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Review

Redox regulation of protein folding in the mitochondrial intermembrane space

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ABSTRACT

Protein translocation pathways to the mitochondrial matrix and inner membrane have been well characterized. However, translocation into the intermembrane space, which was thought to be simply a modification of the traditional translocation pathways, is complex. The mechanism by which a subset of intermembrane space proteins, those with disulfide bonds, are translocated has been largely unknown until recently. Specifically, the intermembrane space proteins with disulfide bonds are imported via the mitochondrial intermembrane space assembly (MIA) pathway. Substrates are imported via a disulfide exchange relay with two components Mia40 and Erv1. This new breakthrough has resulted in novel concepts for assembly of proteins in the intermembrane space, suggesting that this compartment may be similar to that of the endoplasmic reticulum and the prokaryotic periplasm. As a better understanding of this pathway emerges, new paradigms for thiol-disulfide exchange mechanisms may be developed. Given that the intermembrane space is important for disease processes including apoptosis and neurodegeneration, new roles in regulation by oxidation–reduction chemistry seem likely to be relevant.

1. Introduction

The mitochondrion represents a relic of an α-proteobacterium that inhabited the cytosol of the first eukaryote and developed through endosymbiosis [1]. Today, understanding this process and how the mitochondrion has developed within its host eukaryotic cell is an exciting topic that has led to many unexpected findings in mitochondrial biology. The mitochondrion maintains an outer membrane and inner membrane that separate the mitochondrial matrix from the intermembrane space [2]. Like prokaryotes, the inner membrane and matrix are important sites for energy production with the electron transport chain and Kreb's cycle. The outer membrane interfaces with the host cytoplasm; a subset of proteins of this membrane remain prokaryotic in origin (i.e. a handful of prokaryotic β-barrel proteins such as porin have been maintained) [3,4], whereas new pathways have arisen, such as those for mitochondrial fusion and fission and apoptosis.

During the endosymbiotic event, most of the genes from the progenitor mitochondrion moved to the nucleus. As a result of this migration, trafficking pathways to target proteins back to the organelle had to be developed. Many precursors developed targeting sequences; approximately 40% of proteins in the mitochondrial proteome contain the typical targeting sequence that is located at the N-terminus and consists of an amphipathic α-helix that is positively-charged on one face and hydrophobic on the other face [5,6]. Proteins with N-terminal targeting sequences have been easily identified with the aid of computational methods [7,8]. The remainder of the mitochondrial proteome, however, contains targeting information within the mature part of the protein [9]. Many of these proteins reside in the inner and outer membranes as well as the intermembrane space. Therefore, it is typically necessary to experimentally test the localization of bonafide mitochondrial proteins that lack the typical targeting sequences and use approaches such as mutagenesis to identify potential targeting sequences [10]. Importantly, proteomic analysis of the mitochondrion has aided the identification of these proteins that lack obvious targeting information [6].

As a result of these diverse targeting sequences, a wide range of import pathways has developed in the mitochondrion (Fig. 1) [2,9,11]. Most precursors are thought to pass through the translocase of the outer membrane (TOM) complex. However, the insertion of some outer membrane proteins, particularly apoptotic proteins and those that are anchored to the membrane by one transmembrane domain, may engage in the TOM complex without traversing it or may insert independently [12]. After passage through the TOM complex, proteins with a typical N-terminal targeting sequence are then imported across the inner membrane and use the translocase of the inner membrane-23 (TIM23) pathway, in which Tim23 (with a molecular mass of 23 kDa) is one of the major components. In contrast, proteins such as the mitochondrial carrier family and import components Tim17, Tim22, and Tim23 use the TIM22 pathway, in which Tim22 is one of the major components. These translocons share similar themes with a subset of components that function as receptors and a subset that form the translocation channel. Finally, the sorting and assembly machinery (SAM) complex directs the assembly of β-barrel protein such as Tom40 and porin that reside in the outer membrane [13]. After

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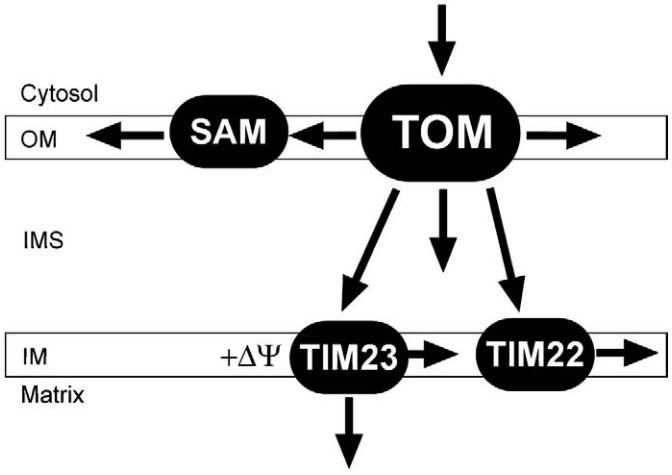

Fig. 1. Schematic of translocation and assembly complexes in the mitochondrion. Nuclear-coded mitochondrial precursors cross the TOM complex. The SAM complex mediates the assembly of outer membrane proteins with complicated topologies. Proteins with a typical N-terminal targeting presequence are imported via the TIM23 complex, whereas inner membrane proteins such as the carrier proteins are imported via the TIM22 complex.

the β-barrel precursors pass through the TOM complex, the β-barrel precursors assemble with the SAM complex from the intermembrane space-side of the membrane.

However, protein import pathways into the intermembrane space remain complicated in that different precursors use different approaches [14]. Proteins that contain a bipartite N-terminal targeting sequence engage the Tim23 translocon and then transfer arrests by the "stop-transfer" domain in the targeting sequence [15]. Translocation depends on the presence of a membrane potential ($\Delta\psi$). The presequence is proteolytically cleaved in two steps by processing peptidases in the mitochondrial matrix and intermembrane space. Representatives include cytochrome b_2, apoptosis inducing factor, and cytochrome *c* peroxidase [15–17]. Other intermembrane space proteins that lack an N-terminal targeting sequence are imported and then fold around cofactors or acquire disulfide bonds [3,14]. Import is independent of the $\Delta\psi$, emphasizing that the inner membrane translocons are bypassed. This class of proteins is small (molecular mass below 22 kDa), and most proteins contain either the CX_3C motif or the CX_9C motif, which form disulfide linkages [18,19]. Characteristic of the CX_3C and CX_9C motifs is that the cysteine residues are separated by three and nine amino acids, respectively. The assembly of these proteins with disulfide bonds represents a new development in mitochondrial biogenesis and will be discussed in detail in this review.

2. Proteins with disulfide bonds in the intermembrane space

The intermembrane space contains a growing list of mitochondrial proteins that potentially contain disulfide bonds (Table 1). In general, disulfide linkages function in both structural and catalytic roles. Disulfide bond formation also serves as a mechanism for the import and assembly of proteins into the intermembrane space [20]. The identification of a subset of proteins with disulfide bonds in the intermembrane space was surprising because the mitochondrial intermembrane space was believed to be a reducing environment like the cytosol and not conducive to disulfide bond formation. However, disulfide bonds can generally be formed in the reducing cytosol as demonstrated by the pox virus that has an entire pathway for synthesizing disulfide bonds in the virion's coat [21]. Thus, the intermembrane space may be an oxidizing environment like the bacterial periplasm and the endoplasmic reticulum [22,23].

2.1. Proteins involved in respiration and oxidative repair

The first proteins that contained disulfide bonds were identified in the cytochrome bc_1 complex (Complex III). The Rieske FeS protein is a redox protein with the highest midpoint potential of the bc_1 complex. A disulfide bridge placed between the two loops of the protein holds the [2Fe–2S] cluster [24,25]. If the disulfide bridge is chemically reduced, reoxidation of the dithiol and the [2Fe–2S] cluster occurs rapidly in *Thermus thermophilus* [26], indicating that the disulfide of the Reiske FeS protein does not require a factor for assembly. Elimination of the disulfide bridge did not impair stability of the FeS cluster, but instead resulted in structural changes in the environment around the FeS cluster. The structural changes specifically affected interactions with ubiquinol and indirectly damaged the ubiquinol oxidation site [27]. Thus the disulfide linkage is requisite for activity of the cytochrome bc_1 complex. In contrast, subunit 8 of the bc_1 complex in mammals (referred to as Qcr6 in yeast) contains disulfide bonds for stability [24,25]. Qcr6 is required for correct complex formation between cytochrome *c* and c_1. Structural studies have shown that mammalian subunit 8 is composed of two long antiparallel alpha-helices that are connected on each end by two disulfide bonds (C24–C68 and C40–C54) [24]. Similarly, the yeast Qcr6 shares an overall general fold, but only one disulfide bond links the antiparallel alpha-helices [25]. Therefore, the bc_1 complex has disulfide bonds that seemingly do not require sulfhydryl oxidases for assembly.

Sco1 (synthesis of cytochrome *c* oxidase) and Sco2 contain single CX_3C motifs and are tethered to the inner membrane, with the bulk of the protein, including the CX_3C motif, facing the intermembrane space [28,31]. Sco1 and Sco2 are copper binding proteins that are required for the assembly of cytochrome oxidase, potentially playing a role in donating copper via the CX_3C motif for cytochrome oxidase maturation [29,32]. Interestingly, Sco1 has a thioredoxin fold and shares a low similarity with peroxiredoxins and thiol:disulfide oxidoreductases, suggesting that Sco1 may perform a catalytic role rather than copper transport role [33]. Indeed, recent crystallization studies have shown that the intermembrane space domain of Sco1 is similar to thioredoxin, with the CX_3C motif positioned analogous to the CX_2C in thioredoxin [31,34]. Sco1 thus may act as a copper-dependent redox switch. Sco2 shares similar properties with Sco1, having similar metal

Table 1
Resident intermembrane space proteins with disulfide linkages

Redox proteins	Function	Reference
Rieske FeS protein	Cytochrome *bc*1 function	[24]
Qcr6	Cytochrome *bc*1 function	[24]
Sod1	Superoxide dismutase	[40]
Ccs1	Sod1p assembly	[40]
Sco1	Cytochrome oxidase assembly	[33]
Sco2	Cytochrome oxidase assembly	[98]
Cox11[a]	Cytochrome oxidase assembly	[58]
Cox12[a]	Cytochrome oxidase assembly	[59]
Cox17	Cytochrome oxidase assembly	[34]
Cox19	Cytochrome oxidase assembly	[60]
Cox23[a]	Cytochrome oxidase assembly	[61]
Pet191[a]	Cytochrome oxidase assembly	[56]
Cmc1[a]	Cytochrome oxidase assembly	[57]
Som1[a]	Inner membrane peptidase subunit	[19]
Tim8	Protein import	[44]
Tim9	Protein import	[99]
Tim10	Protein import	[99]
Tim12	Protein import	[100]
Tim13	Protein import	[44]
Mic14	Unknown function	[19]
Mic17	Unknown function	[19]
Mdm35	Unknown function	[19]
Mia40	Protein import	[20]
Erv1	Sulfhydryl oxidase/FeS cluster export	[73]

[a] Denotes that the disulfide linkages have not been confirmed experimentally, but have been predicted based on homology.

binding and redox properties. Additional structural studies though suggest that Sco2 is less structured that Sco1, and these differences may be important for molecular recognition with different protein partners [30].

The copper/zinc-superoxide dismutase (*SOD1*) and its copper chaperone CCS are distributed between the intermembrane space and the cytosol, in addition to the nucleus and lysosomes [35,36]. Localization of Sod1 to the intermembrane space is to presumably protect against superoxide radicals generated by the respiratory chain [37] and requires the copper chaperone CCS. Structurally, all eukaryotic Sod1 proteins contain one copper, one zinc, and one disulfide bond per monomer [38], which are required for activity. CCS mediates copper insertion into Sod1 by forming a transient heterodimer via a disulfide linkage [36,39]. Interestingly, Sod1 is imported into the intermembrane space in an immature form, lacking copper and zinc and a disulfide bridge [40], and subsequently CCS is required for Sod1 maturation in the intermembrane space [41]. Placement of the disulfide bond in Sod1 is thus required for regulation of enzymatic activity, for prevention of misfolding, and for import into the intermembrane space.

2.2. Proteins with the twin CX_3C motif

The small Tim proteins (Tim8, Tim9, Tim10, Tim12, and Tim13) contain the twin CX_3C motif in which two cysteine residues are separated by three amino acids [42]. Spacing between each CX_3C varies from 11 to 16 amino acids; this motif is unique to the small Tim proteins. This family is conserved from yeast to mammals and plants, but not found in prokaryotes [43]. The small Tim proteins assemble in 70 kDa complexes in the intermembrane space and function as chaperones to guide inner membrane proteins, including those of the mitochondrial carrier family and import components Tim17, Tim22, and Tim23 [44–46]. The three subunits of Tim9 assemble with three subunits of Tim10; similarly Tim8 assembles with Tim13. In addition, a fraction of the Tim9 and Tim10 assemble with Tim12, Tim18, Tim22, and Tim54 at the inner membrane, mediating insertion of the substrate into the inner membrane. Mutations in the human homolog of Tim8, DDP1, result in an X-linked disease deafness-dystonia syndrome [47,48].

The cysteine residues are essential for assembly of the small Tim proteins. A missense mutation in the 4th cysteine of DDP1 results in loss of the DDP1–TIMM13 complex, leading to deafness-dystonia syndrome [48,49]. The twin CX_3C motif has been proposed to coordinate Zn^{2+} by forming a zinc-finger-like structure [50]. The recombinant small Tim monomers bind zinc in a 1:1 ratio [50]; however, the assembled small Tim complexes do not contain zinc, but instead harbor disulfide bonds [44,51,52]. Allen *et al.* studied the folding and assembly requirements of the Tim9–Tim10 complex in detail [52]. Using trypsin digestion and mass spectrometry approaches, Tim10 folds into a similar structure as that predicted from zinc coordination, but differs slightly in that the cysteines pair in juxtaposed disulfide bonds. The inner disulfide bond is formed by C44 and C61, and an outer pair is formed by C40 and C65; the inner thiol linkage is crucial overall for folding and chaperone activity. Zinc might bind to the fully reduced Tim proteins, but does not promote complex formation [52]. Finally, the crystal structure by Gulbis and colleagues confirmed that the small Tim proteins contain juxtapositional disulfide bonds [53]. Interestingly the structure was similar to the bacterial Skp chaperones of the periplasm and the cytosolic prefoldins, although the sequences shared no similarity [54,55].

2.3. Proteins with a CX_9C motif

Like the twin CX_3C motif, another set of intermembrane space proteins contain the twin CX_9C motif in which two cysteine residues are separated by 9 amino acids. This group includes Pet191 [56], Cmc1 [57], Cox11 [58], Cox12 [59], Cox17 [58], Cox19 [60], and Cox23 [61], which are involved in cytochrome oxidase assembly. In addition, Mia40, which is essential for the import of the small Tim proteins and a subset of the CX_9C proteins, contains the same CX_9C motif [62–64]. New members, including a subunit of the inner membrane protease Som1, have been added through a computational method to search the genome for small proteins with a CX_9C motif [19]. Many of these proteins were mislocalized by placement of a GFP on the C-terminus and listed as cytosolic proteins in the databases, but specific biochemical analysis showed that these proteins reside in the intermembrane space. Additional substrates include Mic14, Mic17, and Mdm35; the function of these proteins is not known [19].

Proteins Pet191 [56], Cmc1 [57], Cox11 [58], Cox12 [59], Cox17 [58], Cox19 [60], and Cox23 are required for cytochrome oxidase biogenesis. Cox17 is the best characterized [58]. Structurally, the two α-helices of Cox17 are preceded by two coiled-coil regions. This motif is described as a coiled-coil–helix–coiled-coil–helix (CHCH) domain [65]. Two disulfide bonds (C25–C54 and C35–C44) link the two antiparallel α-helices, keeping them together [34,58]. The disulfide bonds are not essential for physiological function, suggesting a role in structural stabilization. Cox17 contains two additional cysteine residues, C22 and C23, which can either coordinate the copper (I) ion or form a disulfide bond [58]. Thus, Cox17 uses dithiols in its role as a copper chaperone.

Mia40/Tim40 is an essential intermembrane space protein that is tethered to the inner membrane in yeast mitochondria and mediates the import of the small Tim proteins and other CX_9C proteins [19,62–64]. Mia40 is evolutionarily conserved [62–64]; the plant and animal homologs are smaller in molecular mass and lack the transmembrane domain [66]. Mia40 also contains the twin CX_9C motif in addition to an N-terminal CPC motif. Recombinant Mia40 binds copper in addition to zinc [62], suggesting that it might have a structure similar to Cox17. However, Mia40 has also been shown to contain 3 intramolecular disulfide bonds [67]. Specifically, one thiol linkage connects the first two cysteine residues in the CPC motif, whereas the two remaining thiol bonds bridge the cysteine residues of two CX_9C segments. The disulfide bonds in the CX_9C motif most likely stabilize the protein because they were only accessible to reducing agents when treated with denaturant. In contrast, the CPC motif was easily accessible to reducing agents and most likely forms a transient disulfide bond with substrates or Erv1 [67].

2.4. Erv1, a sulfhydryl oxidase

Erv1 is a member of the sulfhydryl oxidase family, which contain flavin adenine dinucleotide (FAD) as a cofactor [68,69]. By definition, sulfhydryl oxidases generate disulfide bonds in proteins using molecular oxygen to produce hydrogen [69]. Erv1, the mitochondrial sulfhydryl oxidase in the intermembrane space, is essential for viability and related to Erv2 of the endoplasmic reticulum [70]. In addition, Erv1 has a human homolog ALR (augmenter of liver regeneration), which suggests that Erv1 plays a critical role in organ development and disease [71]. Like the small Tim proteins and Mia40, Erv1 is conserved in plants, fungi, and animals, but absent in prokaryotes [72,73]. The N-terminal regions of Erv1 and Erv2 are distinct, whereas the C-terminal domain of Erv1 and Erv2 has the redox-active center and the FAD-binding domain and shares a 30% similarity [69,74].

Early studies suggested that Erv1 functioned in an oxidative folding pathway but the substrates were not identified [70]. Erv1 contains 6 cysteine residues, with two CX_2C motifs in yeast [73]. The cysteine residues of the C-terminus coordinate non-covalently bound FAD. Specifically, the C130–C133 disulfide bridge is part of the redox center, whereas the C159–C176 pair stabilizes the FAD-binding domain [73]. In contrast, the N-terminal C30–C33 pair functions as the “shuttle” disulfide to transfer electrons from the substrate to the

C130–C133 pair, before the electrons flow to FAD [75]. In addition, the N-terminus including the C30–C33 pair may be required for Erv1 dimerization. Assembly as a dimer seems to be essential for function because Erv1 of plants lacks the N-terminal cysteine residues, but dimerization is promoted by cysteine residues in the C-terminus [73,76].

The redox potentials of the redox-active centers of Erv1 have been characterized [77,78]. In yeast, the midpoint potentials were measured at −320 mV for the N-terminal C30–C33 pair, −215 mV for the FAD, and −150 mV for the C130–C133 pair [77]. In humans, the midpoint of the FAD was −178 mV and the midpoint potential for the equivalent C130–C133 pair was proposed to be more negative, because it could not be subsequently reduced by the FAD [78].

The first mitochondrial function for Erv1 was described by Lill and colleagues, showing that Erv1 functions in the export of FeS clusters from the mitochondrion to the cytosol [79]. A yeast strain defective in Erv1 function displayed decreased incorporation of ^{55}Fe into cytosolic FeS proteins Leu1 and Rli1, but assembly of mitochondrial FeS proteins was not affected. They postulated that Erv1 functions after the putative FeS cluster exporter Atm1 of the inner membrane and facilitates maturation of cytosolic proteins with FeS clusters. In addition, Erv1 may also play a role in heme export and maturation [77], because cytochrome *c* and cytochrome *c* peroxidase (Ccp1) failed to bind heme in *erv1* mutants. However, a series of papers clearly illustrate that Erv1 primarily functions as a sulfhydryl oxidase with Mia40 and plays a prominent role in import of intermembrane space proteins.

3. A pathway for the formation of disulfide bonds

Erv1 and Mia40 function in a new pathway for the import of the small cysteine-rich proteins into the intermembrane space (Fig. 2) [3,14,20,80]. The molecular details for this pathway are beginning to be dissected. Precursor proteins such as the small Tim proteins and those with a CX_9C motif are imported across the Tom complex in an unfolded and reduced state (Fig. 2) [51,81]. Because the size of the TOM channel is approximately 22 to 26 Å [82,83], the channel can only accommodate two unfolded polypeptide backbones, thereby excluding folded proteins. Thus, the substrates are unfolded during translocation.

Mia40 then forms a transient disulfide bonds with the imported substrates after they cross the TOM complex [20,62–64,84,85]. The interaction between Mia40 and the small Tim proteins is such that Mia40 specifically recognizes and binds to the first cysteine in the small Tim protein, and the first cysteine is essential for efficient import [86,87]. When the first cysteine residue is mutated, the small Tim protein instead diffuses back to the cytoplasm. In addition, the presence of reducing agents decreases the abundance of a Mia40-substrate intermediate [62,63,87], but the interaction is not affected by the presence of metal chelators [87]. Mia40 most likely forms a transient disulfide bond with the N-terminal CPC motif, whereas the thiol bonds in the CX_9C motif seemingly stabilize the protein [67]. Thus, Mia40 serves as a receptor for the cysteine-rich proteins in the intermembrane space.

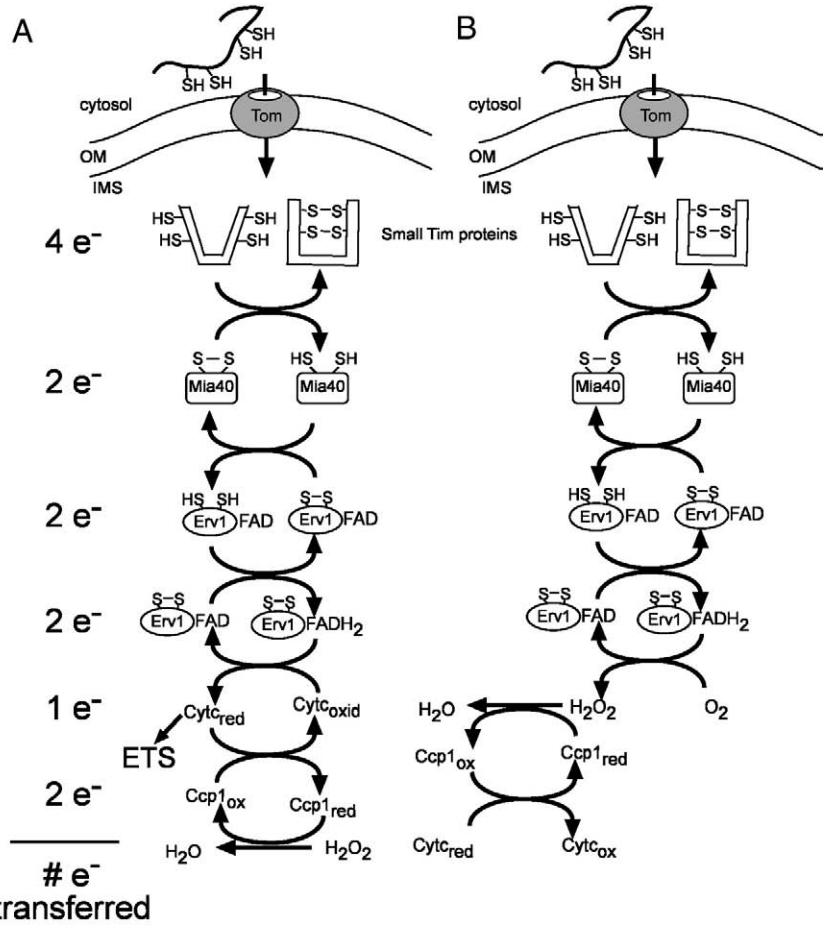

Fig. 2. The Mia40-Erv1 import pathway for CX_3C and CX_9C proteins. The substrates (model substrate is a small Tim protein) are imported in an unfolded, reduced state across the outer membrane (OM). Oxidized Mia40 serves as the import receptor and forms a transient disulfide with the substrate. The substrate leaves in an oxidized state and a series of disulfide exchange reactions continue through Erv1 C130–C133 to FAD. From $FADH_2$, the electrons are passed to different acceptors. (A) Electrons may be shuttled to cytochrome *c* and then to the electron transport chain (ETS) or oxidized Ccp1. (B) Alternatively, electrons may be shuttled to oxygen to generate hydrogen peroxide, which is removed by reduced Ccp1. Ccp1 and cyt *c* can function together to bypass the requirement for the electron transport system. The number of electrons (e^-) that are transferred at each step are indicated. Details have not been elucidated for the specific exchange reactions and additional components most likely participate in this pathway.

After binding to the substrate, Mia40 releases the substrate by an unknown mechanism that may involve the rearrangement of disulfide bonds. The substrate subsequently acquires a mature conformation [87,88]. Mia40 is then reoxidized by the sulfhydryl oxidase Erv1 [20]. Approximately 10% of the Erv1 pool interacts with Mia40 under nonreducing conditions.

It is not clear how the electrons are specifically shuttled through Erv1 from substrates. Typically electrons flow from more negative to positive midpoint potentials. The midpoint potential of Tim9 is −310 mV [89], and electrons may be shuttled to Erv1 via Mia40. If Erv1 functions like most sulfhydryl oxidases [68], the electrons are predicted to flow from C30–C33 (−320 mV) to C130–C133 (−150 mV) and then to FAD (−215 mV). In contrast, the midpoint potentials predict that this is not a favorable electron transfer because electrons are moving to a more negative midpoint potential (i.e., −150 mV→−215 mV). But it is important to note that the midpoint potentials are determined at equilibrium and may be quite different kinetically in the context of the entire pathway. Indeed, prokaryotic DsbB showed a similar trend in that electrons were predicted to flow from more positive to more negative midpoint potentials, but experimental results confirmed this route; and the presence of ubiquinone (+110 mV) as a subsequent electron acceptor was predicted to serve as an electron sink for electrons [90].

Electrons are transferred from Erv1 to several electron acceptors, notably both oxygen and cytochrome *c* (Fig. 2) [77,78,85,91]. Cytochrome *c* seems to compete with oxygen and the electron acceptor may depend upon growth conditions [77]. The electron transport system then serves as an electron acceptor to reoxidize cytochrome *c* [91]. The positive midpoint potentials of cytochrome *c* (+250 mV) and oxygen (+820 mV) may also serve as an electron sink. Erv1 assembles in a 1:1 complex with cytochrome *c* in vivo [77] and Erv1 can directly reduce cytochrome *c* in vitro [77,78,91], which indicates that cytochrome *c* is a preferred electron acceptor. *Saccharomyces cerevisiae* can grow in the absence of a functioning respiratory chain. In this scenario, cytochrome *c* peroxidase (Ccp1) can couple with cytochrome *c* to bypass the requirement for respiration (Fig. 2) [77]. Ccp1 functions as a peroxide scavenger in the intermembrane space [92]. After Erv1 shuttles electrons to oxygen, Ccp1 can remove the generated hydrogen peroxide and then reoxidize cytochrome *c*, thereby bypassing the requirement for a functional electron transport chain. Therefore, the mitochondrion contains a variety of electron acceptors for Erv1.

What is not obvious is how the varying number of electrons are transferred through the series of redox reactions (Fig. 2). Specifically, four electrons are transferred from the small Tim proteins and then two electrons from Erv1 must be transferred to the one-electron acceptor cytochrome *c*. It has been suggested that ALR can accommodate this by holding one electron in the flavin to form a flavin semiquinone [78]. However, a flavin semiquinone was not observed in Erv1 [77], but the species may be short-lived. Additional experiments are required to specifically determine how the electrons are transported from substrates to final electron acceptors. Finally, additional proteins may participate in this pathway.

4. Redox used for assembly of the carrier proteins

The mechanism by which the small Tim proteins escort a hydrophobic precursor from the outer membrane to the inner membrane has been investigated in detail [44]. The small Tim proteins form chaperone-like complexes but the transfer of substrate does not require an energy source such as ATP, and the small Tim proteins are not similar to typical chaperones such as the Hsp60 and Hsp70 families. Instead, the small Tim proteins are structurally similar to that of the prefoldins and bacterial Skp chaperone [53,54,93,94], although the complexes are not similar at the sequence level. The bacterial Skp chaperone assembles as a trimeric periplasmic chaperone that assists outer membrane proteins in their folding and insertion into membranes [54], whereas prefoldin is a hexameric chaperone built from two related classes of subunits that functions in the cytosol of all eukaryotes and archaea to stabilize non-native proteins [93].

However, the mechanism by which the small Tim proteins bind to substrates and release them is not clear. Preliminary studies suggest that redox regulation may play a role in import of the carrier proteins (Fig. 3) [95]. Using *in organello* import assays, a translocation intermediate consisting of the carrier protein and the Tim9–Tim10 complex arrested in the intermembrane space in the presence of oxidant (Fig. 3, Stage 1). When subsequently treated with reductant, the carrier was "chased" into the inner membrane (Fig. 3, Stage 2). The small Tim complex may be disassembled by the presence of reductant, resulting in transfer of the substrate to the insertion complex. Alternatively, the carriers may have increased binding affinity for components such as Tim22 in the 300 kDa insertion complex. The disassembled small Tim proteins may be recycled and reassembled for another round of translocation (Fig. 3, Stage 3).

Other proteins (indicated with a '?' in Fig. 3) most likely facilitate import of the carrier proteins, particularly recycling of the small Tim proteins. Hot13 (Helper of Tim, 13 kDa) was identified as such a protein that bound to the small Tim proteins and facilitated assembly of the 70 kDa small Tim complexes [95]. Hot13 is a zinc-ring finger protein localized to the intermembrane space that contains 11 cysteine residues. Mitochondria lacking Hot13 contained decreased levels of the small Tim proteins. Moreover, the import of the small Tim proteins was not defective, suggesting that Hot13 was required after Mia40 [95]. Hot13 therefore may be a new factor that facilitates recycling of the small Tim proteins during import of the carrier proteins. As with the Mia40-Erv1 import pathway, studies are

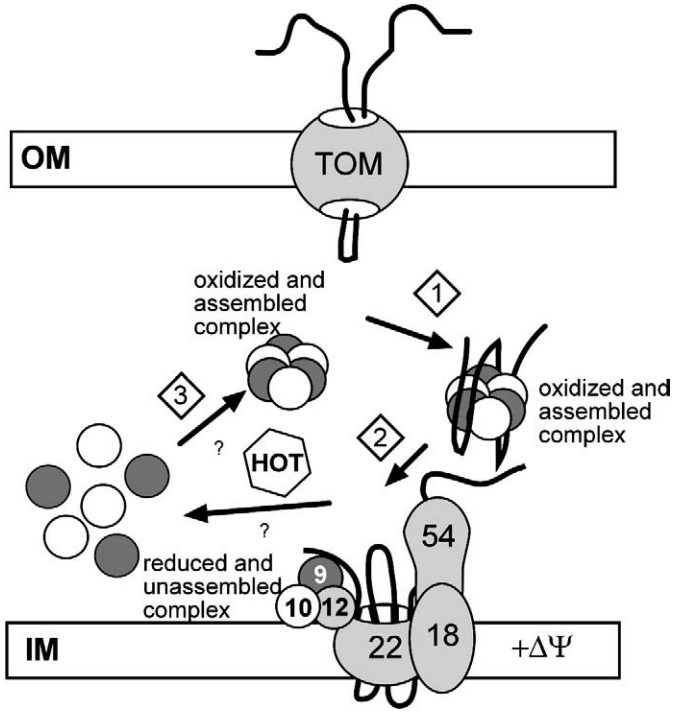

Fig. 3. The import pathway for carrier proteins is redox regulated. In the assembled Tim9–Tim10 complex, the small Tim proteins are oxidized and bind to substrate as it emerges from the TOM complex. In the presence of oxidant, a translocation intermediate bound to the small Tim complexes can be arrested in the intermembrane space (Stage 1). In the presence of reductant, the translocation intermediate is subsequently "chased" and the substrate inserts into the inner membrane (Stage 2). The small Tim proteins are reassembled for another round of import, which may be facilitated by Hot1p (HOT; Stage 3). Detailed mechanistic steps have not been elucidated and additional proteins may participate in this pathway.

required to identify additional components and to understand the import mechanism.

5. Outlook

It is surprising that an import pathway relying on disulfide bridges has been developed considering the redox environment in the intermembrane space is most likely reducing. However, this import mechanism may have evolved when the progenitor mitochondrion was developing during endosymbiosis, because the intermembrane space was derived from the periplasm. It also might have developed as a sorting mechanism to earmark intermembrane space proteins from the collection streaming through the TOM complex destined for the matrix and inner and outer membranes. Like the bacterial periplasm and the endoplasmic reticulum, the mitochondrial intermembrane space now seemingly is a compartment that is amenable to the formation of disulfide bonds. This changes preconceived notions on the function of this compartment. Given that the intermembrane space is critical in apoptotic pathways and proteins such as Pink1 of Parkinson's reside in the intermembrane space [96,97], redox chemistry may potentially play a critical role in these diseases. Future studies should begin to provide insight into the players and mechanisms of redox regulation in the intermembrane space as well as the importance in other pathways such as mitochondrial fusion and fission, apoptosis, and metal trafficking.

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References

[1] M.W. Gray, G. Burger, B.F. Lang, Mitochondrial evolution, Science 283 (1999) 1476–1481.
[2] C.M. Koehler, New developments in mitochondrial assembly, Ann. Rev. Cell Dev. Biol. 20 (2004) 309–335.
[3] S. Kutik, B. Guiard, H.E. Meyer, N. Wiedemann, N. Pfanner, Cooperation of translocase complexes in mitochondrial protein import, J. Cell Biol. 179 (2007) 585–591.
[4] I. Gentle, K. Gabriel, P. Beech, R. Waller, T. Lithgow, The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria, J. Cell Biol. 29 (2004) 29.
[5] A. Sickmann, J. Reinders, Y. Wagner, C. Joppich, R. Zahedi, H.E. Meyer, B. Schonfisch, I. Perschil, A. Chacinska, B. Guiard, P. Rehling, N. Pfanner, C. Meisinger, The proteome of *Saccharomyces cerevisiae* mitochondria, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 13207–13212.
[6] J. Reinders, R.P. Zahedi, N. Pfanner, C. Meisinger, A. Sickmann, Toward the complete yeast mitochondrial proteome: multidimensional separation techniques for mitochondrial proteomics, J. Proteome Res. 5 (2006) 1543–1554.
[7] C. Scharfe, P. Zaccaria, K. Hoertnagel, M. Jaksch, T. Klopstock, M. Dembowski, R. Lill, H. Prokisch, K.D. Gerbitz, W. Neupert, H.W. Mewes, T. Meitinger, MITOP, the mitochondrial proteome database: 2000 update, Nucleic Acids Res. 28 (2000) 155–158.
[8] H. Prokisch, C. Scharfe, D.G. Camp II, W. Xiao, L. David, C. Andreoli, M.E. Monroe, R.J. Moore, M.A. Gritsenko, C. Kozany, K.K. Hixson, H.M. Mottaz, H. Zischka, M. Ueffing, Z.S. Herman, R.W. Davis, T. Meitinger, P.J. Oefner, R.D. Smith, L.M. Steinmetz, Integrative analysis of the mitochondrial proteome in yeast, PLOS Biol. 2 (2004) 795–804.
[9] N. Bolender, A. Sickmann, R. Wagner, C. Meisinger, N. Pfanner, Multiple pathways for sorting mitochondrial precursor proteins, EMBO Rep. 9 (2008) 42–49.
[10] S.J. Habib, W. Neupert, D. Rapaport, Analysis and prediction of mitochondrial targeting signals, Methods Cell Biol. 80 (2007) 761–781.
[11] W. Neupert, J.M. Herrmann, Translocation of proteins into mitochondria, Annu. Rev. Biochem. 76 (2007) 723–749.
[12] D. Rapaport, How does the TOM complex mediate insertion of precursor proteins into the mitochondrial outer membrane? J. Cell Biol. 171 (2005) 419–423.
[13] N. Pfanner, N. Wiedemann, C. Meisinger, T. Lithgow, Assembling the mitochondrial outer membrane, Nat. Struct. Mol. Biol. 11 (2004) 1044–1048.
[14] J.M. Herrmann, K. Hell, Chopped, trapped or tacked-protein translocation into the IMS of mitochondria, Trends Biochem. Sci. 30 (2005) 205–211.
[15] B.S. Glick, A. Brandt, K. Cunningham, S. Muller, R.L. Hallberg, G. Schatz, Cytochromes *c*1 and *b*2 are sorted to the intermembrane space of yeast mitochondria by a stop-transfer mechanism, Cell 69 (1992) 809–822.
[16] K. Esser, B. Tursun, M. Ingenhoven, G. Michaelis, E. Pratje, A novel two-step mechanism for removal of a mitochondrial signal sequence involves the mAAA complex and the putative rhomboid protease Pcp1, J. Mol. Biol. 323 (2002) 835–843.
[17] X. Saelens, N. Festjens, L. Vande Walle, M. van Gurp, G. van Loo, P. Vandenabeele, Toxic proteins released from mitochondria in cell death, Oncogene 23 (2004) 2861–2874.
[18] C.M. Koehler, The small Tim proteins and the twin Cx3C motif, Trends Biochem. Sci. 29 (2004) 1–4.
[19] K. Gabriel, D. Milenkovic, A. Chacinska, J. Muller, B. Guiard, N. Pfanner, C. Meisinger, Novel mitochondrial intermembrane space proteins as substrates of the MIA import pathway, J. Mol. Biol. 365 (2007) 612–620.
[20] N. Mesecke, N. Terziyska, C. Kozany, F. Baumann, W. Neupert, K. Hell, J.M. Herrmann, A disulfide relay system in the intermembrane space of mitochondria that mediates protein import, Cell 121 (2005) 1059–1069.
[21] T.G. Senkevich, C.L. White, E.V. Koonin, B. Moss, A viral member of the ERV1/ALR protein family participates in a cytoplasmic pathway of disulfide bond formation, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 12068–12073.
[22] H. Kadokura, F. Katzen, J. Beckwith, Protein disulfide bond formation in prokaryotes, Annu. Rev. Biochem. 72 (2003) 111–135.
[23] C.S. Sevier, C.A. Kaiser, Conservation and diversity of the cellular disulfide bond formation pathways, Antioxid. Redox Signal. 8 (2006) 797–811.
[24] S. Iwata, M. Saynovits, T.A. Link, H. Michel, Structure of a water soluble fragment of the 'Rieske' iron–sulfur protein of the bovine heart mitochondrial cytochrome *bc*1 complex determined by MAD phasing at 1.5 A resolution, Structure 4 (1996) 567–579.
[25] C. Hunte, J. Koepke, C. Lange, T. Rossmanith, H. Michel, Structure at 2.3 A resolution of the cytochrome *bc*(1) complex from the yeast *Saccharomyces cerevisiae* co-crystallized with an antibody Fv fragment, Structure 8 (2000) 669–684.
[26] J.R. Pedrajas, P. Porras, E. Martinez-Galisteo, C.A. Padilla, A. Miranda-Vizuete, J.A. Barcena, Two isoforms of *Saccharomyces cerevisiae* glutaredoxin 2 are expressed in vivo and localize to different subcellular compartments, Biochem. J. 364 (2002) 617–623.
[27] T. Merbitz-Zahradnik, K. Zwicker, J.H. Nett, T.A. Link, B.L. Trumpower, Elimination of the disulfide bridge in the Rieske iron-sulfur protein allows assembly of the [2Fe–2S] cluster into the Rieske protein but damages the ubiquinol oxidation site in the cytochrome *bc*1 complex, Biochemistry 42 (2003) 13637–13645.
[28] D.M. Glerum, A. Shtanko, A. Tzagoloff, SCO1 and SCO2 act as high copy suppressors of a mitochondrial copper recruitment defect in *Saccharomyces cerevisiae*, J. Biol. Chem. 271 (1996) 20531–20535.
[29] T. Nittis, G.N. George, D.R. Winge, Yeast Sco1, a protein essential for cytochrome *c* oxidase function is a Cu(I)-binding protein, J. Biol. Chem. 276 (2001) 42520–42526.
[30] L. Banci, I. Bertini, S. Ciofi-Baffoni, I. Leontari, M. Martinelli, P. Palumaa, R. Sillard, S. Wang, Human Sco1 functional studies and pathological implications of the P174L mutant, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 15–20.
[31] J.C. Williams, C. Sue, G.S. Banting, H. Yang, D.M. Glerum, W.A. Hendrickson, E.A. Schon, Crystal structure of human SCO1: implications for redox signaling by a mitochondrial cytochrome *c* oxidase "assembly" protein, J. Biol. Chem. 280 (2005) 15202–15211.
[32] E. Sundberg, J.G. Slagter, I. Fridborg, S.P. Cleary, C. Robinson, G. Coupland, ALBINO3, an Arabidopsis nuclear gene essential for chloroplast differentiation, encodes a chloroplast protein that shows homology to proteins present in bacterial membranes and yeast mitochondria, Plant Cell 9 (1997) 717–730.
[33] Y.V. Chinenov, Cytochrome *c* oxidase assembly factors with a thioredoxin fold are conserved among prokaryotes and eukaryotes, J. Mol. Med. 78 (2000) 239–242.
[34] F. Arnesano, E. Balatri, L. Banci, I. Bertini, D.R. Winge, Folding studies of Cox17 reveal an important interplay of cysteine oxidation and copper binding, Structure 13 (2005) 713–722.
[35] M.G. Douglas, B.L. Geller, S.D. Emr, Intracellular targeting and import of an F1-ATPase beta-subunit-beta-galactosidase hybrid protein into yeast mitochondria, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 3983–3987.
[36] S.A. Garland, K. Hoff, L.E. Vickery, V.C. Culotta, *Saccharomyces cerevisiae* ISU1 and ISU2: members of a well-conserved gene family for iron–sulfur cluster assembly, J. Mol. Biol. 294 (1999) 897–907.
[37] F. Zhang, A. Slungaard, G.M. Vercellotti, C. Iadecola, Superoxide-dependent cerebrovascular effects of homocysteine, Am. J. Physiol. 274 (1998) R1704–1711.
[38] D. Bordo, K. Djinovic, M. Bolognesi, Conserved patterns in the Cu,Zn superoxide dismutase family, J. Mol. Biol. 238 (1994) 366–386.
[39] R.K. Lambert, B.R. Wiggs, K. Kuwano, J.C. Hogg, P.D. Pare, Functional significance of increased airway smooth muscle in asthma and COPD, J. Appl. Physiol. 74 (1993) 2771–2781.
[40] L.S. Field, Y. Furukawa, T.V. O'Halloran, V.C. Culotta, Factors controlling the uptake of yeast copper/zinc superoxide dismutase into mitochondria, J. Biol. Chem. 278 (2003) 28052–28059.
[41] Y. Furukawa, A.S. Torres, T.V. O'Halloran, Oxygen-induced maturation of SOD1: a key role for disulfide formation by the copper chaperone CCS, EMBO J. 23 (2004) 2872–2881.
[42] C.M. Koehler, S. Merchant, G. Schatz, How membrane proteins travel across the mitochondrial intermembrane space, Trends Biochem. Sci. 24 (1999) 428–432.

[43] M.F. Bauer, U. Rothbauer, N. Muhlenbein, R.J. Smith, K. Gerbitz, W. Neupert, M. Brunner, S. Hofmann, The mitochondrial TIM22 preprotein translocase is highly conserved throughout the eukaryotic kingdom, FEBS Lett. 464 (1999) 41–47.
[44] S.P. Curran, D. Leuenberger, E. Schmidt, C.M. Koehler, The role of the Tim8p–Tim13p complex in a conserved import pathway for mitochondrial polytopic inner membrane proteins, J. Cell Biol. 158 (2002) 1017–1027.
[45] M. Endres, W. Neupert, M. Brunner, Transport of the ADP/ATP carrier of mitochondria from the TOM complex to the TIM22.54 complex, EMBO J. 18 (1999) 3214–3221.
[46] P. Luciano, S. Geoffroy, A. Brandt, J.F. Hernandez, V. Geli, Functional cooperation of the mitochondrial processing peptidase subunits, J. Mol. Biol. 272 (1997) 213–225.
[47] C.M. Koehler, D. Leuenberger, S. Merchant, A. Renold, T. Junne, G. Schatz, Human deafness dystonia syndrome is a mitochondrial disease, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 2141–2146.
[48] K. Roesch, S.P. Curran, L. Tranebjaerg, C.M. Koehler, Human deafness dystonia syndrome is caused by a defect in assembly of the DDP1/TIMM8a–TIMM13 complex. Hum. Mol. Genet. 11 (2002) 477–486.
[49] K. Roesch, P.J. Hynds, R. Varga, L. Tranebjaerg, C.M. Koehler, The calcium-binding aspartate/glutamate carriers, citrin and aralar1, are new substrates for the DDP1/TIMM8a–TIMM13 complex, Hum. Mol. Genet. 13 (2004) 2101–2111.
[50] C. Sirrenberg, M. Endres, H. Folsch, R.A. Stuart, W. Neupert, M. Brunner, Carrier protein import into mitochondria mediated by the intermembrane proteins Tim10/Mrs11 and Tim12/Mrs5, Nature 391 (1998) 912–915.
[51] H. Lu, S. Allen, L. Wardleworth, P. Savory, K. Tokatlidis, Functional TIM10 Chaperone Assembly Is Redox-regulated in Vivo, J. Biol. Chem. 279 (2004) 18952–18958.
[52] S. Allen, H. Lu, D. Thornton, K. Tokatlidis, Juxtaposition of the two distal "CX3C" motifs via intrachain disulfide bonding is essential for the folding of Tim10, J. Biol. Chem. 278 (2003) 38505–38513.
[53] C.T. Webb, M.A. Gorman, M. Lazarou, M.T. Ryan, J.M. Gulbis, Crystal structure of the mitochondrial chaperone TIM9.10 reveals a six-bladed alpha-propeller, Mol. Cell 21 (2006) 123–133.
[54] T.A. Walton, M.C. Sousa, Crystal structure of Skp, a prefoldin-like chaperone that protects soluble and membrane proteins from aggregation, Mol. Cell 15 (2004) 367–374.
[55] J. Martin-Benito, J. Gomez-Reino, P.C. Stirling, V.F. Lundin, P. Gomez-Puertas, J. Boskovic, P. Chacon, J.J. Fernandez, J. Berenguer, M.R. Leroux, J.M. Valpuesta, Divergent substrate-binding mechanisms reveal an evolutionary specialization of eukaryotic prefoldin compared to its archaeal counterpart, Structure 15 (2007) 101–110.
[56] O. Khalimonchuk, K. Rigby, M. Bestwick, F. Pierrel, P.A. Cobine, D.R. Winge, Pet191 is a cytochrome *c* oxidase assembly factor in *Saccharomyces cerevisiae*, Eukaryot. Cell (in press).
[57] D. Horn, H. Al-Ali, A. Barrientos, Cmc1p is a conserved mitochondrial twin CX9C protein involved in cytochrome *c* oxidase biogenesis, Mol. Cell Biol. 28 (2008) 4354–4364.
[58] L. Banci, I. Bertini, S. Ciofi-Baffoni, A. Janicka, M. Martinelli, H. Kozlowski, P. Palumaa, A structural–dynamical characterization of human Cox17, J. Biol. Chem. 283 (2008) 7912–7920.
[59] A.E. LaMarche, M.I. Abate, S.H. Chan, B.L. Trumpower, Isolation and characterization of COX12, the nuclear gene for a previously unrecognized subunit of *Saccharomyces cerevisiae* cytochrome *c* oxidase, J. Biol. Chem. 267 (1992) 22473–22480.
[60] M.P. Nobrega, S.C. Bandeira, J. Beers, A. Tzagoloff, Characterization of COX19, a widely distributed gene required for expression of mitochondrial cytochrome oxidase, J. Biol. Chem. 277 (2002) 40206–40211.
[61] M.H. Barros, A. Johnson, A. Tzagoloff, COX23, a homologue of COX17, is required for cytochrome oxidase assembly, J. Biol. Chem. 279 (2004) 31943–31947.
[62] N. Terziyska, B. Grumbt, M. Bien, W. Neupert, J.M. Herrmann, K. Hell, The sulfhydryl oxidase Erv1 is a substrate of the Mia40-dependent protein translocation pathway, FEBS Lett. 581 (2007) 1098–1102.
[63] A. Chacinska, S. Pfannschmidt, N. Wiedemann, V. Kozjak, L.K. Sanjuan Szklarz, A. Schulze-Specking, K.N. Truscott, B. Guiard, C. Meisinger, N. Pfanner, Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins, EMBO J. 23 (2004) 3735–3746.
[64] M. Naoe, Y. Ohwa, D. Ishikawa, C. Ohshima, S.I. Nishikawa, H. Yamamoto, T. Endo, Identification of Tim40 that mediates protein sorting to the mitochondrial intermembrane space, J. Biol. Chem. 279 (2004) 47815–47821.
[65] B.A. Westerman, A. Poutsma, E.A. Steegers, C.B. Oudejans, C2360, a nuclear protein expressed in human proliferative cytotrophoblasts, is a representative member of a novel protein family with a conserved coiled coil–helix–coiled coil–helix domain, Genomics 83 (2004) 1094–1104.
[66] S. Hofmann, U. Rothbauer, N. Muhlenbein, K. Baiker, K. Hell, M.F. Bauer, Functional and mutational characterization of human MIA40 acting during import into the mitochondrial intermembrane space, J. Mol. Biol. 353 (2005) 517–528.
[67] B. Grumbt, V. Stroobant, N. Terziyska, L. Israel, K. Hell, Functional characterization of Mia40p, the central component of the disulfide relay system of the mitochondrial intermembrane space, J. Biol. Chem. 282 (2007) 37461–37470.
[68] D.L. Coppock, C. Thorpe, Multidomain flavin-dependent sulfhydryl oxidases, Antioxid. Redox. Signal. 8 (2006) 300–311.
[69] E. Gross, C.S. Sevier, A. Vala, C.A. Kaiser, D. Fass, A new FAD-binding fold and intersubunit disulfide shuttle in the thiol oxidase Erv2p, Nature Struct. Biol. 9 (2002) 61–67.
[70] J. Lee, G. Hofhaus, T. Lisowsky, Erv1p from *Saccharomyces cerevisiae* is a FAD-linked sulfhydryl oxidase, FEBS Lett. 477 (2000) 62–66.
[71] R. Giorda, M. Hagiya, T. Seki, M. Shimonishi, H. Sakai, J. Michaelson, A. Francavilla, T.E. Starzl, M. Trucco, Analysis of the structure and expression of the augmenter of liver regeneration (ALR) gene, Mol. Med. 2 (1996) 97–108.
[72] T. Lisowsky, D.L. Weinstat-Saslow, N. Barton, S.T. Reeders, M.C. Schneider, A new human gene located in the PKD1 region of chromosome 16 is a functional homologue to ERV1 of yeast, Genomics 29 (1995) 690–697.
[73] G. Hofhaus, J.E. Lee, I. Tews, B. Rosenberg, T. Lisowsky, The N-terminal cysteine pair of yeast sulfhydryl oxidase Erv1p is essential for in vivo activity and interacts with the primary redox centre, Eur. J. Biochem. 270 (2003) 1528–1535.
[74] G. Stein, T. Lisowsky, Functional comparison of the yeast scERV1 and scERV2 genes, Yeast 14 (1998) 171–180.
[75] D. Fass, The Erv family of sulfhydryl oxidases, Biochim. Biophys. Acta 1783 (2008) 557–566.
[76] A. Levitan, A. Danon, T. Lisowsky, Unique features of plant mitochondrial sulfhydryl oxidase, J. Biol. Chem. 279 (2004) 20002–20008.
[77] D.V. Dabir, E.P. Leverich, S.K. Kim, F.D. Tsai, M. Hirasawa, D.B. Knaff, C.M. Koehler, A role for cytochrome *c* and cytochrome *c* peroxidase in electron shuttling from Erv1, EMBO J. 26 (2007) 4801–4811.
[78] S.R. Farrell, C. Thorpe, Augmenter of liver regeneration: a flavin-dependent sulfhydryl oxidase with cytochrome *c* reductase activity, Biochemistry 44 (2005) 1532–1541.
[79] H. Lange, T. Lisowsky, J. Gerber, U. Muhlenhoff, G. Kispal, R. Lill, An essential function of the mitochondrial sulfhydryl oxidase Erv1p/ALR in the maturation of cytosolic Fe/S proteins, EMBO Rep. 2 (2001) 715–720.
[80] J.M. Herrmann, R. Kohl, Catch me if you can! Oxidative protein trapping in the intermembrane space of mitochondria, J. Cell Biol. 176 (2007) 559–563.
[81] T. Lutz, W. Neupert, J.M. Herrmann, Import of small Tim proteins into the mitochondrial intermembrane space, EMBO J. 22 (2003) 4400–4408.
[82] M.P. Schwartz, A. Matouschek, The dimensions of the protein import channel in the outer and inner mitochondrial membranes, Proc. Natl. Acad. Sci. U.S.A. 98 (1999) 13086–13090.
[83] K. Hill, K. Model, M.T. Ryan, K. Dietmeier, F. Martin, R. Wagner, N. Pfanner, Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins, Nature 395 (1998) 516–521.
[84] M. Rissler, N. Wiedemann, S. Pfannschmidt, K. Gabriel, B. Guiard, N. Pfanner, A. Chacinska, The essential mitochondrial protein Erv1 cooperates with Mia40 in biogenesis of intermembrane space proteins, J. Mol. Biol. 353 (2005) 485–492.
[85] S. Allen, V. Balabanidou, D.P. Sideris, T. Lisowsky, K. Tokatlidis, Erv1 mediates the Mia40-dependent protein import pathway and provides a functional link to the respiratory chain by shuttling electrons to cytochrome *c*, J. Mol. Biol. 353 (2005) 937–944.
[86] D. Milenkovic, K. Gabriel, B. Guiard, A. Schulze-Specking, N. Pfanner, A. Chacinska, Biogenesis of the essential Tim9–Tim10 chaperone complex of mitochondria: site-specific recognition of cysteine residues by the intermembrane space receptor Mia40, J. Biol. Chem. 282 (2007) 22472–22480.
[87] D.P. Sideris, K. Tokatlidis, Oxidative folding of small Tims is mediated by site-specific docking onto Mia40 in the mitochondrial intermembrane space, Mol. Microbiol. 65 (2007) 1360–1373.
[88] J.M. Müller, D. Milenkovic, B. Guiard, N. Pfanner, A. Chacinska, Precursor oxidation by mia40 and erv1 promotes vectorial transport of proteins into the mitochondrial intermembrane space, Mol. Biol. Cell 19 (2008) 226–236.
[89] B. Morgan, H. Lu, Oxidative folding competes with mitochondrial import of the small Tim proteins, Biochem. J. 411 (2008) 115–122.
[90] T.L. Tapley, T. Eichner, S. Gleiter, D.P. Ballou, J.C. Bardwell, Kinetic characterization of the disulfide bond-forming enzyme DsbB, J. Biol. Chem. 282 (2007) 10263–10271.
[91] K. Bihlmaier, N. Mesecke, N. Terziyska, M. Bien, K. Hell, J.M. Herrmann, The disulfide relay system of mitochondria is connected to the respiratory chain, J. Cell. Biol. 179 (2007) 389–395.
[92] J.E. Erman, L.B. Vitello, Yeast cytochrome *c* peroxidase: mechanistic studies via protein engineering, Biochim. Biophys. Acta 1597 (2002) 193–220.
[93] R. Siegert, M.R. Leroux, C. Scheufler, F.U. Hartl, I. Moarefi, Structure of the molecular chaperone prefoldin: unique interaction of multiple coiled coil tentacles with unfolded proteins, Cell 103 (2000) 621–632.
[94] F.U. Hartl, M. Hayer-Hartl, Molecular chaperones in the cytosol: from nascent chain to folded protein, Science 295 (2002) 1852–1858.
[95] S.P. Curran, D. Leuenberger, E.P. Leverich, D.K. Hwang, K.N. Beverly, C.M. Koehler, The role of Hot13p and redox chemistry in the mitochondrial TIM22 import pathway, J. Biol. Chem. 279 (2004) 43744–43751.
[96] L. Silvestri, V. Caputo, E. Bellacchio, L. Atorino, B. Dallapiccola, E.M. Valente, G. Casari, Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism, Hum. Mol. Genet. 14 (2005) 3477–3492.
[97] H.M. McBride, M. Neuspiel, S. Wasiak, Mitochondria: more than just a powerhouse, Curr. Biol. 16 (2006) R551–R560.
[98] L. Banci, I. Bertini, S. Ciofi-Baffoni, I.P. Gerothanassis, I. Leontari, M. Martinelli, S. Wang, A structural characterization of human SCO2, Structure 15 (2007) 1132–1140.
[99] S.P. Curran, D. Leuenberger, W. Oppliger, C.M. Koehler, The Tim9p–Tim10p complex binds to the transmembrane domains of the ADP-ATP carrier, EMBO J. 21 (2002) 942–953.
[100] C. Baud, C. de Marcos-Lousa, K. Tokatlidis, Molecular interactions of the mitochondrial Tim12 translocase subunit, Protein Pept. Lett. 14 (2007) 597–600.