PHYSICAL CHARACTERIZATION OF RIBOSOMES FROM PURIFIED MITOCHONDRIA OF YEAST

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SUMMARY

Ribosomes from the cytoplasm sedimented in sucrose gradients as 80S monomers whereas mitochondrial ribosomes were 75S. Electrophoretic mobilities of the RNA's purified from isolated ribosomes were calculated to show that cytoplasmic molecules had molecular weights of 1.40×10^6 and 0.77×10^6 daltons, and mitochondrial ribosomal RNA's were 1.25×10^6 and 0.64×10^6 daltons. These RNA's were 26S and 19S for cytoplasmic particles and 25S and 17S for mitochondrial ribosomes, when compared against 23S and 16S *E. coli* RNA's as standards. Mitochondrial polysomes were identified on the bases of sedimentation profiles, dissociation to monomers after brief exposure to RNase, and electron micrographs of thin-sections of mitochondria and cells.

Although several species have been investigated, there have been conflicting reports in each case concerning the physical traits of the mitochondrial ribosomes as compared with particles from the cytoplasm of the same cells. Mitochondrial ribosomes from *Neurospora crassa* have been reported as 73S (1) and 81S (2), with cytoplasmic ribosomes of 77S and 81S, respectively. Vignais, Huet, and André (3) reported the isolation of 77-80S ribosomes from mitochondria of the yeast *Candida utilis*, while Schmitt (4) identified 80S mitochondrial ribosomes in *Saccharomyces cerevisiae*. In both cases, cytoplasmic ribosomes were calculated to be of the 80S type. The identification of polysomes by Küntzel and Noll (1) was based upon sedimentation patterns in sucrose gradients, and by Vignais *et al.* (3) from electron micrographs of mitochondrial thin-sections. In this report we describe the isolation of ribosomes and polysomes from purified mitochondria of *Saccharomyces cerevisiae* The sedimentation behavior of yeast mitochondrial ribosomes and the electrophoretic mobilities of their purified RNA's, as compared with those from cytoplasmic ribosomes, are shown to be at variance with results which have been reported by others (3,4).

MATERIALS AND METHODS

Cells of the diploid strain iso-N (5) were grown at 25° with shaking in a liquid semisynthetic (= SS) medium (5) which contained 10% glucose. Spheroplasts (6) prepared from these glucose-repressed cells (7) were partially derepressed in SS media containing 1 M sorbitol and 2% ethanol for 30 to 90 min at 25° on a rotary shaker. Then the spheroplasts were chilled quickly, collected by low-speed centrifugation, and resuspended for lysis in cold TMK buffer (50 mM Tris, 10 mM $MgCl_2$, and 10 mM KCl, at pH 7.5) containing 0.25 M sorbitol. All subsequent operations were carried out at 0-5°. Spheroplasts were lysed by a 20-sec treatment in a chilled Waring blendor and the resulting brei was centrifuged at 2,000 X *g* for 10 min. A crude mitochondrial fraction then was obtained from the cell-free supernatant fluid by centrifugation at 10,000 X *g* for 15 min. The mitochondria then either were washed 3 times in 0.5 M sorbitol--TMK buffer or they were incubated at room temperature for 10 min with 50 μg/ml bovine pancreatic ribonuclease before washing. The final purified mitochondrial fraction was resuspended in TMK containing 2% Triton X-100 and lysed in a glass tissue grinder. The ribosome suspension from the mitochondrial lysate was obtained by a final centrifugation at 27,000 X *g* for 20 min.

Cytoplasmic ribosomes were obtained by grinding whole glucose-repressed cells with sand in TMK buffer. The material was centrifuged for 5 min at low speed to remove sand and cellular debris, and the supernatant fluid was centrifuged further at 27,000 X *g* for 20 min to remove membranes and large particles.

For zonal separations the suspensions of mitochondrial or cytoplasmic ribosomes were made 2.5% with respect to sucrose and then introduced into a B-XV zonal rotor in a B-35 ultracentrifuge. Separations in swinging bucket rotors were carried out using either the SW25.1 rotor in the Model L (Beckman-Spinco) or the SB283 rotor in the B-35 (International Equipment Co.) ultracentrifuge.

Polyacrylamide gels (3%) were used for electrophoresis according to the method of Peacock and Dingman (8). The gels were stained with methylene blue and analyzed at 606 nm using a Beckman DU spectrophotometer equipped with a gel scanner.

RESULTS AND DISCUSSION

Ribosomes isolated from purified mitochondria showed similar sedimentation profiles both in swinging bucket and zonal rotors (Fig. 1 and 2). In each case most of the ribosomes migrated as a large, slowly-sedimenting monomer popula-

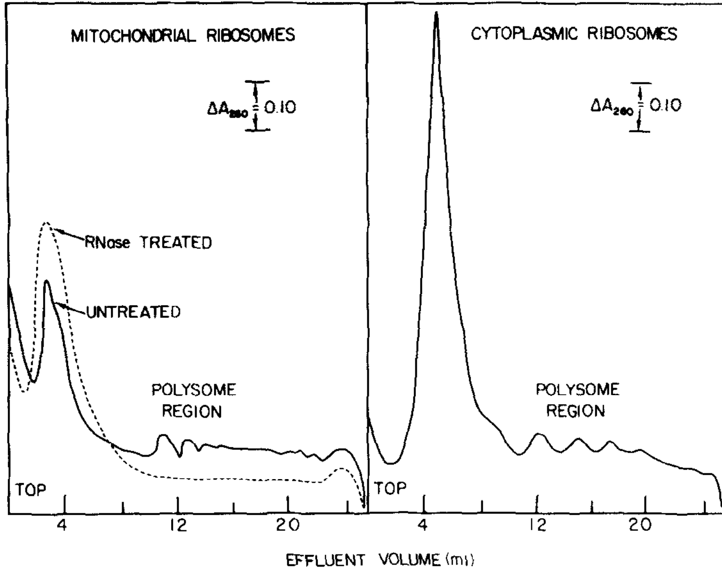


FIGURE 1. Sedimentation profiles of ribosomes isolated from purified mitochondria and from cytoplasm. Identical volumes from the same mitochondrial ribosome suspension either were untreated or RNase (30 µg/ml) was added just prior to centrifugation. All samples (0.2 ml) were layered on 27-ml linear gradients, 15-40% (w/v) sucrose--TMK, and were centrifuged for 3 hr at 25,000 r.p.m. in the Spinco SW25.1 rotor at 4°.

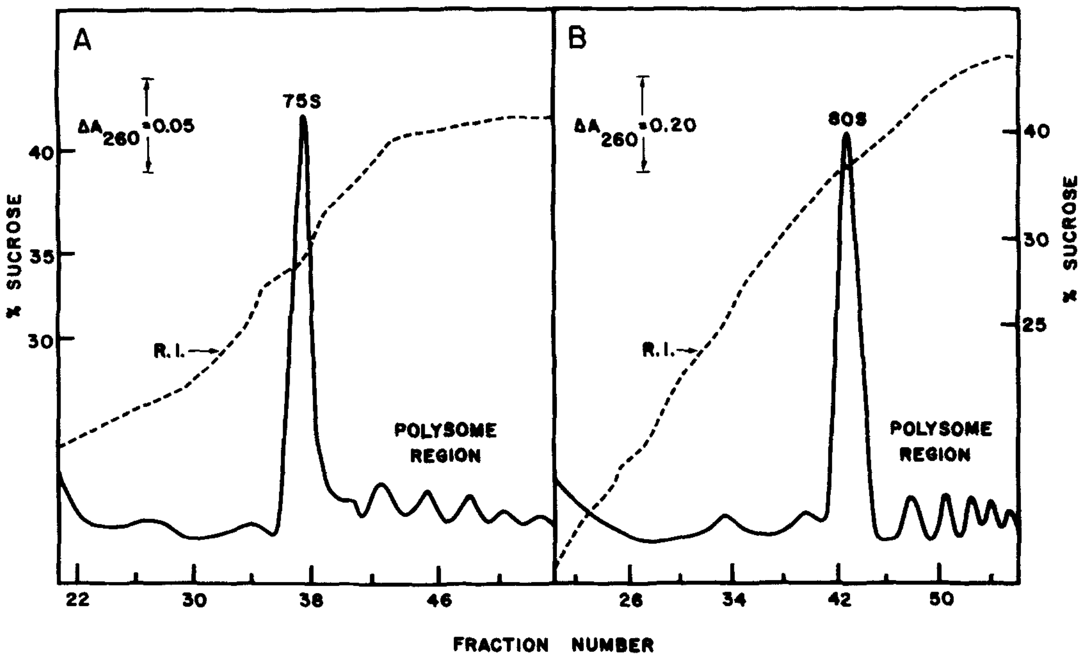


FIGURE 2. Zonal sedimentation profiles of ribosomes isolated from purified mitochondria (A) and from cytoplasm (B). Centrifugation was carried out in the B-XV zonal rotor with 25 ml of ribosome suspension and 500 ml of TMK overlay on 1150-ml isokinetic gradients, 5-55% (w/v) sucrose--TMK. The rotor was centrifuged for 15 hr at 21,500 r.p.m. at 4°. R.I. indicates the refractive index trace.

tion, representing about 25 μg RNA per mg mitochondrial protein. Less than half of the ribosomes migrated more rapidly than the monomer peak as clearly resolved polysome peaks. This behavior was observed whether mitochondria were washed several times, treated with ribonuclease before washing, or isolated under sterile conditions. Further evidence of the presence of polysomes was obtained by comparing the sedimentation profiles of two equal volumes of ribosome suspension obtained from the same particle preparation, one of which was exposed briefly to 30 μg/ml of ribonuclease and the other untreated (Fig. 1). After enzyme treatment there was a concurrent loss of the polysome region and an enhancement of the monomer peak as compared with the untreated sample.

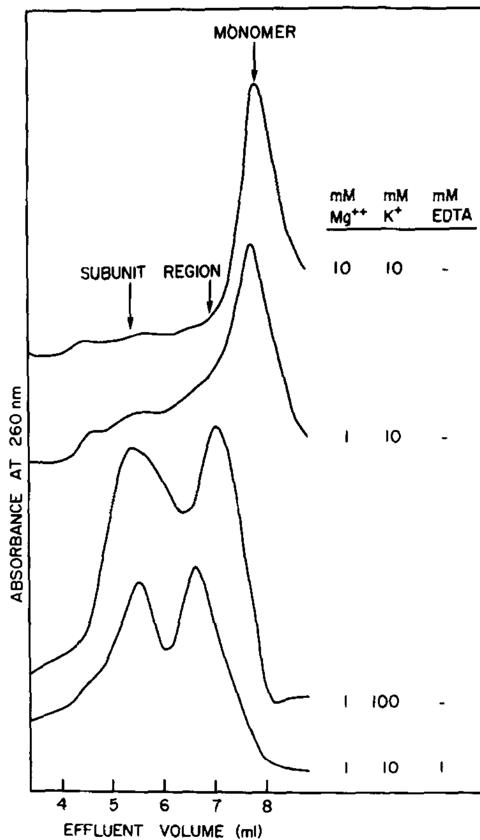


FIGURE 3. Effect of ion concentration on sedimentation behavior of purified mitochondrial ribosomes. Monomers were recovered from zonal gradient fractions by sedimentation in the Type 30 Spinco rotor at 27,500 r.p.m. for 17 hr at 4°. The resulting ribosome pellets were resuspended in TMK and 0.15 ml of the suspension (containing 5 mg RNA per ml) was layered on 11-ml gradients, 0.3-1.4 M sucrose in 50 mM Tris buffer (pH 7.5) with varying ion concentrations (indicated above). The gradients were centrifuged in the SB283 rotor at 35,000 r.p.m. for 3 hr at 4°.

When compared with cytoplasmic ribosomes (Fig. 1 and 2), we found that mitochondrial ribosomes consistently migrated somewhat more slowly. Using standard values of 70S for *E. coli* and 80S for yeast cytoplasmic monomers, we have estimated a value of 75S ± 1 for the mitochondrial ribosome based upon sedimentations in isokinetic sucrose gradients in the B-XV zonal rotor. An intermediate value also was found for *Neurospora crassa* mitochondrial ribosomes as compared with *E. coli* and cytoplasmic particles by Küntzel and Noll (1), but both Vignais *et al.* (3) and Schmitt (4) reported no significant differences between mitochondrial and cytoplasmic ribosomes in the yeasts *Candida* and *Saccharomyces*, respectively.

The sedimentation behavior of mitochondrial ribosomes varied depending upon the ionic strength of the sucrose gradient. Mitochondrial ribosomes were collected from the gradient after zonal centrifugation, sedimented, and re-

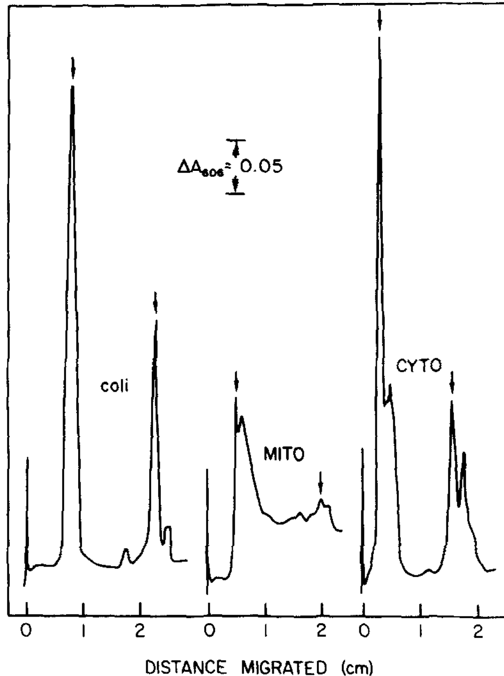


FIGURE 4. Electrophoretic mobility profiles of purified rRNA's from *E. coli*, mitochondrial, and cytoplasmic ribosomes. In each case the ribosomes were recovered from zonal gradient fractions by sedimentation in the Type 30 Spinco rotor at 27,500 r.p.m. for 17 hr at 4°. RNA's were purified by resuspending the ribosome pellets in 0.05 M NaCl--1 mM EDTA (pH 6.2), and treating aliquots of the suspension (0.1 ml containing about 0.75 mg RNA) with 5 µl of 20% sodium dodecyl sulfate. Electrophoresis was carried out in a vertical gel apparatus (E-C Apparatus Co.) at 200 v for 1.5 hr at 5°. Arrows indicate major RNA species.

Abbreviation: rRNA = ribosomal RNA

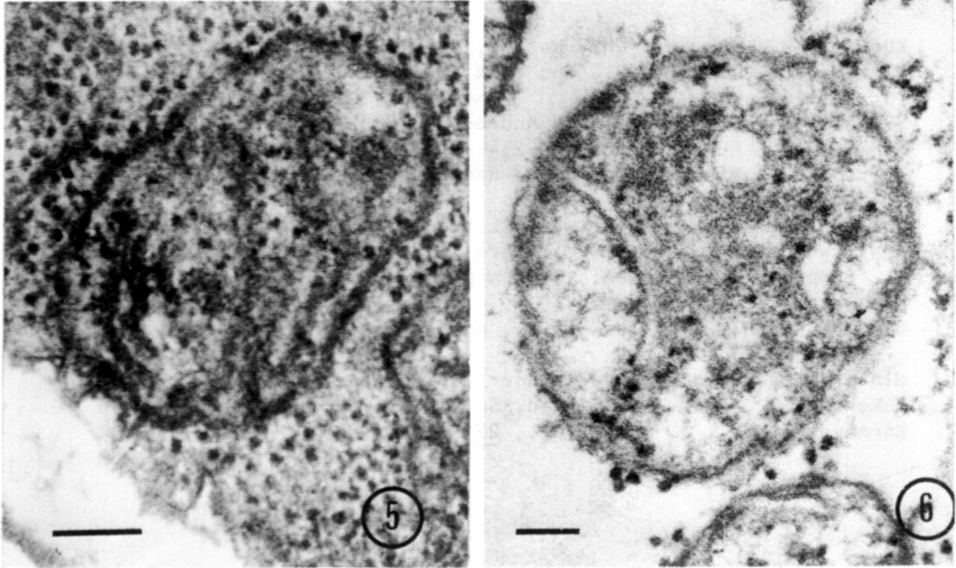
suspended in the TMK isolation buffer, and then layered on sucrose gradients of different ionic concentrations (Fig. 3). Only partial dissociation of monomers into subunits occurred when the Mg^{++} concentration was lowered to 1 mM, but complete dissociation occurred either upon the addition of 1 mM EDTA or a K^+ concentration of 100 mM. Comparable experiments by Schmitt (4) yielded only partial dissociation into subunits even at 500 mM K^+ and 5 mM Mg^{++}.

Differences also were observed in the electrophoretic mobilities of mitochondrial and cytoplasmic ribosomal RNA's (Fig. 4, Table I), whether these were purified from ribosome monomers or from polysomes collected from sucrose gradients. We also found that mitochondrial rRNA was less stable than cytoplasmic rRNA upon storage at -20°. Using the molecular weights calculated (9) from electrophoretic mobility data and sedimentation coefficients of *E. coli* rRNA as 16S and 23S, we found that cytoplasmic rRNA was 19S and 26S whereas mitochondrial rRNA was 17S and 25S for the small and the large subunits, respectively. These values are different from sedimentation coefficients reported by others (10,11) for RNA purified from whole mitochondria rather than from purified ribosomes directly.

We consistently have observed ribosome-like particles in thin-sections of mitochondria in whole cells (Fig. 5) or of isolated mitochondrial fractions (Fig. 6). Such particles often occur in clusters and usually are aligned along the cristae rather than being randomly distributed in the mitochondrial matrix. We also have examined electron micrographs of ribosome suspensions after pos-

TABLE I. *Some Physical Properties of Purified Ribosomal RNA's*

Ribosome source	Mobility ($cm^2 \cdot sec^{-1} \cdot volt^{-1} \times 10^5$)	Molecular weight (daltons X 10^6)	Sedimentation coefficient (S)
E. coli			
large RNA	12.59	1.10	23
small RNA	35.73	0.56	16
Mitochondria			
large RNA	7.87	1.25	25
small RNA	31.17	0.64	17
Cytoplasm			
large RNA	4.41	1.40	26
small RNA	24.87	0.77	19



FIGURES 5 and 6. Electron micrographs of thin-sections through part of a whole cell (Fig. 5) and a purified mitochondrial sample (Fig. 6). Whole cells were fixed in 2% OsO_4 buffered with veronal acetate (pH 7.2); dehydrated and then embedded in Epon 812; and stained with uranyl acetate and lead citrate before scanning. Mitochondrial sediments were fixed in Karnovsky's fluid (12), stained with 2% OsO_4 buffered with veronal acetate (pH 7.2), dehydrated, embedded in Epon 812, and stained with uranyl acetate and lead citrate.

The bar represents 0.1 μ in both photographs.

itive staining with uranyl acetate or negative staining with phosphotungstate. Such particles, like those seen in thin-sections, are slightly smaller than cytoplasmic ribosomes in comparable preparations. These ultrastructure observations are in agreement with the sedimentation data. All the results we have obtained are consistent in showing that ribosomes from mitochondria are somewhat smaller than those from the cytoplasm of the yeast cell, and that they occur in polysome groups.

ACKNOWLEDGMENTS

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