Role of the N- and C-terminal extensions on the activity of mammalian mitochondrial translational initiation factor 3

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ABSTRACT

Mammalian mitochondrial translational initiation factor 3 (IF3mt) promotes initiation complex formation on mitochondrial 55S ribosomes in the presence of IF2mt, fMet-tRNA and poly(A,U,G). The mature form of IF3mt is predicted to be 247 residues. Alignment of IF3mt with bacterial IF3 indicates that it has a central region with 20–30% identity to the bacterial factors. Both the N- and C-termini of IF3mt have extensions of ~30 residues compared with bacterial IF3. To examine the role of the extensions on IF3mt, deletion constructs were prepared in which the N-terminal extension, the C-terminal extension or both extensions were deleted. These truncated derivatives were slightly more active in promoting initiation complex formation than the mature form of IF3mt. Mitochondrial 28S subunits have the ability to bind fMet-tRNA in the absence of mRNA. IF3mt promotes the dissociation of the fMet-tRNA bound in the absence of mRNA. This activity of IF3mt requires the C-terminal extension of this factor. Mitochondrial 28S subunits also bind mRNA independently of fMet-tRNA or added initiation factors. IF3mt has no effect on the formation of these complexes and cannot dissociate them once formed. These observations have lead to a new model for the function of IF3mt in mitochondrial translational initiation.

INTRODUCTION

The synthesis and assembly of the oligomeric complexes in mitochondria involved in electron transport and ATP synthesis require genetic information contained in both the nuclear and mitochondrial genomes. Limited information is available on the mechanism by which the mitochondrially-encoded components in these complexes are synthesized and assembled into the oligomeric complexes in the inner membrane of mitochondria. A number of interesting features distinguish the protein synthesizing system of mammalian mitochondria from other translational systems. Of particular interest is the observation that the mRNAs in this organelle have an almost complete lack of 5' and 3'-untranslated nucleotides. The start codon is generally located within a few nucleotides of the 5' end of the mRNA (1,2). Thus, a Shine/Dalgarino interaction between the mRNA and the 16S rRNA such as observed in prokaryotes is not used in mammalian mitochondrial protein synthesis. Mammalian mitochondrial ribosomes have low sedimentation coefficients (~55S) and consist of 28S and 39S subunits (3). Animal mitochondrial ribosomes are 31% RNA and 69% protein. In contrast, bacterial ribosomes consist of ~67% RNA and 33% protein (4,5).

In bacteria, three translational initiation factors, initiation factors 1, 2 and 3 (IF1, IF2, and IF3), are required for initiation (6–8). No homolog of IF1 has been detected in mammalian mitochondrial systems. However, mitochondrial initiation factor 2 (IF2mt), which promotes the binding of fMet-tRNA to the small subunit of mitochondrial ribosomes has been cloned and characterized (9–15). Recently, the mitochondrial homolog of initiation factor 3 (IF3mt) has been cloned and expressed. In bacterial protein synthesis initiation factor 3 has been assigned a number of discrete functions including (i) dissociation of ribosomes (7,16); (ii) increasing the forward rate constant for codon:anticodon interaction at the P-site (17); (iii) dissociation of fMet-tRNA at AUG codons at the 5' end of leaderless mRNAs (18); (iv) proofreading the selection of the initiator tRNA and an AUG codon at the P-site (19–21) and (v) adjusting the position of the mRNA on the small subunit from a stand-by position to the decoding position (22).

The mature form of IF3mt lacking the predicted mitochondrial import signal, is active in initiation complex
MATERIALS AND METHODS

Materials

Oligonucleotides used for mutagenesis were synthesized at Nucleic Acid Core Facility at the University of North Carolina, Chapel Hill. Bovine mitochondrial 55S ribosomes were prepared as described (29). Mitochondrial 28S and 39S subunits were purified on sucrose gradients (30). Escherichia coli ribosomes were prepared from E. coli W (31,32) and tight couples were collected from a sucrose gradient in the presence of 5 mM MgCl₂ (33). Bovine IF2 mt and E. coli initiation factors were prepared as described (13,23,32). E. coli IF2 was also prepared from an expression construct providing a mixture of the α and β forms of IF2 (A. C. Spencer and L. L. Spremulli, unpublished data). Yeast [³⁵S]Met-tRNA and [¹⁴C]Phe-tRNA were prepared and the [¹⁴C]Phe-tRNA was acetylated as described (32,34). A transcript encoding subunit 2 of bovine cytochrome oxidase was prepared by in vitro transcription (35–37).

Cloning and expression of IF3 mt deletion derivatives

The construct carrying the N-terminal deletion was amplified by PCR using the mature IF3 mt cDNA as template (23), the forward primer GGGAATTCCATATGACCAAGGAAAAGAGA and the reverse primer CCGGGATCCGCCTCGAGCTGATGCAGAACAT. Deletion of the C-terminal extension was carried out using the forward primer CCGGGATCCGCCTCGAGCTGATGCAGAACAT and the reverse primer CCGGGATCCGCCTCGAGCTGATGCAGAACAT and the reverse primer CCGGGATCCGCCTCGAGCTGATGCAGAACAT and the reverse primer CCGGGATCCGCCTCGAGCTGATGCAGAACAT. The double deletion of the N- and C-terminal extensions was obtained using the forward primer GGGAATTCCATATGACCAAGGAAAAGAGA and the reverse primer CCGGGATCCGCCTCGAGCTGATGCAGAACAT and the reverse primer CCGGGATCCGCCTCGAGCTGATGCAGAACAT. These PCR products were digested with Ndel and XhoI and cloned into pET-21(+) (Novagen). This vector provides a sequence encoding six His residues (His-tag) at the C-terminus. The PCR products were transformed into E. coli ER2267 and the nucleotide sequence of the inserted DNA was confirmed. The plasmids were subsequently transformed into E. coli BL21(DE3) for expression.

Purification of IF3 mtFL and deletion derivatives using Ni-NTA

The full-length mature form of human mitochondrial initiation factor 3 (IF3 mtFL) was expressed in E. coli as described (23). The His-tagged protein was purified on Ni-NTA and on S-Sepharose. This later step separates the IF3 mtFL from a 19 kDa degradation product. Ni-NTA preparations of the deletion derivatives did not contain this degradation product and did not require further purification. The N-terminus of the double truncated derivative (IF3 mtΔNC) was sequenced using Edman degradation to ensure that the correct N-terminus was present on the expressed protein. Protein concentrations were determined using the Bradford assay with BSA as a standard (BioRad).

Initiation complex formation assay for IF3 mtFL and its deletion derivatives

The activities of IF3 mtFL and its deletion derivatives were determined by measuring their abilities to stimulate the
binding of $[^{35}S]$fMet-tRNA to either E. coli or mitochondrial ribosomes in filter-binding assays essentially as described previously (23). Reactions (100 μl) on E. coli ribosomes contained 50 mM Tris–HCl, pH 7.6, 1 mM DTT, 80 mM NH₄Cl, 5 mM MgCl₂, 0.25 mM GTP, 12.5 μg poly(A,U,G), 0.06 μM $[^{35}S]$fMet-tRNA, 0.25 μM IF2ₘt, 0.24 μM E. coli 7OS tight couples and varying amounts of IF3ₘt or its deletion derivatives as indicated. For initiation complex formation assays on mitochondrial ribosomes, reaction mixtures (100 μl) contained 50 mM Tris–HCl, pH 7.6, 1 mM DTT, 0.1 mM spermine, 35 mM KCl, 4.5 mM MgCl₂, 0.25 mM GTP, 1 mM DTT, 12.5 μg poly(A,U,G), 0.42 μM IF2ₘt, 0.06 μM $[^{35}S]$fMet-tRNA, 0.05 μM mitochondrial 55S ribosomes and varying amount of IF3ₘt or its deletion derivatives. Reaction mixtures were incubated for 15 min at 37°C then analyzed using a nitrocellulose filter-binding assay as described (9).

Proofreading assays

This assay has been modified from that described in (38,39) for E. coli IF3. A complex carrying $[^{14}C]$AcPhe-tRNA bound to E. coli 30S subunits [AcPhe-tRNA:poly(U):30S] was formed by incubation of activated E. coli 30S subunits (0.08 μM), poly(U) (10 μg) and $[^{14}C]$AcPhe-tRNA (0.3 μM) in a reaction mixture (50 μl) containing 50 mM Tris–HCl, pH 7.6, 0.1 mM spermine, 1 mM DTT, 50 mM NH₄Cl and 15 mM MgCl₂. After incubation at 37°C for 30 min, various amounts of IF3ₘt or buffer (50 μl) were added to the mixture and the incubation was continued for an additional 5 min at 37°C. The mixtures were then diluted 25-fold with pre-warmed dilution buffer (50 mM Tris–HCl, pH 7.6, 1 mM DTT, 50 mM NH₄Cl and 15 mM MgCl₂). The diluted reaction mixtures were incubated for 5 min at 37°C. The amount of initiation complex remaining was determined by a nitrocellulose filter-binding assay. A similar assay was carried out using a complex formed with E. coli 30S subunits (0.08 μM), poly(A,U,G) (12.5 μg) and $[^{35}S]$fMet-tRNA (0.2 μM).

A complex containing $[^{14}C]$AcPhe-tRNA bound to 28S subunits [AcPhe-tRNA:poly(U):28S] was formed by incubation of mitochondrial 28S subunits (0.2 μM) with poly(U) (10 μg) and $[^{14}C]$AcPhe-tRNA (0.3 μM) in a reaction mixture (50 μl) containing 50 mM HEPES–KOH, pH 7.8, 1 mM DTT, 0.1 mM spermine, 35 mM KCl and 25 mM MgCl₂. After incubation at 27°C for 30 min, various amounts of IF3ₘt or compensating buffer (50 μl) were added to the mixture and incubated for 5 min at 27°C. The mixture was then diluted 50-fold with pre-warmed dilution buffer (50 mM HEPES–KOH, pH 7.8, 1 mM DTT, 0.1 mM spermine 35 mM KCl and 25 mM MgCl₂). The reaction mixtures were incubated for an additional 15 min at 27°C. The amount of initiation complex remaining was determined by a nitrocellulose filter-binding assay. Attempts were also made to form a similar complex with mitochondrial 28S subunits (0.2 μM), poly(A,U,G) (12.5 μg) and $[^{35}S]$fMet-tRNA (0.2 μM) and MgCl₂ concentrations ranging from 15 to 50 mM (Results).

Effect of IF3ₘt on the binding of fMet-tRNA to 28S subunits in the presence or absence of mRNA

Reaction mixtures (100 μl) contained various amounts of IF3ₘt and 50 mM Tris–HCl, pH 7.6, 35 mM KCl, 0.1 mM spermine, 1 mM DTT, 7.5 mM MgCl₂, 0.25 mM GTP, 1.25 mM phosphoenolpyruvate, 0.7 U pyruvate kinase, 0.06 μM $[^{35}S]$fMet-tRNA, 0.14 μM IF2ₘt, 0.068 μM 28S subunits and, where indicated, 12.5 μg poly(A,U,G). Reaction mixtures were incubated for 20 min at 27°C and the amount of $[^{35}S]$fMet-tRNA bound to the 28S subunit was determined using a filter-binding assay (9).

Sucrose gradient analysis of initiation complexes

Initiation complexes (200 μl) were assembled as described above containing 0.05 μM 28S subunits or 0.066 μM 39S subunits. Samples were incubated for 20 min at 27°C then applied to a 5 ml sucrose gradient (10–30% sucrose in 50 mM Tris–HCl, pH 7.6, 40 mM KCl, 7.5 mM MgCl₂ and 2 mM DTT). The gradients were subjected to centrifugation for 1 h 45 min at 48 000 r.p.m. in a Beckman SW50.1 rotor. Following centrifugation, gradients were fractionated on an Isco gradient fractionator at a flow rate of 0.8 ml/min. Fractions (0.2 ml) were collected and filtered through nitrocellulose membranes, dried and counted.

RESULTS

Role of the extensions on IF3ₘt in initiation complex formation

Mammalian IF3ₘt has N-terminal and C-terminal extensions just over 30 residues long surrounding the central region homologous to bacterial IF3. To assess the importance of these extensions on the activity of IF3ₘt, three deletion derivatives were constructed (Figure 1A). One derivative lacked the N-terminal extension (IF3ₘtΔN). A second lacked the C-terminal extension (IF3ₘtΔC) while the third lacked both extensions (IF3ₘtΔNC). These derivatives expressed well and were purified from E. coli (Figure 1B).

The activities of the full-length mature IF3ₘt (IF3ₘtFL) and the deletion derivatives were tested in initiation complex formation on bovine mitochondrial 55S ribosomes (Figure 2A). Interestingly, deletion of either the N-terminal or the C-terminal extension in IF3ₘt increased the activity of the factor slightly in promoting the binding of fMet-tRNA to mitochondrial ribosomes. This observation is reminiscent of the effects of removing the long extensions observed in E. gracilis IF3chl. However, removal of the extensions on IF3chl has a significantly larger positive effect (3.5- to 4-fold) on the activity of this factor in initiation complex formation. Removal of the extensions on IF3ₘt also increased the activity of this factor to a small extent in initiation complex formation with the mRNA for subunit 2 of cytochrome oxidase (Figure 2B). Finally, deleting the extensions on IF3ₘt had a slightly positive effect when this factor was tested in initiation complex formation on E. coli 70S ribosomes (Figure 2C) indicating that the effect is not dependent on the interaction of IF3ₘt with mitochondrial ribosomes. Structural information on bacterial IF3 (Figure 2D) suggests that the extensions in IF3ₘt could be positioned to interact with the linker region (Figure 1A). Since the linker is believed to play an important role in the binding of IF3ₘt to the ribosome (27), removal of these extensions may actually increase the binding of IF3ₘt to the small subunit slightly. In this context, it should be noted that the deletion of both extensions on IF3chl increases the
affinity of IF3 for chloroplast 30S subunits ~100-fold (28). Alternatively, the extensions in IF3 may be playing a different role in initiation complex formation.

**Proofreading activity of IF3**

One of the roles assigned to IF3 in initiation is to proofread the selection of fMet-tRNA and the AUG codon in the P-site (20,38,40). This effect appears to occur through conformational changes in the subunit rather than by a direct interaction of IF3 with the fMet-tRNA bound at the P-site (41). One of the classical methods for measuring the proofreading function of IF3 is to test its ability to promote the dissociation of AcPhe-tRNA bound to the small subunit in response to poly(U). When these experiments are carried out with *E. coli* 30S subunits, the AcPhe-tRNA is bound non-enzymatically (in the absence of IF2) using an elevated concentration of Mg2++. As indicated in Figure 3, *E. coli* IF3 effectively dissociates AcPhe-tRNA bound to the 30S subunit but does not dissociate fMet-tRNA bound to the small subunit. When IF3 is tested in this assay, it is quite anemic in dissociating the AcPhe-tRNA (Figure 3). As expected, it does not promote the release of fMet-tRNA. Deletions of both N- and C-terminal extensions had no effect on the response of IF3 in this assay (data not shown). In contrast, deletion of these extensions improved that ability of IF3 to dissociate the 30S:AcPhe-tRNA:poly(U) complex which...
correlated with the improved binding of this factor to chloroplast 30S subunits upon deletion of the extensions (28).

Initial studies to examine the proofreading activity of IF3mt on mitochondrial 28S ribosomal subunits provided some surprises. Non-enzymatic binding of AcPhe-tRNA could be obtained on 28S subunits at 15–25 mM Mg\(^{2+}\) and IF3mt was active in destabilizing these complexes (Table 1). However, the positive control for these experiments should be the stability of complexes formed with fMet-tRNA and the AUG codon in the presence of IF3mt. Non-enzymatic binding of fMet-tRNA occurs readily in the E. coli system as the concentration of Mg\(^{2+}\) is raised. However, essentially no non-enzymatic binding of fMet-tRNA could be detected with 28S subunits even at Mg\(^{2+}\) ion concentrations as high as 40 mM (Table 1). The lack of non-enzymatic binding of fMet-tRNA prevented a true assessment of the ability of IF3mt to proofread initiation complex formation in the mitochondrial system.

**Effect of IF3mt on the binding of fMet-tRNA to 28S subunits in the absence of mRNA**

Control experiments used in analyzing the low numbers obtained in the proofreading experiments lead to the realization that a significant amount of fMet-tRNA binds to mitochondrial 28S subunits in the absence of mRNA. This binding is completely dependent on the presence of IF2mt (data not shown). Interestingly, the message-independent binding of fMet-tRNA to 28S subunits is destabilized by IF3mt (Figure 4). In contrast, IF3mt has no effect on the binding of fMet-tRNA to 28S subunits in the presence of mRNA. The destabilization of fMet-tRNA binding to mitochondrial 28S subunits is in contrast to observations made in E. coli in which IF3 is reported to stabilize the IF2-dependent binding of fMet-tRNA to 30S subunits in the absence of mRNA (42). We have retested this effect using E. coli 30S subunits and have observed that there is some mRNA-independent binding to these small subunits (~10% of the level of binding observed in the presence of poly(A, U, G)]. And, as reported, E. coli IF3 stimulated this binding ~2-fold (data not shown). Interestingly, E. coli IF3 also stimulates the mRNA-independent binding of fMet-tRNA to mitochondrial 28S subunits ~2-fold. Thus, it behaves quite differently in this assay than does IF3mt.

The destabilization of message-independent binding to mitochondrial 28S subunits by IF3mt is quite rapid and is essentially complete within ~20 s (data not shown). IF3mt was able to dissociate fMet-tRNA pre-bound to the 28S subunit in the absence of mRNA indicating that it does not have to be present on the small subunit prior to fMet-tRNA binding to carry out this activity.

Sucrose gradient analysis was used to assess whether the fMet-tRNA bound to mitochondrial 28S subunits in the absence of mRNA could be incorporated into 55S monosomes. As indicated in Figure 5A, fMet-tRNA could be observed bound to 28S subunits when reaction mixtures were incubated in the absence of IF3mt. The presence of IF3mt resulted in a substantial decrease in the amount of fMet-tRNA bound. These experiments confirm that fMet-tRNA binding was observed in the filter-binding assay. As expected, no fMet-tRNA binding was observed to mitochondrial 39S subunits in either the presence or absence of IF3mt (data not shown). About 25% of the fMet-tRNA bound in the absence of mRNA was observed in the 55S region of the gradient after 39S subunits were added indicating that at least a portion of this material could be chased into 55S complexes (Figure 5B). No fMet-tRNA was observed remaining in the 28S region of the gradient. Nitrocellulose filter-binding assays suggest that there is no loss of the fMet-tRNA bound in the absence of mRNA when 39S subunits are added suggesting that the lower yield of 55S complexes observed in the sucrose gradients arises from the reduced stability of these complexes compared with fMet-tRNA bound to 28S subunits directly. The loss of a portion of the fMet-tRNA bound to the subunit upon formation of 55S complexes probably reflects the release of IF2mt upon subunit joining.

| Amino acid-tRNA | aminoacyl-tRNA bound to 28S (pmol) |
|-----------------|-----------------------------------|
| {\[^{14}C\]}AcPhe-tRNA | 0.83 no IF3 added, 0.40 with IF3 added |
| {\[^{35}S\]}fMet-tRNA | 0.007 no IF3 added, 0.003 with IF3 added |

The 28S:poly(U):{\[^{14}C\]}AcPhe-tRNA or 28S:poly(A, U, G):{\[^{35}S\]}fMet-tRNA complexes were formed non-enzymatically and the effect of adding IF3mt was assessed as described in Materials and Methods. A blank representing the retention of label on the filters in the absence of subunits (~0.16 pmol, 80–100 c.p.m., for {\[^{14}C\]}AcPhe-tRNA and ~0.02 pmol for {\[^{35}S\]}fMet-tRNA) has been subtracted from each value.

**Table 1. Non-enzymatic binding of {\[^{14}C\]}AcPhe-tRNA and {\[^{35}S\]}fMet-tRNA to mitochondrial 28S subunits in the presence and absence of IF3**
Since destabilization of mRNA-independent binding of fMet-tRNA to the small subunit is observed in the mammalian mitochondrial system but not in the prokaryotic system, the effects of the N- and C-terminal extensions on IF3mt on this activity were examined. As indicated in Figure 4, both the full-length factor and the derivative lacking the N-terminal extension were active in promoting the release of fMet-tRNA bound to the small subunit in the absence of mRNA. However, when the C-terminal extension was deleted, no destabilization of the fMet-tRNA bound to the small subunit in the absence of mRNA was observed (Figure 4). Deletion of both extensions gave a result identical to that observed when the C-terminal extension alone was deleted (data not shown). This observation suggests that this unusual activity of IF3mt requires the C-terminal extension and suggests that this extension developed on the mammalian mitochondrial factor in order to promote the dissociation of fMet-tRNA bound to the 28S subunit prior to mRNA binding.

The mitochondrial 28S subunit has the ability to bind mRNAs in a sequence independent manner in the absence of any added factors (35,43). The effect of IF3mt on this interaction was tested by monitoring the formation of the complex between 28S subunits and labeled mRNA for subunit 2 of cytochrome oxidase using a nitrocellulose filter-binding assay. IF3mt had no effect on the direct binding of mRNA to 28S subunits (data not shown) indicating that this complex can form in the presence of IF3mt and remains stable in its presence.

DISCUSSION
One of the classical features of bacterial IF3 is the ability to proofread the selection of fMet-tRNA and an AUG (or GUG) codon at the P-site during initiation. Detailed studies of the discrimination of IF3 against non-canonical initiation codons using tRNAs with the characteristic features of the initiator tRNA indicate that this factor recognizes primarily codon-anticodon interactions, at least at the second and third positions of the codon (20). The results reported here leave open the question of whether IF3mt has a proofreading function comparable with that observed with E.coli IF3. One might even question whether proofreading is important in the animal mitochondrial translational system with its limited repertoire of mRNAs to translate. Certain differences must apply to the mitochondrial system since both AUG and AUA serve as methionine codons in this organelle. In humans, 3 of the 13 translational start sites use AUA as the start codon. The basis for the ability of the mammalian fMet-tRNA^Met to read the AUA codon is unclear although it has been postulated that the minor base 5-formyl cytidine has a critical role to play in decoding the AUA triplet (44). In humans, mice and presumably several other mammals, AUU also serves as a start codon for at least one mitochondrial mRNA (45,46).

The same tRNA^Met is used for both initiation and elongation in mammalian mitochondria. This tRNA has retained the classical set of three G:C base pairs at the bottom of the anticodon stem, a characteristic of initiator tRNAs (48,49). The three consecutive G:C pairs are critical for the binding of the initiator tRNA to the P-site during initiation and the anticodon stem is examined by IF3 during translational initiation (19,50). Interestingly, tRNA^Met is the only mammalian mitochondrial tRNA characterized by three consecutive G:C base pairs at the bottom of the anticodon stem suggesting that this feature remains important for the selection of this tRNA for binding to the P-site during initiation (51).

One of the most unusual observations emerging from these studies is that IF3mt has the ability to dissociate the IF2mt-dependent binding of fMet-tRNA to the 28S subunit in the absence of mRNA. Our current working hypothesis to account for this activity is illustrated by the model in Figure 6. In this model, premature binding of fMet-tRNA in the presence of IF2mt would lead to an unproductive complex. IF3mt dissociates this unproductive complex or prevents its formation. In the productive pathway, mRNA binds to the 28S subunit but the subunit is positioned randomly on the message (Step 1). The basis for this idea emerges from the observation that 28S ribosomal subunits bind mRNAs quite tightly (Kd of 25 nM at 50 mM KCl). This binding occurs randomly on the mRNA (35,43). IF3mt is postulated to alter the position of the mRNA, promoting the positioning of the 5' start codon into the P-site.

**Figure 5.** Effect of the addition of 39S subunits on the mRNA-independent binding of [35S]fMet-tRNA formed in the presence and absence of IF3mt. (A) The 28S subunits were incubated with [35S]fMet-tRNA and IF2mt in the absence (closed circles) or presence (open circles) of IF3mt. Reaction mixtures were analyzed by sucrose density gradient centrifugation and the position of the [35S]fMet-tRNA was located by filtering appropriate fractions as described in Materials and Methods. (B) [35S]fMet-tRNA binding was initially carried out with 28S subunits in the presence of IF2mt but in the absence of mRNA. Reactions mixtures were prepared in the absence (closed circles) or presence (open circles) of IF3mt. Following assembly of these complexes, 39S subunits (0.066 μM) were added and the incubation was continued for an additional 5 min at 27°C. The resulting complexes were then analyzed on sucrose gradients.
This idea has precedents in bacterial initiation in which the 5′-untranslated region near the Shine/Dalgarino sequence lies at the junction of the platform and the head of the small subunit (52) (Step 2). IF3 binding near the platform promotes the rearrangement of the mRNA facilitating the correct placement of the AUG start codon in the P-site (22,53). Following the correct positioning of the mRNA, IF2ₘₜ promotes the binding of fMet-tRNA (Step 3). Finally, the 39S subunit joins this complex leading to the release of the initiation factors and the formation of the 55S initiation complex (Step 4).

The data presented here indicate that the N-terminal and C-terminal extensions on IF3ₘₜ are not essential for the activity of this factor in promoting initiation complex formation on mitochondrial 55S ribosomes. However, the C-terminal extension appears to be essential for allowing IF3ₘₜ to dissociate fMet-tRNA bound in the absence of mRNA. In the bacterial system, the order of binding of mRNA and fMet-tRNA to the small subunit appears to be random. Either will also bind to 30S subunits in the absence of the other although with less stability (7,54). We believe that the evolution of the C-terminal extension on mammalian IF2ₘₜ arose as a means to create an ordered pathway for the binding of mRNA prior to the binding of fMet-tRNA during initiation.

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