MITOCHONDRIAL RIBOSOMAL SUBUNITS FROM *SACCHAROMYCES CEREVISIAE* RHO⁻ MUTANTS

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1. Introduction

The mitochondrial DNA of *Saccharomyces cerevisiae* codes for one copy each of 23 S and 16 S ribosomal RNA (rRNA) [1,2]. These RNA molecules are components of the 50 S and 37 S mitochondrial ribosomal (mitoribosomal) subunits, respectively. The two rRNA genes are far distant from each other (about two fifths of the length of the genome) [3–5]. It is possible to delete either one of these genes by rho⁻ mutations [2–4]. We have recently shown that some rho⁻ mutants possess only the 23 S rRNA gene and transcribe it while others possess and transcribe only the 16 S rRNA gene [3,6]. The rho⁻ mutants are fully viable, since in the absence of mitochondrial functions, fermentation ensures the energy supply. Mitochondrial rRNA-less mutants offer a possibility to examine some problems of regulation of ribosome synthesis. In bacterial systems such mutants are obviously lethal. When 23 S rRNA or 16 S rRNA is present, is the 50 S or 37 S mitoribosomal subunits detectable? Available data [7] suggest that most of the mitoribosomal proteins (r-proteins) are synthesized on cytoplasmic ribosomes using nuclear messages. However, it is not known whether there is any contribution of the mitochondrial protein synthesizing system in the production of these proteins.

In this paper we report experiments indicating the presence of mitoribosomal subunits in rho⁻ mutants of defined genetic structure.

2. Materials and methods

2.1. *Preparation of mitoribosomal subunits*

The strains used and their mitochondrial genotypes are the following: IL8-8C (rho⁺) [6], D21 and F11 (rho⁻ C-E) [6], O_IP1 and O_IP2 (rho⁻ P-O_I) [4]. The rho⁺ and rho⁻ strains were grown in a medium containing 1% Yeast Extract (Difco), 1% Bactopeptone (Difco) and 2% D-galactose. Protoplasts were prepared and mitochondria purified as described [6]. For the rho⁺ strain, mitochondria were incubated with puromycin as described by Grivell et al. (8). Mitochondria were suspended at approximatively 10 mg protein per ml in 10 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$. Lysis was achieved by addition of 3% sodium desoxycholate (DOC) (protein to DOC ratio equal to 1.0 or to 0.5 for rho⁺ or rho⁻ strains respectively. The lysates were clarified by centrifugation at 17.5 krpm (Sorvall rotor SS34) for 20 min. The supernatant solutions were layered onto isokinetic sucrose gradients (15–27.5 w/w) made up in 500 mM NH_4Cl, 20 mM Tris–HCl (pH 7.4), 5 or 20 mM $MgCl_2$ and centrifuged in the Beckman SW41 rotor at 40 krpm for 6 h at 4°C. Gradients were fractionated while the absorbance at 260 nm was monitored with a Gilford model density gradient scanner (or with an ISCO model).

2.2. *Analysis of mitochondrial RNA*

Total mitochondrial RNA was extracted from purified mitochondria and analyzed by acrylamide gel electrophoresis as described [6].

3. Results

3.1. *Large mitoribosomal subunit*

We have shown [6] that the rho⁻ mutants D21 and F11 have kept the C-E region of the mitochondrial genome. The 23 S rRNA gene is retained but the 16 S rRNA gene is lost. Mitochondrial RNA was extracted by phenol from these mutants and analyzed by electrophoresis. Figure 1 shows that the 23 S rRNA gene is transcribed normally.

In order to know in what form this 23 S rRNA is present in the mutant mitochondria, the D21 mitochondria were lysed and the lysate centrifuged through a sucrose gradient in the presence of 5 mM $MgCl_2$ (fig.2). We find a material which sediments exactly like the rho⁺ 50 S mitoribosomal subunit. The F11 mutant gives a similar result (not shown). So, the majority if not all of the 50 S r-proteins seems to be synthesized and transferred into the mitochondria to form apparently normal ribosomal subunits. This synthesis requires neither the mitochondrial translation machinery nor the presence of the 37 S subunit. In other words, this synthesis is not induced, via the nucleus, by a protein translated on mitochondrial

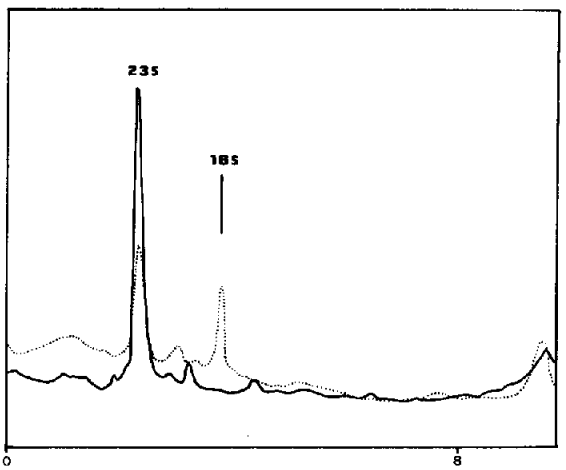

Fig.1. Electrophoretic pattern of mitochondrial RNA from D21 rho⁻ (solid line) and IL8-8C rho⁺ (dotted line). One reference tube with RNA isolated from rho⁺ mitochondria was run in parallel with the rho⁻ RNA. Electrophoresis was performed in 2.4% acrylamide gels (length 10 cm, diam. 0.6 cm) for 3–4 h at room temperature. The electrophoretic flow was from left to right. Ordinate: absorbance at 260 nm, abscissa: distance migrated in cm. The 16 S mitochondrial rRNA position is indicated by a solid line.

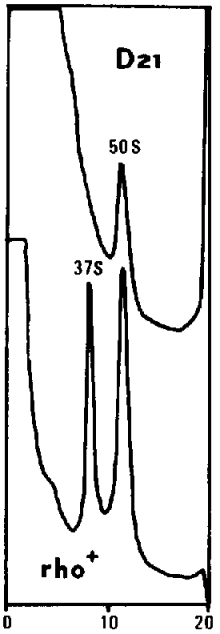

Fig.2. Separation of mitochondrial ribosomal subunits from D21 rho⁻ and IL8-8C rho⁺ by sucrose gradient centrifugation in a buffer containing 5 mM $MgCl_2$. Rho⁺ ribosomes were centrifuged in parallel. The direction of sedimentation was from left to right. Ordinate: absorbance at 260 nm, abscissa: fraction number.

ribosomes. The existence of a mitochondrial RNA inducer remains a possibility since transcription still occurs in rho⁻ mitochondria. The presence of r-proteins might explain why the unused 23 S rRNA is not degraded in rho⁻ mitochondria.

After centrifugation, the D21 mitochondrial lysate shows, in addition to the 50 S subunit, a shoulder of a smaller component (fig.2 upper part). By increasing the concentration of Mg^{2+} in the gradient, this component becomes more apparent (fig.3). This particle may be interpreted according to two hypotheses. First, it is degraded material from 50 S subunits, EDTA used during the preparation of mitochondria may break down the 50 S particles into smaller components (the mitochondrial membranes of rho⁻ mutants are more fragile than those of rho⁺). Second, the particle may represent an incomplete ribosomal subunit in which some r-proteins are missing, since all 50 S r-proteins may not be produced in the same quantity.

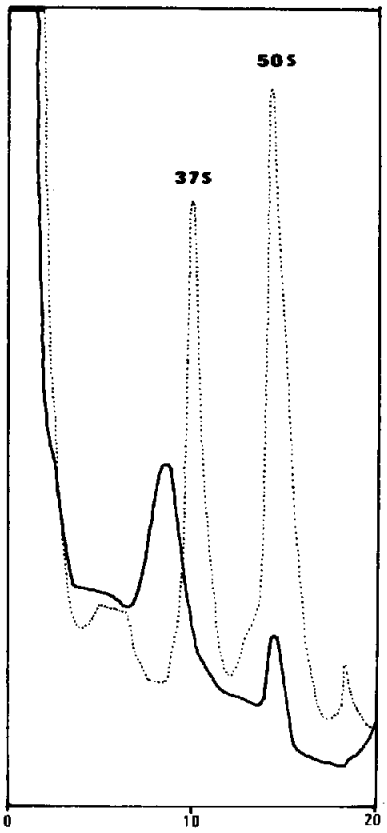

Fig.3. Separation of mitochondrial ribosomal subunits from D21 rho$^-$ (solid line) and IL8-8C rho$^+$ (dotted line) by sucrose centrifugation in a buffer containing 20 mM $MgCl_2$. Rho$^+$ ribosomes were centrifuged in parallel.

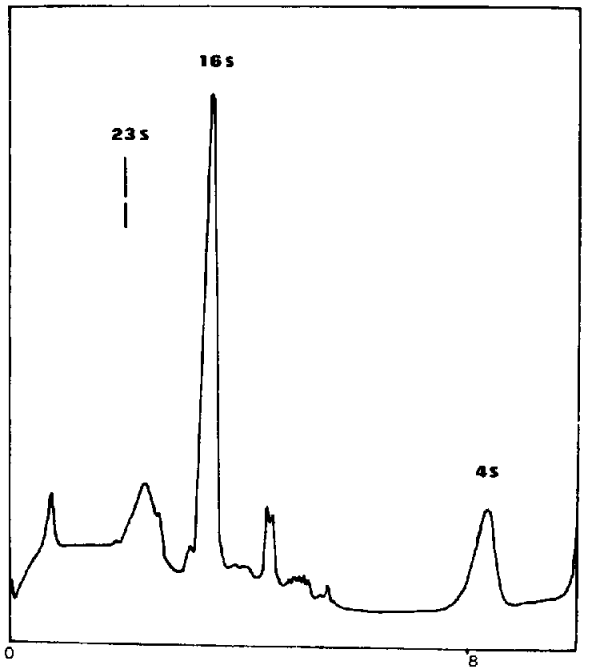

Fig.4. Electrophoretic pattern of mitochondrial RNA of the O_IP1 rho$^-$.

3.2. *Small mitoribosomal subunit*

The two rho$^-$ mutants O_IP1 and O_IP2 have kept the O_I-P region of the genome. They have retained the 16 S rRNA gene and lost the 23 S rRNA gene [3,4]. The 16 S rRNA is appreciably transcribed in these strains (fig.4). The lysate of O_IP1 mitochondria was analyzed by centrifugation through a sucrose gradient with 20 mM $MgCl_2$. The tracing of the density gradient scanner (fig.5) shows a peak with a sedimentation

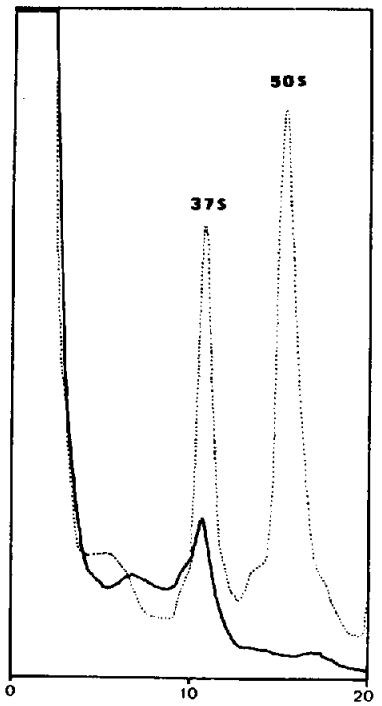

Fig.5. Separation of mitochondrial ribosomal subunits from O_IP1 rho$^-$ (solid line) and I18-8C rho$^+$ (dotted line) by sucrose gradient centrifugation in a buffer containing 20 mM $MgCl_2$. Rho$^+$ ribosomes were centrifuged in parallel.

coefficient of 37 S, identical to that of the small mitoribosomal subunit of normal yeast. The result was very similar for the O_1P2 mutant (not shown).

4. Discussion

The use of rho$^-$ mutants shows that 50 S mitoribosomal subunits are synthesized in the absence of 37 S subunits and conversely. However, we do not know whether some r-proteins are missing in these particles. When the rRNAs are detectable, the majority of r-proteins are present in the yeast rho$^-$ mitochondria but we do not know if these r-proteins are present when the rRNA is absent. Maaløe has proposed a model for the regulation of rRNA gene expression and r-protein gene expression [9,10]. The model assumes that one (or several) of the r-proteins induces rRNA synthesis. The rate of the transcription of r-protein genes would be 'passively' regulated by the availability of RNA-polymerases. We may accept Maaløe hypothesis as a plausible regulatory model for rRNA gene transcription in yeast mitochondria. Experiments will be designed to test such a hypothesis.

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