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SIGNIFICANCE OF 80-S RIBOSOMES ASSOCIATED WITH NEUROSPORA CRASSA MITOCHONDRIA

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The mitochondrial ribosome of Neurospora crassa was first described as having a sedimentation coefficient of 73-S (subunits 50-S and 37-S) compared to 77-S for the cytoplasmic ribosome (subunits 60-S and 37-S) (1). The results of KÜNTZEL and NOLL (1) were accepted for some years. Recently however, DATEMA et al. (2) have described the mitochondrial ribosome as having an S-value of 80. They further stated that the 73-S mitoribosome of Neurospora is an artefact of the isolation procedure. It has been suggested (2) that washing mitochondria with EDTA-containing media results in the loss of essential components giving 73-S ribosomes, whereas isolation of mitochondria in the presence of magnesium and heparin yields intact ribosomes with an S-value of 80. Here we attempt to evaluate the significance of 73-S and 80-S ribosomes occuring in mitochondria isolated by different procedures.

1. Ultracentrifugation of ribosomes from mitochondria isolated in the presence and absence of magnesium ions

Mitochondria were isolated according to four different procedures as outlined in the table. Ribosomes were prepared from each preparation in an identical manner. Ribosomes from $SE_{1.0}T$ mitochondria show a prominent 73-S monomer with 50-S and 37-S subunits and a small amount of dimers (103-S) and trimers (fig. 1). In the case of SAMT ribosomes obtained from mitochondria isolated as described by DATEMA et al. (2), the gradient in addition to the 73-S component obtained in

TABLE

Designition of mitochondrial preparations	$SE_{1.0}T$	SAMT	SME	$SE_{0.1}T$
Isolation medium	A	B	B	C
Wash medium	2 x A	3 x B	2 x A	2 x C

A: 0.44 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.6; B: 0.44 M sucrose, 100 mM NH_4Cl, 10 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.6; C: 0.44 M sucrose, 0,1 mM EDTA, 10 mM Tris-HCl, pH 7.6.

$SE_{1.0}T$ preparations, shows a prominent peak at 79-S with a smaller peak at 109-S (fig. 1). These additional peaks are coincident with mono- and dimers of cytoplasmic ribosomes. In the case of SME mitochondria (isolated in SAMT but twice washed

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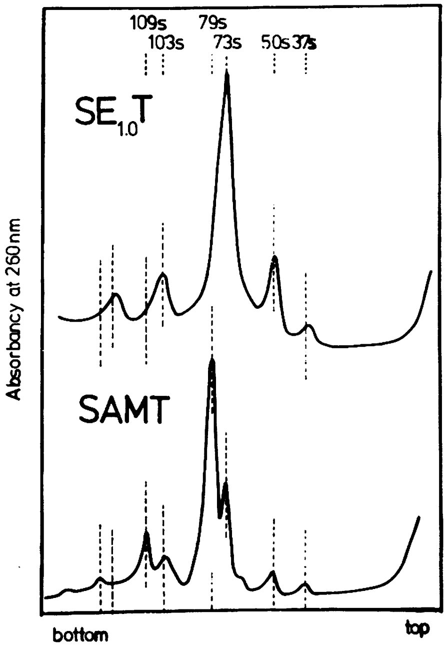
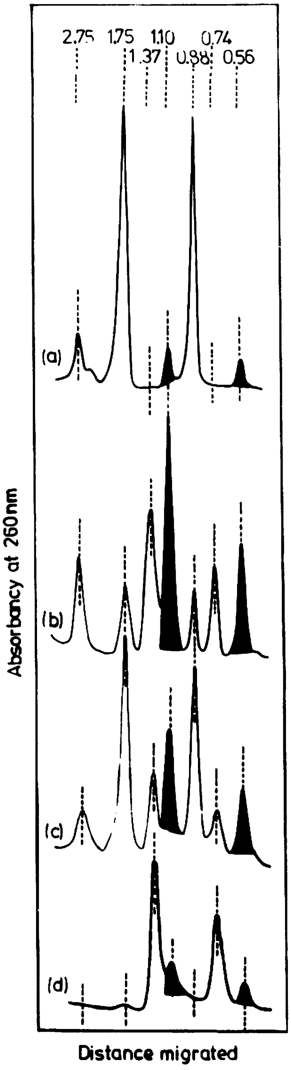
with $SE_{1.0}T$), a profile qualitatively similar to that obtained from SAMT mitochondria is obtained. The amount of subunits is however greater and the content of 73-S, dimers and trimers is lower (not shown).

These data are in agreement with those of DATEMA et al. (2). We however, interpret them in a different manner. We conclude that the 79-S and the 109-S components can be attributed to contaminating cytoplasmic ribosomes.



<u>Fig. 1</u>

Density gradient centrifugation of ribosomes isolated from mitochondria prepared in $SE_{1.0}T$ and SAMT buffer.



<u>Fig. 2</u>

Polyacrylamide gel electrophoresis of high molecular weight RNA extracted from mitochondria, which were isolated in different buffers: (a) $SE_{1.0}T$, (b) SAMT, (c) SME, (d) total cytoplasmic RNA. Apparent molecular weights were determined by comparison with E. coli ribosomal RNA co-electrophoresed with the RNA exracts. The gels were run at 25°C. Apparent molecular weights in daltons by 10^{-6} are shown at the top of the figure.

2. Ribosomal RNA from mitochondria isolated under different conditions

RNA was extracted from the different mitochondrial preparations and subjected to polyacrylamide gel electrophoresis. The $SE_{1.0}T$ preparation (fig. 2a) shows two ribosomal components with apparent molecular weights of 1.75 and 0.88 x 10^6 daltons and a small DNA peak. These components are clearly distinguishable from the cytoplasmic RNAs (apparent molecular weights 1.37 and 0.74 x 10^6 daltons; cf. fig. 2d). SAMT and SME mitochondrial preparations (fig. 2b and 2c) show profiles, which are clearly composites of $SE_{1.0}T$ profile (fig. 2a) and that of the cytoplasmic RNA (fig. 2d). The SAMT profile (fig. 2b) also shows an extremely high DNA content. The individual RNA species are clearly distinguishable by their mobilities relative to E. coli ribosomal RNA (apparent molecular weight 1.1 and 0.56 x 10^6 daltons). The data in fig. 2 firmly support the conclusion that ribosomes from mitochondria isolated in the presence of magnesium consist of a mixture of cytoplasmic and mitochondrial ribosomes.

The presence of cytoplasmic ribosomes in SAMT preparations can be further shown by electrophoretic analysis of the low molecular weight RNAs. $SE_{1.0}T$ mitochondria show only a 4-S component and heating and cooling fails to show any further low molecular weight components. RNA from cytoplasmic ribosomes on the other hand shows only a 5-S RNA and on heating and cooling shows an additional peak of 5.8-S RNA. SAMT low molecular weight RNA shows the presence of 5-S and 4-S peak and on heat treatment shows in addition a 5.8-S peak. The latter peak is characteristic of cytoplasmic ribosomes and its presence in SAMT-RNA we attribute to the presence of cytoplasmic ribosomal RNA (3).

3. Electron microscopic examination of mitochondrial preparations

In view on the heterogeneity of the ribosomes obtained from SAMT mitochondria we compared the SAMT and $SE_{1.0}T$ mitochondrial preparations by electron microscopy.

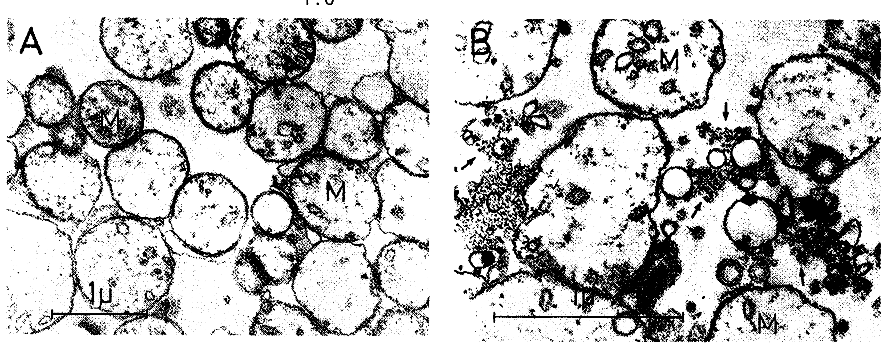


Fig. 3

Electron microscopy of mitochondrial preparations isolated in $SE_{1.0}T$ (A) and SAMT (B). Both fractions were treated with antibodies specific for cytoplasmic ribosomes.
M: mitochondria, C: tentatively identified chromatin; Arrows indicate agglutination of cytoplasmic ribosomes.

$SE_{1.0}T$ mitochondria can be seen to consist of a homogeneous population of organelles with well preserved inner and outer membranes. In contrast, the SAMT preparations are clearly heterogeneous containing much mitochondrial material, with patches of material resembling chromatin, and membrane attached ribosomes. The treatment of SAMT ribosomes with antibodies specific for cytoplasmic ribosomes led to the aggregation of much rough membrane vesicles and to the formation of large clumps of aggregated particles, which have the appearance of cytoplasmic ribosomes (fig. 3B). $SE_{1.0}T$ mitochondria treated with the same antibody show no evidence of either chromatin, rough vesicles or ribosome-like aggregates (fig. 3A). We therefore attribute the ribosomal heterogeneity of SAMT preparations (cf. fig. 1 and fig. 2b) to the presence of contaminating cytoplasmic ribosomes.

4. Effect of heparin on ribosomes from different mitochondrial preparations

DATEMA et al. (2) have stated that the isolation of the mitochondrial 80-S ribosomes requires the presence of magnesium ions and heparin during the lysis of the mitochondria to protect the ribosomes from degradation to the 73-S condition. The effect of heparin in the isolation media for ribosomes is shown in fig. 4

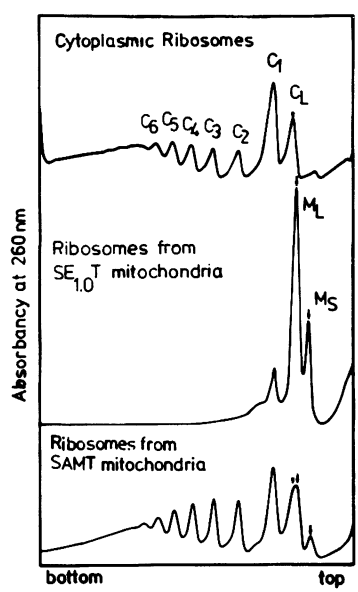


Fig. 4

Density gradient centrifugation of ribosomes isolated in the presence of heparin (0.5 mg/ml isolation medium)

Cytoplasmic ribosomes are effectively protected and only a small amount of 60-S subunit appears in heparin preparations. The effect of heparin on the $SE_{1.0}T$ preparations is quite striking. The ribosomes are almost completely dissociated and mainly subunits are obtained. In SAMT preparations the inclusion of heparin causes the disappearance of the 73-S ribosome with concomitant appearance of 50-S and 37-S subunits leaving the 80-S ribosome intact so that the gradients show only one monomer-type, viz. the cytoplasmic.

5. Immunoprecipitation of ribosomes from different mitochondrial preparations

We have previously described the preparation of specific antibodies against cytoplasmic and mitochondrial ribosomes (4). These antibodies were used to demonstrate in SAMT ribosomal preparations the presence of cytoplasmic and mitochondrial ribosomes. SAMT ribosomes were prepared from cells homogeneously labeled with ^{3}H-leucine. The ribosomes were subjected to density gradient centrifugation. The gradient was divided into 30 fractions and total radioactivity was measured (fig. 5A). Each fraction was then divided into two equal portions. One portion was treated with antibodies against cytoplasmic ribosomes and cold carrier cytoplasmic ribosomes, the other with antibodies against mitochondrial ribosomes and cold carrier 73-S ribosomes. Radioactivities in the precipitates and in the supernatants (fig. 5B and 5C) were dtermined. The antibody against the cytoribosomes completely precipitates the 79-S monomer and the 109-S dimer and leaves the 73-S monomer, the 103-S dimer and the 50-S subunit in the supernatant. Conversely, the antibody against mitochondrial 73-S ribosomes precipitates the 73-S monomer, the 103-S dimer and the 50-S subunit, leaving the 79-S particle in the supernatant.

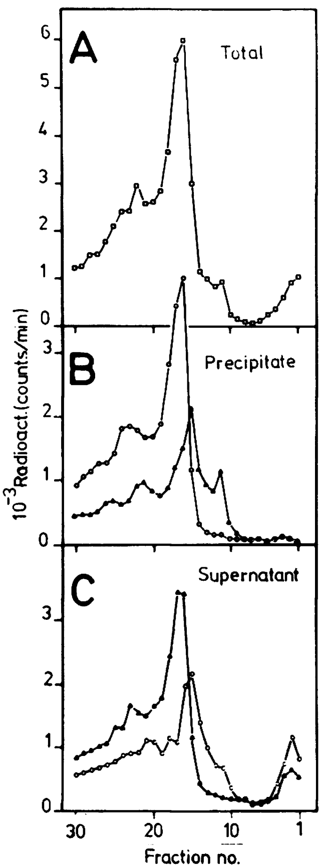


Fig. 5

Immunoprecipitation of mitochondrial and cytoplasmic ribosomes from density gradient fractions of ribosomes isolated from mitochondria prepared in SAMT buffer.

—o——o— : Radioactivity precipitated with antiserum against cytoplasmic ribosomes;

—▲——▲— : Radioactivity precipitated with antiserum against mitochondrial ribosomes.

6. Functional properties of ribosomes of mitochondria prepared in the presence and absence of magnesium ions

We have shown in an earlier publication that mitochondria prepared in $SE_{1.0}T$ under appropriate conditions are able to form subunits 1, 2 and 3 of cytochrome oxidase (5). This shows clearly that the ribosomes in $SE_{1.0}T$ mitochondria are capable of carrying out the synthesis of defined mitochondrial proteins. Since only one type of monomer is found in the $SE_{1.0}T$ mitochondria, viz. the 73-S, we conclude that this is the functional mitochondrial monomer. The isolated 73-S ribosome is able to react with puromycin and to release the nascent chains. In the absence of a crude preparation of G-factor, which may also contain a dissociation factor, after the release the ribosomes remain as 73-S monomer, in its presence they fall apart into 50-S and 37-S subunits.

Conclusions

The data presented clearly show that mitochondrial preparations obtained in the presence of magnesium contain both cytoplasmic and mitochondrial ribosomes. In our view, as first suggested by KÜNTZEL and NOLL (1), the 73-S ribosome is the real functional mitochondrial ribosome.

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