Identification of 12 New Yeast Mitochondrial Ribosomal Proteins
Including 6 That Have No Prokaryotic Homologues*

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Mitochondrial ribosomal proteins were studied best in yeast, where the small subunit was shown to contain about 35 proteins. Yet, genetic and biochemical studies identified only 14 proteins, half of which were predictable by sequence homology with prokaryotic ribosomal components of the small subunit. Using a described affinity purification technique and tagged versions of yeast Ykl155c and Mrp1, we isolated this mitochondrial ribosomal subunit and identified a total of 20 proteins, of which 12 are new. For a subset of the newly described ribosomal proteins, we showed that they are localized in mitochondria and are required for the respiratory competency of the yeast cells. This brings to 26 the total number of proteins described as components of the mitochondrial small ribosomal subunit. Remarkably, almost half of the previously and newly identified mitochondrial ribosomal components showed no similarity to any known ribosomal protein. Homologues could be found, however, in predicted protein sequences from Schizosaccharomyces pombe. In more distant species, putative homologues were detected for Ykl155c, which shares conserved motifs with uncharacterized proteins of higher eukaryotes including humans. Another newly identified ribosomal protein, Ygl129c, was previously shown to be a member of the DAP-3 family of mitochondrial apoptosis mediators.

In yeast mitochondria, the majority of the characterized ribosomal proteins are essential for protein synthesis (for review see Ref. 1). Homologues of about 18 of the 21 prokaryotic small ribosomal subunit proteins can be identified by similarity searches in the yeast complete genomic sequence. However, the number of ribosomal proteins is larger in the small subunit of yeast mitochondria, estimated to be 33 or 36 (2, 3).

Thus far, yeast mitochondrial ribosomal proteins have been identified either by the study of mutant strains with mitochondrial dysfunction (pet mutants; for review see Ref. 4) or by direct biochemical approaches involving isolation of mitochondria, purification of mitochondrial ribosomes, and protein separation followed by microsequencing (5). Mitochondrial ribosome purification has thus far been technically difficult, because the ribosomal proteins represent only 2–3% of the mitochondrial proteins (2). To date, these approaches have identified only a subset of the total number of mitochondrial ribosomal proteins. In yeast, the eukaryote in which most of the studies were performed, only 14 proteins of the small mitochondrial ribosomal subunit have been characterized experimentally (1, 6).

We were originally interested in the study of the yeast YKL155C gene because it was found in a two-hybrid exhaustive screen to interact with the Prp11 splicing factor (7). To test whether this protein was associated with splicing factors under physiological conditions, we used the recently described tandem affinity purification technique (8) that allows the isolation of protein complexes by two successive affinity purification steps under mild conditions. Instead of containing splicing factors, the purified complex associated with Ykl155c was found to consist of the mitochondrial small ribosomal subunit. We took advantage of this rapid and efficient affinity purification strategy to identify and characterize twelve novel proteins of the small mitochondrial ribosomal subunit.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Strains**—Strains used in this study are listed in Table I. Gene deletions were made by replacing the entire open reading frame by a TRP1 or KanMX6 cassette (9). The strains containing green fluorescent protein (GFP)** fusion proteins or TAP-tagged fusion proteins were constructed by genomic insertion of the tag together with the TRP1 marker downstream of the affected genes to obtain C-terminally tagged fusion proteins. The Tag/TRP1 markers were generated by polymerase chain reaction either from pFA6a-GFP/S65Tr-TRP1 for the GFP fusion (9) or pBS1479 for the TAP-tagged fusion proteins (8) using oligonucleotides designed according to the authors. The YGL129C-containing vector pAH028.1R was constructed by cloning a BamHI/NotI polymerase chain reaction fragment of YGL129C into a pCM190-derived plasmid (10).

**Complex Purification**—Complex purification was done essentially by the method described in detail by Rigaut et al. (8) starting with 2 liters of yeast culture. Polycrylamide gradient gel electrophoresis was done in the Tris-Tricine system (11). A gradient of 5–20% acrylamide was used in all cases. Protein bands were visualized by Coomassie Blue G-250 staining (12).

**Mass Spectrometry**—In-gel digestion of the proteins was performed by the protocol of Shevchenko et al. (13), using bovine trypsin (Roche Molecular Biochemicals). The generated peptides were cleaned on a reversed-phase support using Millipore ZipTip C18 or Poros R2 (Perceptive Biosystems). The mixture of peptides was analyzed by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry on a Voyager DE-STR system (Perceptive Biosystems).

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1 The abbreviations used are: GFP, green fluorescent protein; TAP, tandem affinity purification; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; MALDI-TOF, matrix-assisted laser desorption ionization time of flight.

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**Table I**

| Strain name | Genotype | Source |
|-------------|----------|--------|
| MGD353–13D  | MATa, trpl–289, ura3–52, ade2, leu2–3, 112, arg4 | B. Séraphin |
| W303–1A     | MATa, Δtrpl, ura3–1, ade2–1, leu2–3, 112, his3–11, 15 | (27) |
| BMA64       | MATa/MATα, Δtrpl1/Δtrpl1, ura3–1/ura3–1, ade2–1/ade2–1, leu2–3, 112/leu2–3, 112, his3–11, 15/ his3–11, 15 | F. Lacroute |
| LMA31       | BMA64 containing YKL155C/YKL155C ΔKanMX6 | This study |
| LMA21-GFP   | W303 containing YKL155C-GFP/HIS3 | This study |
| LMA19       | MGD353–13D containing YKL155C-TAP/TRP1 | This study |
| LMA119      | MGD353–13D containing MRPL-TAP/TRP1 | This study |
| LMA132      | MGD353–13D containing YLR036C-GFP/TRP1 | This study |
| LMA133      | MGD353–13D containing YGR215W-GFP/TRP1 | This study |
| LMA134      | MGD353–13D containing YNR037C-GFP/TRP1 | This study |
| LMA135–3    | MGD353–13D containing ydr034c–TRP1 | This study |
| LMA136      | MGD353–13D containing YDR041W-GFP/TRP1 | This study |
| LMA137      | MGD353–13D containing YDR175C-GFP/TRP1 | This study |
| LMA139      | MGD353–13D containing YER050C-GFP/TRP1 | This study |
| LMA140      | MGD353–13D containing YJR111W-GFP/TRP1 | This study |
| FYAH018     | MATa/MATα, ura3–52/ura3–52, ΔKanMX6, YLY22/ly2–Δ202, TRP1/trp1–Δ63, HIS3/his3–Δ200, ygl129c–KanMX6/YGL129C | This study |
| FYAH018/028.1R | FYAH018 bearing pAH028.1R | This study |

**FIG. 1.** Proteins that copurify with Ykl155c are components of the small mitochondrial ribosomal subunit. **A**, the proteins that were isolated by tandem affinity purification using either a tagged version of Ykl155c (lane 1) or Mrp1 (lane 2) were separated by a Tris-Tricine 5–20% gradient gel electrophoresis. **MW**, molecular weight marker. Protein band identifications were done by mass spectrometry (see “Experimental Procedures”). **B** and **C**, RNA dot-blot analysis of the RNA that copurifies with Ykl155c (lane 1) or Mrp1 (lane 2) using 2 μg of total RNA as a control (**T**). Hybridization was done using radioactively labeled probes specific for the large subunit 21 S mitochondrial rRNA (**B**) and the small subunit 15 S mitochondrial rRNA (**C**).
The bands were excised from the gel, and the proteins were purified when using Ykl155c-TAP. The intensity of the Ykl155c band appears to be significantly lower in the Mrp1-TAP purification. It is possible that part of the tagged form of Ykl155c is not associated with the ribonucleoprotein complex, and therefore additional free protein was purified when using Ykl155c-TAP.

To search for RNA components in the purified complex, the RNAs extracted from the purified fractions were heated-denatured, spotted on a filter, and hybridized with either a 21 S (Fig. 1B) or a 15 S (Fig. 1C) yeast rRNA-specific probe. As expected, the 15 S probe for the small subunit mitochondrial rRNA hybridized specifically to the RNA associated with the Ykl155c and Mrp1 complexes, whereas the 21 S-specific probe did not.

Finally, we analyzed the cosedimentation of Ykl155c with Mrp1 and the 15 S RNA by ultracentrifugation on a 15–30% sucrose gradient under dissociating conditions (0.5 M salt). Whole yeast soluble crude extracts from the Ykl155c-TAP- and Mrp1-TAP-tagged strains were mixed and loaded on top of the gradient. After ultracentrifugation, the fractions were analyzed for their protein content by denaturing gel electrophoresis and immunoblot specific for the protein A component of the tag. In addition, the RNAs of an aliquot from each fraction were spotted on a filter and probed with the 21 S- or 15 S-specific probe. Cosedimentation of Ykl155c with Mrp1 and the 15 S mitochondrial rRNA is shown in Fig. 3.

In addition to the proteins previously described as components of the mitochondrial small ribosomal subunit, we found 12 proteins that had not been previously biochemically characterized. Only 6 of these 12 proteins had clear known ribosomal homologues (Table II).

The nomenclature of the mitochondrial ribosomal proteins is more as variable as the methods employed for their characterization, but MRP (for mitochondrial ribosomal protein) is the most frequently used. However, this name does not allow one to discriminate between small and large subunit proteins and thus may be misleading. For example, Mrp2 is not the homologue of the prokaryotic S2 ribosomal proteins but belongs to the S14p family. Accordingly, we preferred to designate the newly identified genes RSMxx genes, where RSM stands for ribosomal small subunit of mitochondria, and xx is the number of the corresponding prokaryotic protein family (see Table II). The genes that encode proteins that are not significantly similar to prokaryotic proteins were named with the same prefix RSM followed by a number that begins with 22, because there are 21 prokaryotic small ribosomal subunit protein families. Thus, the proposed name of the YKL155c gene is RSM22.
A large amount of functional data was generated in the course of genetic screenings and functional genomic studies in yeast. Hence, for a number of the identified proteins, some cellular or functional data were already available in public data bases. In addition, for some of the less well characterized proteins, we analyzed the cellular localization of the GFP C-terminal tagged proteins and the respiratory competency of the corresponding homologues Yjr113c and Ydr041w. Mammalian homologues of S7 (19) and S10 (20) were also recently described as components of the bovine small mitochondrial ribosomal subunit. As expected, the fluorescently labeled yeast proteins were not previously experimentally identified as components of the yeast mitochondrial ribosome.

### Table II

Summary of small mitochondrial ribosomal subunit proteins

| Systematic name<sup>a</sup> | Gene name | Prokaryotic family | First direct biochemical identification | Apparent molecular mass<sup>b</sup> | Comments |
|-----------------------------|-----------|--------------------|----------------------------------------|-------------------------------------|----------|
| Potential yeast homologues of known prokaryotic small ribosomal subunit proteins | YNL137c | NAM9 | S4p (28) | Gly<sup>c</sup> (29) | |
| YHL004w | MRP4 | S2p (30) | Gly<sup>c</sup> (30) | 45 | |
| Q0140 | VARI | S3p (31) | Mitochondrially encoded, Gly<sup>c</sup> (32) (33) | 42 | Mitochondrial ribosomal mRNA expression profile (35) |
| YBR251w | MRPS5 | S5p (34) | This study | 37 | Gly<sup>c</sup> (35) |
| YDR337w | MRPS28 | S15p (23) | Gly<sup>c</sup> (23) | 31 | |
| YBR146w | MRPS9 | S9p | This study | 31 | Gly<sup>c</sup> (36) |
| YDR113c | RSM7 | S7p | Mitochondrial localization (this study) | 24 | |
| YDR041w | RSM10 | S10p | Mitochondrial localization (this study) | 21 | |
| YER050c | RSM18 | S18p | This study | 16 | Gly<sup>c</sup> (37) |
| YPR166c | MRP2 | S14p (38) | Gly<sup>c</sup> (38) | 13 | Mitochondrial localization; Gly<sup>c</sup>d (this study) |
| YNR037c | RSM19 | S19p | This study | 8.3 | Mitochondrial localization, Gly<sup>c</sup> (this study) |
| Yeast mitochondrial small subunit proteins with no known ribosomal homologues | YKL155c | RSM22 | None | This study | 68 | Mitochondrial localization, Gly<sup>c</sup> (this study), (37) |
| YGL129c | RSM23 | None | This study | 46 | Mitochondrial localization, Gly<sup>c</sup> (this study), (39); DAP-3 family member (24) |
| YDR175c | RSM24 | None | This study | 41 | Mitochondrial localization (this study), Gly<sup>c</sup> (35), mitochondrial ribosome mRNA expression profile (37) |
| YOR158w | PET123 | None (40) | Gly<sup>c</sup> (41) | 40 | |
| YPL118w | MRP51 | None (6) | Mitochondrial localization, Gly<sup>c</sup> (6) | 39 | |
| YDR347w | MRP1 | None (42) | Mitochondrial localization, Gly<sup>c</sup> (43) | 37 | |
| YIL093c | RSM25 | None | This study | 35 | Mitochondrial localization, Gly<sup>c</sup> (this study) |
| YJR101w | RSM26 | None | This study | 29 | Similar to Mrp1; Gly<sup>c</sup> (44) |
| YGR215w | RSM27 | None | This study | 15 | Mitochondrial localization (this study) |

### Other known or predictable small subunit mitochondrial ribosomal proteins

| Systematic name<sup>a</sup> | Gene name | Prokaryotic family | First direct biochemical identification | Predicted molecular mass<sup>b</sup> | Comments |
|-----------------------------|-----------|--------------------|----------------------------------------|-------------------------------------|----------|
| Potential yeast homologues of known prokaryotic small ribosomal subunit proteins | YNL306w | MRPS38 | S11p (5) | Deletion is lethal (45) |
| YMR118c | None | S17p | None | 22 | Gly<sup>c</sup> (37); mitochondrial ribosome mRNA expression profile (35) |
| YBL090w | MRP21 | S21p (6) | Gly<sup>c</sup> (6) | 20 | |
| YMR158w | None | S8p | None | 18 | |
| YNR036c | None | S12p | None | 17 | |
| YKL005c | MRP17 | S6p (46) | Mitochondrial localization, Gly<sup>c</sup> (46) | 17 | Deletion slows growth on glucose and glycerol; mitochondrial localization (47) |
| YNL081c | None | S13p | None | 16 | |
| YPL013c | None | S16p | None | 14 | |
| Yeast mitochondrial small subunit proteins with no known ribosomal homologues | YHR079c | PPK1/YMS2 | None (5) | Similarity to human protein phosphatase methyl esterase (48) | 40 | |
| YGR084c | MRP13 | None (49) | Deletion has no respiratory effect (49) | 39 | |
| YDL045w-A | MRP10 | None (18) | Mitochondrial localization, Gly- (18) | 14 | |

<sup>a</sup> Systematic names are in bold and underlined when the corresponding proteins were not previously experimentally identified as components of the yeast mitochondrial ribosome.

<sup>b</sup> In each category, proteins were sorted according to their apparent molecular mass determined in this study or predicted molecular mass calculated from the complete sequence.

<sup>c</sup> Gene disruption or deletion hinders growth on glycerol as a unique carbon source (Gly-).

<sup>d</sup> GFP fusion at the C terminus hinders growth on glycerol in a haploid strain.
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Novel Proteins Not Similar to Prokaryotic Ribosomal Components Are Essential for Mitochondrial Function—Given that six newly identified ribosomal proteins have no obvious similarity with previously known ribosomal proteins, it was important to determine the cellular localization and the involvement of these proteins in mitochondrial function. The result of the cellular localization of the GFP C-terminally tagged proteins is shown in Fig. 4 and summarized in Table II. With the exception of Yjr101w, which was not analyzed, all these proteins were unambiguously localized to the mitochondria. Ykl155c, Ygl129c, Ydr175c, and Yjr101w were previously reported in the literature or public data bases to be essential for mitochondrial function (see Table II for references). We confirmed the direct involvement of Ykl155c in mitochondrial function by showing that the defective growth on glycerol resulting from the deletion of its gene could be complemented by a plasmid expressing the wild-type version of the protein (data not shown). For Yil093c, no previously reported functional data were available. We deleted the gene, which proved to be essential for growth on a medium containing glycerol as the sole carbon source (data not shown). For Yil093c, a homologue of the S19 prokaryotic ribosomal proteins. However, in a heterozygous diploid strain, we were able to detect a weak GFP signal that matches the mitochondrial marker (Fig. 4, bottom).

Affinity Purification of the Ribosomal Subunit Is Complementary to Previously Described Methods—The number of protein components of the yeast small mitochondrial ribosomal subunit was estimated by mitochondrial purification, preparative ultracentrifugation, and two-dimensional gel electrophoresis to lie between 33 (2) and 36 (3). We used an affinity purification method with tagged versions of yeast Ykl155c and Mrp1 that allowed us to identify, by mass spectrometry, 20 different proteins of this ribosomal particle. Some of the previously described components of the small subunit were not found (Table II). Our affinity-purified complex is unlikely to correspond to a specific particle, distinct in vivo from the bona fide small mitochondrial ribosomal subunit, because of the identical patterns of proteins obtained with either Ykl155c or Mrp1 (a previously described mitochondrial ribosomal protein). Thus, the discrepancies observed between the presented method and...
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Previously described techniques might result from the loss of specific proteins during the purification procedure or from our inability to identify some of the components. Conversely, at least Ykl155c, with an apparent molecular mass of 68 kDa, is conspicuously absent from the two-dimensional gels previously published for the mitochondrial small ribosomal subunit in which no proteins with an apparent mass above 60 kDa were revealed. The two approaches may thus be regarded as complementary.

Yeast and Mammalian Mitochondrial Ribosomal Proteins—An international consortium for the study of mammalian mitochondrial ribosomal proteins has been created recently (Mammalian Mitochondrial Ribosomal Consortium). A number of mammalian homologues of the prokaryotic ribosomal proteins have been identified by using the bovine ribosome as a source for the isolation and purification of the mitochondrial ribosomes (19–22). Mammalian proteins belonging to prokaryotic families S7 (19), S12 (21), and S10 and S15 (20) were described this way. We were able to identify in yeast, along with other mitochondrial homologues of prokaryotic ribosomal proteins, the corresponding proteins for the S7 (Yjr113c/Rsm7) and S10 family (Ydr041w/Rsm10) and also the previously identified S15 homologue, Mrps28 (23).

The analysis of the bovine and yeast mitochondrial ribosome shows that an important number of proteins have no similarity with known ribosomal proteins from prokaryotes. For this group of yeast proteins, significant similarities can be detected with sequences of the relatively distant fungi Schizosaccharomyces pombe. In some cases, similar proteins from other fungi may also be found, and one example is a hypothetical protein from Neurospora crassa (NCBI GenPept database gi 7899415) that is similar to the yeast Ydr175c/Rsm24 protein. Yjr101w/Rsm26 is similar to the yeast mitochondrial small ribosomal subunit protein Mrp1. Interestingly, both proteins also share similarities with proteins of the superoxide dismutase family.

No prokaryotic homologues of Ykl155c/Rsm22 may be detected, but we found significant similarities of its sequence with hypothetical proteins of yet unknown function in several higher eukaryotes, including humans (Fig. 5). It is tempting to speculate that these proteins are true homologues of the yeast Ykl155c/Rsm22 ribosomal protein and are components of the corresponding mitochondrial ribosomes.

Ygl129c/Rsm23 was found by similarity search to be a member of the DAP-3 family of apoptosis mediator proteins (24). Just like Ygl129c/Rsm23, the mouse mDAP-3 protein is localized into the mitochondrial matrix. Ygl129c/Rsm23 is the yeast sequence most similar to the DAP-3 proteins, but this similarity is weak (17% identity with the mouse mDAP-3). Nevertheless, a BLAST search using the S. pombe Ygl129c homologue sequence (SPBC29A3.15c) also finds the human hDAP-3 sequence. Moreover, mDAP-3 is able to partially complement a mitochondrial DNA loss phenotype observed in yeast strains deleted for YGL129C (24). If Ygl129c/Rsm23 is the true homologue of hDAP-3, these observations would suggest an interesting link between modulation of apoptosis and the mitochondrial protein synthesis machinery.

In conclusion, an efficient affinity purification technique allowed us to characterize novel ribosomal components and to bring to 26 the total number of proteins identified as components of the yeast mitochondrial small ribosomal subunit. These findings and the discovery of the yeast Ykl155c/Rsm22 and Ylg129c/Rsm23 as essential components of the mitochondrial ribosome and members of conserved eukaryotic protein families contribute to extending the classification of mitochondrial ribosomal proteins to three different classes. A first group, which probably exists in every eukaryotic organism, consists of proteins similar to prokaryotic ribosomal components. Another group contain proteins with no prokaryotic homologues but only conserved in related species. Finally, a third group that includes the Ykl155c and Ylg129c protein families comprises mitochondrial ribosomal proteins that are conserved across diverse eukaryotic species.

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