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Identification and comparative analysis of the large subunit mitochondrial ribosomal proteins of *Neurospora crassa*

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Abstract

The mitochondrial ribosome (mitoribosome) has highly evolved from its putative prokaryotic ancestor and varies considerably from one organism to another. To gain further insights into its structural and evolutionary characteristics, we have purified and identified individual mitochondrial ribosomal proteins (mrps) of *Neurospora crassa* by mass spectrometry and compared them with those of the budding yeast *Saccharomyces cerevisiae*. Most of the mrps of the two fungi are well conserved with each other, though the degree of conservation varies to a large extent. One of the *N. crassa* mrps was found to be homologous to yeast Mhr1p that is involved in homologous DNA recombination and genome maintenance in yeast mitochondria.

1. Introduction

The cytoplasmic ribosome is highly conserved in a variety of organisms, reflecting its essential role in the translation of genetic messages. In contrast, the mitochondrial ribosome (mitoribosome) appears to be structurally variable to a considerable extent from one organism to another. The mammalian mitoribosomes, for example, have smaller RNAs and a larger number of proteins than the bacterial counterparts [1-5]. This feature of protein-richness may be explained to indicate that the reduced size of rRNAs is compensated by some protein components that might have been recruited to mitoribosomes during the course of evolution. On the other hand, the mitoribosomes of yeast and other fungi have rRNAs the size of which is comparable to that of *E. coli*, and yet they have a larger number of proteins comparable to those in mammalian mitoribosomes. Furthermore, many of the proteins are unique in each organism and their homologues cannot be found in bacteria [6-8]. Thus, an intriguing question will be what the original functions, if any, of these proteins could have been. Indeed, some of the yeast mitoribosomal proteins (mrps) have been shown to interact with specific mRNA or translational activators of mitochondrial genes, and some others seem to be involved in various cellular functions other than mitochondrial protein synthesis as described by Gan *et al.* [8].

Another interesting aspect of mitoribosomes is that they seem to evolve much more rapidly than their cytoplasmic counterparts. Since the ribosome is an essential subcellular structure consisting of many components, changes in any one of the protein components must be well coordinated with others to maintain the speed and accuracy of translation. This would also apply to most, if not all, of mrps. Therefore, to elucidate the roles of individual mrps, it would be feasible to perform comparative analysis with several related organisms. Until now, however, the mrps of only a few mammals and the budding yeast *S. cerevisiae* have been characterized but the data of closely related organisms are not available. Therefore, to gain further insight into the functions of individual mrps and their evolution, we performed systematic analysis of the mitoribosomes of another ascomycete, *N. crassa*.

The mitoribosome of *N. crassa* contains two rRNAs that are slightly larger than their *S. cerevisiae* counterparts (http://www.rna.icmb.utexas.edu/) and appears to contain about 60 to 70 different proteins [9, 10]. So far, only a few mrp genes of *N.*

crassa have been identified through the genetic and serological analysis of the mitochondrial proteins and some others predicted from the sequence similarity based on the recent genome analysis [11].

2. Materials and Methods

2.1 Strains, media and general procedure

Wild type *N. crassa* strain C1-T10-28a [12] was used for the isolation of mitoribosomes. Growth media and genetic manipulation of *N. crassa* were essentially as described [13].

S. cerevisiae strains with S-tag fused mrps were constructed essentially as described previously [8]. Briefly, the gene for an mrp of RAY3A-D (*leu2*/*leu2, his3*/*his3, ura3*/*ura3, trp1*/*trp1*) cells that carried a pSH*Leu* plasmid containing the corresponding gene for an S-tag fusion mrp was disrupted and an α type haploid disruptant was selected after sporulation and used. Growth media, culture conditions and genetic manipulations for yeast are essentially as described [14].

2.2 Plasmid construction

PCB1003-S plasmid was constructed by inserting a 950 bp *Bam*HI fragment of plasmid pSH*Leu* [8] encoding an S-tag sequence into the *Bam*HI site of the multi-cloning site of pCB1003 [15]. The downstream *Bam*HI site was eliminated by converting it into a *Bam*HI/*Bcl*I hybrid sequence by PCR. All plasmids containing S-tagged mrp genes of *N. crassa* were constructed using PCB1003-S. DNA fragments harboring respective genes without termination codon were amplified by PCR from the genomic DNA of C1-T10-28a cells with appropriate primers to add restriction sites. They were then inserted into PCB1003-S in frame at the multi-cloning site derived from pSH*Le*u. A yeast plasmid for the expression of each of the S-tagged mrp genes was constructed starting with pSH*Le*u as described previously [8].

2.3. Purification of mitochondrial ribosomes and analysis of mrps

N. crassa mitochondria were purified from about 20 g wet-weight cells of strain C1-T10-28a grown in 600 ml of Vogel's medium at 25 ºC for 50 hours. Cells were homogenized with 50 g of quartz sand (Wako Pure Chemical, Japan) and 15 ml of Isolation buffer (0.44M sucrose, 50mM Tris-HCl pH8.0, 1 mM EDTA) in a mortar. After removing cell debris and quartz sand, mitochondria were precipitated by centrifuging at 18,000 g for 17 min and then dissolved in 15 ml of 70 % sucrose solution (in 10 mM Tris-HCl pH 8.0, 0.1 mM EDTA). Mitochondria were purified by

centrifuging through overlaid 44 % sucrose in a Beckman SW50.1 rotor at 45,000 rpm for 2 hours and re-suspended in 4 ml of $HKCT_{500/50}$ solution containing calcium chloride [16]. Mitoribosomes were then isolated from the purified mitochondria, separated into subunits and their proteins were analyzed essentially as described before [8] except that buffers developed by Lambowitz were used to prevent nuclease activity [16]. Yeast mitoribosomes were purified and mrps were analyzed as described previously [8].

2.4. Mass spectrometry

Mass spectrometric analysis was performed essentially as described [8]. Trypsin as well as lysyl endopeptidase were used to digest proteins.

2.5. S-tag assay

The concentration of S-tagged proteins was measured by using an S-Tag Rapid Assay Kit (Novagen, Madison USA). The procedure provided by the manufacture was mostly followed except that the reaction time was for 10 min. The ribonuclease activity was measured by the absorption at 280 nm that reflects the break down of poly(C) into acid-soluble nucleotides. The large subunits of mitoribosome isolated from cells expressing an S-tagged mrp were dissolved in 0.2 % SDS/20 mM Tris-HCl (pH 8.0) at a concentration of about 0.0005 OD_{280}/μl. No effect of SDS to the ribonuclease activity was observed when 2 μl of samples were used in a 100-μl volume of assay mixture containing S-protein and poly(C). In the case of S-tagged MrpL12, about 0.0002 OD_{280} of mitoribosomal large subunits was used.

3. **Results and Discussion**

3.1. Identification of N. crassa mrps by mass spectrometry

Mitoribosomes were purified and separated into subunits as described in Materials and Methods. The isolated large subunits were subjected to SDS-PAGE and proteins were separated into seventeen fractions according to their molecular mass. Proteins in each fraction were analyzed by peptide mass fingerprinting using the MASCOT program and a *Neurospora*-protein database, Assembly Version 3 and 7 [11]. In this way more than thirty proteins were identified. Most of the identified proteins were found to be homologous to the large subunit mrps of yeast (Table 1). However, one protein, termed ncu00103.2, showed a significant degree of similarity to yeast Mhr1 (Table 2) that was reported to be involved in mitochondrial homologous DNA recombination [17] and maintenance of the mitochondrial genome [18]. Mhr1 was also reported to be present in RNA pol II holoenzyme functioning as a direct transcriptional repressor [19]. Despite extensive analyses [6-8], this protein has so far not been detected in the mitoribosome of yeast. In addition, several proteins with similarity to yeast small subunit mrps and to cytoplasmic ribosomal proteins were identified.

3.2. Localization of the identified proteins to mitoribosomal subunits

Subsequently, we examined whether those proteins that are apparently not homologous to yeast large subunit mrps are indeed localized in the mitoribosome of *N. crassa* or not. For this purpose, the respective gene was cloned into plasmid pCB1003-S to attach an S-tag peptide at the C-terminus of the encoded protein and the resultant plasmid was introduced into *N. crassa* C1-T10-28a cells by transformation. Clones harboring the tagged protein genes were isolated by selecting Hygromycin B resistance and analyzed by PCR with appropriate primers. Subsequently, mitoribosomes were purified, their subunits separated by sucrose density gradient centrifugation and analyzed by SDS-PAGE followed by Western blotting to detect each of the S-tagged proteins.

As shown in Figs. 1-A and B, S-tagged ncu05552.2 and ncu00103.2 proteins were detected in fractions containing the large subunit, although the rest of the proteins examined were not detected in this way. Consequently, we added the former two as

large subunit mrps of *N. crassa* (Table 1).

The apparent molecular mass of ncu00103.2 as measured by SDS-PAGE was about 10 kDa larger than the calculated value due to the added tag sequence [8]. In contrast, the molecular mass of ncu05552.2 was estimated to be 54 kDa that was much smaller than expected (Table 1). Perhaps, this was due to wrong assignment of the translational initiation codon for NCU05552.2. Indeed, we were able to detect only fragments derived from the C-terminal half of its predicted protein (data not shown) by the mass spectrometric analysis. Moreover, the molecular mass calculated for the protein starting from the third methionine situated at the 126th amino acid residue was 49.6kDa, which corresponds well to that of the detected protein. The protein predicted in this way can be aligned well and shows 15 % identity with the large subunit mrp of *S. cerevisiae*, MrpL13 (Table 2). Therefore, we would like to propose that NCU05552.2 should start with its third methionine codon. The nucleotide sequence surrounding the third methionine codon appears to support this hypothesis (data not shown).

3.3 Comparison of protein components of the mitoribosoms of N. crassa and S. cerevisiae

In *S. cerevisiae*, 41 proteins of the large subunit of mitoribosome have so far been experimentally identified and three were predicted from their sequence similarity to the respective bacterial ribosomal proteins [8]. Of the 41 proteins, 23 are homologous to bacterial ribosomal proteins, while 18 appear to be unique to mitoribosome. Ten proteins of the latter category seem to be specific to fungi, because no clear counterparts of them have been found in mammalian mitoribosomes. As described above, mass spectrometric analysis of the proteins of the large subunit of *N. crassa* mitoribosome led to the identification of 28 homologues of the yeast large subunit mrps.

We surveyed the *N. crassa* genome database [11] to search for putative mrps and also performed BLAST to search for the *N. crassa* homologues of the individual mrps of *S. cerevisiae*. In this way 13 additional proteins were obtained as candidates for large subunit mrps of *N. crassa*. Comparison of the *N. crassa* mrps thus identified with yeast mrps clearly indicated that the protein components of mitoribosome are well conserved in the two fungal species. However, alignment of the homologous proteins of *N. crassa*, *S. cerevisiae* and *E. coli* revealed varying degrees of similarity in different proteins and regional differences in some mrps. Most mrps are larger than their bacterial

counterparts with the presence of sequences preceding and/or following the "core-sequence", or the sequence homologous to the corresponding bacterial ribosomal proteins. The N-terminal and/or C-terminal extra-stretches are less conserved even between *N. crassa* and *S. cerevisiae* (Table 2), indicating that the core-sequence is likely to be functionally more important and bears more constraints to the evolutionary changes. Alternatively, they might have evolved to fit to the structure of mitoribosome in each species that consists of different rRNAs and protein molecules. Earlier, Li *et al.* [20] showed from the analysis of chimeric genes that the *S. cerevisiae* mrp homologue of bacterial S15 ribosomal protein termed MrpS28 has an amino-terminal sequence that is absent from bacterial proteins but is essential for the function of mitoribosome. Similarly, the less conserved amino-terminal region of yeast S12 homologue, termed Ynr036p, seems to have an organism-specific function [21]. Therefore, it will be interesting to investigate further whether the extra-stretches of individual mrps have some species-specific functions in *N. crassa* and *S. cerevisiae* or not.

3.4. Mitoribosomal localization of Mhr1 protein in S. cerevisiae

Since protein ncu00103.2 was newly identified as an mrp in *N. crassa*, we subsequently examined whether its yeast homologue Mhr1 is also localized on the mitoribosome or not. A genomic DNA fragment containing the yeast *MHR1* gene was PCR amplified from the wild-type strain RAY3A-D of yeast and cloned into plasmid pSH*Leu* to fuse the gene with an S-tag peptide. Transformants were isolated and their proteins of mitoribosomal subunits analyzed by Western blotting. As shown in Fig. 1-C, the tagged Mhr1 was detected in the large subunit fraction, indicating that this protein is indeed present on the mitoribosome in *S. cerevisiae* as well. Although the reason why our previous analysis of yeast mrps [6] failed to detect Mhr1 is not clear, a possible explanation may be that Mhr1 was not well separated from (an)other mrp(s) by two-dimensional gel electrophoresis and hence the amino acid sequences required to identify the gene could not be obtained. A more recent analysis by tag-mediated affinity purification supports the localization of Mhr1 on the mitoribosomal large subunit [22].

It has been shown that almost all mrps of *S. cerevisiae* are essential for the mitochondrial function as summarized by Gan *et al.* [8] and that inefficient protein synthesis caused by deletion or mutation in one or more of the mrp genes is known to induce a rho^0 mutation, rendering cells completely defective in the mitochondrial

function. In this connection the fact that Mhr1 protein was found associated with the mitoribosome is very intriguing, because this protein has been reported to be important for the replication and distribution of the mitochondrial genome [17, 18, 23]. Involvement of some mrps in the maintenance of mtDNA was further suggested in the recent investigation of the highly conserved gene termed *MMF1* of *S. cerevisiae* [24].

3.5. Relative amount of Mhr1 and MrpL12 proteins in the large subunit of yeast mitoribosome

Finally, to investigate whether the majority of mitoribosomes contain one copy of Mhr1 protein like most mrps or whether Mhr1 exists on the mitoribosome only during a limited period of time as a factor-like molecule, we attached an S-tag sequence to the C-terminus of Mhr1 as well as of some yeast mrps to estimate the stoichiometry of each tagged protein. The S-tag peptide interacts with S-protein specifically and forms an active ribonuclease. Therefore, the amount of an S-tag fusion protein could be measured by assaying the ribonuclease activity upon addition of S-protein [25]. For this analysis, we first constructed yeast strains that produce only S-tagged Mhr1 or one of the S-tagged mrps as described in Materials and Methods. The large subunit of mitoribosome was then purified from each strain and the ribonuclease activity was measured (Table 3).

The results showed that the amount of Mhr1 relative to MrpL1, a yeast mrp of L1 ribosomal protein family, was 0.93 and that of Img1and MrpL51 was 0.66 and 1.1, respectively. Protein Img1 is a yeast mrp of L19 ribosomal protein family and MrpL51 is a mitoribosome-specific protein conserved in mammals and other organisms [4]. Therefore, we would like to include Mhr1 in the list of yeast large subunit mrps in spite of the fact that it has a function in homologous recombination and that it seems to exist in the nucleus in addition to mitochondria [19]. The reason why the relative amount of Img1 estimated in this analysis is significantly lower than others is not clear. Perhaps, Img1 might dissociate from the mitoribosome more easily than other mrps during purification, or the lower ribonuclease activity might be due to the conformation of S-tagged Img1 protein within the mitoribosome.

In this analysis we included a mitochondrial nucleoid protein Ygr068 (Mnp1) of *S. cerevisiae*, because its predicted primary structure shows an apparent similarity to bacterial ribosomal protein L7/12 despite that no previous investigations detected its

association with yeast mitoribosome [6, 8]. It was also reported that a majority of this protein exists in the mitochondrial nucleoid rather than mitoribosome [26]. The relative amount of Ygr068 on the large subunit of mitoribosome as calculated from the ribonuclease activity was 3.3 and Western blot analysis indicated its localization on the large subunit of mitoribosome (Fig. 1D). We, therefore, concluded that Ygr068 is indeed a yeast mrp corresponding to bacterial L7/12 ribosomal protein and, therefore, would like to name its gene *MRPL12* rather than *MNP1*. The results of S-tag assays mentioned above indicated that the yeast mitoribosome contains four copies of this protein as in the case of bacterial ribosomes. The reason why our systematic analysis by mass spectrometry of *S. cerevisiae* as well as *N. crassa* mitoribosomes failed to detect this protein is not clear. They might be more loosely bound to mitoribosomes in comparison to bacterial L7/12 protein or corresponding mammalian mrp [3, 4] and came off during the purification of mitoribosomes more easily, whereas the S-tagged MrpL12 (Ygr068) protein might be more tightly bound to the large subunit of mitoribosome.

The ORF ncu06469.2 encodes a protein that is an *N. crassa* homologue of MrpL12. We were able to detect an S-tagged protein derived from it in the mitochondrial fraction but not on the isolated mitochondrial large subunit (data not shown). This might be due to the nature of the protein or due to differences in the preparation procedure of mitoribosomes. It is also conceivable that the natural untagged ncu06469.2 protein might have been preferentially incorporated into mitoribosomes, because with *N. crassa* it is difficult to obtain a mutant producing only the tagged protein and hence we used cells containing the genes for both S-tagged and wild type ncu06469.2 proteins. Since *N. crassa* ncu06469.2 and *S. cerevisiae* MrpL12 proteins are highly conserved (48% identical), we believe that both are localized on the mitoribosomal large subunit and involved in protein synthesis. Also, it could be that the protein is associated with the mitochondrial nucleoid and play a role in maintaining the mitochondrial DNA as well [26].

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References

[1] O'Brien, T.W. (1971) The general occurrence of 55 S ribosomes in mammalian liver mitochondria. J. Biol. Chem. 246, 3409-3417.

[2] Koc, E.C., Burkhart, W., Blackburn, K., Moseley, A. & Spremulli, L.L. (2001) The small subunit of the mammalian mitochondrial ribosome. Identification of the full complement of ribosomal proteins present. J. Biol. Chem. 276, 19363-19374.

[3] Koc, E.C., Burkhart, W., Blackburn, K., Moyer, M.B., Schlatzer, D.M., Moseley, A. & Spremulli, L.L. (2001). The large subunit of the mammalian mitochondrial ribosome. Analysis of the complement of ribosomal proteins present. J. Biol. Chem. 276, 43958-43969.

[4] Suzuki, T., Terasaki, M., Takemoto-Hori, C., Hanada, T., Ueda, T., Wada, A. & Watanabe, K. (2001) Structural compensation for the deficit of rRNA with proteins in the mammalian mitochondrial ribosome. Systematic analysis of protein components of the large ribosomal subunit from mammalian mitochondria. J. Biol. Chem. 276, 21724-21736.

[5] Suzuki, T., Terasaki, M., Takemoto-Hori, C., Hanada, T., Ueda, T., Wada, A. & Watanabe, K. (2001) Proteomic analysis of the mammalian mitochondrial ribosome. Identification of protein components in the 28 S small subunit. J. Biol. Chem. 276, 33181-33195.

[6] Kitakawa, M., Graack, H.R., Grohmann, L., Goldschmidt-Reisin, S., Herfurth, E., Wittmann-Liebold, B., Nishimura, T. & Isono, K. (1997) Identification and characterization of the genes for mitochondrial ribosomal proteins of *Saccharomyces cerevisiae*. Eur. J. Biochem. 245, 449-456.

[7] Saveanu, C., Fromont-Racine, M., Harington, A., Ricard, F., Namane, A. & Jacquier, A. (2001) Identification of 12 new yeast mitochondrial ribosomal proteins including 6 that have no prokaryotic homologues. J. Biol. Chem. 276, 15861-15867

[8] Gan, X., Kitakawa, M., Yoshino, K., Oshiro, N., Yonezawa, K. & Isono, K. (2002) Tag-mediated isolation of yeast mitochondrial ribosome and mass spectrometric identification of its new components. Eur. J. Biochem. 269, 5203-5214.

[9] Lambowitz, A.M., LaPolla, R.J. & Collins, R.A. (1979) Mitochondrial ribosome assembly in *Neurospora*. Two-dimensional gel electrophoretic analysis of mitochondrial ribosomal proteins. J. Cell Biol. 82, 17-31.

[10] van den Bogert, C. & de Vries, H. (1976) The mitochondrial ribosomes of *Neurospora crassa*. II. Comparison of the proteins from *Neurospora crassa* mitochondrial ribosomes with ribosomal proteins from *Neurospora* cytoplasm, from rat liver mitochondria and from bacteria. Biochim. Biophys. Acta. 442, 227-238.

[11] Galagan, J.E. *et al*. (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422, 859-868.

[12] Tamaru, H. & Inoue, H. (1989) Isolation and characterization of a laccase-derepressed mutant of *Neurospora crassa*. J. Bacteriol. 171, 6288-6293.

[13] Davis, R.H. & de Serres, F.J. (1970) Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. 17, 79-143.

[14] Burke, D., Dawson, D. & Strearns, T. (2000) Methods in yeast genetics. A Cold Spring Harbor Laboratory Course Manual 2000 edition; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

[15] Carroll, A.M., Sweigard, J.A. & Valent, B. (1994) Improved Vectors for Selecting Resistance to Hygromycin. Fungal Genet. Newsl. 41, 22.

[16] Lambowitz, A.M. (1979) Preparation and analysis of mitochondrial ribosomes. Methods Enzymol. 59, 421-433.

[17] Ling, F., Morioka, H., Ohtsuka, E. & Shibata, T. (2000) A role for MHR1, a gene required for mitochondrial genetic recombination, in the repair of damage spontaneously introduced in yeast mtDNA. Nucleic Acids Res. 28, 4956-4963.

[18] Berger, K.H. & Yaffe, M.P. (2000) Mitochondrial DNA inheritance in *Saccharomyces cerevisiae*. Trends Microbiol. 8, 508-513.

[19] Traven, A., Staresincic, L., Arneric, M. & Sopta, M. (2002) The yeast protein Xtc1 functions as a direct transcriptional repressor. Nucleic Acids Res. 30, 2358-2364.

[20] Li, Y., Huff, M.O., Hanic-Joyce, P.J. & Ellis, S.R. (1993) Derivatives of the yeast mitochondrial ribosomal protein MrpS28 replace ribosomal protein S15 as functional components of the *Escherichia coli* ribosome. J Mol. Biol. 233, 606-614.

[21] Fujita, K, Horie, T. & Isono, K. (2001) Cross-genomic analysis of the translational systems of various organisms. J. Ind. Microbiol. Biotechnol. 27 (3), 163-169.

[22] Gavin A.C., Bosche M., Krause R., Grandi P., Marzioch M., Bauer A., Schultz J., Rick J.M., Michon A.M., Cruciat C.M., Remor M., Hofert C., Schelder M., Brajenovic M., Ruffner H., Merino A., Klein K., Hudak M., Dickson D., Rudi T., Gnau V., Bauch A., Bastuck S., Huhse B., Leutwein C., Heurtier M.A., Copley R.R., Edelmann A., Querfurth E., Rybin V., Drewes G., Raida M., Bouwmeester T., Bork P., Seraphin B., Kuster B., Neubauer G. & Superti-Furga G. (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415, 141-147.

[23] Ling, F. & Shibata, T. (2004) Mhr1p-dependent Concatemeric Mitochondrial DNA Formation for Generating Yeast Mitochondrial Homoplasmic Cells. Mol. Biol. Cell 15, 310-322.

[24] Accardi, R., Oxelmark, E., Jauniaux, N., de Pinto, V., Marchini, A. & Tommasino, M. (2004) High levels of the mitochondrial large ribosomal subunit protein 40 prevent loss of mitochondrial DNA in null mmf1 *Saccharomyces cerevisiae* cells.

Yeast 21, 539-548.

[25] Raines R.T., McCormick M., Van Oosbree T.R. & Mierendorf R.C. (2000) The S·Tag fusion system for protein purification. Methods Enzymol. 326, 362-376.

[26] Sato, H. & Miyakawa I. (2004) A 22 kDa protein specific for yeast mitochondrial nucleoids is an unidentified putative ribosomal protein encoded in open reading frame YGL068W. Protoplasma 223, 175-182.

[27] Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G. & Gibson, T.J. (1998) Multiple sequence alignment with Clustal X. Trends Biochem. Sci. 23, 403-405.

Table 1. Summary of large subunit mrps in *N. crassa*. Genes for proteins identified by mass finger printing analysis are listed. Predicted genes from the sequence similarity to yeast or bacterial ribosomal proteins are shown in parenthesis. / indicates that no gene corresponding to bacterial and/or yeast mitochondrial ribosomal protein genes was found by the standard BLAST analysis of the genome sequence. Molecular mass (*M*) is calculated from full-length protein sequence. Yeast and human homologues are listed.

N.crassa		prokaryotic	*S. cerevisiae*		Human	
gene	*M* (kDa)	family	gene	*M* (kDa)	gene[a]	*M* (kDa)
homologues of prokaryotic ribosomal proteins						
NCU08893.2	33.3	L1	*MRPL1*	31.0	MRP-L1	34.5
(NCU02757.2)	39.0	L2	*RML2*	43.8	MRP-L2	38.4
NCU06749.2	41.2	L3	*MRPL9*	29.8	MRP-L3	38.6
NCU01474.2	38.8	L4	*YmL6*	32.0	MRP-L4	34.9
NCU04171.2	39.1	L5	*MRPL7*	33.1		
(NCU04068.2)	22.2	L6	*MRPL6*	23.9		
(NCU06469.2)	18.4	L7/12	*MRPL12(YGL068w)*	20.7	MRP-L7	21.4
/		L9	/		MRP-L9	30.2
(NCU04412.2)	37.3	L10	*MRPL11*	28.5	MRP-L10	29.3
NCU04336.2	16.6	L11	*MRPL19*	16.7	MRP-L11	20.7
NCU07852.2	20.3	L13	*MRPL23*	18.5	MRP-L13	20.7
(NCU08552.2)	13.7	L14	*MRPL38*	14.9	MRP-L14	15.9
NCU05714.2	33.9	L15	*MRPL10*	36.4	MRP-L15	33.4
(NCU06768.2)	27.4	L16	*MRPL16*	26.5	MRP-L16	28.5
NCU01023.2	23.2	L17	*MRPL8*	27.0	MRP-L17	20.0
/		L18	/		MRP-L18	20.6
NCU06450.2	28.2	L19	*IMG1*	19.4	MRP-L19	33.5
/		L20	/		MRP-L20	17.4
NCU00977.2	23.9	L21	*MRPL49*	25.4	MRP-L21	22.8
NCU05813.2	41.5	L22	*MRPL22*	35.0	MRP-L22	26.2
NCU08329.2	26.0	L23	*MRP20*	30.6	MRP-L23	17.8

/		L24	/		MRP-L24	24.9
/		L25	/			
NCU08861.2	49.4	L27	*MRP7*	43.3	MRP-L27	16.1
NCU08410.2	31.5	L28	*MRPL24*	30.1	MRP-L28	30.2
/		L29	/			
NCU06786.2	18.2	L30	*MRPL33*	9.5	MRP-L30	18.6
(NCU04138.2)	23.7	L31	*MRPL36*	22.2		
(NCU03516.2)	14.1	L32	*MRPL32*	21.5	MRP-L32	21.4
/		L33	*MRPL39*	8.0	MRP-L33	7.6
(NCU03638.2)	14.8	L34	*(YDR115w)*	12.1	MRP-L34	10.2
/		L35	/		MRP-L35	21.5
(NCU06038.2)	13.9	L36	*(YPL183W-A)*	10.7	MRP-L36	11.8
proteins unique to mitochondria						
NCU05552.2	63.1		*MRPL13*	31.5		
NCU01473.2	46.3		*MRPL35*	42.8	MRP-L38	44.6
NCU08299.2	48.0		*MRPL3*	44.0	MRP-L44	37.5
NCU01485.2	47.4		*MRPL40*	33.8		
NCU07560.2	41.3		*MRPL17*	32.2	MRP-L46	31.7
(NCU08684.2)	41.0		*MRPL28*	17.4		
NCU00211.2	36.4		*MRPL31*	15.5		
NCU05287.2	32.9		*MRPL4*	37.0	MRP-L47	29.6
NCU00103.2	32.5		*MHR1*	26.9		
NCU01650.2	18.9		*MRP49*	16.0		
NCU09826.2	29.6		*MRPL15*	28.2		
NCU04225.2	25.4		*MRPL20*	22.4		
NCU03094.2	24.8		*MRPL37*	12.0	MRP-L54	15.8
NCU02804.2	18.8		*MRPL25*	18.6		
(NCU05890.2)	18.2		*IMG2*	16.4	MRP-L49	19.2
NCU07549.2	15.1		*MRPL51*	16.1	MRP-L43	21.9
(NCU07057.2)	11.8		*MRPL27*	16.5	MRP-L41	15.4
(NCU00952.2)	11.1		*MRPL44*	11.5		
/			*MRPL50*	16.3		

[a] Protein names are from [3]. Only those related to bacterial and/or

yeast mitochondrial ribosomal proteins are listed

Table 2. Similarity of large subunit mrps in *N. crassa* and yeast. Percentages of amino acid residues identical between *N. crassa* and yeast mrps in the region aligned by CLUSTAL X [27] are listed.

homologues of prokaryotic ribosomal proteins						proteins unique to mitochondria		
gene	family[a]	identity (%)	identity in regions (%)[b]			gene	family[a]	identity (%)
			N-terminus	Core	C-terminus			
NCU08893.2	L1	23	14	26	-	**NCU05552.2**	MRPL13	15
NCU06749.2	L3	37	14	46	-	**NCU01473.2**	MRPL35	23
NCU01474.2	L4	30	18	32	-	**NCU08299.2**	MRPL3	41
NCU04171.2	L5	28	19	34	-	**NCU01485.2**	MRPL40	27
NCU06469.2	L7/12	48	25	54	-	**NCU07560.2**	MRPL17	28
NCU04336.2	L11	53	-	53	-	**NCU00211.2**	MRPL31	41
NCU07852.2	L13	43	-	48	14	**NCU05287.2**	MRPL4	34
NCU05714.2	L15	38	13	44	18	**NCU00103.2**	MHR1	18
NCU01023.2	L17	32	-	45	21	**NCU01650.2**	MRP49	13
NCU06450.2	L19	23	19	25	-	**NCU09826.2**	MRPL15	26
NCU00977.2	L21	27	15	38	-	**NCU04225.2**	MRPL20	27
NCU05813.2	L22	26	16	20	29	**NCU03094.2**	MRPL37	35
NCU08329.2	L23	25	-	37	14	**NCU02804.2**	MRPL25	41
NCU08861.2	L27	21	10.7	44	15	**NCU07549.2**	MRPL51	45
NCU08410.2	L28	29	19.3	51	20			
NCU06786.2	L30	44	-	48	36			

[a] Homologues of *E. coli* or *S. cerevisiae* are shown

[b] "N-terminus" and "C-terminus" are regions that are aligned between *N. crassa* and *S. cerevisiae* but not found in *E. coli* proteins. "Core" is the region where the corresponding *E. coli* ribosomal protein is aligned

Table 3. Relative amount of Mhr1 and MrpL12 in yeast mitoribosomal large subunit. Mitoribosomal large subunits containing each of the indicated S-tagged proteins were purified. The concentration of S-tagged protein was measured by assaying the ribonuclease activity that was indicated as the increase in absorbance at 280 nm (ΔOD_{280}). Values were normalized to that obtained with 0.001 OD_{280} of mitoribosomal large subunits in 100 μl assay volume. The amount of protein relative to MrpL1 (3rd column) indicates the ratio of normalized increase in absorbance between each S-tagged protein and MrpL1.

protein	ΔOD_{280}[a]	relative to MrpL1[b]	stdv[c]
MrpL1	1.09	1.0	-
Mhr1	1.06	0.93	0.33
ImgI	0.63	0.66	0.23
MrpL51	1.03	1.1	0.35
MrpL12	3.38	3.3	1.70

[a] Mean of more than ten independent assays.

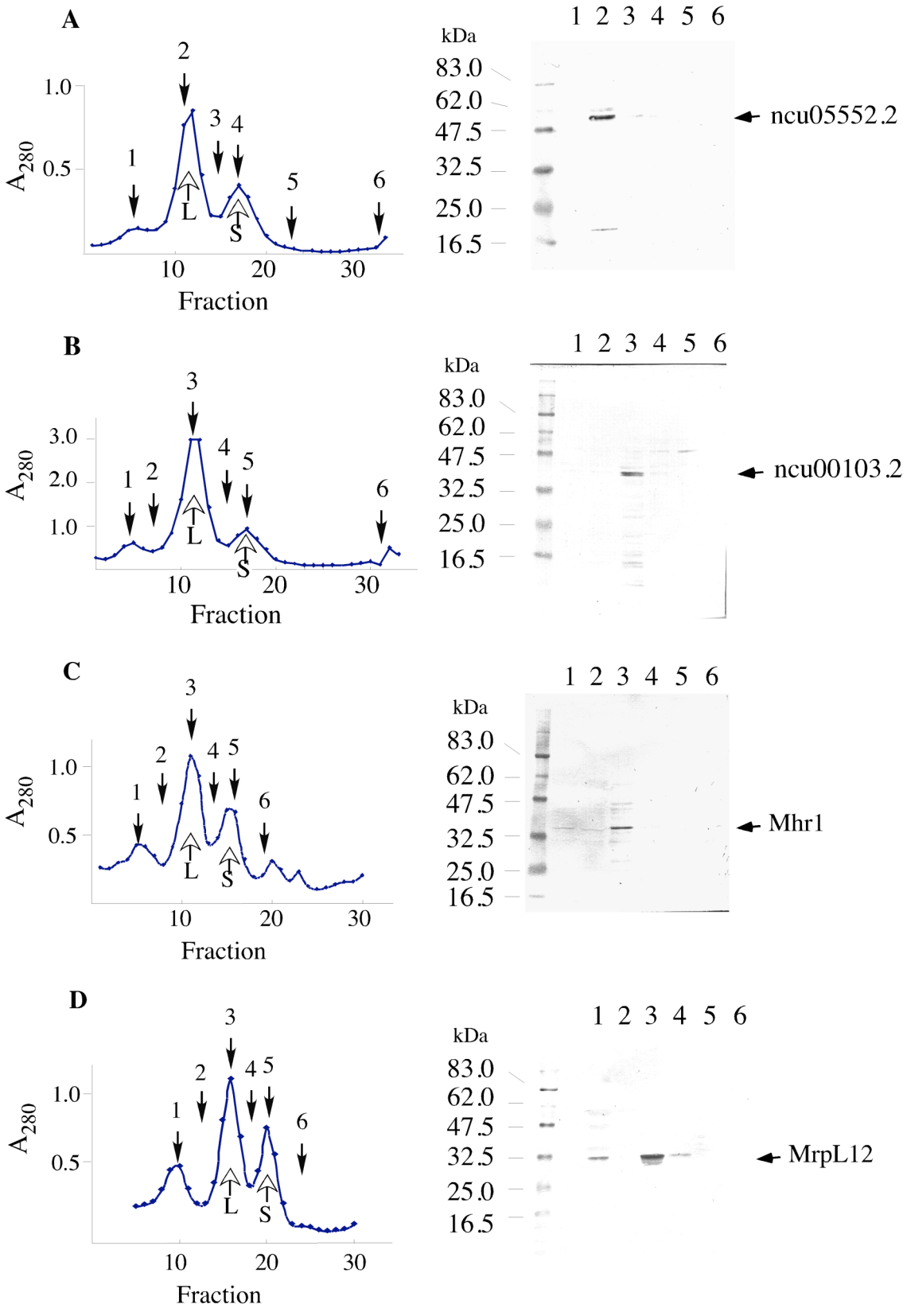
[b] Mean of ratios calculated for each assay.

[c] Standard deviation of the protein amount relative to MrpL1.

Legends to Figure:

Fig. 1. Subunit localization of mrps. Mitoribosomes containing each of the S-tagged mrps indicated were purified from cells of *N. crassa* (A, B) and *S. cerevisiae* (C, D) and subunits were separated by sucrose density gradient centrifugation. Proteins in each fraction were acetone-precipitated, separated by SDS-PAGE and analyzed by Western blotting. About a half (A) or a quarter (B, C and D) of the precipitated proteins were subjected to SDS-PAGE. The profile of sucrose density gradient centrifugation is shown in the left side panel. The small (S) and large (L) subunit peaks are indicated by upward arrows and fractions examined by Western blot analysis (right side panel) are numbered. The left most lane in the right side panel shows size markers.

Fig. 1


A
B
C
D
A280
Fraction
kDa
83.0
62.0
47.5
32.5
25.0
16.5
1 2 3 4 5 6
ncu05552.2
ncu00103.2
Mhr1
MrpL12
L
S