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Diversity and Evolution of Mitochondrial Translation Apparatus

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Abstract—The evolution of mitochondria has proceeded independently in different eukaryotic lines, which is reflected in the diversity of mitochondrial genomes and mechanisms of their expression in eukaryotic species. Mitochondria have lost most of bacterial ancestor genes by transferring them to the nucleus or eliminating them. However, mitochondria of almost all eukaryotic cells still retain relatively small genomes, as well as their replication, transcription, and translation apparatuses. The dependence on the nuclear genome, specific features of mitochondrial transcripts, and synthesis of highly hydrophobic membrane proteins in the mitochondria have led to significant changes in the translation apparatus inherited from the bacterial ancestor, which retained the basic structure necessary for protein synthesis but became more specialized and labile. In this review, we discuss specific properties of translation initiation in the mitochondria and how the evolution of mitochondria affected the functions of main factors initiating protein biosynthesis in these organelles.

INTRODUCTION

Mitochondria are double-membrane organelles of eukaryotic cells; their ancestors are Alphaproteobacteria related to the modern group Rickettsiales [1]. The role of mitochondria in the cell activity cannot be overestimated. Besides respiration, they are involved in the metabolism of amino acids and nucleotides and biogenesis of lipids and iron–sulfur (Fe-S) clusters, as well as a serve as a key cell signaling hub and act as apoptosis regulators [2-4]. As a result of evolution, mitochondria have lost most genes of their bacterial ancestor that were either fully lost or transferred to the nuclear genome [5]. The overwhelming majority of mitochondrial proteins are encoded by the nuclear genome, synthesized in the cytosol, and imported to the mitochondria.

Mitochondria had appeared before the common trunk of the evolutionary tree of ancient eukaryotes divided into branches giving rise to new types. This can also account for the existing diversity of mitochondrial genomes, which are different in structure, size, and organization of mitochondrial gene expression in different groups of eukaryotes. Most animals, except for some arthropods, mollusks, and nematodes, have extremely compact circular mitochondrial genomes (~16 kb) with a limited number of noncoding sequences [6]. At the same time, mitochondrial genomes of plants are much larger (up to several hundreds of kb) and differ greatly in size even in closely related species [7]. For example, the mitochondrial genome of *Silene latifolia* consists of a single chromosome (253 kb), while the genome of *Silene conica* mitochondria consists of 128 chromosomes (from 44 to 192 kb in size). Representatives of the order Trypanosomatida have an unusual mitochondrial genome organization. Trypanosomal mitochondrial DNA (mtDNA) is a network of numerous maxi- and mini-rings that form catenanes. The maxi-rings contain most of the genetic information, while the mini-rings encode

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small RNAs [8]. mtDNA encodes transport and ribosomal RNAs, proteins involved in the replication, expression of mitochondrial genome and mitochondrial translation, as well as subunits of oxidative phosphorylation complexes. The number of protein-coding genes varies from two (*Cox1* and *Cox3*) in *Chromera velia* to 67 in *Reclimonas americans*, although most mitochondrial genomes contain 12 to 20 genes [5, 9]. Despite a small number of genes left in mtDNA, mitochondria maintain fully functional transcriptional and translational machinery which represents a simplified version of bacterial protein synthesis apparatus that has been supplemented in the process of evolution with new features and factors unique for each eukaryotic species.

EVOLUTION OF MITOCHONDRIAL RIBOSOMES

Translation in the mitochondria is performed by special mitochondrial ribosomes (mitoribosomes) — large RNA–protein complexes converting genetic information encoded in mRNA into proteins. Similar to all ribosomes, mitoribosomes consist of the large and small subunits. The data of structural and phylogenetic analysis of mitoribosomal RNA and mitoribosomal proteins indicate that mitochondrial ribosomes have evolved from similar organelles of an alphaproteobacteria-like ancestor of mitochondria [10]. Mitoribosomes are extremely heterogeneous with respect to the rRNA content. The total mass of rRNA in mitoribosomes varies from 0.5 MDa in *Caenorhabditis elegans* to 1.6 MDa in *Neurospora crassa*, which is comparable to the rRNA content in a bacterial ribosome (1.4 MDa) [11]. Bilaterally symmetrical Metazoa, including mammals, have significantly lesser amount of mitochondrial rRNA. For example, RNA makes 25-30% of mammalian mitoribosomes vs. 60-70% of bacterial ribosomes [11]. In contrast to bacterial and cytoplasmic ribosomes, mitoribosomes of most organisms have lost the 5S rRNA, although some organisms retained it. According to the structural studies, 5S rRNA was found in the mitoribosomes of *Arabidopsis thaliana* [12]; noncanonical 5S rRNAs were discovered in *Chlamydomonas reinhardtii* [13] and *Plasmodium falciparum* [14]. In mammals, the large mitoribosomal subunit contains mitochondrial tRNA^{Val} or tRNA^{Phe} that take the place of 5S rRNA [15].

The evolution of mitochondrial rRNAs has proceeded in different directions — both degradation and expansion. For example, RNA of the yeast ribosomal large subunit contains a considerable amount of additional sequences compared to the bacterial 16S rRNA. All these sequences localize to the ribosome periphery; some of them are involved in the formation of the stalk required for the association of yeast mitoribosome with the surface of the inner mitochondrial membrane [16].

As a result of rRNA reorganization during evolution, the mitoribosome has lost some bacterial proteins. Thus, reduction of the h8, h9, and h44 loops in the 18S rRNA of *Arabidopsis* mitoribosome small subunit has resulted in the loss of ribosomal protein S20. The loss of 5S rRNA in yeast coincided with the loss of the large subunit proteins bL25m and uL18m [9]. In general, in the course of evolution, the protein content of the mitochondrial ribosome increased in most eukaryotic lineages.

Some mitoribosomal proteins have greater molecular masses and are characterized by the presence of N- or C-terminal extensions compared to their bacterial homologs. In some cases, this is necessary to compensate for the loss of the rRNA structural domains and to stabilize the structure of the ribosome. Many evolutionarily new mitoribosomal proteins have been recruited from the cytoplasm. Most often, they are not incorporated in the mitoribosomal core but localize mainly at the mitoribosome periphery [11, 17]. Some of these proteins, e.g., mS39, mL41, and mL46, are conserved among eukaryotes, while other are species-specific [18]. Many acquired mitoribosomal proteins are bifunctional. For example, the small subunit protein mS29 participates in the Dap3-induced apoptosis and exhibits the GTPase activity [19].

Such diversity of mitochondrial ribosomes might be accounted for the above-described diversity of mitochondrial genomes and reflect their specialization for the synthesis of a small number of mitochondrially encoded proteins.

SPECIALIZATION OF TRANSLATION INITIATION PROCESSES

Mitochondrial protein-coding genes mostly encode subunits of oxidative phosphorylation complexes. However, the majority of subunits of respiratory chain complexes are encoded in the nucleus, synthesized in the perimitochondrial regions of the cytosol, and imported into the mitochondria. Correct assembly of respiratory chain complexes requires precise coordination of mitochondrial translation and biosynthesis of mitochondrial proteins in the cytosol. In all likelihood, the key stage of such regulation is translation initiation, which is significantly different in mitochondria compared to bacteria.

In bacteria, translation begins with the mRNA binding to the small ribosomal subunit and selection of the correct start codon, which is performed by the initiation factors IF1, IF2, and IF3. IF3 binds to the small ribosomal subunit, thus preventing its reassociation with the 50S subunit. This results in the formation of a pool of free 30S subunits necessary for the translation initiation [20]. IF1 blocks the A-site of the small ribosomal subunit, thus facilitating positioning of the initiator tRNA at the peptidyl site (P-site). Next, the ternary complex

formed by IF2, GTP, and initiator aminoacyl-tRNA (fMet-tRNA) binds to the small ribosomal subunit. The start codon is positioned at the P-site due to the recognition of the Shine−Dalgarno (SD) sequence within the mRNA by the anti-SD sequence of 16S rRNA. IF2 in a complex with GTP promotes insertion of the initiator tRNA into the P-site. At the same time, IF3 discriminates against elongator tRNA and stimulates formation of the preinitiation complex by increasing the rate of the codon−anticodon interaction. IF3 also contributes to the dissociation of the pseudo-initiation complexes (formed by tRNAs different from the initiator tRNA) and noncanonical initiation complexes (containing start codons different from AUG, GUG, and UUG). Correct codon−anticodon interactions result in the association of 30S and 50S subunits, dissociation of IF3, GTP hydrolysis by IF2, and release of IF2 and IF1 [21].

In addition to the canonical pathway described above, bacteria have two more mechanisms of translation initiation (Fig. 1). One of them is the read-through translation that occurs when after termination of translation of the first cistron in a polycistronic mRNA, the ribosome does not dissociate into subunits but continues to scan mRNA until reaching the next start codon [22]. IF3 plays a key role in this mechanism of translation initiation.

Beside mRNAs containing the 5' untranslated regions (5'-UTRs) with the SD sequences, bacteria also have the so-called leaderless mRNAs lacking the 5'-UTRs [23]. In these mRNAs, the start codon is located either directly at the 5'-end or at a short distance from it. In this case, translation begins with the binding start codon by the associated 70S ribosome. *In vitro* studies have shown that although initiation factors do not play a key role in this mechanism, their presence stimulates translation initiation. Some leaderless mRNAs require initiation factors for the translation initiation [24].

A distinctive feature of mitochondrial mRNAs is the presence of start codons different from AUG [25]. In mammals, the synthesis of NADH dehydrogenase subunit 2 (ND2) starts with AUU, while the synthesis of ND1, ND3, and ND5 starts with AUA. All mammalian mitochondrial mRNAs are characterized by very short 5'-UTRs (only 1 to 3 nucleotides long) or their absence, whereas mRNAs in yeast and plant mitochondria have large 5'-terminal extensions [26]. Also, all mitochondrial mRNAs and rRNAs of the small ribosomal subunit lack the SD and anti-SD sequences, respectively [27]. These features of mitochondrial mRNAs indicate that the mechanism of translation initiation in mitochondria differs from the canonical translation initiation in bacteria.

First and foremost, there are differences in the number and functions of initiation factors. IF2 is the key factor in the translation initiation in bacteria. It binds the initiator fMet-tRNA and correctly positions it at the P-site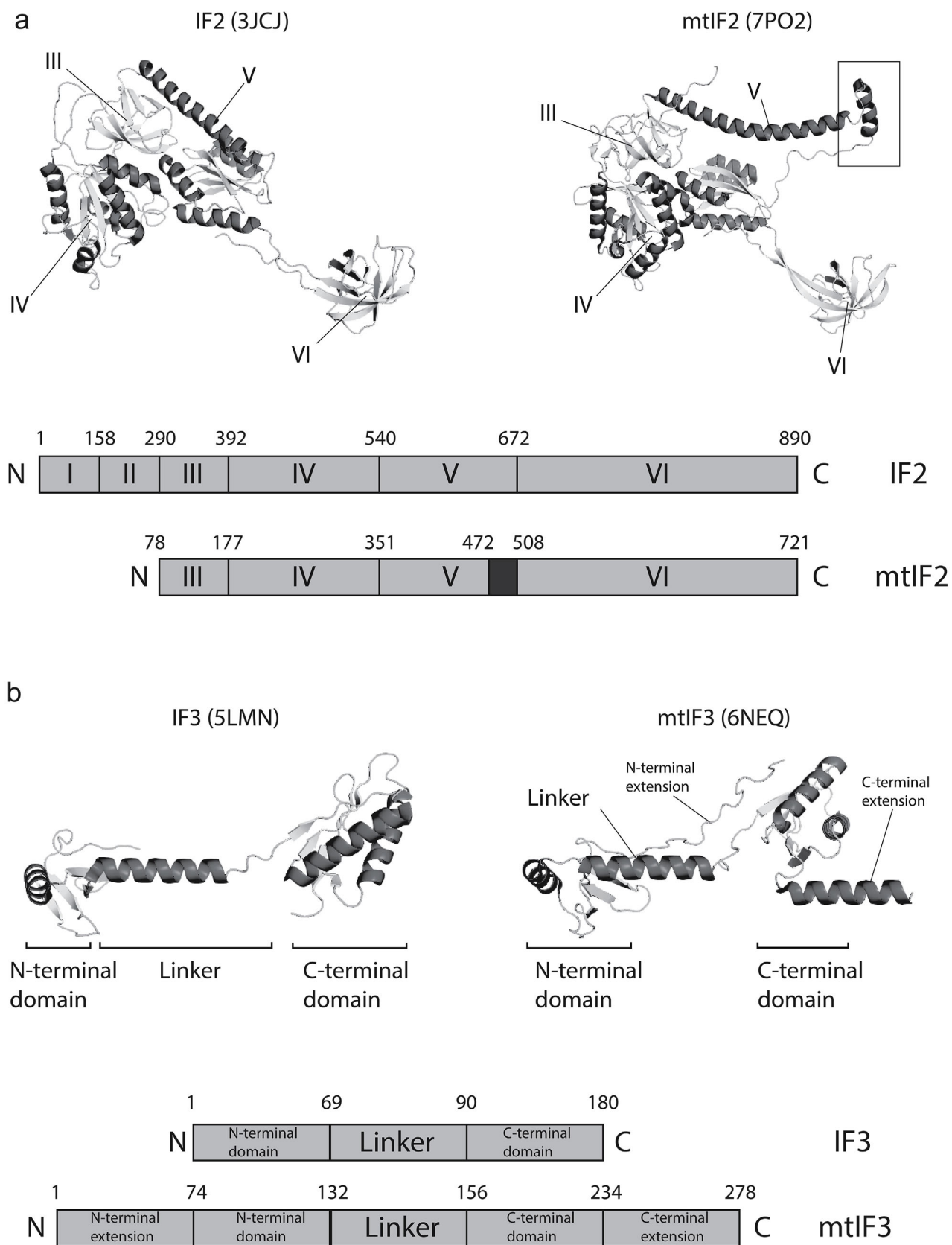 of the small subunit, thus increasing the rate and accuracy of translation initiation [28]. It is the only initiation factor whose homologs have been found in the mitochondria of all eukaryotes. Bacterial IF2 consists of the N-terminal domains I and II involved in the binding with the small ribosomal subunit, domain III with yet unknown function, highly conserved GTPase domain IV, and two C-terminal domains (V and VI). Mitochondrial initiation factors 2 (mtIF2) lacks the first two domains but the rest of its domain structure (domains III-VI) is similar to that of bacterial IF2 (Fig. 2a). It should be emphasized that in contrast to bacterial IF2, in which the binding to the small ribosomal subunit is determined by the two N-terminal domains only, efficient interaction between mtIF2 and mammalian mitoribosomal 28S subunit requires preservation of its intact multidomain structure [29]. mtIF2 also has an insert between domains V and VI, whose length and sequence varies between the species. This sequence interacts with the ribosomal A-site similar to bacterial IF1, which has no homologs in the mitochondria [30]. Thus, the functions of bacterial factors IF1 and IF2 in the mitochondria are performed by mtIF2, which is corroborated by the fact that mtIF2 can substitute for IF1 and IF2 in *Escherichia coli* cells [31] and by the data of structural studies of the interaction between mtIF2 and *E. coli* ribosome [32]. Despite the functional and structural similarity, the mechanisms of action of bacterial IF2 and mtIF2 are different. Similar to bacteria, translation of leaderless mRNA in mammalian mitochondria can be initiated by the full ribosome, although mtIF2 presumably plays an important role in this process [33]. For example, deletions in the mtIF2-encoding gene in HEK293T cells led to the complete termination of the mitochondrial translation.

Mitochondrial translation in yeast is quite different. Although the mechanism of its initiation remains unclear, it is known that the role of mtIF2 varies depending on a species. For example, deletion of the *mtIF2* gene in *Schizosaccharomyces pombe* leads to a decrease in the efficiency of translation of all mRNAs [34], while in *Saccharomyces cerevisiae* all except Cytb and Atp9 [35].

Mitochondrial translation initiation factor 3 (mtIF3) exhibits low homology between different species and with bacterial IF3 (20-25% homology). Nevertheless, the data of *in vitro* studies indicate that mtIF3 is functionally similar to bacterial IF3 [36]. mtIF3, like bacterial IF3, consists of two N- and C-terminal domains connected by a linker but has additional sequences (approximately 30 amino acid residues) at both ends of the molecule (Fig. 2b). In contrast to bacterial IF3, which binds to the 30S ribosome through the C-terminal domain [37], both N- and C-terminal domains of mtIF3 interact with the small mitoribosomal 28S subunit, while the terminal extensions prevent the attachment of the large 39S subunit [38, 39].
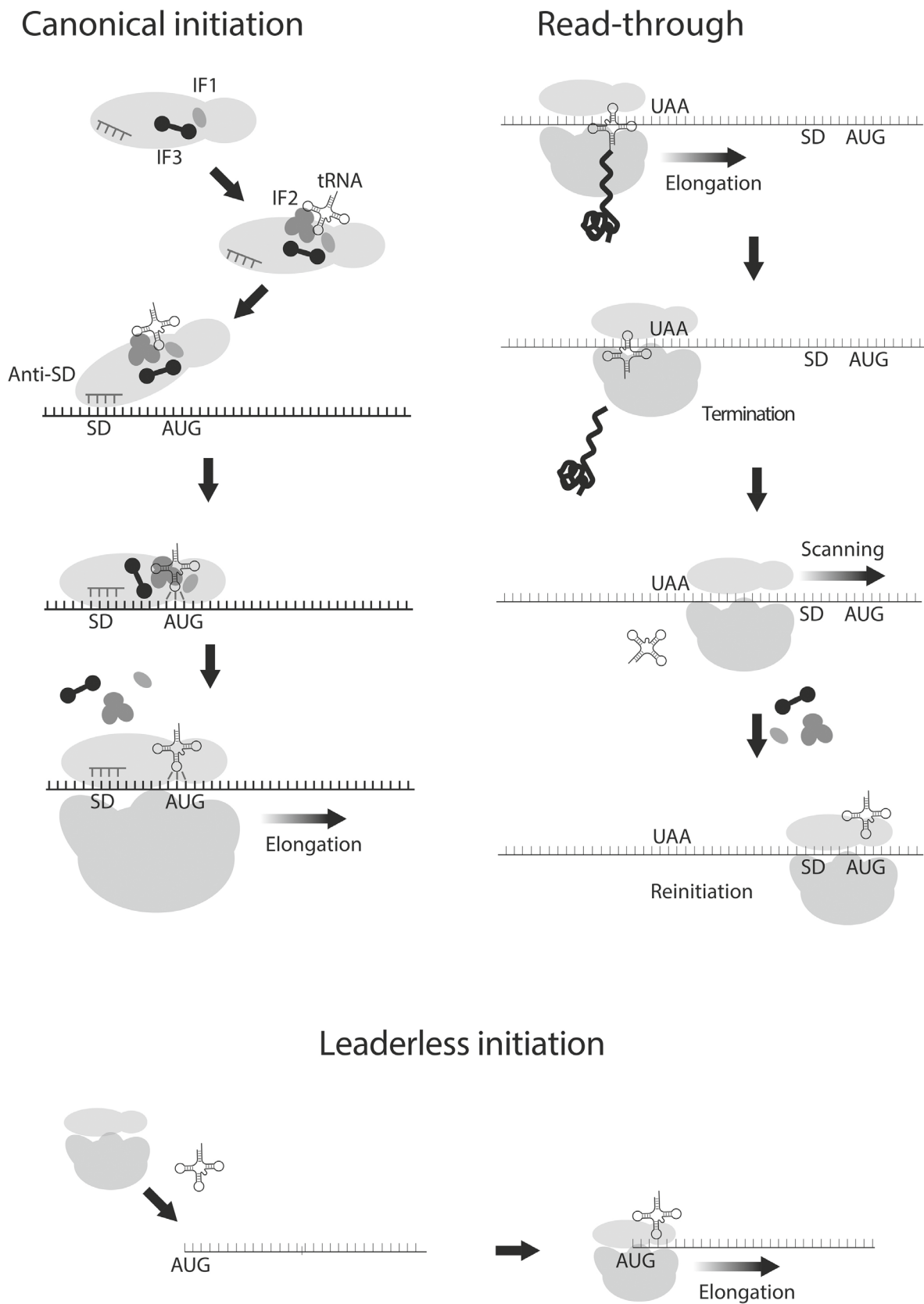
Fig. 1. Translation initiation in bacteria can proceed by three mechanisms. In canonical initiation, the SD sequence of mRNA is recognized by the anti-SD sequence of 16S rRNA. In the read-through translation, the ribosome does not dissociate into subunits after reaching the stop codon (UAA) but continues mRNA scanning until it encounters the next start codon (AUG). The mechanism, by which intact ribosome recognizes the start codon and initiates translation on leaderless mRNAs, remains unclear.

a

IF2 (3JCJ)

mtIF2 (7PO2)

1	158	290	392	540	672	890
N	I	II	III	IV	V	VI	C	IF2

78	177	351	472	508	721
N	III	IV	V		VI	C	mtIF2

b

IF3 (5LMN)

mtIF3 (6NEQ)

Linker

N-terminal extension

C-terminal extension

N-terminal domain   Linker   C-terminal domain

N-terminal domain   C-terminal domain

1	69	90	180
N	N-terminal domain	Linker	C-terminal domain	C	IF3

1	74	132	156	234	278
N	N-terminal extension	N-terminal domain	Linker	C-terminal domain	C-terminal extension	C	mtIF3

Fig. 2. Domain organization and structure of bacterial initiation factors and their homologs from mammalian mitochondria. a) The structures of bacterial IF2 (PDB ID: 3JCJ) and mtIF2 (PDB ID: 7PO2). mtIF2 lacks two N-terminal domains and has an insert between domains V and VI, which interacts with the ribosomal A-site similarly to bacterial IF1 (indicated with a square). b) The structures of bacterial IF3 (PDB ID: 5MLN) and mtIF3 (PDB ID: 6NEQ).

Deletion of the terminal extensions affects the functioning of mammalian mtIF3. Thus, deletion of the C-terminal extension impaired the ability of mtIF3 to destabilize noncanonical or pseudo-initiation complexes in *E. coli* translation system, while deletion of the N-terminal extension increased several folds the protein affinity to the 39S subunit.

In *S. cerevisiae*, deletion of the terminal extensions in Aim23p (yeast mitochondrial homolog of IF3) caused the loss of the protein activity, resulting in the phenotype analogous to the phenotype of cell fully deficient by this protein [40]. These data indicate that the mechanisms of action of mammalian Aim23p and mtIF3 (IF3 homologs) are different despite their common function, which was confirmed by the gene deletion experiments. Deletion of the IF3 gene in *E. coli* cells is lethal [41]. The results of deletions of genes for the mitochondrial homologs of IF3 vary between different organisms. Deletion of the mtIF3 gene in *S. pombe* yeast had no effect on cell growth and mitochondrial translation [34]. At the same time, deletion of the Aim23 gene in *S. cerevisiae* impaired cell growth on the media with a nonfermentable carbon source, indicating a decrease in mitochondrial function. However, mitochondrial dysfunction in this case was associated not with the reduced efficiency of mitochondrial translation but with the imbalance, as there was a significant decrease in the synthesis of the cytochrome *c* oxidase complex components and upregulation of synthesis of ATP synthase subunits [42]. Later, it was shown that, at least in the case of Cox2 biosynthesis, such effect could be explained by the fact that Aim23p interacts with the protein Pet111 [43], which increases efficiency of the *Cox2* mRNA translation. Deletion of the mtIF3 gene in some human cell lines also led to the mitochondrial dysfunction because of reduced synthesis of Atp6 protein [33, 44]. It should be noted that despite the slight effect of mtIF3 on the mitochondrial translation in cell lines, deletion of the *mtIF3* gene in mammals is lethal at the level of the organism. Homozygous *mtIF3* knockout mice die at the embryo stage, while heterozygous animals were characterized by the development of progressive cardiomyopathy. Studying the profile of mitochondrial translation in cardiac muscle cells of these animals showed reduced synthesis of ND3 [45]. Summarizing the above data, mtIF3 acts as a tissue-specific regulator of translation of certain mRNAs in mammalian mitochondria, although its mechanism of action is still unclear. In the case of bicistronic *Atp8–Atp6* mRNA, mtIF3 might participate in the read-through initiation similarly to bacterial IF3. In support of this assumption, recent analysis of ribosome distribution on the mitochondrial mRNA in *mtIF3*-deficient HEK293 cells demonstrated changes in occupancy of mitoribosomes on the bicistronic *Atp8–Atp6* mRNA. The number of ribosomes on the mRNA fragment corresponding to the Atp6-encoding region decreased, while the number of ribosomes on the coding region for Atp8 increased [33].

Therefore, it can be concluded that mitochondria have inherited a set of translation initiation factors from their bacterial precursor. These factors have retained most of their original "bacterial" functions, as confirmed by different studies performed mainly in heterologous systems. However, mitochondrial initiation factors could acquire additional functions associated with the evolution of mRNAs, mitochondrial genetic code, and mitoribosomes.

SPECIALIZATION OF mRNA RECRUITMENT MECHANISMS

At the first stages of translation initiation, mRNA is recruited to the ribosome for the start codon selection. In bacteria, this process is based on the complementary interaction between the SD sequence located upstream of the start codon and the anti-SD sequence of the 16S rRNA. All eukaryotic mitochondrial mRNAs lack the SD sequence, and some of them lack the 5'-UTR, i.e., sequences that could have been involved in the factor-dependent regulation of the start codon selection.

The presence of elongated 5'-terminal extensions in the *S. cerevisiae* mitochondrial mRNAs in the absence of any ribosome binding sites should cause difficulties in the start codon selection. In the case of cytoplasmic translation, this problem is solved by the presence of 5'-cap, to which the preinitiation complex binds and scans the 5'-UTR for the start codon [46]. Mitochondria lack the factors for the scanning mechanism, and mitochondrial mRNAs are not capped. Instead, yeasts have a set of the so-called translational activators (table) that bind to the 5'-UTRs and mitoribosome subunits [47, 48], thus contributing to the selection of correct start codon. Cryo-electron microscopy data show that the region near the peptide exit tunnel of the yeast mitoribosome can be a common platform for various activators [49]. Most of these translational activators belong to the family of pentatricopeptide (PPR) proteins characterized by RNA binding activity.

All proteins encoded in the mitochondrial genome of *S. cerevisiae*, except for Var1, are the core components of respiratory chain complexes required to provide the energy demands of cells [58]. Beside mitochondrially encoded proteins, oxidative phosphorylation complexes contain the products of cytosolic protein translation and, therefore, assembly of these complexes requires very fine tuning, on one hand, and sufficient plasticity, on the other hand, to adapt to rapidly changing energy demands of the cells. To satisfy these requirements, some translational activators form feedback loops that coordinate the synthesis of mitochondrial polypeptides and assembly of functionally active respiratory chain complexes.

Translational activators in the mitochondria of *S. cerevisiae*

Regulated mRNA	Activator	PPR	Mechanism of action	Homolog in *Homo sapiens*
Cox1	Pet309	PPR+	Pet309 binds the 5'-UTR of *Cox1* mRNA, contributing to the synthesis of full-size Cox1 protein [50]; deletion of the *Pet309* gene leads to the cessation of Cox1 synthesis; correct binding of the ribosome on the mRNA is also regulated by the Mss51 protein; deletion of the *Mss51* gene leads to changes in the pattern of interaction between Pet309 and mRNA and to the synthesis of a new protein (15 kDa) [51]	LRPPRC
	Mss51	PPR–		Zmynd17
Cox2	Pet111	PPR+	binds *Cox2* mRNA at two sites (closer to the 5' end and near the start codon), preventing formation of secondary structures inhibiting translation [52]	–
Cox3	Pet54 Pet122 Pet494	PPR– PPR– PPR–	Pet54 directly binds to the 5'-UTR of *Cox3* mRNA, contributing to formation of the Pet122–Pet54–Pet494 activation complex for the efficient synthesis of Cox3 protein [53, 54]	– – –
Cytb	Cbs1 Cbs2 Cbp1 Cbp3 Cbp6	PPR– PPR– PPR+ PPR– PPR–	Cbs1 binds to the 5'-UTR of *Cytb* mRNA, contributing to its further binding with the large ribosomal subunit in the presence of Cbs2 and Cbp1; at the same time, Cbs1 acts as an inhibitor preventing the start of translation; translation block is eliminated when Cbs1 is displaced from the mRNA–ribosome complex by Cbp3–Cbp6 proteins [55]	– – – UQCC1 UQCC2
Atp6/8	Atp22	PPR+	although *Atp6* and *Atp8* are components of a bicistronic transcript, deletion of the gene encoding Atp22 affects the synthesis of Atp6 only [56]; the mechanism of Atp22 influence on the *Atp6* mRNA translation remains unclear	–
Atp9	Aep1/Nca1 Aep2/Atp13	PPR+ PPR+	mechanism of action remains unclear [56]	– –
Var1	Sov1	PPR+	Sov1 binds *Var1* mRNA due to the presence of PPR domains, providing its efficient translation; the mechanism of action remains unclear [57]	–

For example, the synthesis of apocytochrome *b* depends on five activators (Cbs1, Cbs2, Cbp1, Cbp3, and Cbp6). While Cbs1, Cbs2, and Cbp1 bind the 5'-UTR of the *Cytb* mRNA and recruit it to the large mitoribosomal subunit by binding with the polypeptide exit tunnel. Next, Cbs1 bound to the 5'-UTR of the *Cytb* mRNA is displaced by the Cbp3–Cbp6 complex, and the 5'-UTR becomes available for the binding with the small mitoribosomal subunit for translation initiation. The newly synthesized protein binds to the Cbp3–Cbp6 complex involved in the assembly of the coenzyme Q–cytochrome *c* reductase complex [55]. The synthesis of the cytochrome *c* oxidase Cox1 subunit in *S. cerevisiae* is regulated in a similar way. Translational activator Pet309 binds the 5'-UTR of the *Cox1* mRNA in the presence of Mss51 protein. Next, Mss51 in a complex with other factors binds the nascent polypeptide and participates in the assembly of cytochrome *c* oxidase [59]. Impaired assembly leads to the accumulation of immature intermediates associated with Mss51; as a result, the pool of free Mss51 decreases, which leads to the reduced efficiency of *Cox1* mRNA translation initiation [60].

In mammals, mitochondrial mRNAs are leaderless; almost all of them contain a start codon directly

at the 5'-end. According to the cryo-electron micros-copy data, the binding of these mRNAs involves the small ribosomal subunit protein mS39 located at the entrance to the mRNA tunnel. With its PPR domain, mS39 binds mRNA at the U-rich region in the immediate proximity of the seventh codon of mRNA. Such region is conserved in most of 11 mammalian mitochondrial mRNAs [30]. For efficient mRNA binding, mammalian mitoribosome also interacts with the LRPPRC−SLIRP protein complex. This complex binds to the mitoribo-somal protein mS39, providing mRNA recruitment and regulating its translation rate. Thus, the LRPPRC−SLIRP complex can be a universal translational activa-tor in mammalian mitochondria [61, 62]. In addition, fine translation regulation in mammals, like in yeast, is provided by specific activator proteins. The first identi-fied protein with such function was TACO1. Mutations in its gene were found in some patients with the Leigh syndrome and impaired function of the cytochrome *c* oxidase complex. The latter fact is related to the specific effect of TACO1 on the synthesis of mitochondrially en-coded Cox1 [63], the core component of cytochrome *c* oxidase. It has been shown that TACO1 has several binding sites in the 5' region of the *Cox1* mRNA, as well as displays the affinity to the mitochondrial ribosome. It binds to the small subunit and, although to a lesser extent, interacts with the associated mitoribosome [64]. Therefore, TACO1 can contribute to the recruitment of the *Cox1* mRNA to the ribosome and regulate transla-tion initiation, as well as influence the elongation stage.

Another translational activator in mammalian mi-tochondria is PTCD2, which is capable of specifically regulating the synthesis of Cox3 subunit of the cyto-chrome *c* oxidase complex [65]. PTCD2 is associated with the monosome, which indicates that its regulato-ry mechanism is also based on the binding of the *Cox3* mRNA to the mitoribosome. No activators have been identified for other mRNAs in mammalian mitochon-dria so far.

Similar to yeast mitochondrial mRNAs, plant mRNAs have 5'-UTRs. About half of plant mitochon-drial mRNAs have an A-rich sequence (AxAAA) located 19 nucleotides upstream of the start AUG codon. Rec-ognition of this sequence can involve mitoribosomal protein mS83 from the PPR family [12]. However, the presence of some mitochondrial mRNAs lacking the AxAAA sequence suggests an existence of alternative mechanisms for the mRNA recruitment in plant mito-chondria. Unlike yeasts, which have no mitoribosomal proteins of the PPR family, plant mitoribosomes con-tain numerous PPR proteins. Mammals have only two of them: mS39 and mS27. Most plant mitoribosomal PPR proteins perform only structural functions, except for the ribosomal protein PPR1. Deletion of its gene has a significant effect on translation of all mitochon-drial mRNAs [66]. However, it is not improbable that

some mitoribosomal PPR proteins can play the role of translational activators, similarly to the activators in yeast and mammals. The candidates for translational activators are proteins from the Rf-PPR family, such as recently described RFL8. This protein specifically acti-vates translation of the *ccmFN2* mRNA encoding one of the polypeptides of the heme−lyase complex [67].

SPECIALIZATION OF MEMBRANE PROTEIN SYNTHESIS

Genes that are almost always present in the mito-chondrial DNA encode the main subunits of respiratory chain complexes: ND1, ND2, ND3, ND4, ND4L, ND5, and ND6 of NADH dehydrogenase; apocytochrome *b* of coenzyme Q−cytochrome *c* reductase; Cox1, Cox2, and Cox3 of cytochrome *c* oxidase; and subunits Atp6 and Atp9 of ATP synthase. However, there are some ex-ceptions. For example, yeast have completely lost the NADH dehydrogenase complex and, consequently, re-spective mitochondrial genes [68].

Mitochondrially encoded subunits of the respiratory chain complexes are extremely hydrophobic proteins, so their synthesis has to be coupled with the incorporation of the growing polypeptide into the inner mitochon-drial membrane [69]. Evolution of the mitochondrial genome has proceeded toward the increase in the con-tent of genes for hydrophobic proteins, which resulted in the respective specialization of mitoribosomes. The exit tunnel for the growing polypeptide has undergone sub-stantial changes [70]. In mammalian mitoribosomes, it is lined with proteins containing more hydrophobic amino acids compared to the bacterial ribosomes to pro-vide correct protein folding. The tunnel of the large mi-toribosomal subunit of *S. cerevisiae* is characterized by two unique adaptations. First, the entrance to the tunnel is narrower due to the formation of an additional base pair in the 21S rRNA and, second, there is a mitochon-dria-specific extension of the uL23m protein resulting in the exit tunnel bending. Mitoribosomes are tethered to the mitochondrial inner membrane. In mammals, the "anchor" is the mL45 protein [30] located in the area of the nascent polypeptide exit tunnel on the large ribo-somal subunit. In yeast, mitoribosomes are attached to the membrane by the Mba1 protein and the extension segment 96-ES1 of the large subunit rRNA [71].

CONCLUSIONS

All processes in the mitochondria depend on pro-teins encoded by the nuclear genome. In addition, mi-tochondrial translation is associated with the assembly and functioning of respiratory chain complexes. It is reasonable to suggest that mitochondrial translation had

to adapt to the evolutionary changes in both mitochondrial and nuclear genomes, as well as to the evolution of respiration [11]. Such adaptation has resulted in the formation of unique mitochondrial ribosomes specialized for the synthesis of hydrophobic proteins and in the emergence of unique regulatory mechanisms ensuring adaptation of protein synthesis to the cell metabolic status.

Evolution of regulatory processes has led to the appearance of factors affecting the synthesis of particular mRNAs. The presence of these regulatory factors located at the periphery of mitoribosomes suggests an existence of different subpopulations of mitoribosomes specialized for the biosynthesis of some particular mitochondrial genome-encoded proteins or their groups. The phenomenon of specialization is also known for cytoplasmic ribosomes [72, 73], although in this case, it is achieved by modification of rRNAs or ribosomal proteins or the use of various paralogs of ribosomal proteins.

The factors regulating translation of mitochondrial mRNAs not only form feedback loops but also participate in the mRNA recruitment to the ribosome, as well as determine selection of the start codon. It is possible that such expansion of functions of regulatory factors has also led to changes in the role of translation initiation factors, while their universal functions have become less in demand.

However, such assumptions can be made only for some well-studied model species, such as yeast or mammals, whereas mitochondrial translation machinery in most organisms remains a mystery.

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