respectively.

The electrophoretic patterns of poly(A)-containing and -lacking RNAs are presented in Figure 3. The poly(A)-containing RNA migrates as 9–16 Sϵ, while the poly(A)-lacking RNA contains 8–12 Sϵ species. The size distribution of mt poly(A)-containing RNA is in agreement with the pulse-labeled mt polysomal mRNA reported previously (Avadhani et al., 1974). In addition, similar heterogeneous populations of

TABLE II: Hybridization of Mitochondrial Poly(A) Lacking mRNA with Nuclear and Mitochondrial DNA.[a]

RNA Hybridized to	cpm of RNA Used	Competing RNA	^{3}H cpm Hybridized	% RNA Hybridized
nDNA	4260		298	7.0
nDNA	8520		533	6.2
mtDNA	4260		3982	93.5
mtDNA	8520		7912	92.8
mtDNA	4260	mt rRNA (30 μg)	3940	92.5
mtDNA	4260	mt rRNA (60 μg)	3856	90.4
mtDNA	4260	Cyto rRNA (100 μg)	3914	91.2
mtDNA	4260	mt poly(A) containing RNA (5 μg)	3780	88.7

[a] Details of hybridization were as described under the Experimental Procedures. Figures in parentheses represent the amounts of competing RNA used. The poly(A)-lacking RNA (21 300 cpm/μg) was prepared as described in Figures 2 and 3.

TABLE III: Synthesis of Oligo(dT)-Cellulose-Bound cDNA to Mitochondrial Poly(A) Containing RNA.[a]

Volume of Reaction (ml)	Amount of Poly(A) Containing RNA (μg)	^{3}H cpm Incorporated
0.2	1	37 600
0.4	2	64 060
0.6	3	109 200
0.2	0.0	101
0.4	0.0	117

[a] The composition of reaction mixture and conditions of reaction were as described under the Experimental Procedures excepting that [^{3}H]TTP (New England Nuclear, 10 Ci/mmol) was added to each tube at the level of 250 μCi/ml. Radioactivity determinations were as described under the Experimental Procedures and in Venetianer and Leder (1974).

poly(A)-containing RNA were reported in HeLa mt system (Ojala and Attardi, 1974). The pattern of poly(A)-lacking mRNA, however, is somewhat different from polysome associated RNA synthesized by isolated mitochondria that did not bind to oligo(dT)-cellulose (Avadhani et al., 1974).

The genetic origin of mt poly(A)-lacking mRNA was determined by hybridization with nDNA and mt DNA. As shown in Table II, only 6–7% of labeled RNA hybridizes with nDNA, while over 90% of the RNA radioactivity hybridizes with mt DNA. The extent of hybridization is not affected by the addition of cytoplasmic or mt rRNAs, as well as mt poly(A)-containing RNA. These results indicate that mt poly(A)-lacking RNA is probably the product of mt genome and that it has sequence properties different from mt rRNA or mt poly(A)-containing RNA.

Homology Studies Using Solid Phase Bound cDNA. The results of DNA–RNA hybridization showing little homology between mt poly(A)-containing and -lacking mRNA were further investigated using solid phase bound cDNA (Venetianer and Leder, 1974). The control experiments of Table III indicate that the extent of [^{3}H]dTTP incorporation into the cold CH_3COOH precipitable or 0.1 N NaOH resistant fraction is directly proportional to the size of the reaction mixture with other conditions remaining identical. Also, the incorporation is totally dependent on the addition of poly(A)-containing mRNA. Although not reported here, the presence of all four nucleotides, enzyme, and oligo(dT) primer supplied as oligo(dT)-cellulose were all necessary for the activity as previously observed by Venetianer and Leder (1974). Along side this control experiment, a pilot reaction was run using identical reaction mixture (8 ml of final volume and 40 μg of poly(A)-containing RNA purified from total mt) except that unlabeled nucleotides were used. Assuming that the rate of cDNA synthesis in the pilot reaction is comparable to that of control (Table III), roughly 8.5–9.0 μg of oligo(dT)-cellulose-bound cDNA was formed. These results directly agree with those of Venetianer and Leder (1974) who observed that the extent of cDNA synthesis was about 15–20% of the input mRNA.

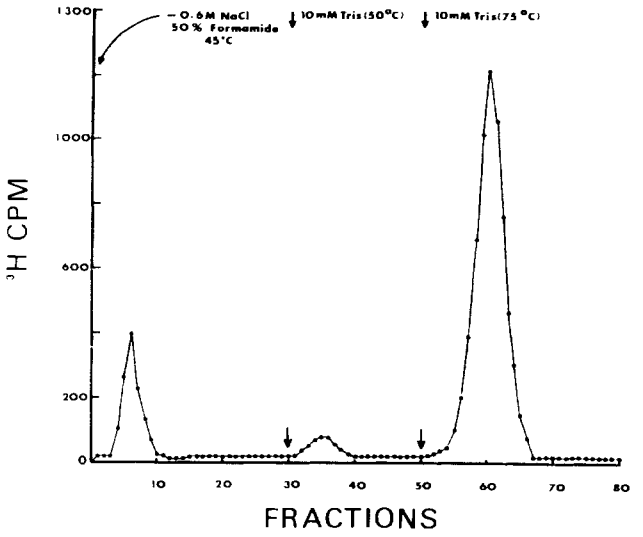

FIGURE 4: Sequence homology between mt poly(A)-containing mRNA and solid phase bound cDNA. Poly(A)-containing mRNA was prepared from mt polysomes as described in Figure 2 and purified as described in Figure 3. An RNA sample containing 8600 ^{3}H cpm in 2.0 ml of 10 mM Tris-HCl (pH 7.5), 0.6 M NaCl, and 50% formamide was applied to the column and washed with 13 ml of the same buffer at 45 °C. The column was then washed with 10 ml of 10 mM Tris-HCl (pH 7.5) at 50 °C and finally with 15 ml of 10 mM Tris-HCl (pH 7.5) at 75 °C. Fractions of 0.5 ml were collected in the scintillation vials and counted with 10 mM Cab-O-Sil mixture.

If indeed the enzyme copied the mt poly(A) containing RNA to form cDNA, the solid phase-bound product should exhibit exclusive sequence specificity for poly(A)-containing RNA from mt polysomes. To verify this possibility, about 0.4 μg of mt poly(A)-containing mRNA, chemically labeled with [^{3}H]dimethyl sulfate, was layered on the cDNA column and analyzed for complementarity. Under the chromatographic conditions used, the unbound RNA elutes out at 45 °C in the application buffer. RNA bound nonspecifically elutes at room temperature (25 °C) in 10 mM Tris buffer (see Venetianer and Leder, 1974). Loose hybrids, because of random complementarity or RNA bound due to oligo(dT) and poly(A) interactions, elute at 50 °C in 10 mM Tris buffer. Only the RNA strands tightly hybridized to cDNA elute above 70 °C. As seen in Figure 4, over 85% of the mt poly(A)-containing RNA specifically hybridizes with the cDNA indicating a high degree of complementarity.

The sequence specificity of the solid phase bound cDNA was further verified by analyzing various cellular RNAs. As shown in Table IV, less than 3% of mt rRNA, or cytoplasmic rRNA, and about 5% of cytoplasmic poly(A) containing RNA hybridize with cDNA. These results clearly show that the cDNA is specific for mt poly(A) containing RNA.

TABLE IV: Extent of Complementarity of Solid Phase-Bound cDNA to Various Cellular RNAs.[a]

RNA Tested	% RNA Binding to cDNA	
	Loose Hybrids	Specific Hybrids
Cytoplasmic rRNA	1.6	1.9
Cytoplasmic poly(A) containing RNA	2.3	3.2
mt rRNA	1.5	2.1
mt poly(A) containing RNA	4.5	82.4

[a] Cytoplasmic RNAs were prepared from Ehrlich ascites cells labeled with ^{32}P as described earlier (Lewis et al., 1976). Release of high Mg^{2+} polysomes with puromycin, separation of ribosome particles and isolation of poly(A)-containing RNA on oligo(dT)-cellulose were all as described under the Experimental Procedures. Mt rRNA was prepared as described in Figure 1. For details of chromatography see Figure 4.

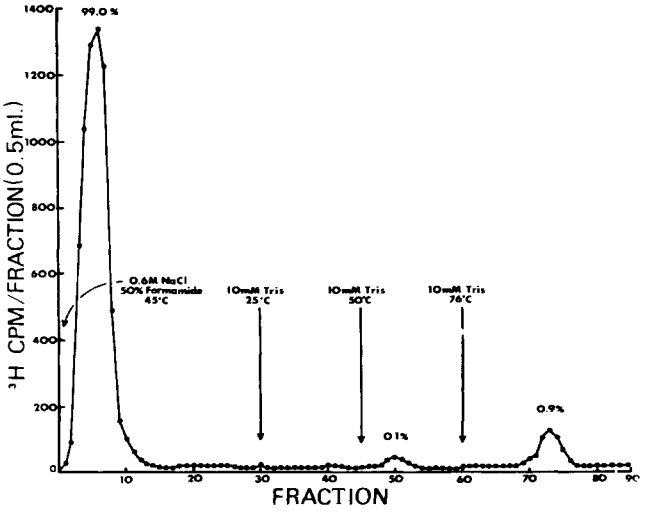

FIGURE 5: Analysis of poly(A)-lacking mRNA on the cDNA column. The poly(A)-lacking mRNA was prepared and purified as described in Figure 3 and under Experimental Procedures. RNA (8500 cpm) was layered on the cDNA column and analyzed as described in Figure 4 except that an additional washing with 7.5 ml of 10 mM Tris-HCl (pH 7.5) at 25 °C was introduced.

After having shown the specificity of the cDNA preparation, we used this procedure to determine the extent of sequence homology between the mt poly(A)-containing and -lacking mRNAs. As shown in Figure 5, about 99% of the input poly(A)-lacking mRNA counts elute at 45 °C in the application buffer, while the remaining 1% of the RNA forms either loose or tight hybrids with the cDNA. Although not shown here, the percent of binding did not significantly increase when the amount of RNA analyzed was reduced (up to tenfold). These results demonstrate that the majority of mt poly(A)-lacking mRNAs have sequence properties distinctly different from the mt poly(A)-containing mRNA.

Discussion

The present studies were undertaken to provide unequivocal evidence for the presence or absence of poly(A)-lacking mRNA species in mammalian mitochondria. Because of the difficulty in obtaining high specific activity mt mRNA in sufficient quantities, chemical labeling with [^3H]dimethyl sulfate was used throughout these experiments. The use of chemical labeling also eliminates the uncertainty of the uniformity of labeling generally experienced in the pulse-labeling technique. It is, therefore, apparent that labeled mRNAs used in these studies closely resemble the steady-state population of mt mRNAs. It was also essential to ensure that the chemical labeling procedure causes minimal degradation of mRNAs. As shown in Figure 1, the radioactivity patterns of mt total RNA and rRNA closely resemble the optical density profiles indicating minimal degradation of RNA during labeling. Further, the conditions of polysome isolation and release of mRNA do not cause any detectable cleavage of mt rRNA strands (see Figure 1B) suggesting that no extensive degradation of mRNAs may occur during isolation. The release of mRNA by puromycin appears to be quantitative, since over 96% of the poly(A) estimated in polysomal RNA was recovered in the puromycin released fraction. Assuming that the poly(A)-lacking mRNAs react similarly to puromycin treatment, it appears that mRNA molecules used in these studies are true representatives of the mRNA pools associated with the polysome complex.

As analyzed on oligo(dT)-cellulose column (Figure 2) about 49% of the puromycin released mRNAs do not contain poly(A) runs. The poly(A)-containing and -lacking mRNAs show a marked difference in their electrophoretic mobility; the former migrating as 9–16 Sϵ, and the latter 8–12 Sϵ. However, since there is a significant overlap in the patterns, it was possible that some or all of the poly(A) lacking species were either degradation or deadenylation products of mt poly(A)-containing RNA. The hybridization results presented in Table II indicate that the mt poly(A)-lacking species may have sequence properties different from the poly(A)-containing species, since a tenfold higher concentration of the oligo(dT)-bound fraction from total mt RNA did not show significant competition with the poly(A)-lacking species. The hybridization results also show that most of the poly(A)-lacking mRNAs are the transcription products of the mt genome. Due to the limited availability of competing RNA, further proof was sought using the solid phase-bound cDNA technique. This method has several advantages over DNA–RNA hybridization. For example, the bound cDNA can be repeatedly used for several experiments, and also, the problem of loose-association due to random complementarity can be easily detected and avoided in this procedure. The results clearly show that cDNA prepared against poly(A)-containing RNA purified from total mt RNA shows high sequence specificity for mt poly(A)-containing RNA (Figure 4), and exhibits low-sequence homology with mt rRNA, cytoplasmic rRNA, and cytoplasmic poly(A)-containing RNA (Table IV). In keeping with the DNA–RNA hybridization results, mt poly(A)-lacking mRNAs show less than 1% homology with cDNA. These results provide definitive evidence for the existence of discrete poly(A)-lacking mRNAs in Ehrlich ascites mitochondria. In keeping with our previous (Avadhani et al., 1974) and present findings, Kisselev et al. (1975) have recently shown that only about 30% of RNA released from rat liver mt polysomes bind to oligo(dT)-cellulose implying that the rest of the mRNAs may lack poly(A).

Poly(A)-lacking mRNAs believed to be histone messengers were first reported by Adesnik and Darnell (1972) and Greenberg and Perry (1972). Recently, poly(A)-lacking mRNA species coding for non-histone proteins have been discovered by Nemer et al. (1975), and by Milcarek et al. (1974). More recent experiments of Greenberg (1975) show that up to 40% of cytoplasmic polysome-associated mRNAs in animal cells might lack poly(A). Although the exact role of

mt poly(A)-lacking mRNA is unknown, our results show that as in the cytoplasmic system, the mt protein synthesis involves the translation of both poly(A)-containing and poly(A)-lacking mRNAs.

Acknowledgments

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