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Review

The MIA pathway: A tight bond between protein transport and oxidative folding in mitochondria

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ABSTRACT

Many newly synthesized proteins obtain disulfide bonds in the bacterial periplasm, the endoplasmic reticulum (ER) and the mitochondrial intermembrane space. The acquisition of disulfide bonds is critical for the folding, assembly and activity of these proteins. Spontaneous oxidation of thiol groups is inefficient *in vivo*, therefore cells have developed machineries that catalyse the oxidation of substrate proteins. The identification of the machinery that mediates this process in the intermembrane space of mitochondria, known as MIA (mitochondrial intermembrane space assembly), provided a unique mechanism of protein transport. The MIA machinery introduces disulfide bonds into incoming intermembrane space precursors and thus tightly couples the process of precursor translocation to precursor oxidation. We discuss our current understanding of the MIA pathway and the mechanisms that oversee thiol-exchange reactions in mitochondria.

1. Introduction

The creation of a disulfide bond requires the oxidation of two cysteine thiol groups and the simultaneous release of two electrons. This seemingly simple chemical reaction plays a fundamental role in protein biochemistry by regulating protein structure, stability and function. *In vivo*, this process is catalysed by a variety of thiol–redox enzymes, which permit fast and reversible thiol–disulfide exchange. Disulfide bond generating machineries have been described for the bacterial periplasm (DsbB–DsbA), the ER (Ero1–PDI), and most recently for the mitochondrial intermembrane space (Erv1–Mia40) (Fig. 1) (reviewed in Refs. [1–7]). Here we will discuss the mitochondrial specific oxidation machinery and its essential role in maintaining the integrity of the intermembrane space of mitochondria.

2. Various translocation and sorting pathways drive biogenesis of mitochondrial precursor proteins

A distinctive feature of the mitochondrial protein oxidation pathway is that it serves to transport and accumulate proteins in the intermembrane space of mitochondria. This does not appear to be the case in the bacterial periplasm or ER where oxidative folding follows translocation of the protein into an environment favourable for disulfide bond formation [1–4]. Given this unique role of disulfide bond formation in the localization of intermembrane space precursors, it is worthwhile to briefly describe protein import into mitochondria and the machineries that execute this process. The functioning of mitochondria is intimately linked to the successful import and assembly of the entire mitochondrial proteome [8–11]. The nuclear and mitochondrial genome contribute to building the protein content of mitochondria, but the mitochondrial genome codes only for a few largely hydrophobic proteins that form the cores of the inner membrane embedded respiratory complexes. These proteins are co-translationally inserted into the inner mitochondrial membrane with help of export machineries, such as OXA (Fig. 2). The components of export machineries are conserved hydrophobic proteins belonging to the YidC/Oxa1/Alb3 protein family and are present in bacteria, mitochondria and chloroplasts to facilitate the association of bacterial or organellar translating ribosomes to membranes [12]. However, the greater majority of all mitochondrial proteins are nuclear encoded. Even in a simple eukaryotic organism like the Baker's yeast, *Saccharomyces cerevisiae*, up to 1000 proteins are encoded by the nuclear genome, synthesized on cytosolic ribosomes and need to be efficiently targeted and integrated into one of the four mitochondrial subcompartments: the outer membrane, intermembrane space, inner membrane or matrix [13]. The import of all these premature precursor proteins is facilitated by organelle specific targeting elements and highly dynamic mitochondrial import and assembly machineries (Fig. 2) [8–11].

Abbreviations: ERV1, essential for respiration and vegetative growth; MIA, mitochondrial intermembrane space assembly; PDI, protein disulfide isomerase; SAM, sorting and assembly machinery; TIM, translocase of the inner membrane; $\Delta\psi$, membrane potential; TOM, translocase of the outer membrane

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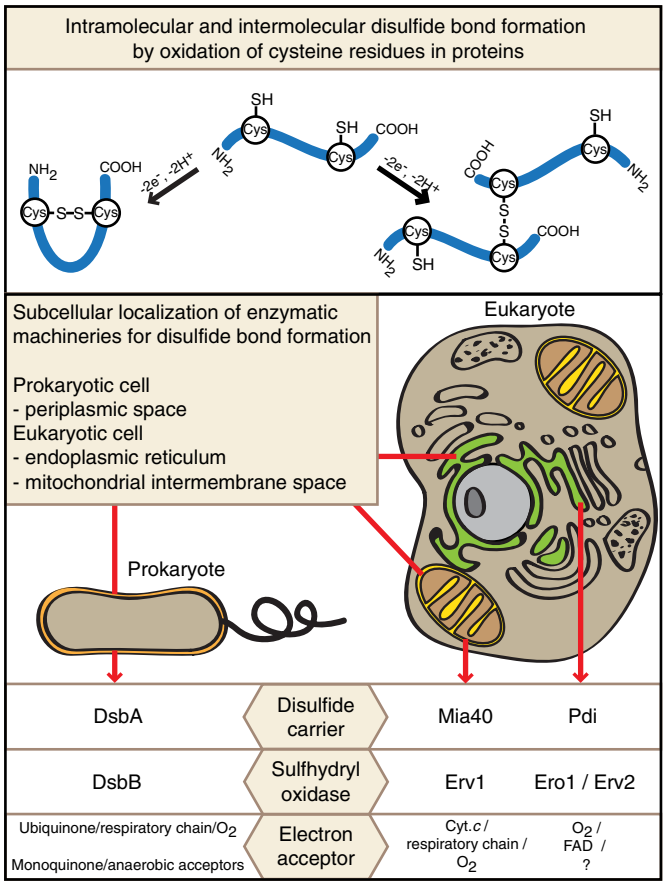

Fig. 1. Disulfide bond formation in cells. A pair of reduced cysteine residues can be oxidized to form covalent bonds within or between proteins. Disulfide bond generating reactions are enzymatically catalysed *in vivo* and are restricted to specific cellular compartments: the endoplasmic reticulum, the intermembrane space of mitochondria and the periplasmic space of bacteria. The key enzymatic components and electron acceptors are listed for each cellular compartment.

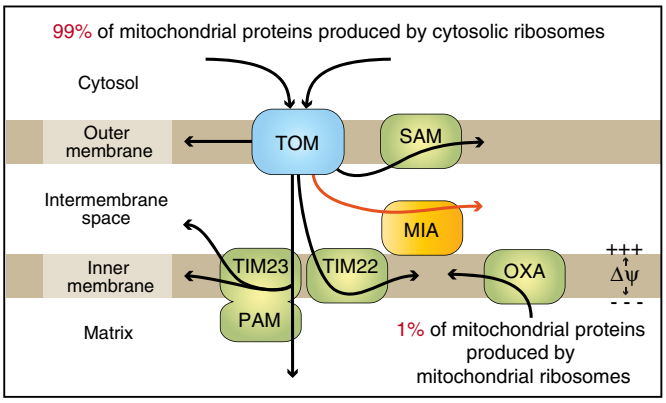

Fig. 2. Schematic representation of protein import pathways into mitochondria. Precursor proteins synthesized in the cytosol employ a common entry gate across the outer mitochondrial membrane — the TOM channel. Subsequently, they utilize different sorting pathways depending on their target location within mitochondria. Precursors destined to the outer membrane are sorted and embedded in the outer membrane by the SAM machinery. Translocation across or insertion into the inner mitochondrial membrane requires an electrochemical potential across the inner mitochondrial membrane ($\Delta\psi$). Matrix proteins employ the TIM23 complex together with the PAM motor for translocation across the inner membrane. Insertion of proteins integral to the inner membrane is mediated by the TIM23 complex or TIM22 complex. To end up in the intermembrane space, some precursors follow the stop-transfer pathway mediated by TIM23. A class of small intermembrane space proteins utilizes a unique mechanism of protein trapping by oxidative folding governed by the MIA pathway. A limited number of inner membrane proteins are synthesized in the mitochondrial matrix and are co-translationally inserted into the inner membrane by specialized machineries such as OXA.

Nearly all mitochondrial precursors are imported into the organelle *via* the Translocase of the Outer Membrane (TOM complex), which is often referred to as the general entry gate into mitochondria (Fig. 2) [9–11,14]. Following their passage *via* the TOM complex import pathways branch out into defined biogenesis pathways, directed by interplay between specific sorting signals and translocation machineries. The most well characterized import pathway into mitochondria is governed by the TIM23 machinery (translocase of the inner mitochondrial membrane) and is followed by precursors residing in the mitochondrial matrix and some members of the inner membrane. These precursor proteins contain an N-terminal positively-charged and cleavable extension termed a presequence. Presequence containing precursors are directed from the TOM complex to the TIM23 translocase for their translocation across, or membrane insertion with the help of the electrochemical potential of the inner mitochondrial membrane (Fig. 2). Translocation of precursors across the inner mitochondrial membrane into the matrix requires the PAM (presequence translocase-associated motor) complex, which has mtHsp70 as its major player and provides energy in the form of ATP as an additional driving force [9,10,15,16].

Proteins belonging to the outer membrane, inner membrane and intermembrane space are typically characterized by the presence of internal targeting elements that are scattered throughout the length of the protein. For instance, outer membrane β-barrel proteins utilize a specific import signal in the last β-strand of the precursors (β-signal) that directs them to the outer membrane SAM (sorting and assembly machinery) complex for integration and assembly into functional complexes (Fig. 2) [14,17,18]. Polytopic inner membrane proteins belonging to the carrier family possess multiple internal targeting signals, which largely overlap with the hydrophobic transmembrane segments of these proteins. These precursors are sorted to an alternative translocase, the TIM22 or Carrier Translocase for insertion into the inner membrane, which is supported by the presence of the electrochemical inner membrane potential (Fig. 2) [9,19,20].

In the case of intermembrane space proteins two alternative modes of import have thus far been described. First, a subset of intermembrane space precursors follows the presequence pathway governed by TIM23. The precursors contain a bipartite signal consisting of an N-terminal presequence and a hydrophobic sorting signal. This hydrophobic anchor halts translocation of the precursor in the TIM23 translocase and facilitates lateral release of the precursor into the inner membrane and resembles the mechanism used by TIM23 for integration of presequence-containing inner membrane proteins [9,10,15,21]. Following proteolytic cleavage and removal of the presequence in the matrix, a second proteolytic cleavage on the intermembrane space side leads to release of a soluble intermembrane space protein.

The second route to the intermembrane space of mitochondria does not involve an electrochemical potential nor TIM translocases of the inner membrane, but rather relies on protein oxidative folding. The MIA (mitochondrial intermembrane space assembly) pathway has been discovered most recently and its mode of action differs significantly from the typical import pathways into mitochondria. Perhaps the most exclusive feature of this pathway compared to other mitochondrial import pathways and also other cellular translocation systems is the use of cysteine chemistry to stabilize translocation intermediates and mature folded proteins *via* disulfide bonds. Disulfide bond formation by MIA leads to the trapping of proteins in the intermembrane space of mitochondria [4,9,22–26]. Herein, we will describe the mechanisms and molecular machines that tightly link oxidative folding to protein transport into the mitochondrial intermembrane space.