# THE MITOCHONDRIAL RIBOSOME FROM *LOCUSTA MIGRATORIA*: DISSOCIATION INTO SUBUNITS

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## 1. Introduction

Mitochondrial ribosomes from locust thorax muscles have been identified recently in our laboratory by specific labelling of the adhering nascent peptide chains and have been isolated from mitochondria freed of contaminating cytoplasmic ribosomes [1]. Now data are presented which show that these 60 S mitochondrial ribosomes can be dissociated into subunits of 40 S and 25 S. Gel electrophoretic patterns of the proteins from these subunits are clearly different from each other and from their cytoplasmic counterparts.

## 2. Materials and methods

### 2.1. *Preparation of ribosomes and ribosomal subunits*

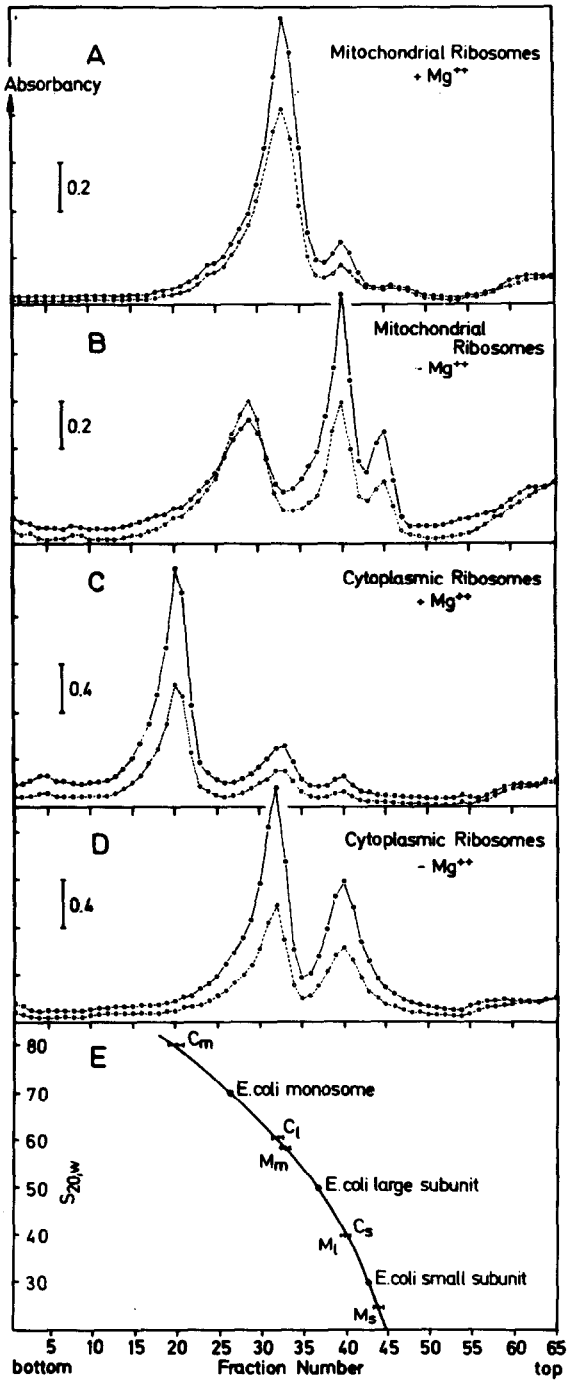
Mitochondria were prepared by homogenization of muscles in SET buffer (0.3 M sucrose, 0.05 M EDTA, 0.01 M triethanolamine, pH 7.2) containing 0.02 mg/ml subtilopeptidase A [2]. The homogenate was centrifuged (10 min; 10,000 *g*), the pellet was washed once with SET, resuspended in SET and centrifuged (45 min; 70,000 *g*; Spinco SW 25.2 rotor) through a three step sucrose density gradient (10 ml 53%, 10 ml 50% and 30 ml 35% sucrose, in 0.05 M EDTA, 0.01 M triethanolamine buffer, pH 7.2). Mitochondria were collected from the interphase between the 35% and 50% sucrose and diluted with two volumes of AMT (0.1 M $NH_4Cl$, 0.01 M $MgCl_2$, 0.03 M tris buffer, pH 7.6). The mitochondria were centrifuged (15 min; 10,000 *g*) and the pellet was lysed by resuspension in 2.5% Triton X 100 in AMT (5–10 mg protein/ml). After a clarifying spin (30 min; 24,000 *g*) the lysate was layered over 1.5 ml 25% sucrose in AMT and centrifuged in a Spinco 40 rotor (120 min; 100,000 *g*). The ribosomal pellet was rinsed with and resuspended in AMT. The suspension was subjected to a 10 min 20,000 *g* centrifugation and the clear supernatant (0.6–0.8 ml, absorbancy ranging from 3.0 to 9.0 at 260 nm) was placed on a convex sucrose density gradient as described before [1].

Undissociated ribosomes were prepared employing a gradient made from AMT, while subunits were prepared using a gradient made without added magnesium. Fractions corresponding to absorbancy peaks (fig. 1) were pooled, diluted with a 20 fold volume AMT and centrifuged for 16 hr at 144,000 *g*.

Cytoplasmic ribosomes were prepared by the same procedure, except that SET buffer was replaced by SMT (0.3 sucrose, 0.01 M $MgCl_2$, 0.01 M triethanolamine buffer, pH 7.2) and that treatment with subtilopeptidase A and the sucrose step gradient were omitted. In this case the whole pellet of the 10 min 10,000 *g* centrifugation of the muscle homogenate was lysed with Triton-AMT. The very small amount of mitochondrial ribosomes present in such preparations of cytoplasmic ribosomes can be neglected [1–3]. All steps were carried out at 0–4°.

Radioactively labelled *E. coli* B ribosomes were prepared from cells grown in the presence of phosphate-P-32 [4].

The convex sucrose density gradients were cali-




brated by co-centrifugation of P-32-labelled *E. coli* ribosomes (60,000 cpm/$A_{260\ nm}$ unit) assuming $s_{20,w}$ values of 70 for the monomers and of 50 and 30 for subunits (see fig. 1E).

### 2.2. *Gel electrophoresis of ribosomal proteins*

For disc electrophoresis the pellets from the 16 hr 144,000 *g* centrifugation were treated with 12 M acetic acid, 0.1 M $MgCl_2$ for 2 hr at 0°, then centrifuged to remove the precipitated RNA. The supernatant was made 6 M with urea and applied to 7.5% polyacrylamide gels which contained 6 M acetic acid and 6 M urea as described by Takayama [5]. For flat gel electrophoresis the pellets were dried in acetone and ether, dissolved in phenol/formic acid/water and applied to 7.5% polyacrylamide gels as described by Sebald [6].

## 3. Results and discussion

### 3.1. *Gradient centrifugation of mitochondrial and cytoplasmic ribosomes*

The sedimentation profile of undissociated ribosomes isolated from mitochondria freed from cytoplasmic ribosomes is shown in fig. 1A. The absorbancy pattern (260 nm and 280 nm) displays a main peak at fraction 33 which can be attributed to the mitochondrial monomeric ribosome [1]. This corresponds to a sedimentation coefficient of 60 S. In addition, two minor peaks in fractions 40 and 45 were observed. The ratio of absorbancies at 260 to 280 nm in the peak fraction is below the range which is to be expected for a ribosomal particle (1.4–1.6). It should be further noted that in the first fractions of the peak (29–31) this ratio is much lower (1.1–1.2).

Fig. 1. Sucrose density gradient centrifugation of mitochondrial and cytoplasmic ribosomes. •——•: absorbancy at 260 nm; ○ - - - ○: absorbancy at 280 nm.
(A) undissociated mitochondrial ribosomes ($Mg^{2+}$ containing gradient)
(B) dissociated mitochondrial ribosomes ($Mg^{2+}$ free gradient)
(C) undissociated cytoplasmic ribosomes ($Mg^{2+}$ containing gradient)
(D) dissociated cytoplasmic ribosomes ($Mg^{2+}$ free gradient)
(E) calibration of the gradients with *E. coli* standard. The S values employed in the graph are mean values from 3–7 gradients. The bars show the deviation range.

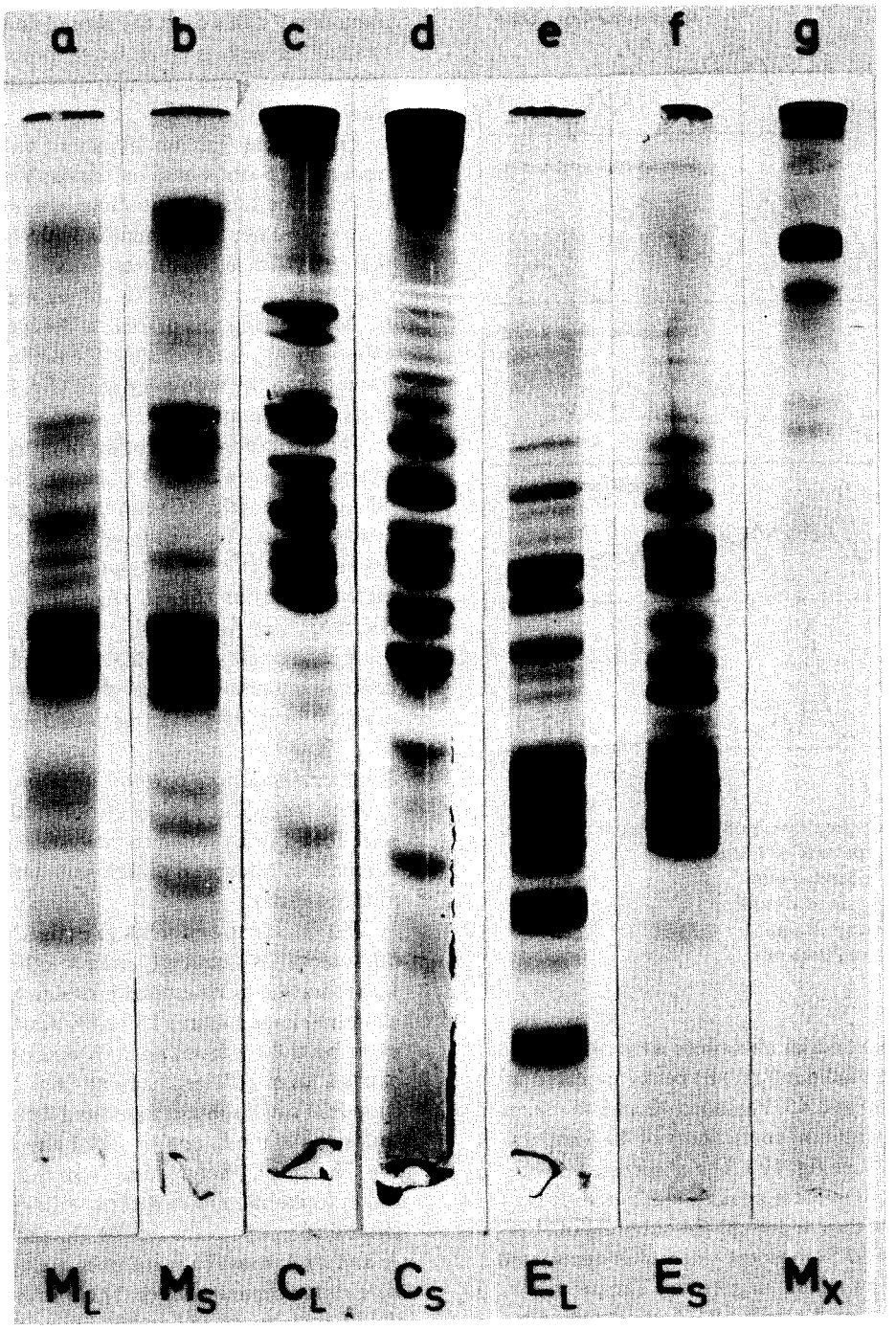



Fig. 2. Disc electrophoresis of ribosomal proteins from locust and *E. coli*. (a) mitochondrial large subunit ($M_L$); (b) mitochondrial small subunit ($M_S$); (c) cytoplasmic large subunit ($C_L$); (d) cytoplasmic small subunit ($C_S$); (e) *E. coli* large subunit ($E_L$); (f) *E. coli* small subunit ($E_S$); (g) $M_X$ particle.

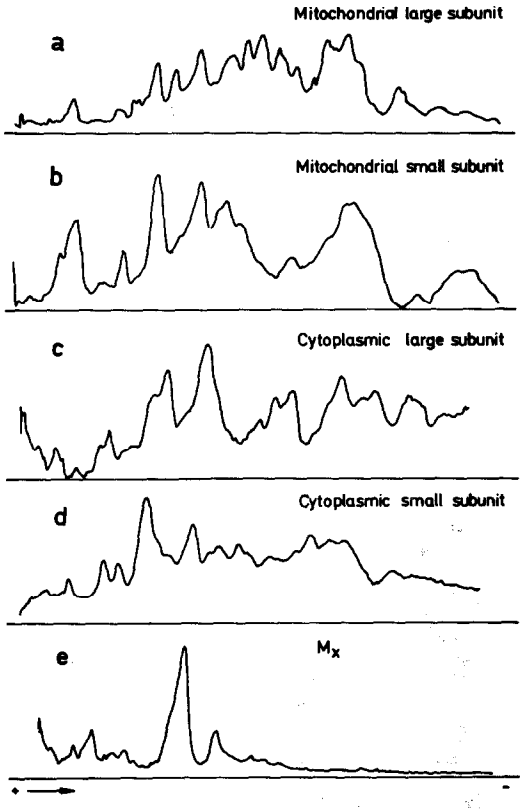



Fig. 3. Flat gel electrophoresis of proteins from ribosomes and from the $M_X$ particle of *Locusta*.
(a) mitochondrial large subunit
(b) mitochondrial small subunit
(c) cytoplasmic large subunit
(d) cytoplasmic small subunit
(e) $M_X$ particle

When mitochondrial ribosomes were centrifuged on a $Mg^{2+}$ free gradient (fig. 1B) peaks appeared in fractions 29, 40 and 45. Positions 40 and 45 correspond to sedimentation coefficients of 40 S and 25 S, respectively (see fig. 1E). The absorbancy ratios 260 nm/280 nm in these peaks are 1.7–1.8. It is concluded that they represent the subunits of the mitochondrial 60 S ribosome which was dissociated in the absence of added magnesium. The proportion of large to small subunit RNA as estimated by planimetry of the areas under the $A_{260\ nm}$ curve amounts to 2.1–2.3.

On the other hand the two minor peaks in fig. 1A are ascribed to free subunits accompanying the monomer. In this case, the proportion of the large to small subunits is much larger, probably because of incomplete sedimentation of the small subunits from the lysate.

The peak at fraction 29 cannot be attributed to residual 60 S ribosomes on the basis of its sedimentation properties. Furthermore, the absorbancy ratio at 260 nm to 280 nm (0.8–0.9) indicates that this particle consists essentially of protein (nucleic acid content = 20–30 μg/mg protein). In the following this particle is designated '$M_X$'. Obviously $M_X$ is responsible for the low 260 nm/280 nm ratios in the monomer peak (fig. 1A), especially in its first fractions.

The corresponding profiles of cytoplasmic ribosomes are shown in fig. 1 C and D. The cytoplasmic monomer (fig. 1C) exhibits a higher S coefficient (80 S, see fig. 1E) than the mitochondrial counterpart. The subunits which arise after complete dissociation (fig. 1D) have sedimentation coefficients of 61 S and 40 S (fig. 1E). The ratio of large to small subunit RNA is 2.3. In the magnesium containing gradient the subunits are visible as minor peaks (fig. 1C, fractions 33 and 40).

### 3.2. *Electrophoretic patterns of ribosomal proteins*

Both methods of gel electrophoresis employed were found to be suited to analysis of the very low amounts of ribosomal proteins obtained from locust mitochondria.

The disc electrophoresis [5] (fig. 2) is similar to other methods frequently used in other laboratories for separation of ribosomal proteins. With the mitochondrial large subunit 15 bands can be discriminated by this method (fig. 2a), in contrast to 21 bands with *E. coli* large subunit (fig. 2f). The mitochondrial small subunit exhibits 13 electrophoretic bands, while the *E. coli* small subunit has 15 bands (fig. 2b and g). The proteins from the mitochondrial and cytoplasmic subunits show different electrophoretic band patterns (cf. fig. 2a and b with fig. 2c and d). It must be mentioned that the samples were run in separate tubes. Therefore the possibilities of comparison of band positions are restricted.

With the horizontal electrophoresis method [6] (fig. 3) several samples were run simultaneously in one gel. The restriction in this system lies in the greater tendency towards aggregation. The densito-

grams obtained with this method again show differences in the band patterns derived from the various subunits.

Thus it is concluded that the mitochondrial and cytoplasmic large and small subunits consist of different proteins. Different protein compositions of mitochondrial and cytoplasmic ribosomes have been observed in fungi [7], plants [8] and protozoa [9]. Until now differences in the subunit proteins have been shown only with Neurospora [10].

### 3.3. *Particle* $M_X$

Gel electrophoresis of the proteins from $M_X$ exhibits one major band (comprising about 50% of total $M_X$) and some minor bands (figs. 2g, 3e). The pattern obtained from mitochondrial 60 S peak material strongly resembles that of the $M_X$ peak. This can be explained by the fact that in the magnesium free gradient the proportion of protein present in $M_X$ surpasses the amount of protein in both ribosomal subunits by a factor of 7–10. The origin and function of $M_X$ is not known, and it remains an open question whether its occurrence is unique to locust mitochondria.

## 4. Conclusions

The data presented here, together with earlier observations on pulse labelling of nascent peptide chains [1] provide conclusive evidence that the mitochondrial 60 S particle of *Locusta* is indeed a genuine monomeric ribosome. It is active in poly-U directed polyphenylalanine synthesis [11], and differs from its cytoplasmic counterpart with respect to sedimentation coefficients and protein composition. Moreover, it seems highly probable that the small RNA species reported [2, 3] are derived from relatively small mitochondrial subunits.

The first indications for the existence of 60 S ribosomes came from work with rat liver mitochondria [12, 13]. This 60 S mitochondrial ribosome was first isolated and characterized as a monomeric ribosome from Xenopus oocyte [14]. Recently, low S coefficients of mitochondrial ribosomes were confirmed in vertebrate cell cultures [14–19]. Minor peaks with still lower S coefficients accompanying the 60 S monomer were observed and have been ascribed to mitochondrial ribosomal subunits [14, 16–19]. Houssais [16] was able to observe dissociation of 61 S mitochondrial ribosomes of mouse cells into 46 S and 34 S subunits on a gradient 1 mM in magnesium.

In addition to these reports, our results sustain the emerging concept that mitochondria of higher animals possess a special class of ribosomes characterized by a sedimentation constant of about 60 S. They show that this concept holds also for invertebrate animals. Finally, they suggest that the 'miniribosome' from Locusta is composed of 'mini-subunits'.

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