PROTEIN SYNTHESIS WITH ISOLATED MITOCHONDRIAL POLYSOMES

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A technique has been described for the isolation of intact mitochondrial polysomes which are active in protein synthesis. Mitochondrial ribosomes, derived from the polysomes, are of 71S particles and have 32S and 50S subunits. These subunits can initiate protein synthesis using mitochondrial RNA as a template. It is, thus, evident that mitochondria contain active messengers in the process of translation. The translational machinery in this organelle is prokaryotic in nature.

Mitochondria appear to contain an autonomous protein-synthesizing system (1). These organelles contain tRNA, f-Met-tRNA (2-4), and specific peptide-chain elongation factors (5). Mitochondrial lysates are known to incorporate amino acids (6,7). Further, various mitochondrial ribosomes have been reported to initiate protein synthesis on synthetic and viral RNA templates (8-10). Nevertheless, to date, no isolation of active mitochondrial polysomes or mitochondrial m-RNA dependent ribosomes has been reported.

The present paper reports the isolation of mitochondrial polysomes which participate directly in protein synthesis without the necessity of an exogenous m-RNA being added to the system. Data presented here also show the presence of a prokaryotic-type protein synthesis in the polysomal and ribosomal systems.

MATERIALS AND METHODS

Organism: A streptomycin-bleached, chloroplast-lacking mutant of Euglena gracilis (SM-L1) was grown in a defined medium containing 0.2 M ethanol as carbon source (11).

Isolation of Mitochondria: Cells were washed twice with KTM 15 buffer (25 mM KCl, 50 mM Tris-HCl, 15 mM $MgCl_2$, pH 7.6) containing 0.25M sucrose and resuspended in twice their volume with the same buffer. A crude yet RNase specific protein fraction from rat liver (12) and heparin (13) were used as RNase inhibitors and were added to the cell suspension at the final concentrations of 1.0 mg/ml and 500 µg/ml respectively. Mitochondria were isolated by a glass-bead grinding technique and purified by differential centrifugation as described (14).

Isolation of Polysomes: The final mitochondrial pellet was suspended in twice its volume with KTM 15 buffer containing 0.25M sucrose. Rat liver isolate and heparin were added as above. Mitochondria were lysed with 0.3% sodium deoxycholate. The lysate was centrifuged at 15,000 X g for 10 min. The supernatant (2.3 ml) was layered over 1.0 ml of 2.0M sucrose in KTM 15 buffer and centrifuged at 405,000 X G (max.) for 2.5 hr at 0-2°C. The polysomal pellet was rinsed 3-4 times with KTM 15 buffer and was suspended in the same buffer at a final concentration of 2 mg RNA/ml (1.0 O.D. unit at 260 nm = 50 µg RNA).

Preparation of Ribosomes: The polysomal pellet was rinsed and suspended with KTM.2 buffer (25 mM KCl, 50 mM Tris-HCl, 0.2 mM $MgCl_2$, pH 7.6) containing 0.25M sucrose and incubated in an ice bath for 2 hr. The resultant ribosomes and subunits were pelleted at 405,000 X g (max.) for 2 hr and suspended in KTM.2 buffer at a concentration of 2.0 mg RNA/ml.

Sucrose Density-Gradient Centrifugation: Exponential gradients (15) of 10-34% sucrose (12 ml volume) were used for these experiments. The layering volume was 0.1 ml and contained about 3 units of 260 nm-absorbing material. Gradients were centrifuged at 210,000 X G (max.) for 90 min at 0-2°C and analysed in a Gilford Densitometer, Model 2000, with the help of an ISCO gradient fractionator, Model 182.

In vitro Protein Synthesis: The pH 5.4 enzymes were prepared from the S-200 fractions of Euglena cytoplasm, mitochondria, E. coli A19 and rat liver cytoplasm (16) and were added to the system at a concentration of 2.5 O.D.-260 nm-absorbing units/tube. The ribosomal system contained ribosomes equivalent to 100 µg RNA and 80 µg of phenol-extracted (17) total mitochondrial RNA. Protein synthesis assays were run at 37°C for 30 min in 0.2 ml final volumes. Other conditions were as described earlier (18,19) excepting that 1.0 µCi of ^{14}C amino acid mix (New England Nuclear, 1.8 µCi/mg) was added to each tube.

RESULTS AND DISCUSSION

The mitochondrial polysomal fraction isolated by the present technique contains highly polymerized particles as indicated by at least 6 peaks of U.V. absorbing ribosomal forms on a sucrose gradient (Fig. 1A). RNase treatment degrades the polymeric particles to mono-ribosomes as expected (Fig. 1B). Further, low concentrations of Mg^{+2}, such as 0.2 mM, dissociates the polysomes into 71S ribosomes and subunits of 50S and 32S (Fig. 1C). The ribosomal preparations yield practically no U.V. absorbing material in the 87S region of the gradient (Fig. 1C). Cytoplasmic ribosomes (87S) are known to be less sensitive to low concentrations of Mg^{+2} ions as compared to their mitochondrial counterparts

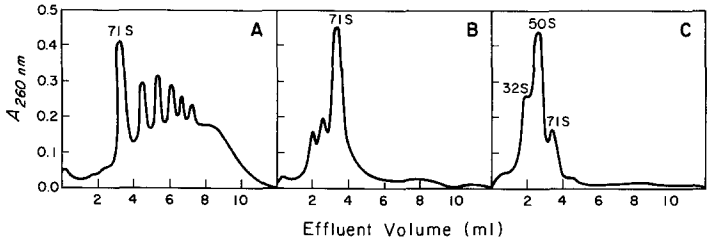

Fig. 1. Sucorse density gradient analysis of: (A) Mitochondrial polysomes; (B) Polysomes treated with 2 µg RNase (bovine pancreatic, Nutritional Biochemicals)/500 µg polysomal RNA for 15 min at 0-4°C; and (C) Mitochondrial ribosomal preparation. S values were determined using E. coli and rat liver ribosomes as markers. Other details were as described in the Methods.

(20,21). Thus, the mitochondrial polysomes in these experiments appear to be free of cytoplasmic components.

Another criterion for characterizing polysomes is met by the ability of these mitochondrial isolates to participate directly in protein synthesis (Table I). The activity of these polysomes was dependent upon the addition of pH 5.4 enzymes (Table I), and ATP and GTP. On the other hand, mitochondrial ribosomes similarly supplied with pH 5.4 enzymes ATP, GTP, etc., exhibit little activity in the absence of added messenger RNA (18,19). Phenol-extracted mitochondrial RNA serves as an efficient message in these mitochondrial ribosomes (Table I). Both the polysomal and ribosomal systems are inhibited by streptomycin and not by cycloheximide (Table I) as expected (5-9).

The mitochondrial ribosomal system requires pH 5.4 enzymes either from mitochondria or from E. coli for complete activity (Table II). The similar fraction from Euglena cytoplasm* is 50% efficient, while the rat liver* cytoplasmic fraction is only 12% efficient (Table II). Similar results were observed with mitochondrial polysomes. These results support the findings that mitochondrial protein-synthesizing systems require prokaryotic-type tRNA (2-4) and enzymes (8-10). The presence of mitochondrial-specific peptide-chain elongation factors, different from cytoplasmic but similar to bacterial factors, has been reported in yeast (5).

The problem of cross contamination by cytoplasmic components has impeded studies on the molecular aspects of protein synthesis in various cellular compartments including mitochondria (1). The one claim (22) of isolation of mitochondrial ribosomal aggregates capable of participating directly in protein synthesis has been disputed (1). Other available evidence is indirect, for example, labeling the

*The decreased efficiency observed with the Euglena cytoplasmic and rat liver pH 5.4 enzymes was not because of their inactivation during isolation or storage since both the fractions showed high activity in the Euglena cytoplasmic system.

Table I. *In vitro* Protein Synthesis by Mitochondrial Polysomes and Ribosomes.

System	Description	CPM/0.2 ml*
Polysomal	Complete	18,750
	-Polysomes	1,640
	-pH 5.4 enzymes	1,790
	+Streptomycin	6,570
	+Cycloheximide	16,835
Ribosomal	Complete	11,580
	-Template RNA	1,970
	-Ribosomes	1,275
	-pH 5.4 enzymes	1,760
	-ATP and GTP	2,680
	+Streptomycin	2,096
	+Cycloheximide	10,470

*Values are mean of triplicate tubes. Assays were run as described in the Methods. For this experiment, pH 5.4 enzyme fraction from mitochondria was used. Streptomycin (Nutritional Biochemical Corp.) and cycloheximide (Sigma) when added were at the concentration of 50 µg/0.2 ml system.

Table II. Effect of pH 5.4 Enzymes from Various Sources on the Activity of Mitochondrial Ribosomal System.

Source of Enzymes	% Activity
Mitochondria	100
E. coli	115
Euglena cytoplasm	49
Rat liver	12

Assays were carried out as in Table I. The ribosomal system (see Table I) using mitochondrial pH 5.4 enzymes was considered to be 100% active. Relative activities are given for the other pH 5.4 enzymes.

mitochondrial components in the presence of specific cytoplasmic inhibitors. The present experiments show the possibility of isolation of intact mitochondrial polysomes, active in protein synthesis. These polysomes are free of cytoplasmic components as seen by their specific dissociation in a low Mg^{+2} buffer, degradation pattern with RNase, and insensitivity to cycloheximide for protein synthesis. Further, these results can not reflect polysomes derived from component plastids for the colorless *Euglena* (SM-L1) does

not contain proplastids. Also, protein synthesis with mitochondrial mRNA and ribosomes requires the tRNA and enzymes either from mitochondria or from E. coli. Similar specificity of tRNA and enzyme requirements have been reported in various systems (5,8-10) using non-mitochondrial templates.

In conclusion, the present results provide direct evidence for the presence of active mRNA and occurrence of active translation in mitochondria. This system should provide a direct method for obtaining mitochondrial mRNA for further studies on in vitro protein synthesis in this organelle.

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