FEBS Letters xxx (2014) xxx–xxx

journal homepage: www.FEBSLetters.org

Review

Mitochondrial protein translocases for survival and wellbeing

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ARTICLE INFO

Article history:
Received 28 April 2014
Revised 15 May 2014
Accepted 15 May 2014
Available online xxxx

Edited by Wilhelm Just

Keywords:
Cancer
HIF1α
Mitochondrial disease
Neurodegeneration
p53
Protein biogenesis and import

ABSTRACT

Mitochondria are involved in many essential cellular activities. These broad functions explicate the need for the well-orchestrated biogenesis of mitochondrial proteins to avoid death and pathological consequences, both in unicellular and more complex organisms. Yeast as a model organism has been pivotal in identifying components and mechanisms that drive the transport and sorting of nuclear-encoded mitochondrial proteins. The machinery components that are involved in the import of mitochondrial proteins are generally evolutionarily conserved within the eukaryotic kingdom. However, topological and functional differences have been observed. We review the similarities and differences in mitochondrial translocases from yeast to human. Additionally, we provide a systematic overview of the contribution of mitochondrial import machineries to human pathologies, including cancer, mitochondrial diseases, and neurodegeneration.

1. Introduction

Mitochondria are semi-autonomous organelles that possess their own DNA and translational machinery. This is the legacy from their mitochondrial ancestor, the endosymbiotic proteobacterium [1]. Mitochondrial DNA encodes only a handful of proteins that are central to the process of oxidative phosphorylation. Therefore, the mitochondrial proteome, which consists of approximately 800 (in yeast) to 1500 (in mammals) proteins, needs to be substantially complemented with nuclear-encoded proteins [2,3]. Upon synthesis on cytosolic ribosomes, immature mitochondrial precursor proteins require chaperone-assisted transfer to the mitochondrial surface. The sorting of precursors within mitochondria is an intricate process because of the complex architecture of the organelle [4]. Mitochondria are surrounded by two membranes that are highly divergent with regard to their function and lipid and protein composition. Both the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) define two aqueous mitochondrial subcompartments, the intermembrane space (IMS) and the matrix. To reach their destined mitochondrial milieu, precursor proteins are driven by various import machineries to different mitochondrial compartments. This trafficking is based on sorting signals included within the precursors' primary or secondary structures [5–8].

2. Principles of mitochondrial protein import: lessons from yeast

Extensive studies of mitochondrial protein import using the model yeast *Saccharomyces cerevisiae* uncovered the components of different translocases and provided detailed mechanistic and topological information about their function and interplay [5–8]. The translocase of the outer mitochondrial membrane (TOM) complex is a general entry gate for mitochondrial precursor proteins that are synthesized in the cytosol (Fig. 1A). Its components recognize a precursor protein and pass it to specialized import machineries. The precursors of β-barrel proteins are sorted to the OMM by sorting and assembly machinery (SAM; Fig. 1A). The import of small IMS proteins with multiple cysteine residues occurs because of the thiol-disulfide exchange via the mitochondrial intermembrane space assembly (MIA) pathway (Fig. 1B). The translocase of the inner mitochondrial membrane 23 (TIM23) complex directs presequence-containing precursors to the matrix, IMM, or IMS (Fig. 1C). Metabolite carriers that reside in the IMM and contain multiple internal transmembrane signals are embedded into the

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http://dx.doi.org/10.1016/j.febslet.2014.05.028
0014-5793/

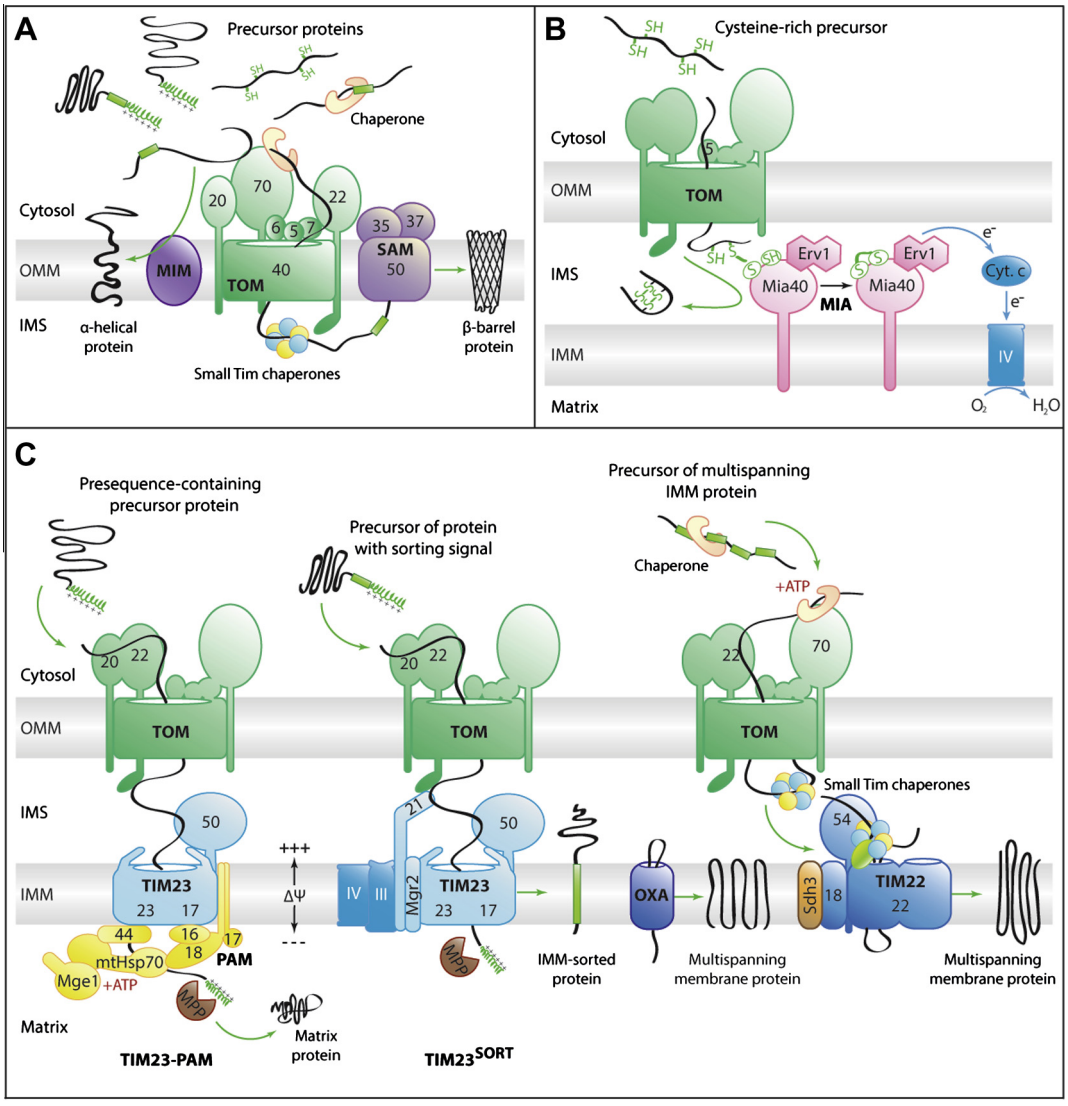

Fig. 1. Protein transport pathways into yeast *Saccharomyces cerevisiae* mitochondria. (A) General entry gate for mitochondrial precursor proteins and sorting into the outer mitochondrial membrane (OMM). Mitochondrial proteins are synthesized in the cytosol in precursor forms. Their delivery to the mitochondrial surface is assisted by cytosolic chaperones that maintain precursor proteins in an import-competent state. Precursors enter mitochondria through the translocase of the outer membrane (TOM), the general entry gate, and are further directed to their final mitochondrial destinations with assistance from specific sorting machineries. Precursor proteins that contain α-helical transmembrane segments are inserted into the OMM by the insertase of the mitochondrial outer membrane (MIM) for assembly. More complex precursors with β-barrel topology are transported via TOM into the intermembrane space (IMS) and handed over to the sorting and assembly machinery (SAM) with the help of small Tim chaperone complexes. (B) Oxidation-driven import into the intermembrane space (IMS). Cysteine-rich precursor proteins are delivered into the IMS via the mitochondrial intermembrane space assembly (MIA) pathway. Emerging from the TOM complex, they transiently bind Mia40 via disulfide bonds. Mia40 oxidizes and folds precursor proteins. The reoxidation of Mia40 is mediated by Erv1, which shuttles released electrons via cytochrome *c* to cytochrome *c* oxidase (complex IV). (C) Translocation to the matrix and inner mitochondrial membrane (IMM). Presequence-containing precursors are delivered to the matrix and inner membrane in an inner membrane electrochemical potential ($\Delta\psi$)-dependent manner via the presequence translocase TIM23. TIM23 is associated with the presequence translocase-associated motor (PAM) and transfers precursor proteins into the matrix in an ATP-dependent manner. The insertion of presequence-containing precursors into the inner membrane is driven by the $TIM23^{SORT}$ complex, marked by the presence of Tim21. Upon delivery into the matrix, the presequences are recognized and cleaved by matrix-processing peptidase (MPP). The hydrophobic precursors of carrier proteins are guided through the IMS by small TIM chaperone complexes that deliver precursors to the carrier translocase of the inner membrane, the TIM22 complex. The membrane insertion of carrier precursors via the TIM22 complex is driven by the electrochemical potential across the inner membrane. The mitochondrial protein export machinery (OXA) participates in the biogenesis of some cytosol-translated multispanning membrane proteins that are targeted to the IMM via the TIM22 and TIM23 translocases. e^-, electron.

IMM by the carrier translocase of the inner mitochondrial membrane 22 (TIM22) complex [5–8] (Fig. 1C).

2.1. Yeast outer membrane translocase: a closer look

All mitochondrial precursors enter the organelle in an unfolded, import-competent state via the TOM complex [9–11] (Fig. 1A). The central component and a channel-forming subunit of the TOM complex is a membrane β-barrel protein, Tom40. Additional subunits perform important functions in maintaining the architecture of the TOM complex and passage of precursor proteins. Tom20 and Tom70 are membrane-anchored proteins that are loosely associated with the TOM complex. Through their cytosol-exposed C-terminal domains, they serve as receptors for various classes of incoming precursor proteins. Tom22 is a core subunit of TOM and performs two essential functions. First, Tom22 maintains the architecture of the TOM complex. Second, it provides further binding sites for mitochondrial precursor proteins on both sides of the

Please cite this article in press as: Sokol, A.M., et al. Mitochondrial protein translocases for survival and wellbeing. FEBS Lett. (2014), http://dx.doi.org/10.1016/j.febslet.2014.05.028