Protein transport into mitochondria

Johannes M Herrmann and Walter Neupert*

Mitochondria are made up of two membrane systems that subdivide this organelle into two aqueous subcompartments: the matrix, which is enclosed by the inner membrane, and the intermembrane space, which is located between the inner and the outer membrane. Protein import into mitochondria is a complex reaction, as every protein has to be routed to its specific destination within the organelle. In the past few years, studies with mitochondria of *Neurospora crassa* and *Saccharomyces cerevisiae* have led to the identification of four distinct translocation machineries that are conserved among eukaryotes. These translocases, in a concerted fashion, mediate import and sorting of proteins into the mitochondrial subcompartments.

Addresses
Adolf-Butenandt-Institut für Physiologische Chemie, Goethestrasse 33, 80336 München, Germany
*e-mail: neupert@bio.med.uni-muenchen.de

Current Opinion in Microbiology 2000, **3**:210–214

1369-5274/00/$ – see front matter

Abbreviations

MSF	mitochondrial import stimulating factor
MTS	matrix-targeting signal
TIM	translocase of the inner membrane
TOM	translocase of the outer membrane

Introduction

Eukaryotic cells are subdivided into different compartments that play unique roles in growth and cellular metabolism, and contain specific collections of enzymes that catalyse requisite chemical reactions. Since nearly all proteins are synthesised in the cytoplasm, complex protein targeting systems exist that deliver every single polypeptide to its specific destination. Typically, these targeting systems comprise three components. First, signals on the proteins to be sorted that address it to the target compartment. Second, chaperoning factors that ensure a loosely folded, transport-competent conformation and the accessibility of the signals. Third, membrane localised translocation machineries that are composed of two elements: receptors that specifically recognise the pertinent polypeptides, and channels that promote preprotein insertion into or translocation across lipid bilayers. These translocation machineries are not just pore-forming structures but rather active devices that have to mediate uni-directional translocation by coupling transport to exergonic reactions such as ATP hydrolysis or protein folding.

Here, we report on recent advances in the understanding on the process of protein transport to mitochondria. For more detailed reviews, see [1–3].

The main road: protein transport to the mitochondrial matrix

Mitochondrial targeting signals

Proteins destined to be imported into mitochondria (preproteins) are typically synthesised as precursors carrying amino-terminal extensions. These presequences are both necessary and sufficient for mitochondrial targeting [4] and usually are proteolytically removed following translocation into the matrix. These 'matrix-targeting signals' (MTSs) comprise some 20 to 60 amino acid residues that have the potential to form amphiphilic α-helices with one hydrophobic and one positively charged face [5,6]. The extent of helicity, hydrophobicity and positive charge varies significantly among different presequences and may have to be balanced *in vivo* to minimise nonspecific membrane binding [7]. Although MTSs are usually found at the amino terminus of preproteins, other arrangements have been described recently: the mitochondrial DNA-helicase Hmi1 has a cleavable carboxy-terminal MTS, which mediates import in a reverse carboxy- to amino-terminal direction [8•]. Alternatively, a segment of positively charged amino acid residues located directly carboxy-terminal of a single transmembrane domain can form a hairpin-like structure that mimics a typical amphiphilic presequence and functions as an internal MTS [9]. Depending on the position of the MTS, preproteins are thought to enter mitochondria either in a linear fashion (where the amino or the carboxyl terminus can be first) or as a loop (where the internal MTS kink would be first). A number of mitochondrial preproteins are lacking MTS-like sequences and harbour largely unidentified signals within their sequences.

During or following synthesis in the cytosol, mitochondrial preproteins are bound by chaperones that maintain them in a loosely folded translocation-competent conformation. These chaperones include members of the Hsp70 family as well as specific factors, such as the mitochondrial import stimulating factor (MSF), that recognise mitochondrial presequences [10–12]. At the level of the mitochondrial surface, preproteins are recognised by receptors that are part of the TOM complex (for translocase of the outer membrane).

The translocase of the outer membrane: the TOM complex

The TOM complex is a multisubunit translocation machinery. It comprises an array of import receptors and a protein-conducting channel. The receptors appear to guide the preproteins to the channel by sequential interactions with negatively charged binding sites of increasing affinities [13,14•].

The recent purification of the TOM complex from *Neurospora crassa* and analysis by blue native gel electrophoresis of detergent solubilised mitochondrial

membranes of yeast have provided insights in the composition, structure and channel functions of the TOM complex [15••,16•,17]. After isolation from *Neurospora* mitochondria, a holo complex of about 490 kDa can be dissociated into the receptors Tom20 and Tom70, and the core complex (or 'general insertion pore') consisting of Tom40, Tom22, Tom7 and Tom6. The four latter subunits form a structure of 12 nm × 7 nm (diameter in the membrane plane) × 7 nm (height). Two pores of about 2.1 nm diameter are most likely to represent the protein-conducting channels. Tom40 is predicted to traverse the outer membrane as a series of β strands [18]. It is the only essential TOM subunit in yeast and is most likely to be the main element of the translocation pore [19•,20]. All other TOM subunits are predicted to traverse the outer membrane by helical transmembrane segments. In *Saccharomyces cerevisiae*, deletion of Tom22 leads to subcomplexes comprising Tom40, Tom7, Tom6, and Tom5 (for which no homologue has been found so far in *N. crassa*). These subcomplexes are still functional in protein import, although, in this case, recruitment of preproteins to the translocation pore might not be mediated by receptor components [20]. Thus, Tom22 might form a link from the general insertion pore to the receptor components Tom20 and Tom70. However, the finding that newly imported Tom40 subunits can integrate into pre-existing TOM complexes [21], and the different crosslinking behaviour of Tom40 in the absence and presence of preproteins [22] favours a more flexible and dynamic architecture of the TOM complex.

Following translocation through the TOM channel, preproteins bind to the intermembrane space-exposed face of the complex presumably by specific interactions with Tom40 [23] and/or Tom22 [14•,24]. How integral outer membrane proteins leave the TOM complex and integrate into the lipid bilayer is poorly understood.

The route into the mitochondrial matrix: the TIM23 complex

After translocation into the intermembrane space, preproteins interact with the TIM23 complex. This translocase of the inner membrane (TIM) consists of Tim17, Tim23, Tim44 and presumably an as yet unidentified 14 kDa subunit. The integral membrane proteins Tim23 and Tim17 are related in their sequence and form the protein-conducting channel of the inner membrane [25,26]. The hydrophilic matrix protein Tim44 is associated both with Tim23/Tim17 [25,27] and with lipids of the inner membrane [28]. These three subunits seem to be present in a 2:2:2 ratio but hardly anything is known on the structure of the complex [29]. Tim23 of yeast forms dimers in the presence of a membrane potential [30]. Dimerisation is mediated by leucine zippers in the amino-terminal domain of Tim23. Binding of preproteins causes dissociation of the Tim23 dimer and initiates translocation of the preprotein across the inner membrane. In addition to dimerisation of the TIM23 complex, the membrane potential may generate an electrophoretic driving force to translocate the typically positively charged presequences into the matrix. In the matrix, the incoming preprotein is bound by the chaperone mt-Hsp70, which is associated with Tim44 in an ATP-dependent manner. It has been suggested that Tim44 is not an essential structural element of the translocation pore, but is crucial for efficient interaction of the incoming polypeptide with mt-Hsp70 [31]. Upon preprotein binding to mt-Hsp70, ATP-hydrolysis occurs, leading to a tight association of mt-Hsp70 to the preprotein and to the dissociation of mt-Hsp70 from Tim44 [32]. The nucleotide exchange factor Mge1 facilitates the release of the nucleotide from mt-Hsp70 [33]. Two mt-Hsp70 molecules are attached to the Tim44 dimer. This is proposed to allow a 'hand-over-hand'-like binding mode of mt-Hsp70 to the incoming polypeptide and to efficiently prevent the backsliding of the preprotein [29].

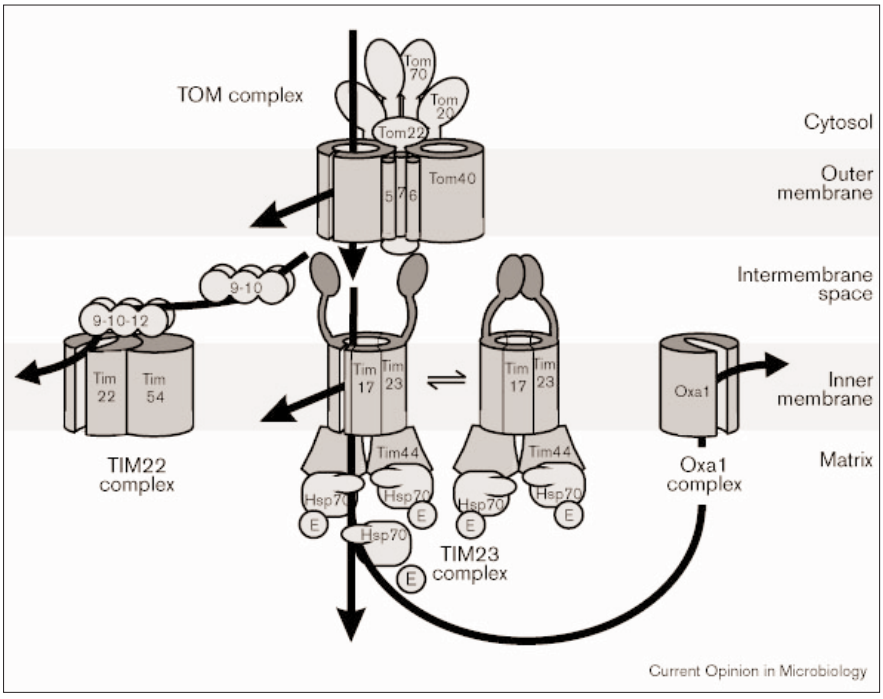
The question how mt-Hsp70 generates the translocation force for protein import has been addressed in several studies, and two alternative models have been suggested. mt-Hsp70 functions as a ratchet that transforms the bidirectional movement of the preprotein resulting from Brownian motion into an uni-directional translocation into the matrix. According to the 'Brownian ratchet hypothesis' this mechanism is sufficient to import even folded proteins into mitochondria. The increased ATP-requirement observed for folded preproteins is thought to be due to the need of a smaller step size of the ratchet under these conditions [34]. Alternatively, a conformational change in the mt-Hsp70 molecule has been proposed to generate a power stroke that would pull the preprotein across the inner membrane [35,36]. However, there is no direct evidence for a pulling function of mt-Hsp70. As the preproteins reach the mitochondrial matrix, mt-Hsp70 and other chaperones mediate their folding and assembly (for review see [2]).

Different roads lead to the same destination: protein sorting to the intermembrane space and the inner membrane

Although the intermembrane space contains only few proteins, several pathways into this subcompartment have been described. Most intermembrane proteins do not contain classic MTSs and require only the TOM complex to reach the intermembrane space. However, some examples, such as cytochrome b_2, contain amino-terminal targeting signals. Import of these proteins depends also on the TIM23 complex. For detailed discussion see [37].

In the past few years, three different pathways have been described that lead to a localisation of proteins in the inner membrane. First, proteins can be imported as described above for matrix proteins but become arrested at the level of the TIM23 complex and are laterally inserted into the lipid bilayer. This 'stop-transfer mechanism' [38,39] seems to be typical for monotopic membrane proteins whose amino terminus faces the matrix [40,41]. At least for one of these proteins, D-lactate dehydrogenase, a cluster of

Figure 1



The four translocation machineries identified in mitochondria. The receptors, Tom70 and Tom20, of the TOM complex recognise MTSs on proteins targeted to the mitochondria and guide the preproteins to the core complex (Tom40, Tom22, Tom7, Tom6 and Tom5). Once translocated into the innermembrane space, the preproteins targeted for the matrix interact with the TIM23 complex, causing the dissociation of Tim23 dimer and translocation of the preprotein across the inner membrane. In the matrix, the prepeptide is bound by the mt-Hsp70 chaperone which becomes dissociated from Tim44. Preproteins can be inserted into the inner membrane by several different pathways. First, preprotein translocation across the inner membrane becomes arrested in the TIM23 complex and the preprotein is inserted in the lipid bilayer. Second, preproteins in the matrix can be reinserted into the inner membrane by the Oxa1 complex. Third, after translocation through the TOM complex, a Tim9/Tim10 soluble complex binds preproteins and transfers them to the TIM22 complex (Tim9/Tim10/Tim12/Tim22/Tim54), which mediates the insertion of the preproteins into the inner membrane. E, Mge1.

charges located directly carboxy-terminal of the transmembrane segment is essential for translocation arrest and/or membrane integration [41]. However, the process by which preproteins leave the TIM23 channel and insert into the membrane is poorly understood.

Secondly, proteins can be completely transported into the matrix from where they reinsert into the inner membrane. Thus, the domains of these proteins that are exposed in the intermembrane space have to traverse the inner membrane twice. This reinsertion process resembles the membrane insertion of mitochondrially encoded proteins. Signals and machinery mediating this process seem to be conserved from the bacterial progenitors of mitochondria. This route was therefore named the 'conservative sorting' pathway [42]. Examples include the mono- and polytopic membrane proteins whose amino termini face the intermembrane space [41,43,44]. To date the inner membrane protein Oxa1 is the only identified component of this insertion complex. In addition, homologs of the general protein translocation machinery of bacterial inner membrane, the Sec complex (see review by Bernstein, this issue, pp 203–209), seem to be present in mitochondria of some primitive eukaryotes [45]. Oxa1 is conserved from bacteria to chloroplasts and mitochondria, but only little is known about the function and structure of this translocation machinery [46,47,48••]. As far as known no soluble protein is exported across the inner membrane by the Oxa1 complex. However, this translocase is able to facilitate the export of hydrophilic protein domains of considerable length. In one example, even the fusion of the 45 kDa protein Arg8 to the intermembrane space domain of cytochrome oxidase subunit 2 led to an efficient export of this 61 kDa sequence across the inner membrane in an Oxa1-dependent manner [49]. Thus, the Oxa1 machinery is not simply an insertion facilitator for hydrophobic segments but has the capacity to mediate the penetration of long and hydrophilic polypeptides across the inner membrane.

A third pathway into the inner membrane was discovered only recently. This import route is used by polytopic membrane proteins that do not contain classic MTSs but internal targeting signals [50–52]. Members of the solute carrier family such as the ATP/ADP carrier (AAC) belong to this group. These proteins are recognised on the surface of mitochondria by the Tom70 receptor before they are handed over to Tom20/Tom22 and the TOM channel. As the precursors are partially translocated through the TOM channel, they are pulled into the intermembrane space by binding to a soluble 70 kDa complex composed of Tim9 and Tim10. Then they are transferred to the 300 kDa TIM22 complex, which consists of the peripheral Tim9/Tim10/Tim12 complex and the integral inner membrane proteins Tim22 and Tim54 [53••,54–56,57••]. In the presence of a membrane potential, the TIM22 complex mediates the insertion of the preproteins into the inner membrane [56]. Recently, a second soluble intermembrane space complex formed by Tim8 and Tim13 was described which also seems to be involved in mitochondrial protein import. The exact function of Tim8 and Tim13, however, is unknown [58,59]. Both soluble intermembrane space complexes might play a chaperone-like function and prevent misfolding and aggregation of hydrophobic inner

membrane proteins as they cross the intermembrane space. In addition, they might act as trapping devices that support uni-directional protein translocation across the outer membrane in a ratchet-like mechanism analogous to the role of mt-Hsp70 for protein translocation into the matrix.

Conclusions

In summary, four protein translocation machineries have been described in mitochondria: the TOM, the TIM23, the TIM22 and the Oxa1 complexes (see Figure 1). They mediate sorting and transport of preproteins into the mitochondrial subcompartments in a concerted action. There is no evidence for a direct physical interaction of these complexes. However, a functional and kinetic coupling is likely for the translocation of preproteins across TOM and TIM complexes since TIM subunits and mt-Hsp70 provide the energy for efficient preprotein translocation across the TOM channel. In addition, it was reported recently that import of the hydrophobic protein Coq2 is affected by both Tim10 and Tim23 mutations and might therefore require components of both TIM complexes [59]. Subunits of these translocation complexes do not show any significant homology to prokaryotic proteins and obviously originate after the uptake of mitochondrial ancestors by eukaryotic host cells. In contrast, the Oxa1 translocase appears to act independently of other translocases and represents a phylogenetically old translocation system that mitochondria inherited from their prokaryotic progenitors.

Update

It has recently been shown that the *E. coli* homologue of Oxa1 (now called YidC) cooperates with the Sec machinery and is involved in insertion of hydrophobic proteins into membranes [60].

Acknowledgement

We thank B Westermann for critical comments on the manuscript.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Schatz G: **The protein import system of mitochondria.** *J Biol Chem* 1996, **50**:31763-31766.

2. Neupert W: **Protein import into mitochondria.** *Annu Rev Biochem* 1997, **66**:863-917.

3. Pfanner N, Craig EA, Hönlinger A: **Mitochondrial preprotein translocase.** *Annu Rev Dev Biol* 1997, **13**:25-51.

4. Hurt EC, Pesold-Hurt B, Schatz G: **The aminoterminal region of an imported mitochondrial precursor polypeptide can direct cytoplasmic dihydrofolate reductase into the mitochondrial matrix.** *EMBO J* 1984, **3**:3149-3156.

5. Von Heijne G: **Mitochondrial targeting sequences may form amphiphilic helices.** *EMBO J* 1986, **5**:1335-1342.

6. Roise D, Theiler F, Horvath SJ, Tomich JM, Richards JH, Allison DS, Schatz G: **Amphiphilicity is essential for mitochondrial presequence function.** *EMBO J* 1988, **7**:649-653.

7. Ni L, Heard TS, Weiner H: ***In vivo* mitochondrial import.** *J Biol Chem* 1999, **274**:12685-12691.

8. • Lee CM, Sedman J, Neupert W, Stuart RA: **The DNA helicase, Hmi1p, is transported into mitochondria by a C-terminal cleavable targeting signal.** *J Biol Chem* 1999, **274**:20937-20942.

This is the first report demonstrating a cleavable carboxy-terminal mitochondrial-targeting signal on an authentic precursor protein.

9. Fölsch H, Guiard B, Neupert W, Stuart RA: **Internal targeting signal of the BCS1 protein: a novel mechanism of import into mitochondria.** *EMBO J* 1996, **15**:479-487.

10. Deshaies RJ, Koch BD, Werner-Washburne M, Craig EA, Schekman R: **A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides.** *Nature* 1988, **332**:800-805.

11. Murakami H, Pain D, Blobel G: **70-kD heat shock-related protein is one of at least two distinct cytosolic factors stimulating protein import into mitochondria.** *J Cell Biol* 1988, **107**:2051-2057.

12. Hachiya N, Mihara K, Suda K, Horst M, Schatz G, Lithgow T: **Reconstitution of the initial steps of mitochondrial protein import.** *Nature* 1995, **376**:705-709.

13. Mayer A, Neupert W, Lill R: **Mitochondrial protein import: reversible binding of the presequence at the trans side of the outer membrane drives partial translocation and unfolding.** *Cell* 1995, **80**:127-137.

14. • Komiya T, Rospert S, Koehler C, Looser R, Schatz G, Mihara K: **Interaction of mitochondrial targeting signals with acidic receptor domains along the protein import pathway: evidence for the 'acid chain' hypothesis.** *EMBO J* 1998, **17**:3886-3898.

By *in vitro* binding experiments, the authors show that components of the TOM complex differ in their affinities for mitochondrial-targeting sequences. On the basis of this observation, the authors propose the 'acid chain' hypothesis, according to which preproteins interact in a sequential order with binding sites of increasing affinity which leads to a translocation of the preproteins through the TOM channel.

15. •• Künkele K-P, Heins S, Dembowski M, Nargang FE, Benz R, Thieffry M, Walz J, Lill R, Nussberger S, Neupert W: **The preprotein translocation channel of the outer membrane of mitochondria.** *Cell* 1998, **93**:1009-1019.

The authors purify the TOM complex from *Neurospora crassa*. After reconstitution into liposomes, this translocase mediates insertion of proteins into and tranlocation across the lipid bilayer. Electron microscopy and particle analysis reveals insights in the stucture and architecture of the translocase of the mitochondrial outer membrane.

16. • Ahting U, Thun C, Hegerl R, Typke D, Nargang FE, Neupert W, Nussberger S: **The TOM core complex: the general protein import pore of the outer membrane of mitochonria.** *J Cell Biol* 1999, **147**:959-968

In this study the authors report on the isolation and the structure of the TOM core complex, which has all characteristics of the general insertion pore. Electron tomography exhibits two open pores traversing these core complexes.

17. Dekker PJ, Ryan MT, Brix J, Müller H, Hönlinger A, Pfanner N: **Preprotein translocase of the outer mitochondrial membrane: molecular dissection and assembly of the general import pore complex.** *Mol Cell Biol* 1998, **18**:6515-6524.

18. Mannella C, Neuwald A, Lawrence C: **Detection of likely beta-strand regions in sequences of mitochondrial pore proteins using the Gibbs sampler.** *J Bioenerg Biomembr* 1996, **28**:163-169.

19. • Hill K, Model K, Ryan MT, Dietmeier K, Martin F, Wagner R, Pfanner N: **Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins.** *Nature* 1998, **395**:516-521.

The authors over-express Tom40 in bacteria and reconstitute it into liposomes leading to a pore forming complex that mimics the TOM machinery in several characteristics.

20. van Wilpe S, Ryan MT, Hill K, Maarse AC, Meisinger C, Brix J, Dekker JT, Moczko M, Wagner R, Meijer M, Guiard B, Hönlinger A, Pfanner N: **Tom22 is a multifunctional organizer of the mitochondrial preprotein translocase.** *Nature* 1999, **401**:485-489.

21. Rapaport D, Neupert W: **Biogenesis of Tom40, core component of the TOM complex in mitochondria.** *J Cell Biol* 1999, **146**:321-331.

22. Rapaport D, Künkele K-P, Dembowski M, Ahting U, Nargang F, Neupert W, Lill R: **Dynamics of the TOM complex of mitochondria during binding and translocation of preproteins.** *Mol Cell Biol* 1998, **18**:5256-5262.

23. Rapaport D, Neupert W, Lill R: **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* 1997, **272**:18725-18731.

24. Bolliger L, Junne T, Schatz G, Lithgow T: **Acidic receptor domains on both sides of the outer membrane mediate translocation of precursor proteins into yeast mitochondria.** *EMBO J* 1995, **14**:6318-6326.

25. Berthold J, Bauer MF, Schneider H-C, Klaus C, Dietmeier K, Neupert W, Brunner M: **The MIM complex mediates preprotein translocation across the mitochondrial inner membrane and couples it to the mt-Hsp70/ATP driving system.** *Cell* 1995, **81**:1085-1093.

26. Ryan KR, Leung RS, Jensen RJ: **Characterisation of the mitochondrial inner membrane translocase complex: the Tim23p hydrophobic domain interacts with Tim17p but not with other Tim23p molecules.** *Mol Cell Biol* 1998, **18**:178-187.

27. Maarse AC, Blom J, Grivell LA, Meijer M: **MPI1, an essential gene encoding a mitochondrial membrane protein, is possibly involved in protein import into yeast mitochondria.** *EMBO J* 1992, **11**:3619-3628.

28. Weiss C, Oppliger W, Vergères G, Demel R, Jenö P, Horst M, de Kruijff B, Schatz G, Azem A: **Domain structure and lipid interaction of recombinant yeast Tim44.** *Proc Natl Acad Sci USA* 1999, **96**:8890-8894.

29. Moro F, Sirrenberg C, Schneider H-C, Neupert W, Brunner M: **The TIM17·23 preprotein translocase of mitochondria: composition and function in protein transport of mitochondria.** *EMBO J* 1999, **18**:3667-3675.

30. Bauer MF, Sirrenberg C, Neupert W, Brunner M: **Role of Tim23 as voltage sensor and presequence receptor in protein import into mitochondria.** *Cell* 1996, **87**:33-41.

31. Bömer U, Maarse AC, Martin F, Geissler A, Merlin A, Schönfisch B, Meijer M, Pfanner N, Rassow J: **Separation of structural and dynamic functions of the mitochondrial translocase: Tim44 is crucial for the inner membrane import sites in translocation of tightly folded domains, but not of loosely folded preproteins.** *EMBO J* 1998, **17**:4226-4237.

32. Schneider H-C, Westermann B, Neupert W, Brunner M: **The nucleotide exchange factor MGE exerts a key function in the ATP-dependent cycle of mt-Hsp70-Tim44 interaction driving mitochondrial protein import.** *EMBO J* 1996, **15**:5796-5803.

33. Westermann B, Prip-Buus C, Neupert W, Schwarz E: **The role of the GrpE homologue, Mge1p, in mediating protein import and folding in mitochondria.** *EMBO J* 1995, **14**:3452-3460.

34. Schneider H-C, Berthold J, Bauer MF, Dietmeier K, Guiard B, Brunner M, Neupert W: **Mitochondrial Hsp70/MIM44 complex facilitates protein import.** *Nature* 1994, **371**:768-774.

35. Glick BS: **Can Hsp70 proteins act as force-generating motors?** *Cell* 1995, **80**:11-14.

36. Voisine C, Craig EA, Zufall N, von Ahsen O, Pfanner N, Voos W: **The protein import motor of mitochondria: unfolding and trapping of preproteins are distinct and separable functions of matrix Hsp70.** *Cell* 1999, **97**:565-574.

37. Stuart RA, Fölsch H, Gruhler A, Neupert W: **Sorting of proteins to the mitochondrial intermembrane space.** In *Advances Molecular Cell Biology.* Edited by Hartl FU. London: Jai-Press; 1996:193-220.

38. Kaput J, Goltz S, Blobel G: **Nucleotide sequence of the yeast nuclear gene for cytochrome c peroxidase precursor. Functional implications of the presequence for protein transport into mitochondria.** *J Biol Chem* 1982, **257**:15054-15058.

39. Van Loon AP, Schatz G: **Transport of proteins to the inner membrane: the "sorting" domain of the cytochrome c1 presequence is a stop-transfer sequence for the mitochondrial inner membrane.** *EMBO J* 1987, **6**:2441-2448.

40. Miller BR, Cumsky MG: **An unusual mitochondrial import pathway for the precursor to yeast cytochrome c oxidase subunit Va.** *J Cell Biol* 1991, **112**:833-841.

41. Rojo EE, Guiard B, Neupert W, Stuart RA: **Sorting of D-lactate dehydrogenase to the inner membrane of mitochondria: analysis of topogenic signal and energetic requirements.** *J Biol Chem* 1998, **273**:8040-8047.

42. Hartl FU, Schmidt B, Wachter E, Weiss H, Neupert W: **Transport into mitochondria and intramitochondrial sorting of the Fe/S protein of ubiquinol-cytochrome c reductase.** *Cell* 1986, **47**:939-951.

43. Rojo EE, Stuart RA, Neupert W: **Conservative sorting of F_0-ATPase subunit 9: export from matrix requires delta pH across inner membrane and matrix ATP.** *EMBO J* 1995, **14**:3445-3451.

44. Herrmann JM, Neupert W, Stuart RA: **Insertion into the mitochondrial inner membrane of a polytopic protein, the nuclear encoded Oxa1p.** *EMBO J* 1997, **16**:2217-2226.

45. Lang BF, Burger G, O'Kelly CJ, Cedergren R, Golding GB, Lenieux C, Sankoff D, Turmel M, Gray MW: **An ancestral mitochondrial DNA resembling a eubacterial genome in miniature.** *Nature* 1997, **387**:493-497.

46. Bauer M, Behrens M, Esser K, Michaelis G, Pratje E: **PET1402, a nuclear gene required for proteolytic processing of cytochrome oxidase subunit 2 in yeast.** *Mol Gen Genet* 1994, **245**:272-278.

47. Bonnefoy N, Chalvet F, Hamel P, Slominski PP, Dujardin G: ***OXA1*, a *Saccharomyces cerevisiae* nuclear gene whose sequence is conserved from prokaryotes to eukaryotes controls cytochrome oxidase biogenesis.** *J Mol Biol* 1994, **239**:201-212.

48. •• Hell K, Herrmann JM, Pratje E, Neupert W, Stuart RA: **Oxa1p, an essential component of the N-tail protein export machinery in mitochondria.** *Proc Natl Acad Sci USA* 1998, **95**:2250-2255.
This study identifies Oxa1 as a component of the protein export machinery of the inner membrane. Cross-linking and co-precipitation experiments exhibit a direct contact of Oxa1 with exported sequences.

49. He S, Fox TD: **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* 1997, **8**:1449-1460.

50. Pfanner N, Hoeben P, Tropschug M, Neupert W: **The carboxyterminal two-thirds of the ADP/ATP carrier polypeptide contains sufficient information to direct translocation into mitochondria.** *J Biol Chem* 1987, **262**:14851-14854.

51. Smagula CS, Douglas MG: **ADP/ATP carrier of *S. cerevisiae* contains a mitochondrial import signal between amino acids 72 and 111.** *J Cell Biochem* 1988, **36**:323-328.

52. Palmisano A, Zara V, Hönlinger A, Vozza A, Dekker PJ, Pfanner N, Palmieri F: **Targeting and assembly of the oxoglutarate carrier: general principles for biogenesis of carrier proteins of the mitochondrial inner membrane.** *Biochem J* 1998, **333**:151-158.

53. •• Koehler CM, Jarosch E, Tokatlidis K, Schmid K, Schweyen RJ, Schatz G: **Import of mitochondrial carrier proteins mediated by essential proteins of the intermembrane space.** *Science* 1998, **279**:369-373.
Tim10 and Tim12 are identified as intermembrane space components essential for mitochondrial import of members of the carrier family.

54. Koehler CM, Merchant S, Oppliger W, Schmid K, Jarosche E, Dolfini L, Junne T, Schatz G, Tokatlidis K: **Tim9p, an essential partner subunit of Tim10p for the import of mitochondrial carrier proteins.** *EMBO J* 1998, **17**:6477-6486.

55. Adam A, Endres M, Sirrenberg C, Lottspeich F, Neupert W, Brunner M: **Tim9, a new component of the TIM22·54 translocase in mitochondria.** *EMBO J* 1999, **18**:313-319.

56. Sirrenberg C, Bauer MF, Guiard B, Neupert W, Brunner M: **Import of carrier proteins into the mitochondrial inner membrane mediated by Tim22.** *Nature* 1996, **384**:582-585.

57. •• Sirrenberg C, Endres M, Fölsch H, Stuart RA, Neupert W, Brunner M: **Carrier protein import into mitochondria mediated by the intermembrane proteins Tim10/Mrs11p and Tim12/Mrs5p.** *Nature* 1998, **391**:912-915.
The metal-binding proteins Tim10 and Tim12 interact sequentially with carrier proteins and mediate their transport across the intermembrane space to Tom22, a component of the inner membrane insertion complex.

58. Koehler CM, Leuenberger D, Merchant S, Renaold A, Junne T, Schatz G: **Human deafness dystonia syndrome is a mitochondrial disease.** *Proc Natl Acad Sci USA* 1999, **96**:2141-2146.

59. Leuenberger D, Bally NA, Schatz G, Koehler CM: **Different import pathways through the mitochodrial intermembrane space for inner membrane proteins.** *EMBO J* 1999, **18**:4816-4822.

60. Scotti PA, Urbanus ML, Brunner J, de Gier JL, von Heijne G, van der Does C, Driessen AJM, Oudega B, Luirink J: **YidC, the *Escherichia coli* homologue of mitochondrial Oxa1p, is a component of the Sec translocase.** *EMBO J* 2000, **19**:542-549.