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Review

# Mitochondrial translation initiation machinery: Conservation and diversification☆

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ABSTRACT

The highly streamlined mitochondrial genome encodes almost exclusively a handful of transmembrane components of the respiratory chain complex. In order to ensure the correct assembly of the respiratory chain, the products of these genes must be produced in the correct stoichiometry and inserted into the membrane, posing a unique challenge to the mitochondrial translational system. In this review we describe the proteins orchestrating mitochondrial translation initiation: bacterial-like general initiation factors mIF2 and mIF3, as well as mitochondria-specific components – mRNA-specific translational activators and mRNA-nonspecific accessory initiation factors. We consider how the fast rate of evolution in these organelles has not only created a system that is divergent from that of its bacterial ancestors, but has led to a huge diversity in lineage specific mechanistic features of mitochondrial translation initiation among eukaryotes.


## 1. Introduction

The mitochondria of eukaryotic cells provide energy via the process of oxidative phosphorylation, perform fatty acid, heme and iron-sulfur cluster biosynthesis, and coordinate programmed cell death [1]. According to the generally accepted endosymbiotic theory, the ancestor of these organelles was a free-living bacterium that survived engulfment to become incorporated as an obligate endosymbiont within the cytoplasm of the host cell [2]. During the course of evolution, most of the mitochondrial protein-coding genes have been transferred to the nuclear genome. However, a few genes have been retained in the genome of the modern organelle. The gene complement can differ species to species, but mostly codes for ribosomal RNAs, tRNAs and membrane components of the electron transport chain. The mitochondrial genome encodes just 8 proteins in yeast [3], and 13 in humans [4]. The presence of a protein-coding genome, although small, necessitates the preservation of a functional translation apparatus in mitochondria.

The mitochondrial protein synthesis system has a similar architecture to that of its bacterial relatives, with the translational cycle subdivided into four universal steps: initiation, elongation, termination and recycling. Although there are many conserved aspects, mitochondrial translation is characterized by a number of distinctive features that set it apart from bacteria [5]. The mitochondrial ribosome is characterized by a higher protein content in comparison with the bacterial counterpart [6]. The mitochondrial genetic code deviates from the standard, with differences in codon usage accompanied by a reduction in number and modifications of mitochondrial tRNAs [7].

One of the most dramatic differences between mitochondrial and bacterial translation is in the translational factors orchestrating the process, especially initiation factors. In bacteria, there are three universally present initiation factors, IFs: IF1, IF2, and IF3 [8]. Mitochondrial IF2 (mIF2) is universally present, mIF3 is near-universal, with a handful of exceptions, and mIF1 is universally lacking [9]. Finally, there is a large group of lineage specific mitochondrial translational activators, the majority of


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which have been identified in the yeast *Saccharomyces cerevisiae* [9,10]. In this review we summarize the current knowledge about protein factors involved in mitochondrial initiation by contrasting it with the ancestral bacterial system and paying special attention to lineage specific features.

## 2. Mitochondrial initiation factor 2 (mIF2)

### 2.1. General characteristics of the bacterial ortholog

IF2 is a translational GTPase that orchestrates initiator tRNA selection and ribosomal subunit joining (for review see Ref. [11]). The latter activity is conserved among IF2 and its orthologs in the eukaryotic cytoplasmic translation system (eIF5B) and archaea (aIF5B) [12]. IF2 consists of six domains numbered from I to VI (Fig. 1). Domain IV is a GTPase, and domain VI directly interacts with the initiator Met-tRNA$_i^{Met}$ [13].

### 2.2. Functions of mIF2

The first function of mIF2 is selection of the initiator tRNA. Unlike in bacteria, in human mitochondria one methionine tRNA species acts both as initiator tRNA and elongator tRNA [14]. A fraction of the Met-tRNA$^{Met}$ is formylated, leading to an increase in tRNA affinity to mIF2, accompanied with a decrease in affinity to EF-Tu – a translational GTPase delivering elongator tRNAs during the elongation stage. This ensures that formylated fMet-tRNA$^{Met}$ specifically participates in the initiation of translation [15]. This dual use of Met-tRNA$^{Met}$ is not limited to mammals; the single celled excavate parasite *Trypanosoma brucei*, which imports all its mitochondrial tRNAs, also formylates just a subset of Met-tRNA$^{Met}$ molecules for use in initiation [16].

In yeast mitochondria, the situation is more similar to the bacterial system in that there are two tRNA$^{Met}$ species, initiator (tRNA$_i^{fMet}$) and elongator (tRNA$^{Met}$) [17]. As in the mammalian system, formylation of Met-tRNA$_i^{fMet}$ in *S. cerevisiae* increases its affinity to mIF2 [18]. In *Escherichia coli*, disruption of the *fmt* gene coding for Met-tRNA$_i^{fMet}$ formyltransferase abolishes initiator tRNA formylation, severely impairing bacterial growth [19], whereas in *Pseudomonas aeruginosa* the growth effect is only moderate [20]. A deletion of the equivalent gene *FMT1* in *S. cerevisiae* does not lead to a significant impairment of mitochondrial translation and yeast growth [21]. Moreover, replacement of *S. cerevisiae* mIF2 with its bovine ortholog in the context of the *FMT1* deletion also does not result in any visible defects of mitochondrial translation [22], suggesting that the relative insensitivity to formylation of initiator tRNA is a general feature of mIF2.

The relative insensitivity of *S. cerevisiae* to *FMT1* deletion has been suggested to be due to the participation of an accessory protein Aep3p in the process of initiator tRNA selection in *S. cerevisiae* mitochondria [23]. Simultaneous disruption of both *FMT1* and *AEP3* genes leads to a synthetic respiratory defect – a phenotype even more severe than that seen in *fmt*-deficient *E. coli* [23]. In vitro experiments have shown that complex formation between Aep3p and mIF2 promotes the binding of Met-tRNA$_i^{fMet}$ – but not of fMet-tRNA$_i^{fMet}$ – to mIF2, thus promoting Met-tRNA$_i^{fMet}$ use in initiation. Moreover, the genome of apicomplexan *Toxoplasma gondii* does not encode the FMT gene, suggesting that in this organism initiation naturally uses an unformylated initiator tRNA [24].

The second activity of IF2 and e/aIF5B – their role in ribosomal subunit joining – has not yet been experimentally investigated for mIF2. This is due to an absence of a suitable sophisticated mitochondrial in vitro translational system. Given that subunit joining is a universally conserved function of both bacterial (IF2) as well as eukaryotic and archaeal (e/aIF5B) orthologs, it is most likely that

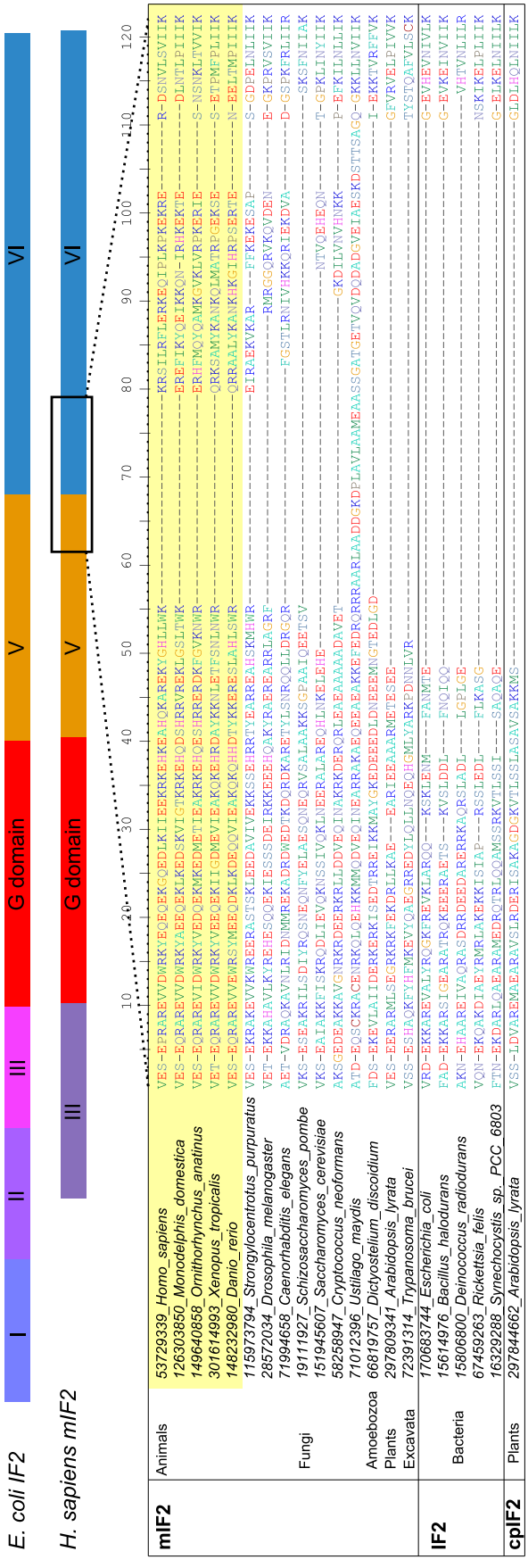


**Fig. 1.** Domain organization of IF2 and mIF2. Location and sequence alignment of the mIF2 insertion region is shown for a set of representative species. The yellow highlighting shows the taxonomic limits of the conserved insertion region. See Ref. [9] for a larger alignment.