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THE BIOGENESIS OF MITOCHONDRIA

Transcriptional, Translational and Genetic Aspects

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FINE STRUCTURE OF MITOCHONDRIAL RIBOSOMES OF LOCUST FLIGHT MUSCLE

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From the mitochondria of *Locusta mirgatoria* thoracic muscle, ribosomes were obtained which are clearly distinct from their cytosolic counterparts. They exhibit a sedimentation constant of 60S, dissociate into subunits of 40S and of 25S and contain RNA molecules of 0.52 and $0.28.10^6$ (1-4). In contrast, cytosolic ribosomes display sedimentation constants of 80S and dissociate into subunits with 60S and 40S and contain RNA molecules of 1.5 and $0.7.10^6$. It was shown that also the protein parts of both ribosome types are different (2).

SECTIONS OF MUSCLE TISSUE.

In sections of muscle tissue from *Locusta Migratoria* cytoplasmic ribosomes lie between myofibrils and are densely packed around the mitochondria (Fig. 1. They have a mean diameter of 180-200 Å. In the mitochondria, corresponding particles are smaller (mean diameter about 130 Å) and less abundant. They are scattered irregularly in the matrix and show no preferential arrangement along mitochondrial membranes. The difference in the diameters between cytosolic and mitochondrial ribosomes points to a volume difference of 3:1 which agrees well with the difference of the molecular weights of the corresponding RNA-molecules. Similar dimensions for intramitochondrial ribosomal particles have also been observed for vertebrate tissues (5). After fixation in potassium permanganate or

Fig. 1. Section of the dorsal longitudinal thoracic muscle from a locust 2 days after imaginal moult. Fixation in 3 % Glutaraldehyde and 2 % osmium tetroxide; section stained with uranyl acetate and lead citrate; x 90,000. CR: cytosolic ribosomes; G: glycogen particles; MR: mitochondrial ribosomes.

digestion with RNAse cytosolic and mitochondrial ribosomes are not visible and can be discriminated by this behaviour from glycogen particles. Glycogen particles are located in the I-band of myofilaments and measure about 230 Å (Fig. 1).

ISOLATED RIBOSOMES FROM LOCUST MUSCLE.

In a previous communication (2) it was shown that undissociated mitochondrial ribosomes from *Locusta* are heavily contaminated by a protein particle, provisionally called "Mx". In order to characterize isolated mitochondrial ribosomes, this protein impurity has to be removed. This was accomplished by freezing and thawing of the mitochondrial lysate at elevated concentrations of ammonium chloride. As shown in Fig. 2 this treatment leads to a partial dissociation of the ribosomes (most of the small subunits being lost during sedimentation of the particles). Parallel to the dissociation, however, the amount of the protein contamination in the residual monosomes is diminished. This is apparent from the decreased absorbancy at 280 nm.

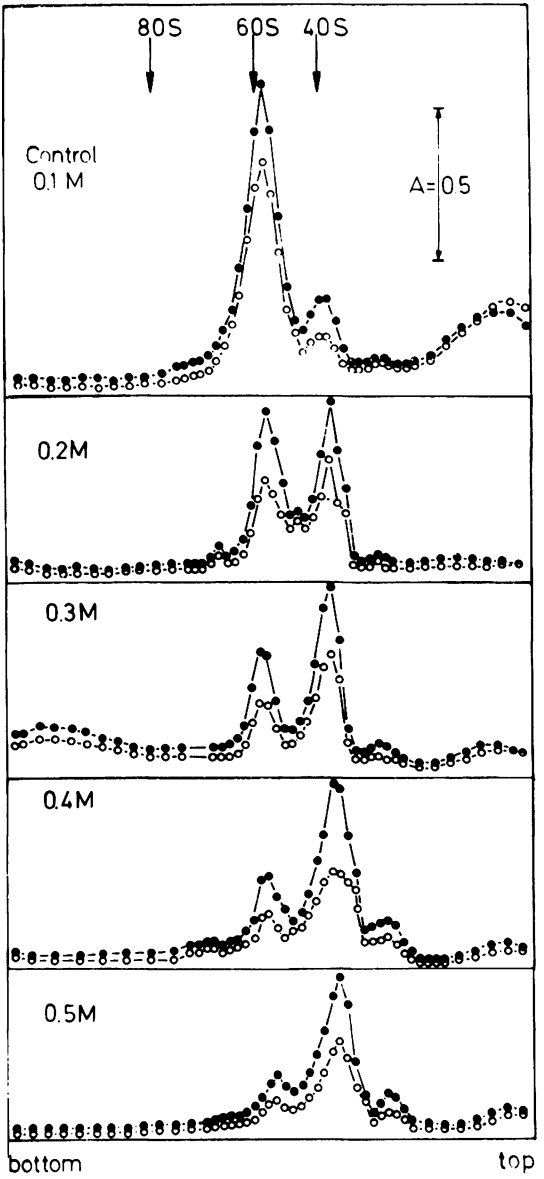

Fig. 2. Gradient profiles of mitochondrial ribosomes from Locusta migratoria after freezing and thawing the mitochondrial lysate in different concentrations of ammonium chloride. Preparation of ribosomes and gradient centrifugation was performed as described (2) except that the molarity of ammonium chloride in the mitochondrial lysate was changed as indicated in the graphs and that the lysate was frozen for 12-18 h at -80°C.

At a concentration of 0.3 M ammonium chloride contamination with "Mx" is neglegible and on the other hand enough undissociated mitochondrial ribosomes are left to perform electron microscopic studies.

Section of cytoplasmic ribosomal pellets and of pellets of purified mitochondrial ribosomes show a homogeneous distribution of particles without contamination by other cell constituents (Fig. 3A and B). In

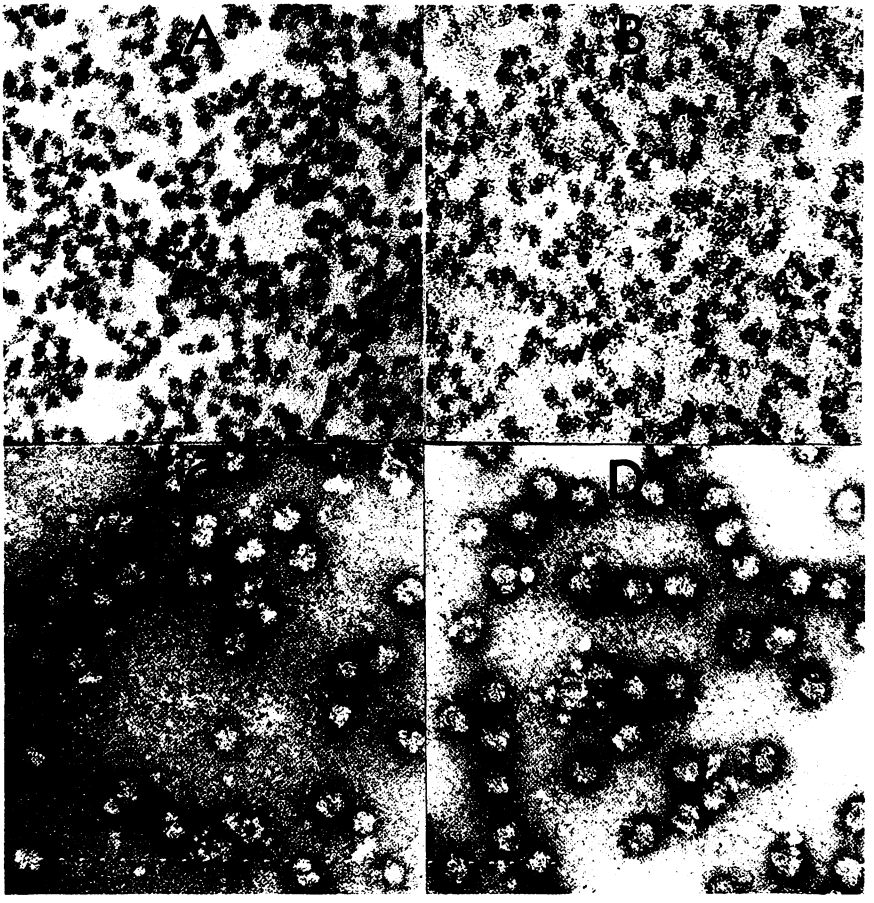

Fig. 3. Fields of positively and negatively stained mitochondrial and cytosolic ribosomes from Locusta thoracic muscle. A and B: positively stained sections of ribosomal pellets. Fixation and staining as in Fig. 1; x 120,000. A: purified mitochondrial ribosomes; B: cytosolic ribosomes. C and D: unfixed ribosomal particles after negative staining; x 150,000. C: mitochondrial ribosomes collected from the 60S region of a sucrose gradient as shown in Fig. 2 (0.3 M); D: cytosolic 80S ribosomes.

these sections cytosolic ribosomes exhibit a mean diameter of 200 Å, whereas mitochondrial ribosomes measure about 180 Å.

For negative staining of undissociated mitochondrial ribosomes, the "Mx"-free 60S peak of a sucrose gradient containing mitochondrial ribosomes prepared by freezing in 0.3 M ammonium chloride, is pooled and dialysed against a buffer containing 1 M methanol (6) 2 mM Mg^{2+} and 30 mM Tris; pH 7.6. Cytosolic ribosomes are collected from the 80S peak of a corresponding sucrose gradient and processed in the same way. The optical density of the preparations was adjusted to 0.2-0.4 A_{260}-units/ml and the negative staining was performed according to Nonomura *et al.* (7).

In Fig. 3C and D fields of mitochondrial and cytosolic ribosomes are shown side by side. Both are homogenous preparations containing particles of homogenous size. Frequently in both types of particles the cleft can be seen which separates large and small subunit. More similarities become evident if the particles are properly orientated. For comparison we have selected (Fig. 4) pictures which correspond to the pictures which have been described by Nonomura *et al.* (7) as "frontal view" and "lateral view" in the case of rat liver cytosolic ribosomes. In the "frontal view" the ribosomes show an oval outline, the small subunit extending along the whole width of the ribosome. Frequently in this profile a dense spot is to be observed off center on the cleft between small and large subunit (Fig. 4A). "Lateral views" (Fig. 4B) are more rarely seen, especially in the case of mitochondrial ribosomes from *Locusta*. They are represented by kidney shaped profiles with the small subunit sitting asymmetrically and seen from their small end. Other profiles are present in the fields, however, and unambiguous interpretation is difficult and must await the results of tilting experiments in progress.

In any case the galeries show that cytosolic and mitochondrial ribosomes from *Locusta* display in principle the same profiles. So the nature of the 60S particle from the locust flight muscle mitochondria as a

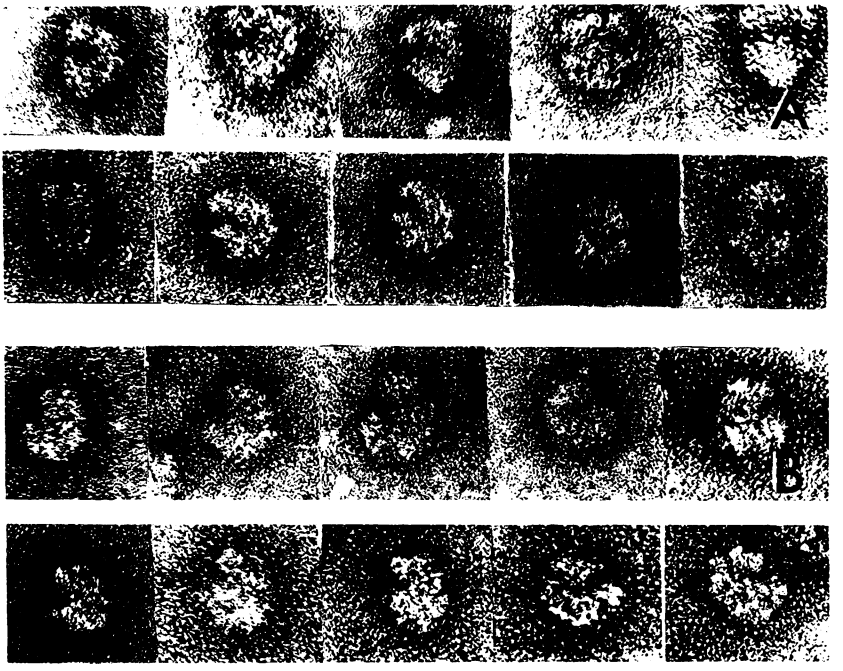

Fig. 4. Selected images of negatively stained mitochondrial and cytosolic ribosomes; x 400,000. A: frontal views, upper row cytosolic ribosomes, lower row mitochondrial ribosomes. B: lateral views, upper row cytosolic ribosomes, lower row mitochondrial ribosomes.

complete ribosome is substantiated by the negative staining experiments. This confirms and extends earlier attempts to demonstrate by negative staining the ribosomal nature of 60S particles from mitochondria of other higher organisms (8,9).

Beside obvious similarities between cytosolic and mitochondrial ribosomes from *Locusta* there are also indications of different morphological features. For example, as mentioned before, "lateral views" are more seldom observed in the case of mitochondrial ribosomes than in the preparations of cytosolic ribosomes. Also the pictures of the frontal views show differences. In no case a small subunit partition can be identified with certainty in the mitochondrial ribosomes. A small subunit partition, however, is clearly seen in cytosolic ribosomes. Most of the pictures of mitochondrial ribosomes show also the cleft between the subunits only in one half of the particle.

The dimensions of the mitochondrial ribosomes after

negative staining are smaller than the dimensions of the cytosolic ribosomes (Table I). This holds especially for the total heigth and for the width along the cleft between the subunits. Both types of ribosomes appear as slightly prolate elipsoids, the cytosolic ribosomes measuring about 300 x 260 x 240 Å, whereas the mitochondrial ribosomes measure 270 x 210 x 210 Å. It is obvious that after isolation of the ribosomes, the difference in the dimensions between cytosolic and mitochondrial ribosomes is always lesser than between extra- and intramitochondrial ribosomal particles in tissue sections. The reason for this discrepancy is not clear. It may well be due to an artifact produced by one of the preparation techniques or to a different behaviour of the mitochondrial ribosomes after isolation from the cell environment.

SUBUNITS OF MITOCHONDRIAL RIBOSOMES.

Demonstration of the isolated subunits from the mitochondrial ribosomes was possible after dissociation

TABLE I

DIMENSIONS OF NEGATIVELY STAINED RIBOSOMES FROM *LOCUSTA MIGRATORIA* THORACIC MUSCLE.
Given are the dimensions in Å units ± S.D. 50 measurements were averaged for each value, only 20 in the case of lateral views of mitochondrial ribosomes.

	Cyto-Ribosomes	Mito-Ribosomes
Frontal view		
total height	294 ± 18	271 ± 18
height of large subunit	185 ± 16	159 ± 16
height of small subunit	106 ± 15	103 ± 13
width along the cleft between the subunits	245 ± 21	210 ± 18
Lateral view		
total height	294 ± 22	268 ± 25
height of large subunit	191 ± 16	157 ± 23
height of small subunit	104 ± 16	110 ± 19
width of large subunit	257 ± 16	215 ± 28
width of small subunit	145 ± 19	140 ± 28

in 4.0 mM EDTA and subsequent fixation of the subunits according to Subramanian (10). The subunits were separated by density gradient centrifugation and the particles from the 40S peak and from the 25S peak of the gradient were collected separately and processed as described for the undissociated ribosomes. Fields

of negatively stained large and small subunits of the mitochondrial ribosomes from *Locusta* are shown side by

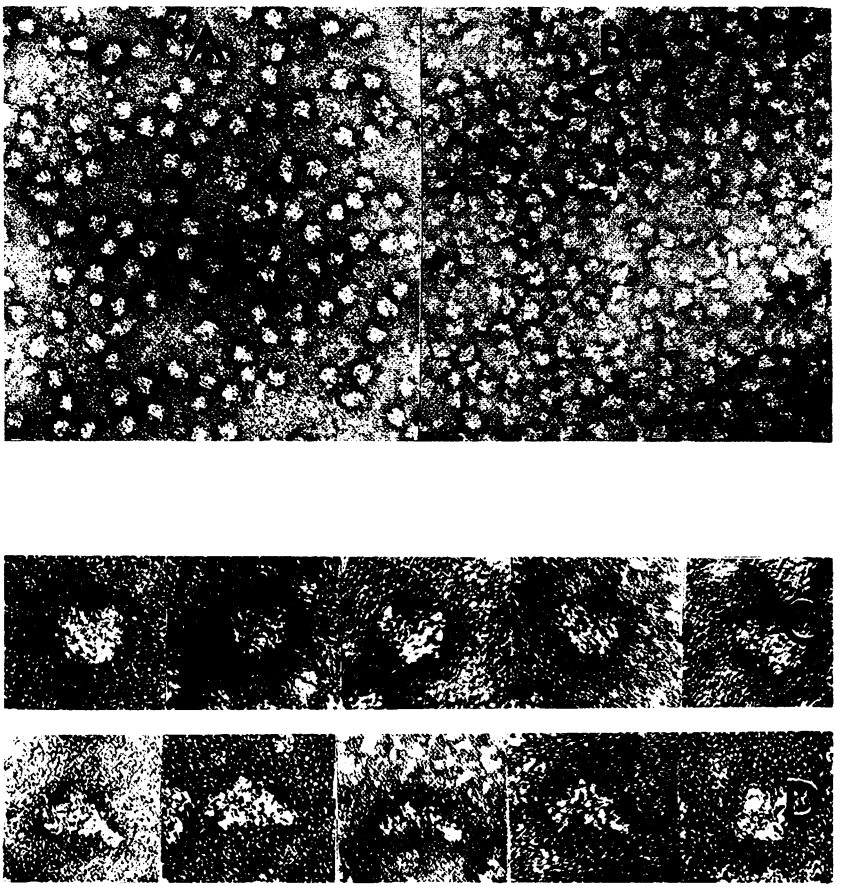

Fig. 5. Subunits of mitochondrial ribosomes from *Locusta migratoria*. The ribosomes were partly dissociated by incubation at 4° C in 4.0 mM EDTA and without magnesium. The suspension was fixed with glutaraldehyde according to (10) and was placed immidiately after fixation on a sucrose gradient. The peaks in the gradient corresponding to the particles sedimenting with 40S and 25S were collected and processed as described in the text. A: general view of a field of large (40S) subunits; x 112,000. B: general view of a field of small (25S) subunits; x 112,000. C: selected images of large subunits; x 400,000. D: selected images of small subunits; x 400,000.

side in Fig. 5A and B. Clearly each of the fields contains a homogeneous particle fraction and evidently the morphological features of the particles obtained from the 40S peak are different from the particles in the field obtained from the 25S peak. The pictures of the large subunits (Fig. 5C) coincides well with those described for ribosomes of other origin (7,11,12). They show a rounded or semicircular profile. One side of the particles seems to be flattened or concave, Frequently on this concave side a dense spot is observed, which may correspond to a shallow groove on the surface of the particle. A knoblike projection as described for other mitochondrial large subunits (11) could not be identified with certainty.

The small subunit shows elongated profiles as is to be expected from the pictures of small subunits from other ribosomal types. However, in contrast to small subunits from rat liver cytosol (7) or from *E.coli* (12,13) the profiles are not rounded and the small subunit partition is absent or at least difficult to demonstrate. Instead angular profiles prevail: tetragonal, triangular or slightly concave forms are the most abundant. In this respect the morphological features of the 25S small subunit from the 60S mitochondrial ribosome of locusts coincide possibly with the features of the 36S small subunit from the 72S mitochondrial ribosome of yeast (11).

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