RIBOSOMAL RNA AND RIBOSOMES FROM MITOCHONDRIA OF NEUROSPORA CRASSA

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Experimental evidence from many sources indicates that mitochondria are semi-autonomous organelles containing DNA, which probably serves as a genetic determinant for some aspects of mitochondrial function.[1] In *Neurospora crassa*, mitochondria have been shown to have a DNA with a pattern of replication[2] and a buoyant density[3] different from nuclear DNA. In addition, a DNA-dependent RNA polymerase with special properties[3] and transfer RNA's (tRNA) with amino acid acceptor function[4] have been demonstrated in these organelles. One of these tRNA's was found exclusively in the mitochondrial fraction.[4]

In this paper, we report the isolation and characterization of mitochondrial ribosomal RNA's (rRNA) and their association with mitochondrial ribosomes. As in the case of the other elements probably involved in gene expression in *Neurospora* mitochondria, these RNA's and ribosomes have unique properties which differentiate them from cytoplasmic ribosomes and establish them as true mitochondrial components.

Materials and Methods.—Strains: The following strains of *Neurospora crassa* were used: EM 5256 (wild-type) and STL-7 (lysine$^-$). These strains were used interchangeably.

Buffer solutions: TM: 0.01 *M* $MgCl_2$, 0.01 *M* Tris-Cl (pH 7.6); TMK: 0.05 *M* KCl, 0.01 *M* Tris-Cl (pH 7.6), $MgCl_2$ concentration in parentheses, e.g., TMK (2 mM); TMAm: 0.03 *M* NH_4Cl, 0.02 *M* Tris-Cl (pH 7.6), $MgCl_2$ concentration in parentheses.

Growth of cells and preparation of cell fractions: Methods for the growth of *Neurospora* and preparation of mitochondria by isopycnic centrifugation in sucrose gradients have been described previously.[5] For large-scale preparation of mitochondrial RNA, cells were grown for 16–18 hr at 25°C under forced aeration in 5-liter carboys containing 3 liters of minimal medium. Labeled RNA was obtained from cultures to which carrier-free H^3-uracil (generally labeled, New England Nuclear Corp.) was added to the growth medium.

Mitochondrial fractions, obtained from sucrose gradients, were diluted with either 8% sucrose-1 mM EDTA (for RNA) or TM, TMK (2 mM), or TMAm (2 mM) buffer (for ribosomes) and centrifuged for 20 min at 40,000 rpm (Spinco no. 40 rotor). The resulting pellet was resuspended in a small volume of buffer and the suspension used for RNA isolation or preparation of mitochondrial ribosomes.

To prepare mitochondrial ribosomes, the mitochondria, in TMK (2 mM) or TMAm (2 mM) buffer (10–15 mg protein/ml), were lysed by adding 10% DOC (pH 8) (sodium deoxycholate, Matheson, Coleman and Bell) to a final concentration of 0.5%. The ribosomes were then separated from the clear red lysate either by directly layering the lysate on a sucrose gradient (described in Fig. 2) or by diluting with TMK (2 mM)-0.5% DOC and centrifuging for 30 min at 25,000 rpm (Spinco no. 40.3 rotor). In the latter case, the supernatant was removed and resedimented at 39,000 rpm (Spinco no. 40.3 rotor) for 120 min. This ribosomal pellet was rinsed with TMK (2 mM) and then resuspended by homogenization in a small volume of TMK (2 mM) buffer. The cloudy suspension was clarified by centrifugation at 2300 rpm (International Centrifuge) for 15 min. When RNA was to be extracted from ribosomes, PVS (polyvinyl sulfate, potassium salt, General Biochemicals) was added to all solutions at a concentration of 20 μg/ml.

To prepare cytoplasmic ribosomes, cells were ground with sand in TM, TMK (10 mM), or TMAm (10 mM) buffer. After a centrifugation at 20,000 rpm (Spinco no. 40 rotor) for 20 min, the supernatant was centrifuged for 120 min at 40,000 rpm (Spinco no. 40 rotor). The ribosome pellet was resuspended in either of the above buffers and the solution was clarified by centrifugation at 20,000 rpm (Spinco no. 40.3 rotor) for 15 min.

RNA isolation: For nucleotide analysis and determination of sedimentation coefficients, RNA was isolated directly from cytoplasmic ribosomes or mitochondrial fractions by a modification of the method of Stanley and Bock.[6] The ribosomal suspension in TM buffer was made 0.5% in SDS (sodium dodecyl sulfate, Matheson, Coleman and Bell) and 0.1% in Macaloid (American Tansul Co., Houston, Texas) and the mixture was shaken at 30°C for 5 min. The mitochondria in TM buffer were made 2% in SDS and 0.1% in Macaloid and shaken at 40°C for 5 min. From this stage on, both preparations were treated identically. The mixtures were chilled to 0°C and shaken for 5 min with an equal volume of buffer-equilibrated phenol (500 gm, Mallinckrodt)-cresol (70 ml, Eastman)[7] at 4°C. The phases were separated by centrifugation, and the extraction was repeated three more times with fresh Macaloid and phenol-cresol. Dissolved phenol-cresol was removed by several ether extractions and residual ether was removed with a stream of nitrogen. This step was always completed on the same day the hyphae were harvested. The resulting RNA solution was stored at −20°C.

Carrier cytoplasmic ribosomal RNA was prepared by LiCl precipitation[8] of cytoplasmic ribosomes.

Nucleotide composition determination: RNA was precipitated with 0.25 *M* $HClO_4$, and the precipitate dissolved in 0.3 *N* KOH at a concentration of about 0.5 mg/ml and hydrolyzed for 18 hr at 37°C. After hydrolysis the solution was acidified with 0.25 *M* $HClO_4$. The ensuing precipitate was removed by centrifugation at 0°C and the supernatant was adjusted to pH 7.5–9.0 and stored at −20°C.

The nucleotides were analyzed on a Dowex 1-formate column, 30 × 1.8 mm (Bio-Rad AG 1-×8, 200–400 mesh). Hydrolyzed RNA (0.3 mg) was applied to the top of the column and the nucleotides were eluted stepwise with water, 0.05 *M* formic acid, 0.5 *M* formic acid, 0.01 *M* formic acid-0.03 *M* ammonium formate, and 2.0 *M* formic acid. Base ratios were calculated from absorbancy measurements of each fraction in 0.1 *M* sodium phosphate (pH 7.0) using a Cary 14 recording spectrophotometer. The micromolar extinction coefficients at pH 7 for λ_{max} used were: CMP ϵ = 12.7, AMP ϵ = 14.4, UMP ϵ = 9.9, GMP ϵ = 12.2.

Sucrose density gradient centrifugation: Prior to nucleotide composition determinations, fast and slow components present in phenol-cresol extracts of cytoplasmic ribosomes and mitochondria were separated by means of a preparative 5–20% sucrose gradient (25.1 ml) containing 0.05 *M* sodium acetate, 0.05 *M* NaCl (pH 5.2). The gradients were prepared by the method of Britten and Roberts.[9] The material was centrifuged for 18 hr at 25,000 rpm (SW-25.1 rotor), 4°C. After centrifugation, fractions were collected dropwise through a hole punched in the bottom of the tube. Peaks were identified by absorbancy measurements at 260 mμ. Tubes containing a particular peak were pooled and the RNA was ethanol-precipitated. Several other types of sucrose gradients (4.6 ml) were used for analyzing ribosomal particles or isolated RNA, and details are given in the legends of the figures.

Analytical zone sedimentation: Sedimentation measurements of RNA and ribosomes were made in a Spinco model E ultracentrifuge equipped with ultraviolet (UV) optics.[10, 11] Pictures were taken at 2-min intervals and UV photographs were scanned using a Joyce-Loebl microdensitometer. Sedimentation was performed at 28,000 rpm for ribosomes and 44,000 rpm for RNA. A rotor temperature between 19 and 20°C was used in all experiments; during one run the temperature never varied more than ±0.3°C. The following bulk solutions were used: (1) for RNA, high salt solution: 0.5 *M* NaCl, 0.01 *M* sodium citrate, 0.01 *M* sodium phosphate (pH 6.79); for RNA, low salt solution: 50% D_2O-0.05 *M* sodium acetate, 0.05 *M* NaCl (pH 5.2); (2) for ribosomes: 50% D_2O-TMAm (2 mM). Correction factors for η and ρ of the high salt solution and η_{H_2O} and ρ_{H_2O} at the experimental temperature were taken from the International Critical Tables, 1926–1933; correction factors for the D_2O solutions were obtained from Studier:[12] η = 1.119, ρ = 1.055. All sedimentation coefficients reported are corrected to $s_{20,w}$ values using the above correction factors, and $\bar{\nu}$ = 0.53 for RNA,[6] $\bar{\nu}$ = 0.66 for ribosomes.[13]

Analytical methods: Protein was determined by the method of Lowry *et al.*[14] using bovine serum albumin as a standard. RNA was estimated by the orcinol method[15] with yeast RNA as a standard.

Results.—Mitochondrial RNA: When RNA, isolated by phenol-cresol extraction of the mitochondrial fraction, was examined by preparative zonal centrifugation

in sucrose gradients, results similar to those shown in Figure 1 (cpm curve) were obtained. Omission of Macaloid or treatment with RNase during RNA preparation resulted in a total loss of the rapidly sedimenting bands. Treatment of the RNA extract with chromatographically purified pancreatic DNase (20 μg/ml, 20 min at 37°C) did not alter the sedimentation pattern.

As indicated in Figure 1, the mitochondrial RNA sedimented as three components: two resembling ribosomal RNA's and a third sedimenting in the 4–5*S* region. The approximate ratios of the three components, determined from the areas under the peaks, were 1.5:1.0:0.5. This held true whether the distribution was monitored by absorbancy at 260 mμ or by the radioactivity of H^3-uracil incorporated into RNA.

A sucrose gradient profile of labeled mitochondrial RNA cosedimented with cytoplasmic rRNA, prepared by identical procedures, is shown in Figure 1. It is clear that the fast component of mitochondrial ribosomal-type RNA sediments at a slightly slower rate than the corresponding cytoplasmic fast rRNA. Similar experiments carried out with ribosomal RNA from *Escherichia coli* showed equivalent sedimentation rates for *E. coli* rRNA's and labeled mitochondrial RNA.

A more quantitative estimate of the sedimentation velocity of cytoplasmic and mitochondrial RNA's was made by zonal sedimentation in the analytical ultracentrifuge. The results are shown in Table 1. Under the same low-salt conditions of Figure 1, the $s_{20,w}$ values of the fast RNA components from cytoplasmic ribosomes and from mitochondria are very different and a small, but statistically significant, difference is always encountered between the slow RNA components. Under high-salt conditions, in which intermolecular hydrogen bonding is minimized, the mitochondrial RNA components are still clearly distinguishable from cytoplasmic rRNA on the basis of their $s_{20,w}$ values.

When nucleotide compositions of purified fast and slow components from mito-

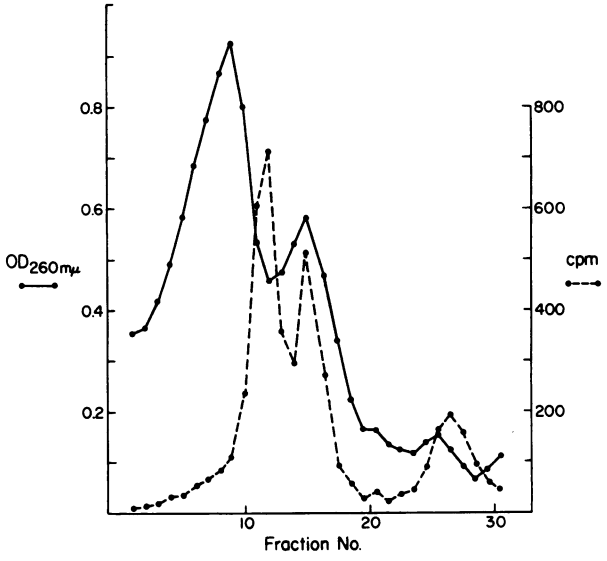

FIG. 1.—Cosedimentation pattern of cytoplasmic ribosomal RNA and H^3-uracil-labeled mitochondrial RNA. Phenol-extracted rRNA from cytoplasmic ribosomes (15.4 OD_{260} units) and phenol-extracted RNA from the mitochondrial fraction (0.29 OD_{260} units, 5900 cpm) were mixed and layered on a linear 5–20% sucrose gradient containing 0.05 *M* sodium acetate, 0.05 *M* NaCl, pH 5.2. Centrifugation was carried out at 39,000 rpm (Spinco SW-39 rotor) for 5 hr at 4°C. Fractions, collected dropwise from a hole punched in the bottom of the tube, were each diluted to 1 ml for measurement of absorbancy at 260 mμ and determination of radioactivity by scintillation counting in Bray's solution.[16] The bottom of the gradient is at fraction no. 1.

TABLE 1

$s_{20,w}$ VALUES OF RNA'S EXTRACTED FROM CYTOPLASMIC RIBOSOMES AND FROM MITOCHONDRIAL FRACTIONS

Source of RNA	Bulk Solution: High salt	Bulk Solution: Low salt
Cytoplasmic ribosomes		
Fast component	28.7 ± 0.2 (10)*	27.9 ± 0.1 (3)
Slow component	19.2 ± 0.1 (5)	18.0 ± 0.2 (3)
Mitochondria		
Fast component	28.2 ± 0.1 (3)	24.5 ± 0.2 (3)
Slow component	20.6 ± 0.1 (3)	18.9 ± 0.1 (3)

Values are mean ± standard error.
* Numbers in parentheses represent the number of determinations. About 0.05 OD_{260} units were used for each determination.

chondria and from cytoplasmic ribosomes were determined, a further difference between these RNA's was found (Table 2).

A major portion of the total mitochondrial RNA can be accounted for by these ribosomal-type RNA's. Mitochondrial RNA prepared by phenol extraction, although quite variable in yield, constitutes at least 50 per cent and sometimes up to 70 per cent of the total mitochondrial RNA determined by the orcinol color reaction (65 μg_{ave} (range: 44 to 80 μg)/mg mitochondrial protein). Contamination by cytoplasmic RNA is negligible since this value for total mitochondrial RNA is not diminished if the intact mitochondria are treated with pancreatic RNase (50 μg/ml, 30 min at 4°C) before their purification on a sucrose gradient.

Furthermore, a direct test of cytoplasmic contamination can be made by mixing a crude unlabeled mitochondrial fraction with H^3-uracil-labeled cytoplasmic ribosomes obtained from an equivalent cell mass. From this mixture, the mitochondria were isolated by the usual isopycnic centrifugation procedure. The RNA's of the mitochondrial and ribosomal fractions were extracted, hydrolyzed in 0.3 *N* KOH, and their specific activities (cpm/OD_{260}) compared. The results indicated that contamination of mitochondrial RNA by cytoplasmic RNA did not exceed 2 per cent.

Mitochondrial ribosomes: When DOC-lysed mitochondria were layered on a sucrose gradient and centrifuged, three species of ribonucleoprotein particles were found. A typical UV profile of such a gradient is seen in Figure 2. From experiments using H^3-uracil-labeled mitochondria, about 70–80 per cent of the radioactivity incorporated into trichloroacetic acid-precipitable material was recovered in these three peaks. Although the ratios of peaks I and II varied considerably from one preparation to another, peak II was the major component in all cases.

Identification of these peaks as ribonucleoprotein particles was supported by

TABLE 2

NUCLEOTIDE COMPOSITION OF RNA'S EXTRACTED FROM CYTOPLASMIC RIBOSOMES AND FROM MITOCHONDRIAL FRACTIONS

Source of RNA	C	A	U	G	G + C
	(Moles per cent)				
Cytoplasmic ribosomes					
Fast component (6)*	21.9 ± 0.2	24.8 ± 0.1	23.9 ± 0.1	29.4 ± 0.2	51.3 ± 0.3
Slow component (5)	21.6 ± 0.1	25.3 ± 0.1	25.4 ± 0.3	27.7 ± 0.4	49.3 ± 0.4
Mitochondria					
Fast component (4)	15.0 ± 0.4	33.9 ± 0.1	31.9 ± 0.4	19.1 ± 0.4	34.1 ± 0.5
Slow component (5)	16.0 ± 0.1	31.8 ± 0.1	31.7 ± 0.3	20.4 ± 0.5	36.4 ± 0.5

Values are mean ± standard error.
* Numbers in parentheses represent the number of determinations.

radioactivity profiles of gradients on which DOC-lysates of H^3-uracil- or H^3-lysine-labeled mitochondria were layered. In a similar experiment performed with C^{14}-lecithin-labeled mitochondria (obtained from cells grown in the presence of C^{14}-choline[5]), all the radioactivity remained at the top of the gradient.

Cosedimentation experiments of cytoplasmic ribosomes with mitochondrial lysates indicated that peak I had the same mobility as 81S[17] cytoplasmic ribosomes, and similar experiments with *E. coli* indicated that peak II sedimented slightly faster than the 50S *E. coli* ribosomal subunit.

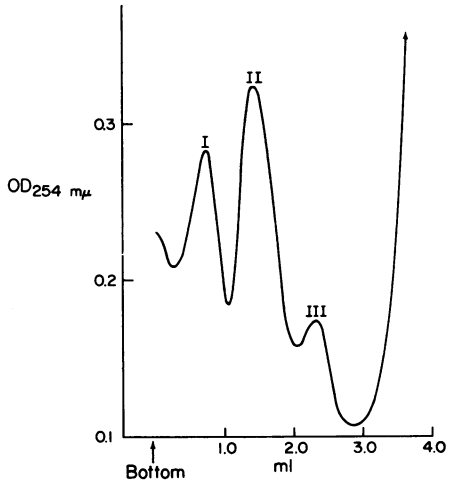

FIG. 2.—Sedimentation pattern of ribosomal particles from mitochondria. DOC-lysed mitochondria, prepared in the presence of PVS, were layered on a linear 5–20% sucrose gradient containing TMK (2 mM) buffer and PVS (20 μg/ml) and centrifuged at 37,000 rpm (SW-39 rotor) for 120 min at 4°C. The gradient was analyzed in a flow cell by recording of absorbancy at 254 mμ (10-mm light path) using an Isco density gradient fractionator.

Peaks I, II, and III were shown to be of mitochondrial origin by an analysis of their RNA's. In these experiments, PVS was present in all solutions. Fractions corresponding to these peaks were pooled separately, and their RNA's extracted and cosedimented on a sucrose gradient with cytoplasmic ribosomal RNA (Fig. 3). The pattern obtained from peak I (Fig. 3I) is similar to that obtained when purified mitochondrial RNA is cosedimented with cytoplasmic ribosomal RNA (Fig. 1): 25 and 19S RNA are present in a radioactivity ratio of approximately 2:1. Furthermore, peak III contains only 19S RNA and peak II contains primarily 25S RNA (the shoulder on the lighter side of the peak is probably due to breakdown of 25S RNA). From these observations we conclude that peaks III and II represent the small and large subunit, respectively, of peak I, which is the mitochondrial ribosomal monomer.

Ribosomes could also be collected from the mitochondrial lysate by centrifugation for 120 minutes at 39,000 rpm (no. 40.3 rotor). The pellet contained all the ribosomal RNA expected from a determination of the total RNA present. Resuspension of the pellet, however, did not result in the complete recovery of this RNA as ribosomes. At most, 50 per cent of the material was in the form of ribosomes or ribosomal subunits, the remainder being present as large aggregates which could be sedimented in very low centrifugal fields. These aggregates were probably larger aggregates of mitochondrial ribosomes which were not readily resuspended. They did not give a polysome profile upon centrifugation in a 15–30 per cent sucrose gradient. All RNA from such a resuspended pellet was of the mitochondrial type, i.e., 25 and 19S, whether it was extracted from the ribosome or ribosomal subunit fractions or from the large aggregate fraction.

The sensitivity of these ribosomes in TMK buffer to Mg^{++} ions also differentiates them from cytoplasmic ribosomes. Mitochondrial ribosomes, prepared in the presence of 2 mM $MgCl_2$, had a distribution pattern on sucrose gradients similar

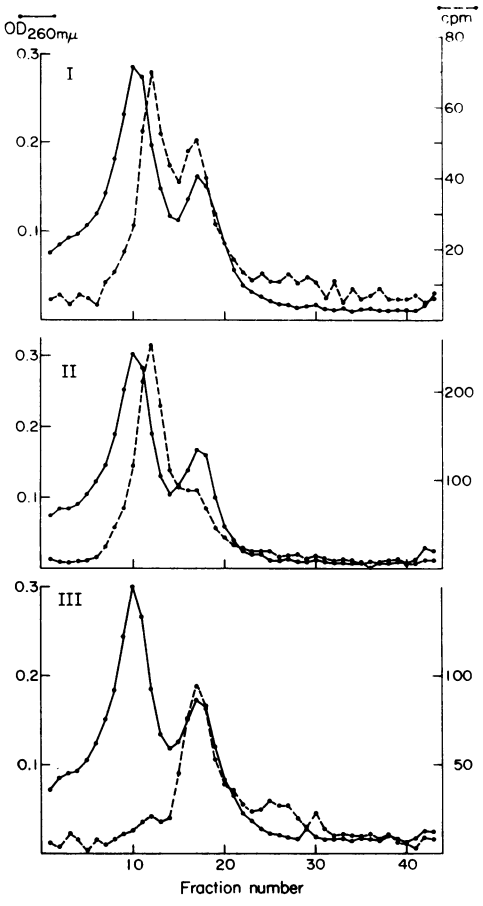

FIG. 3.—Cosedimentation patterns of cytoplasmic ribosmal RNA (OD) and H^3-uracil-labeled RNA (cpm) extracted from the peaks shown in Fig. 2. Tubes containing a particular peak were pooled, carrier cytoplasmic ribosomal RNA (about 6 OD_{260} units) was added, and the RNA precipitated by the addition of 2.5 vol of cold ethanol at −20°C. The precipitate was resuspended in a small volume of TM buffer containing PVS (20 μg/ml) and SDS was added to a final concentration of 1%. The solution was layered on a 5–20% sucrose gradient, centrifuged, and analyzed as in Fig. 1.

to that shown in Figure 2. Most of the ribosomes were present as subunits; in fact, the major component was again the large subunit, peak II. If sedimented ribosomes were resuspended in buffer containing 4 mM $MgCl_2$ and centrifuged on a gradient containing 4 mM Mg^{++}, the subunit peaks decreased in area while the monomer peak increased. Cytoplasmic ribosomes, on the other hand, dissociated only when the Mg^{++} concentration in the preparative media was lowered to 0.1 mM.

Approximate sedimentation coefficients were obtained by direct calculations from the position of the peaks in sucrose gradients.[18] Assuming the monomer peak to be 81*S* (based on the results of the cosedimentation experiments with cytoplasmic ribosomes), the large and small subunits had sedimentation rates of 61 and 47*S*, respectively.

A more precise characterization of mitochondrial ribosomes and their subunits was attempted by determining their $s_{20,w}$ values in the analytical ultracentrifuge. Since these ribosomes could only be obtained in relatively small amounts, the values were determined by analytical zone sedimentation. Under our conditions the following $s_{20,w}$ values were obtained: 89.5 ± 0.2 (value given as mean ± standard error) for the monomer, 59.4 ± 0.8 for the large subunit, 52.5 ± 0.5 for the small subunit. Under the same conditions, the $s_{20,w}$ value of cytoplasmic ribosomes was 89.8 ± 0.9 and that of the *E. coli* monomer was 78.0. It appears, then, that mitochondrial and cytoplasmic ribosomes have very similar sedimentation rates. Our values are higher than previously published $s^0_{20,w}$ values (81.9 for *Neurospora* cytoplasmic ribosomes, 69.1 for *E. coli* ribosomes)[17] obtained by the more conventional method of sedimentation analysis using schlieren optics with extrapolation to infinite dilution.

When thin sections of a mitochondrial ribosome pellet were examined in the electron microscope, small dense particles, about 160 Å in diameter, were seen.

In negatively stained preparations of a resuspended pellet, two major classes of particles were observed. The dimension of the majority of particles was 180 Å in diameter; a smaller particle, more irregular in form, was about 140 Å in diameter. Occasionally, a monomer which consisted of one large and one small particle was seen.

Discussion and Summary.—Our results indicate that the mitochondria of *Neurospora crassa* contain ribosomes and ribosomal subunits. These ribosomes are unique and differ from the cytoplasmic particles by the physical and chemical properties of their RNA's and by their sensitivity to Mg^{++} ions. They contain 25 and 19*S* RNA, and the best estimate of the sedimentation coefficients of the ribosome monomer and its large and small subunits is 81, 61, and 47*S*.

Since the report by Hogeboom, Schneider, and Palade[19] that rat liver mitochondria contain RNA, there have been many estimates of mitochondrial RNA content in various cell types. These estimates, as well as recent reports of the isolation of ribosomes from rat liver mitochondria,[20, 21] may be complicated by unknown levels of contamination by cytoplasmic ribosomes; no unique properties were shown to be associated with the particles isolated from mitochondrial fractions. Many attempts to characterize the sedimentation coefficients of mitochondrial RNA's have failed to demonstrate rapidly sedimenting species[22, 23] and in one case somewhat lower *s* values have been reported for yeast mitochondrial RNA.[24] Such findings could result from nuclease activity in mitochondria. *N. crassa* mitochondria have been shown to have an endonuclease,[25] and in our own experience the presence of a ribonuclease inhibitor is essential for isolation of high-molecular-weight RNA. Wintersberger[26] has reported the isolation of high-molecular-weight RNA from yeast mitochondria. The ratio of the 23*S* to the 16*S* peak appears to be similar to that which we obtained from *Neurospora* mitochondria. We have confirmed the finding by Pollard *et al.*[27] that base composition differences exist between cytoplasmic and mitochondrial RNA's.

The isolation of a 55*S* ribosome from rat liver mitochondria has been recently reported.[28] This ribosome could not be dissociated, nor was its RNA characterized. Although we have isolated a particle with a similar sedimentation coefficient, the finding that only 25*S* RNA was associated with it led us to conclude that it was the large subunit of the mitochondrial ribosome.

Small granules have been seen in electron micrographs of mitochondria[29–32] and are clearly visible in *Neurospora* mitochondria.[33] Our results support their identification as mitochondrial ribosomes.

In vitro studies of isolated mitochondria have shown that they are capable of incorporating amino acids into protein fractions. Mitochondria contain DNA,[3] a DNA-dependent RNA polymerase,[3] amino-acid-activating enzymes,[34, 35] and tRNA's.[4] The characterization of mitochondrial ribosomes from *Neurospora* adds another element to the description of the components necessary for autonomous protein synthesis in this organelle. Our preparations of mitochondrial ribosomes may make it possible to study amino acid incorporation in simplified submitochondrial systems.

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[1] Granick, S., and A. Gibor, in *Progress in Nucleic Acid Research*, ed. J. N. Davidson and W. E. Cohn (New York: Academic Press, 1967), vol. 6, p. 143.
[2] Reich, E., and D. J. L. Luck, these PROCEEDINGS, **55**, 1600 (1966).
[3] Luck, D. J. L., and E. Reich, these PROCEEDINGS, **52**, 931 (1964).
[4] Barnett, W. E., and D. H. Brown, these PROCEEDINGS, **57**, 452 (1967).
[5] Luck, D. J. L., *J. Cell Biol.*, **16**, 483 (1963).
[6] Stanley, W. M., Jr., and R. M. Bock, *Biochemistry*, **4**, 1302 (1965).
[7] Kirby, K. S., *Biochem. J.*, **96**, 266 (1965).
[8] Barlow, J. J., A. P. Mathias, and R. Williamson, *Biochem. Biophys. Res. Commun.*, **13**, 61 (1963).
[9] Britten, R. J., and R. B. Roberts, *Science*, **131**, 32 (1960).
[10] Vinograd, J., R. Bruner, R. Kent, and J. Weigle, these PROCEEDINGS, **49**, 902 (1963).
[11] Studier, F. W., *J. Mol. Biol.*, **11**, 373 (1965).
[12] Studier, F. W., personal communication.
[13] Petermann, M., *The Physical and Chemical Properties of Ribosomes* (Amsterdam: Elsevier, 1964).
[14] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[15] Mejbaum, W., *Z. Physiol. Chem.*, **258**, 117 (1939).
[16] Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).
[17] Taylor, M. M., and R. Storck, these PROCEEDINGS, **52**, 958 (1964).
[18] Nomura, M., B. D. Hall, and S. Spiegelman, *J. Mol. Biol.*, **2**, 306 (1960).
[19] Hogeboom, G. H., W. C. Schneider, and G. E. Palade, *J. Biol. Chem.*, **172**, 619 (1948).
[20] Elaev, N. R., *Biokhim.*, **31**, 234 (1966).
[21] Rabinowitz, M., L. DeSalle, J. Sinclair, R. Stirewalt, and H. Swift, *Federation Proc.*, **25**, 581 (1966).
[22] Truman, D. E. S., and A. Korner, *Biochem. J.*, **84**, 40P (1962).
[23] Kroon, A. M., in *Regulation of Metabolic Processes in Mitochondria*, ed. J. M. Tager, S. Papa, E. Quagliariello, and E. C. Slater (Amsterdam: Elsevier, 1966), p. 397.
[24] Rogers, P. J., B. N. Preston, E. B. Titchener, and A. W. Linnane, *Biochem. Biophys. Res. Commun.*, **27**, 405 (1967).
[25] Linn, S., and I. R. Lehman, *J. Biol. Chem.*, **241**, 2694 (1966).
[26] Wintersberger, E., in *Regulation of Metabolic Processes in Mitochondria*, ed. J. M. Tager, S. Papa, E. Quagliariello, and E. C. Slater (Amsterdam: Elsevier, 1966), p. 439.
[27] Pollard, C. J., A. Stemler, and D. F. Blaydes, *Plant Physiol.*, **41**, 1323 (1966).
[28] O'Brien, T. W., and G. F. Kalf, *J. Biol. Chem.*, **242**, 2172, 2180 (1967).
[29] Mundkur, B., *Exptl. Cell Res.*, **25**, 1 (1961).
[30] André, J., and V. Marinozzi, *J. Microscopie*, **4**, 615 (1965).
[31] Watson, M. L., and W. G. Aldridge, *J. Histochem. Cytochem.*, **12**, 96 (1964).
[32] Swift, H., B. J. Adams, and K. Larsen, *J. Roy. Microscop. Soc.*, **83**, 161 (1964).
[33] Luck, D. J. L., *J. Cell Biol.*, **24**, 445 (1965).
[34] Das, H. K., S. K. Chatterjee, and S. C. Roy, *Biochim. Biophys. Acta*, **87**, 478 (1964).
[35] Craddock, V. M., and M. V. Simpson, *Biochem. J.*, **80**, 348 (1961).