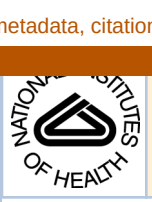



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# Contacts between mammalian mitochondrial translational initiation factor 3 and ribosomal proteins in the small subunit

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## Abstract

Mammalian mitochondrial translational initiation factor 3 ($IF3_{mt}$) binds to the small subunit of the ribosome displacing the large subunit during the initiation of protein biosynthesis. About half of the proteins in mitochondrial ribosomes have homologs in bacteria while the remainder are unique to the mitochondrion. To obtain information on the ribosomal proteins located near the $IF3_{mt}$ binding site, cross-linking studies were carried out followed by identification of the cross-linked proteins by mass spectrometry. $IF3_{mt}$ cross-links to mammalian mitochondrial homologs of the bacterial ribosomal proteins S5, S9, S10, and S18-2 and to unique mitochondrial ribosomal proteins MRPS29, MRPS32, MRPS36 and PTCD3 (Pet309) which has now been identified as a small subunit ribosomal protein. $IF3_{mt}$ has extensions on both the N- and C-termini compared to the bacterial factors. Cross-linking of a truncated derivative lacking these extensions gives the same hits as the full length $IF3_{mt}$ except that no cross-links were observed to MRPS36. IF3 consists of two domains separated by a flexible linker. Cross-linking of the isolated N- and C-domains was observed to a range of ribosomal proteins particularly with the C-domain carrying the linker which showed significant cross-linking to several ribosomal proteins not found in prokaryotes.

### Keywords

Initiation factor 3; mitochondria; Cross-linking; ribosome; ribosomal protein; protein synthesis; mammal

## 1. Introduction

Mammalian mitochondria synthesize thirteen polypeptides encoded by the mitochondrial genome. All of these proteins are subunits of the oligomeric complexes required for



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oxidative phosphorylation. The synthesis of these proteins is carried out by specialized ribosomes that associate with the inner membrane of the mitochondrion [1]. Mammalian mitochondrial ribosomes are 55S particles and consist of 28S small subunits (SSU) and 39S large subunits (LSU) [2]. The protein and RNA content of these particles is quite different from that of bacterial ribosomes. The 28S subunit contains approximately 29 proteins of which 14 are homologs of bacterial ribosomal proteins while 15 are classified as mitochondrial specific ribosomal proteins [3]. The 39S subunit contains about 50 proteins of which 28 are homologs of bacterial ribosomal proteins and 22 of which do not have bacterial homologs. The rRNAs in the mammalian mitochondrial ribosome are considerably shorter than bacterial rRNAs and have little primary sequence conservation with other rRNAs. Cryo-electron microscopy indicates that the core of the mitochondrial ribosome is decorated with many proteins that have no bacterial homologs [4]. Therefore, the interactions of the ribosome with translational factors are expected to show novel differences compared to those occurring in bacterial systems.

Two translational initiation factors have been identified in mammalian mitochondria. Initiation factor 2 ($IF2_{mt}$) stimulates the binding of fMet-tRNA to the P-site of the small subunit [5,6]. Mitochondrial initiation factor 3 ($IF3_{mt}$) promotes the dissociation of the mitochondrial ribosome [7] and reduces the binding of fMet-tRNA to the small subunit in the absence of mRNA [8]. No factor equivalent to IF1 has been observed in mammalian mitochondria and a short segment in $IF2_{mt}$ is thought to play the role of IF1 in this system [9,10].

$IF3_{mt}$ has a central region with homology to the bacterial factors with additional N-terminal and C-terminal extensions (Figure 1). Like *Escherichia coli* IF3, the homology domain is divided into two segments (N-terminal domain and C-terminal domain) separated by a flexible linker [11]. The roles of $IF3_{mt}$ and its terminal extensions and domains in initiation complex formation have been investigated [7,8,11–13]. The three-dimensional structure of $IF3_{mt}$ is still unknown but the N-domain has been modeled based on the structure of the N-domain of *Bacillus stearothermophilus* of IF3 and the NMR structure of the C-domain of mouse $IF3_{mt}$ without the C-terminal extension has been reported. The mature form of $IF3_{mt}$ (after removal of the predicted mitochondrial import signal) binds to the 28S subunit tightly with a Kd of about 30 nM. The C-terminal region binds almost as tightly to the small subunit with a Kd of about 60 nM [13]. This interaction is considerably stronger than that observed with the isolated C-domain of bacterial IF3 [14]. Unlike bacterial IF3, the N-terminal region of $IF3_{mt}$ can also bind independently to the 28S subunit with a Kd of 240 nM.

A number of biochemical and biophysical studies suggest that *E. coli* IF3 interacts with 30S subunits in the region of the platform, cleft, and the head, facing the 50S subunit [15–19]. The C-domain of IF3 is predicted to bind on the platform side of the 30S subunit near G700 in the 16S rRNA [20]. The platform region of the small subunit where the C-domain binds is one of the highly conserved regions between bacterial and mitochondrial ribosomes [4,21]. However, the edges of the platform do contain proteins that are specific to the mitochondrial ribosome, and it is possible that $IF3_{mt}$ makes contacts with these proteins. The N-domain may interact with the A790 region in close proximity to the P-site close to the head of the SSU although no consensus exists for the placement of the N-domain on the small subunit [16,20]. The head of the 28S subunit lacks a number of the bacterial ribosomal protein homologs and appears to contain several proteins specific to mitochondrial ribosomes. In the present study we have examined the nearest neighbor proteins of $IF3_{mt}$ on the 28S subunit using chemical cross-linking followed by mass spectrometry.

## 2. Materials and Methods

### 2.1 Materials

High purity grade chemicals were purchased from Sigma-Aldrich or Fisher Scientific. Dimethyl suberimidate (DMS) was purchased from Pierce. Bovine mitochondria and mitochondrial ribosomes (55S) and yeast [$^{35}$S]fMet-tRNA were prepared as described [22–24]. In order to purify small ribosomal subunit (28S), 55S ribosomes were prepared from crude mitochondrial ribosomes following the standard sucrose density gradient procedure [25]. The purified 55S ribosomes were dissociated into 28S and 39S subunits in buffer containing low $Mg^{2+}$ concentrations. The subunits were then separated on linear sucrose gradients as described [25]. Fractions corresponding to the 28S subunits were combined and collected by high speed centrifugation. These preparations were adjusted to 20 mM $Mg^{2+}$ to convert any contaminating 39S subunits into 55S monosomes. The 28S subunits were then purified on a further linear sucrose gradient at 20 mM $Mg^{2+}$ and again collected by high speed centrifugation. The 28S subunit preparations were greater than 95 % pure having only about 2–5 % contamination of 39S subunits.

Full length mature human $IF3_{mt}$, the C-domain with the linker region of $IF3_{mt}$ ($IF3_{mt}$(C+L)), the N-domain $IF3_{mt}$ with the linker ($IF3_{mt}$(N+L)), and a derivative of $IF3_{mt}$ lacking the N- and C-terminal extensions $IF3_{mt}$($IF3_{mt}$ΔNC) (Fig. 1) were cloned, expressed, and purified as described previously [8,12,13]. All of these derivatives carry a C-terminal $His_6$-tag.

### 2.2 Identification of ribosomal proteins near $IF3_{mt}$ and its domains by dimethyl suberimidate (DMS) cross-linking followed by mass spectrometry

A sample of 28S ribosomal subunits (20 pmol in 100 μL final volume) was mixed with 10-fold excess of $IF3_{mt}$ (2 μM) or with one of the IF3 derivatives at the following levels; a 10-fold excess of $IF3_{mt}$ΔNC (2 μM), a 15-fold molar excess (3 μM) $IF3_{mt}$(C+L) or a 20-fold excess (4 μM) $IF3_{mt}$(N+L) in cross-linking buffer (20 mM HEPES-KOH, pH 7.6, 50 mM KCl, 10 mM $MgCl_2$, and 1 mM Tris(2-carboxyethyl) phosphine hydrochloride, TCEP) and incubated for 20 min at room temperature. A 50-fold molar excess of DMS with respect to protein concentration was added to the above reaction mixtures and incubation was continued for 1 h at room temperature. The reaction was stopped by the addition of 5 μL of 100 mM Tris-HCl, pH 7.6. The cross-linked products were separated from free $IF3_{mt}$ by layering the reaction mixture on a 30% sucrose cushion (7 mL) in buffer containing 20 mM Tris-HCl, pH 7.6, 50 mM KCl, 5 mM $MgCl_2$, and 1 mM TCEP. The centrifuge tube was then filled with approximately 2 mL of the buffer above. Samples were subjected to centrifugation at 42,000 rpm for 12 h in a Beckman Ti70.1 rotor. The pellet was dissolved in 100 μL of Buffer A (20 mM HEPES-KOH, pH 7.6, 500 mM $NH_4Cl$, 1 mM TCEP and 8 M urea) and incubated for 30 min at room temperature. After incubation, 20 μL Ni-NTA resin in Buffer A was added to the solution followed by rocking for 1 h at 4 °C. The ribosomal proteins cross-linked to $IF3_{mt}$ or its domains bound to the Ni-NTA and unbound proteins were removed by three washes of 400 μL each with Buffer A and two washes of 400 μL each with Buffer B (20 mM HEPES-KOH, pH 7.6, 500 mM $NH_4Cl$, and 1 mM TCEP). The bound proteins were eluted with two aliquots of 50 μL each of Buffer C (20 mM HEPES-KOH, pH 7.6 and 250 mM imidazole) which were combined for subsequent analysis. A control sample for the identification of ribosomal proteins bound nonspecifically to the Ni-NTA resin was prepared in an identical manner except that $IF3_{mt}$ was omitted from the incubation mixture.

The cross-linked proteins from both the control sample and the $IF3_{mt}$ cross-linked samples were digested with 50 ng trypsin overnight at 37 °C [26]. The digested samples were lyophilized using a speed vacuum concentrator. The peptides were dissolved in 20 μL of 5%

acetonitrile and 0.1% formic acid solution and then subjected to tandem mass spectrometry analysis as described previously [26,27]. Tandem MS spectra obtained from peptide fragmentation by collision-induced dissociation (CID) were acquired using a capillary liquid chromatography - nanoelectrospray ionization - tandem mass spectrometry (LC/MS/MS) system that consisted of a Surveyor HPLC pump, a Surveyor Micro AS autosampler, and an LTQ linear ion trap mass spectrometer (ThermoFinnigan). The spectra (peaks) were analyzed using a site-licensed Mascot database searching program. Individual ion scores of greater than 35 indicate identity or extensive sequence homology ($p<0.05$) when MS/MS data are queried against a database consisting of mitochondrial ribosomal protein sequences [28]. A cut-off score of about 50 was used in the current analysis to ensure rigor in the identification of the cross-linked proteins.

## 3. Results

### 3.1. $IF3_{mt}$ derivatives used

As indicated above (Fig. 1) mammalian $IF3_{mt}$ contains a central region homologous to the bacterial factors. This region is divided into N- and C-domains connected by a linker region. The homology domain is preceded by an N-terminal extension of about 30 amino acids and followed by a C-terminal extension which is also about 30 residues long. The N- and C-terminal domains allow the protein to achieve an extended conformation spanning as much as 95Å (Fig. 1). In order to explore the ribosomal proteins at or near the $IF3_{mt}$ binding site on the mitochondrial 28S subunit, four different $IF3_{mt}$ derivatives were used; full length $IF3_{mt}$ (lacking only the predicted mitochondrial import signal), a derivative lacking the N- and C-terminal extensions ($IF3_{mt}\Delta NC$) and, therefore, consisting of the region homologous to the bacterial factors, the N-domain with the linker ($IF3_{mt}(N+L)$) or the C-domain with linker ($IF3_{mt}(C+L)$) (Fig. 1). The linker was included in the N- and C-domain constructs since it improves the binding of these regions to the small subunit [13].

### 3.2. Identification of 28S ribosomal proteins located at or near the binding site for $IF3_{mt}$

To identify ribosomal proteins near the $IF3_{mt}$ binding site, a cross-linking strategy was used (Fig. 2). In this approach, $IF3_{mt}$ or its derivatives was incubated with 28S subunits under conditions in which the majority of the subunits would have $IF3_{mt}$ bound [12]. $IF3_{mt}$ binds to the 28S subunit with a Kd of 20–30 nM. Removal of the N- and C-terminal extensions has no effect on the strength of this interaction [12]. $IF3_{mt}$ or its derivatives were cross-linked to the small subunit using DMS. The cross-linked complexes were separated from unbound $IF3_{mt}$ by sedimentation through a sucrose cushion. The complexes were solubilized using buffer containing 8M urea and $IF3_{mt}$ and ribosomal proteins cross-linked to $IF3_{mt}$ were selectively retained on Ni-NTA resins through the $His_6$-tag on $IF3_{mt}$. The proteins eluted from the Ni-NTA resin were digested with trypsin and identified by LC/MS/MS. The cross-linking experiment was repeated five independent times with different preparations of material to ensure the accurate identification of the proteins present. A summary of the results is presented in Table 1 with more detail provided in Tables 2 and 3.

Full-length $IF3_{mt}$ and the ΔNC derivative were cross-linked to a number of small subunit ribosomal proteins (Table 2). There were strong cross-links to 4 proteins with homologs in bacterial systems (MRPS5, MRPS9, MRPS10 and MRPS18-2). Peptides from MRPS18-2 were occasionally observed in control samples analyzed in the absence of $IF3_{mt}$ (Table 2) although many more peptides were consistently observed in samples cross-linked in the presence of $IF3_{mt}$. Hence, we believe that the cross-links to MRPS18-2 represent a meaningful location of this protein close to the $IF3_{mt}$ binding site. Four proteins without homologs in bacterial systems (MRPS29, MRPS32, MRPS36, and PTCD3) are also convincingly cross-linked to $IF3_{mt}$ (Table 2). Of these, only PTCD3 gave two peptides in

control samples (Table 2) and these were observed in only a few analyses. Hence, we believe that PTCD3 is located on the small ribosomal subunit near the binding site for $IF3_{mt}$. It should be noted that PTCD3 was originally identified as a pentatricopeptide repeat domain protein associated with the small subunit of the mammalian mitochondrial ribosome [29]. Decreasing the levels of PTCD3 reduces mitochondrial protein synthesis without effecting mRNA levels. The current work suggests that this protein is an integral component of the 28S subunit which may be located close to the head and platform of the subunit.

To determine which proteins on the 28S subunit are close to the binding region of the N- and C-domains of $IF3_{mt}$, cross-linking studies were carried out with the individual domains (Fig. 1). The linker improves the strength of the interaction between these domains and the small subunit and was retained in both of the constructs used. The C-domain with the linker (C+L construct) binds to the 28S subunit with a Kd of about 60 nM while the N+L binds with a Kd of 240 nM [13]. As indicated in Table 3, little cross-linking was observed to the N-terminal region. One peptide was observed from MRPS10 which was also observed cross-linking to full-length $IF3_{mt}$. The low level of cross-linking with this sample probably reflects its weaker binding to the 28S subunit. The C-domain with the linker was cross-linked to several ribosomal proteins with bacterial homologs including MRPS5, MRPS9, MRPS10, and MRPS18-2. All of these proteins were observed to be cross-linked to $IF3_{mt}$ or the ΔNC derivative. In addition, $IF3_{mt}$ (C+L) was cross-linked to several proteins which do not have homologs in bacterial systems including MRPS29, MRPS32, MRPS36, and PTCD3.

The specificity of the cross-linking observed was evaluated by examining the MS data for other ribosomal proteins (Supplementary Table S1). Of the approximately 30 proteins in the 28S subunit, no peptides were observed for 15 of them. Peptides were detected from six ribosomal proteins not listed in Table 1 but cross-linking to these proteins was not observed with any consistency and generally gave peptides with poor ion scores. Thus, we believe that the cross-linked proteins observed here represent proteins at or near the $IF3_{mt}$ binding site in the 28S subunit.

## 4. Discussion

Cross-linking studies using *E. coli* IF3 indicate that this factor is located close to a number of ribosomal proteins including S12 in the upper portion of the body, S2, S3, S7, S13 and S19 in the head and S11, S18 and S21 in the platform [30,31]. Dallas and Noller [16] using RNA cleavage by Fe(II)-derivatized IF3 have shown that the C-domain of this factor is located on the platform. They also suggest that the N-domain may be wedged between the platform and head close to the E-site.

No cross-links between $IF3_{mt}$ and ribosomal protein S12 located in the body of the SSU were observed despite this protein being a major cross-linking partner of bacterial IF3. This difference could be due to the size and high Lys and Arg content of the mitochondrial S12 which would make its detection by LC/MS/MS challenging. Rather, cross-links were observed to MRPS5 in the body of the small subunit. This protein forms part of the mRNA tunnel in the small subunit. Mitochondrial MRPS5 is about twice the size of its bacterial counterpart and may have regions exposed on the interface side of the small subunit.

A number of cross-links between bacterial IF3 and small subunit are observed to proteins located in the head of the small subunit. The contacts between IF3 and the head to the 30S subunit appear to be enriched in protein-protein interactions while considerable contacts between the C-domain of IF3 and the platform are meditated through RNA-protein interactions [16]. The head and the neck of the SSU have eight proteins S2, S3, S7, S9, S10, S13, S14 and S19 in the bacterial ribosome, and five (MRPS2, MRPS7, MRPS9, MRPS10

and MRPS14) of these have been retained in the mammalian mitochondrial ribosome. *E. coli* IF3 can be cross-linked to S2, S3, S7, S13 and S19 in the head region (Fig. 3A). Of these proteins, S2 is located on the back of the head close to the platform; S7 is found in the head just above the platform; S3 is largely on the back of the head and S13 and S19 are on the interface side of the head. It should be noted that a number of these proteins have finger-like extensions that penetrate a considerable distance into the structure of the subunit. Of the proteins found in the head of the small subunit that cross-link to bacterial IF3, S3, S13 and S19 are absent in the mammalian mitochondrial ribosome. Further, no cross-links are observed to S2, S3 or S7. These observations indicate that the contacts between bacterial IF3 and proteins of the small subunit are quite different than those observed with the mitochondrial factor.

In the case of $IF3_{mt}$, cross-links are observed with MRPS9 and MRPS10 in the head (Fig. 3B). Both MRPS9 and MRPS10 are about twice the size of their bacterial homologs. MRPS9 is located largely on the solvent side of the head but has a long extension that approaches the P-site. MRPS10 is also located primarily on the back of the head; however, the larger size of the mitochondrial homolog and the truncation of rRNA in the small subunit may allow this protein to interact with $IF3_{mt}$ if the factor is bound on the interface side of the small subunit (Fig. 3B). It should be noted the MRPS10 was the only ribosomal protein providing a clear cross-link to the N-domain of $IF3_{mt}$ suggesting that the N-terminal domain of this factor is located in contact with the head of the small subunit. This observation is in agreement with the proximity of *E. coli* IF3 near nucleotide 790 close to the decoding site on the small subunit [20].

The platform of the small subunit of bacterial ribosomes contains six proteins (S6, S8, S11, S15, S18, and S21). *E. coli* IF3 can be cross-linked to three of the platform proteins, S11, S18, and S21 (Fig. 3A). The platform of the mammalian mitochondrial ribosome is the most conserved region of the small subunit and has homologs of all of these proteins except S8 [3,21]. CryoEM studies [4] indicate that edges of the platform are decorated with ribosomal proteins specific for the mitochondrial ribosome. $IF3_{mt}$ and the C-terminal domain cross-link strongly to MRPS18-2 in the platform. Surprisingly, no cross-links are observed to other platform proteins. The lack of cross-linking to MRPS6 and MRPS21 could be due to the low molecular weights and high Lys and Arg contents of these proteins leading in peptides difficult to be detected by mass spectrometry after trypsin digestion. However, cross-links to MRPS11 and MRPS15 would, in principle have been readily detected if they had occurred. This observation and the lack of conserved cross-links to proteins in the head suggest that the contacts between $IF3_{mt}$ and the 28S subunit of the mitochondrial ribosome are quite different from the contacts between bacterial and the 30S subunit of the prokaryotic ribosome.

A number of cross-links were obtained between $IF3_{mt}$ and proteins that do not have homologs in bacterial ribosomes. The C-domain and full length $IF3_{mt}$ cross-linked to non-homolog proteins MRPS32 and MRPS36, therefore, these proteins may be located at the edge of the platform. Cross-links were also obtained with MRPS29. MRPS29 is also referred to as DAP3 and is believed to play a role in mitochondrially-mediated apoptosis [32–35].This cross-link was somewhat surprising since immune electron microscopy has suggested that MRPS29 is located on the solvent side of the small subunit. MRPS29 is large for a ribosomal protein (nearly 44 kDa) and may have extensions that penetrate a considerable distance from the body of the protein.

Of considerable interest are the strong cross-links between $IF3_{mt}$ and its C-domain to the protein designated PTCD3. This pentatricopeptide domain protein is known to associate with the small subunit of mitochondrial ribosomes [29]. Lowering PTCD3 levels in

osteosarcoma cells has been shown to decrease mitochondrial protein synthesis. Our recent analysis has shown that PTCD3 is a small subunit ribosomal protein in mammals (E. Koc, *manuscript in preparation*). The location of this protein in the mitochondrial small subunit is not known. However, its extensive cross-linking to $IF3_{mt}$ and mitochondrial mRNAs (*unpublished observations*) suggest that it may be at least partially located at the interface side of the 28S subunit.

**Highlights**

- Thirteen proteins are synthesized by a specialized protein biosynthetic system in mitochondria.
- Mitochondrial ribosomes have many proteins with no homologs in other systems.
- Ribosomal proteins at the binding site of initiation factor 3 were identified.
- IF3mt has very different contacts with the ribosome than observed with bacterial IF3.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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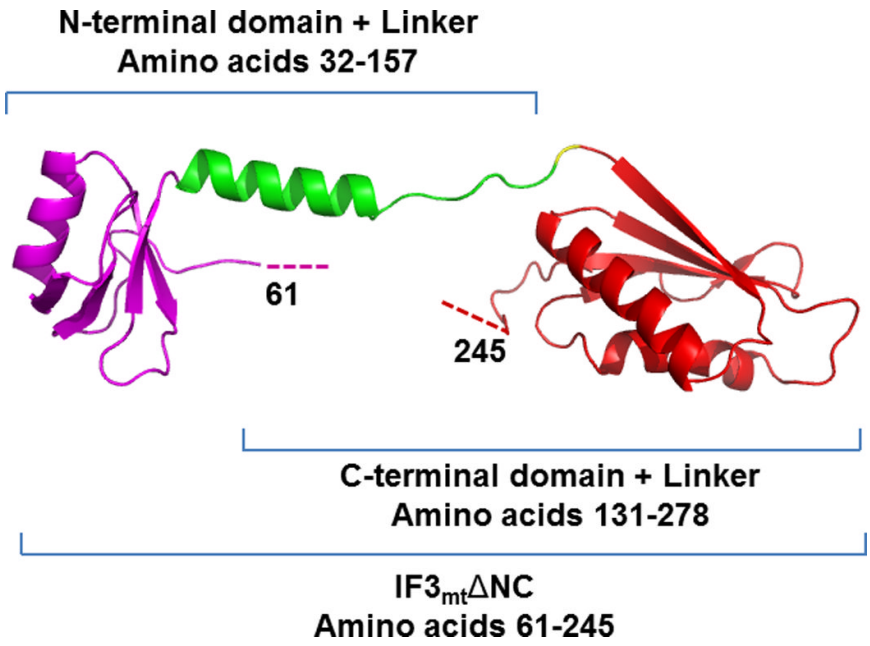


**Figure 1. Domain organization of bacterial IF3 and human IF3$_{mt}$ and its truncated C- and N-domains**

**A** three dimensional model of IF3$_{mt}$ was developed [7] based on the structure of the N-domain of *Bacillus stearothermophilus* of IF3 (PDB coordinates 1TIG) and the NMR structure of the C-domain of mouse IF3$_{mt}$ without the C-terminal extension (PDB coordinates 2CRQ). The figure shown represents the predicted structure of IF3$_{mt}$ΔNC (residues 61–245) since the N-and C-terminal extensions could not be modeled. The regions included in the domains are indicated above and below the model. Again these constructs contain the N-and C-terminal extensions which are not shown. The N-terminal domain contains residues 32–157 (including the linker) beginning with the first amino acid of the predicted mature form of the protein. The C-terminal construct contains resides 131–278 (including the linker) of IF3$_{mt}$. The dashed lines indicate the extensions which are not modeled.

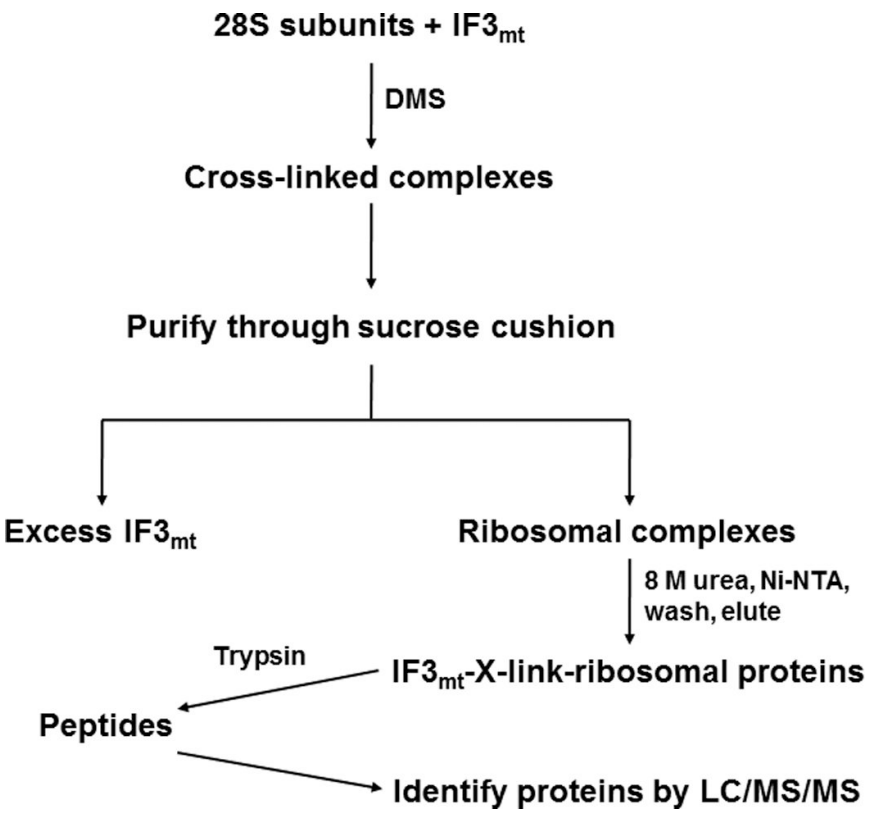


**Figure 2. Strategy used to identify ribosomal proteins near the $IF3_{mt}$ binding site**

$IF3_{mt}$ and its domains were incubated with 28S subunits and cross-linked to nearby proteins using DMS as described in Materials and Methods. Cross-linked complexes were purified by centrifugation through a sucrose cushion to remove excess free $IF3_{mt}$. The ribosomes were then denatured and ribosomal proteins cross-linked to $IF3_{mt}$ and its domains were recovered on Ni-NTA resin. Cross-linked proteins were digested with trypsin and identified by LC/MS/MS.

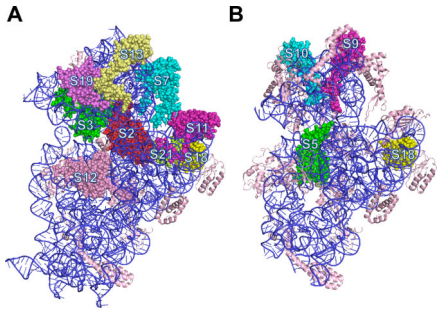


**Figure 3. Binding site of IF3 on the small ribosomal subunit**

(A) Model of the 30S ribosomal subunit (PDB 3R8O) with proteins cross-linking to *E. coli* IF3 shown as space-filled while other ribosomal proteins are shown as pink ribbons. The rRNA is shown as blue. (B) Representation of the bacterial 30S subunit showing the proteins cross-linking to $IF3_{mt}$ that have homologs in the mammalian mitochondrial 28S subunit. The regions of the rRNA missing in the mammalian mitochondrial ribosome have been manually removed from the coordinates of the subunit. Proteins cross-lining to $IF3_{mt}$ are space filled while other ribosomal proteins are shown as pink ribbons.

## Table 1

Summary of the ribosomal proteins cross-linked to $IF3_{mt}$ or its derivatives

| $IF3_{mt}$ Derivative | Cross-linking ribosomal proteins | Bacterial homolog |
|---|---|---|
| Full length | MRPS10 | S10 |
| | MRPS18-2 | S18 |
| | MRPS29 | None |
| | MRPS32 | None |
| | MRPS36 | None |
| | PTCD3 | None |
| ΔNC | MRPS5 | S5 |
| | MRPS9 | S9 |
| | MRPS10 | S10 |
| | MRPS18-2 | S18 |
| | MRPS29 | None |
| | MRPS32 | None |
| | PTCD3 | None |
| N-domain | MRPS10 | S10 |
| C-domain | MRPS5 | S5 |
| | MRPS9 | S9 |
| | MRPS10 | S10 |
| | MRPS18-2 | S18 |
| | MRPS29 | None |
| | MRPS32 | None |
| | MRPS36 | None |
| | PTCD3 | None |

## Table 2

Ribosomal proteins cross-linking to full-length and ΔNC $IF3_{mt}$.*

| **Full-length $IF3_{mt}$** | | | | |
|---|---|---|---|---|
| **Protein** | **Sequence** | **Score** | **m/z** | **Mr (expt)** |
| MRPS10 | NLPEGVAMEVTK | 65 | 644.7 | 1287.4 |
| | PVWETTPEEK | 58 | 672.5 | 1342.9 |
| | KPVWETTPEEKGDSKS | 84 | 909.3 | 1816.6 |
| | AVLDSYEYAFAVLAAK | 110 | 830.6 | 1659.1 |
| | DLTKPTITISDEPDLYK | 115 | 1026.3 | 2050.7 |
| | DLTKPTITISDEPDLYKR | 113 | 736.1 | 2205.4 |
| MRPS18-2 | EESGPPPESMPK | 64 | 643.1 | 1172.5 |
| | VPLTAPTEATSTEQAGPQSAL | 115 | 1035.7 | 2069.4 |
| | YLDSEEYHNR | 66 | 662.8 | 1323.7 |
| | DHGLLSYHIPQVEPR | 87 | 881.3 | 1760.7 |
| | LYQGHLREESGPPPESMPK | 80 | 1077.0 | 2152.1 |
| | ARDHGLLSYHIPQVEPR | 58 | 664.2 | 1989.7 |
| MRPS29 | NATDAVGIVLK | 59 | 550.5 | 1098.9 |
| | KAYLPQELLGK | 62 | 630.7 | 1259.4 |
| | KPALELLHYLK | 56 | 442.0 | 1323.1 |
| | GSPLAEVVEQGIMR | 73 | 751.1 | 1500.2 |
| | LLVAVDGVNALWGR | 57 | 743.2 | 1484.5 |
| MRPS32 | VELALTSDAR | 60 | 539.0 | 1076.0 |
| MRPS36 | SAGLPSHTSSISQHSK | 80 | 812.8 | 1623.7 |
| | LVSQEEIEFIQR | 78 | 746.2 | 1490.3 |
| | KLVSQEEIEFIQR | 92 | 810.5 | 1619.0 |
| | DNPKPNVSEVLR | 61 | 684.7 | 1367.4 |
| PTCD3 | ASSSPAQAVEVVK | 76 | 636.8 | 1271.6 |
| | VAVLQALASTVHR | 70 | 682.8 | 1363.6 |
| | LTADFTLSQEQK | 79 | 690.8 | 1379.6 |
| | NELLNEFMDSAK | 55 | 705.7 | 1409.4 |
| | DPDDDMFFQSAMR | 92 | 787.4 | 1572.8 |
| | DEGADIAGTEEVVIPK | 101 | 822.2 | 1642.5 |
| | LTADFTLSQEQK | 64 | 690.6 | 1379.2 |
| **ΔNC $IF3_{mt}$** | | | | |
| **Protein** | **Sequence** | **Score** | **m/z** | **Mr (expt)** |
| MRPS5 | VSGSVNMLSLTR | 93 | 633.1 | 1264.1 |
| | KDPEPEDEVPDIKLDWDDVK | 53 | 794.5 | 2380.4 |
| MRPS9 | LSDQDYAQFIR | 79 | 678.1 | 1354.3 |
| | TANAEAVVYGHGSGK | 68 | 730.6 | 1459.2 |
| | HLANMMGEDPETFTQEDVDR | 55 | 778.6 | 2332.8 |
| MRPS10 | NLPEGVAMEVTK | 60 | 645.0 | 1288.0 |
| | KPVWETTPEEK | 57 | 672.1 | 1342.2 |

| | | | | |
|---|---|---|---|---|
| | DLTKPTITISDEPDTLYKR | 75 | 735.6 | 2203.9 |
| MRPS18-2 | EESGPPPESMPK | 51 | 642.6 | 1283.1 |
| | YLDSEEYHNR | 72 | 662.8 | 1323.6 |
| | VPLTAPTEATSTEQAGPQSAL | 63 | 1034.9 | 2067.9 |
| | LYQGHLREESGPPPESMPK | 53 | 718.0 | 2151.0 |
| | RLYQGHLR | 51 | 348.2 | 1041.6 |
| | DHGLLSYHIPQVEPR | 51 | 880.7 | 1759.3 |
| | APPEDDSLPPIPVSPYEDEPWK | 59 | 1240.1 | 2478.2 |
| MRPS29 | NATDAVGIVLK | 82 | 550.7 | 1099.3 |
| | ESTEKGSPLAEVVEQGIMR | 50 | 687.1 | 2058.4 |
| | KPALELLHYLK | 56 | 442.3 | 1323.8 |
| | GSPLAEVVEQGIMR | 65 | 743.0 | 1484.0 |
| MRPS32 | VELALTSDAR | 68 | 537.7 | 1073.4 |
| | SEHLEQGPMIEQLSK | 94 | 863.1 | 1724.2 |
| PTCD3 | NELLNEFMDSAK | 61 | 705.5 | 1409.1 |
| | ASSSPAQAVEVVK | 54 | 636.6 | 1271.1 |
| | VAVLQALASTVHR | 76 | 682.4 | 1362.7 |
| | LTADFTLSQEQK | 68 | 690.7 | 1379.5 |
| | DPDDDMFFQSAMR | 84 | 787.9 | 1573.8 |
| | DEGADIAGTEEVVIPK | 61 | 821.6 | 1641.3 |

**−IF3$_{mt}$ control**

| **Protein** | **Sequence** | **Score** | **m/z** | **Mr (expt)** |
|---|---|---|---|---|
| MRPS18-2 | EESGPPPESMPK | 60 | 643.1 | 1284.3 |
| | YLDSEEYHNR | 65 | 663.5 | 1324.9 |
| | DHGLLSYHIPQVEPR | 85 | 881.4 | 1760.7 |
| | VPLTAPTEATSTEQAGPQSAL | 91 | 1035.6 | 2069.3 |
| | APPEDDSLPPIPVSPYEDEPWK | 91 | 1239.8 | 2477.7 |
| PTCD3 | VAVLQALASTVHR | 59 | 682.9 | 1363.8 |
| | NELLNEFMDSAK | 68 | 706.3 | 1410.6 |

*Many of the peptides listed in the sample cross-linked to IF3$_{mt}$ were observed in multiple samples. They are listed only once for simplicity.

## Table 3

Cross-linking to the N- and C-terminal domains of $IF3_{mt}$.*

| **N-domain + Linker** | | | | |
|---|---|---|---|---|
| **Protein** | **Sequence** | **Score** | **m/z** | **Mr(expt)** |
| MRPS10 | DLTKPTITISDEPDTLYKR | 61 | 736.1 | 2205.2 |
| **C-domain + Linker** | | | | |
| **Protein** | **Sequence** | **Score** | **m/z** | **Mr(expt)** |
| MRPS5 | LIGIKDMYAKVSGSVNMLSLTR | 48 | 810.8 | 2429.3 |
| | KDPEPEDEVPDIKLDWDDVK | 63 | 794.2 | 2379.7 |
| MRPS9 | LSDQDYAQFIR | 70 | 678.2 | 1354.3 |
| | WLIKEELEEMLVEK | 74 | 596.9 | 1787.7 |
| | HLANMMGEDPETFTQEDVDR | 63 | 778.8 | 2333.5 |
| MRPS10 | LSVLVK | 51 | 329.5 | 657.0 |
| | NLPEGVAMEVTK | 67 | 644.8 | 1287.6 |
| | AVLDSYEYFAVLAAK | 86 | 830.6 | 1659.3 |
| | DLTKPTITISDEPDTLYKR | 92 | 735.7 | 2204.1 |
| | LEQLPEHIKKPVWETTPEEK | 51 | 810.9 | 2429.8 |
| MRPS18-2 | PLTAPTEATSTEQAGPQSAL | 107 | 1035.6 | 2069.2 |
| | YLDSEEYHNR | 60 | 662.9 | 1323.8 |
| | DHGLLSYHIPQVEPR | 72 | 880.6 | 1759.1 |
| | VPLTAPTEATSTEQAGPQSAL | 70 | 690.2 | 2067.6 |
| | APPEDDSLPPIPVSPYEDEPWK | 61 | 827.9 | 2480.6 |
| MRPS29 | KAYLPQELLGK | 51 | 630.3 | 1258.5 |
| | KPALELLHYLK | 77 | 441.9 | 1322.6 |
| | FDQPLEASIWLK | 63 | 723.7 | 1445.3 |
| | GSPLAEVVEQGIMR | 60 | 743.0 | 1484.1 |
| | KGSPLAEVVEQGIMR | 8 | 687.1 | 2058.3 |
| MRPS32 | SEHLEQGPMIEQLSK | 80 | 863.1 | 1724.2 |
| MRPS36 | SAGLPSHSSVISQHSK | 37 | 542.0 | 1623.1 |
| | LVSQEEIEFIQR | 70 | 746.5 | 1491.0 |
| | KLVSQEEIEFIQR | 78 | 810.2 | 1618.4 |
| | SAGLPSHTSSISQHSK | 45 | 812.8 | 1623.6 |
| PTCD3 | PQIWK | 50 | 579.2 | 1156.3 |
| | VAVLQALASTVHR | 71 | 682.7 | 1363.5 |
| | LTADFTLSQEQK | 69 | 690.5 | 1379.1 |
| | NELLNEFMDSAK | 62 | 705.8 | 1409.5 |
| | SDLKEEILMLMAR | 92 | 774.6 | 1547.3 |
| | DPDDDMFFQSAMR | 91 | 787.2 | 1572.4 |
| | GSSLIIYDIMDEITGK | 73 | 877.6 | 1753.3 |
| | AHTQALSMYTELLNNR | 84 | 931.6 | 1861.2 |
| | DLELAYQVHGLLNTGDNR | 95 | 1014.4 | 2026.7 |
| | TFSPKDPDDDMFFQSAMR | 68 | 1068.1 | 2134.2 |

| |
|---|
| **Control for Domains** |
| No peptides observed above the cut-off score set at 50 |

*Many of the peptides listed in the sample cross-linked to $IF3_{mt}$ were observed in multiple samples. They are listed only once for simplicity.