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Review

Mitochondrial protein import: Common principles and physiological networks ☆

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ABSTRACT

Most mitochondrial proteins are encoded in the nucleus. They are synthesized as precursor forms in the cytosol and must be imported into mitochondria with the help of different protein translocases. Distinct import signals within precursors direct each protein to the mitochondrial surface and subsequently onto specific transport routes to its final destination within these organelles. In this review we highlight common principles of mitochondrial protein import and address different mechanisms of protein integration into mitochondrial membranes. Over the last years it has become clear that mitochondrial protein translocases are not independently operating units, but in fact closely cooperate with each other. We discuss recent studies that indicate how the pathways for mitochondrial protein biogenesis are embedded into a functional network of various other physiological processes, such as energy metabolism, signal transduction, and maintenance of mitochondrial morphology. This article is part of a Special Issue entitled: Protein Import and Quality Control in Mitochondria and Plastids.

1. Introduction

Mitochondria are ubiquitous organelles surrounded by two membranes, the outer and the inner membrane, which confine two aqueous compartments, the matrix and the intermembrane space (IMS). Tubular invaginations of the inner mitochondrial membrane form the cristae, which harbor the enzyme complexes of the oxidative phosphorylation system. In addition to their central role in ATP synthesis mitochondria accommodate central metabolic pathways, like the Krebs cycle and the β-oxidation of fatty acids. They provide cells with a large number of metabolites, such as amino acids and steroids, and are involved in the formation of heme and iron-sulfur clusters. Based on proteomic analyses it has been estimated that mitochondria contain ~1500 different proteins in mammals and ~1000 different proteins in yeast [1–3]. Because of their endosymbiotic origin mitochondria still contain their own small genome encoding for a limited amount of proteins that are mostly subunits of respiratory chain complexes and the F_1F_o-ATPase synthase. Thus, nearly all mitochondrial proteins are encoded by nuclear genes, synthesized as precursor forms on cytosolic ribosomes and subsequently transported into the organelle. Our knowledge on the general principles of protein import into mitochondria mainly originates from genetic and biochemical studies with the model organism baker's yeast (*Saccharomyces cerevisiae*). Most of the mechanisms described in the following were initially discovered in *S. cerevisiae*, but the vast majority of the protein machineries involved were later found to be highly conserved in higher eukaryotes (Fig. 1). It is widely accepted that import of precursor proteins into mitochondria generally occurs in a post-translational manner. For some proteins, like Sod2 or fumarase, however, there are clear indications for a co-translational import mechanism [4,5]. Cytosolic ribosomes translating mRNAs for mitochondrial precursor proteins have been found in proximity to the outer mitochondrial membrane [6–8]. Specific signals within both the 3′ untranslated and the coding regions of these mRNAs have been shown to mediate their targeting [9–12]. In yeast, recruitment of mRNAs to the mitochondrial surface involves the Puf3 protein and the outer membrane precursor protein receptors Tom20 and Tom70 [13–16].

Precursor protein targeting to mitochondria and sorting to distinct mitochondrial subcompartments requires the presence of specific import signals within the transported polypeptides (Fig. 1). The most frequently found mitochondrial import signal is an N-terminal extension termed presequence. These presequences are amphipathic α-helical segments with a net positive charge and show a prevalent length distribution of 15 to 55 amino acids [17]. In general, N-terminal presequences are proteolytically removed after import by the mitochondrial processing peptidase and other proteases [18,19]. An interesting exception is the helicase Hmi1, which is channeled into mitochondria by a presequence-like structure at its C-terminus [20]. Often less well defined internal signal

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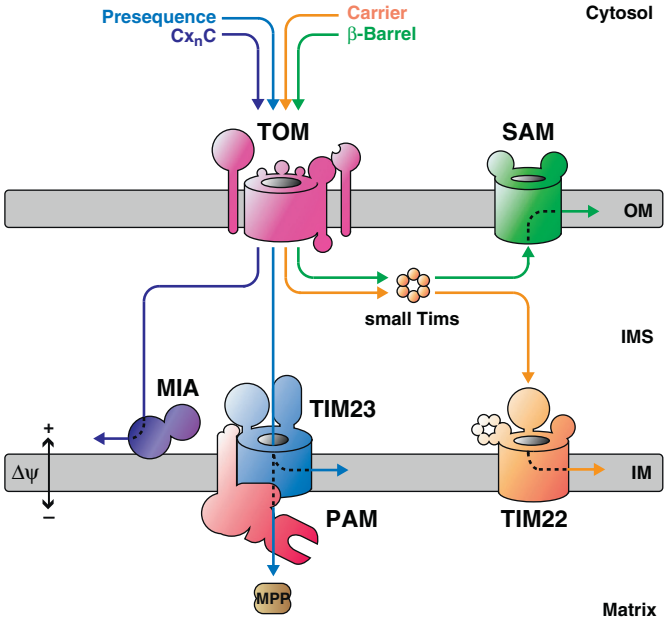

Fig. 1. Different targeting signals direct nuclear encoded precursor proteins on specific transport routes to their final localization within mitochondria. After translocation of precursors through the general translocase of the outer membrane (TOM complex), distinct downstream import pathway diverge in the intermembrane space (IMS): Biogenesis of β-barrel proteins of the outer membrane (OM) requires the small Tim chaperones of the IMS and the sorting and assembly machinery (SAM). Proteins of the IMS that contain cysteine-rich signals (Cx_nC) are imported via the mitochondrial intermembrane space import and assembly (MIA) pathway. Carrier proteins of the inner membrane (IM) are transported with the help of the small Tims and the translocase of the inner membrane 22 (TIM22 complex). Presequence-containing proteins are inserted into the inner membrane or imported into the matrix by the translocase of the inner membrane 23 (TIM23 complex; presequence translocase). Matrix translocation requires the activity of the presequence translocase-associated import motor (PAM). Presequences are proteolytically removed by the mitochondrial processing peptidase (MPP) upon import. Δψ, membrane potential across the inner mitochondrial membrane.

sequences direct the transport of other mitochondrial proteins. Different forms of these internal import signals target the precursors to diverse destinations within mitochondria and will be further discussed in the respective sections of this review.

To prevent misfolding and aggregation, hydrophobic segments of mitochondrial precursor proteins are shielded from the aqueous cytosolic milieu by dedicated chaperones that escort them to the organelle's surface [21–23]. The outer membrane receptors Tom20 and Tom70 serve as initial docking sites for precursor proteins and function as quality control checkpoints, which only permit access to mitochondria, if a given protein contains an appropriate targeting signal. Except for some α-helical outer membrane proteins, virtually all precursors initially enter mitochondria by passing a common entry gate formed by the Translocase of the Outer Membrane (TOM complex). Upon translocation across the outer membrane different transport routes exist that are specifically required for transport of a subset of precursor proteins to their final destination (Fig. 1). Many IMS proteins are imported by the Mitochondrial Intermembrane space import and Assembly (MIA) machinery, which couples sorting of client proteins to their oxidative folding through a disulfide relay mechanism. Outer membrane β-barrel proteins are bound by small Tim chaperones of the IMS that transfer precursors to the Sorting and Assembly Machinery (SAM), where they are integrated into the outer membrane. Mitochondrial metabolite carrier proteins are also guided by small Tim chaperones through the IMS and subsequently integrated into the inner membrane by the Translocase of the Inner Membrane 22 (TIM22 complex) in a membrane potential (Δψ)-driven manner. Presequence-containing precursor proteins are directly passed on from the TOM complex to the Translocase of the Inner Membrane 23 (TIM23 complex; presequence translocase) without the need for soluble IMS chaperones. Dependent on the presence or absence of additional import signals, the TIM23 complex mediates either the translocation of presequence-carrying precursors into the matrix or their lateral sorting into the inner membrane. Whereas lateral membrane integration depends on Δψ as the sole energy source, complete import into the matrix additionally requires the ATP-driven Presequence translocase-Associated import Motor (PAM).

2. Protein translocation across the outer membrane: the TOM complex

The TOM complex is a particularly fascinating protein translocase, as it mediates the transport of various different types of precursors with highly diverse import signals across the outer membrane and then selectively distributes them to multiple downstream protein sorting machineries. Tom40, the central component of the TOM complex, is integrated into the outer membrane in a β-barrel conformation and forms aqueous pores, through which mitochondrial precursor proteins pass [24–26]. Additional subunits support or modulate the quaternary structure of the TOM complex and/or function as receptors: Tom20, Tom70/Tom71, Tom22, and the small Tom proteins Tom5, Tom6 and Tom7. The primary receptors Tom20 and Tom70/Tom71 selectively bind to different subsets of mitochondrial precursor proteins [27]. Tom20 mainly recognizes N-terminal presequences by binding the hydrophobic face of their amphipathic α-helical conformation [28,29]. Precursor proteins with hydrophobic internal targeting signals are preferentially bound by Tom70 and Tom71 [30,31]. Tom70 and Tom71 have high sequence homology and overlapping functions, however Tom71 is expressed only in small amounts [32–34]. Apart from their role in protein import, the Tom70 and Tom71 receptors have been suggested to participate in mitochondrial morphology maintenance by recruiting the morphogenesis factor Mfb1 to the organellar surface [35]. The central receptor Tom22 is critical for the integrity of the TOM complex and exposes presequence binding domains to both the cytosol and the IMS [36–39]. Tom5 is thought to assist the transfer of precursor proteins from Tom22 to the Tom40 channel and to support the biogenesis of Tom40 [40,41]. Tom6 and Tom7 antagonistically regulate the dynamic assembly of the TOM complex: Whereas Tom6 promotes TOM biogenesis through its association with early assembly intermediates, Tom7 destabilizes both intermediate and mature TOM complex forms, likely to facilitate the incorporation of newly imported subunits [42–45]. Interestingly, the assembly and activity of the TOM complex was recently shown to be controlled by cytosolic protein kinases: Whereas casein kinase 2 (CK2) phosphorylates Tom22 to facilitate its biogenesis, the receptor activity of Tom70 is decreased through phosphorylation by protein kinase A [46,47]. These findings demonstrate that the functional state of the TOM complex is intimately linked to large-scale regulatory circuits of cellular physiology.

The pathway of precursor passage through the TOM complex is best understood for presequence-containing proteins. After their initial recognition by the Tom20 receptor, precursors bind to cytosolic domains of Tom22 and Tom5, engage a polar slide formed within the pore of Tom40 and finally contact an acidic binding site on the trans side of the TOM complex formed by Tom40, Tom7 and the IMS domain of Tom22 [36,37,48–51]. The increasing affinity of these interactions is considered to drive the inward-directed movement of precursors. For presequence-containing proteins, the transport across the outer membrane is tightly coupled to the translocation across or into the inner membrane via the TIM23 machinery through a direct hand-over of substrates as soon as they emerge from the TOM complex [52,53].

3. Biogenesis of outer membrane β-barrel proteins: the SAM complex

The presence of membrane proteins with a β-barrel conformation is a key feature of the outer membrane of Gram-negative bacteria. In