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The mitochondrial import machinery: preprotein-conducting channels with binding sites for presequences

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Abstract

Mitochondrial preproteins with amino-terminal presequences must cross two membranes to reach the matrix of the organelle. Both outer and inner membranes contain hydrophilic high-conductance channels that are responsible for selective translocation of preproteins. The channels are embedded in dynamic protein complexes, the TOM complex of the outer membrane and the TIM23 complex of the inner membrane. Both channel-forming proteins, Tom40 and Tim23, carry specific binding sites for presequences, but differ in their pore size and response to a membrane potential. Studies with the TOM machinery show that other subunits of the translocase complex also provide specific binding sites for preproteins, modulate the channel activity and are critical for assembly of the channel.
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Keywords: Mitochondrion; Protein sorting; Protein translocation; TOM complex; Channel; Presequence

1. Introduction

The biogenesis of mitochondria strictly depends on the synthesis of precursor proteins on cytosolic polysomes. More than 98% of mitochondrial proteins are encoded by nuclear genes and synthesized in the cytosol. The typical mitochondrial preprotein is synthesized with an amino-terminal extension of about 20-amino-acid residues (10–80 residues), termed presequence. Mitochondrial presequences form positively charged amphipathic α-helices and contain specific information for directing proteins to the mitochondrial surface and across both mitochondrial membranes into the matrix of the organelle [1–6]. After recognition of the presequence by receptors proteins [see article by Endo and Kohda in this issue], the preprotein is translocated across the outer membrane via the general import pore (GIP) (Fig. 1). The GIP forms the central part of a dynamic multi-subunit machinery, termed the translocase of the outer membrane (TOM), which will be the major focus of this review. The presequence is then pulled across the inner membrane by the electrical membrane potential $\Delta\psi$. The translocase of the inner membrane (TIM) provides a specific channel for presequence-containing preproteins, the TIM23 complex. The mitochondrial heat shock protein 70 (mtHsp70) forms the ATP-dependent core of the import pore that promotes completion of preprotein transport into the matrix [see article by Voos and Röltzers in this issue]. The mitochondrial processing peptidase (MPP) cleaves off the presequence and the protein is folded to its active form, in several cases supported by folding helpers such as heat shock protein 60 (Hsp60) or prolyl isomerases [7–9].

Besides cleavable preproteins with a presequence, mitochondria import many other types of preproteins, carrying targeting signals at different locations within their primary structure. The metabolite carriers of the inner membrane form a large family of proteins that are typically synthesized without a presequence, yet contain multiple targeting signals distributed over the mature protein part [10]. Practically all preproteins are translocated across the outer membrane via the GIP, while the inner membrane contains distinct translocases for different types of preproteins [see articles by Jensen and Dunn, and Stuart in this issue]. This means that cleavable preproteins and carrier preproteins use the same

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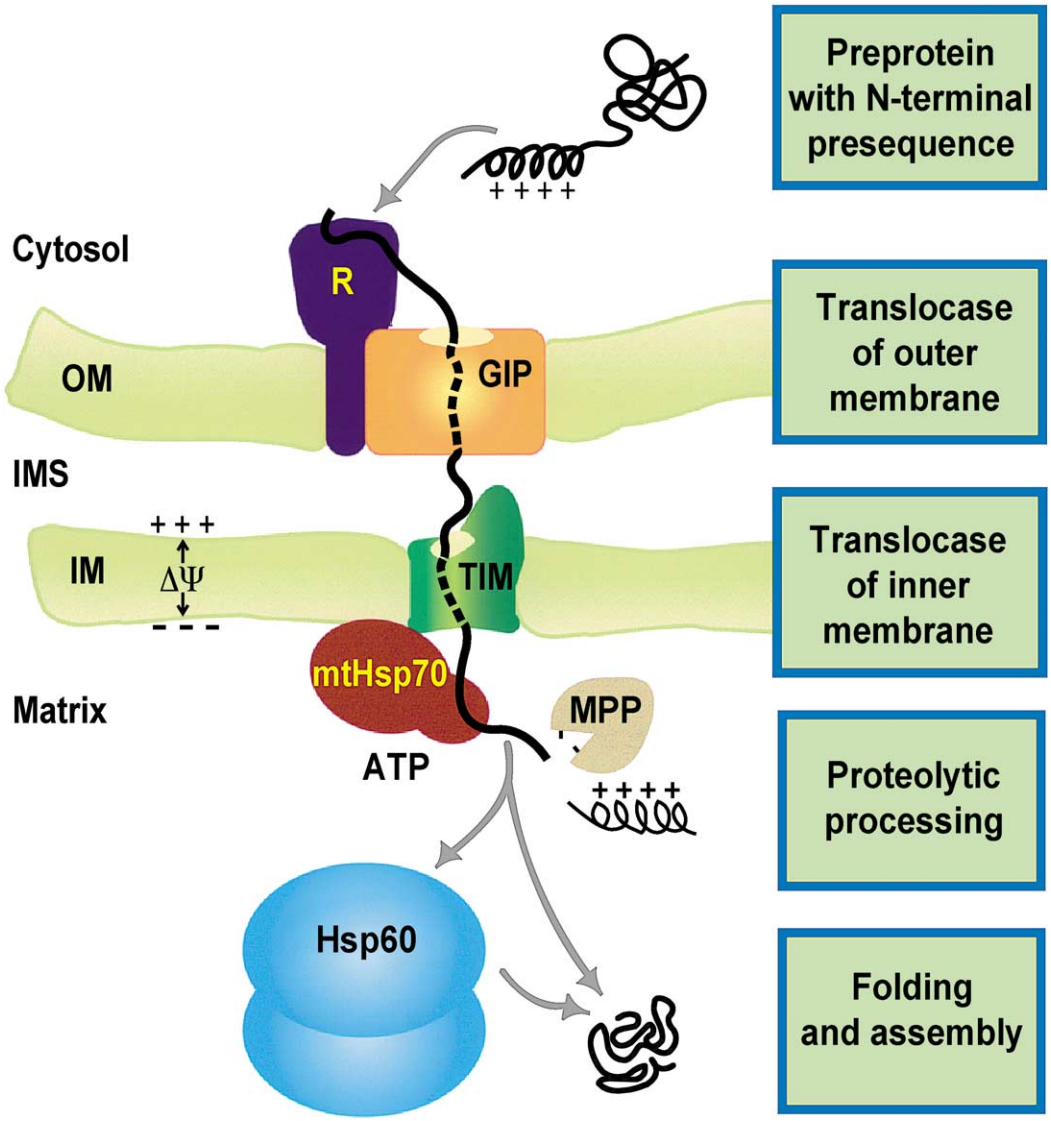

Fig. 1. The import pathway of a presequence-containing preprotein into mitochondria. The positively charged presequence is recognized by receptors (R) on the mitochondrial surface, and the preprotein is translocated through the GIP across the outer membrane (OM). After crossing the intermembrane space (IMS), the preprotein is translocated across the inner membrane (IM) by the TIM. Transport requires the membrane potential ($\Delta\psi$) across the inner membrane and the ATP-dependent action of mtHsp70. The presequence is cleaved off by the MPP. Hsp60 and further folding helpers assist in folding and assembly to the mature form.

channel of the outer membrane, although their mechanism of translocation differs.

2. The translocase of the outer mitochondrial membrane

The preprotein translocase of the outer mitochondrial membrane consists of at least seven different subunits that are named Tom with a number indicating the approximate molecular mass in kDa [11]. While Tom20 and Tom70 function as initial receptors for cleavable preproteins and carrier preproteins, respectively, five other Tom proteins form the stable core of the translocase, the 400 kDa GIP complex [12–16]. This GIP complex consists of Tom40, Tom22 and the three small Tom proteins, Tom7, Tom6 and Tom5 (Fig. 2). All subunits have been identified in both the yeast *Saccharomyces cerevisiae* and the filamentous fungus *Neurospora crassa* with the exception of Tom5 that has not been found in *Neurospora* so far.

2.1. Tom40 is the pore-forming subunit

A central question has been which subunits of the TOM machinery form the channel for translocation of preproteins. The answer turned out to be quite simple, that is only one of the seven Tom proteins is needed to form a specific channel for preproteins. Tom40, the only Tom protein that is strictly essential for viability of yeast under all growth conditions, was originally identified by chemical cross-linking to a preprotein arrested across the mitochondrial membranes

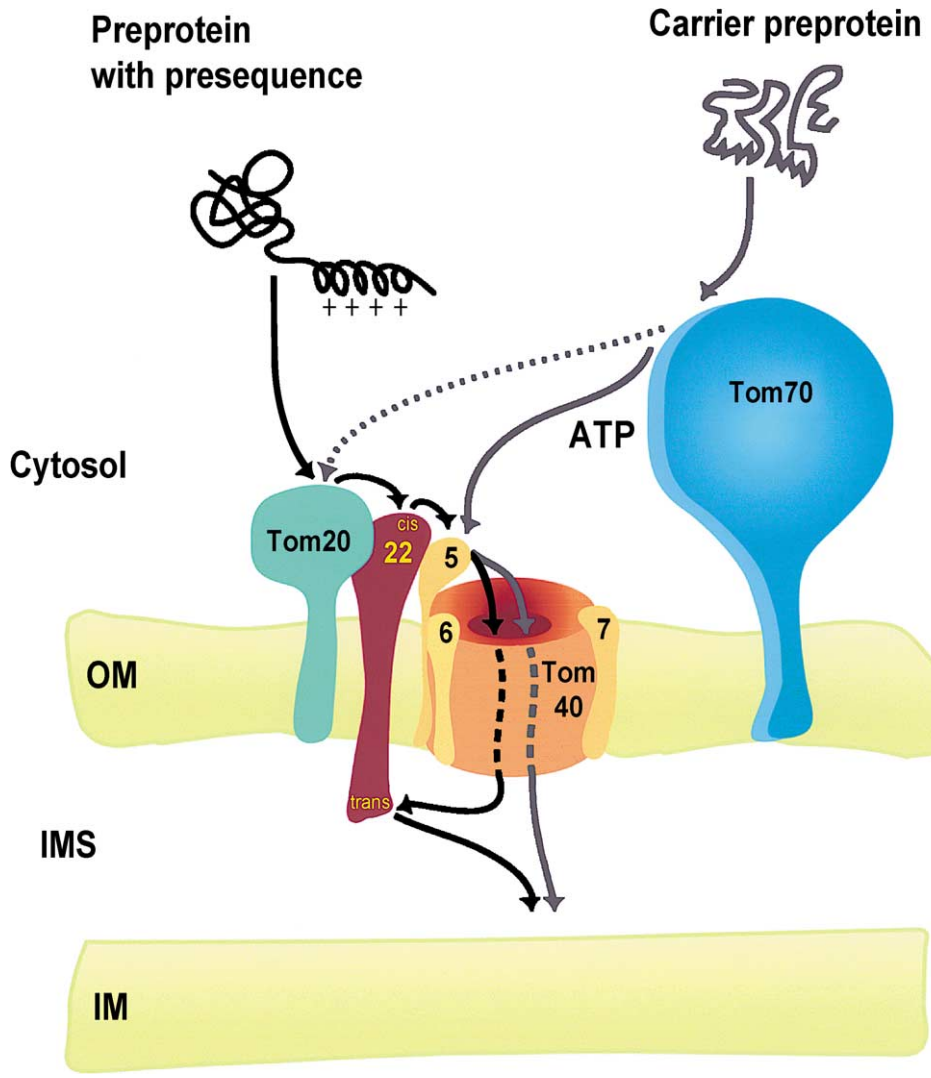

Fig. 2. The preprotein translocase of the outer mitochondrial membrane (TOM). Preproteins with a presequence are guided across the outer membrane (OM) by a chain of binding sites, formed by the receptors Tom20 and Tom22, the small protein Tom5, the channel Tom40 and the intermembrane space (IMS) domain of Tom22. Tom6 and Tom7 modulate assembly and dissocation of the translocase. Carrier preproteins with internal targeting information preferentially use the dimeric receptor Tom70, but are directed to the same import channel Tom40. A complete TOM complex contains two to three translocation pores (not shown) formed by multiple Tom40 molecules.

and by its presence in the TOM complex [17–19]. When Tom40 was expressed in *Escherichia coli* cells and reconstituted into liposomes, a cation-selective high-conductance channel was measured upon fusion of the liposomes with a planar lipid bilayer [20]. This channel exhibited the typical characteristics of the TOM channel observed in mitochondrial outer membranes and in purified TOM complexes, demonstrating that Tom40 is the pore-forming subunit of the TOM machinery [13–15,20]. In particular, the purified and reconstituted Tom40 exhibited a specific binding site for mitochondrial presequences [20] comparable to the interaction of a presequence with Tom40 observed in the mitochondrial membranes [21]. The cation-selectivity of Tom40 fits well to the high abundance of positively charged amino acid residues in mitochondrial presequences [22]. Addition of a synthetic peptide corresponding to the presequence of cytochrome *c* oxidase subunit IV to reconstituted Tom40 strongly increased the frequency of channel gating, while a control peptide with the same number of positively charged residues but without mitochondrial targeting function did not [20].

The characteristics of the channel formed by Tom40 are related to the peptide-sensitive channel (PSC) of large conductance that was characterized by electrophysiological techniques and localized to the mitochondrial outer membrane. PSC is cation-selective and reacts with mitochondrial presequence peptides [23,24]. An unresolved difference is that PSC is affected by the non-mitochondrial basic peptide dynorphin B [25], while Tom40 does not show a specific reactivity with dynorphin B [20].

Tom40 shows a weak similarity to the most abundant pore-forming protein of the mitochondrial outer membrane,

porin, which is also known as the voltage-dependent anion-selective channel (VDAC). In particular, secondary structure predictions indicate a high content of ß-structure for both proteins [20,26,27], indicating that they may form a ß-barrel-like structure observed with bacterial porins [28]. The pore size of reconstituted Tom40 was determined by polymers of different size, leading to the assessment of an effective diameter of ~ 20–22 Å [20]. This agrees well with electron micrographic analysis of the purified TOM complex that showed stain-filled pits with a diameter of ~ 20 Å [13]. Moreover, the pore size of the TOM channel in intact mitochondria was assessed by the use of preproteins with covalently attached gold clusters of defined diameter. While gold clusters of 26 Å could not be translocated across the outer membrane, preproteins with 20-Å gold clusters were efficiently imported [29].

The typical preprotein with amino-terminal presequence is translocated across the mitochondrial membranes as linear chain [30], which may be in an extended conformation or an α-helical conformation. A pore with a diameter of ~ 20–22 Å can easily accommodate an α-helical polypeptide chain, even two polypeptide chains would fit into such a pore, raising the question why the pore has to be so large. The explanation was found by analysis of the import pathway of the carrier preproteins that are not imported as linear chains, but probably in partially folded forms [31–34]. Indeed, carrier preproteins cross the outer membrane neither with the amino-terminus first nor with the carboxy-terminus first. This could be directly shown by attaching tightly folded domains to both ends of the preprotein of the ADP/ATP carrier (AAC) [34]. The enzyme dihydrofolate reductase (DHFR) is stabilized by the specific ligand methotrexate (MTX) and thus cannot be unfolded by the mitochondrial import machinery. A DHFR–AAC–DHFR construct was arrested across the mitochondrial outer membrane in the presence of methotrexate such that both DHFR moieties were exposed on the mitochondrial surface (Fig. 3). At the same time, a middle portion of the AAC could be chemically cross-linked to the tiny Tim proteins in the intermembrane space, demonstrating that AAC had trav-

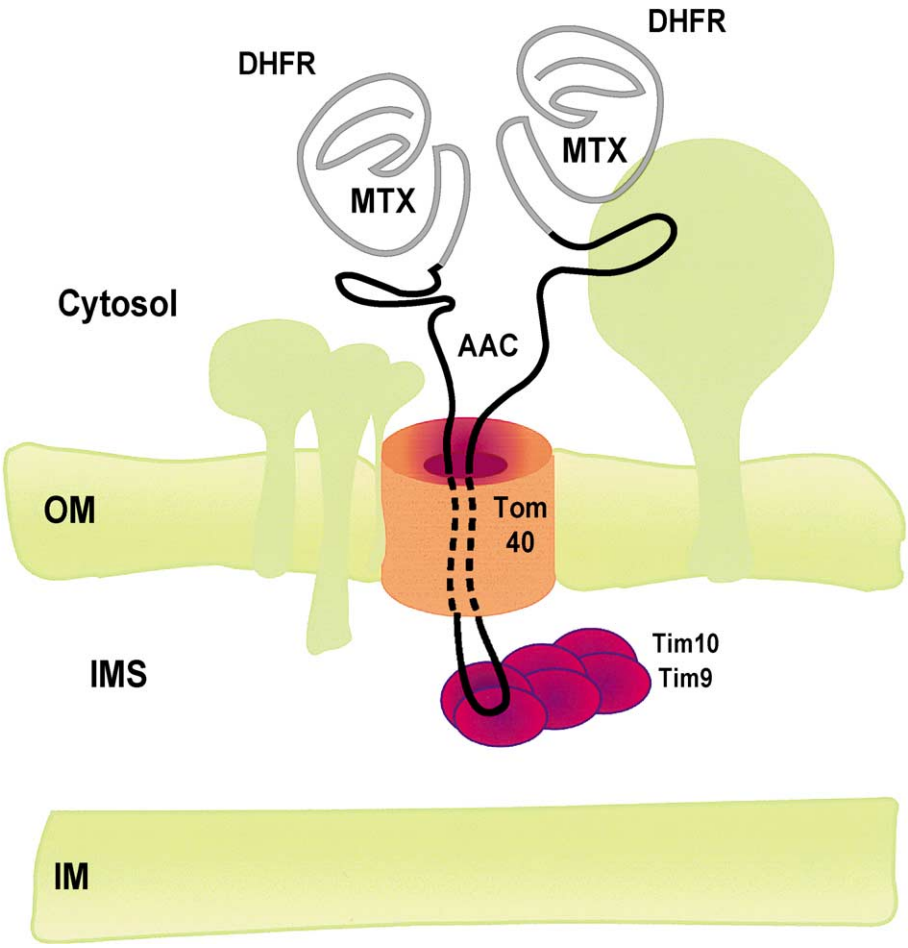

Fig. 3. The preprotein of the ADP/ATP carrier crosses the outer membrane in a loop formation. Schematic representation of an experiment conducted by Wiedemann et al. [34]. A fusion protein consisting of the AAC and two molecules of DHFR on both ends was accumulated across the mitochondrial outer membrane (OM). The specific ligand MTX stabilized the DHFR moieties and prevented their translocation through the Tom40 channel. Since the AAC could be chemically cross-linked to tiny Tim proteins in the intermembrane space (IMS), the preprotein spanned the outer membrane with at least two segments, i.e. in a loop formation.

ersed the outer membrane with an internal segment first, i.e. in a loop formation [34].

2.2. Organization of the general import pore (GIP) complex

The GIP complex contains ~6–8 molecules Tom40, ~3–4 molecules Tom22, and ~2 molecules of each of the small Tom proteins (Tom5, Tom6, Tom7) [12–15]. The GIP complex shows an unusual high stability: the association of the five components Tom40, Tom22, Tom7, Tom6 and Tom5 is resistant to treatment with high concentrations of urea or alkaline pH. Tom20 is more loosely associated with the GIP complex and readily released [16]. The basic units are formed by dimers of Tom40 that are assembled to the larger GIP complex of 400 kDa by the help of Tom6 and Tom22. In the absence of Tom6 or Tom22, the GIP complex dissociates into small complexes of ~100 kDa, consisting of a dimer of Tom40 and the small Tom proteins [12,15,35,36].

It is unknown how the pores are exactly formed. A possible scenario is that a pore is located within a dimer (or monomer) of Tom40. Alternatively, a pore may be situated between several Tom40 dimers. Electron micrographic analysis of the isolated TOM complex revealed two to three stain-filled pits that probably resemble import channels for preproteins [13,14]. Electrophysiological studies with purified TOM complexes indicated the presence of two active channels [24] that function in a coupled, simultaneous manner [16]. Tom22 plays an important role in controlling the gating of the channel. While the TOM channel analyzed from wild-type mitochondrial outer membranes is mainly in a closed state, the channel formed by purified Tom40 as well as the channel observed in outer membranes from a yeast mutant lacking Tom22 are largely in the open state [15,20]. Tom22, thus, not only functions as receptor for preproteins, but is also an organizer of the GIP complex [15].

A presequence-containing preprotein interacts with a number of specific binding sites during its transfer across the outer membrane (Fig. 2). After recognition by the receptors Tom20 and Tom22, the preprotein is transferred to Tom5 that is in close proximity to the Tom40 channel [37]. Tom5 may be considered as a receptor at the third level and facilitates the insertion of preproteins into the import pore. Some special preproteins destined for the intermembrane space even do not use any of the classical import receptors like Tom20, Tom22 or Tom70, yet only depend on Tom5 and Tom40 for their transfer across the outer membrane [38]. The presequences are then recognized by Tom40 and, upon passage through the GIP, interact with a *trans* binding site on the intermembrane space side of the outer membrane [39–42]. The intermembrane space domain of Tom22 has been shown to function as a binding site for presequences [39,42,43]. In summary, a presequence can interact with at least five different binding sites on its route across the outer membrane. Since presequences are positively charged and several Tom proteins contain patches of negatively charged residues, ionic forces seem to be important to guide presequence-containing preproteins across the membrane ("acid chain hypothesis") [37,43–45]. However, recent studies show that other noncovalent forces, including hydrophobic interactions, are also involved in the transient contacts between preproteins and Tom components [16,46,47]. We thus propose the "binding chain hypothesis" where preproteins are guided across the mitochondrial outer membrane via transient contacts with a chain of binding sites, involving each kind of noncovalent interaction [5,16].

2.3. Assembly of the GIP complex

All Tom proteins (as well as all Tim proteins) are themselves encoded by nuclear genes and synthesized as preproteins in the cytosol. This means that mitochondria must import all components of their protein import machinery. Most Tom proteins do not possess classical import signals and thus their mechanism of targeting and membrane insertion is not readily apparent. The most detailed information is available on the import and assembly pathway of the essential component Tom40 (Table 1). Like all other Tom proteins, Tom40 is synthesized without cleavable extension. Targeting of the Tom40 precursor to mitochondria involves at least two import receptors, Tom20 and Tom22 [36,48,49]. The preprotein then assembles with Tom5, forming a 250 kDa complex (assembly intermediate I). Surprisingly, this complex is peripherally membrane-associated on the *trans* side of the outer membrane, i.e. the 250 K complex is not accessible to added proteases from the cytosolic side, but only from the intermembrane space side [36]. This implies that the preprotein is translocated across the outer membrane and inserts into the membrane from the intermembrane space side, possibly resembling the mechanism how bacterial porins are inserted into the outer membrane from the periplasmic side. It is unknown if and which additional components are present in the 250 K complex. This intermediate matures to a smaller complex of 100 kDa (assembly intermediate II). The 100 K complex additionally contains Tom6 and is membrane-integrated. Finally Tom22 assembles with the 100 K complex, leading to formation of the mature 400 K GIP complex [36].

Table 1
Targeting and assembly pathway of Tom40 into the GIP complex [36]

1. Synthesis of the Tom40 preprotein on cytosolic polysomes without cleavable extension.
2. The Tom40 preprotein is recognized by the receptors Tom20 and Tom22 on the mitochondrial surface.
3. The preprotein is translocated across the outer membrane and associates with Tom5 to form a 250 K complex exposed to the intermembrane space (assembly intermediate I).
4. Formation of a membrane-integrated 100 K complex, containing (at least) Tom40, Tom6 and Tom5 (assembly intermediate II).
5. Upon assembly of Tom22, the mature 400 K GIP complex is formed.
6. Tom7 plays modulatory roles by facilitating the release of receptors from the Tom40 preprotein and by promoting a continuous exchange between the mature 400 K complex and the assembly intermediate of 100 K (intermediate II).

Tom7 plays a modulatory role in assembly and dissociation of the TOM machinery [50] and is required at two stages of the assembly pathway of Tom40. First, Tom7 supports the release of the import receptors Tom20 and Tom22 from the preprotein. Secondly, Tom7 promotes an exchange between the mature 400 K GIP complex and the 100 K assembly intermediate II [36]. The current evidence suggests that a small fraction of the mature 400 K complex continuously dissociates into the 100 K complex by release of Tom22 and subsequently is rebuilt to the mature complex. Tom7 seems to facilitate both dissociation and assembly of the 400 K complex. Why is a cycling between a mature complex and its late assembly intermediate useful? It has been observed that preexisting TOM complexes can incorporate individual new subunits, for example radiolabeled Tom40 can assemble into preexisting unlabeled GIP complexes and thereby replaces at least one of the unlabeled Tom40 subunits [12,36,49,51]. We propose that the new subunits do not directly replace "old" subunits at the level of the 400 K complex, but that the new subunits follow the import and assembly pathway via the intermediates I and II. The replacement of old subunits occurs at the level of intermediate II, followed by assembly into the mature complex. We speculate that an exchange between a mature complex and its late assembly intermediate may represent a general feature of large protein complexes.

Tom40 probably contains distinct signals for targeting and assembly. While the amino-terminal and carboxy-terminal segments of Tom40 are dispensable for targeting of the preprotein to mitochondria, a region in the amino-terminal portion of Tom40 is required for assembly into the GIP complex [49,51]. Since purified Tom40 undergoes homo-oligomerization also in the absence of this amino-terminal region [52], this region is likely involved in the assembly of Tom40 with other Tom proteins.

3. The presequence translocase of the inner membrane

The presequence translocase of the inner membrane, termed the TIM23 complex, consists of two integral membrane proteins, Tim23 and Tim17, and a peripherally attached motor system (Fig. 4) [the motor, comprising mtHsp70, its membrane anchor Tim44 and co-chaperones, will be discussed in the article by Voos and Röltzers in this issue]. The membrane-embedded domains of Tim23 and Tim17 are homologous, however, they cannot substitute for each other since each of these proteins is essential for viability of yeast [53–56]. Tim23 and Tim17 are stably associated in a 90 K core complex and are responsible for formation of the import channel(s) across the inner membrane [57–63]. The TIM23 complex may contain two copies of the various subunits. At least for Tim23 and Tim44 it could be shown that they can form homodimers [63,64]. The possible presence of several additional subunits in the TIM23 complex has been reported, however, none of these components has been characterized on a molecular level [57,59,60,63]. In addition to its membrane domain, Tim23 carries a hydrophilic domain exposed to the intermembrane space. This domain has the potential to dimerize and bind presequences [43,64]. Tim23 may thus possess receptor-like properties at the inner membrane.

Electrophysiological studies indicated the presence of a characteristic channel activity in the mitochondrial inner membrane, termed the multiple conductance channel (MCC), that was affected by presequence peptides [65,66]. Moreover, the activity of MCC could be related to the presequence translocase, suggesting that the mitochondrial inner membrane may contain a hydrophilic preprotein-conducting channel [67]. Which subunits of the translocase are needed to form a channel? While it has been generally assumed that Tim23 and Tim17 are so tightly associated that both proteins together should form the channel, recent studies indicate that Tim23 alone is responsible for forming the MCC [68]. When purified Tim23 was reconstituted into liposomes, it formed a cation-selective high-conductance channel with similar characteristics as the MCC of the inner membrane [68]. The opening of the Tim23 channel is strongly stimulated by a membrane potential—in contrast to Tom40 of the outer membrane [20]. The membrane potential across the inner membrane, thus, likely plays a dual role in translocation of preproteins into the matrix by exerting an electrophoretic effect on the positively charged presequences [69] and by activation of the channel protein Tim23 [64,68].

The pore diameter of Tim23 is narrower than that of Tom40. With an inner diameter of $\sim$ 13 Å, Tim23 can accommodate one polypeptide chain in α-helical conformation, but not two α-helices, suggesting that translocation across the inner membrane requires stronger unfolding of a polypeptide chain than translocation across the outer membrane [29,68]. The membrane domain of Tim23 forms the channel, while a high-affinity effect of a presequence peptide on the channel activity is mediated by the amino-terminal domain located in the intermembrane space. The combined action of both domains of Tim23 thus explains how presequence recognition at the inner membrane is efficiently coupled to channeling of the preprotein across the membrane [68].

The exact function of Tim17 is unknown. Due to its homology with Tim23, it may be expected that it forms a channel by itself. Since Tim17 lacks the amino-terminal presequence-binding domain, a putative Tim17 channel probably has a lower presequence specificity. We speculate that Tim17 could be involved in the translocation of special preproteins across or into the inner membrane (Fig. 4).

4. Perspectives

A major aim will be to obtain detailed structural information on the presequence-sensitive channels of the mitochondrial outer and inner membranes. How can the leakage

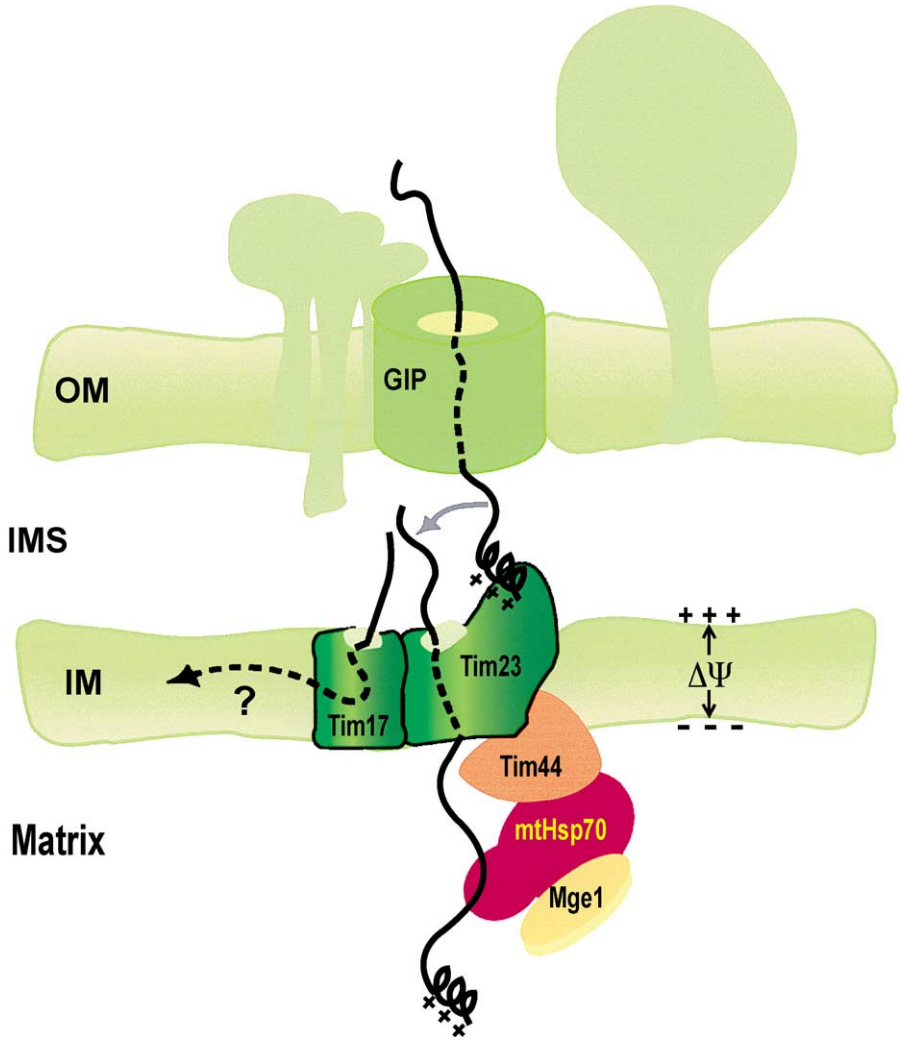

Fig. 4. The presequence translocase of the inner mitochondrial membrane (TIM). The presequence translocase (TIM23 complex) of the inner membrane (IM) contains (at least) two integral membrane proteins, Tim23 and Tim17, and a peripheral motor system (Tim44, mtHsp70 and the co-chaperone Mge1). After translocation through the general import pore (GIP) of the outer membrane (OM), the presequences cross the intermembrane space (IMS) and are recognized by the amino-terminal domain of Tim23. The channel(s) across the inner membrane are formed by Tim23 and possibly also by Tim17. The membrane potential ($\Delta\psi$) across the inner membrane activates the Tim23 channel and exerts an electrophoretic effect on the positively charged presequences. The TIM23 complex is probably present as dimer.

of other molecules be prevented during the translocation of preproteins? This problem is of particular importance for the TIM channel since a major leakage of ions would lead to a dissipation of the membrane potential and thus block further protein import.

The studies with the TOM machinery indicate that the import pore complex is not simply a static channel, but possesses dynamic properties. Similar properties may also apply to the TIM23 complex since larger complexes containing Tim23 and Tim17 have been observed besides the 90 K core complex [61,63]. Which functions of the import machineries depend on the dynamic properties? This may include the assembly of the translocases and the sorting of preproteins into the membranes. It is conceivable that the translocation channels may open laterally to release preproteins into the lipid phase of the membranes.

The energetics of preprotein translocation across the outer membrane is only partly understood. While the chain of binding sites will be able to guide the translocation of a preprotein across the membrane, it does not provide the energy to render the net translocation directional. A major driving force is surely the coupling to the presequence translocase of the inner membrane once the presequence can sense the membrane potential. However, proteins of the outer membrane and some intermembrane space proteins are imported independently of the presence or absence of a membrane potential. In these cases, the energy gained by folding of the proteins to their mature forms once they have reached their functional destination may be used to drive translocation. Additionally, it is possible that an energy-providing process operates at the outer membrane that is still unknown.

Finally, it will be of great importance to identify further channel proteins involved in mitochondrial protein import. A first candidate is Tim22 of the second translocase of the inner membrane that mediates the insertion of hydrophobic proteins such as carrier proteins [70] [see article by Jensen and Dunn in this issue]. Tim22 is homologous to Tim23 and Tim17 and those three Tim proteins share significant similarity with OEP16, an amino acid transporter of the chloroplast outer envelope [71]. Tim22 may thus form a hydrophilic channel. The Oxa1 protein of the inner membrane is involved in protein export from the matrix of mitochondria [72,73]. Oxa1 or proteins cooperating with it are possible candidates for export channels.

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References

[1] G. Schatz, B. Dobberstein, Common principles of protein translocation across membranes, Science 271 (1996) 1519–1526.
[2] W. Neupert, Protein import into mitochondria, Ann. Rev. Biochem. 66 (1997) 863–917.
[3] R.E. Jensen, A.E. Johnson, Protein translocation: is Hsp70 pulling my chain? Curr. Biol. 9 (1999) R779–R782.
[4] C.M. Koehler, Protein translocation pathways of the mitochondrion, FEBS Lett. 476 (2000) 27–31.
[5] N. Pfanner, A. Geissler, Versatility of the mitochondrial protein import machinery, Nat. Rev., Mol. Cell Biol. 2 (2001) 339–349.
[6] K.N. Truscott, N. Pfanner, W. Voos, Transport of proteins into mitochondria, Rev. Physiol., Biochem. Pharmacol. 143 (2001) 81–136.
[7] J. Ostermann, A.L. Horwich, W. Neupert, F.-U. Hartl, Protein folding in mitochondria requires complex formation with hsp60 and ATP hydrolysis, Nature 341 (1989) 125–129.
[8] J. Rassow, K. Mohrs, S. Koidl, I.B. Barthelmess, N. Pfanner, M. Tropschung, Cyclophilin 20 is involved in mitochondrial protein folding in cooperation with molecular chaperones hsp70 and hsp60, Mol. Cell. Biol. 15 (1995) 2654–2662.
[9] S. Rospert, R. Looser, Y. Dubaquie, A. Matouschek, B.S. Glick, G. Schatz, Hsp60 independent protein folding in the matrix of yeast mitochondria, EMBO J. 15 (1996) 764–774.
[10] J. Brix, S. Rüdiger, B. Bukau, J. Schneider-Mergener, N. Pfanner, Distribution of binding sequences for the mitochondrial import receptors Tom20, Tom22, and Tom70 in a presequence-carrying preprotein and a non-cleavable preprotein, J. Biol. Chem. 274 (1999) 16522–16530.
[11] N. Pfanner, M.G. Douglas, T. Endo, N.J. Hoogenraad, R.E. Jensen, M. Meijer, W. Neupert, G. Schatz, U.K. Schmitz, G.C. Shore, Uniform nomenclature for the protein transport machinery of the mitochondrial membranes, Trends Biochem. Sci. 21 (1996) 51–52.
[12] P.J.T. Dekker, M.T. Ryan, J. Brix, H. Müller, A. Hönlinger, N. Pfanner, Preprotein translocase of the outer mitochondrial membrane: molecular dissection and assembly of the general import pore complex, Mol. Cell. Biol. 18 (1998) 6515–6524.
[13] K.P. Künkele, S. Heins, M. Dembowski, F.E. Nargang, R. Benz, M. Thieffry, J. Walz, R. Lill, S. Nussberger, W. Neupert, The preprotein translocation channel of the outer membrane of mitochondria, Cell 93 (1998) 1009–1019.
[14] U. Ahting, C. Thun, R. Hegerl, D. Typke, F.E. Nargang, W. Neupert, S. Nussberger, The TOM core complex: the general protein import pore of the outer membrane of mitochondria, J. Cell Biol. 147 (1999) 959–968.
[15] S. van Wilpe, M.T. Ryan, K. Hill, A.C. Maarse, C. Meisinger, J. Brix, P.J.T. Dekker, M. Moczko, R. Wagner, M. Meijer, B. Guiard, A. Hönlinger, N. Pfanner, Tom22 is a multifunctional organizer of the mitochondrial preprotein translocase, Nature 401 (1999) 485–489.
[16] C. Meisinger, M.T. Ryan, K. Hill, K. Model, J.H. Lim, A. Sickmann, H. Müller, H.E. Meyer, R. Wagner, N. Pfanner, Protein import channel of the outer mitochondrial membrane: a highly stable Tom40–Tom22 core structure differentially interacts with preproteins, small Tom proteins, and import receptors, Mol. Cell. Biol. 21 (2001) 2337–2348.
[17] D. Vestweber, J. Brunner, A. Baker, G. Schatz, A 42 K outer-membrane protein is a component of the yeast mitochondrial protein import site, Nature 341 (1989) 205–209.
[18] K.P. Baker, A. Schaniel, D. Vestweber, G. Schatz, A yeast mitochondrial outer membrane protein essential for protein import and cell viability, Nature 348 (1990) 605–609.
[19] M. Kiebler, R. Pfaller, T. Söllner, G. Griffiths, H. Horstmann, N. Pfanner, W. Neupert, Identification of a mitochondrial receptor complex required for recognition and membrane insertion of precursor proteins, Nature 348 (1990) 610–616.
[20] K. Hill, K. Model, M.T. Ryan, K. Dietmeier, F. Martin, R. Wagner, N. Pfanner, Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins, Nature 395 (1998) 516–521.
[21] D. Rapaport, W. Neupert, R. Lill, Mitochondrial protein import. Tom40 plays a major role in targeting and translocation of preproteins by forming a specific binding site for the presequence, J. Biol. Chem. 272 (1997) 18725–18731.
[22] G. von Heijne, J. Steppuhn, R.G. Herrmann, Domain structure of mitochondrial and chloroplast targeting peptides, Eur. J. Biochem. 180 (1989) 532–545.
[23] J.F. Chich, D. Goldschmidt, M. Thieffry, J.P. Henry, A peptide sensitive channel of large conductance is localized on mitochondrial outer membrane, Eur. J. Biochem. 196 (1991) 29–35.
[24] K.P. Künkele, P. Juin, C. Pompa, F.E. Nargang, J.P. Henry, W. Neupert, R. Lill, M. Thieffry, The isolated complex of the translocase of the outer membrane of mitochondria: characterization of the cation-selective and voltage-gated preprotein-conducting pore, J. Biol. Chem. 273 (1998) 31032–31039.
[25] P. Juin, M. Thieffry, J.-P. Henry, F.M. Valette, Relationship between peptide-sensitive channel and the mitochondrial outer membrane protein translocation machinery, J. Biol. Chem. 272 (1997) 6044–6050.
[26] C.A. Mannella, A.F. Neuwald, C.E. Lawrence, Detection of likely transmembrane β-strand regions in sequence of mitochondrial pore proteins using the Gibbs sampler, J. Bioenerg. Biomembranes 28 (1996) 163–169.
[27] K. Gabriel, S.K. Buchanan, T. Lithgow, The alpha and the beta: protein translocation across mitochondrial and plastid outer membranes, Trends Biochem. Sci. 26 (2001) 36–40.
[28] M.S. Weiss, U. Abele, J. Weckesser, W. Welte, E. Schiltz, G.E. Schulz, Molecular architecture and electrostatic properties of a bacterial porin, Science 254 (1991) 1627–1630.
[29] M.P. Schwartz, A. Matouschek, The dimensions of the protein import channels in the outer and inner mitochondrial membranes, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 13086–13090.
[30] J. Rassow, F.-U. Hartl, B. Guiard, N. Pfanner, W. Neupert, Polypeptides traverse the mitochondrial envelope in an extended state, FEBS Lett. 275 (1990) 190–194.
[31] N. Pfanner, W. Neupert, Distinct steps in the import of ADP/ATP carrier into mitochondria, J. Biol. Chem. 262 (1987) 7528–7536.
[32] N. Pfanner, M. Tropschung, W. Neupert, Mitochondrial protein im-

port: nucleoside triphosphates are involved in conferring import-competence to precursors, Cell 49 (1987) 815–823.
[33] M. Endres, W. Neupert, M. Brunner, Transport of ADP/ATP carrier of mitochondria from the TOM complex to the TIM22•54 complex, EMBO J. 18 (1999) 3214–3221.
[34] N. Wiedeman, N. Pfanner, M.T. Ryan, The three modules of ADP/ATP carrier cooperate in receptor recruitment and translocation into mitochondria, EMBO J. 20 (2001) 951–960.
[35] D. Rapaport, K.P. Kunkele, M. Dembowski, U. Ahting, F.E. Nargang, W. Neupert, R. Lill, Dynamics of the TOM complex of mitochondria during binding and translocation of preproteins, Mol. Cell. Biol. 18 (1998) 5256–5262.
[36] K. Model, C. Meisinger, T. Prinz, N. Wiedeman, K.N. Truscott, N. Pfanner, M.T. Ryan, Multistep assembly of the preprotein import channel of the mitochondrial outer membrane, Nat. Struct. Biol. 8 (2001) 361–370.
[37] K. Dietmeier, A. Hönlinger, U. Bömer, P.J.T. Dekker, C. Eckerskorn, F. Lottspeich, M. Kübrich, N. Pfanner, Tom5 functionally links mitochondrial preprotein receptors to the general import pore, Nature 388 (1997) 195–200.
[38] M. Kurz, H. Martin, J. Rassow, N. Pfanner, M.T. Ryan, Biogenesis of Tim proteins of the mitochondrial carrier import pathway: differential targeting mechanisms and crossing-over with the main import pathway, Mol. Biol. Cell 10 (1999) 2461–2474.
[39] L. Bolliger, T. Junne, G. Schatz, T. Lithgow, Acidic receptor domains on both sides of the outer membrane mediate translocation of precursor proteins into yeast mitochondria, EMBO J. 14 (1995) 6318–6326.
[40] A. Mayer, W. Neupert, R. Lill, Mitochondrial protein import: reversible binding of the presequence at the trans side of the outer membrane drives partial translocation and unfolding, Cell 80 (1995) 127–137.
[41] T. Kanamori, S. Nishikawa, I. Shin, P.G. Schultz, T. Endo, Probing the environment along the protein import pathway in yeast mitochondria by site-specific photocrosslinking, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 485–490.
[42] M. Moczko, U. Bömer, M. Kübrich, N. Zufall, A. Hönlinger, N. Pfanner, The intermembrane space domain of mitochondrial Tom22 functions as a trans binding site for preproteins with N-terminal targeting sequence, Mol. Cell. Biol. 17 (1997) 6574–6584.
[43] T. Komiya, S. Rospert, C. Koehler, R. Looser, G. Schatz, K. Mihara, Interaction of mitochondrial targeting signals with acidic receptor domains along the protein import pathway: evidence for the "acid chain" hypothesis, EMBO J. 17 (1998) 3886–3898.
[44] A. Hönlinger, M. Kübrich, M. Moczko, F. Gärtner, L. Mallet, F. Bussereau, C. Eckerskorn, F. Lottspeich, K. Dietmeier, M. Jacquet, N. Pfanner, The mitochondrial receptor complex: Mom22 is essential for cell viability and directly interacts with preprotein, Mol. Cell. Biol. 15 (1995) 3382–3389.
[45] G. Schatz, Just follow the acid chain, Nature 388 (1997) 121–122.
[46] Y. Abe, T. Shodai, T. Muto, K. Mihara, H. Torii, S. Nishikawa, T. Endo, D. Kohda, Structural basis of presequence recognition by the mitochondrial protein import receptor Tom20, Cell 100 (2000) 551–560.
[47] M. Yano, N. Hoogenraad, K. Terada, M. Mori, Identification and functional analysis of human Tom22 for protein import into mitochondria, Mol. Cell. Biol. 20 (2000) 7205–7213.
[48] P. Keil, A. Weinzierl, M. Kiebler, K. Dietmeier, T. Sollner, N. Pfanner, Biogenesis of the mitochondrial receptor complex: two receptors are required for binding of MOM38 to the outer membrane surface, J. Biol. Chem. 268 (1993) 19177–19180.
[49] D. Rapaport, W. Neupert, Biogenesis of Tom40, core component of the TOM complex of mitochondria, J. Cell Biol. 146 (1999) 321–331.
[50] A. Hönlinger, U. Bömer, A. Alconada, C. Eckerskorn, F. Lottspeich, K. Dietmeier, N. Pfanner, Tom7 modulates the dynamics of the mitochondrial outer membrane translocase and plays a pathway-related role in protein import, EMBO J. 15 (1996) 2125–2137.
[51] D. Rapaport, R.D. Taylor, M. Kaser, T. Langer, W. Neupert, F.E. Nargang, Structural requirements of Tom40 for assembly into preexisting TOM complexes of mitochondria, Mol. Biol. Cell 12 (2001) 1189–1198.
[52] D.M. Gordon, J. Wang, B. Amutha, D. Pain, Self-association and precursor binding of *Saccharomyces cerevisiae* Tom40p, the core component of the protein translocation channel of the mitochondrial outer membrane, Biochem. J. 356 (2001) 207–215.
[53] J.L.T. Emtage, R.E. Jensen, MAS6 encodes an essential inner membrane component of the yeast mitochondrial protein import pathway, J. Cell Biol. 122 (1993) 1003–1012.
[54] P.J.T. Dekker, P. Keil, J. Rassow, A.C. Maarse, N. Pfanner, M. Meijer, Identification of MIM23, a putative component of the protein import machinery of the mitochondrial inner membrane, FEBS Lett. 330 (1993) 66–70.
[55] K.R. Ryan, M.M. Menold, S. Garret, R.E. Jensen, SMS1, a high-copy suppressor of the yeast mas6 mutant, encodes an essential inner membrane protein required for mitochondrial protein import, Mol. Biol. Cell 5 (1994) 529–538.
[56] A.C. Maarse, J. Blom, P. Keil, N. Pfanner, M. Meijer, Identification of the essential yeast protein MIM17, an integral mitochondral inner membrane protein involved in protein import, FEBS Lett. 394 (1994) 215–221.
[57] K.R. Ryan, R.E. Jensen, Mas6 can be cross-linked to an arrested precursor and interacts with other proteins during mitochondrial protein import, J. Biol. Chem. 268 (1993) 23743–23746.
[58] M. Kübrich, P. Keil, J. Rassow, P.J.T. Dekker, J. Blom, M. Meijer, N. Pfanner, The polytopic mitochondrial inner membrane proteins MIM17 and MIM23 operate at the same preprotein import site, FEBS Lett. 349 (1994) 222–228.
[59] J. Blom, P.J.T. Dekker, M. Meijer, Functional and physical interactions of components of the yeast mitochondrial inner membrane import machinery (MIM), Eur. J. Biochem. 232 (1995) 309–314.
[60] J. Berthold, M.F. Bauer, H.-C. Schneider, C. Klaus, K. Dietmeier, W. Neupert, M. Brunner, The MIM complex mediates preprotein translocation across the mitochondrial inner membrane and couples it to the mt-Hsp70/ATP driven system, Cell 81 (1995) 1085–1093.
[61] P.J.T. Dekker, F. Martin, A.C. Maarse, U. Bömer, H. Müller, B. Guiard, M. Meijer, J. Rassow, N. Pfanner, The Tim core complex defines the number of mitochondrial translocation contact sites and can hold arrested preproteins in the absence of matrix Hsp70–Tim44, EMBO J. 16 (1997) 5408–5419.
[62] K.R. Ryan, R.S. Leung, R.E. Jensen, Characterization of the mitochondrial inner membrane translocase complex: the Tim23p hydrophobic domain interacts with Tim17p but not with other Tim23p molecules, Mol. Cell. Biol. 18 (1998) 178–187.
[63] F. Moro, C. Sirrenberg, H.C. Schneider, W. Neupert, M. Brunner, The TIM17-23 preprotein translocase in mitochondria: composition and function in protein transport into the matrix, EMBO J. 18 (1999) 3667–3675.
[64] M.F. Bauer, C. Sirrenberg, W. Neupert, M. Brunner, Role of Tim23 as a voltage sensor and presequence receptor in protein import into mitochondria, Cell 87 (1996) 33–41.
[65] T.A. Lohret, K.W. Kinally, Targeting peptides transiently block a mitochondrial channel, J. Biol. Chem. 270 (1995) 15950–15953.
[66] K.W. Kinally, T.A. Lohret, M.L. Campo, C.A. Mannella, Perspectives on the mitochondrial multiple conductance channel, J. Bioenerg. Biomembranes 28 (1996) 115–123.
[67] T.A. Lohret, R.E. Jensen, K.W. Kinally, Tim23, a protein import component of the mitochondrial inner membrane is required for normal activity of the multiple conductance channel, MCC, J. Cell Biol. 137 (1997) 377–386.
[68] K.N. Truscott, P. Kovermann, A. Geissler, A. Merlin, M. Meijer, A.J.M. Driessen, J. Rassow, N. Pfanner, R. Wagner, A presequence- and voltage-sensitive channel of the mitochondrial preprotein translocase formed by Tim23, Nat. Struct. Biol. 8 (2001) 674–682.
[69] J. Martin, K. Mahlke, N. Pfanner, Role of an energized inner membrane in mitochondrial protein import: $\Delta\Psi$ drives the movement of presequences, J. Biol. Chem. 266 (1991) 18051–18057.

[70] C. Sirrenberg, M.F. Bauer, B. Guiard, W. Neupert, M. Brunner, Import of carrier proteins into the mitochondrial inner membrane mediated by Tim22, Nature 384 (1996) 582–585.
[71] J. Rassow, P.J.T. Dekker, S. van Wilpe, M. Meijer, J. Soll, The preprotein translocase of the mitochondrial inner membrane: function and evolution, J. Mol. Biol. 286 (1999) 105–120.
[72] S. He, T.D. Fox, Membrane translocation of mitochondrially coded Cox2p: distinct requirements for export of N- and C-termini and dependence on the conserved protein Oxa1p, Mol. Biol. Cell 8 (1997) 1449–1460.
[73] K. Hell, W. Neupert, R.A. Stuart, Oxa1p acts as a general membrane insertion machinery for proteins encoded by mitochondrial DNA, EMBO J. 20 (2001) 1281–1288.