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RNA SYNTHESIS IN MITOCHONDRIA ISOLATED FROM RAT LIVER

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Abstract. Mitochondrial RNA (mtRNA) was synthesized from purine and pyrimidine nucleosides in coupling with oxidative phosphorylation using isolated mitochondria. The *in vitro* synthesized mtRNA was adenine-uracil rich and sedimented at about 20 S by sucrose density gradient centrifugation. A major part of the newly synthesized mtRNA was shown to be poly (A)-containing RNA by the resistance to the digestion with pancreatic RNase and RNase T_1 and the affinity to poly (U)-Sepharose columns or Millipore filters.

In recent years several investigations (1–4) have reported the existence of RNA bonding covalently about 4 S poly (A) chain at its 3′-terminus in mitochondrial fraction isolated from cells labeled with ^{3}H-nucleosides in culture medium, and it seems that this poly (A)-containing RNA is synthesized in mitochondria. However, the synthesis site of the poly (A)-containing RNA is obscure, since poly (A) polymerase has been identified in cytosolic (5, 6) and microsomal (6) fractions. There are several conflicting reports on the synthesis of poly (A)-containing RNA using mitochondria isolated from Ehrlich ascites tumor (4, 7), yeast (8, 9) and rat liver (10). Recently, Rose and Jacob (11) reported that rat liver mitochondria were able to add poly (A) chain having 20 to 23 AMP units to endogenous RNA, and this poly (A) chain was much smaller compared with that in mitochondrial RNA (mt RNA) synthesized *in vivo* (1–4). The specific activity of label in RNA synthesized in these *in vitro* experiments was generally very low. Therefore, there seems to be great difficulty in the critical characterization of RNA synthesized by isolated mitochondria.

Inaba and Oda (12) previously reported that mitochondria isolated from rat liver are capable of synthesizing purine and pyrimidine nucleoside triphosphates from corresponding nucleosides in coupling with oxidative phosphorylation. The present paper introduces an approach to the study of RNA synthesis in isolated mitochondria using this *in vitro* system coupled with oxidative phosphorylation and reports the partial characterization of this newly synthesized RNA.

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MATERIALS AND METHODS

Materials. ^{32}Pi (carrier free) and ^{3}H-adenosine (1-3 Ci/m mole) were obtained from the Japan Atomic Energy Institute and the Radiochemical Center (England), respectively. Purine and pyrimidine nucleosides were obtained from Nakarai Pure Chemicals, Japan. Nucleotides and Dowex I (×2) resin were purchased from Sigma Chemical Co. Poly (U)-Sepharose 4B was obtained from Pharmacia Fine Chemicals.

Preparation of mitochondria. Mitochondria were prepared from the liver of young male rats (Donryu strain) by a modification of the method of Hogeboom (13), which involves one more treatment of the homogenate on 0.34 M sucrose phase and two more washing with 0.25 M sucrose containing 5mM Tris-HCl (pH 7.4) buffer at the final step.

Labeling of mitochondria. Unless otherwise mentioned, mitochondria (100mg protein) were suspended in 50ml of a buffer containing 5 mM Tris-HCl (pH 7.4), 170 mM sucrose, 2 mM $MgCl_2$, 0.1 mM EDTA, 10 mM potassium α-ketoglutarate, 2mM ^{32}P-labeled phosphate buffer (200 mCi/m mole, pH 7.4), 0.1% bovine serum albumin, 2 mM adenosine, 1 mM guanosine, 1 mM cytidine and 1 mM uridine. Incubation was carried out at 25°C for 60 min in a shaking water bath. The reaction was then stopped by adding an equal volume of cold 20 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose and 0.05% bovine serum albumin, and the mixture was centrifuged at 10,000×g for 10min and the precipitate was used for extracting RNA.

Extraction of RNA. Mitochondria were dissolved in sodium dodecyl sulfate (SDS) buffer (1% SDS, 100 mM NaCl, 10 mM EDTA, 20 mM Tris-HCl, pH 7.4, and 0.004% polyvinyl sulfate), and RNA was extracted by a modification of the SDS-phenol method (14). This mtRNA fraction was separated into salt soluble RNA and salt insoluble RNA (SI-mtRNA) using the hypertonic salt fractionation method (15) in the presence of 0.004% polyvinyl sulfate (PVS). The SI-mtRNA fraction was applied on the gel filtration of Sephadex G-50 column and eluted with 0.1 M acetate buffer (pH 5.4) containing 0.004% PVS.

Sucrose density gradient of RNA. An appropriate amount of RNA was layered on 5 ml of 5 to 20% sucrose gradient in 0.1 M acetate buffer, pH 5.5. A Spinco rotor SW 39 was used at 34,000 rev/min for 300 min at 4°C, and the gradient was usually fractionated into 20 tubes.

Ion exchange chromatography. RNA was hydrolyzed at 37°C for 20 hr with 0.3 N NaOH, and the mononucleotides were applied on a Dowex 1 (×2) formate form column (200-400 mesh, 0.8 cm×25 cm). Elution was performed by passing the following fluids in succession into a 100 ml mixing chamber containing 50 ml of distilled water: 30 ml of distilled water, 30 ml of 1 N formic acid and 240 ml of 4 N formic acid. The elution rate was 12 ml/hr, and 3 ml fractions were collected.

Detection of poly (A)-containing RNA. Poly (A) sequence in the SI-mtRNA was detected by affinity chromatography (16) and by the Millipore filter method (17).

Radioactivity. Measurements were carried out in Bray's dioxane scintillator solution with a Packard Tri-Carb scintillation counter (Model 3320). In ion exchange chromatography, a 2 ml portion of ^{32}P-labeled fraction was dried under an infrared lamp, and the radioactivity was estimated with a gas-flow counter (Aloka Inc., Japan).

RESULTS

Sucrose density gradient centrifugation was carried out to verify the size of SI-mtRNA. Fig. 1 shows the sedimentation profile of SI-mtRNA isolated from mitochondria after labeling for 120 min at 25°C. A prominent radioactive peak was recognized at about 20 S. A similar sedimentation profile was also observed in SI-mtRNA isolated from mitochondria labeled for 60 min at 25°C. These SI-mtRNA had specific complementarity with mitochondrial DNA in molecular hybridization, using Gillespie and Spiegelman's method (18) (unpublished data).

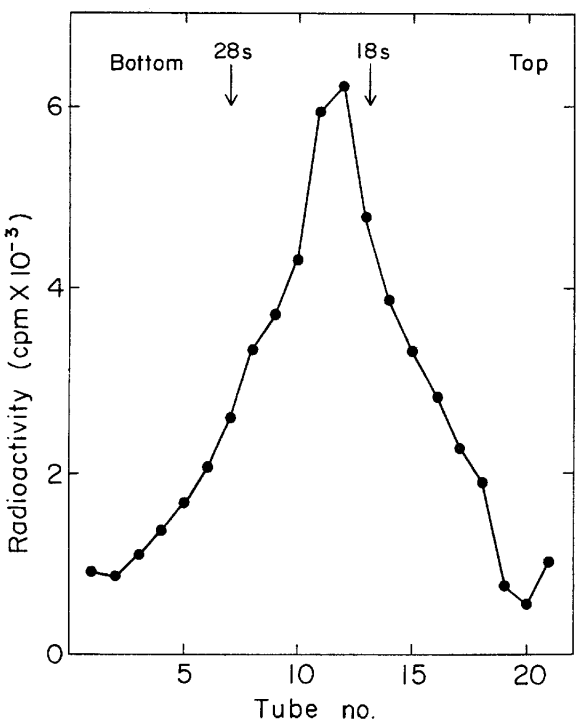

Fig. 1. Sucrose gradient centrifugation of *in vitro* synthesized SI-mtRNA. The reaction mixture containing 100 mg protein of mitochondria was incubated for 120 min at 25°C. Extraction and purification of mtRNA were carried out as described in the text. 28 S and 18 S rRNAs were used as markers (arrows).

TABLE 1. NUCLEOTIDE COMPOSITION OF *in vitro* SYNTHESIZED SI-mtRNA

Incubation time (25°C)	^{32}P distribution (%)			
	AMP	CMP	GMP	UMP
60 min	33.5	17.1	23.7	25.8
120 min	31.3	14.7	23.6	30.6

To analyze the nucleotide composition of the ^{32}P-labeled mtRNA, the RNA was hydrolyzed and the hydrolysate was fractionated with Dowex 1 column chromatography. The newly synthesized SI-mtRNA was adenine-uracil rich, namely, the AMP + UMP/GMP + CMP ratio was 1.45 in the labeling for 60 min and 1.62 in the labeling for 120 min (Table 1).

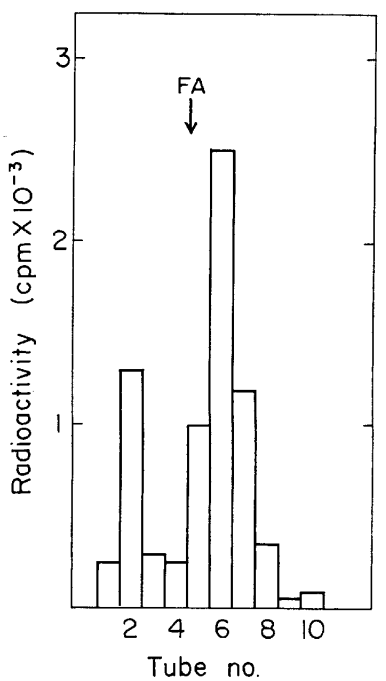

Fig. 2. Affinity chromatogram of *in vitro* synthesized SI-mtRNA on poly (U)-Sepharose 4B column. Mitochondria (72 mg protein) were suspended in 50 ml of 5mM Tris-HCl buffer (pH 7.4) containing 170 mM sucrose, 2 mM $MgCl_2$, 0.1 mM EDTA, 10 mM potassium α-ketoglutarate, 2 mM phosphate buffer (pH 7.4), 2 μM ^{3}H-labeled adenosine (5 mCi/μ mole), 1 mM guanosine, 1 mM cytidine, 1 mM uridine and 0.1% BSA. Incubation was carried out at 25°C for 60 min. ^{3}H-labeled SI-mtRNA was extracted as described as in the text. ^{3}H-labeled SI mtRNA (8100 cpm/0.1 ml) was applied on a poly (U)-Sepharose 4B column (0.5×3 cm). Elution was carried out by passage through the following media: (a) 4 ml of 25% formamide containing 10 mM EDTA, 0.7 M NaCl and 50 mM Tris-HCl (pH 7.5) and (b) 4 ml of 90% formamide (FA) containing 10 mM EDTA, 0.2% laurylsarcosine and 10 mM potassium phosphate buffer (pH 7.5) The elution rate was 4 ml/hr, and 1 ml fractions in (a) and 0.25 ml fractions in (b) were collected.

Two different methods were used to detect whether the poly (A) sequence is contained in newly synthesized SI-mtRNA.

The mtRNA showed a strong affinity to the poly (U)-Sepharose column (Fig. 2).

TABLE 2. EFFECT OF RNASE TREATMENT ON THE BINDING OF *in vitro* SYNTHESIZED SI-mtRNA TO MILLIPORE-FILTERS

RNase treatment	^{32}P counts bound to the filter**	
	TCA treatment (cpm)	Without TCA treatment (cpm)
None	908	744
RNase A* (10 μg/ml)	136	120
RNase A (30 μg/ml)	116	124
RNase A (60 μg/ml)	124	120
RNase A (100 μg/ml) and RNase T_1* (20 unit/ml)	76	104

* Pancreatic RNase and T_1 RNase were products of Boehringer.

** HA WPO24, Millipore Filter Corp.

^{32}P-labeled SI-mtRNA was isolated from mitochondria after incubation for 60 min at 25°C, as described in the text, and brought in contact with Millipore filters (11). RNase treatment of samples was carried out for 20 min at 30°C in 50 mM Tris-HCl (pH 7.6), 50 mM KCl and 1 mM $MgCl_2$. Values are expressed in cpm after correction for background (12-20 cpm).

The newly synthesized SI-mtRNA was also retained on nitrocellulose membrane without pretreatment with trichloroacetic acid and showed resistance to digestion by pancreatic RNase and RNase T_1 (Table 2). These results appear to show that the poly (A) sequence is contained in the SI-mtRNA synthesized by isolated mitochondria.

DISCUSSION

The present study together with our previous report (12) indicates that isolated mitochondria are able to synthesize RNA from purine and pyrimidine nucleosides in coupling with oxidative phosphorylation. This evidence implies that mitochondria are capable of an autonomous energy supply for biogenesis, as aerobic bacteria. The present reaction mixture system was useful for estimating the nucleotide composition of mtRNA synthesized *in vitro*.

Recently, several investigators (7, 8, 10) reported that isolated mitochondria were able to synthesize poly (A)-containing RNA. Aujame and Freeman (7) reported that poly (A)-containing RNA synthesized by isolated mitochondria sedimented at about 18 S with liver cytosolic rRNA which was contained in their preparation of mtRNA. The reasons for the conflict between their results and

those reported by Avadhani, Lewis and Rutman (4) using the same mitochondrial preparation are not known.

The present study provides partial characterization of the poly (A) sequence in mtRNA synthesized by isolated mitochondria.

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REFERENCES

1. Hirsch, M. and Penman, S.: Mitochondrial polyadenylic acid-containing RNA: Localization and characterization. *J. Mol. Biol.* **80**, 379-391, 1973.
2. Hirsch, M. and Penman, S.: Post-transcriptional addition of polyadenylic acid to mitochondrial RNA by a cordycepin-insensitive process. *J. Mol. Biol.* **83**, 131-142, 1974.
3. Ojala, D. and Attardi, G.: Identification of discrete polyadenylate-containing RNA components transcribed from HeLa cell mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* **71**, 563-567, 1974.
4. Avadhani, N. G., Lewis, F. S. and Rutman, R. J.: Messenger ribonucleic acid metabolism in mammalian mitochondria. Quantitative aspects of structural information coded by the mitochondrial genome. *Biochemistry* **13**, 4638-4645, 1974.
5. Edmonds, M. and Abrams, R.: Polynucleotide biosynthesis: Formation of a sequence of adenylate units from adenosine triphosphate by an enzyme from thymus nuclei. *J. Biol. Chem.* **235**, 1142-1149, 1960.
6. Wilkie, N. M. and Smellie, R. M. S.: Chain extension of ribonucleic acid by enzymes from liver cytoplasm. *Biochem. J.* **109**, 485-494, 1968.
7. Aujame, L. and Freeman, K. B.: The synthesis of polyadenylic acid containing ribonucleic acid by isolated mitochondria from Ehrlich ascites cells. *Biochem. J.* **156**, 499-506, 1976.
8. Cooper, C. S. and Avers, C. J.: Evidence of involvement of mitochondrial polysomes and messenger RNA in the synthesis of organelle proteins. In *The Biogenesis of Mitochondria*, ed. A. M. Kroon and C. Saccone, Academic Press, New York, pp. 289-303, 1974.
9. Groot, G. S. P., Flavell, R. A., Van Ommen, G. J. B. and Grivell, L. A.: Yeast mitochondrial RNA does not contain poly (A). *Nature* **252**, 167-169, 1974.
10. Cantatore, P., De Giorgi, C. and Saccone, C.: Synthesis of poly (A) containing RNA in isolated mitochondria from rat liver. *Biochem. Biophys. Res. Commun.* **70**, 431-437, 1976.
11. Rose, K. M. and Jacob, T.: Poly (adenylic acid) synthesis in isolated rat liver mitochondria. *Biochemistry* **15**, 5046-5052, 1976.
12. Inaba, K. and Oda, T.: Phosphorylation of purine and pyrimidine nucleosides by isolated liver mitochondria. *Acta Med. Okayama* **29**, 367-375, 1975.
13. Hogeboom, G. H.: Fractionation of cell components of animal tissue. In *Method in Enzymology*, Vol. 1, ed. S. P. Colowick and N. O. Kaplan, Academic press, New York and London, pp. 16-19, 1955.
14. McCarthy, B. J. and Hoyer, B. H.: Identity of DNA and diversity of messenger RNA molecules in normal mouse tissues. *Proc. Natl. Acad. Sci. USA* **52**, 915-922, 1964.
15. Crestfield, A. M., Smith, K. C. and Allen, F. W.: The preparation and characterization of ribonucleic acids from yeast. *J. Biol. Chem.* **216**, 185-193, 1955.
16. Lindberg, U. and Persson, T.: Isolation of mRNA from KB-cells by affinity chromatography on polyuridylic acid covalently linked to Sepharose. *Eur. J. Biochem.* **31**, 246-254, 1972.
17. Lee, S. Y., Mendecki, J. and Brawerman, G.: A polynucleotide segment rich in adenylic acid in the rapidly labeled polyribosomal RNA component of mouse sarcoma 180 ascites cells. *Proc. Natl. Acad. Sci. USA* **68**, 1331-1335, 1971.
18. Gillespie, D. and Spiegelman, S.: A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *J. Mol. Biol.* **12**, 829-842, 1965.