SEDIMENTATION PROPERTIES OF MITOCHONDRIAL AND CYTOPLASMIC RIBOSOMAL RNA'S FROM *NEUROSPORA**

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Protein synthesis is known¹⁻¹⁴ to occur within mitochondria, and several components of their translational apparatus have been isolated and characterized.^{8, 15-21} O'Brien and Kalf^{19, 20} found that ribosomes of rat liver mitochondria have a sedimentation coefficient of 55S, in contrast to the cytoplasmic (microsomal) 78S ribosomal monomeric units. Similarly, Rogers *et al.*²¹ reported that yeast mitochondria contain ribosomal RNA's (rRNA's) (with $s_{20,w}^0$ values of 12.7, 17.8, and 22.4) that are distinct from the major class of rRNA's isolated from whole yeast cells.

In previous reports¹⁶⁻¹⁷ we demonstrated that *Neurospora* mitochondria contain a full complement of tRNA's and aminoacyl-RNA synthetases. In addition, the mitochondrial phenylalanyl-, aspartyl-, and leucyl-RNA synthetases and their respective tRNA's were shown to be uniquely associated with this organelle.

In the present report, rRNA's from *Neurospora* mitochondria have been compared with *Neurospora* cytoplasmic and *E. coli* rRNA's by sucrose density-gradient centrifugation. Data are presented which demonstrate that the sedimentation properties of mitochondrial rRNA's are distinctly different from those of cytoplasmic rRNA's but similar to those of *E. coli* rRNA's.

Experimental Procedures.—*Strains:* *Neurospora crassa* wild-type strain OR23-la and *E. coli* strain B were used.

Preparation of mitochondrial and cytoplasmic fractions: Hyphae in the exponential phase of growth were collected from enriched Vogel's synthetic medium.²³ Mitochondria were prepared as described previously^{16, 17} by methods²² of Hall and Greenawalt followed by zonal sucrose gradient centrifugation. After removal of the nuclei and debris from the disrupted hyphae by low-speed centrifugation ($1500 \times g$), the mitochondria were pelleted by centrifugation at $8000 \times g$. The supernatant from the latter centrifugation was recentrifuged at $30,000 \times g$ for 30 min to remove residual mitochondria; the resulting supernatant is considered as the cytoplasmic fraction. Mitochondria from the $8000 \times g$ pellet were resuspended and reisolated by zonal centrifugation prior to use.

Preparation of rRNA's: (a) *P³²-labeled Neurospora whole-cell rRNA:* Hyphae in the exponential phase of growth were collected from Vogel's²³ synthetic medium modified to contain one tenth the amount of phosphate and supplemented with 50 mc of carrier-free, *P³²*-inorganic phosphate per liter. The hyphae were suspended in 4 vol of 0.1 *M* Tris-HCl buffer (pH 7.5) containing 0.01 *M* magnesium acetate and 0.001 *M* ethylenediaminetetraacetate (EDTA) (suspending buffer), and were disrupted by passage through a French pressure cell at 10,000 psi pressure. After centrifugation at $30,000 \times g$ for 30 min to remove cellular debris, the ribosomal pellets were prepared by layering the supernatant over 5 ml of suspending buffer made to 1 *M* sucrose and by centrifuging at $105,000 \times g$ for 2 hr in the model L-2 Spinco centrifuge.

Dissociated rRNA's were prepared for sucrose gradient analyses by resuspending the ribosomal pellets in suspending buffer and adjusting to 0.5% sodium dodecyl sulfate (SDS), then incubating at room temperature for ~2 min and immediately layering on the sucrose gradients.

(b) *P³²-labeled E. coli rRNA:* Cells were harvested from low phosphate synthetic medium,²⁴ supplemented with 50 mc of carrier-free *P³²*-inorganic phosphate per liter. rRNA's were prepared as described above for *Neurospora*.

(c) *Nonradioactive cytoplasmic rRNA*: Ribosomal pellets from the cytoplasmic fraction were prepared as described above, resuspended in TMS (0.1 M Tris-HCl buffer, pH 7.5, containing 0.005 M MgCl_2 and 0.001 M Spermidine), made to 0.5% SDS, and shaken with an equal volume of phenol for 1 hr at 4°C. After centrifugation, the aqueous phase was removed and the nucleic acids precipitated by the addition of 0.1 vol of 1 M NaCl and 3 vol of ethanol. Residual phenol was removed by two additional ethanol precipitations from TMS, and the final precipitate was dissolved in 0.1 M Tris-HCl buffer (pH 7.5) containing 0.01 M NaCl and 0.001 M EDTA. This solution was made to 1.3 M NaCl, and after ~12 hr at 4°C, the precipitated rRNA's²⁵⁻²⁷ were collected by centrifugation.

(d) *Mitochondrial rRNA*: Freshly prepared mitochondria were adjusted to 0.1 M potassium acetate (pH 5.0) and 3 vol of ethanol added. After ~12 hr at -20°C, the precipitated material was collected by centrifugation, resuspended in 0.1 M Tris-HCl buffer (pH 7.5) containing 1.0% SDS, 0.01 M magnesium acetate, and 0.001 M EDTA, and shaken with an equal volume of phenol at 4°C for 2 hr. After centrifugation, the aqueous phase was removed and the nucleic acids precipitated by the addition of 0.1 vol of 1 M NaCl and 3 vol of ethanol. Mitochondrial rRNA's were then isolated by salt precipitation as described above. In a typical mitochondrial preparation, containing ~1.5 gm of protein, the yield of rRNA was ~15 mg.

Methylated albumin kieselguhr (MAK) chromatography: MAK was prepared as described by Mandel and Hershey.²⁸ Nucleic acid preparations were applied to MAK columns (1.5 × 3.0 cm) and eluted with linear KCl gradients from 0.5 M (70 ml) to 1.7 M (70 ml) containing 0.05 M potassium phosphate buffer, pH 6.8.

To obtain complete elution of the nucleic acids, the column was washed with 1 M NH_4OH . The material eluting with NH_4OH has been found to be predominantly rRNA (Dure and Medina, in preparation).

Sucrose density-gradient analyses: Linear density gradients were formed between 6 and 22% sucrose in 0.01 M sodium acetate buffer (pH 6.0) containing 0.001 M NaCl, 0.001 M EDTA, and 0.05% SDS. After layering the sample on the gradients, they were centrifuged at 25,000 rpm in the Spinco SW25 rotor at 4°C for 19 hr.

The gradients were monitored for absorbance at 254 m μ by utilizing an ISCO gradient fractionator and fraction collector. A time delay device was used to equate precisely the absorbance profile with the fractions collected, and 0.1-ml aliquots were used with the filter paper disk method of Bollum²⁹ to determine radioactivity.

FIG. 1.—Elution profiles of mitochondrial nucleic acids from MAK. (A) *Neurospora* mitochondrial nucleic acids were prepared and eluted from MAK columns as described in *Experimental Procedures*. (B) The elution profile of the 1 M NaCl insoluble nucleic acid fraction of mitochondria (see *Experimental Procedures*).

Results and Discussion.—The elution profile of mitochondrial nucleic acids from MAK is shown in Figure 1A; it is apparent that the three major classes of nucleic acid—tRNA, DNA, and rRNA—are present³⁰⁻³² in extracts from these organelles. For the present analyses, rRNA's were prepared from total mitochondrial nucleic

acid preparations by NaCl precipitation. Figure 1B shows the MAK elution profile of the 1 M NaCl insoluble material.

These mitochondrial nucleic acids are referred to as "ribosomal" RNA's because of their similarity in behavior to known ribosomal RNA's in (1) their chromatographic properties on MAK,³⁰⁻³² (2) their insolubility in 1 M NaCl,²⁵⁻²⁷ and (3) their sedimentation properties.

Sucrose density-gradient centrifugation has been used to compare the sedimentation properties of the mitochondrial rRNA's with *Neurospora* cytoplasmic and *E. coli* rRNA's. The data in Figures 2A and B show that the heavy rRNA com-

FIG. 2.—Sucrose density-gradient profiles of ribosomal RNA's. (A) Five A_{254} units of *Neurospora* mitochondrial rRNA mixed with *Neurospora* cytoplasmic P^{32} -rRNA. (B) Five A_{254} units of *Neurospora* mitochondrial rRNA and *E. coli* P^{32} -rRNA. See *Experimental Procedures* for details. (C) Five A_{254} units of *Neurospora* cytoplasmic rRNA mixed with *Neurospora* cytoplasmic P^{32} -rRNA. (D) Five A_{254} units of *E. coli* rRNA mixed with *E. coli* P^{32} -rRNA.

ponents of the mitochondria and the cytoplasm are very different, whereas the sedimentation profiles for mitochondrial and *E. coli* rRNA's are similar. Figures 2C and D show essentially perfect coincidence between radioactivity and absorbance profiles when different preparations of the same material are mixed and analyzed. It should be noted that the radioactive material does not contribute to absorbance in these analyses.

The difference in the heavy rRNA components from *Neurospora* mitochondria and cytoplasm may also be seen in absorbance profiles alone, when the two preparations are mixed and sedimented (Fig. 3A). The profile for a mixture of mitochondrial and *E. coli* rRNA's (Fig. 3B), however, reveals only two peaks, the

sharpness and symmetry of which indicate the close similarity of sedimentation properties of the species within each peak. It should be kept in mind that sterile conditions are used in the isolation of mitochondria and that assays for bacterial contamination are routinely made at each stage of purification.¹⁷

FIG. 3.—Sucrose density-gradient profiles for ribosomal RNA's. (A) 2.5 A₂₆₀ units of *Neurospora* cytoplasmic rRNA mixed with 2.5 A₂₆₀ units of *Neurospora* mitochondrial rRNA and *Neurospora* cytoplasmic P³²-rRNA. (B) 2.5 A₂₆₀ units of *Neurospora* mitochondrial rRNA mixed with 2.5 A₂₆₀ units of *E. coli* rRNA and *E. coli* P³²-rRNA.

The sedimentation properties of the two *E. coli* rRNA's have been examined in many laboratories and have been assigned *S* values of approximately 16 and 23. Assuming the latter value of 23S for the heavy *E. coli* rRNA and using the equations of Martin and Ames,³³ we calculated the sedimentation coefficients of the *Neurospora* cytoplasmic rRNA's as 16 and 25.6S.

The observations reported here are in agreement with the recent report of Rogers *et al.*,²¹ who found yeast mitochondrial rRNA's to be very similar to those from *E. coli* and quite different from yeast cytoplasmic rRNA's. We have not, however, detected a third, very light mitochondrial rRNA component (12.7S) as have Rogers and co-workers. A related observation has been reported by Stutz and Noll,³⁴ who found the rRNA's of chloroplasts to be of the bacterial type (i.e., 16 and 23S).

The concept which arises from this and many previous reports is (1) that within mitochondria (and perhaps within chloroplasts) the complete sequence of transcriptional and translational events occurs, and (2) that the macromolecules involved are unique to the organelle and distinct from those found in and used by the cytoplasm.

Summary.—*Neurospora* mitochondrial rRNA's were isolated and characterized by sucrose density-gradient centrifugation. They were found to sediment at rates indistinguishable from those of *E. coli* 16 and 23S rRNA's. In contrast, the calculated *S* values for light and heavy *Neurospora* cytoplasmic rRNA's were 16 and 25.6S, respectively. Thus, the sedimentation behavior of *Neurospora* mitochondrial rRNA's is different from that of cytoplasmic rRNA's but similar to that of rRNA's of bacterial origin.

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