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

BY LEON S. DURE,† J. L. EPLER, AND W. EDGAR BARNETT

DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF GEORGIA, ATHENS, AND
BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE

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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.9, 11-21 O'Brien and Kalf19, 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 OR23-1a 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 previously16-17 by methods23 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 X g), the mitochondria were pelleted by centrifugation at 8000 X g. The supernatant from the latter centrifugation was recentrifuged at 30,000 X g for 30 min to remove residual mitochondria; the resulting supernatant is considered as the cytoplasmic fraction. Mitochondria from the 8000 X g pellet were resuspended and reisolated by zonal centrifugation prior to use.

Preparation of rRNA's: (a) $P^{32}$-labeled Neurospora whole-cell rRNA: Hyphae in the exponential phase of growth were collected from Vogel's synthetic medium modified to contain one tenth the amount of phosphate and supplemented with 50 mc of carrier-free, $P^{32}$-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 X 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 X 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) $P^{32}$-labeled E. coli rRNA: Cells were harvested from low phosphate synthetic medium,24 supplemented with 50 mc of carrier-free $P^{32}$-inorganic phosphate per liter. rRNA's were prepared as described above for Neurospora.

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(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 MgCl₂ 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’s 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. Nucleic acid preparations were applied to MAK columns (1.5 X 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 NH₄OH. The material eluting with NH₄OH 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 mμ 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 Bollum to determine radioactivity.

**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 present in extracts from these organelles. For the present analyses, rRNA's were prepared from total mitochondrial nucleic acids using the MAK method.

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**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).**
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,20-22 (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-

![Figure 2](image)

**Fig. 2.**—Sucrose density-gradient profiles of ribosomal RNA's. (A) Five A260 units of Neurospora mitochondrial rRNA mixed with Neurospora cytoplasmic P32-rRNA. (B) Five A260 units of Neurospora mitochondrial rRNA and E. coli P32-rRNA. See Experimental Procedures for details. (C) Five A260 units of Neurospora cytoplasmic rRNA mixed with Neurospora cytoplasmic P32-rRNA. (D) Five A260 units of E. coli rRNA mixed with E. coli P32-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

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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