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Part G: Bioenergetics: Biogenesis of Mitochondria, Organization, and Transport

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[8] Mitochondrial Ribosomes of *Neurospora crassa*: Isolation, Analysis, and Use

By W. NEUPERT, F. MILLER, R. MICHEL, and G. HALLERMAYER

Introduction

Mitochondrial ribosomes are central constituents of the mitochondrial transcription-translation system which is linked to the mitochondrial DNA.[1-5] The functional and structural characteristics of *Neurospora* mitochondrial ribosomes have been intensively investigated. They were the first mitochondrial ribosomes to be isolated and positively characterized as mitochondrial.[6,7] They represent a good system for the study of mitochondrial ribosomes and their reactions from a variety of reasons: (a) they can be isolated in high quantities from fast-growing *Neurospora* cells; (b) they can be obtained in pure condition, since it is possible to remove cytoplasmic ribosomes and membraneous material; (c) mutations in *Neurospora* affecting ribosome structure and functions are available. On the other hand, there are a number of allied problems in the biogenesis of mitochondria in which a knowledge of mitochondrial ribosomes is a prerequisite to a meaningful experimental approach.

[1] P. Borst and L. A. Grivell, *FEBS Lett.* **13**, 73 (1971).
[2] P. Borst, *Annu. Rev. Biochem.* **41**, 333 (1972).
[3] T. W. O'Brien and D. E. Matthews, *Handb. Genet.* **5**, 535 (1976).
[4] N. G. Avadhani, F. S. Lewis, and R. J. Rutman, *Sub-Cell. Biochem.* **4**, 93 (1975).
[5] W. Neupert, *in* "Horizons in Biochemistry and Biophysics" (E. Quagliariello, F. Palmieri, and T. P. Singer, eds.), Vol. III, p. 257. Addison-Wesley, Reading, Massachusetts, 1977.
[6] H. Küntzel and H. Noll, *Nature (London)* **215**, 1340 (1967).
[7] M. R. Rifkin, D. D. Wood, and D. J. L. Luck, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1025 (1967).

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Isolation of Mitochondrial Ribosomes

Growth of Neurospora Cells and Isolation of Mitochondria. Growth of mycelia and procedures to break cells are described in a separate article of this volume.[8] *Neurospora* cells should be grown until they reach early or middle log phase. The cells are harvested by filtration and then washed two times with ice-cold isolation medium (about 10 ml/g wet weight). The medium for isolation of mitochondria contains EDTA to avoid massive contamination of mitochondria by cytoplasmic ribosomes.

Sucrose, 0.44 *M*
EDTA, 0.002 *M*
Tris-HCl, pH 7.5, 0.01 *M*

Mitochondria are isolated from the cell homogenate according to the flow diagram shown in Fig. 1. They should be washed in isolation medium at least three times, by gently resuspending them in a glass-Teflon potter and resedimenting them, to reduce the amount of contaminating cytoplasmic ribosomes to less than 2–4%.

All chemicals should be reagent grade and all operations should be carried out at 0°–4°.

Isolation of Ribosomes. The final mitochondrial pellet is resuspended in AMT buffer to a protein concentration of 1–2 mg/ml; 0.15 volume of 20% Triton X-100 (Sigma Chemical Co., St. Louis, Missouri) in AMT is added.

NH_4Cl, 0.1 *M*
$MgCl_2$, 0.01 *M*
Tris-HCl, pH 7.5, 0.03 *M*

The cleared suspension is centrifuged for 20 min at 48,000 g_{max}. Usually a small pellet containing mainly cell walls and unbroken cells is discarded. The ribosomes are pelleted from the lysate by centrifuging the supernatant through a cushion of 1.4 *M* sucrose in AMT. In a 10- to 13-ml centrifuge tube, a 2-ml cushion is adequate. Centrifugation depends on rotor and centrifuge used. Using the Spinco 50Ti rotor, 2 hr at 50,000 rpm (226,000 g_{max}) give a good ribosomal pellet. Use of a sucrose cushion greatly improves the purity of the ribosomes. In the absence of a cushion, the ribosomal pellet contains considerable amounts of yellow membraneous material.

Centrifugation of the clarified mitochondrial lysate can also be performed in 1.5-ml reaction tubes made of polyethylene (e.g., Eppendorf-Gerätebau, Hamburg, Germany) when small amounts of ribosomes are to be isolated. The tubes are placed in adaptors which fit into a Spinco 50Ti

[8] W. Sebald, S. Werner, and H. Weiss, this volume [5].

rotor and which have about half the height of a 13.5-ml tube. The holes of the adaptors are filled with water before setting in the tubes, so that they are floating. The tubes may be run with or without caps.

After centrifuging down the ribosomes, the supernatant is removed by suction. The surface of the colorless ribosomal pellet is gently rinsed by carefully overlaying about 1 ml of AMT buffer. Care is taken that no residue of the lysate remains at the wall of the centrifuge tubes. Usually the tubes are placed upside down on filter paper for about 5 min. The ribosomes are resuspended in AMT buffer (about 0.1 ml per 10-ml tube) by gently shaking the tubes for about 5 min. The ribosomal suspension is collected and subjected to a clarifying spin for 5 min at 10,000 g_{max}. This clarifying spin gives only a pellet when ribosomes are aggregated or contaminated by membraneous material.

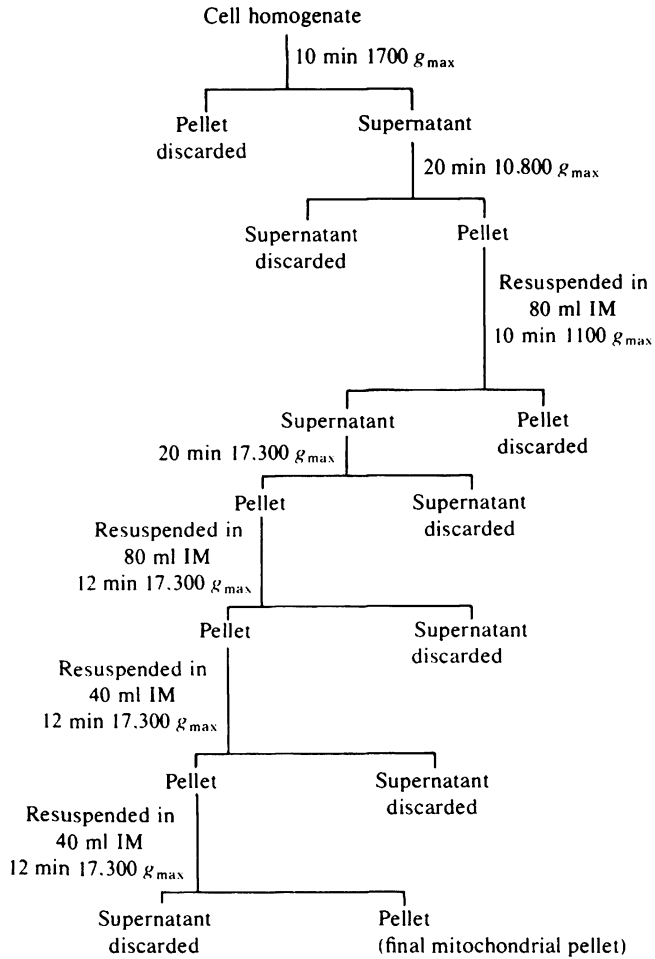
The uv spectrum of the supernatant is recorded, and the amount of ribosomes is calculated from the extinction at 260 nm. The amount of ribosomes is usually expressed in A_{260} units. One A_{260} unit is the quantity of ribosomes contained in 1 ml of solution which has an absorbance of 1 when measured in a 1-cm path length cell. One A_{260} unit corresponds to 40 μg of ribosomal RNA. The average yield of mitochondrial ribosomes is about 0.5 A_{260} per milligram of mitochondrial protein. One milligram of mitochondrial protein is usually obtained from 0.5 g hyphae (wet weight). Mitochondrial ribosomes tend to aggregate especially after collection of ribosomes by centrifugation and may therefore be partly lost. Also, raising the temperature above 0°–4° leads to aggregation. Furthermore, the ribosomes have a tendency to stick to the walls of the cellulose nitrate centrifuge tubes.

Dissociation of Mitochondrial Ribosomes into Subunits. Several simple and rapid methods have been described for the preparation of ribosomal subunits.

a. Mitochondrial ribosomes are isolated as described above with the exception that heparin (sodium salt, grade II, Sigma Chemical Co., St. Louis, Missouri) is added to the AMT buffer used for the isolation of ribosomes from the isolated mitochondria.[9] Concentration of heparin is 0.5 mg/ml. Centrifugation of the mitochondrial lysate for 4 hr is advisable to avoid loss of slowly sedimenting subunits. Gradient centrifugation shows that practically all ribosomes are dissociated to 50 S and 37 S subunits (see Fig. 2B).

b. Ribosomes are taken up in AT buffer (0.01 *M* Tris-HCl, 0.1 *M* NH_4Cl, pH 7.5) and layered on the gradient, the upper part of which

[9] R. Michel, G. Hallermayer, M. A. Harmey, F. Miller, and W. Neupert, *in* "Genetics and Biogenesis of Chloroplasts and Mitochondria" (T. Bücher *et al.*, eds.), p. 725. North-Holland Publ., Amsterdam, 1976.


Cell homogenate
10 min 1700 g_{max}
Pellet discarded
Supernatant
20 min 10.800 g_{max}
Supernatant discarded
Pellet
Resuspended in 80 ml IM 10 min 1100 g_{max}
Supernatant
20 min 17.300 g_{max}
Pellet discarded
Pellet
Resuspended in 80 ml IM 12 min 17.300 g_{max}
Supernatant discarded
Pellet
Resuspended in 40 ml IM 12 min 17.300 g_{max}
Supernatant discarded
Pellet
Resuspended in 40 ml IM 12 min 17.300 g_{max}
Supernatant discarded
Pellet (final mitochondrial pellet)

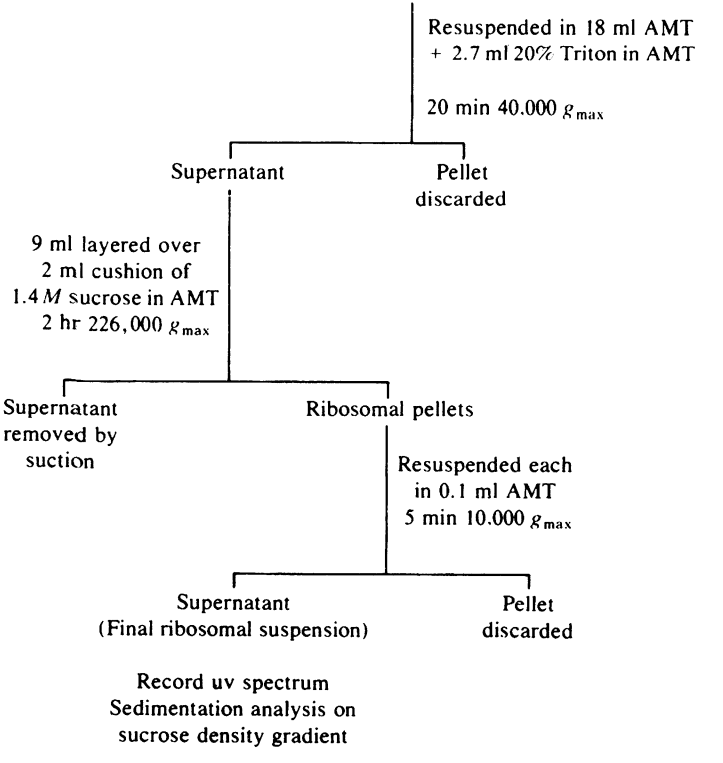


FIG. 1. Flow diagram for the isolation of mitochondrial ribosomes. *Neurospora* cells (15 g wet weight) from a 20-liter culture are homogenized in a bottom-driven macerator with 150 ml of isolation medium (IM) (0.44 *M* sucrose, 2 m*M* EDTA, 0.03 *M* Tris-HCl, pH 7.5) and put through a grind mill. AMT: 0.1 *M* NH_4Cl, 0.01 *M* $MgCl_2$, 0.03 *M* Tris-HCl, pH 7.5.

consists of a layer of SAMT buffer (0.20 *M* sucrose, 0.01 *M* Tris-HCl, 0.1 *M* NH_4Cl, 0.1 m*M* EDTA, pH 7.5). The rest of the gradient consists of 0.3–0.9 *M* sucrose in AMT buffer. Centrifugation is carried out for 3–4 hr (see below). The brief passage through the EDTA layer dissociates the ribosomes into subunits which then separate on the Mg^{2+} containing gradient. A 1.5-ml SAMT layer is adequate for 10- to 12-ml centrifuge tubes.[10]

c. A suspension of mitochondrial ribosomes in AMT buffer is subjected four times to a freezing-thawing treatment. For freezing, the suspension is put into a deep freeze box and is allowed to cool to −20°C. The sample is thawed by letting it adjust to 4°C.[11]

II. Analysis of Mitochondrial Ribosomes

Sucrose Density Gradient Centrifugation. Further separation of mitochondrial ribosomes is carried out by sucrose density gradient centrifugation. Linear or convex (isokinetic) sucrose gradients ranging from 0.3 to 1.0 *M* sucrose in AMT are prepared according to established procedures.[12] About 0.05 to 0.2 ml of the ribosome suspension is layered on the gradient. Centrifugation in a Spinco SW41 rotor at 40,000 rpm (286,000 g_{max}) for 3–4 hr is appropriate for the analysis of monomers and subunits, centrifugation for 45–60 min for the analysis of polymeric forms. Gradients are monitored by pumping the content of the centrifuge tubes through a quartz flow cell and recording the absorption at 260 nm. Usually gradients are pierced at the bottom and 60% sucrose is pumped into the tubes. One to 5 A_{260} units of ribosomes give good absorption profiles when a 0.5-cm path length cell is used and the absorption scale is 0–1.

Calibration of gradients is usually carried out by cocentrifugation of *E. coli* ribosomes prepared according to established procedures.[13] In special cases, cocentrifugation of radioactively labeled *E. coli* ribosomes is very useful, since minute amounts of *E. coli* ribosomes can be employed which do not disturb the absorption profiles of the *Neurospora* ribosomes.

A typical gradient profile obtained according to the described procedure is shown in Fig. 2A. The mitochondrial ribosomes contain a prominent 73 S monomer peak and clearly discernable subunits (50 S and 37 S). Polymeric forms are also found. They exist in decreasing amounts from dimers to heptamers. The percentage of polymers, monomers, and subunits can be estimated from the A_{260} profiles. The total polymeric forms

[10] R. Michel and W. Neupert, *Eur. J. Biochem.* **36**, 53 (1973).
[11] S. Werner, personal communication.
[12] H. Noll, *Nature (London)* **215**, 360 (1967).
[13] M. W. Nirenberg, Vol. 6, p. 17.

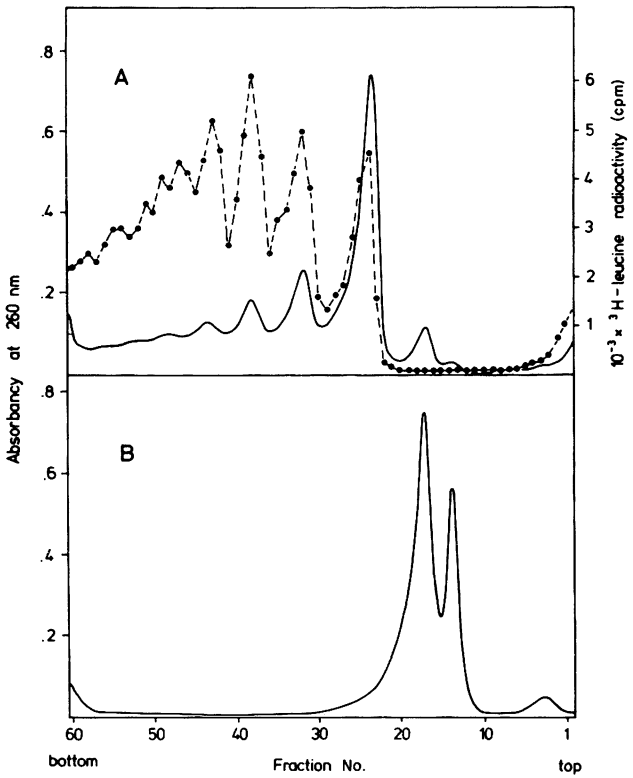


FIG. 2. Analysis of mitochondrial ribosomes by sucrose density gradient centrifugation. (A) Mitochondrial ribosomes prepared according to the flow diagram in Fig. 1. Before isolating ribosomes, *Neurospora* cells were treated with cycloheximide and ^{3}H-leucine to label nascent polypeptide chains (see text). (B) Mitochondrial ribosomes dissociated into subunits by heparin (see text). Absorbancy at 260 nm (——); radioactivity (-●--●-).

account for 40–50% of total ribosomes, and monomers account also for some 40–50%. Subunits contribute some 10%.

The relatively low percentage of polymers in mitochondrial ribosome preparations is in sharp contrast to cytoplasmic ribosomes, where up to 95% of the total ribosomes may be isolated as polymers. Such yields of cytoplasmic polymers require the presence of heparin[9]; without heparin most of the ribosomes are usually recovered as monomers.[6,10,14] In the case of mitochondrial ribosomes addition of heparin results in the dissociation of polymeric and monomeric forms into subunits.[9] A gradient profile of mitochondrial ribosomal subunits obtained by heparin treatment is shown in Fig. 2B.

[14] F. A. M. Alberghina and S. R. Suskind, *J. Bacteriol.* **94**, 630 (1967).

Mitochondrial ribosomes have been described to dissociate at much higher levels of Mg^{2+} than their cytoplasmic counterparts.[15] If the Mg^{2+} concentration is lowered to 0.1 m*M* dissociation takes place, whereas cytoplasmic ribosomes begin to dissociate at concentrations of 0.01 m*M*. Mitochondrial polymeric ribosomes show a high tendency to aggregate and may be lost when temperature and pH are not carefully controlled. These polymers are not converted to monomers by the action of ribonuclease and may represent aggregates linked via their nascent polypeptide chains.[10] A similar characteristic has been described for HeLa cell mitochondrial ribosomes.[16]

Nascent polypeptide chains on mitochondrial ribosomes can be specifically labeled with radioactive amino acids *in vivo*. A culture of *Neurospora* cells is incubated with cycloheximide (0.1 mg/ml) for 2 min, then ^{3}H-leucine (1 μCi/ml, specific radioactivity 10–60 mCi/mmole) is added and incorporation is allowed to proceed for 5 min. Then the culture is cooled to 0° by adding 2 volumes of ice-water mixture, and mitochondrial ribosomes are isolated. The selective labeling of nascent polypeptide chains is possible since (a) cycloheximide specifically blocks cytoplasmic translation but not mitochondrial translation,[17,18] and (b) the mitochondrial ribosomal proteins are synthesized on cytoplasmic ribosomes.[19–21]

The isolated ribosomes are subjected to density gradient centrifugation, and the gradient content after passing the absorbance monitor is collected with a fractionation collector. Radioactivity in the fractions is determined by liquid scintillation counting. Nascent polypeptide chains are mainly (about 90%) associated with the polysome portion of the ribosomes (Fig. 2A).

Electron Microscopic Analysis of Mitochondrial Ribosomes. POSITIVE STAINING. Mitochondrial ribosomes can be demonstrated in thin sections of *Neurospora* cells or of isolated mitochondria. Pellets of cells or mitochondria are fixed with 3% glutaraldehyde in 0.1 *M* cacodylate buffer, pH 7.4, for 6–12 hr. The pellets are then rinsed three times with 0.29 *M* sucrose, 0.2 *M* cacodylate buffer, pH 7.4, and postfixed in 1% OsO_4 for 1 hr in 0.29 *M* sucrose, 10 m*M* $MgCl_2$, 0.1 *M* cacodylate buffer, pH 7.2. Samples are dehydrated in ethanol and embedded in Epon 812. Thin sections are contrasted with 7% magnesium uranyl acetate and lead citrate. Figure 3 shows a thin section of a cell of the wall-less "slime"

[15] H. Küntzel, *J. Mol. Biol.* **40**, 315 (1969).
[16] D. Ojala and G. Attardi, *J. Mol. Biol.* **65**, 273 (1972).
[17] W. Sebald, A. J. Schwab, and T. Bücher, *FEBS Lett.* **4**, 243 (1969).
[18] E. S. Hawley and J. W. Greenawalt, *J. Biol. Chem.* **254**, 3574 (1969).
[19] W. Neupert, W. Sebald, A. J. Schwab, A. Pfaller, and T. Bücher, *Eur. J. Biochem.* **10**, 585 (1969).
[20] H. Küntzel, *Nature (London)* **222**, 142 (1969).
[21] P. M. Lizardi and D. J. L. Luck, *J. Cell Biol.* **54**, 56 (1972).

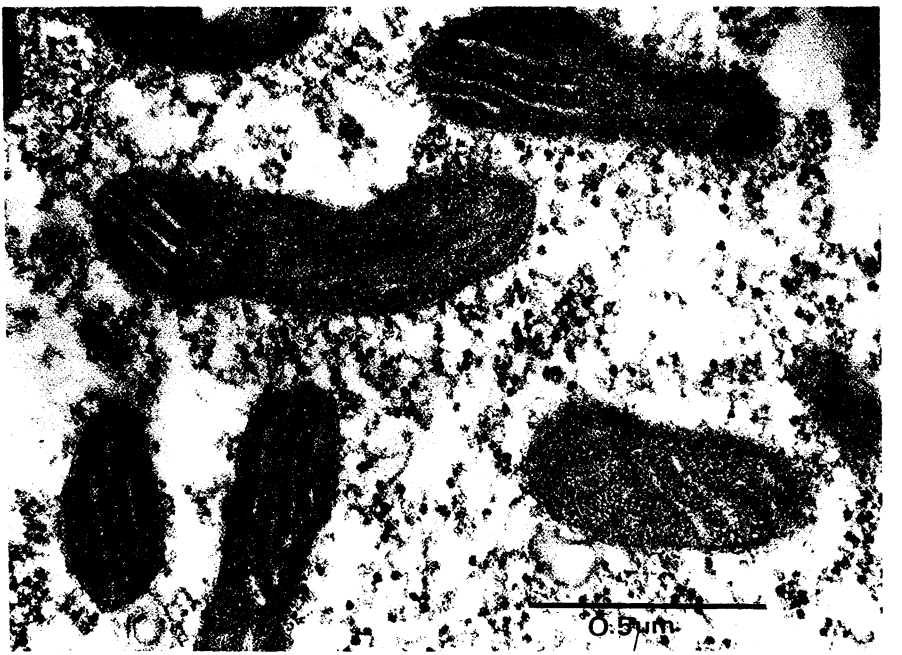


FIG. 3. Small field from the cytoplasm of a wall-less mutant of *Neurospora* (Slime arg-1A). Mitochondrial ribosomes are abundant and arranged mainly in rows along the periphery of the inner membrane. Cytoplasmic ribosomes are also visible.

mutant of *Neurospora*, in which mitochondrial ribosomes can be better demonstrated than in the wild type. Ribosomes often show a polysomelike arrangement, characteristically along the peripheral inner mitochondrial membrane and not regularly distributed over the whole matrix area. With yeast, similar techniques were employed and similar results were obtained.[22,23]

NEGATIVE STAINING. The physical dimensions may be measured by analyzing electron micrographs of negatively stained ribosomes.[24] The shapes observed are projections and depend on the orientation of the ribosomes on the grid. For negative staining, ribosomes in AMT buffer are dialyzed for 16–18 hr against a buffer containing 1 *M* methanol, 0.002 *M* $MgCl_2$, and 0.03 *M* Tris–HCl, pH 7.6, according to Vasiliev.[25] Holey parlodion films are prepared according to Drahos and Delong,[26] picked up

[22] P. V. Vignais, B. J. Stevens, J. Huet, and J. André, *J. Cell Biol.* **54**, 468 (1972).
[23] K. Watson, *J. Cell Biol.* **55**, 721 (1972).
[24] Y. Nonomura, G. Blobel, and D. Sabatini, *J. Mol. Biol.* **60**, 303 (1971).
[25] V. D. Vasiliev, *FEBS Lett.* **14**, 203 (1971).
[26] V. Drahos and A. Delong, *Nature (London)* **186**, 104 (1960).

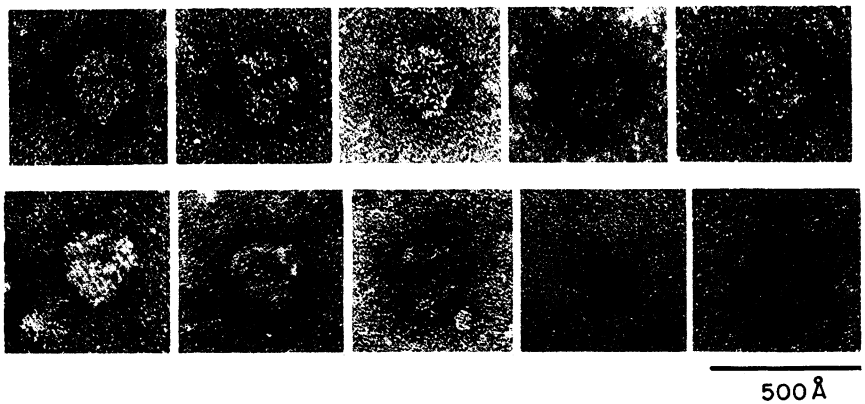


FIG. 4. Selected images of negatively stained mitochondrial and cytoplasmic ribosomes from *Neurospora crassa*. Upper row: mitochondrial ribosomes. Lower row: cytoplasmic ribosomes. The first four images in each row represent frontal views, the last one a lateral view. The groove between small subunits (top) and large subunit (bottom) can be seen in frontal views of either type of ribosome. Lateral views in both cases show the asymmetric arrangement of the small subunit with respect to the large subunit. × 400,000.

on copper grids (300 mesh), and reinforced by a thick layer of carbon. The grids are covered with a second parlodion film, and a thin layer of carbon is again evaporated on to them at 10^{-5} torr. The grids are then rinsed for 2 days in amyl acetate to remove the parlodion films. A drop of the unfixed ribosome suspension is deposited on the grid and removed with filter paper after 1–5 min. A drop of 2% unbuffered uranyl acetate is immediately placed on the grid, before the film has dried. 0.5 μl of 0.015% octadecanol in hexane (w/v) is applied to the surface of the stained droplet and the stain withdrawn with filter paper. Micrographs from such preparations are taken at direct magnifications of 80×10^3 to 100×10^3. Figure 4 shows a gallery of selected images of mitochondrial and cytoplasmic ribosomes. Frontal views and lateral views according to the definition of Nonomura *et al.*[24] can be discriminated in either case.

Table I contains the average dimensions along the main axes of the ribosomes.

Immunological Studies of Neurospora Ribosomes. Specific antibodies may be readily prepared against mitochondrial ribosomes. Such antibodies show no cross-reaction with cytoplasmic ribosomes, and antibodies against cytoplasmic ribosomes do not react with mitochondrial ribosomes.[9,27] Antibodies are raised in rabbits in the following manner. Approximately 30–40 A_{260} units of ribosomes in 0.5 ml AT buffer are emulsified with an equal volume of complete Freund's adjuvant (Difco,

[27] G. Hallermayer and W. Neupert, *FEBS Lett.* **41**, 264 (1974).

TABLE I
PROPERTIES OF MITOCHONDRIAL AND CYTOPLASMIC RIBOSOMES FROM NEUROSPORA CRASSA

Property	Mitochondrial ribosomes	Reference	Cytoplasmic ribosomes	Reference
Sedimentation coefficient ($s_{20,w}$)				
Monomer	73	6	77	6
			78	40, 41
			81	7
Dimer	103	6	108	6
			115	14
Large and small subunit	50 + 37	6, 15	60 + 37	6, 15
			56 + 36	41
Dimensions (Negative staining)				
Main axes[a] (Å)	273 ± 18 × 222 ± 18		250 ± 17 × 213 ± 16	
RNA components				
Sedimentation coefficient	23 + 16.2	6	25.8 + 16.5	6
$s_{20,w}$-units	23 + 16	34	25.6 + 16	34
Molecular weights (dalton × 10^6)	1.28 + 0.74	19	1.28 + 0.64	19
			1.29 + 0.69	41
(Gel electrophoresis at 5°C)				
5 S RNA	Not detected	9, 31	Present	9, 31
5.8 S RNA	Not detected	9, 31	Present	9, 31
G + C content (%)	38	6	49	6
	35	7	50	7
Methylation of large and small subunit RNA (methyl groups per 100 nucleotides)	0.13 + 0.067	4[2]	1.3 + 1.5	42
Number of ribosomal proteins in large and small subunits	30 + 23	21, 43	31 + 21	39
	31 + 30	39		
Dissociating levels of magnesium ions (at 50 m*M* NH_4Cl) (m*M*)	0.1	15	0.01	15
Antibiotic sensitivity				
Chloramphenicol	+	17, 18	−	17, 18
Cycloheximide (Actidione)	−	17, 18	+	17, 18
Anisomycin	−	21	+	21

[a] Mean values and standard deviations (N = 65) of height × width measured on frontal views.

Detroit, Michigan) and injected subcutaneously into four different positions of the neck region of the rabbit. Four injections at intervals of 10 days have been found to yield satisfactory titers. For immunoprecipitation, antiserum or γ-globulins (prepared by ammonium sulfate precipitation, redissolved in AMT and dialyzed against AMT for 10–15 hr) are added to the ribosomes in AMT buffer containing 1% Triton X-100 (about 2 A_{260} units/ml). Two hundred microliters of antiserum is usually sufficient to precipitate 2 A_{260} units of ribosomes. Precipitates are centrifuged, washed three times with 1% Triton X-100 in AMT, and washed once with AMT buffer.

Such antibodies can specifically precipitate mitochondrial ribosomes from mixtures of cytoplasmic and mitochondrial ribosomes.[9] They can, furthermore, be used to precipitate ribosomal proteins.

Antibodies can be employed to study problems, such as phylogenetic affinities between related species, pool sizes of free mitochondrial ribosomal proteins, and cellular transport of such proteins.

Isolation and Analysis of Ribosomal RNA Components. The RNA components can be extracted by standard extraction procedures either from isolated ribosomes or from isolated mitochondria. However, it is very difficult to obtain undegraded RNA from isolated ribosomes.[6] Therefore, ribosomal RNA is usually isolated from whole mitochondria. Also in this case, it is advisable to add nuclease inhibitors, such as diethyl pyrocarbonate.[28] The method of Parish and Kirby[29] modified by Leaver and Ingle[30] and the method of Solymosy *et al.*[28] appear to give the best results with respect to integrity and yield of ribosomal RNA.[9,31–33]

The molecular dimensions are usually determined by (a) sucrose density gradient centrifugation,[6,7,34] and (b) polyacrylamide gel electrophoresis, essentially according to the methods described by Peacock and Dingman[35] and by Loening.[36] Both procedures must be carried out under conditions in which the mitochondrial RNA does not unfold, or else under conditions in which complete denaturation is induced.[37] The ribosomal RNA components found are listed in Table I. The guanine

[28] F. Solymosy, I. Fedorcsak, A. Gulyas, G. L. Farkas, and L. Ehrenberg, *Eur. J. Biochem.* **5**, 520 (1968).

[29] J. H. Parish and K. S. Kirby, *Biochim. Biophys. Acta* **129**, 554 (1966).

[30] C. J. Leaver and J. Ingle, *Biochem. J.* **123**, 235 (1971).

[31] P. M. Lizardi and D. J. L. Luck, *Nature (London), New Biol.* **229**, 140 (1971).

[32] Y. Kuriyama and D. J. L. Luck, *J. Mol. Biol.* **73**, 425 (1973).

[33] H. Küntzel, Z. Barath, I. Ali, J. Kind, and H.-H. Althaus, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1574 (1973).

[34] L. S. Dure, J. L. Epler, and W. E. Barnett, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1883 (1969).

[35] A. C. Peacock and C. W. Dingman, *Biochemistry* **7**, 668 (1968).

[36] U. E. Loening, *Biochem. J.* **113**, 131 (1969).

[37] L. Reijnders, P. Sloof, and P. Borst, *Eur. J. Biochem.* **35**, 266 (1973).

and cytosine content of the mitochondrial RNA is low compared to that of the cytoplasmic ribosomal RNA,[1,6,7] and this feature may be exploited in the resolution of mixtures of cytoplasmic and mitochondrial ribosomal RNA's. The cytoplasmic and mitochondrial RNA's have very similar electrophoretic mobility when electrophoresed at 4°. However, if the temperature of electrophoresis is raised to 25°, the mitochondrial RNA partially unfolds and exhibits a reduced electrophoretic mobility.

Analysis of Mitochondrial Ribosomal Proteins. Several methods can be employed for the separation of mitochondrial ribosomal proteins. The most satisfactory is two-dimensional electrophoresis as described by Kaltschmidt and Wittmann[38] and employed by van den Bogert and de Vries.[39] One-dimensional electrophoresis in urea gels or in sodium dodecyl sulfate-urea gels combined with isoelectric focusing can also give good resolution as demonstrated by Lizardi and Luck.[21] Separation on polyacrylamide gradient gels has also been shown to be a powerful and convenient technique.[40] For special purposes chromatography on carboxymethyl cellulose columns may be a useful method.[20]

The number of proteins found in large and small subunits of mitochondrial 73 S ribosomes by various authors is presented in Table I.[21,39,43]

Analysis of Mitochondrial Ribosomal Function. Assays for the functional activity of isolated mitochondrial ribosomes are few and very limited. There are no published data on the translation of mitochondrial messenger RNA or messenger RNA from other sources by purified 73 S ribosomes of *Neurospora crassa*. Only partial reactions of protein synthesis have been demonstrated, i.e., (a) poly(U)-directed synthesis of polyphenylalanine[44], (b) binding of formylmethionyl-tRNA[45], (c) synthesis of formylmethionylpuromycin[45], and (d) the "fragment reaction" (peptidyltransferase activity).[46] The activity of the poly(U)-directed system was found to be very low, and also the "fragment reaction" showed much lower levels when compared to *E. coli* ribosomes. Significantly, both of these functions are inhibited by antibiotics which react with prokaryotic ribosomes, e.g., chloramphenicol (see Table I).

[38] E. Kaltschmidt and H. G. Wittmann, *Anal. Biochem.* **36,** 401 (1970).
[39] C. van den Bogert and H. de Vries, *Biochim. Biophys. Acta* **442,** 227 (1976).
[40] M. M. Taylor and R. Storck, *Proc. Natl. Acad. Sci. U.S.A.* **52,** 958 (1964).
[41] P. Cammarano, A. Felsani, A. Romeo, and F. A. M. Alberghina, *Biochim. Biophys. Acta* **308,** 404 (1973).
[42] A. M. Lambowitz and D. J. L. Luck, *J. Biol. Chem.* **10,** 3081 (1976).
[43] A. M. Lambowitz, N.-H. Chua, and D. J. L. Luck, *J. Mol. Biol.* **107,** 223 (1976).
[44] H. Küntzel, *FEBS Lett.* **4,** 140 (1969).
[45] F. Sala and H. Küntzel, *Eur. J. Biochem.* **15,** 280 (1970).
[46] H. de Vries, E. Agsteribbe, and A. M. Kroon, *Biochim. Biophys. Acta* **246,** 111 (1971).

III. Use of *Neurospora* Mitochondrial Ribosomes

The ribosomes of *Neurospora* mitochondria may be used in an approach to a number of fundamental problems related to biogenesis and autonomy of mitochondria, such as the following.

1. Analysis and characterization of mitochondrial messenger RNA
2. Studies on the mechanism of mitochondrial translation and the mechanism of antibiotic action on mitochondrial biogenesis
3. Studies on the interaction of mitochondrial ribosomes and mitochondrial membranes and the mechanism of insertion of mitochondrial translation products into the mitochondrial membrane
4. Studies on the assembly of mitochondrial ribosomes and on the transport of mitochondrial proteins from the cytoplasm across the mitochondrial membranes
5. Studies on the genetic basis of defects of mitochondrial ribosomes phenotypically characterized by deficiencies in respiratory complexes.

Comments

In a recent series of papers one group of workers has reported the isolation of a special type of *Neurospora* mitochondrial ribosome having a sedimentation coefficient of 80 S.[39,47,48] The authors have expressed the view that this is the real functional mitochondrial ribosome, whereas the 73 S ribosome arises by degradation of this 80 S ribosome. The 80 S ribosome differs from the 73 S ribosome in many respects. In a detailed study of *Neurospora* ribosomes[9,49] we were unable to detect an 80 S mitochondrial ribosome. Rather we have observed that under the conditions described for the isolation of mitochondrial 80 S ribosomes, the 73 S mitochondrial ribosomes were heavily contaminated by cytoplasmic 78–79 S cytoplasmic ribosomes. In our view, the above-mentioned authors have not excluded the possibility that the 80 S ribosome is a cytoplasmic contaminant, a possibility strongly favored by our own findings and those of Lizardi and Luck.[31]

[47] R. Datema, E. Agsteribbe, and A. M. Kroon, *Biochim. Biophys. Acta* **335**, 386 (1974).

[48] A. M. Kroon, P. Terpstra, M. Holtrop, H. de Vries, C. van den Bogert, J. de Jonge, and E. Agsteribbe, *in* "Genetics and Biogenesis of Chloroplasts and Mitochondria" (T. Bücher *et al.*, eds.), p. 685. North-Holland Publ., Amsterdam, 1976.

[49] R. Michel, G. Hallermayer, M. A. Harmey, F. Miller, and W. Neupert, *Biochim. Biophys. Acta* **478**, 316 (1977).