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SUPPLEMENTARY PAPERS

STRUCTURE OF CYTOPLASMIC 80 S AND MITOCHONDRIAL 60 S RIBOSOMES FROM LOCUSTA MIGRATORIA

F. Miller, W. Kleinow and W. Neupert

Institut für Zellbiologie und Institut für Physiologische Chemie,
Universität München, Germany

Cytoplasmic (80 S) and mitochondrial (60 S) ribosomes have been isolated from locust flight muscle (Fig. 1A,B). Mitochondrial ribosomes were found to be contaminated by a protein particle which is not disintegrated during lysis of mitochondria with Triton X 100 (1). Purification of mitochondrial ribosomes could be achieved by lysis of mitochondria in 0.055 M $MgCl_2$. This method results in a partial dissociation of mitochondrial ribosomes (Fig. 1B).

Unfixed cytoplasmic and mitochondrial ribosome monomers were examined by electron microscopy after negative staining with uranyl acetate. Cytoplasmic ribosomes measure 294 x 245 x 257 Å; mitochondrial ribosomes show dimensions of 271 x 210 x 215 Å. From these values a volume ratio of cytoplasmic : mitochondrial ribosomes of 1,5 : 1 was estimated. Despite their different sedimentation constants mitochondrial ribosomes after negative staining show a morphology similar to cytoplasmic ribosomes. Both types of particles show bipartite profiles which correspond to the frontal and lateral views described by Nonomura et al. (2) for cytoplasmic rat liver ribosomes. In frontal views the small subunit (SS) is visible over its entire length and appears oblong; the large subunit (LS) is roughly triangular with curved sides. In cytoplasmic ribosomes (Fig.2) the subunits are separated partially or completely by a dense band; the SS is partitioned into two parts of unequal size by a faint dense line perpendicular to the dense band; where this line abuts on the horizontal band, a dense spot is found off center, either to the right or to the left side. In mitochondrial ribosomes (Fig.3) the partition of the SS is absent, the horizontal band is not visible over the entire width, but ends also in a dense spot.

The lateral views of cytoplasmic (Fig.2) and mitochondrial (Fig.3) ribosomes are similar; the SS appears roundish and is displaced either to the right or to the left side of the LS. The two enantiomorphic forms occur with equal frequency, but lateral views are rarely found in preparations of mitochondrial ribosomes.

Subunits of mitochondrial ribosomes (40 S and 25 S) were obtained by incubation either at 37°C in a magnesium-free buffer or at 4°C in a buffer containing no magnesium but 0.004 M EDTA prior to fixation in glutaraldehyde (Fig.1 C). Mitochondrial LS (40S) show profiles with one flattened or concave side, where stain sometimes accumulates in a shallow groove, and two convex sides (Fig.4). Roughly circular profiles also occur.

Mitochondrial SS (25 S) display elongated angular profiles. Often the particles are curved with one pointed and one blunted end (Fig.5). A small subunit partition could not be observed.

Our findings show that 60 S ribosomes occur not only in vertebrates (3,4) but also in mitochondria of invertebrates; thus, 60 S ribosomes may be peculiar to mitochondria of metazoa. The small sedimentation constant may be explained either by a small particle weight or by a relative large specific partial volume (5).

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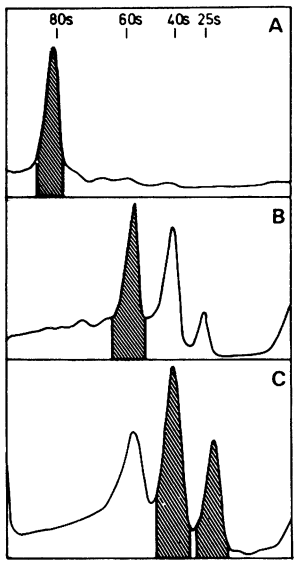

Fig. 1 Gradient profiles (absorbance at 260 nm) of cyto- and mitoribosomes from Locusta migratoria. Sucrose gradients were made as described (1) in AMT (0.1 M NH_4Cl, 0,01 M $MgCl_2$, 0.03 M Tris-HCl, ph 7.2) or AT (0.1 M NH_4Cl, 0,03 M Tris-HCl, pH 7.2) and centrifuged for 5 hrs at 41 000 rpm in a Spinco SW 41 Ti rotor. The dark areas represent fractions used for electron microscopy.
A) Cytoplasmic monoribosomes (80 S) in AMT.
B) Mitochondrial ribosomes (60 S) in AMT. The ribosomes were prepared by lysis of mitochondria in a medium containing 0,055 M $MgCl_2$; hence, they are partly dissociated.
C) Subunits (40 S and 25 S) of mitoribosomes in AT. The preparation was fixed with glutaraldehyde after incubation under dissociating conditions. Some undissociated ribosomes persist after this treatment.

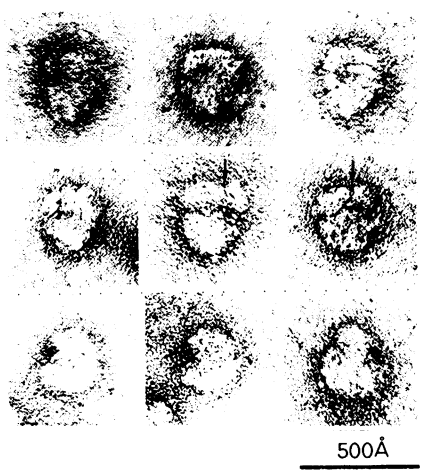

Fig. 2 Cytoplasmic ribosomes. Frontal views (top two rows) and lateral views (bottom row). Arrows mark partition in small subunit.

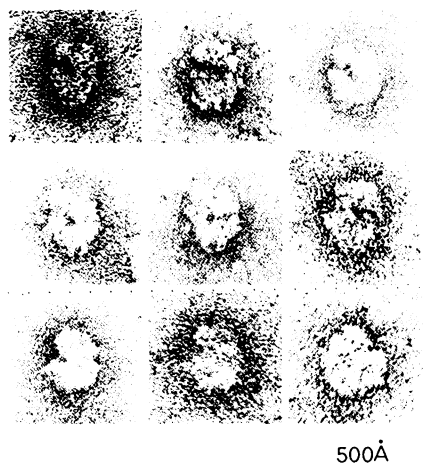

Fig. 3 Mitochondrial ribosomes. Frontal views (top two rows) and lateral views (bottom row).

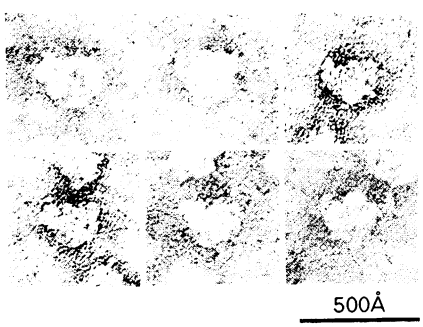

Fig. 4 Large subunits of mitochondrial ribosomes.

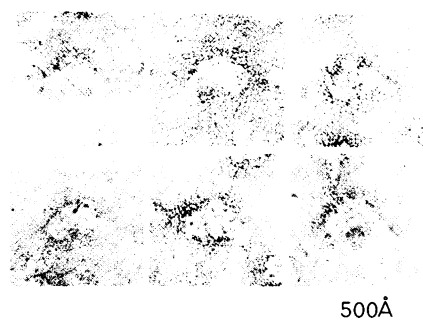

Fig. 5 Small subunits of mitochondrial ribosomes.