CORE PARTICLES AND PROTEINS FROM MITOCHONDRIAL RIBOSOMES OF YEAST

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We have described [1] the isolation procedure and some properties of a mitochondrial 72 S ribosome from *Saccharomyces cerevisiae*. This 72 S ribosome, which could be separated from cytoplasmic 80 S ribosomes, was easily dissociated into a 38 S, eucaryotic-type, small ribosomal subunit and a 50 S, procaryotic-type, large subunit. Mito-ribosomes were extracted in pure form only at high ionic strength from EDTA-washed mitochondria. Mitochondrial fractions which were not treated with EDTA contained, in addition, 80 S cytoplasmic membrane-bound ribosomes, which could less easily be dissociated than 'free' cytoplasmic ribosomes [1, 2]. These results and those of Küntzell [3] demonstrated the presence of 72–73 S ribosomes in the mitochondria of two eucaryotic microorganisms (yeast and Neurospora), these ribosomes being thus distinct both from animal and bacterial ribosomes. This finding is in contradiction with descriptions of 80 S [4–6] and 75 S [7] mito-ribosomes which are probably obtained when unsuitable preparation methods are used for mitochondria or ribosomes [1]. It seems that mito-ribosomes from several animal cells (rat liver, HeLa, *Xenopus laevis*) are smaller than those we have characterized in yeast. They sediment at 55–60 S [8–11]. Only for *Xenopus laevis* [11], have 43 and 32 S ribosomal subunits been characterized.

To further distinguish the mitochondrial ribosomes from their cytoplasmic counterparts, we have studied the extraction of their proteins and analysed them on polyacrylamide gels. There is only one report of analysis of mito-ribosomal proteins, by Küntzell [12]. In this case, completely different elution patterns from CMC columns were found for cyto- and mito-ribosomal proteins of *Neurospora crassa*. A more exhaustive study has been published for chloroplastic ribosomal proteins. In these cases, different patterns were clearly found for cyto- and chloroplastic ribosomal proteins [13–16] as well between *E. coli* and chloroplast ribosomal proteins [14]. This is contrary to the finding of nearly identical patterns for cytoplasmic ribosomes from different organs of the same plant [13] or animal [17–19]. It must be remembered, however, that chloroplast ribosomes are of the procaryotic type [16].

A detailed investigation on the mode of extraction of mito- and chloroplastic ribosomal proteins has not yet been published. It is an interesting problem to study because procaryotic ribosomes treated with high concentrations of monovalent ions show, in the presence of decreasing concentrations of magnesium, a progressive loss of proteins leading to slower sedimenting 'core' particles. On the contrary, eucaryotic ribosomes are unstable in these conditions and are dissociated into soluble proteins and precipitated ribosomal RNA. We have studied the behaviour of yeast mito-ribosomes, in presence of 2 M LiCl and 50 mM magnesium, in a way similar to previous studies made on *E. coli* ribosomes [20–24].

Fig. 1 shows the sedimentation patterns of mito-ribosomes, membrane-bound and 'free' cytoplasmic ribosomes; and of *E. coli* ribosomes, before (upper graph) and after (lower graph) incubation for 6 hr in 10 mM tris-Cl (pH 7.5), 50 mM Mg^{2+}, 2 M LiCl. Cytoplasmic ribosomes clearly give only small peaks of ribonucleoproteins and there is no evidence of 'core' particles. Membrane-bound cyto-ribosomes give two small peaks of 'core' particles, which probably arise from contaminating mito-ribosomes. *E. coli* and mito-ribosomes are both transformed into 40–41 S and 24–25 S 'core' particles, arising from the large and the small subparticles, respectively. The sedimentation coefficients were calculated by cosedimenting

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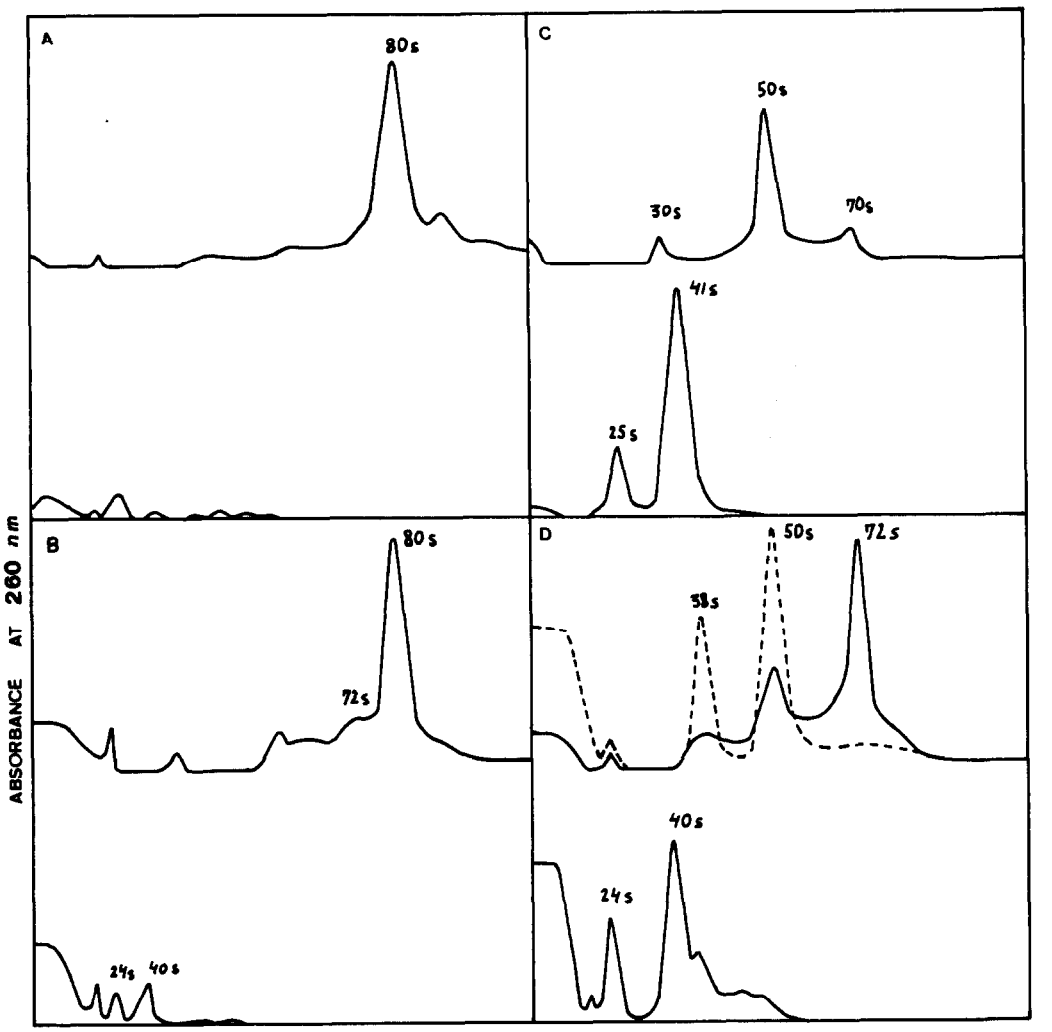

Fig. 1. Sucrose gradient analysis of 'core' particles. Preparation of ribosomes and analysis was performed as previously described [1].

(A) Upper graph: cytoplasmic 'free' ribosomes, washed with 10 mM tris-Cl (pH 7.5), 14 mM Mg^{2+}, 600 mM NH_4Cl, 1% triton, analysed in 10 mM tris-Cl (pH 7.5), 5 mM Mg^{2+}. Lower graph: same ribosomes after 6 hr incubation, at 0°, in 10 mM tris-Cl (pH 7.5), 2 M LiCl, 50 mM Mg^{2+}, analysed in the same solution.

(B) Upper graph: membrane-bound ribosomes (extracted from mitochondrial fraction not washed with EDTA) analysed in 10 mM tris-Cl (pH 7.5) 5 mM Mg^{2+}. Lower graph: same ribosomes after incubation, as in (A).

(C) Upper graph: *E. coli* ribosomes analysed in 10 mM tris-Cl (pH 7.5), 5 mM Mg^{2+}, 100 mM KCl. Lower graph: same ribosomes, after incubation, as in (A).

(D) Upper graph: (——) mito-ribosomes analysed in 10 mM tris-Cl (pH 7.5), 5 mM Mg^{2+}; (- - - -) mito-ribosomes analysed in 10 mM tris-Cl (pH 7.5), 5 mM Mg^{2+}, 100 mM KCl. Lower graph: same ribosomes, after incubation, as in (A).

E. coli ribosomal and 'core' particles in 10 mM tris-Cl (pH 7.5), 0.5 mM Mg^{2+} and, then, using the 'core' particles' S value as a standard (results not shown). The fact that 'core' particles can be produced from mito-ribosomes suggests that the mode of biogenesis of these ribosomes may be similar to that in *E. coli*, in which assembly from RNA and proteins is in two steps (review by Nomura [25]). It serves to further differen-

tiate mito- from cyto-ribosomal subunits. Preliminary experiments suggest that the scheme of transformation of bacterial ribosomes into slower sedimenting 'core' particles observed upon lowering the Mg^{2+} concentration at constant LiCl concentration also applies to mito-ribosomes (results not shown). At low Mg^{2+} concentration, however, the yield of core particles does not reach 100%, probably because of nuclease action, and this makes a detailed study difficult. Thus it appears that the eucaryotic-type S value (38 S) of the small mito-ribosomal subparticle constrasts with its 'procaryotic' transformation into a 'core' particle. The 50 S mito-subparticle exhibits procaryotic-type properties; on the one hand it is transformed into a 'core' particle, and on the other hand the sensitivity of mitochondrial proteins synthesis to chloramphenicol and erythromycin is localised on this subunit [26]. These observations and the unusual base composition of mitochondrial ribosomal RNA [27] called for further study of the mito-ribosomal proteins.

Fig. 2 shows the polyacrylamide gel patterns of mito-, *E. coli*- and cyto- 'free' and membrane-bound, ribosomal proteins. These patterns are clearly completely different from each other although some bands exhibit a similar migration velocity (not necessarily meaning identical proteins). To obtain a better resolution the analysis of proteins was extended to the comparison of the individual subunits. The implications of the above findings will be discussed later.

The comparison of the cytoplasmic 'free' and membrane-bound total ribosomal proteins reveals that the two patterns are very similar but at least two different protein bands of the 'free' ribosomes are not found in the membrane-bound particles. In addition three bands from the membrane-bound proteins (among which one possibly arising from contaminating mito-ribosomes) are not found in their 'soluble' counterparts. These results indicate that the different dissociability [1, 2] and membrane-affinity of the two types of ribosomes could, at least partly, be explained by a different protein composition. Differences in ribosomal protein patterns were also very recently found between 'free' and membrane-bound ribosomes from chick embryo cells by Fridlender and Wettstein [28]. These observations do not rule out the possibility that the specific role played by membrane-bound ribosomes in animal cells [29], which seem to produce only some specific proteins for export, could result from a dual specificity at the level of the ribosome and at that of initiation factors. The discovery of membrane-bound ribosomes in yeast suggests that proteins other than export-proteins are produced on them. An attractive hypothesis would be the specific production by these ribosomes of proteins for the mitochondria and peroxisomes.

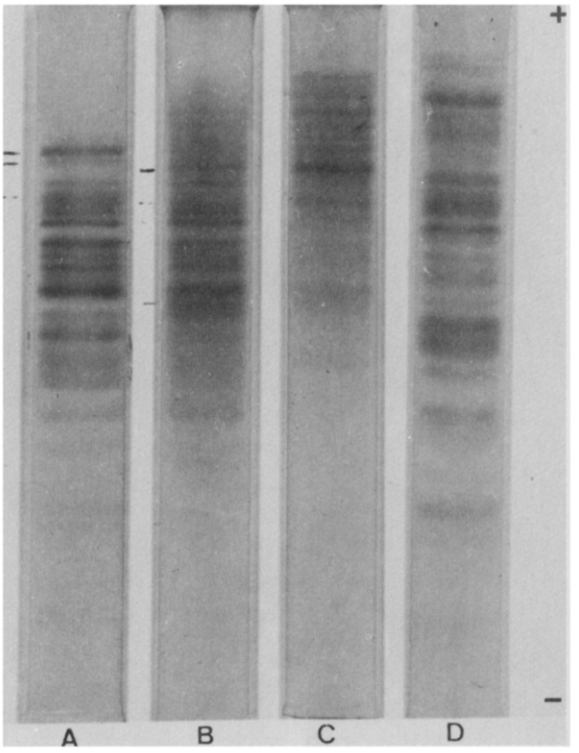

Fig. 2. Polyacrylamide gel analysis of total ribosomal proteins. Proteins were extracted from each class of ribosome s by the LiCl–urea method (Spitnik-Elson [31]). The precipitated RNA was sedimented and the supernatant protein was dialised against 7 M urea–60 mM acetic acid (pH 4.5) at 3–5°. 50–100 μg protein was layered on polyacrylamide gels prepared from the same stock solution, according to Leboy, Cox and Flaks [32]. Electrophoresis was continued until solvent front had just migrated down the gel. Distances of migration of the same bands in different tubes of gel were identical. Protein bands were stained by the method of Chrambach et al. [33].
(A) Proteins from cytoplasmic 'free' ribosomes, washed with same solution as in fig. 1A;
(B) Proteins from membrane-bound ribosomes;
(C) Proteins from mito-ribosomes;
(D) Proteins from *E. coli* ribosomes.

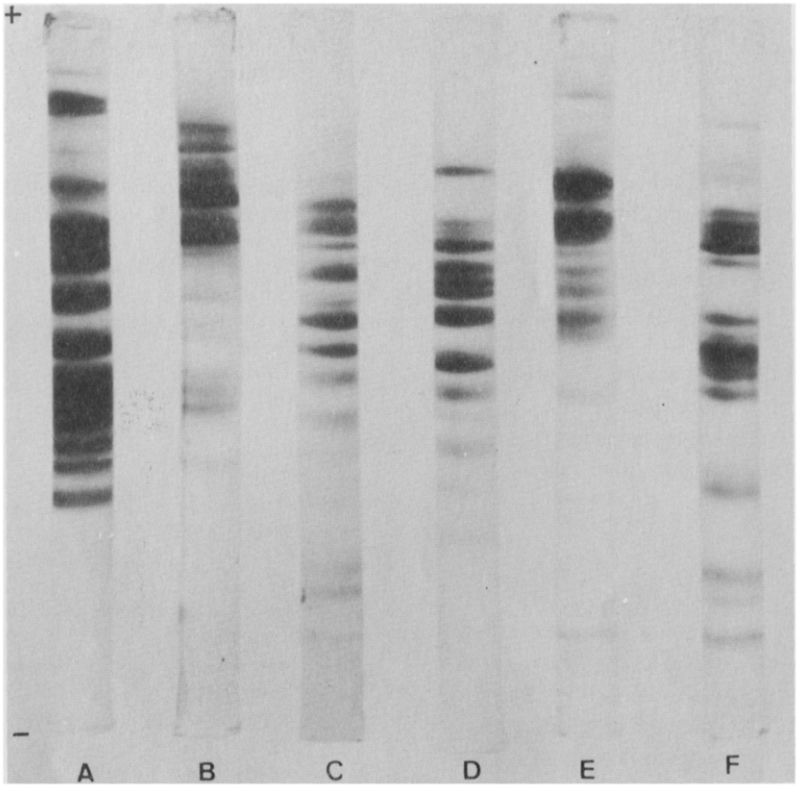

Fig. 3. Polyacrylamide gel analysis of proteins from ribosomal subunits. Ribosomes were prepared and purified as described in fig. 2. Mito- and cyto-ribosomes were dissociated on SW 27 rotor of Spinco, on sucrose gradients containing respectively 10 mM tris-Cl (pH 7.5), 5 mM Mg^{2+}, 100 mM KCl; and 10 mM tris-Cl (pH 7.5), 1 mM Mg^{2+}, 100 mM KCl. Pure subunits were collected and sedimented. Ribosomal proteins were extracted with the RNase method of Osawa, Otaka, Itoh and Fukui [34], applied and run on polyacrylamide gels as described in fig. 2.
(A) Proteins from the 30 S *E. coli* ribosomal subunit;
(B) Proteins from the 38 S mito-ribosomal subunit;
(C) Proteins from the 38 S cyto-ribosomal subunit;
(D) Proteins from the 60 S cyto-ribosomal subunit;
(E) Proteins from the 50 S mito-ribosomal subunit;
(F) Proteins from the 50 S *E. coli* ribosomal subunit.

Mito- and cyto-ribosomes were dissociated on sucrose gradients and sedimented in pure form. Fig. 3 demonstrates that there is hardly any protein in common in mito-, cyto- or *E. coli* subparticles. The proteins from the large and small subunits are also distinct. There is no greater similarity between 50 S *E. coli* and mito-protein patterns than between 38 S mito and 30 S *E. coli* patterns. It appears thus that the eucaryotic-type sedimentation coefficient of the small mito-ribosomal subunit is solely a 'macroscopic' peculiarity and is not reflected in the physical properties and protein composition of the subunit. The chloramphenicol and erythomycin sensitivity of mitochondrial protein synthesis suggeted a similar site of interaction of these 50 S bacterial subunit inhibitors with the mito-subparticle. Our finding of different ribosomal proteins does not exclude this possibility nor that mito- and *E. coli* ribosomal proteins could be exchanged in ribosome reconstruction experiments. Nomura, Traub and Beckmann [30] indeed found that only part of the protein and RNA interact to give an active ribosome; thus, different gel patterns of

proteins do not necessarily mean different interaction sites.

Despite the fact that their ribosomal RNA is coded by mitochondria DNA [27] we have found that cycloheximide inhibits the synthesis of mito- and cyto-ribosomal proteins to the same extent. There is no significant inhibition of the synthesis of mito-ribosomal proteins by chloramphenicol (results to be published elsewhere in more detail), demonstrating thus that all or nearly all mito-ribosomal proteins are synthesized on cyto-ribosomes. A similar result has been published for *Neurospora crassa* by Küntzell [12]. This last result does not resolve the problem of the genetic origin of mito-ribosomal proteins as this inhibition could come from a cytoplasmic ribosome-inhibited lecture of exported mito-messenger RNA.

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