Characterization of Mitochondrial Ribosomes from Yeast

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ABSTRACT A method for the preparation of mitochondrial ribosomes from yeast is presented. Mitochondrial ribosomes differ from their cytoplasmic counterpart in nucleotide composition, electrophoretic mobility of a number of their respective ribosomal proteins, and thermal denaturation profiles. The mitochondrial and cytoplasmic ribosomes have identical sedimentation coefficients (80 S) and dissociate into 60S and 40S subunits. These findings demonstrate that, in yeast, mitochondrial ribosomes are distinct from cytoplasmic ribosomes.

Nuclear and cytoplasmic genes (1, 2) govern mitochondrial functions in yeast. Only a few of the gene products of mitochondrial DNA have been identified (3, 4). The presence of a protein-synthesizing system in mitochondria, like that in bacteria and apparently different from the mammalian type present in the cytoplasm (5), is suggested by the following observations. Various antibiotics, such as chloramphenicol, inhibit in vivo and in vitro only the protein synthesis in the mitochondria of yeast (6). Mutants resistant to these antibiotics show non-Mendelian or cytoplasmic inheritance (2). fMet-tRNA, the chain-initiating tRNA in bacteria (7), has been detected in the mitochondria of yeast (8). Cytoplasmic ribosomal RNA fails to form complementary hybrids with mitochondrial DNA (9), and the ribosomal-like RNA extracted from isolated mitochondrial particles hybridizes readily only with mitochondrial DNA (10). Finally, the two high molecular weight species of RNA from mitochondria each have lower sedimentation values than the corresponding RNA from cytoplasmic ribosomes (10-12).

In Neurospora crassa, mitochondrial ribosomes and their subunits have been isolated and characterized, and differences between the mitochondrial and cytoplasmic ribosomal proteins have been detected chromatographically (13). This paper describes the isolation and physical characterization of ribosomal particles from yeast mitochondria. Their sedimentation properties and their types of RNA and protein indicate that yeast mitochondrial ribosomes are distinct from those present in the cytoplasm.

MATERIALS

Chemicals were obtained from the following: glusulase, from Endo Laboratories; Renografin (N,N'-dicetyl-3,5-diamino-2,4,6-triiodobenzoate), from E. R. Squibb; galactose, from Pfannstiel Laboratories, Nonidet-West (N-P40), from Shell Oil; acrylamide, bis (N,N'-methylene bisacrylamide) and tetramethylrhodamine (TEMED), from Eastman Kodak Co.; RNase-free sucrose from Schwarz BioResearch, Inc.; toluidine blue and amido black, from Aniline Dye Co.

METHODS

Organism and culturing conditions

A wild type homothallic diploid strain of Saccharomyces cerevisiae (Y55) was grown with vigorous aeration at 30°C in a New Brunswick fermentor, with a synthetic medium containing 2% galactose as the carbon source to derepress mitochondrial synthesis (H. B. Lukins et al., manuscript in preparation). When the optical density (600 nm) of the suspension reached 8 units, the cells were chilled by the addition of crushed ice and harvested by centrifugation in a Sharples continuous-flow centrifuge. The cells (yield about 200 ml of well-packed cells) were used immediately for preparation of mitochondria.

Isolation of mitochondria

Freshly harvested yeast cells were transformed into spheroplasts, and crude mitochondrial fractions were prepared, as described by Lamb et al. (14). The mitochondrial suspension was layered on a 12–26% linear Renografin gradient containing 0.6 M sorbitol and centrifuged as described by Schatz et al. (15). Mitochondrial fractions between the densities of 1.2 and 1.15 were pooled, pelleted by centrifugation, and washed twice to remove Renografin (which absorbed UV radiation strongly). The final preparation (purified mitochondria) was free of contaminating protoplasts and nuclei. These freshly prepared mitochondria were then quick-frozen in Dry-Ice–alcohol and stored at −20°C.

Isolation of mitochondrial ribosomes

The purified mitochondria were thawed and carefully suspended by homogenization in TMK buffer (50 mM Tris-HCl (pH 7.4)–10 mM MgCl₂–25 mM KCl) containing 10 mM dithiothreitol and 0.5% Nonidet. To release the ribosomes, purified mitochondria were disrupted by slowly passing the suspension through a French press at 8000 psi. The homogenate was centrifuged in a Ti 50 rotor (Spinco L2-65B) at 30,000 × g for 30 min; the clear bright-yellow supernatant was centrifuged for 1.5 hr at 105,000 × g. The yellow pellet (crude mitochondrial ribosomes) was suspended by homogenization in TMK buffer and subjected to centrifugation in a 10–40% linear sucrose gradient in a SW 25.1 (Spinco) rotor at 23,000 rpm for 8 hr at 4°C. The primary monosome peak was pooled; the ribosomes were collected by centrifugation and recentrifuged on a similar sucrose gradient. The pooled ribosomal material was then washed twice with buffer. The final clear pellet (purified mitochondrial ribosomes) was quick-frozen and stored at −80°C in a Revco freezer until further use. Identical procedures were used to isolate cytoplasmic
Mitochondrial Ribosomes from Yeast

Ribosomes from the 105,000 × g pellet prepared from glucose-grown cells.

Ribosomal proteins
Pure mitochondrial and cytoplasmic ribosomes were washed twice with 0.5 M NH₄Cl-0.1 M MgCl₂-TK buffer. Ribosomal proteins were prepared from both preparations and subjected to acrylamide gel electrophoresis according to the method of Gesteland and Staehelin (16) after dialysis of ribosomal proteins against 4 M urea and TMK buffer containing 10 mM mercaptoethanol.

Ribosomal RNA (rRNA)
Purified cytoplasmic and mitochondrial ribosomes were treated with LiCl (17). The rRNA fraction was extracted twice with phenol and several times with ether, and the rRNA was precipitated with 70% ethanol.

Mitochondrial RNA
Mitochondrial RNA was extracted with 2% sodium dodecyl sulfate in TM buffer (no KCl) and deproteinized by phenol. This RNA was electrophoresed according to the method of Peacock and Dingman (18). Ribosomal RNA was removed from the acrylamide gels by electrophoresis and analyzed for base composition.

Chemical determinations
Protein and RNA content of the ribosomal fractions were determined by the method of Lowry et al. (19) and by the orcinol (20) method, respectively. Bovine serum albumin and commercial yeast RNA were used as standards.

RESULTS
As mitochondrial ribosomes are purified the RNA:protein ratio rises appreciably and approaches that of purified cytoplasmic ribosomes. The initial mitochondrial fractions contained 19 μg of RNA per mg of proteins. The first crude ribosomal pellet was composed of 120 μg/ml protein. Purified ribosomes contained 900 μg/ml protein—equivalent to 47% RNA, in contrast to 50% for cytoplasmic ribosomes. The A₂₆₀/A₆₀₀ ratio of purified mitochondrial ribosomes is only slightly less (1.88) than that observed with cytoplasmic ribosomes (1.92). The adsorption spectra of purified cytoplasmic and mitochondrial ribosomes are identical and typical of ribosomal particles. The absorption maxima and minima for both are 268 and 242 nm.

Sedimentation properties of mitochondrial ribosomes
Sedimentation of the mitochondrial ribosomal fraction on a linear sucrose gradient yields a major monosome peak at approximately 80 S (Fig. 1). The absorbance at the top of the tube is due to dithiothreitol. Preliminary experiments indicated that the monosomes readily dissociate in a medium low in magnesium.

The sedimentation values of mitochondrial and cytoplasmic ribosomes were determined by analytical ultracentrifugation. Schlieren photographs show one predominant species for each. A mixture of the two types of ribosomes does not resolve into distinct sedimenting species. From these data the corrected s₂₀,w value of each was calculated as 80 S.

Subunits of mitochondrial ribosomes were formed by suspending the ribosomes according to the method of Schmitt (21) in TM buffer containing 0.3 M KCl. The sedimentation values calculated for these subunits were 60 and 40 S.

Thermal transitions of cytoplasmic and mitochondrial ribosomes
If the structure or composition of the two types of ribosomes differed, one might expect this to be reflected in their thermal transitions. In TK buffer containing 5 mM MgCl₂, both purified ribosome preparations showed a biphasic change in hyperchromicity with increasing temperature. The hyperchromic shifts and Tm values were: mitochondrial ribosomes 87°C and 52.5°C and cytoplasmic ribosomes 55% and 58°C (inset Fig. 2). The changes in hyperchromicity can be more readily seen if one compares the differential changes in absorbance with temperature (Fig. 3). Mitochondrial ribosomes have maximal hyperchromic change at 49.5°C, cytoplasmic ribosomes at 60.5°C. Lesser changes in hyperchromicity occur for mitochondrial ribosomes at 58.5°C and for cytoplasmic ribosomes at 50.5°C.

Species of RNA in mitochondrial ribosomes
Table 1 summarizes the base composition of RNA extracted from pure cytoplasmic and mitochondrial ribosomes. The G+C content of total cytoplasmic rRNA is 52.6%, in agreement with previous observations that both 28S and 18S rRNA from yeast contain approximately equal proportions of each nucleotide (9). In sharp contrast, total mitochondrial rRNA, as well as the ribosomal subunits, contain approximately equal proportions of purine and pyrimidine bases but have considerably lower G+C contents (30.2-33.3%). These findings provide, for the first time, proof that the bulk of the mitochondrial RNA previously isolated (11, 12) is ribosomal.

In addition to differences in base composition, rRNA from pure mitochondrial and cytoplasmic ribosomes differ with respect to migration in acrylamide gels (Fig. 3). Mitochondrial rRNA species migrate more slowly than their cytoplasmic rRNA counterparts in 2.5% acrylamide gels.

Comparison of ribosomal proteins
Cytoplasmic and mitochondrial ribosomes differ in their RNA species (as well as in their physical properties). To further compare these particles, we extracted the predominant protein species present in cytoplasmic and mitochondrial mono-

![Fig. 1. Centrifugation of mitochondrial ribosomes (monosomes) on sucrose gradients. Crude mitochondrial ribosomes were layered on a 10–40% linear sucrose gradient and centrifuged at 23,000 rpm for 8 hr in a Spinco SW 25.1 rotor at 4°C. 30-drop fractions were collected by siphoning from the bottom of the tube.](image-url)
The ribosomal RNA subunits were separated by gel electrophoresis. The RNA, either total or extracted from the gels, was precipitated with alcohol, dissolved in 0.05 M ammonium acetate buffer (pH 5.5) containing 10^{-3} M EDTA, and then dialyzed against 0.05 M ammonium acetate buffer for 5 hr to remove Mg^{2+} ions and EDTA. RNA was precipitated with alcohol, dissolved with NH_{4}OAc buffer, and digested with T_{1} and T_{2} RNase for 3 hr at 30°C. The digest was lyophilized overnight to remove NH_{4}OAc. The RNA digest was then dissolved in 0.1 M phosphate buffer, pH 3.5, and the relative amount of each base (with a standard error of ±3%) was determined by measuring the area under each nucleotide peak eluted at 70°C by a linear gradient of 0.1-0.3 M potassium phosphate, pH 3.5, in a Picker nucleotide analyzer.

The ribosomal RNA subunits were separated by gel electrophoresis (Fig. 4). Eighteen major bands were observed in each preparation, which is probably a minimal estimate if we remember the findings on bacterial ribosomes (22). Although most of the bands from both ribosomal preparations have similar migration rates, six of the major mitochondrial protein bands appear to have unique positions in which there are no counterpart bands from cytoplasmic ribosomal proteins.

**DISCUSSION AND CONCLUSIONS**

Based on differences in the sensitivity to antibiotics of the protein-synthesizing activity, as well as the composition and properties of bulk mitochondrial RNA, it is probable that mitochondria from yeast contain ribosomes distinct from those in the cytoplasm. Isolation, and thereby characterization, of mitochondrial ribosomes is complicated by the fact that these ribosomes comprise a very small portion (less than 5%) of the total in the cell. The characteristics of these ribosomes and compared them by polyacrylamide gel electrophoresis (Fig. 4). Eighteen major bands were observed in each preparation, which is probably a minimal estimate if we remember the findings on bacterial ribosomes (22). Although most of the bands from both ribosomal preparations have similar migration rates, six of the major mitochondrial protein bands appear to have unique positions in which there are no counterpart bands from cytoplasmic ribosomal proteins.

**TABLE 1. Base composition of ribosomal RNA**

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>C</th>
<th>A</th>
<th>U</th>
<th>G</th>
<th>G+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic</td>
<td>Total</td>
<td>26.0</td>
<td>22.6</td>
<td>24.7</td>
<td>26.6</td>
<td>52.6</td>
</tr>
<tr>
<td></td>
<td>18S</td>
<td>26.5</td>
<td>22.5</td>
<td>25.0</td>
<td>25.0</td>
<td>51.5</td>
</tr>
<tr>
<td></td>
<td>2S</td>
<td>25.9</td>
<td>24.1</td>
<td>22.1</td>
<td>27.8</td>
<td>55.7</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>Total</td>
<td>12.5</td>
<td>38.1</td>
<td>34.5</td>
<td>17.7</td>
<td>30.2</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>12.9</td>
<td>33.3</td>
<td>36.4</td>
<td>17.4</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>16.1</td>
<td>32.6</td>
<td>34.0</td>
<td>17.2</td>
<td>33.3</td>
</tr>
</tbody>
</table>

The bulk of the RNA isolated from yeast mitochondria has a value characteristic of rRNA (25 S and 15 S). Assuming these to be rRNA, we adopted the following criteria to monitor the isolation of "mitochondrial ribosomes": These ribosomes should contain RNA species analogous in (i) size and (ii) base composition to the major RNA species in mitochondria, and (iii) support bacterial-like protein synthesis. In the present paper the first two of these criteria have been met for the isolation of ribosomes from yeast mitochondria. Some of these data have been reported elsewhere (23). Organellar ribosomes differ from cytoplasmic ribosomes in number of organisms (Table 2). Our findings that mitochondrial and cytoplasmic ribosomes of yeast sediment at 80 S is in agreement with the value reported by Schmitt (21) and Vignais et al. (24). In low concentrations of Mg^{2+}, both ribosomes dissociate into 60S and 40S subunits. Schmitt (21) reported that in high KCl concentrations, 60S subunits were transformed into 50S particles. There are conflicting reports on the size of ribosomes from Neurospora mitochondria (13, 25). Chloroplast ribosomes from Chlamydomonas (26) and tobacco (27) are 70 S.

The size of organellar ribosomal RNA is as yet unclear. In sucrose gradients and ultracentrifugation studies, the mitochondrial RNA of yeast and Neurospora appear bacterial-like in size (Table 2). However, under various conditions of acrylamide gel electrophoresis, mitochondrial rRNA from yeast migrates more slowly than cytoplasmic rRNA. These differences could reflect conformational rather than molecular weight differences between the RNA species. Various factors, including the buffer used for RNA isolation and the ionic strength of the buffer used during electrophoresis, are known to affect the rate of RNA migration. Differences have been reported in the base composition, molecular weight, and thermal denaturation of cytoplasmic and mitochondrial rRNA in Aspergillus nidulans (29).

Ribosomes from mitochondria and cytoplasm clearly differ in a number of properties. First, in Neurospora (13) and in yeast (this paper), mitochondrial and cytoplasmic ribosomes have different patterns of ribosomal proteins. Second, in yeast, mitochondrial rRNA has a lower G+C content than does cytoplasmic rRNA. Finally, the melting profiles of the

**TABLE 2. Comparison of ribosomes from various organisms**

<table>
<thead>
<tr>
<th>Component</th>
<th>Yeast (21, 24)</th>
<th>Neurospora (13, 25)</th>
<th>Chlamydomonas (26)</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribosomes</td>
<td>80</td>
<td>80</td>
<td>77, 81</td>
<td>80</td>
</tr>
<tr>
<td>Large subunit*</td>
<td>60</td>
<td>50, 60</td>
<td>50</td>
<td>47</td>
</tr>
<tr>
<td>Small subunit*</td>
<td>40</td>
<td>30, 38-40</td>
<td>37</td>
<td>31</td>
</tr>
<tr>
<td>rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large RNA*</td>
<td>28</td>
<td>22-25</td>
<td>28</td>
<td>25.1</td>
</tr>
<tr>
<td>Small RNA*</td>
<td>18</td>
<td>15</td>
<td>18</td>
<td>17.6</td>
</tr>
<tr>
<td>% G+C</td>
<td>52.6</td>
<td>30.2</td>
<td>49.3-51.3</td>
<td>34-36</td>
</tr>
<tr>
<td>Antibiotic sensitivity†</td>
<td>Cyc</td>
<td>CAP</td>
<td>Cyc</td>
<td>CAP</td>
</tr>
</tbody>
</table>

* S_{40, w}.
† Cyc = cycloheximide; CAP = chloramphenicol.
The cytoplasmic inheritance have been systems. Various antibiotic-resistant to both the Mg++ requirements, systems are inhibited by cycloheximide, yeast ribosomal subunits from bacteria. Both Neurospora (13) and mitochondrial ribosomes chloroamphenicol, this is similar their stability toward Mg++. This reflects both protein-RNA changes accompanying two ribosome preparations are different. The conformational changes accompanying thermal denaturation of ribosomes reflect both protein–RNA interactions and RNA composition (28).

Although the sedimentation characteristics of mitochondrial ribosomes differ from those from bacteria, both have similar stability toward Mg++. In general, organelle ribosomes require a higher Mg++ concentration for their stability than their cytoplasmic counterpart (13, 25, 27). 50S subunits from Neurospora mitochondrial ribosomes (13) are sensitive to chloroamphenicol; this is similar to the behavior of 50S ribosomal subunits from bacteria. Both Neurospora (13) and yeast cytoplasmic cell-free protein-synthesizing systems are inhibited by cycloheximide, whereas the mitochondrial systems are sensitive to chloroamphenicol.

Studies indicate that fMet-tRNA, some of the T-factors, Mg++ requirements, and antibiotic sensitivities are common to both the bacterial and mitochondrial protein-synthesizing systems. Various antibiotic-resistant mutants that show cytoplasmic inheritance have been reported in yeast (2). With the present improvements in the isolation of mitochondrial ribosomes and factors, it is now possible to determine directly the molecular nature of the antibiotic mutants. Such experiments are in progress.

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