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Review

# Co-translational membrane insertion of mitochondrially encoded proteins

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ABSTRACT

The components of the mitochondrial proteome represent a mosaic of dual genetic origin: while most mitochondrial proteins are encoded by nuclear genes and imported into the organelle following synthesis in the cytosol, a small number of proteins is encoded by the mitochondrial genome. Though small in number, mitochondrial translation products are vital for cellular functionality as these proteins represent the core subunits of the respiratory chain and the ATPase which produce the vast majority of the cellular ATP. Mitochondrial translation products are almost exclusively highly hydrophobic polypeptides which are inserted into the inner membrane in the course of their synthesis. The machinery that mediates membrane insertion in mitochondria is deduced from that of their bacterial ancestors and hence shows profound similarities to the insertion machinery of prokaryotes. However, the specialization on the production of a very small set of translation products drove a severe reduction in the complexity of this system. The insertase Oxa1 forms the central component of the insertion machinery. Oxa1 directly binds to mitochondrial ribosomes and, together with the inner membrane protein Mba1, aligns the polypeptide exit tunnel of the ribosome with the insertion site at the inner membrane. The specific hallmarks and the critical components of this machinery are discussed in this review article.



## 1. Introduction

Eukaryotic cells contain two distinct translation systems, one in the cytosol and one in mitochondria. The cytosolic translation machinery was studied intensively over the last decades and is understood to high resolution. In contrast, only little knowledge exists about the synthesis of mitochondrially encoded proteins. The reason why the mitochondrial translation system attracted relatively little attention may be the misconception that it would closely resemble that of bacteria. On the one hand, the mitochondrial translation system clearly shows its bacterial origin in many aspects. In particular the catalytic properties of mitochondrial and bacterial ribosomes are presumably similar. This is evident from the fact that the proteins and rRNA domains that contribute to decoding and peptide bond formation share high degrees of similarity between mitochondria and bacteria. Moreover, translation factors for initiation, elongation and release are conserved, and it was shown that several mitochondrial factors can functionally replace their homologs in bacteria [1,2,3]. On the other hand, except for the catalytic regions, mitochondrial ribosomes significantly differ from those of bacteria. During the more than two billion years of mitochondrial evolution, the RNA content of mitochondrial ribosomes was largely reduced from about 65% in bacteria to about 31% in mitochondria of mammals [4,5]. This dramatic loss of RNA was accompanied by a considerable increase in protein mass so that the ribosome of mammalian mitochondria is – in its dimensions – larger than its counterparts in the cytosol of bacteria and eukaryotes. This change also involves the alteration of the polypeptide exit tunnel which, at least in mammalian mitochondrial ribosomes, shows a unique architecture [4], presumably due to the specialization of the mitochondrial ribosome on the synthesis of hydrophobic membrane proteins. The components which are relevant for the co-translational membrane integration of mitochondrial translation products will be described in the following, and their function and relevance will be discussed.

## 2. Mitochondrially encoded proteins

During evolution of the eukaryotic cell, most genes of the mitochondrial ancestor were either lost or transferred to the nuclear genome [6]. The development of mitochondrial import machineries made this gene transfer possible and led to the present-day situation in which about 98% of all mitochondrial proteins are encoded by nuclear genes [7–9]. Nevertheless, a small set of genes obviously resisted the transfer to the nucleus in all eukaryotic lineages (Fig. 1). The genes which are (almost) consistently present in mitochondrial genomes encode the central membrane-embedded reaction centers of the respiratory chain subunits (Fig. 2): ND1, ND2, ND3, ND4, ND4L, ND5 and ND6 of NADH dehydrogenase (complex I), cytochrome *b* of cytochrome *c* reductase (complex III), Cox1, Cox2 and Cox3 of cytochrome *c* oxidase (complex IV), and Atp6 and Atp9 of the ATP synthase (complex V). Only in a few examples some of these genes are

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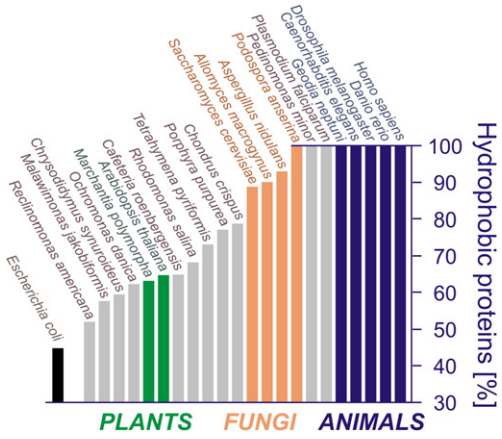



**Fig. 1.** Mitochondrial genomes mainly code for hydrophobic proteins. The percentage of membrane proteins of all gene products encoded by the genomes of mitochondria (of the indicated organisms) and *E. coli* was calculated. Transmembrane domains were predicted using the TMpred algorithm [84].

missing from mitochondrial genomes, for example upon complete loss of complex I in fungi (Fig. 2) or in the extremely reduced genomes of some protists and unicellular algae [10–12].

While these hydrophobic subunits of the respiratory chain represent the only genes of mitochondrial genomes in animals, additional proteins like ribosomal subunits, enzymes and assembly factors are mitochondrially encoded in other kingdoms. Fig. 1 shows that during evolution of eukaryotes the percentage of mitochondrial genes encoding hydrophobic proteins steadily increased and in strongly deviated eukaryotes like *Apicomplexae* and animals all genes for hydrophilic proteins are gone.

It remains a puzzling question why – after the transfer of hundreds of genes – the last few mitochondrial genes obviously had to be retained within the organelle despite the enormous effort for the cell: in order to express this handful of genes in mitochondria literally hundreds of proteins need to be synthesized in the cytosol and imported [13]. Two mutually non-exclusive explanations for the presence of DNA in mitochondria were put forward which both are supported by experimental evidence: (1) the extreme hydrophobicity of some proteins might prevent their efficient import from the cytosol to the inner membrane. This clearly could explain why mitochondrial genomes encode primarily hydrophobic proteins. Experiments in which cytochrome *b*, a protein normally encoded by mitochondrial DNA, was expressed as fusion to a mitochondrial presequence indeed revealed an unproductive aggregation of this protein in the cytosol [14]. However, experiments with allotopically expressed Atp6 from a nuclear gene in human cells suggest that protein import of this hydrophobic is possible, though extremely inefficient [15]. (2) An alternative explanation for the retention of mitochondrial DNA is the ability of this system to couple synthesis and assembly in order to regulate gene expression. Such an elegant regulatory circuit exists for expression of Cox1 in yeast mitochondria [16–19] which is explained in detail in Fig. 3. A similar, though still uncharacterized feedback control mechanism might exist for the expression of other proteins, for example for Atp6 [20].

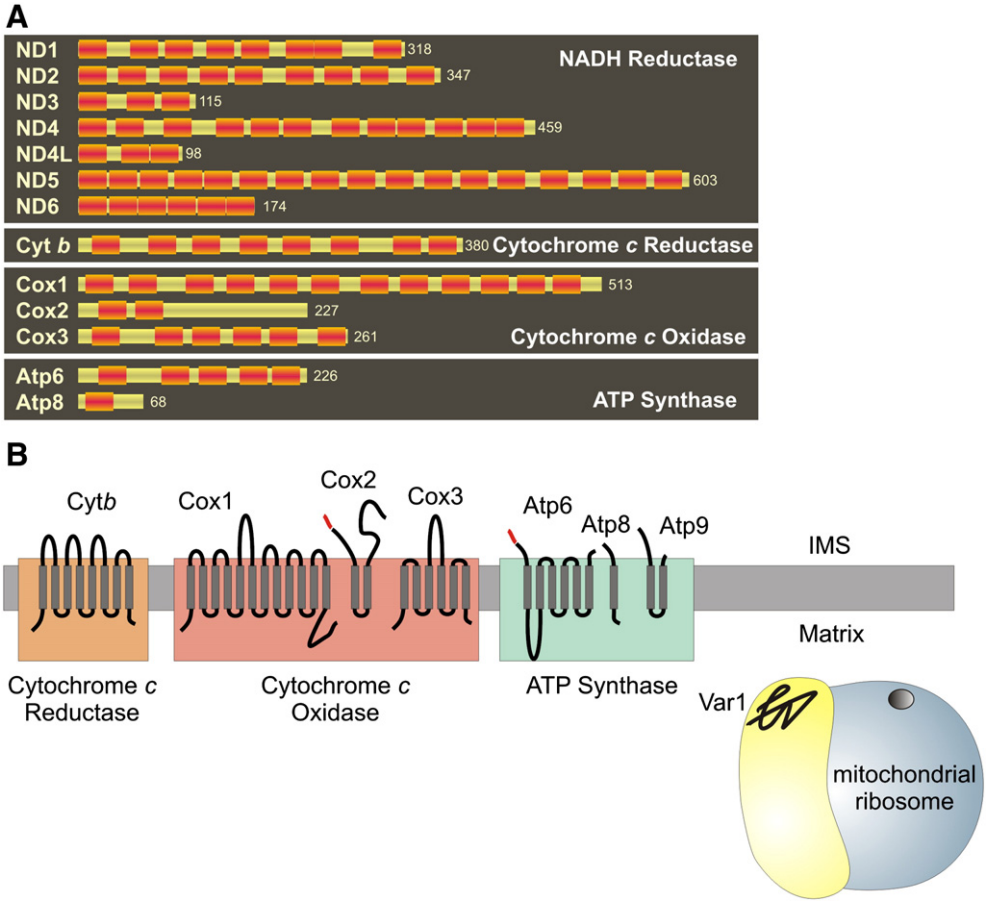



**Fig. 2.** Mitochondrially encoded proteins in human and baker's yeast. (A) Mitochondrially encoded proteins of humans. Numbers refer to the amino acid residues of the proteins. Transmembrane domains in the proteins are indicated as red boxes. (B) Yeast mitochondrial DNA codes for eight proteins which represent subunits of cytochrome *c* reductase (cytochrome *b*), cytochrome *c* oxidase (Cox1; Cox2; Cox3), the ATP synthase complex (Atp6, Atp8, Atp9), and the ribosome (Var1). Cox2 and Atp6 are synthesized with N-terminal leader peptides which are removed by proteolytic cleavage of the inner membrane peptidase (Imp1/Imp2) and the Atp23 protease, respectively [85-87].

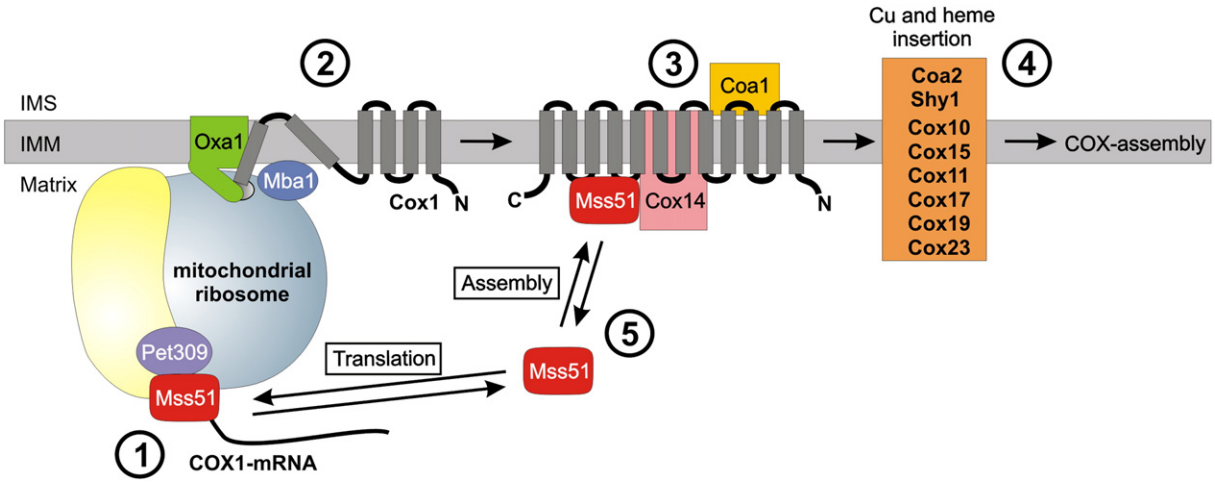



**Fig. 3.** Regulation of Cox1 synthesis. The protein Mss51 plays a central role in this process as this translational activator connects Cox1 synthesis to Cox1 assembly. Together with Pet309, Mss51 mediates translation of *COX1* mRNA (1). Newly synthesized Cox1 is co-translational inserted into the inner mitochondrial membrane with the help of Oxa1 and Mba1 (2). Newly inserted Cox1 sequesters the Mss51 protein into a complex with Cox14 and Coa1 (3). This binding of Mss51 to Cox1 thereby prevents Mss51 to activate the synthesis of additional Cox1. Cox1 is further matured by the addition of the co-factors copper (aided by Cox15, Cox17, Cox19, and Cox23) and heme (involving Cox10 and Cox15), and the protein can continue to assemble into the functional cytochrome *c* oxidase complex (4). This assembly liberates Mss51 from the newly synthesized Cox1 (5), thus enabling Mss51 to activate translation of the *COX1* mRNA (1). By this mechanism the level of *de novo* synthesis of Cox1 is adjusted to amounts that can be successfully assembled.

## 3. The mitochondrial protein insertion machinery

### 3.1. Topogenesis of polytopic membrane proteins

As depicted in Figs. 1 and 2, mitochondrially encoded membrane proteins adopt a high degree of variety of different topologies. Most of them are polytopic membrane proteins containing up to seventeen (ND5) transmembrane segments while only one is monotopic (Atp8). Most mitochondrially encoded proteins have rather small hydrophilic regions between the transmembrane segments, and particularly the loops exposed to the intermembrane space are typically very short. The exception here is subunit 2 of Complex IV (Cox2) that contains a large hydrophilic domain that faces the intermembrane space. The topogenesis of Cox2 requires the activity of several factors and is depicted in Fig. 4.

Topogenesis of polytopic membrane proteins is directed by a number of different factors. Generally, all mitochondrially encoded membrane proteins follow the positive-inside rule. Like for bacterial inner membrane proteins, loops and termini of mitochondrially encoded proteins residing in the matrix carry a net positive charge, while they are negative in the intermembrane space. The outward positive electrochemical gradient generated by the respiratory chain can be used to actively promote translocation of negatively charged segments or to impede translocation of positively charged residues. For conservatively sorted, nuclear encoded proteins, *i.e.* proteins that insert into the inner membrane following import into the matrix, it was shown that topogenesis depends on the charges flanking the transmembrane domain [21,22]. Thus, charges obviously function as topogenic signals which determine the orientation of a transmembrane segment. In addition, the transfer of negative charges to the positively charged intermembrane space appears to drive the insertion reaction. Upon partial dissipation of the membrane potential, the post-translational membrane translocation of the negatively charged domains is blocked, whereas uncharged regions still can cross the membrane. In contrast, the insertion of mitochondrially encoded proteins does not strictly depend on the membrane

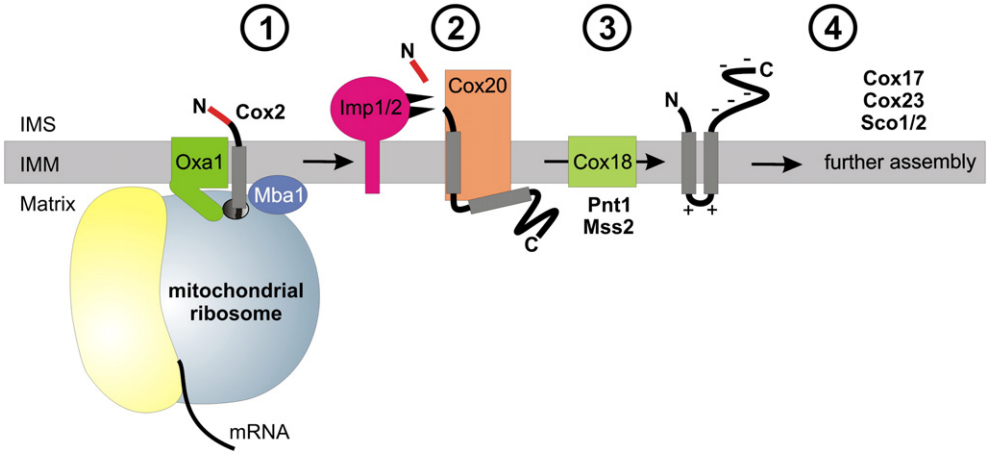



**Fig. 4.** Topogenesis of Cox2. Cox2 is co-translationally inserted into the inner mitochondrial with the help of Oxa1 and Mba1 (1). Following transport to the intermembrane space, the N-terminal leader peptide (red) of Cox2 is removed by the intermembrane peptidase (Imp1/Imp2) (2). The inner membrane protein Cox20 binds to the precursor form of Cox2 and facilitates its proteolytic maturation. Next, the second transmembrane segment and the large hydrophilic C-terminus are inserted and translocated, respectively, a reaction requiring Cox18, Mss2 and Pnt1 (3). During the subsequent translocation of the C-terminus of Cox2, copper is inserted into this domain, a reaction depending on Cox17, Cox19, Cox23, Sco1, and Sco2. Finally, Cox2 assembles together with other subunits into a functional cytochrome *c* oxidase complex (4).

potential, so that even in the presence of the ionophore valinomycin, these proteins can reach their correct topology. It remains unclear whether the mitochondrial ribosome directly contributes to the translocation of a polypeptide across the inner membrane.

In addition to proteinaceous factors, phospholipids influence the insertion process directly by an interaction with topogenic signals in the membrane protein. Transient binding of positively charged amino acid residues to negatively charged phospholipids (phosphatidylglycerol and cardiolipin) prevents the transfer of positively charged domains across the inner membrane. Accordingly, down-regulation of Pgs1, the enzyme catalyzing the terminal step in phosphatidylglycerol synthesis, interferes with the biogenesis of mitochondrially encoded proteins [23]. The precise role of phospholipids in the translocation reaction is still poorly understood.

### 3.2. Protein insertion by SecYEG and YidC in bacteria

The mitochondrial insertion machinery developed from that of eubacteria and still shows a number of similarities. In bacteria, the biogenesis of most polytopic membrane proteins occurs in a co-translational reaction (for details see the review by Arnold Driessen in this issue). A hydrophobic segment is recognized by the signal recognition particle (SRP) and targeted via an interaction with the SRP-receptor to the inner membrane. There, the ribosome nascent chain complex is transferred to the SecYEG translocon, and the SRP and SRP-receptor are released. The SecYEG translocon allows protein translocation across and protein insertion into the bacterial inner membrane [24]. The protein conducting channel is established by SecY. This polytopic membrane protein spans the membrane ten times with the N- and C-terminus facing the cytosol. SecY is organized into N- and C-terminal domains, comprising transmembrane segments 1–5 and 6–10, respectively [25]. Both domains are connected by a periplasmic loop between transmembrane 5 and 6, yielding an overall structure that resembles a clamshell with a central funnel-like pore. Sorting of preproteins for insertion or translocation occurs at the level of the SecYEG complex and involves distinct configurations of the channel. In the case of insertion, a lateral gate is established in the translocon that allows release of transmembrane segments into the lipid bilayer. The transmembrane segments are inserted into the membrane either on their own or in a pairwise configuration [26]. In many cases the substrates are passed on from the SecYEG complex to the integral membrane protein YidC which facilitates their correct membrane integration and presumably contributes to the folding of transmembrane proteins to their correct structure [27]. Moreover, YidC is able to catalyze the insertion of a number of membrane proteins in a SecYEG-independent reaction [28,29,30]. Substrates that are inserted directly by YidC lack highly charged hydrophilic domains on the periplasmic side of the membrane and are generally small proteins (one or two transmembrane segments) that assemble into larger complexes. Hence, bacterial YidC is sufficient for the integration of membrane segments into the membrane, but the transfer of more complex, charged domains across the membrane requires a functional SecYEG complex.

A dedicated quality control system surveys the newly synthesized polypeptides. In bacteria, this system contains, among others, the AAA protease FtsH, an ATP-dependent metalloprotease [31]. FtsH is an endopeptidase with a special ability to dislocate membrane protein substrates out of the membrane, for which its own membrane-embedded nature is essential. FtsH degrades misassembled membrane proteins, contributing to their quality maintenance.

### 3.3. Protein insertion by the mitochondrial Oxa1 complex

Presumably due to the specialization on the synthesis of a very small number of translation products, the mitochondrial insertion machinery appears to be much simpler than the bacterial one: mitochondria of plants, fungi and animals contain neither a signal recognition particle, nor a SecYEG complex [32]. Only very archaic marine protists like the jakobid *Reclinomonas americana* appear to contain a SecYEG complex of which the hydrophobic SecY subunit is encoded on the mitochondrial genome [33] (Fig. 1).

In mitochondria, the YidC homolog Oxa1 appears to play the central role in membrane insertion of translation products [34]. Oxa1 was initially identified in 1994 as a component required for the topogenesis of Cox2 [35,36]. Cox2 represents the best studied membrane protein because in fungi it is synthesized with a leader peptide which allows to use the processing of the protein as an indication for its membrane insertion (Fig. 4). It rapidly became clear that Oxa1 is a member of a large protein family, known as the YidC/Alb3/Oxa1 family. Members of this family are present in the inner membranes of bacteria (YidC), chloroplasts (Alb3) and mitochondria (Oxa1). All members of this family contain a core hydrophobic domain of five transmembrane segments exhibiting the catalytic activity [30,37]. This domain can be functionally exchanged between the different members of the protein family [38–42].

Members of the YidC/Alb3/Oxa1 family catalyze insertion of proteins. It was shown for both YidC and Oxa1 that they form homo-oligomeric complexes [43]. X-ray scattering of 2D-cristals shows a dimeric structure of YidC in which the subunits exhibit an antiparallel orientation. Based on cryo-EM reconstruction of recombinant Oxa1 bound to *E. coli* ribosomes, it was suggested that the Oxa1-dimer represents a SecY-like structure [44]. Similar to the ten transmembrane segments of SecY, a dimer of Oxa1 would contribute ten transmembrane helices that may form a clam-like structure. This assembly could allow the formation of a protein conducting channel as well as a lateral release of transmembrane segments into the lipid bilayer.

In mitochondria, Oxa1 contacts nascent polypeptides very early during their synthesis [45] [34]. This contact of Oxa1 with its substrates is facilitated by the direct binding of Oxa1 to the mitochondrial ribosome. Oxa1 contains a C-terminal extension that is both required and sufficient for binding to the ribosome [46,47]. In addition to its insertase activity, YidC/Alb3/Oxa1 proteins presumably contribute to the folding and assembly of membrane proteins [27]. For example, Oxa1 and YidC play a role in the assembly of the $F_0$ sector of the mitochondrial ATPase which might be different to their function in protein insertion [48,29]. In both systems, Oxa1/YidC interacts directly with an assembly intermediate of the c subunit of the $F_0$ sector (Atp9) and promotes its conversion into a fully assembled ATPase complex.

Although Oxa1 clearly plays a crucial role in protein insertion into the inner membrane, it is likely that it cooperates with other components in this process. This is for example indicated by the fact that, except for Cox2, all translation products integrate into the membrane also in the absence of Oxa1, albeit with reduced efficiencies. Therefore, several genetic screens were performed to identify additional components of the protein insertion machinery. One of these screens showed that mutations in the cytochrome $c_1$ subunit of cytochrome *c* reductase can suppress the defects of Oxa1-deficient mutants thus restoring the ability to insert mitochondrially encoded proteins [49]. These point mutations introduce a positive charge in the transmembrane segment of cytochrome $c_1$ and it was suggested that this positive charge might be utilized to facilitate the translocation of negatively charged stretches to the intermembrane space. Whether this point mutant can act on its own or cooperates with a yet undiscovered component of the insertion machinery is not clear.

### 3.4. Cox18, a mitochondrial Oxa1 homolog specifically required for insertion of Cox2

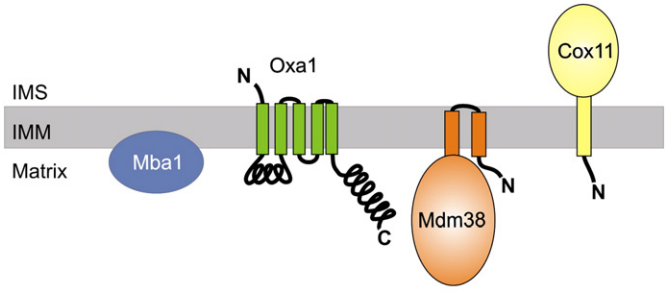
In order to identify novel insertion components, genetic screens were designed by the group of Tom Fox, using a fusion of the matrix

protein Arg8 to the C-terminus of Cox2 [50]. Mutants defective in the export of this region accumulate Arg8 in the matrix and allow the cells to grow on arginine-deficient medium. This elegant screen led to the identification of three components, Cox18, Pnt1 and Mss2, each necessary for the export of the Arg8 domain [51,52,53].

Cox18 (also termed Oxa2 in *N. crassa*) is a member of the YidC/Alb3/Oxa1 family and contains the conserved insertase domain, but – unlike Oxa1 – lacks a C-terminal ribosome binding site [39]. Upon expression in *E. coli*, Cox18 can complement YidC-deficient strains indicating that it exhibits insertase activity and is a *bona fide* member of the YidC/Alb3/Oxa1 protein family [54]. Biochemical evidence suggests that Cox18 plays an important post-translational role in the assembly of cytochrome *c* oxidase [55]. Analysis in mitochondria of *N. crassa* indicated that Cox18 can bind to Cox2 and Cox3 after completion of their synthesis [56]. It is not entirely clear why mitochondria contain two functionally distinct members of the YidC/Alb3/Oxa1 family. Even upon overexpression of Oxa1, yeast cells still require Cox18 for assembly of cytochrome *c* oxidase. Importantly, the C-terminus of Cox2 is properly translocated in this case suggesting that Cox18 is not absolutely required for the export reaction but may have a further function in a later step of complex IV assembly [57]. The molecular functions of Pnt1 and Mss2 are still unknown, but they likely contribute to the export of the C-terminus of Cox2 to the intermembrane space [52,58].

## 4. Coupling translation to membrane integration

In contrast to cytosolic ribosomes of pro- and eukaryotes, mitochondrial ribosomes are permanently associated with the inner membrane, at least in yeast [59,60]. The tight membrane binding presumably reflects their specialization on the production of hydrophobic membrane proteins. In mammalian mitochondria, ribosomes might be less tightly bound to the inner membrane but are still co-isolated with membrane vesicles after fractionation of actively translating ribosomes. The tethering of the ribosomes to the membrane obviously made an SRP in the mitochondria obsolete and caused a major change in the structure of mitochondrial ribosomes, in particular in the region of the polypeptide exit tunnel [4]. How mitochondrial ribosomes are bound to the membrane is not entirely clear but several components were identified that physically connect ribosomes to the inner membrane (Fig. 5).




| | Mba1 | Oxa1 | Mdm38 | Cox11 |
|---|---|---|---|---|
| MW (kDa) | 28 | 40 | 58.6 | 34 |
| pI | 10.00 | 10.18 | 5.7 | 9.6 |

**Fig. 5.** Biogenesis factors interacting with the mitochondrial ribosome. Mba1 and Oxa1 play a direct role in the insertion process. Oxa1 mediates the insertion and translocation of mitochondrially encoded proteins. To interact with the mitochondrial ribosome, Oxa1 contains a C-terminal α-helical domain that is sufficient to interact with ribosomes. Mba1 is a ribosome receptor that helps to align the ribosomal exit tunnel to the insertion site of the inner membrane. Mdm38 interacts similarly with the ribosome; its function however remains unclear. Cox11 is a factor that helps to insert copper into Cox1. The topologies of the indicated proteins are depicted, together with the molecular masses and pIs of the mature proteins.

### 4.1. Oxa1 couples translation to membrane insertion

The C-terminus of Oxa1 forms a roughly 100 amino acid residue long, positively charged region which faces the matrix. This region presumably forms a amphipatic α-helix with one positively charged and one hydrophobic surface and shows the hallmark of a coiled-coil structure [47]. This domain is sufficient and necessary for tight ribosome binding and tethers the Oxa1 insertase to mitochondrial ribosomes [46,47]. Oxa1 contains an additional matrix-exposed loop (between transmembrane segment 1 and 2) that might contribute to ribosome binding. Cross-linking experiments indicated that Oxa1 binds in close proximity to the polypeptide exit tunnel of the ribosome, suggesting that the newly synthesized proteins might directly engage with Oxa1 as soon as they emerge from the ribosome (see below).

### 4.2. Mba1, a mitochondrial ribosome receptor

Oxa1 is supported in the insertion of mitochondrially encoded proteins by the peripheral membrane protein Mba1 [45,59]. Mba1 is part of a large protein family that includes the mammalian ribosomal proteins Mrpl45. Like Oxa1, Mba1 binds directly to the large subunit of the mitochondrial ribosome where it is in close proximity to short nascent polypeptides as they emerge from the ribosome. Mba1 presumably functions as receptor that helps to align the ribosomal exit tunnel and the insertion site of the inner membrane. Hence, the simultaneous deletion of Mba1 and the C-terminal ribosome binding domain of Oxa1 greatly impairs co-translational membrane insertion of nascent chains and leads to a respiration-deficient phenotype [59]. In such double mutants, mitochondrially encoded proteins accumulate in non-productive form at the matrix side of the inner membrane and are bound by the matrix chaperone Hsp70 [59] that under normal conditions does not interact with the hydrophobic translation products in mitochondria [61].

Interestingly, Mba1 was initially identified together with Oxa1 as high copy suppressors of a deletion mutant of *YTA10* [62]. Yta10 is part of the m-AAA protease that is homologous to the bacterial FtsH. Like FtsH, the mitochondrial AAA-proteases (the matrix-exposed m-AAA protease and the intermembrane space-exposed i-AAA protease) are crucial for the protein homeostasis of the inner membrane [63]. Experimental evidence suggests that they survey the newly made polypeptides very early during their synthesis, as short nascent chains can be efficiently cross-linked to Yta10 [45]. Moreover, the m-AAA protease is involved in the processing of the ribosomal protein MrpL32, presumably to restrict the assembly of mitochondrial ribosomes to the region underneath the inner membrane [64].

### 4.3. Mdm38/LETM1, a ribosome-interacting protein associated with Wolf-Hirschhorn-Syndrome

Mdm38/LETM1 is a third membrane protein that binds to mitochondrial ribosomes [65,66]. In contrast to Oxa1 and Mba1 which are positively charged and hence probably bind also to RNA-motifs on the ribosome, Mdm38 is negatively charged. Moreover, this membrane protein is highly conserved among eukaryotes. Deletion of the human homolog LETM1 is associated with the Wolf-Hirschhorn-Syndrome, a fatal neurodegenerative disorder [67]. Absence of Mdm38 changes the potassium homeostasis in the matrix which has direct consequences for mitochondrial morphology and apoptosis signaling [68]. Moreover, a recent study suggests that LETM1 functions as a $H^+/Ca^{2+}$ antiporter in the inner membrane [69]. Whether Mdm38 is involved in two independent functions, ion homeostasis and ribosome binding, or whether one of these activities is only indirectly associated with the protein is not clear.

### 4.4. Cox11, a ribosome-associated metallation factor for Cox1

Cox11 is an inner membrane protein with a conserved IMS domain which catalyzes the insertion of copper into the Cu(B) site of Cox1 [70,71]. Co-migration experiments suggest that Cox11 is associated with mitochondrial ribosomes, presumably to facilitate the co-translational metallation of Cox1 [72]. Where Cox11 binds to the ribosome and whether this interaction is relevant for co-translational protein insertion is not clear.

### 4.5. Translational activators bind mRNAs to the inner membrane

Not only the mitochondrial ribosome, but also mRNAs are bound to the inner membrane. In yeast mitochondria, a number of so-called translational activators were identified which are essential for translation and mRNA stability. These factors are membrane-associated proteins or protein complexes which bind mRNAs presumably via interactions with the 5′ untranslated regions [73–77]. Thereby, each gene appears to have its exclusive set of translational regulators. The recent discovery of TACO1, a translational activator specific for Cox1 in mammalian mitochondria, suggests that animals might utilize similar translational control mechanisms as fungi [78].

## 5. The ribosomal tunnel exit of mitochondria

The polypeptide exit tunnel (PET) of the ribosome is the site where nascent polypeptides are exposed to a hydrophilic environment for the first time. Hence it is an important site where the fate of the newly synthesized proteins is determined. Multiple factors interact with the newly made proteins. They are recruited to the PET by binding to ribosomal proteins and RNA-structures. In bacteria, the rim of the PET is formed by RNA and a group of four ubiquitously conserved ribosomal proteins (L22, L23, L24 and L29). The arsenal of ligands of the PET includes deformylases, processing peptidases, chaperones, targeting factors and components of the insertion machinery. Importantly, L23 is involved in almost all interactions with ribosome-associated biogenesis factors studied so far that include in bacteria SRP [79,80], the molecular chaperone trigger factor [80,81], YidC [44] and the SecYEG translocon [82]. This concentration of many factors binding to the same site implies that they interact with the ribosome in an exclusive, often sequential manner according to the stage of protein biogenesis.

The ligands of the mitochondrial exit tunnel and their dynamics are far less known. Cryo-EM reconstruction indicates that the PET of mitochondrial ribosomes differs considerably from the bacterial structure [4]. In proximity to the standard PET, mitochondrial ribosomes have an additional opening referred to as the polypeptide accessible site. Whether this region really forms a tunnel or just represents a region of low electron density is unclear, but it was speculated that such a tunnel might be used by a subset of proteins allowing them to interact with specific biogenesis factors.

Mitochondria contain homologs of the conserved ribosomal proteins surrounding the exit tunnel in bacteria, L22, L23, L24 and L29, which in yeast are named Mrpl22, Mrp20, Mprl40 and Mrpl4, respectively (Fig. 6). Due to additional flanking sequences all these mitochondrial proteins are larger than their bacterial counterparts. The significance of these extension domains is not known. They may simply have a stabilizing function compensating for the low content of rRNA and/or serve as docking sites for translation and assembly factors.

The only protein for which the binding site on the ribosome was mapped is Oxa1 [46]. Cross-linking studies revealed that Oxa1 binds to short nascent chains, supporting a function of the protein during co-translational protein insertion [34,45]. Co-migration on sucrose gradients showed that Oxa1 interacts with the large ribosomal subunit [47]. Chemical cross-linking and pull-down experiments identified the binding site of Oxa1 in proximity of Mrp20, the homolog of the bacterial L23 protein, and of Mrpl40, the homolog of L24 [83] (Steffi Gruschke and Martin Ott, unpublished). The binding of Oxa1 to the ribosome is salt-sensitive and likely involves an interaction of the C-terminal, positively charged α-helical domain of Oxa1 with the negatively charged 21S RNA, because limited RNAse treatment readily

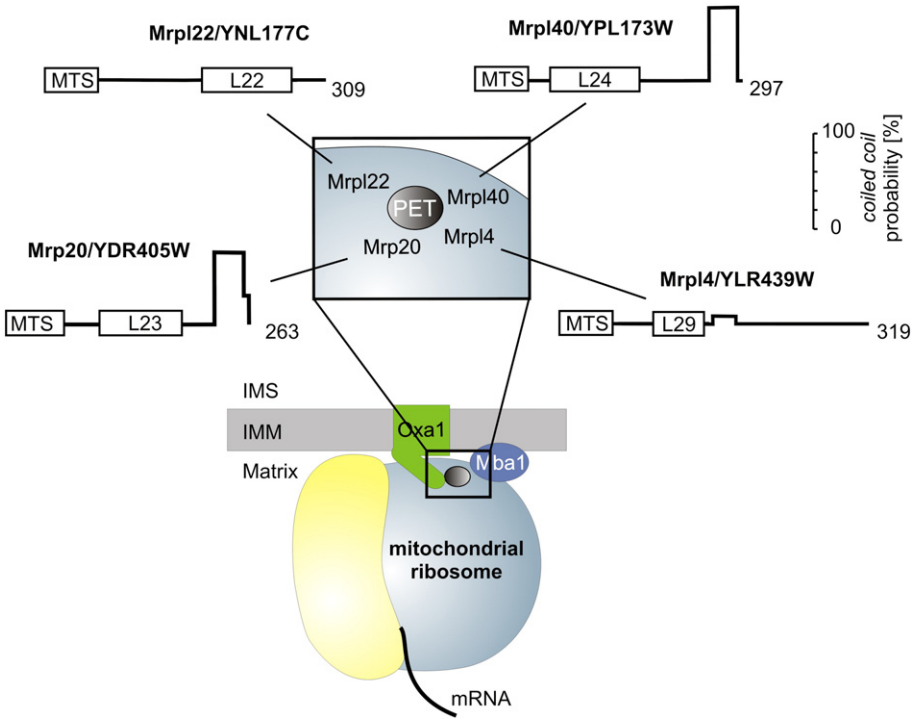



**Fig. 6.** The ribosomal exit tunnel is formed by conserved proteins. In bacteria, the rim of the PET is formed by RNA and a group of four ubiquitously conserved ribosomal proteins (L22, L23, L24 and L29). Homologs of these proteins are present in mitochondrial ribosomes. Schematic representations of the yeast homologs are depicted. They contain, in addition to the regions homologous to the bacterial counterparts (marked by boxes), mitochondrial targeting signals (MTS) at their N termini and C- or N-terminal extension domains. Some of these extension domains show a high probability to form coiled-coil structures which were calculated on the basis of a prediction algorithm [88].

releases Oxa1 from the ribosome while Mrp20 is not liberated [59]. This binding site of Oxa1 at the L23/L24 surface was recently confirmed in high resolution cryo-EM studies with bacterial ribosomes [44]. The interaction of Oxa1 with the ribosome is not subject to an obvious regulation, as no changes in the cross-linking efficiency to Mrp20 [46] or in ribosome association were observed when translation (and hence membrane insertion of newly synthesized proteins) are inhibited by puromycin treatment [59].

## 6. Outlook

Although the presence of DNA and ribosomes in mitochondria is known for more than 40 years, surprisingly little is known about the organization and regulation of mitochondrial protein expression. This might partially be explained by the fact that no cell-free system could be established so far in which mitochondrial translation can be analyzed *in vitro*. It is often assumed that the mitochondrial system simply resembles that of bacteria. However, a number of observations suggest pronounced differences between mitochondria and bacteria. This is especially evident for translational control in mitochondria which is carried out by translational activators, a system not found in bacteria. Similarly, the mitochondrial protein insertion machinery is strikingly different from the bacterial system because of the lack of the central protein translocase, the SecYEG translocon.

The assembly of mitochondrially encoded subunits into functional respiratory chain complexes proceeds through multiple steps that are catalyzed by a variety of different biogenesis factors. Hence, it is conceivable that synthesis of mitochondrially encoded subunits and their assembly with nuclear encoded, imported proteins are coordinated processes that occur in specific locations in the inner membrane.

Such a scenario of a spatial organization of the inner membrane allowing optimal assembly of respiratory chain complexes is supported by numerous observations. First, the translational activators for the mitochondrially encoded complex IV subunits Cox1, Cox2 and Cox3 form a complex in the inner membrane [75]. This suggest that this organization might allow to channel the assembly process in a way that subunits destined for one complex are synthesized and inserted in close proximity to each other. It is moreover feasible that these organized ribosomes recruit a special set of assembly factors, hence facilitating the assembly process. This notion is supported by the observation that the insertion of co-factors into mitochondrially encoded subunits can occur co-translationally, as suggested by the interaction of Cox11 with mitochondrial ribosomes. Third, insertion components as well as the mitochondrial quality control system transiently interact with the newly synthesized polypeptides. All these important interactions have to be organized in a spatial and temporal manner. It is feasible that the ribosome plays an important role as a platform to coordinate the early steps in the assembly of the respiratory complexes. It will be exciting in the future to shed some light on the organization of the mitochondrial translation machinery and its organizational role for the biogenesis of the respiratory chain.

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