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Review

Cooperation of protein machineries in mitochondrial protein sorting

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ABSTRACT

The function of mitochondria depends on the import of proteins, which are synthesized as precursors on cytosolic ribosomes. The majority of the precursor proteins are sorted into the mitochondrial subcompartments via five distinct routes. Recent studies revealed that molecular cooperation between protein machineries is a central feature of mitochondrial protein biogenesis. First, coupling to various partner proteins affects the substrate specificity of translocases and single translocation steps. Second, there is a substantial cooperation between different protein translocases in the import of specific precursor proteins. Third, protein transport is intimately linked to processing, folding and assembly reactions. Fourth, sorting of precursor proteins is functionally and physically connected to protein machineries, which fulfill central functions for respiration, maintenance of membrane architecture and form contacts to the endoplasmic reticulum. Therefore, we propose that the protein transport systems are part of a complicated protein network for mitochondrial biogenesis.

1. Introduction

Mitochondria fulfill essential functions for the survival of the cell. They supply ATP for the cellular metabolism, which is generated by energy conversion via oxidative phosphorylation. Biosynthesis pathways for amino acids, lipids, heme and the formation of iron–sulfur clusters are located to mitochondria. Additionally, mitochondria provide a signaling platform for apoptosis. Due to the versatile functions, defects in mitochondrial biogenesis cause numerous severe diseases [1,2]. Within the cell mitochondria form a dynamic tubular network, which constantly undergoes fusion and fission [3]. To maintain the tubular structure a close contact to the endoplasmic reticulum (ER) is important [4,5]. In bakers yeast *Saccharomyces cerevisiae* the ER-mitochondria encounter structure (ERMES) forms a molecular bridge between both organelles [4]. Mitochondria have two membranes: the outer and the inner membrane. The inner membrane can be dissected into two parts: the inner boundary membrane and the cristae membrane. The inner boundary membrane is in close proximity to the outer membrane and forms several contact sites to outer membrane proteins. The cristae membrane forms large invaginations of the inner membrane, which protrude into the mitochondrial matrix. Protein complexes of the respiratory chain are enriched in the cristae membranes [6]. The mitochondrial contact site and cristae organizing system (MICOS) is a large protein complex that is important to form the complex architecture of the inner membrane [7–10].

Mitochondria contain about 1000 different proteins in yeast and 1500 proteins in human to carry out the various metabolic and signaling functions as well as to preserve mitochondrial architecture and gene expression [11–13]. Due to their endosymbiotic origin mitochondria contain their own circular genome. Mitochondrial DNA encodes for eight proteins in yeast and thirteen proteins in humans. Most mitochondria-encoded proteins are central components of the respiratory chain complexes. Thus, the vast majority of mitochondrial proteins are synthesized on cytosolic ribosomes as precursors. The precursors contain internal or cleavable signals that target them to the mitochondrial surface. The translocase of the outer membrane (TOM complex) forms the general entry gate for the precursor proteins. After passage through the TOM channel different protein machineries sort the precursor proteins into the mitochondrial subcompartments (Fig. 1). So far, five main protein sorting pathways into mitochondria have been identified in yeast mitochondria [14–18]: First, precursor proteins containing a cleavable presequence are transported into the mitochondrial matrix and inner membrane via the presequence translocase (TIM23 complex). Second, the carrier translocase (TIM22 complex) mediates the insertion of inner membrane metabolite carriers. Third, the mitochondrial intermembrane space import and assembly machinery (MIA) drives import of proteins with cysteine-rich motifs. Fourth, the sorting and assembly machinery (SAM complex) inserts precursors of proteins with a transmembrane β-barrel domain into the outer membrane. Finally, outer membrane proteins with multiple α-helical transmembrane segments are not imported via the TOM channel, but integrated into the target membrane by the mitochondrial import

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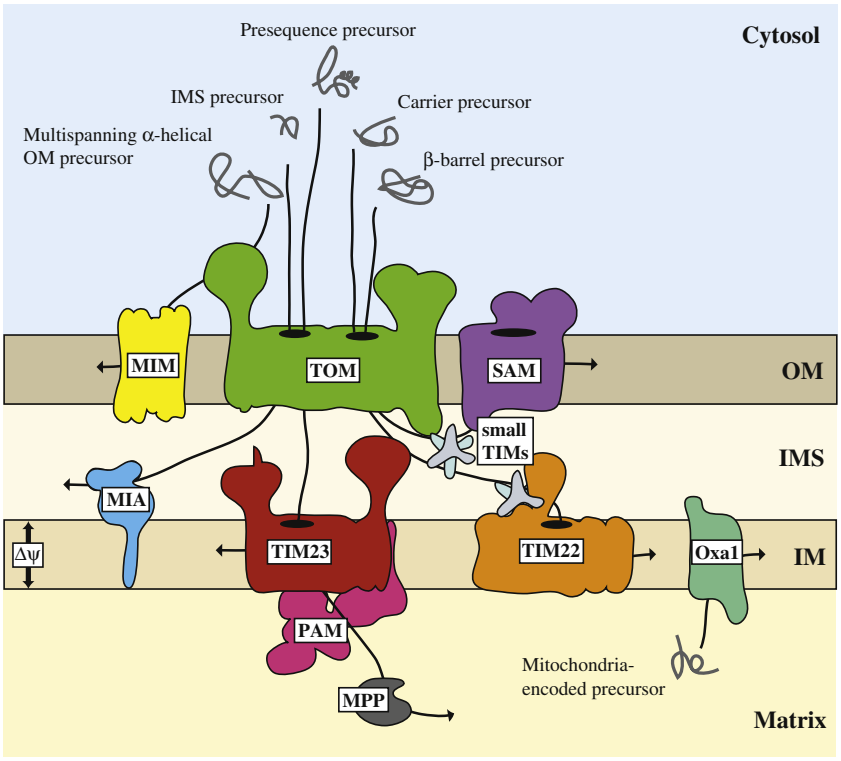

Fig. 1. Five import routes for mitochondrial precursor proteins. The translocase of the outer membrane (TOM complex) forms the general entry gate for mitochondrial precursors. After passage of the precursors through the TOM complex different sorting pathways branch off. Precursor proteins with a cleavable presequence are sorted into the inner membrane or mitochondrial matrix via the presequence translocase (TIM23 complex). The presequence translocase associated motor (PAM) provides the driving force to complete translocation into the matrix. The presequence is removed by the mitochondrial processing peptidase (MPP). Precursors of carrier proteins contain internal signal sequences and are guided by the small TIM proteins through the intermembrane space to the carrier translocase (TIM22 complex), which inserts these precursors into the inner membrane. The carrier and the presequence pathway depend on the membrane potential across the inner membrane ($\Delta\psi$). Proteins with conserved cysteine-rich motifs are sorted into the intermembrane space by the intermembrane space import and assembly (MIA) pathway. The small TIM proteins also transfer precursors of β-barrel proteins to the sorting and assembly machinery (SAM), which mediates their folding and membrane integration. Precursors of outer membrane proteins with multiple α-helical membrane spans are recognized by Tom70 and integrated into the target membrane by the mitochondrial import (MIM) machinery. Oxa1 (oxidase assembly) mediates the insertion of mitochondria-encoded proteins into the inner membrane. OM, outer membrane; IMS, intermembrane space; IM, inner membrane.

(MIM) machinery. Mitochondria-encoded proteins are inserted into the inner membrane by the oxidase assembly (Oxa1) machinery [19,20]. Initially, it was believed that the distinct protein translocases are largely independent of each other. Yet in the last few years, import studies employing a wide variety of precursor proteins and analysis of molecular interaction partners of protein translocases revealed an extensive cooperation of protein machineries involved in mitochondrial protein sorting. Furthermore, mitochondrial protein import is directly linked to other processes like generation and maintenance of membrane architecture or respiration. We propose that molecular cooperation of protein machineries and the functional link to other processes is a central feature of mitochondrial protein biogenesis.

2. The TOM complex — platform for entry and control

The TOM complex forms the entry gate for most of the mitochondrial precursor proteins (Fig. 1). The TOM complex consists of seven subunits [21–25]. The receptor proteins Tom20, Tom70 and Tom22 bind to incoming precursor proteins [26–34]. The β-barrel protein Tom40 forms the protein-conducting channel [35–37]. The three small Tom proteins Tom5, Tom6 and Tom7 are involved in stability and assembly of the TOM complex and may support precursor protein transfer towards the translocation pore [38–45]. The TOM complex initiates sorting of precursor proteins into the mitochondrial subcompartments by cooperation with other protein translocases [41,46–49]. This sorting step may be supported by interaction to the central component of the MICOS complex, Mic60 [7,50,51]. Interestingly, the small TOM subunits Tom5 and Tom7 associate with proteins involved in ER-mitochondria contact sites [52–54]. Thus, the TOM complex is closely linked to protein machineries, which are important to provide membrane contacts and to maintain mitochondrial morphology (Fig. 2).

The TOM complex cooperates with cytosolic factors in protein import. Such targeting factors bind to precursor proteins after their synthesis is complete and target them to the TOM receptors on the mitochondrial surface (Fig. 2). So far, a few targeting factors have been described [55–64]. For most of these cytosolic proteins it is not clear whether they are specifically required for transport of single precursor proteins or play a more general role in protein targeting. Molecular chaperones like the cytosolic Hsp70 in yeast and Hsp90 in human support targeting of hydrophobic precursor proteins by keeping the client protein in an import-competent unfolded state [59,60,65,66]. Tom70 recognizes these precursor-loaded chaperones on the mitochondrial surface [59,60]. The chaperones may also support first translocation steps [67]. Systematic identification of factors involved in targeting of mitochondrial precursor proteins will be crucial to shed light on the mechanism of cytosolic protein transport to mitochondria.

Whereas the chaperone-mediated targeting of precursors reflects post-translation protein transport, several observations point to the presence of a co-translational protein import mechanism into mitochondria as well (Fig. 2). First, cytosolic ribosomes were found at the outer mitochondrial membrane [68]. Second, mRNAs encoding for mitochondrial precursor proteins are enriched on mitochondria [69]. Tom20 was reported to mediate the localization of these mRNAs to mitochondria [70]. In the targeting process the presequence is synthesized and after emerging from the exit tunnel mediates the binding of the ribosome-nascent chain complex to mitochondria [71]. In this line,