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# The effect of mutated mitochondrial ribosomal proteins S16 and S22 on the assembly of the small and large ribosomal subunits in human mitochondria

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## Abstract

Mutations in mitochondrial small subunit ribosomal proteins MRPS16 or MRPS22 cause severe, fatal respiratory chain dysfunction due to impaired translation of mitochondrial mRNAs. The loss of either MRPS16 or MRPS22 was accompanied by the loss of most of another small subunit protein MRPS11. However, MRPS2 was reduced only about 2-fold in patient fibroblasts. This observation suggests that the small ribosomal subunit is only partially able to assemble in these patients. Two large subunit ribosomal proteins, MRPL13 and MRPL15, were present in substantial amounts suggesting that the large ribosomal subunit is still present despite a nonfunctional small subunit.

### Keywords

Mitochondria; ribosome; ribosomal subunit; respiratory chain complexes; ribosomal proteins

## 1. Introduction

The 16.5 kb human mitochondrial genome encodes 22 tRNAs, 2 rRNAs and thirteen polypeptides. These proteins, which are inserted into the inner membrane and assembled with nuclearly encoded polypeptides, are essential components of the mitochondrial respiratory chain complexes (MRC) I, III, IV and V. The synthesis of these 13 proteins requires a unique, mitochondrial translational system including the unusual ribosomes found within this organelle. Mammalian mitochondrial ribosomes are 55S particles and consist of a 28S small subunit and a 39S large subunit (Pel et al., 1994). The small subunit includes a 12S rRNA and an estimated 29 proteins while the large subunit contains a 16S rRNA and about 48 proteins (Koc et al., 2000; Koc et al., 2001b). Approximately half of the mitochondrial ribosomal proteins (MRPs) have homologs in bacteria while the remainder are unique to mitochondria (Sharma et al., 2003). Some of the human MRP's have no counterparts even in yeast and their functions are largely unknown. A number of the MRPs that lack bacterial homologs appear to have taken on novel roles in mammalian cells. For example, both MRPS27 and MRPS29 appear

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to have a role in apoptosis (Koc et al., 2001c; Mukamel et al., 2004) and several others have a role in cell cycle control (Chen et al., 2007; Kim et al., 2005; Yoo et al., 2005). Both of the ribosomal subunits are approximately 2/3 protein and 1/3 RNA, a ratio which is the opposite to the ratio found in prokaryotic ribosomes. Of note, while rRNAs are encoded by mitochondrial DNA, all the MRPs are nuclear encoded and imported from the cytosol requiring coordination between mitochondrial transcription and cytosolic translation.

A number of mitochondrial diseases result from mutations leading to defective mitochondrial protein synthesis and, hence, to respiratory chain deficiencies (Enriquez et al., 1995; King et al., 1992). These disorders present a wide variety of clinical symptoms generally involving tissues with high energy requirements. Defects in mitochondrial protein synthesis may arise from mutations in either the nuclear or mitochondrial genomes. Diseases associated with mutations in mitochondrial DNA, especially those affecting the tRNA genes and the12S rRNA gene, have been extensively studied (Jacobs, 2003; Schon, 2000). However, much less is known about a new expanding group of disorders encompassing nuclear mutations in the mitochondrial translational machinery (Edvardson et al., 2007; Scheper et al., 2007).

In recent studies (Miller et al., 2004; Saada et al., 2007) mutations in the nuclear genes encoding two mitochondrial small subunit ribosomal proteins were identified. The hallmarks that led to their detection were severe respiratory chain dysfunction encompassing MRC complexes I, III, IV and V, normal levels of complex II, normal mitochondrial DNA, normal mitochondrial mRNAs and 16S rRNA and a significant decrease in the abundance of 12S rRNA. Since rRNA is stable only when incorporated in a ribosomal structure, it was hypothesized that these defects would lead to an unstable mitochondrial ribosomal small subunit while the large subunit was expected to be intact.

A homozygous nonsense mutation (Arg111Stop) in the mitochondrial small subunit ribosomal protein S16 (MRPS16) has been associated with agenesis of corpus callosum, dysmorphism and fatal neonatal lactic acidosis (Miller et al., 2004). A second, homozygous, missense mutation (R170H) in the gene encoding another small subunit ribosomal protein MRPS22 has been associated with edema, fatal cardiomyopathy and tubulopathy (Saada et al., 2007). In contrast to MRPS16 which is highly conserved and essential for the ribosomal small subunit assembly, MRPS22 has no counterpart in bacteria or yeast and its function is obscure. The availability of fibroblasts from these patients provided a unique tool for examining the human mitochondrial ribosome. In the present study we have examined the impact of MRPS16 or MRPS22 mutations on the small and large ribosomal subunits in order to expand our knowledge of the human mitochondrial ribosomal assembly.

## 2. Material and Methods

### 2.1 Materials

Regular chemicals were purchased from Sigma-Aldrich or Fisher Scientific. ECL western blotting analysis kits were purchased from GE Healthcare. ECL films were obtained from GE Healthcare and Kodak. Protein free blocking solution was purchased from Pierce Technologies. PVDF membranes were purchased from Millipore Corporation. Restriction enzymes were purchased from New England Biolabs. Digested plasmids and PCR products were purified using the Qiagen Gel Extraction/PCR Purification Kit. The cDNAs encoding human mitochondrial ribosomal proteins MRPS2 (ATCC number MGC-16952), MRPS11 (ATCC number MGC-40231), MRPL13 (ATCC number MGC-33899) and MRPL15 (ATCC MGC-4898) were obtained from American Type Culture Collection (ATCC). Tissue culture media were from Biological Industries (Beit Hemek Israel). “Assays-on-Demand Gene Expression Products” were from Applied Biosystems and used with ImProm-II reverse transcriptase (Promega).

### 2.2 Cloning, expression and purification of MRPS2, MRPS11, MRPL13 and MRPL15

The cDNAs were amplified out of the stock pCMV-SPORT6 vectors using forward and reverse primers with NdeI and XhoI restriction sites (underlined), respectively, as follows: MRPS2 forward, GGAATTCCATATGATCCGCGAGTCGGAGGAC; MRPS2 reverse, AACCGCTCGAGCAGGGAATGGCTCATGTCA; MRPS11 forward, GGAATTCCATATGCCGGCCGGGACCATCTGC; MRPS11 reverse, AACCGCTCGAGCAGCTTCCGAGCCTTCCT; MRPL13 forward, GGAATTCCATATGCAGGGATTACATAAACCTGTG; MRPL13 reverse, AACCGCTCGAGTAGCCGATAATCTTCAGGT; MRPL15 forward, GGAATTCCATATGTGTGGCAGAGGCCATAAA; MRPL15 reverse, AACCGCTCGAGTGAGGTATAATACTTAAG.

The PCR products were digested with NdeI and XhoI and ligated into the pET21b(+) plasmid, which provided a C-terminal $His_6$-tag. The ligated plasmids were transformed into Stratagene's *E. coli* DH5α cells. Candidate plasmids were confirmed by sequencing and transformed into E. *coli* BL21 (DE3) RIL cells (Stratagene) for expression (Koc et al., 2002).

The purification of the expressed proteins on Ni-NTA resins was carried out following the QIAexpressionist denaturing protocol as described (Qiagen). Proteins were concentrated to a final volume of approximately 200 μL by placing the samples into dialysis tubing and layering them with solid PEG 8000.

### 2.3 Antibody Production against Mammalian MRPS2, MRPS11, MRPS16, MRPL13, and MRPL15

Using the immunoadjuvant TiterMax Gold, a 250 μL emulsion was prepared for each factor that contained 125 μL TiterMax and 125 μL of the concentrated protein (~250 μg). Each emulsion was divided into two 100 μL fractions and injected into New Zealand White Rabbits to produce the respective antibodies. The rabbits were injected at 0, 4 and 8 weeks and blood was collected to test the serum at each injection time. The final bleed was performed at 16 weeks at which time the rabbits were exsanguinated. The specificity of the antibodies was verified by Western blotting as described (Ma et al., 1996; Spencer et al., 2005). Antibodies against MRPS16 were prepared in rabbits using the peptide CPRDGRFVEQLGSYDPLPNSHGEKL conjugated to KHL as antigen (Sigma-Aldrich Israel).

### 2.4 Tissue cultures and mitochondrial preparation

Fibroblast cultures were established from forearm biopsies (with informed consent). The cells were gown in DMEM with 4.5 g glucose/L supplemented with 10% fetal calf serum, 50 μg/mL uridine and 110 μg/mL pyruvate in an atmosphere of 5% $CO_2$ at 37 °C. Prior to mitochondrial isolation, the cells were incubated for 30 min in the presence of 100 μg/mL chloramphenicol in the growth medium. Mitochondria were isolated from ten T75 tissue culture flasks as follows; after two washes with phosphate buffered saline, the cells were collected by scraping them into 10 mL of isolation buffer (320 mM sucrose, 2 mM EGTA, 0.1 mg/mL sodium heparin in 5 mM Tris-HCl, pH 7.3) and homogenized by nitrogen cavitation at 450 psi (pressure bomb from Parr Instrument Moline IL) followed by a 10 min centrifugation at 2,000 × g to pellet nuclei and unlysed cells.

The mitochondrial enriched fraction was obtained by centrifuging the supernatant at 16,000 × g for 10 min, the resulting pellet was re-suspended in isolation buffer containing 0.02% digitonin and centrifuged for 10 min at 12,000 × g. The mitochondrial pellets were stored at −70 °C until use.

### 2.5 Determination of MRPS16 and MRPS22 mRNA levels

For the preparation of cDNA total mRNA (prepared from $2\times 10^6$ cells) was reverse transcribed using ImProm-II reverse transcriptase as per the manufacturer's instructions. The cDNA was used in a PCR reaction to amplify and detect the expression of specific nucleic acid sequences using the "Assays-on-Demand Gene Expression Products". β-actin served as an endogenous control and MRPS16 or MRPS22 as the target cDNAs. The human cytoplasmic large subunit ribosomal protein (RPLP0) was added as an internal control target. The levels of all the cDNAs were equalized and real-time PCR reactions were performed using each probe/primer set separately. The experiment included mRNA prepared from 4 normal fibroblast controls and 2 patient fibroblast cell preparations performed in duplicate. The relative expression of the target transcript was calculated with the comparative Ct method (Applied Biosystems User Manual).

### 2.6 Isolation of human mitochondrial ribosomes from mitochondria and detection of mitochondrial ribosomal proteins using Western blotting

Partially purified mitochondrial ribosomes were prepared from control and patient mitochondria as described (Spremulli, 2007). In brief, mitochondrial pellets (50 mg) from control fibroblasts, the MRPS16-mutated patient fibroblasts or the MRPS22-mutated patient fibroblasts were suspended in 500 μL of Base Buffer (20 mM HEPES-KOH, pH 7.6, 20 mM $MgCl_2$, 40 mM KCl, 2 mM dithiothreitol, 1.6% Triton X-100, 10% sucrose and 0.1 mM phenylmethylsulfonyl fluoride) and centrifuged for 10 min at 13,000 rpm in an Eppendorf Centrifuge. The supernatants were layered on a 3 mL cushion of 30% sucrose and the tube was filled with Base Buffer. Samples were centrifuged for 18 h at 48,000 rpm in a Beckman Type 70.1 Ti rotor. Pellets containing ribosomes or partially assembled ribosomal particles were dissolved in 25 μL 2x sodium dodecyl sulfate (SDS) gel loading dye (100 mM Tris-HCl, pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol and 100 mM β-mercaptoethanol). A portion of each sample (20 μL) was analyzed by SDS-polyacrylamide gel electrophoresis on a 15% gel. Proteins were transferred to PVDF membranes and probed with the indicated antibodies using the Enhanced Chemiluminescence (ECL) Western blotting method as described (GE Healthcare, Amersham product booklet). Band intensities were analyzed using the UNSCAN-IT software. Membrane stripping procedures were performed based on Amersham ECL Western blotting product booklet.

In separate experiments whole mitochondrial pellets (15 mg each) were dissolved in 30 μL 2x SDS gel loading dye for determining the total proteins in healthy and patient mitochondria. A portion of each dissolved pellet (10 μL) was diluted to 20 μL by adding 2x SDS dye and heated for 5 min. Additional β-mercaptoethanol (100 mM) was added before loading onto the SDS gel. The levels of specific proteins were analyzed by ECL Western blotting.

## 3. Results

### 3.1 Levels of mRNAs in MRPS16 and MRPS22 fibroblasts

The deleterious mutation observed in the MRPS16 gene due to the nonsense mutation of the Arg111Stop codon to a stop codon, was predicted to lead to the premature termination of the MRPS16 protein. The presence of MRPS16 mRNA was not examined in the original studies. We, therefore, measured the level of the MRPS16 mRNA by real-time PCR in cells from the patient and from control fibroblasts. These studies indicated that, in the patient's cells, the MRPS16 mRNA was decreased to 13% of the levels found in normal fibroblasts while the level of MRPS22 mRNA was unaffected (Table 1). The reduction in the level of the MRPS16 mRNA is presumably a result of nonsense mediated RNA decay (Khajavi et al.,2006). This observation is somewhat surprising since the nonsense mutation is located in the third and last exon of the MRPS16 gene. Nonsense mutations in the last exon are generally not good substrates for nonsense- mediated decay. Hence, the reason for the decreased levels of the

MRPS16 mRNA are not clear. The missense mutation in MRPS22 detected in the patients did not lead to a significant decrease in the levels of either MRPS22 or MRPS16 mRNA (Table 1).

### 3.2 Effects of the mutation in MRPS16 on the levels of MRPS16 and of other ribosomal proteins

The effect of the mutation in MRPS16 on the presence and stability of several other proteins of the small subunit was examined using specific antibodies. For these experiments, the mitochondria were extracted with Triton X-100 in order to free the ribosomes from the membranes. These extracts were centrifuged through a sucrose cushion to pellet ribosomes and partially assembled ribosomal particles, which were then analyzed for the presence of ribosomal proteins using Western blotting. As indicated in Fig. 1, MRPS16 is readily detected in ribosomal fractions from the control fibroblasts. As expected from the premature stop codon and the mRNA quantitation, no MRPS16 was detected in the mitochondrial ribosomal fraction from the patient carrying the mutated MRPS16. The predicted, truncated form encompassing the N-terminus of MRPS16 was not observed on the Western blot suggesting that this fragment was degraded.

In order to examine the assembly of the small ribosomal subunit in the mitochondria of the MRPS16-mutated patient, Western analysis of ribosomal fractions from control and MRPS16-mutated mitochondria were examined for the presence of two additional small subunit ribosomal proteins (MRPS2 and MRPS11). As indicated in Fig. 1, the ribosomal fraction from this patient still retained about 60% of the control levels of MRPS2. This observation suggests that the stability of MRPS2 is not strongly affected by the defect in MRPS16, thus, it is likely that MRPS2 is present in partially assembled ribosomal particles in the cells of the patient. Due to limitations of the amount of material available from primary fibroblasts, it was not possible to obtain an S-value for the partially assembled ribosomal particles present by centrifuging them through a sucrose gradient. Notably, we intentionally chose to perform these studies with primary (untransformed) fibroblasts, in order to reduce potential artifacts from transformed cells; thus the amount of starting material was limited. Despite this limitation, valuable information could be derived from the patient's cells. Of note here is that MRPS2 from the patient's cells could be sedimented through a sucrose cushion suggesting that it is present in a reasonably large macromolecular complex.

In contrast to MRPS2, the level of MRPS11 was significantly reduced (over 80 %) in the fibroblasts from the MRPS16-mutated patient (Fig. 1) indicating that the stable assembly of MRPS11 into a macromolecular complex with other ribosomal components is quite dependent on the presence of MRPS16. The reduction in the levels of MRPS11 in the ribosomal fraction could be due to the degradation of this protein when not assembled into the ribosome. Alternatively, MRPS11 could have remained un-assembled in the soluble fraction or in sub-ribosomal particles that did not sediment in the sucrose cushion. To distinguish between these possibilities, whole mitochondrial pellets from control and patient fibroblasts were directly solubilized in SDS gel loading buffer and then subjected to Western analysis using the anti-MRPS11 antibodies. While this protein was detected in whole mitochondria from control cells, it was present in only in small amounts in the patient fibroblasts (data not shown). This observation suggests that MRPS11 was not assembled into ribosomal particles in the absence of MRPS16 and was subsequently degraded.

The fate of the large ribosomal subunit in the absence of a properly assembled small subunit was examined by looking for the presence of two large subunit ribosomal proteins, MRPL13 and MRPL15. These proteins, homologs of the bacterial protein L13 and L15, are located on the side and back of the large subunit respectively. As indicated in Fig. 1, both MRPL13 and MRPL15 are present at the same levels in the control and patient fibroblasts. This observation

is in agreement with the presence of normal levels of the 16S large subunit rRNA in the patient mitochondria (Miller et al., 2004).

### 3.3 Effect of Mutation of MRPS22

MRPS22 is a protein specific to mitochondrial ribosomes that does not have a homolog in bacterial, eukaryotic cytoplasmic or yeast mitochondrial ribosomes. Still it is an essential component of the mammalian mitochondrial ribosome, since a missense mutation at position 170 changing a conserved Arg residue to His is lethal in humans (Saada et al., 2007). Nothing is currently known about the location of MRPS22 in the ribosome or the role it may play in ribosome assembly or function. However, the mutation of this protein causes a dramatic reduction in the level of the 12S rRNA suggesting that it plays an important role in the stability or assembly of the small subunit in human mitochondria (Saada et al., 2007). To examine the effect of the mutation in MRPS22 on the presence of other ribosomal proteins, ribosomal fractions prepared from control and MRPS22 cells were analyzed by Western blotting. The presence of MRPS22 *per se* could not be tested due to the lack of available antibodies. However, it was possible to examine these preparations for three small subunit ribosomal proteins. Mitochondria of the MRPS22 patient show an 80–90% decrease in the levels of both MRPS11 and MRPS16 respectively (Fig. 2). This observation strongly suggests that MRPS22 plays an important role in the assembly and stability of the mitochondrial ribosome. In its absence, several ribosomal proteins cannot assemble into sub-ribosomal particles.

In contrast, significant amounts (about 60%) of MRPS2 are observed in the fibroblasts of the MRPS22 patient. This observation is consistent with the finding obtained from the MRPS16-mutated patient and suggests that a macromolecular complex containing MRPS2 can assemble in the absence of normal ribosome assembly in human mitochondria.

Both large subunit ribosomal proteins tested (MRPL13 and MRPL15) are present in significant amounts in the MRPS22-mutated patient mitochondria indicating that the large subunit is still present in these cells as well (Fig. 2).

## 4. Discussion

Current knowledge on the structure and assembly of the mammalian mitochondrial ribosome is limited; thus the study of human ribosomes defective in specific MRPs can potentially contribute significant new information. MRPS16 is the homolog of the bacterial protein S16. It is essential for viability in *E. coli* (Persson et al., 1995) and is clearly also essential in the human mitochondrial ribosome. S16 is located on the lower bottom of the ribosome on the solvent side of the small subunit (Fig. 3A). In the *E. coli* ribosome S16 primarily makes contact with a number of helical segments of the 16S rRNA (Wimberly et al., 2000). However, over half of these segments are missing in the mitochondrial ribosome. This region, like much of the mitochondrial ribosome as a whole, is rich in proteins and MRPS16 may make a number of protein:protein contacts that do not occur in the bacterial ribosome.

In the assembly of the bacterial 30S subunit, S16 does not initially interact with the naked rRNA, but requires the prior binding of S4 and, to some extent S20, before incorporation into the small subunit (Fig. 3B). Mammalian mitochondria do not contain proteins homologous to either S4 or S20 and, hence, the incorporation of MRPS16 into the small subunit must be dependent on other proteins (Koc et al., 2001a). A good candidate is the mitochondrial small subunit specific protein MRPS22. The data presented here show clearly that MRPS16 is barely detectable in fibroblasts of patients with defects in MRPS22.

MRPS11 and MRPS2 are the homologs of the corresponding bacterial proteins S11 and S2. S11 is located on the platform of the small subunit (Fig. 3A) where is makes a number of

contacts with other ribosomal proteins. S2 is found on the solvent side of the small subunit at the contact between the head and the body. Both S11 and S2 are tertiary RNA binding proteins that do not interact with the small subunit rRNA until a number of other proteins have assembled on the RNA. The data presented here clearly indicates that MRPS11 does not assemble well into ribosomal particles or sub-particles in either the MRPS16 or the MRPS22 mutated ribosomal particles. This observation is in contrast to observations made with respect to the assembly of the bacterial ribosome. In bacteria (Fig. 3B), assembly of S11 into the small subunit requires S15 followed by S6 and S18, which help create the platform on the small subunit in conjunction with the 5′ domain of the bacterial ribosomal small subunit rRNA (Culver,2003). This process does not require the presence of S16 in the body of the subunit. The failure of MRPS11 to be stably incorporated into a ribosomal-like particle in the absence of MRPS16 is intriguing and suggests that the proper formation of the platform of the small subunit of the mitochondrial ribosome requires the proper assembly and organization of the body of this particle.

In contrast to MRPS11, MRPS2 is able to assemble into a rapidly sedimenting particle to a significant extent in the absence of either MRPS16 or MRPS22. This observation agrees with the assembly process of the bacterial ribosome in which S2 incorporation is basically independent of S16 (Fig. 3B). Independent assembly of MRPS2 may reflect the ability of the head/neck of the small subunit encompassing the 3′ major domain of the rRNA to assemble into an independent subcomplex containing a number of ribosomal proteins including S2 (Samaha et al., 1994). The formation of this complex may account for the presence of MRPS2 in the mutated cell lines.

Finally, the results presented here suggest that the large subunit can be assembled and remain stable in the absence of a functional small subunit of the mitochondrial ribosome corroborating the previous assumption based on the presence of the 16S rRNA. This observation suggests that there is no regulatory cross-talk monitoring the levels of the large and small subunits in the mitochondrion. Since the rRNAs in both subunits are co-transcribed, such a regulatory mechanism would presumably not be necessary. Examining the ribosomes from patients with defective MRP's provides a unique tool to study the human mitochondrial translation system and ribosomal assembly in particular. An expanded knowledge of this machinery may advance research towards developing therapeutic options.

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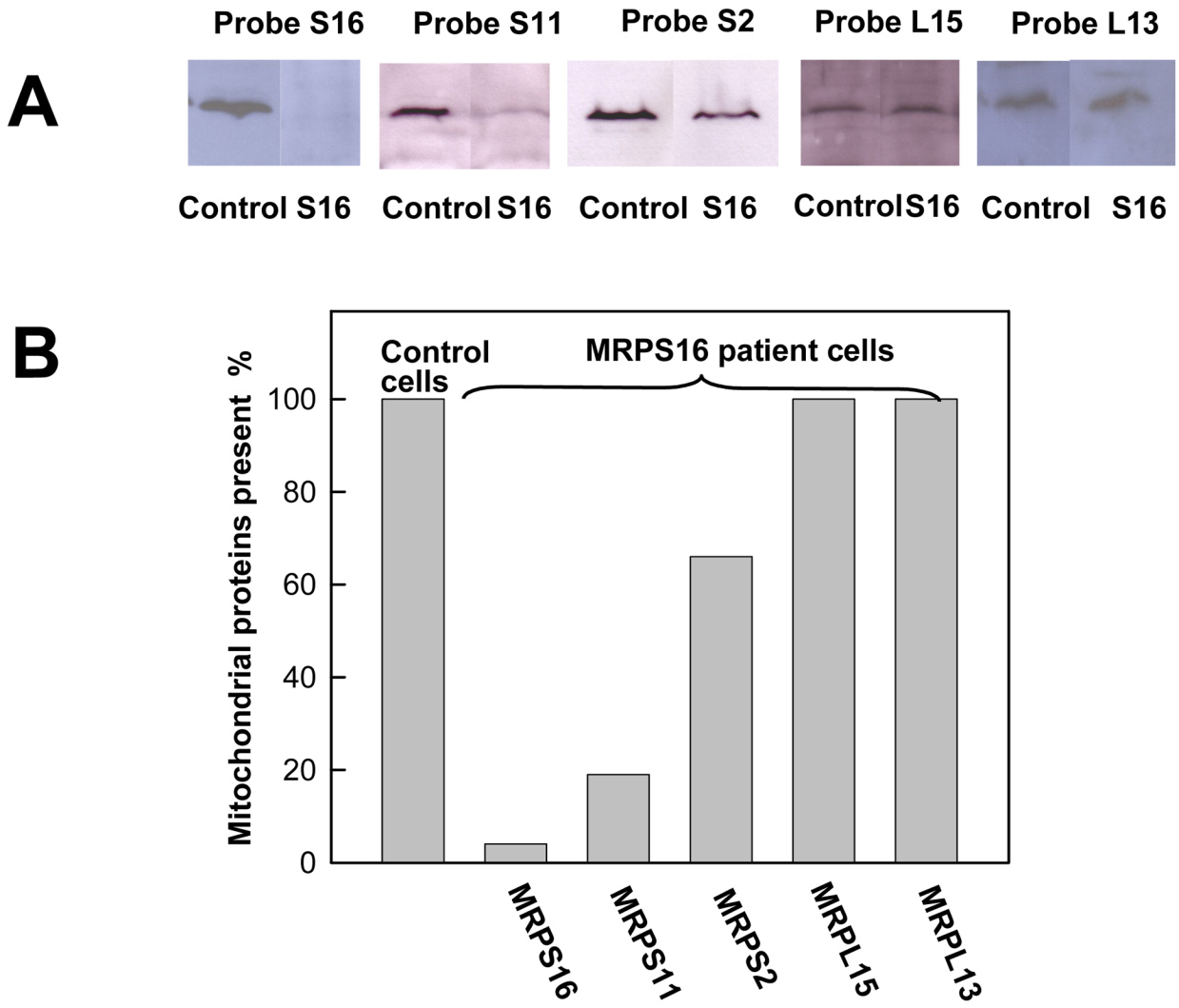


**Figure 1.**

Identification of human mitochondrial ribosomal proteins in control and MRPS16 patient cells. (A) The levels of patient mitochondrial ribosomal proteins MRPS16, MRPS2, MRPS11, MRPL13 and MRPL15 from healthy control and MRPS16 cells were determined by Western blotting using antibodies against the specified protein and detected by ECL. The background of the blots varied due the type of the film used, the exposure time and the number of times the membrane was re-probed. In each case, control and patient mitochondria were examined under the same conditions. The antibody used is indicated as the probe above the given lanes. (B) Quantification of the amount of the indicated protein present in fibroblast mitochondria from the MRPS16 patient compared to the amount present in control cells. Relative band intensity was determined using the UNSCAN-IT software.

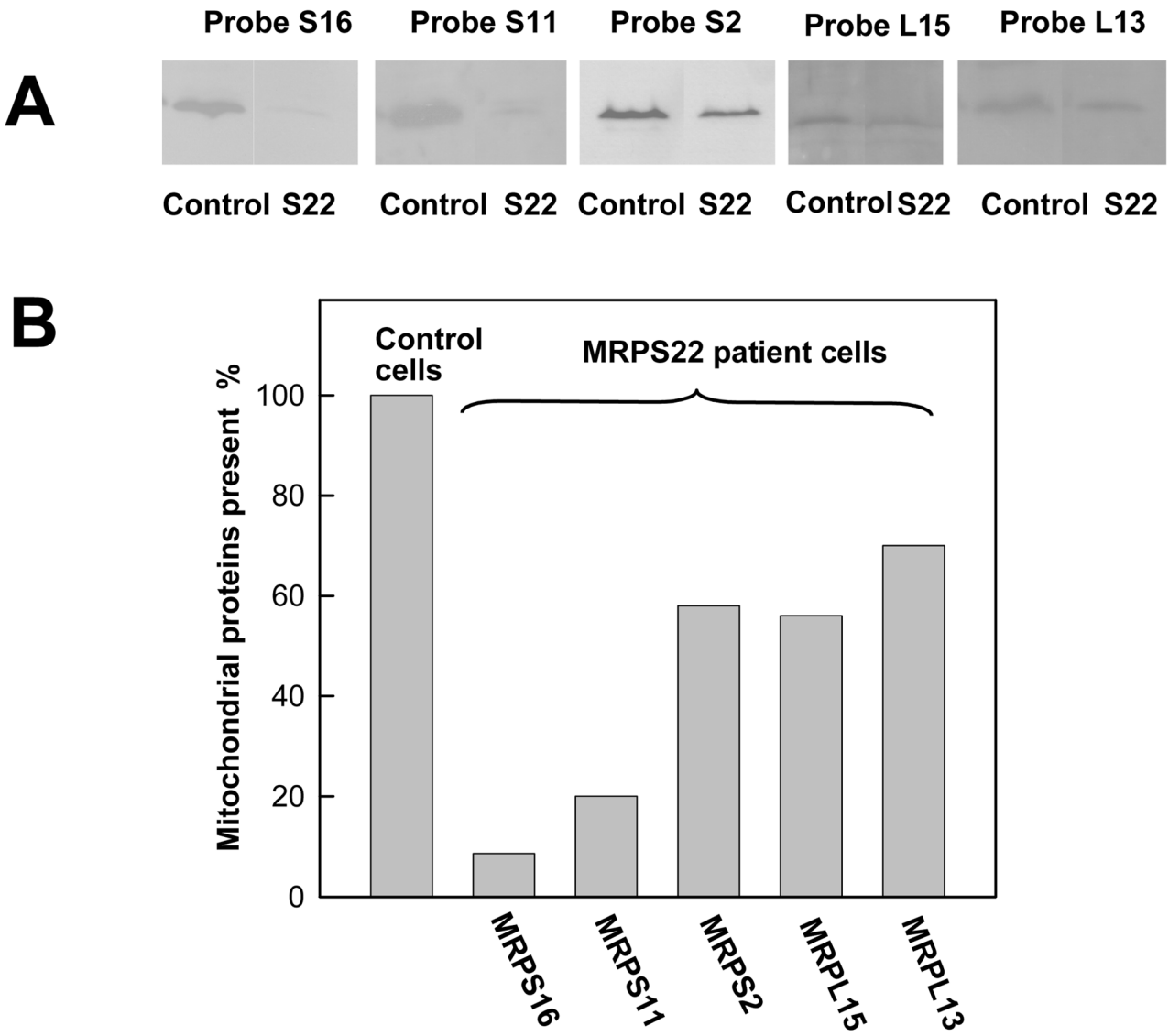


**Figure 2.**

Identification of human mitochondrial ribosomal proteins of control and MRPS22 patient cells. (A) The levels of mitochondrial ribosomal proteins MRPS16, MRPS2, MRPS11, MRPL13 and MRPL15 were examined in mitochondria derived from control and MRPS22 cells using Western blotting and the indicated antibody as a probe. The backgrounds on the blots depended on the type of film used, and the number of times the membranes were stripped for reprobing. (B) Quantification of the amount of the indicated protein present in fibroblast mitochondria from the MRPS16 patient compared to the amount present in control cells. Relative band intensities were determined using the SCAN-IT software.

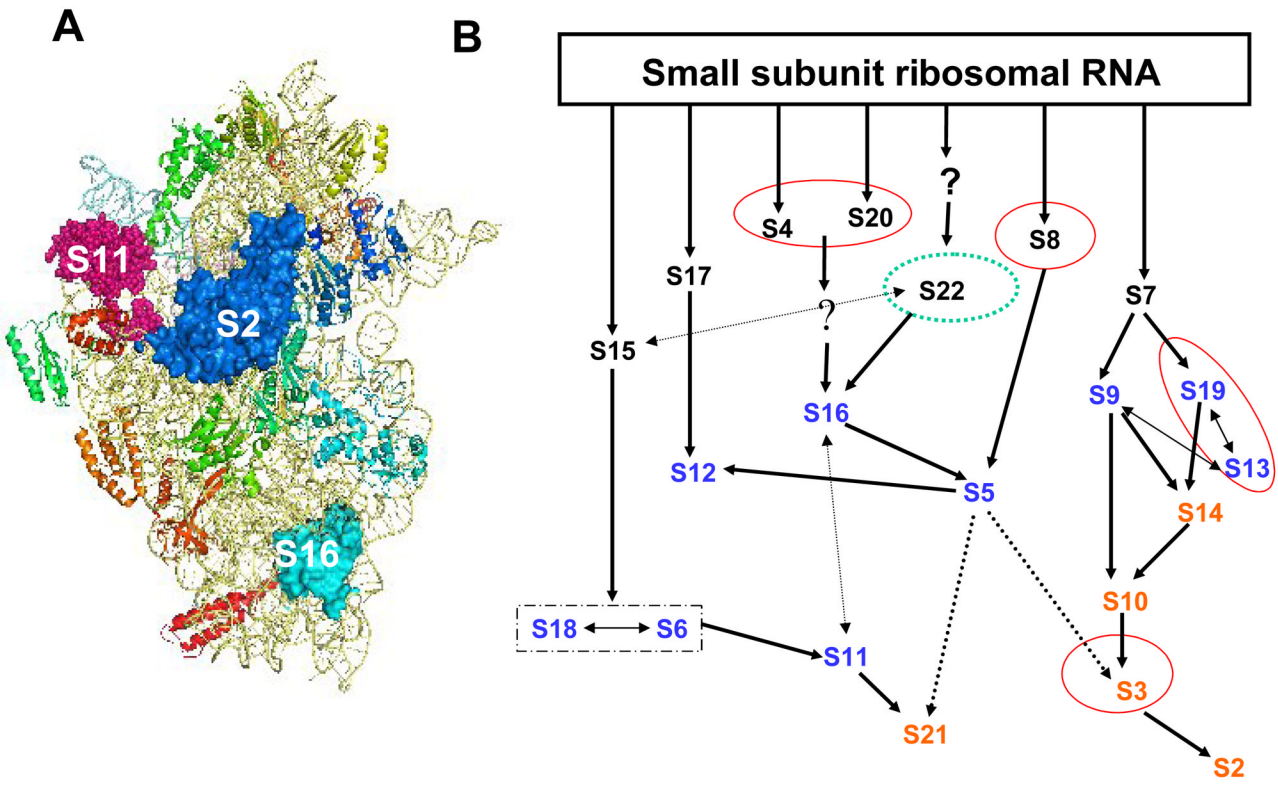


**Figure 3.**

The small subunit of the bacterial ribosome and its assembly. (A) The positions of S16, S11 and S2 in the small subunit of the bacterial ribosome are indicated. The model is based on the pdb coordinates of the 30S subunit from *Thermus thermophilus* (2J00) and displayed using Pymol (DeLano, W.L. The PyMOL Molecular Graphics System (2002) on World Wide Web http://www.pymol.org). The rRNA is shown in gold, S16 is space-filled in turquoise, S11 is space-filled in red and S2 is in dark blue. Other ribosomal proteins are shown as ribbon structures. (B) Modified assembly map for the 30S subunit from *E. coli* indicating the positions in the assembly observed with bacterial S16, S11 and S2. This map is based on an updated map presented in (Culver, 2003). Red circles indicate proteins that are not present in the mammalian mitochondrial ribosome. Mammalian mitochondrial ribosomal protein MRPS22 is shown in the map but its position is clearly hypothetical. Possible connections to other proteins are shown in dotted lines. The proteins that are primary RNA binding proteins in *E. coli* are in black; secondary RNA binding proteins are in blue while tertiary RNA binding proteins are red.

**Table 1**

Quantification of MRPS16 and MRPS22 transcripts[1]

| Fibroblasts | Relative amount of mRNA present | | |
|---|---|---|---|
| | RPLP0 | MRPS 16 | MRPS 22 |
| MRPS16 patient | 0.84 | 0.13 | 0.99 |
| MRPS22 patient | 0.85 | 0.73 | 0.81 |
| Controls (n=4) | 1.0 | 1.0 | 1.0 |

[1] The levels of mRNA's were determined by quantitative RT-PCR and quantified relative to β-actin levels in 4 normal control fibroblasts.