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Review

Protein transport machineries for precursor translocation across the inner mitochondrial membrane

Karina Wagner [a,b], David U. Mick [a,b,c], Peter Rehling [c,*]

[a] Institut für Biochemie und Molekularbiologie, ZBMZ, Universität Freiburg, D-79104 Freiburg, Germany
[b] Fakultät für Biologie, Universität Freiburg, D-79104 Freiburg, Germany
[c] Abteilung Biochemie II, Universität Göttingen, D-37073 Göttingen, Germany

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ABSTRACT

The mitochondrial inner membrane has a central function for the energy metabolism of the cell. The respiratory chain generates a proton gradient across the inner mitochondrial membrane, which is used to produce ATP by the F_1F_o-ATPase. To maintain the electrochemical gradient, the inner membrane represents an efficient permeability barrier for small molecules. Nevertheless, metabolites as well as polypeptide chains need to be transported across the inner membrane while the electrochemical gradient is retained. While specialized metabolite carrier proteins mediate the transport of small molecules, dedicated protein translocation machineries in the inner mitochondrial membrane (so called TIM complexes) transport precursor proteins across the inner membrane. Here we describe the organization of the TIM complexes and discuss the current models as to how they mediate the posttranslational import of proteins across and into the inner mitochondrial membrane.

1. Introduction

Mitochondria of eukaryotic cells originated from α-proteobacteria, which lost most of the protein encoding genes to the nucleus of their host cell over time (for review see [1]). Consequently, mitochondrial genomes usually encode only a small subset of mostly hydrophobic proteins. In the yeast *S. cerevisiae* only eight and in human thirteen proteins are synthesized within the mitochondria. Since most of the approximately 1000 mitochondrial proteins are translated in the cytosol, mitochondria require sophisticated machineries that are competent of importing these proteins into the four mitochondrial subcompartments (outer membrane, intermembrane space, inner membrane, and matrix). Moreover, the proteins that are synthesized in the cytosol require targeting signals, which direct them not only to mitochondria but also to their final destination inside the organelle. The mitochondrial ancestors, α-proteobacteria, already possessed machineries for export of proteins from the cytosol into and across the plasma membrane to the periplasmic space [2]. Some of these machineries were maintained in the mitochondria during evolution (i.e. the export machinery for mitochondrial-encoded proteins) whereas others had to evolve for the import of proteins into the organelle.

2. Import pathways into mitochondria — an overview of the players

To traverse the outer and inner mitochondrial membranes precursor proteins need the assistance of membrane embedded translocase machineries. These multi-protein complexes usually contain signal receptors and form pores in the membrane through which the precursor proteins are transported. The process of precursor translocation is energy dependent and the translocases help to translate the driving energies into a vectorial movement of the polypeptide chain [3–6].

The TOM complex (translocase of the outer mitochondrial membrane) is the main entrance into mitochondria. It contains receptors that expose domains at the cytosolic surface of the membrane for binding of incoming precursor proteins [2–6]. The receptors pass the precursors on to Tom40, which forms the protein-conducting pore of the complex. Upon exit from the Tom40 channel, proteins use different transport machineries depending on their final destination and topology. Prior to their insertion into the outer membrane β-barrel proteins are transported into the intermembrane space by the TOM complex and subsequently sorted into the outer membrane by the SAM complex (*s*orting and *a*ssembly *m*achinery) (Fig. 1A). In the intermembrane space a small TIM chaperone complex facilitates the transport of the β-barrel precursor proteins from the TOM to the SAM complex [3,5,7]. The Sam50 protein is related to the bacterial Omp85 and forms a channel in the outer membrane that is closely associated with Sam35, which recognizes the β-signal [2–5,8]. The molecular mechanism by which the

* Corresponding author. Tel.: +49 551 39 5947; fax: +49 551 39 5979.
E-mail address: peter.rehling@medizin.uni-goettingen.de (P. Rehling).

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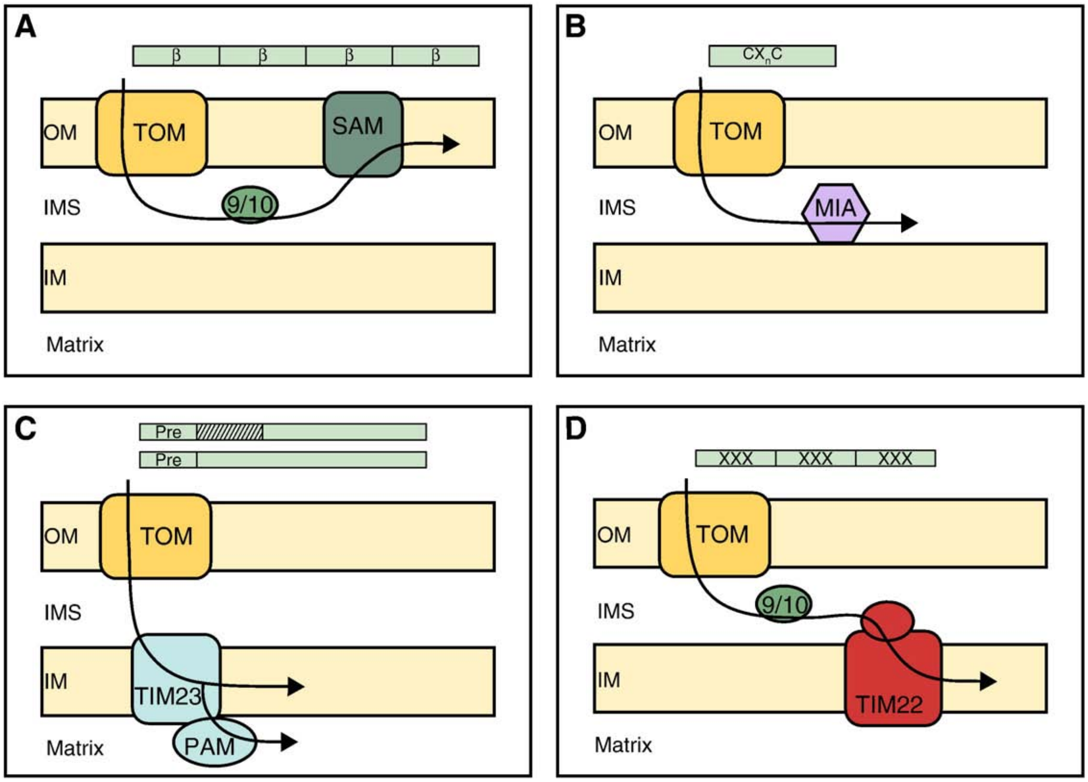

Fig. 1. Import pathways into mitochondria. (A) β-barrel proteins pass the outer membrane via the TOM machinery and are guided by the small Tim chaperones to the SAM machinery. (B) Preproteins containing cysteine motifs (usually $n=3$ or 9) are imported via the TOM complex and the MIA machinery. (C) Presequence-containing preproteins require the TOM and TIM23 complexes for transport into or across the inner membrane. Hydrophobic sorting signals direct precursor proteins laterally into the inner membrane Pre, presequence; hydrophobic sorting signal, hatched. (D) Carrier pathway proteins use the TOM and small Tim proteins for transport to the TIM22 translocase, which inserts them into the inner mitochondrial membrane. OM, outer membrane; IM, inner membrane; IMS, intermembrane space.

SAM machinery inserts β-barrel proteins into the outer membrane is still enigmatic. Moreover, the recent finding that assembly of the α-helical Tom22 protein also depends on the SAM complex suggests that the complex fulfills more functions than anticipated [9].

A set of proteins of the intermembrane space requires the MIA machinery (mitochondrial intermembrane space import and assembly) for import and folding. Substrates of the MIA machinery are proteins that contain cysteine-rich motives such as the small Tim chaperones of the intermembrane space (Fig. 1B). Together with the sulfhydryl oxidase Erv1, Mia40 catalyzes the formation of disulfide bonds in its substrates [10–12].

Depending on the precursors' targeting signals, membrane translocation across the inner mitochondrial membrane can be carried out by one of two distinct translocases: the TIM23 or the TIM22 complex (translocase of the inner mitochondrial membrane) (Fig. 1C and D). While both inner membrane translocases differ with respect to their substrates, the Tim22 and Tim23 proteins that form the protein-conducting pores of the TIM complexes display sequence similarity and only a single common ancestor (Tim22) is present in early eukaryotes with so called degenerate mitochondria [13].

3. Transport pathway of carrier proteins into the inner membrane

Although the inner mitochondrial membrane represents a stringent permeability barrier, mitochondria need to constantly exchange metabolites such as ATP, phosphate, carnitine/acylcarnitine, oxoglutarate/malate, and many others with the cytosol [14]. To pass such metabolites across the inner membrane, specialized carrier proteins are necessary that mediate the transport into and out of the mitochondrial matrix. These metabolite carrier proteins are inserted into the inner membrane of mitochondria through a complex transport route that has been termed the carrier pathway and which involves the TIM22 translocase complex in the inner mitochondrial membrane.

3.1. Substrates of the carrier translocase complex

Substrates of the carrier translocase (TIM22 complex) are especially hydrophobic proteins, which possess several transmembrane segments. These proteins are translated in the cytosol as mature-sized proteins and possess internal targeting signals, which are distributed over the length of the precursor (Fig. 1D). Several of such signals are usually present in a single precursor molecule. Most substrates of the carrier translocase belong to the family of metabolite carriers. Beside their functional similarities, members of the carrier protein family share structural features. Carrier proteins are built of three modules of approximately similar length, each of which consists of two transmembrane segments that are connected by a hydrophilic loop (Fig. 2A).

Thus, they form six transmembrane α-helices in the lipid phase of the inner membrane [15]. It is currently assumed, that other carrier proteins display a similar structure and that they associate into dimers in the inner mitochondrial membrane [16]. In addition to the carrier proteins, also other polytopic inner membrane proteins are transported along the carrier transport route, among them are Tim23 and Tim22 [17,18]. In contrast to carrier proteins, Tim22 and Tim23 possess only four predicted transmembrane spans. After their insertion into the lipid phase, Tim23 and Tim22 expose their N- and C-termini into the intermembrane space. Little is known about the targeting information in these proteins, however, it appears that for import of Tim23 into mitochondria, transmembrane helices one and four are important. For translocation across the outer membrane a N-terminal segment of Tim23 is critical, to which the non-essential small Tim