Transport of Proteins into Mitochondria

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Abstract

Mitochondria are essential organelles of eukaryotic cells. They consist of hundreds of different proteins that exhibit crucial activities in respiration, catabolic metabolism and the synthesis of amino acids, lipids, heme and iron-sulfur clusters. With the exception of a handful of hydrophobic mitochondrially encoded membrane proteins, all these proteins are synthesized on cytosolic ribosomes, targeted to receptors on the mitochondrial surface, and transported across or inserted into the outer and inner mitochondrial membrane before they are folded and assembled into their final native structure. This review article provides a comprehensive overview of the mechanisms and components of the mitochondrial protein import systems with a particular focus on recent developments in the field.

Keywords ER-SURF · Mitochondria · Protein import · Targeting signals

1 Introduction

In 1980, Günther Blobel published a theoretical paper on "intracellular protein topogenesis" in which he coined the fundamental terminology of protein translocation [1]. For example, he defined four different classes of topogenic signals as determinants for the specific intracellular localization of each protein: (i) signal (or targeting) sequences that initiate protein translocation to compartments such as the endoplasmic reticulum (ER), the mitochondria or chloroplasts, (ii) stop-transfer signals that interrupt these translocation reactions to mediate lateral membrane insertion; (iii) sorting sequences that bind targeting factors (such as importins for nuclear import, although they were not discovered at that time), and (iv) insertion sequences that integrate proteins into membranes. Moreover, he defined the terminology for co-translational (for translocation into the ER and the bacterial inner membrane and for the insertion of mitochondrial translation products) and post-translational translocation (for import into mitochondria, chloroplasts and peroxisomes) as well as different topological classes of membrane proteins, in particular monotopic (membrane-associated), bitopic (single spanning) and polytopic (multispanning) proteins. This conceptual study paved the way to decades of intensive research by the protein translocation community that elucidated in impressive detail the individual translocation processes, their constituents, the underlying biophysical and energetic mechanisms and the regulatory principles that adapt their activities to cellular needs. Nevertheless, the recent discussions about the temporal and mechanistic coordination of the synthesis and the translocation of mitochondrial precursor proteins, i.e. whether mitochondrial import is post- or co-translational, shows the importance of Günther Blobel's definitions for these categories and that many details of the protein translocation processes still await to be discovered.

Mitochondria consist of two membranes the outer and inner membrane which enclose two aqueous compartments: the intermembrane space (IMS) and the matrix [2]. The IMS and inner membrane are functionally and structurally separated into regions forming the cristae, invaginations that harbor predominantly the complexes of the respiratory chain [3–8], and the region that is underlying the outer membrane and crucial for the exchange of metabolites, proteins and lipids between mitochondria and the rest of the cell [9–11].

Mitochondria contain a small genome for the expression of a few proteins (13 in humans, 8 in baker's yeast). These proteins are almost exclusively very hydrophobic membrane proteins which form the reaction centers of the respiratory chain. Owing to their extreme hydrophobicity, these proteins

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cannot be synthesized in the cytosol as precursors but are integrated into the inner membrane co-translationally by mitochondrial ribosomes, which are tightly bound to the inner membrane [4, 12, 13]. All other mitochondrial proteins, roughly 800 in yeast and 1500 in human cells [14, 15] are nuclear-encoded, synthesized on cytosolic ribosomes and imported into mitochondria. Particularly the early steps in mitochondrial protein targeting are poorly understood. Several recent studies reported that the accumulation of mitochondrial precursor proteins in the cytosol leads to severe stress conditions which induces several distinct, though overlapping signaling responses that were named mitochondrial precursor overaccumulation stress (mPOS), unfolded protein response activated by mistargeting of proteins (UPRam), mitochondrial unfolded protein response (mtUPR) and mitochondrial compromised protein import response (mitoCPR) [16–20]. In the cytosol, mitochondrial precursor proteins are prone to aggregate and have been found to associate with aggregates such as those formed by poly-Q proteins [21–23]. Hence, the targeting to mitochondria needs to be efficient and tightly regulated. In this review, an overview is provided about how mitochondrial precursor proteins are targeted to, imported into and distributed within mitochondria.

2 Protein Targeting to Mitochondria

In general, protein targeting can be divided into four distinct reactions (Fig. 1a): precursor proteins are (1) synthesized on ribosomes, (2) recognized by general or specific targeting factors, (3) targeted to the respective membrane of destination, and finally (4) translocated across or inserted into the membrane at the target compartment [24]. Since proteins are imported into mitochondria in an unfolded conformation, chaperones and other folding factors assist in these reactions to maintain precursors import-competent and even actively support their targeting and binding to receptor proteins on the mitochondrial outer membrane [25–27].

Mitochondrial precursors are synthesized with *targeting signals* which direct them to the different mitochondrial subcompartments (Fig. 1b). The majority of mitochondrial proteins are synthesized with an N-terminal *matrix-targeting sequence (MTS)*. These signals are about 8 to 80 residues in length, lack negative residues, are rich in hydroxylated residues such as serines and threonines, form amphipathic α-helices with one positively charged and one hydrophobic face and drive import into the matrix. Thus, they are mainly found on matrix and inner membrane proteins, but in some cases can also be present in proteins of the IMS and even the outer membrane [28–32]. Interestingly, a recent study shows that proteins of the matrix and the inner membrane often contain additional *internal MTS-like structures (iMTS-L)* that help to maintain them in an import-competent state (Fig. 1b) [33, 34].
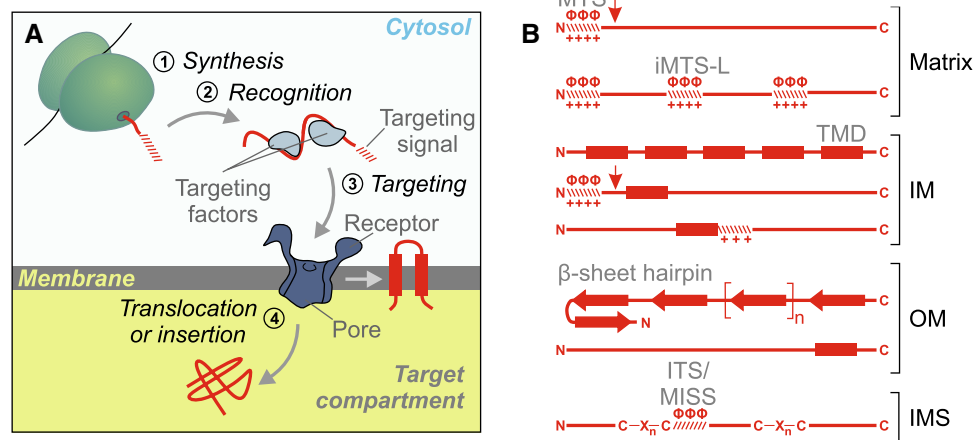

Fig. 1 Schematic flow of protein targeting. **a** Protein targeting follows 4 steps. (1) Proteins are (1) synthesized on cytosolic ribosomes, (2) recognized by targeting factors and chaperones which prevent their misfolding and aggregation, (3) targeted to their destination membrane, and (4) recognized and threaded through translocation pores in their target membrane to reach their respective compartment. **b** Targeting signals in mitochondrial proteins. Matrix proteins contain presequences or MTSs that form an amphipathic alpha helix with a hydrophobic (ϕ) and a positively (+) charged site. The import efficiency can be improved by iMTS-L sequences in the mature part of these proteins. But MTSs are not the only signals for mitochondrial targeting and a number of further examples are shown here and described in the text. Arrowheads indicate sites for proteolytic processing. *MTS* matrix-targeting sequence, *iMTS-L* internal MTS-like structure, *TMD* transmembrane domain, *IM* inner membrane, *OM* outer membrane, *IMS* intermembrane space

A large group of multispanning inner membrane proteins, in particular carriers and core components of the inner membrane translocases, lack N-terminal targeting signals but use *internal signals* that are in proximity to their transmembrane domains (TMD) (Fig. 1b) [35]. Some monotopic inner membrane proteins, such as the yeast Bcs1, Mdj2, Tim14/Pam18 or the mammalian DAKAP1 [36, 37] also employ internal signals. In this case, an internal TMD is followed by a positively charged stretch. Presumably, this region forms a hairpin-like loop that mimics an MTS.

Proteins of the outer membrane normally lack N-terminal MTSs and use heterogeneous signals to be targeted to mitochondria. A number of proteins are anchored to the outer membrane by C-terminal TMDs (Fig. 1b). These tail-anchored proteins (TA-proteins) can be distributed between mitochondria, ER and peroxisomes. Mitochondrial specificity can be achieved by a lower hydrophobicity of the TMD and a less charged C-terminus compared to peroxisomal TA-proteins [26, 38, 39]. In addition, the TMDs of mitochondrial TA-proteins are shorter than those of ER proteins [40]. Also the composition of the membrane is probably critical for the localization of TA-proteins. In yeast, the ergosterol content influenced the targeting of TA-proteins [41]. TA-anchored ER proteins that were erroneously targeted to the mitochondria are removed by the AAA protein Msp1 on the outer membrane [42, 43]. Similar to TA-proteins but inverse in their final topology, signal-anchored proteins use an N-terminal TMD to be targeted to the outer mitochondrial membrane. Tom20 and Tom70 are examples for signal-anchored proteins [44]. Many outer membrane proteins belong to the group of β-barrel proteins. These proteins presumably use β-sheet hairpin structures as targeting signals

to reach the mitochondrial outer membrane [45]. Additional signals mediate the insertion from the intermembrane space into the outer membrane of β-barrel proteins which are conserved between prokaryotes and eukaryotes [46, 47].

Most proteins of the IMS are of low molecular mass (7–20 kDa) and either coordinate cofactors or contain structural disulfide bonds, features that are critical for their targeting. As a consequence, cysteine residues often play crucial roles as part of targeting signals in these proteins. In particular, so-called *ITS/MISS sequences* were shown to be necessary and sufficient for IMS targeting (Fig. 1b). These sequences form internal amphipathic helices in direct proximity to a cysteine residue, which is essential for mitochondrial import [48, 49]. These signals are found in twin-Cx_9C and small Tim proteins [50–54].

The presence of N-terminal MTSs can be predicted by algorithms [55, 56]. However, the prediction programs do not recognize other targeting signals, making it often difficult to predict the mitochondrial localization of proteins of the outer membrane, the IMS and the inner membrane. Nevertheless, in the last decade, the mitochondrial proteome was studied into detail, which gave rise to datasets of mitochondrial proteins and their sub-organellar localization [2, 14, 52, 57–59].

2.1 Post-translational Targeting: Chaperone-guided Targeting

Our understanding of the early steps in the targeting of mitochondrial precursors is very limited. Several cytosolic factors are critical for the targeting of mitochondrial precursor proteins (Fig. 2a). Cytosolic chaperones in particular, such
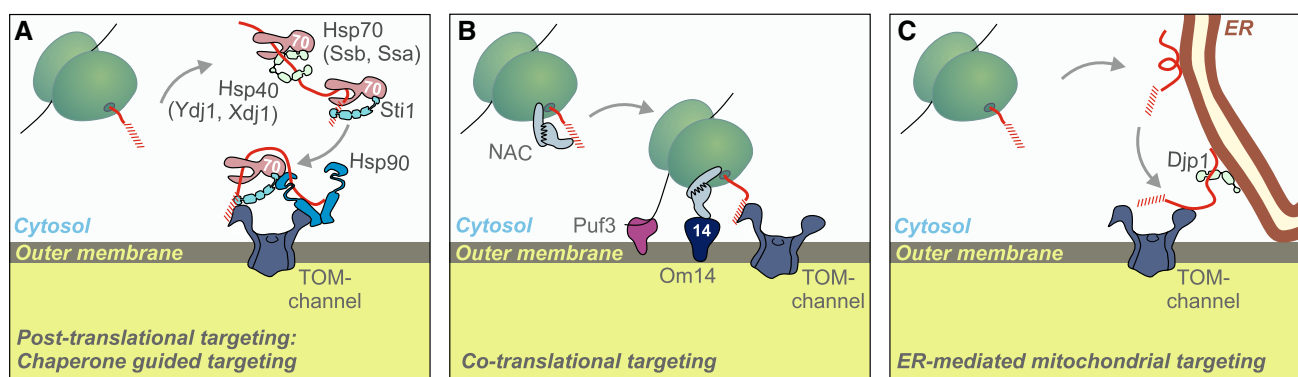

Fig. 2 Protein targeting to the mitochondrial outer membrane. **a** Chaperone-guided post-translational targeting of mitochondrial proteins. After their synthesis, precursors are bound by chaperones of the Hsp70 family and their cofactors. The Hsp90 co-chaperone Sti1 binds the MTS and recruits Hsp70 to the precursor. **b** Certain mitochondrial proteins might be imported co-translationally. In some cases, the mRNA-binding factor Puf3 on the mitochondrial surface facilitates this reaction. The ribosomal associated NAC complex can bind to the mitochondrial outer membrane protein Om14 to tether the ribosome to the outer membrane. *NAC* nascent polypeptide-associated complex. **c** ER-mediated mitochondrial protein targeting: ER-SURF. Precursor proteins bind to the ER membrane where the J protein Djp1 recognizes the mitochondrial proteins. The mitochondrial proteins are handed over to the mitochondrial outer membrane in order to be imported

as Ssa1 or Ydj1, have been proposed to target proteins to mitochondria, owing to the observation that mutants in these components accumulate precursor proteins in the cytosol [60, 61]. However, this accumulation could also, at least in part, be explained by the critical role of chaperones in the degradation of cytosolic precursors [62].

In yeast, cytosolic co-chaperones of the Hsp40 family (also called J proteins), in particular Ydj1, Xdj1 and Sis1, recognize mitochondrial precursors during or subsequently to their synthesis and recruit cytosolic Hsp70 chaperones (Ssa1–Ssa4) [27, 63, 64]. Moreover, Hsp90 and its cofactors, such as the yeast Sti1, bind to precursors in the cytosol [25, 65]. At least in mammalian cells, these general chaperones are assisted by precursor-binding proteins, such as ubiquilin, which are presumably particularly important for the targeting of hydrophobic precursors [66]. Cytosolic chaperones have been proposed to facilitate post-translational targeting by several mechanisms: they maintain precursors unfolded and import-competent, they prevent their aggregation and degradation by the ubiquitin-proteasome system and they can directly bind to mitochondrial surface receptors, in particular to Tom70, and thus promote the targeting of precursor proteins. Although it is well accepted that chaperones play a crucial role in precursor targeting, their precise contribution as targeting factors or in keeping precursor proteins import-competent as well as their substrate spectrum is unclear. Thus, whether they exhibit a general function for all precursors or are only critical for specific, aggregation-prone preproteins has to be shown in the future.

2.2 Co-translational Targeting

There is very good evidence that the bulk of mitochondrial protein import occurs in a post-translational manner [67, 68]. However, a number of inner membrane proteins have been reported to be imported co-translationally. Since the early 2000s the intracellular distribution of mRNAs has been studied by microscopy and fractionation techniques. In yeast, specific mitochondrial mRNAs, such as those encoding the inner membrane protein Oxa1, were reported to be enriched on the mitochondrial surface by binding to the mitochondrial surface protein Puf3 and components of the TOM complex [69–72]. In addition, the yeast nascent polypeptide-associated complex (NAC) was suggested to interact with the mitochondrial outer membrane protein Om14, thereby targeting cytosolic ribosomes to mitochondria [73, 74]. Indeed, NAC is important for mitochondrial protein targeting (Fig. 2b). The loss of NAC leads to ER localization of mitochondrial proteins [75]. Moreover, proximity-specific ribosomal profiling data show that certain mitochondrial (membrane) proteins are translated at the mitochondrial outer membrane, clearly demonstrating that co-translational targeting exists [76, 77]. In addition, the mitochondrial matrix protein fumarase has been shown as the first protein to be imported in a co-translational fashion in vitro and in vivo in yeast [78–80]. To what extent co-translational import is used is not clear.

2.3 ER-mediated Mitochondrial Targeting

A recent study screened for factors that are necessary for efficient targeting of the mitochondrial inner membrane protein Oxa1 in yeast and identified a novel targeting pathway that was named ER-SURF (ER surface-mediated protein targeting) (Fig. 2c). The surface of the ER plays an important and active role in the intracellular targeting of precursors from ribosomes to mitochondria. The J protein Djp1 was shown to serve as an important component of this pathway which seems to be predominantly relevant for the targeting of hydrophobic precursors of membrane proteins [81, 82]. A close cooperation of the ER and mitochondria for the exchange of lipids and calcium was documented before, processes that are facilitated by physical contact sites of both organelles such as those formed by the ER mitochondria encounter structure (ERMES) complex of yeast cells [10, 83–86]. In mammalian cells, the ER and mitochondrial membrane often can form an extended contact region, named MAM (mitochondria-associated membranes). However, whether ERMES or MAM are critical sites for the precursor transfer from ER to mitochondria in ER-SURF is not known.

The observation that the ER surface plays a facilitating role in mitochondrial preprotein import came as a surprise because the ER is considered as an adverse off-site target for mitochondrial proteins, particularly under stress conditions [75, 87, 88]. RNA localization analyses surprisingly revealed that mRNAs for many mitochondrial proteins are located on the ER surface suggesting that these proteins are intentionally synthesized on the ER from which they are handed over to mitochondria [77, 89]. However, the details of the ER-SURF pathway are not well understood and still await elucidation.

The diverse targeting mechanisms described here are not mutually exclusive and appear to operate in parallel, each being used by different proteins to varying degrees. This use of several targeting mechanisms resembles the targeting of ER proteins that employ multiple sorting pathways [24, 90, 91]. The use of parallel pathways provides redundancy and possibly a more stable situation that is robust even under variable growth conditions.

3 Recognition at the Mitochondrial Outer Membrane

Receptors on the mitochondrial surface recognize mitochondrial precursors. These receptors are part of the translocase of the outer membrane (TOM) complex and direct their

substrates into the protein-conducting channel that is formed by the β-barrel protein Tom40. Two receptor systems act in parallel (Fig. 3a): One receptor system is formed by Tom20 and Tom22 which are tightly associated to the TOM complex to bind N-terminal MTSs of matrix-targeted proteins as well as β-barrel proteins [92–94]. Tom70 (and, in baker's yeast, its paralog Tom71) is more loosely and perhaps dynamically associated with the TOM core complex. It binds preferentially to the internal targeting sequences of carrier proteins and multi-spanning proteins of the outer membrane but also to the iMTS-L sequences in matrix proteins. Thereby Tom70 recruits them to the outer membrane and maintains these bound precursors unfolded and import-competent [25, 33, 95] (Fig. 3b). Tom70 not only recognizes presequences but also chaperones of the Hsp70 and Hsp90 systems and its different binding sites cooperate to keep precursors unfolded and to feed them into the import machinery (Fig. 3a) [25, 33, 96]. Tom70 belongs to the family of tetratricopeptide repeat (TPR) proteins. Many TPR proteins serve as co-chaperones that assist Hsp70 and Hsp90 chaperones during protein (un) folding [97, 98].

Although Tom20 preferentially binds to presequences and Tom70 to internal targeting sequences [99, 100], in vivo both receptors are able to functionally replace each other. Single mutants are viable, though their mitochondria are not fully functional, but double mutants that lack both receptors are inviable. Although these receptors, in particular Tom70, are only found in fungi and animals but not in many other eukaryotic groups, the concept of two cooperative receptors that differ in their substrate spectrum might be rather universal and was developed several times independently in the eukaryotic lineage [101–103].

4 Protein Import into Mitochondria

The TOM complex serves as the general entry gate into mitochondria. In its fully active form, it contains three Tom40 β-barrel channels that are connected by a central cluster of Tom22 subunits. The small TOM proteins Tom5, Tom6 and Tom7 regulate the assembly and further stabilize the TOM complex [92, 94]. Due to a dynamic reorganization of the TOM complex, smaller TOM complexes with only two Tom40 β-barrel channels also exist, which have been proposed to be important for the biogenesis of new TOM complexes and for regulation of mitochondrial translocation. Phosphorylation of the TOM complex plays a further regulatory role to adapt the translocation efficiency of cells to different growth conditions or cell cycle stages [104, 105]. The mechanism by which precursors traverse the outer membrane is not well understood, but there is evidence that several binding sites along the import route drive translocation by sequential interactions [94, 106]. It was suggested that these binding sites increase in affinity to guide proteins to the translocase of the inner membrane (TIM) complex, but the evidence for such a mechanism is not strong [107]. In any case, the trans-site of the TOM complex serves as a branch point of several import routes from which precursors need to be sorted to further destinations such as the matrix, the inner membrane, the IMS and, for β-barrel proteins, via the IMS into the outer membrane. Since many excellent review articles described the details of these import routes and the machineries involved in great detail [108, 109] we will give here only a general overview.
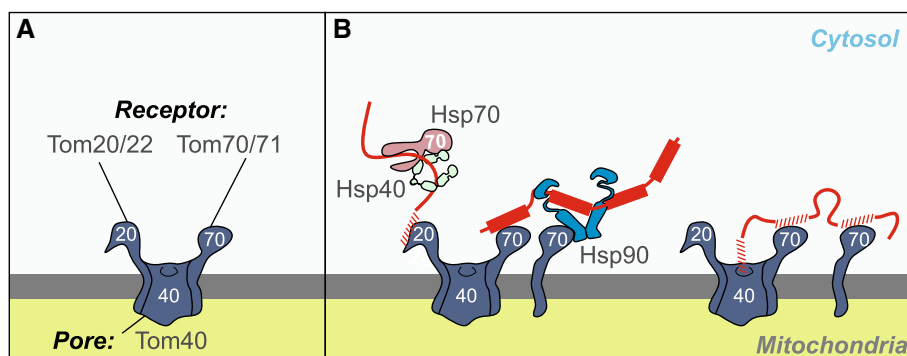

Fig. 3 Receptors of the TOM-channel. **a** The mitochondrial translocase of the outer membrane (TOM) is a protein complex consisting of a pore forming β-barrel protein Tom40. Tom20 and Tom22, as well as Tom70 and its paralog Tom71 form the receptors of the TOM complex. **b** Tom20 recognizes predominantly N-terminal presequences whereas Tom70 binds internal targeting signals, such as those present in carrier proteins. Tom70 cooperates with chaperones such as Hsp90 and binds iMTS-Ls of proteins. Thereby it maintains proteins import-competent

4.1 Presequence-mediated Import by the TIM23 Translocase

The majority of mitochondrial proteins use the "classical" import pathway, the import through the presequence translocase (Fig. 4a). These precursor proteins are recognized by Tom20/Tom22 and translocated in an unfolded state through the Tom40 pore. The MTS facilitates the binding to Tom20/Tom22 [99, 110] and the translocation through the TOM pore after which they bind to the trans-site of the TOM complex which is in part formed by the IMS domain of Tom22. Tom22 binds with low affinity to Tim21 [111], a subunit that is important to recruit the TIM23 complex that serves as the presequence translocase in the inner membrane. The presequence is then handed over to Tim50, a membrane-anchored TIM23 subunit with a large hydrophilic domain exposed into the IMS [112, 113]. Tim50 and the N-terminal region of Tim23 pass the precursor on to the TIM23 pore that is formed by its two multispanning inner membrane subunits Tim23 and Tim17 [114–118]. Since the respiratory chain pumps protons from the matrix into the IMS, the inner membrane facing the IMS is positively charged whereas the membrane side facing the matrix is negatively charged. The positively charged MTS presumably makes use of this gradient to be translocated by an electrophoresis-like reaction through the TIM23 channel [119, 120].

The import motor, also called the PAM machinery (presequence translocase-associated motor), is docked onto the matrix side of the TIM23 complex. Its active player is the matrix Hsp70 chaperone (mtHsp70, Ssc1 in yeast and mortalin in humans), which binds the incoming polypeptides

and thereby prevents backsliding [96]. According to the Brownian ratchet hypothesis, which today is well-accepted in the field, Brownian movement is believed to direct the precursor further into the matrix, so that another Hsp70 can associate with it. In addition to the random Brownian movement, "entropic pulling" might support protein translocation using a force of entropic origin [121]. The Tim44 subunit serves as the Hsp70 recruitment site on the import channel that ensures the close proximity of Hsp70 to the import pore [122–124], and several regulatory proteins control the ATPase cycle of Hsp70 that is essential for the import reaction [122, 125, 126]. Following translocation, the MTSs are removed by the mitochondrial processing peptidase (MPP) and rapidly degraded. In the matrix, protein folding is supported by Hsp70 and other chaperones [28, 127].

Proteins with a single TMD can use a stop-transfer mechanism to be laterally released into the inner membrane. As proposed in Günther Blobel's review of 1980, these proteins contain stop-anchor signals which mediate their translocation arrest and prevent their further translocation into the matrix [128–130].

4.2 Import and Membrane-insertion of Carrier Proteins by the TIM22 Translocase

Carrier proteins use an alternative inner membrane translocase, the TIM22 complex, to be inserted into the inner membrane (Fig. 4b). They are initially recognized predominantly by Tom70 on the outer membrane which, together with Hsp70 and Hsp90 chaperones keeps them unfolded and prevents their aggregation [25, 131]. Following translocation
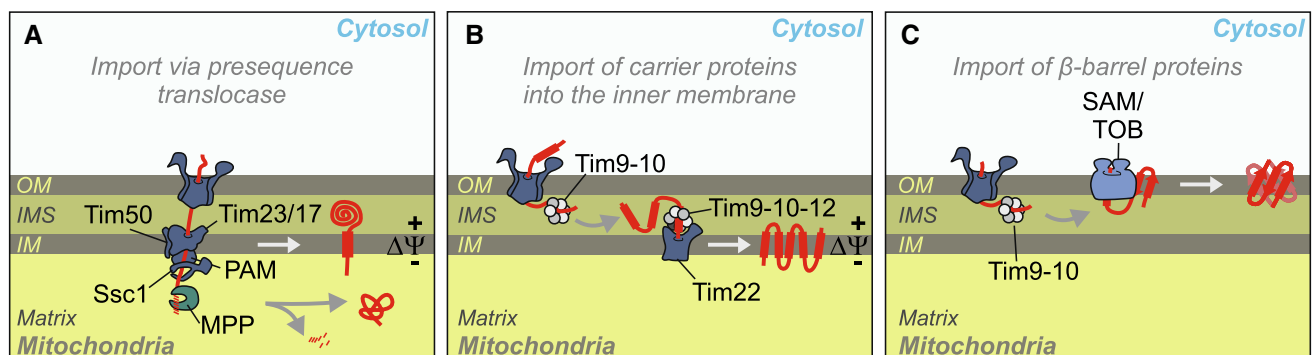

Fig. 4 Different import routes lead to the matrix, the inner membrane or the outer membrane. **a** Matrix targeting by the presequence translocase. Proteins with an N-terminal MTS are translocated through the TOM complex. In the IMS, Tim50 recognizes the emerging MTS and guides the polypeptide into the translocation pore of the TIM23 complex. The membrane potential (ΔΨ) facilitates the transport of the MTS across the inner membrane. The import motor or PAM complex employs the mitochondrial Hsp70 Ssc1 to complete the translocation into the matrix in an ATP-dependent reaction. The mitochondrial processing peptidase (MPP) removes the MTS from most precursors. Stop-transfer sequences can laterally insert translocation

intermediates into the inner membrane. **b** Carrier proteins use the TIM22 complex. Carrier proteins are polytopic membrane proteins which are ushered by the Tim9–Tim10 hexamer through the IMS and inserted into the inner membrane by the TIM22 complex in a membrane potential-dependent reaction. **c** Import of β-barrel proteins by the SAM/TOB complex. Presumably owing to their bacterial origin, β-barrel proteins are inserted into the outer membrane from the IMS. Small Tim proteins escort these hydrophobic proteins to the SAM/TOB complex which mediates their integration. *OM* outer membrane, *IM* inner membrane, *IMS* intermembrane space

through the TOM channel, they bind to a soluble chaperone complex in the IMS, the hexameric Tim9–Tim10 complex that consists of three Tim9 and three Tim10 subunits [132–135]. This step is independent of the membrane potential [35]. Inside of the IMS, carriers are passed on to a second hexameric chaperone, the Tim9–Tim10–Tim12 complex, that consists of three Tim9, two Tim10 and one Tim12 protein and that is tightly bound to Tim22, the core subunit of the TIM22 complex [136, 137]. Several accessory membrane proteins assist Tim22 in its role as membrane insertase for carriers such as Tim54, Tim18 and, in humans, Tim29 and acylglycerol kinase [138–142].

4.3 The Insertion of β-Barrel Proteins into the Outer Membrane by the SAM/TOB Complex

β-Barrel proteins of the outer membrane, such as Tom40 or Por1, are presumably recognized by specific β-hairpin structures [27, 45, 143] before they are translocated through the TOM channel and bound by the Tim9–Tim10 complex (Fig. 4c). From there, they are passed on to the SAM (sorting and assembly machinery) or TOB (topogenesis of β-barrel proteins) complex in the outer membrane [133, 143]. Precursors are integrated into the outer membrane by the essential protein Sam50 (also Tob55 or Omp85), that itself is a β-barrel protein. Sam50 can presumably open its barrel structure on one side in order to release its substrates into the lipid bilayer [143–145].

The mechanisms by which TA proteins are recognized and inserted into the mitochondrial outer membrane are largely elusive. However, in vitro studies suggest that some of them can insert without a specific insertion machinery [39].

4.4 Mia40-mediated Import into the Intermembrane Space

Many small proteins of the IMS use the mitochondrial disulfide relay or MIA machinery (Fig. 5) [146]. These proteins are characterized by conserved cysteine motifs [50, 147]. They traverse the TOM channel and bind the IMS protein Mia40 (called CHCHD4 in humans), which prevents their backsliding into the cytosol. Mia40, the core component of the MIA import machinery, is an oxidoreductase with a hydrophobic pocket. For a long time it was believed that Mia40 predominantly or exclusively binds its substrates via a mixed disulfide bond, an interaction that would trap them in the IMS [146, 148–151]. However, more recent studies suggest that the hydrophobic binding pocket of Mia40 mediates the crucial binding for protein translocation and its oxidoreductase activity is only used for oxidative folding of its substrates after and independent from their import reaction [54, 152]. For some substrates, such as the
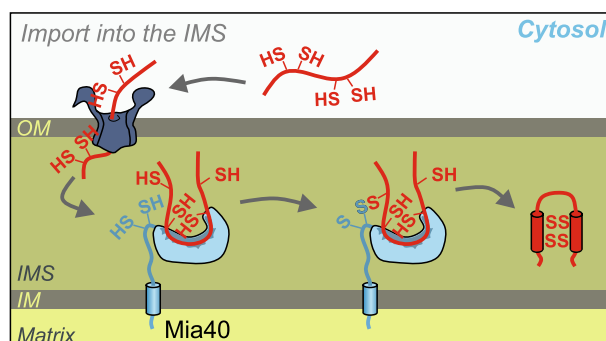

Fig. 5 The MIA import pathway. Proteins of the IMS usually have conserved cysteine patterns that serve as Mia40 recognition signals. After being transported through the TOM channel, these proteins are bound by Mia40 via its hydrophobic pocket. Subsequently, Mia40 mediates their oxidative folding which prevents their re-translocation into the cytosol

human inner membrane protein MICU1, Mia40-mediated oxidation even occurs independent from their import [153].

5 The Mitochondrial Ultrastructure: Organization of Protein Import Machineries

Mitochondria are complex organelles with a highly organized ultrastructure. Many components, such as the TOM complexes in the outer membrane, are positioned at specific locations and define specific functional subdomains of the organelle [154]. This is particularly true for the structures in the inner membrane. Here the mitochondrial contact sites and cristae organization system (MICOS) complex plays a crucial role as heterooligomeric organizing structure [5, 155]. MICOS bends the inner membrane to form its characteristic cristae junctions [156]. In addition, the MICOS complex coordinates the distribution of many inner membrane proteins such as the enzymes of the respiratory chain and of the import machinery. The MICOS subunit Mic60 was shown to interact with the TOM complex and Mia40 [157–159]. This interaction positions Mia40 in direct proximity to the TOM pore to allow efficient import into the IMS [160]. In a similar way, MICOS might form contacts with the SAM/TOB complex to facilitate the biogenesis of β-barrel proteins [157] (Fig. 6).

The outer and inner membranes are further connected by contacts between the TOM complex and the TIM23 translocase in the inner membrane, in particular between the IMS domains of Tom22 and Tim50. These contacts are believed to align both translocases so that precursors are passed through both structures in a coordinated fashion [161].

The cytosolic side of the TOM complex also plays a crucial role as a contact-forming structure. Tom70 forms an
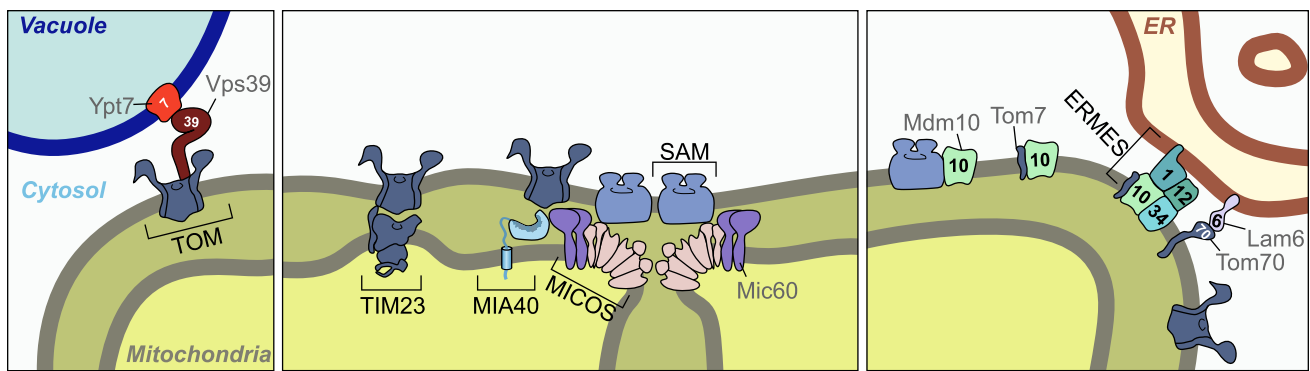
Fig. 6 The import machineries are integrated into highly organized structures. The different translocation complexes are organized in super-complexes. TOM and TIM23 are aligned via an interaction of Tim50 with Tom22. This interaction leads to contacts between the outer and inner membranes. The MICOS complex is crucial for the formation of cristae junctions and the characteristic inner membrane architecture of mitochondria. The MICOS subunit Mic60 exposes a large domain to the IMS that forms critical contacts with Mia40 and the TOM complex. The TOM subunits Tom7 and Tom70 are involved in the formation of contact sites of mitochondria with the ER and Tom40 with the vacuolar membrane

ER contact site together with the ER protein Lam6, and the TOM complex also plays a role for the ERMES complex [162–165]. In addition, the TOM complex interacts with Vps39 and Ypt7 to form contacts with the vacuole [165, 166]. While these organelle contacts play a well-established role of the sorting of lipids, their contribution to the intracellular protein translocation is less clear and an exciting aspect to study in the future. In his perspicacious review, Günther Blobel defined the underlying rules and signals of intracellular protein topogenesis, but the example of mitochondrial biogenesis impressively demonstrates the complexity of protein sorting in a living cell. There is still a multitude of biology out there that awaits discovery.

Compliance with Ethical Standards

References

1. Blobel G (1980) Intracellular protein topogenesis. Proc Natl Acad Sci USA 77(3):1496–1500
2. Vögtle F-N, Burkhart JM, Gonczarowska-Jorge H et al (2017) Landscape of submitochondrial protein distribution. Nat Commun 8(1):290. https://doi.org/10.1038/s41467-017-00359-0
3. Paumard P, Vaillier J, Coulary B et al (2002) The ATP synthase is involved in generating mitochondrial cristae morphology. EMBO J 21(3):221–230. https://doi.org/10.1093/emboj/21.3.221
4. Pfeffer S, Woellhaf MW, Herrmann JM et al (2015) Organization of the mitochondrial translation machinery studied in situ by cryoelectron tomography. Nat Commun 6:6019. https://doi.org/10.1038/ncomms7019
5. Hessenberger M, Zerbes RM, Rampelt H et al (2017) Regulated membrane remodeling by Mic60 controls formation of mitochondrial crista junctions. Nat Commun 8:15258. https://doi.org/10.1038/ncomms15258
6. Tarasenko D, Barbot M, Jans DC et al (2017) The MICOS component Mic60 displays a conserved membrane-bending activity that is necessary for normal cristae morphology. J Cell Biol 216(4):889–899. https://doi.org/10.1083/jcb.201609046
7. Rampelt H, van der Laan M (2017) The Yin & Yang of mitochondrial architecture—interplay of MICOS and F1Fo-ATP synthase in cristae formation. Microb Cell 4(8):236–239. https://doi.org/10.15698/mic2017.08.583
8. Eydt K, Davies KM, Behrendt C et al (2017) Cristae architecture is determined by an interplay of the MICOS complex and the F1FO ATP synthase via Mic27 and Mic10. Microb Cell 4(8):259–272. https://doi.org/10.15698/mic2017.08.585
9. Harner ME, Unger A-K, Izawa T et al (2014) Aim24 and MICOS modulate respiratory function, tafazzin-related cardiolipin modification and mitochondrial architecture. Elife 3:e01684. https://doi.org/10.7554/eLife.01684
10. Kawano S, Tamura Y, Kojima R et al (2018) Structure-function insights into direct lipid transfer between membranes by Mmm1-Mdm12 of ERMES. J Cell Biol 217(3):959–974. https://doi.org/10.1083/jcb.201704119
11. Herrmann JM, Riemer J (2010) The intermembrane space of mitochondria. Antioxid Redox Signal 13(9):1341–1358. https://doi.org/10.1089/ars.2009.3063
12. Möller-Hergt BV, Carlström A, Stephan K et al (2018) The ribosome receptors Mrx15 and Mba1 jointly organize cotranslational insertion and protein biogenesis in mitochondria. Mol Biol Cell 29(20):2386–2396. https://doi.org/10.1091/mbc.E18-04-0227
13. Kummer E, Leibundgut M, Rackham O et al (2018) Unique features of mammalian mitochondrial translation initiation revealed by cryo-EM. Nature 560(7717):263–267. https://doi.org/10.1038/s41586-018-0373-y

14. Morgenstern M, Stiller SB, Lübbert P et al (2017) Definition of a high-confidence mitochondrial proteome at quantitative scale. Cell Rep 19(13):2836–2852. https://doi.org/10.1016/j.celrep.2017.06.014

15. Calvo SE, Clauser KR, Mootha VK (2016) MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. Nucleic Acids Res 44(D1):D1251–D1257. https://doi.org/10.1093/nar/gkv1003

16. Wrobel L, Topf U, Bragoszewski P et al (2015) Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. Nature 524(7566):485–488. https://doi.org/10.1038/nature14951

17. Weidberg H, Amon A (2018) MitoCPR-A surveillance pathway that protects mitochondria in response to protein import stress. Science 360(6385). https://doi.org/10.1126/science.aan4146

18. Wang X, Chen XJ (2015) A cytosolic network suppressing mitochondria-mediated proteostatic stress and cell death. Nature 524(7566):481–484. https://doi.org/10.1038/nature14859

19. Labbadia J, Brielmann RM, Neto MF et al (2017) Mitochondrial stress restores the heat shock response and prevents proteostasis collapse during aging. Cell Rep 21(6):1481–1494. https://doi.org/10.1016/j.celrep.2017.10.038

20. Fiorese CJ, Schulz AM, Lin Y-F et al (2016) The transcription factor ATF5 mediates a mammalian mitochondrial UPR. Curr Biol 26(15):2037–2043. https://doi.org/10.1016/j.cub.2016.06.002

21. Gruber A, Hornburg D, Antonin M et al (2018) Molecular and structural architecture of polyQ aggregates in yeast. Proc Natl Acad Sci USA 115(15):E3446–E3453. https://doi.org/10.1073/pnas.1717978115

22. Liu W, Duan X, Fang X et al (2018) Mitochondrial protein import regulates cytosolic protein homeostasis and neuronal integrity. Autophagy 14(8):1293–1309. https://doi.org/10.1080/15548627.2018.1474991

23. Papsdorf K, Kaiser CJO, Drazic A et al (2015) Polyglutamine toxicity in yeast induces metabolic alterations and mitochondrial defects. BMC Genom 16:662. https://doi.org/10.1186/s12864-015-1831-7

24. Aviram N, Schuldiner M (2017) Targeting and translocation of proteins to the endoplasmic reticulum at a glance. J Cell Sci 130(24):4079–4085. https://doi.org/10.1242/jcs.204396

25. Young JC, Hoogenraad NJ, Hartl FU (2003) Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70. Cell 112(1):41–50. https://doi.org/10.1016/S0092-8674(02)01250-3

26. Cichocki BA, Krumpe K, Vitali DG et al (2018) Pex19 is involved in importing dually targeted tail-anchored proteins to both mitochondria and peroxisomes. Traffic. https://doi.org/10.1111/tra.12604

27. Jores T, Lawatscheck J, Beke V et al (2018) Cytosolic Hsp70 and Hsp40 chaperones enable the biogenesis of mitochondrial β-barrel proteins. J Cell Biol. https://doi.org/10.1083/jcb.201712029

28. Vögtle F-N, Wortelkamp S, Zahedi RP et al (2009) Global analysis of the mitochondrial N-proteome identifies a processing peptidase critical for protein stability. Cell 139(2):428–439. https://doi.org/10.1016/j.cell.2009.07.045

29. Sinzel M, Tan T, Wendling P et al (2016) Mcp3 is a novel mitochondrial outer membrane protein that follows a unique IMP-dependent biogenesis pathway. EMBO Rep 17(7):965–981. https://doi.org/10.15252/embr.201541273

30. Doyle SR, Kasinadhuni NRP, Chan CK et al (2013) Evidence of evolutionary constraints that influences the sequence composition and diversity of mitochondrial matrix targeting signals. PLoS ONE 8(6):e67938. https://doi.org/10.1371/journal.pone.0067938

31. Geissler A, Krimmer T, Bömer U et al (2000) Membrane potential-driven protein import into mitochondria. The sorting sequence of cytochrome b(2) modulates the deltapsi-dependence of translocation of the matrix-targeting sequence. Mol Biol Cell 11(11):3977–3991. https://doi.org/10.1091/mbc.11.11.3977

32. Huang S, Taylor NL, Whelan J et al (2009) Refining the definition of plant mitochondrial presequences through analysis of sorting signals, N-terminal modifications, and cleavage Motifs1WOA. Plant Physiol 150(3):1272–1285. https://doi.org/10.1104/pp.109.137885

33. Backes S, Hess S, Boos F et al (2018) Tom70 enhances mitochondrial preprotein import efficiency by binding to internal targeting sequences. J Cell Biol 217(4):1369–1382. https://doi.org/10.1083/jcb.201708044

34. Boos F, Mühlhaus T, Herrmann J (2018) Detection of internal matrix targeting signal-like sequences (iMTS-Ls) in mitochondrial precursor proteins using the TargetP prediction tool. Biol Protoc. https://doi.org/10.21769/BioProtoc.2474

35. Wiedemann N, Pfanner N, Ryan MT (2001) The three modules of ADP/ATP carrier cooperate in receptor recruitment and translocation into mitochondria. EMBO J 20(5):951–960. https://doi.org/10.1093/emboj/20.5.951

36. Fölsch H, Guiard B, Neupert W et al (1996) Internal targeting signal of the BCS1 protein: a novel mechanism of import into mitochondria. EMBO J 15(3):479–487

37. Ma Y, Taylor SS (2008) A molecular switch for targeting between endoplasmic reticulum (ER) and mitochondria: conversion of a mitochondria-targeting element into an ER-targeting signal in DAKAP1. J Biol Chem 283(17):11743–11751. https://doi.org/10.1074/jbc.M710494200

38. Costello JL, Castro IG, Camões F et al (2017) Predicting the targeting of tail-anchored proteins to subcellular compartments in mammalian cells. J Cell Sci 130(9):1675–1687. https://doi.org/10.1242/jcs.200204

39. Kemper C, Habib SJ, Engl G et al (2008) Integration of tail-anchored proteins into the mitochondrial outer membrane does not require any known import components. J Cell Sci 121(Pt 12):1990–1998. https://doi.org/10.1242/jcs.024034

40. Marty NJ, Teresinski HJ, Hwang YT et al (2014) New insights into the targeting of a subset of tail-anchored proteins to the outer mitochondrial membrane. Front Plant Sci 5:426. https://doi.org/10.3389/fpls.2014.00426

41. Krumpe K, Frumkin I, Herzig Y et al (2012) Ergosterol content specifies targeting of tail-anchored proteins to mitochondrial outer membranes. Mol Biol Cell 23(20):3927–3935. https://doi.org/10.1091/mbc.E11-12-0994

42. Chen Y-C, Umanah GKE, Dephoure N et al (2014) Msp1/ATAD1 maintains mitochondrial function by facilitating the degradation of mislocalized tail-anchored proteins. EMBO J 33(14):1548–1564. https://doi.org/10.15252/embj.201487943

43. Okreglak V, Walter P (2014) The conserved AAA-ATPase Msp1 confers organelle specificity to tail-anchored proteins. Proc Natl Acad Sci USA 111(22):8019–8024. https://doi.org/10.1073/pnas.1405755111

44. Ahting U, Waizenegger T, Neupert W et al (2005) Signal-anchored proteins follow a unique insertion pathway into the outer membrane of mitochondria. J Biol Chem 280(1):48–53. https://doi.org/10.1074/jbc.M410905200

45. Jores T, Klinger A, Groß LE et al (2016) Characterization of the targeting signal in mitochondrial β-barrel proteins. Nat Commun 7:12036. https://doi.org/10.1038/ncomms12036

46. Gratzer S (1995) Mas37p, a novel receptor subunit for protein import into mitochondria. J Cell Biol 129(1):25–34. https://doi.org/10.1083/jcb.129.1.25

47. Imai K, Fujita N, Gromiha MM et al (2011) Eukaryote-wide sequence analysis of mitochondrial β-barrel outer

membrane proteins. BMC Genom 12:79. https://doi.org/10.1186/1471-2164-12-79

48. Sideris DP, Petrakis N, Katrakili N et al (2009) A novel inter-membrane space-targeting signal docks cysteines onto Mia40 during mitochondrial oxidative folding. J Cell Biol 187(7):1007–1022. https://doi.org/10.1083/jcb.200905134

49. Milenkovic D, Ramming T, Müller JM et al (2009) Identification of the signal directing Tim9 and Tim10 into the intermembrane space of mitochondria. Mol Biol Cell 20(10):2530–2539. https://doi.org/10.1091/mbc.E08-11-1108

50. Longen S, Bien M, Bihlmaier K et al (2009) Systematic analysis of the twin cx(9)c protein family. J Mol Biol 393(2):356–368. https://doi.org/10.1016/j.jmb.2009.08.041

51. Gabriel K, Milenkovic D, Chacinska A et al (2007) Novel mito-chondrial intermembrane space proteins as substrates of the MIA import pathway. J Mol Biol 365(3):612–620. https://doi.org/10.1016/j.jmb.2006.10.038

52. Vögtle F-N, Burkhart JM, Rao S et al (2012) Intermembrane space proteome of yeast mitochondria. Mol Cell Proteom 11(12):1840–1852. https://doi.org/10.1074/mcp.M112.021105

53. Peleh V, Zannini F, Backes S et al (2017) Erv1 of Arabidop-sis thaliana can directly oxidize mitochondrial intermembrane space proteins in the absence of redox-active Mia40. BMC Biol 15(1):106. https://doi.org/10.1186/s12915-017-0445-8

54. Peleh V, Cordat E, Herrmann JM (2016) Mia40 is a trans-site receptor that drives protein import into the mitochondrial intermembrane space by hydrophobic substrate binding. Elife 5:e16177. https://doi.org/10.7554/eLife.16177

55. Emanuelsson O, Nielsen H, Brunak S et al (2000) Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. J Mol Biol 300(4):1005–1016. https://doi.org/10.1006/jmbi.2000.3903

56. Claros MG, Vincens P (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. Eur J Biochem 241(3):779–786

57. Ohlmeier S, Kastaniotis AJ, Hiltunen JK et al (2004) The yeast mitochondrial proteome, a study of fermentative and respiratory growth. J Biol Chem 279(6):3956–3979. https://doi.org/10.1074/jbc.M310160200

58. Hung V, Zou P, Rhee H-W et al (2014) Proteomic mapping of the human mitochondrial intermembrane space in live cells via ratiometric APEX tagging. Mol Cell 55(2):332–341. https://doi.org/10.1016/j.molcel.2014.06.003

59. Hung V, Lam SS, Udeshi ND et al (2017) Proteomic mapping of cytosol-facing outer mitochondrial and ER membranes in living human cells by proximity biotinylation. Elife 6:e24463. https://doi.org/10.7554/eLife.24463

60. Caplan AJ, Cyr DM, Douglas MG (1992) YDJ1p facilitates poly-peptide translocation across different intracellular membranes by a conserved mechanism. Cell 71(7):1143–1155

61. Deshaies RJ, Koch BD, Werner-Washburne M et al (1988) A sub-family of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. Nature 332(6167):800–805. https://doi.org/10.1038/332800a0

62. Lee DH, Sherman MY, Goldberg AL (1996) Involvement of the molecular chaperone Ydj1 in the ubiquitin-dependent degrada-tion of short-lived and abnormal proteins in Saccharomyces cer-evisiae. Mol Cell Biol 16(9):4773–4781

63. Sahi C, Kominek J, Ziegelhoffer T et al (2013) Sequential dupli-cations of an ancient member of the DnaJ-family expanded the functional chaperone network in the eukaryotic cytosol. Mol Biol Evol 30(5):985–998. https://doi.org/10.1093/molbev/mst008

64. Opaliński Ł, Song J, Priesnitz C et al (2018) Recruitment of cytosolic J-proteins by TOM receptors promotes mitochondrial protein biogenesis. Cell Rep 25(8):2036–2043.e5. https://doi.org/10.1016/j.celrep.2018.10.083

65. Hoseini H, Pandey S, Jores T et al (2016) The cytosolic cochaperone Sti1 is relevant for mitochondrial biogenesis and morphology. FEBS J 283(18):3338–3352. https://doi.org/10.1111/febs.13813

66. Itakura E, Zavodszky E, Shao S et al (2016) Ubiquilins chap-erone and triage mitochondrial membrane proteins for degra-dation. Mol Cell 63(1):21–33. https://doi.org/10.1016/j.molcel.2016.05.020

67. Gold VAM, Chroscicki P, Bragoszewski P et al (2017) Visuali-zation of cytosolic ribosomes on the surface of mitochondria by elect ron cryo-tomography. EMBO Rep 18(10):1786–1800. https://doi.org/10.15252/embr.201744261

68. Wienhues U, Becker K, Schleyer M et al (1991) Protein folding causes an arrest of preprotein translocation into mitochondria in vivo. J Cell Biol 115(6):1601–1609

69. Gadir N, Haim-Vilmovsky L, Kraut-Cohen J et al (2011) Localization of mRNAs coding for mitochondrial proteins in the yeast Saccharomyces cerevisiae. RNA 17(8):1551–1565. https://doi.org/10.1261/rna.2621111

70. Saint-Georges Y, Garcia M, Delaveau T et al (2008) Yeast mitochondrial biogenesis: a role for the PUF RNA-binding protein Puf3p in mRNA localization. PLoS One 3(6):e2293. https://doi.org/10.1371/journal.pone.0002293

71. Eliyahu E, Pnueli L, Melamed D et al (2010) Tom20 medi-ates localization of mRNAs to mitochondria in a translation-dependent manner. Mol Cell Biol 30(1):284–294. https://doi.org/10.1128/MCB.00651-09

72. Zabezhinsky D, Slobodin B, Rapaport D et al (2016) An essen-tial role for COPI in mRNA localization to mitochondria and mitochondrial function. Cell Rep 15(3):540–549. https://doi.org/10.1016/j.celrep.2016.03.053

73. Lesnik C, Cohen Y, Atir-Lande A et al (2014) OM14 is a mito-chondrial receptor for cytosolic ribosomes that supports co-translational import into mitochondria. Nat Commun 5:5711. https://doi.org/10.1038/ncomms6711

74. Ponce-Rojas JC, Avendaño-Monsalve MC, Yañez-Falcón AR et al (2017) αβ'-NAC cooperates with Sam37 to mediate early stages of mitochondrial protein import. FEBS J 284(5):814–830. https://doi.org/10.1111/febs.14024

75. Gamerdinger M, Hanebuth MA, Frickey T et al (2015) The principle of antagonism ensures protein targeting specificity at the endoplasmic reticulum. Science 348(6231):201–207. https://doi.org/10.1126/science.aaa5335

76. Williams CC, Jan CH, Weissman JS (2014) Targeting and plas-ticity of mitochondrial proteins revealed by proximity-specific ribosome profiling. Science 346(6210):748–751. https://doi.org/10.1126/science.1257522

77. Jan CH, Williams CC, Weissman JS (2014) Principles of ER cotranslational translocation revealed by proximity-specific ribosome profiling. Science 346(6210):1257521. https://doi.org/10.1126/science.1257521

78. Karniely S, Regev-Rudzki N, Pines O (2006) The prese-quence of fumarase is exposed to the cytosol during import into mitochondria. J Mol Biol 358(2):396–405. https://doi.org/10.1016/j.jmb.2006.02.023

79. Yogev O, Karniely S, Pines O (2007) Translation-coupled translocation of yeast fumarase into mitochondria in vivo. J Biol Chem 282(40):29222–29229. https://doi.org/10.1074/jbc.M704201200

80. Sass E, Karniely S, Pines O (2003) Folding of fumarase during mitochondrial import determines its dual targeting in yeast. J Biol Chem 278(46):45109–45116. https://doi.org/10.1074/jbc.M302344200

81. Hansen KG, Aviram N, Laborenz J et al (2018) An ER sur-face retrieval pathway safeguards the import of mitochondrial

membrane proteins in yeast. Science 361(6407):1118–1122. https://doi.org/10.1126/science.aar8174

82. Papic D, Elbaz-Alon Y, Koerdt SN et al (2013) The role of Djp1 in import of the mitochondrial protein Mim1 demonstrates specificity between a cochaperone and its substrate protein. Mol Cell Biol 33(20):4083–4094. https://doi.org/10.1128/MCB.00227-13

83. Jeong H, Park J, Jun Y et al (2017) Crystal structures of Mmm1 and Mdm12-Mmm1 reveal mechanistic insight into phospholipid trafficking at ER-mitochondria contact sites. Proc Natl Acad Sci USA 114(45):E9502–E9511. https://doi.org/10.1073/pnas.1715592114

84. Hirabayashi Y, Kwon S-K, Paek H et al (2017) ER-mitochondria tethering by PDZD8 regulates Ca2 + dynamics in mammalian neurons. Science 358(6363):623–630. https://doi.org/10.1126/science.aan6009

85. John Peter AT, Herrmann B, Antunes D et al (2017) Vps13-Mcp1 interact at vacuole-mitochondria interfaces and bypass ER-mitochondria contact sites. J Cell Biol 216(10):3219–3229. https://doi.org/10.1083/jcb.201610055

86. Kornmann B, Currie E, Collins SR et al (2009) An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 325(5939):477–481. https://doi.org/10.1126/science.1175088

87. Costa EA, Subramanian K, Nunnari J et al (2018) Defining the physiological role of SRP in protein-targeting efficiency and specificity. Science 359(6376):689–692. https://doi.org/10.1126/science.aar3607

88. Vitali DG, Sinzel M, Bulthuis EP et al (2018) The GET pathway can increase the risk of mitochondrial outer membrane proteins to be mistargeted to the ER. J Cell Sci 131(10):jcs211110. https://doi.org/10.1242/jcs.211110

89. Kaewsapsak P, Shechner DM, Mallard W et al (2017) Live-cell mapping of organelle-associated RNAs via proximity biotinylation combined with protein-RNA crosslinking. Elife 6:e29224. https://doi.org/10.7554/eLife.29224

90. Aviram N, Ast T, Costa EA et al (2016) The SND proteins constitute an alternative targeting route to the endoplasmic reticulum. Nature 540(7631):134–138. https://doi.org/10.1038/nature20169

91. Schuldiner M, Metz J, Schmid V et al (2008) The GET complex mediates insertion of tail-anchored proteins into the ER membrane. Cell 134(4):634–645. https://doi.org/10.1016/j.cell.2008.06.025

92. Bausewein T, Mills DJ, Langer JD et al (2017) Cryo-EM structure of the TOM core complex from *Neurospora crassa*. Cell 170(4):693–700.e7. https://doi.org/10.1016/j.cell.2017.07.012

93. Model K, Meisinger C, Kühlbrandt W (2008) Cryo-electron microscopy structure of a yeast mitochondrial preprotein translocase. J Mol Biol 383(5):1049–1057. https://doi.org/10.1016/j.jmb.2008.07.087

94. Shiota T, Imai K, Qiu J et al (2015) Molecular architecture of the active mitochondrial protein gate. Science 349(6255):1544–1548. https://doi.org/10.1126/science.aac6428

95. Yamamoto H, Fukui K, Takahashi H et al (2009) Roles of Tom70 in import of presequence-containing mitochondrial proteins. J Biol Chem 284(46):31635–31646. https://doi.org/10.1074/jbc.M109.041756

96. Backes S, Herrmann JM (2017) Protein translocation into the intermembrane space and matrix of mitochondria: mechanisms and driving forces. Front Mol Biosci 4:83. https://doi.org/10.3389/fmolb.2017.00083

97. Melin J, Kilisch M, Neumann P et al (2015) A presequence-binding groove in Tom70 supports import of Mdl1 into mitochondria. Biochim Biophys Acta 1853(8):1850–1859. https://doi.org/10.1016/j.bbamcr.2015.04.021

98. Fan ACY, Kozlov G, Hoegl A et al (2011) Interaction between the human mitochondrial import receptors Tom20 and Tom70 in vitro suggests a chaperone displacement mechanism. J Biol Chem 286(37):32208–32219. https://doi.org/10.1074/jbc.M111.280446

99. Abe Y, Shodai T, Muto T et al (2000) Structural basis of presequence recognition by the mitochondrial protein import receptor Tom20. Cell 100(5):551–560. https://doi.org/10.1016/S0092-8674(00)80691-1

100. Yamamoto H, Itoh N, Kawano S et al (2011) Dual role of the receptor Tom20 in specificity and efficiency of protein import into mitochondria. Proc Natl Acad Sci USA 108(1):91–96. https://doi.org/10.1073/pnas.1014918108

101. Perry AJ, Hulett JM, Likić VA et al (2006) Convergent evolution of receptors for protein import into mitochondria. Curr Biol 16(3):221–229. https://doi.org/10.1016/j.cub.2005.12.034

102. Garg S, Stölting J, Zimorski V et al (2015) Conservation of transit peptide-independent protein import into the mitochondrial and hydrogenosomal matrix. Genome Biol Evol 7(9):2716–2726. https://doi.org/10.1093/gbe/evv175

103. Mani J, Desy S, Niemann M et al (2015) Mitochondrial protein import receptors in Kinetoplastids reveal convergent evolution over large phylogenetic distances. Nat Commun 6:6646. https://doi.org/10.1038/ncomms7646

104. Harbauer AB, Opalińska M, Gerbeth C et al (2014) Mitochondria. Cell cycle-dependent regulation of mitochondrial preprotein translocase. Science 346(6213):1109–1113. https://doi.org/10.1126/science.1261253

105. Schmidt O, Harbauer AB, Rao S et al (2011) Regulation of mitochondrial protein import by cytosolic kinases. Cell 144(2):227–239. https://doi.org/10.1016/j.cell.2010.12.015

106. Esaki M, Shimizu H, Ono T et al (2004) Mitochondrial protein import. Requirement of presequence elements and tom components for precursor binding to the TOM complex. J Biol Chem 279(44):45701–45707. https://doi.org/10.1074/jbc.M404591200

107. Komiya T, Rospert S, Koehler C et al (1998) Interaction of mitochondrial targeting signals with acidic receptor domains along the protein import pathway: evidence for the 'acid chain' hypothesis. EMBO J 17(14):3886–3898. https://doi.org/10.1093/emboj/17.14.3886

108. Chacinska A, Koehler CM, Milenkovic D et al (2009) Importing mitochondrial proteins: machineries and mechanisms. Cell 138(4):628–644. https://doi.org/10.1016/j.cell.2009.08.005

109. Neupert W, Herrmann JM (2007) Translocation of proteins into mitochondria. Annu Rev Biochem 76:723–749. https://doi.org/10.1146/annurev.biochem.76.052705.163409

110. Melin J, Schulz C, Wrobel L et al (2014) Presequence recognition by the tom40 channel contributes to precursor translocation into the mitochondrial matrix. Mol Cell Biol 34(18):3473–3485. https://doi.org/10.1128/MCB.00433-14

111. Albrecht R, Rehling P, Chacinska A et al (2006) The Tim21 binding domain connects the preprotein translocases of both mitochondrial membranes. EMBO Rep 7(12):1233–1238. https://doi.org/10.1038/sj.embor.7400828

112. Shiota T, Mabuchi H, Tanaka-Yamano S et al (2011) In vivo protein-interaction mapping of a mitochondrial translocator protein Tom22 at work. Proc Natl Acad Sci USA 108(37):15179–15183. https://doi.org/10.1073/pnas.1105921108

113. Mokranjac D, Paschen SA, Kozany C et al (2003) Tim50, a novel component of the TIM23 preprotein translocase of mitochondria. EMBO J 22(4):816–825. https://doi.org/10.1093/emboj/cdg090

114. Ramesh A, Peleh V, Martinez-Caballero S et al (2016) A disulfide bond in the TIM23 complex is crucial for voltage gating and mitochondrial protein import. J Cell Biol 214(4):417–431. https://doi.org/10.1083/jcb.201602074

115. Demishtein-Zohary K, Günsel U, Marom M et al (2017) Role of Tim17 in coupling the import motor to the translocation channel of the mitochondrial presequence translocase. Elife 6:e22696. https://doi.org/10.7554/eLife.22696

116. Demishtein-Zohary K, Marom M, Neupert W et al (2015) GxxxG motifs hold the TIM23 complex together. FEBS J 282(11):2178–2186. https://doi.org/10.1111/febs.13266

117. Schendzielorz AB, Schulz C, Lytovchenko O et al (2017) Two distinct membrane potential-dependent steps drive mitochondrial matrix protein translocation. J Cell Biol 216(1):83–92. https://doi.org/10.1083/jcb.201607066

118. Meinecke M, Wagner R, Kovermann P et al (2006) Tim50 maintains the permeability barrier of the mitochondrial inner membrane. Science 312(5779):1523–1526. https://doi.org/10.1126/science.1127628

119. Lytovchenko O, Melin J, Schulz C et al (2013) Signal recognition initiates reorganization of the presequence translocase during protein import. EMBO J 32(6):886–898. https://doi.org/10.1038/emboj.2013.23

120. Chacinska A, Lind M, Frazier AE et al (2005) Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17. Cell 120(6):817–829. https://doi.org/10.1016/j.cell.2005.01.011

121. Los Rios P de, Ben-Zvi A, Slutsky O et al (2006) Hsp70 chaperones accelerate protein translocation and the unfolding of stable protein aggregates by entropic pulling. Proc Natl Acad Sci USA 103(16):6166–6171. https://doi.org/10.1073/pnas.0510496103

122. Ting S-Y, Yan NL, Schilke BA et al (2017) Dual interaction of scaffold protein Tim44 of mitochondrial import motor with channel-forming translocase subunit Tim23. Elife 6:e23609. https://doi.org/10.7554/eLife.23609

123. Miyata N, Tang Z, Conti MA et al (2017) Adaptation of a genetic screen reveals an inhibitor for mitochondrial protein import component Tim44. J Biol Chem 292(13):5429–5442. https://doi.org/10.1074/jbc.M116.770131

124. Banerjee R, Gladkova C, Mapa K et al (2015) Protein translocation channel of mitochondrial inner membrane and matrix-exposed import motor communicate via two-domain coupling protein. Elife 4:e11897. https://doi.org/10.7554/eLife.11897

125. D'Silva PD, Schilke B, Walter W et al (2003) J protein cochaperone of the mitochondrial inner membrane required for protein import into the mitochondrial matrix. Proc Natl Acad Sci USA 100(24):13839–13844. https://doi.org/10.1073/pnas.1936150100

126. Krayl M, Lim JH, Martin F et al (2007) A cooperative action of the ATP-dependent import motor complex and the inner membrane potential drives mitochondrial preprotein import. Mol Cell Biol 27(2):411–425. https://doi.org/10.1128/MCB.01391-06

127. Burkhart JM, Taskin AA, Zahedi RP et al (2015) Quantitative profiling for substrates of the mitochondrial presequence processing protease reveals a set of nonsubstrate proteins increased upon proteotoxic stress. J Proteome Res 14(11):4550–4563. https://doi.org/10.1021/acs.jproteome.5b00327

128. Ieva R, Schrempp SG, Opaliński L et al (2014) Mgr2 functions as lateral gatekeeper for preprotein sorting in the mitochondrial inner membrane. Mol Cell 56(5):641–652. https://doi.org/10.1016/j.molcel.2014.10.010

129. Bömer U, Meijer M, Guiard B et al (1997) The sorting route of cytochrome b2 branches from the general mitochondrial import pathway at the preprotein translocase of the inner membrane. J Biol Chem 272(48):30439–30446. https://doi.org/10.1074/jbc.272.48.30439

130. Rojo EE, Guiard B, Neupert W et al (1998) Sorting of D-lactate dehydrogenase to the inner membrane of mitochondria. Analysis of topogenic signal and energetic requirements. J Biol Chem 273(14):8040–8047

131. Zanphorlin LM, Lima TB, Wong MJ et al (2016) Heat shock protein 90 kDa (Hsp90) has a second functional interaction site with the mitochondrial import receptor Tom70. J Biol Chem 291(36):18620–18631. https://doi.org/10.1074/jbc.M115.710137

132. Webb CT, Gorman MA, Lazarou M et al (2006) Crystal structure of the mitochondrial chaperone TIM9.10 reveals a six-bladed alpha-propeller. Mol Cell 21(1):123–133. https://doi.org/10.1016/j.molcel.2005.11.010

133. Baker MJ, Webb CT, Stroud DA et al (2009) Structural and functional requirements for activity of the Tim9–Tim10 complex in mitochondrial protein import. Mol Biol Cell 20(3):769–779. https://doi.org/10.1091/mbc.E08-09-0903

134. Hasson SA, Damoiseaux R, Glavin JD et al (2010) Substrate specificity of the TIM22 mitochondrial import pathway revealed with small molecule inhibitor of protein translocation. Proc Natl Acad Sci USA 107(21):9578–9583. https://doi.org/10.1073/pnas.0914387107

135. Weinhäupl K, Lindau C, Hessel A et al (2018) Structural basis of membrane protein chaperoning through the mitochondrial intermembrane space. Cell 175(5):1365–1379.e25. https://doi.org/10.1016/j.cell.2018.10.039

136. Sirrenberg C, Endres M, Fölsch H et al (1998) Carrier protein import into mitochondria mediated by the intermembrane proteins Tim10/Mrs11 and Tim12/Mrs5. Nature 391:912. https://doi.org/10.1038/36136

137. Callegari S, Richter F, Chojnacka K et al (2016) TIM29 is a subunit of the human carrier translocase required for protein transport. FEBS Lett 590(23):4147–4158. https://doi.org/10.1002/1873-3468.12450

138. Kang Y, Baker MJ, Liem M et al (2016) Tim29 is a novel subunit of the human TIM22 translocase and is involved in complex assembly and stability. Elife. https://doi.org/10.7554/eLife.17463

139. Pacheu-Grau D, Callegari S, Emperador S et al (2018) Mutations of the mitochondrial carrier translocase channel subunit TIM22 cause early-onset mitochondrial myopathy. Hum Mol Genet 27(23):4135–4144. https://doi.org/10.1093/hmg/ddy305

140. Kang Y, Stroud DA, Baker MJ et al (2017) Sengers syndrome-associated mitochondrial acylglycerol kinase is a subunit of the human TIM22 protein import complex. Mol Cell 67(3):457–470.e5. https://doi.org/10.1016/j.molcel.2017.06.014

141. Vukotic M, Nolte H, König T et al (2017) Acylglycerol kinase mutated in sengers syndrome is a subunit of the TIM22 protein translocase in mitochondria. Mol Cell 67(3):471–483.e7. https://doi.org/10.1016/j.molcel.2017.06.013

142. Gebert N, Gebert M, Oeljeklaus S et al (2011) Dual function of Sdh3 in the respiratory chain and TIM22 protein translocase of the mitochondrial inner membrane. Mol Cell 44(5):811–818. https://doi.org/10.1016/j.molcel.2011.09.025

143. Höhr AIC, Lindau C, Wirth C et al (2018) Membrane protein insertion through a mitochondrial β-barrel gate. Science 359(6373):eaah6834. https://doi.org/10.1126/science.aah6834

144. Wiedemann N, Kozjak V, Chacinska A et al (2003) Machinery for protein sorting and assembly in the mitochondrial outer membrane. Nature 424:565. https://doi.org/10.1038/nature01753

145. Becker T, Guiard B, Thornton N et al (2010) Assembly of the mitochondrial protein import channel: role of Tom5 in two-stage interaction of Tom40 with the SAM complex. Mol Biol Cell 21(18):3106–3113. https://doi.org/10.1091/mbc.E10-06-0518

146. Mesecke N, Terziyska N, Kozany C et al (2005) A disulfide relay system in the intermembrane space of mitochondria that mediates protein import. Cell 121(7):1059–1069. https://doi.org/10.1016/j.cell.2005.04.011

147. Banci L, Bertini I, Ciofi-Baffoni S et al (2012) Structural characterization of CHCHD5 and CHCHD7: two atypical human twin CX9C proteins. J Struct Biol 180(1):190–200. https://doi.org/10.1016/j.jsb.2012.07.007

148. Hofmann S, Rothbauer U, Mühlenbein N et al (2005) Functional and mutational characterization of human MIA40 acting during import into the mitochondrial intermembrane space. J Mol Biol 353(3):517–528. https://doi.org/10.1016/j.jmb.2005.08.064

149. Klöppel C, Suzuki Y, Kojer K et al (2011) Mia40-dependent oxidation of cysteines in domain I of Ccs1 controls its distribution between mitochondria and the cytosol. Mol Biol Cell 22(20):3749–3757. https://doi.org/10.1091/mbc.E11-04-0293

150. Terziyska N, Grumbt B, Kozany C et al (2009) Structural and functional roles of the conserved cysteine residues of the redox-regulated import receptor Mia40 in the intermembrane space of mitochondria. J Biol Chem 284(3):1353–1363. https://doi.org/10.1074/jbc.M805035200

151. Fischer M, Horn S, Belkacemi A et al (2013) Protein import and oxidative folding in the mitochondrial intermembrane space of intact mammalian cells. Mol Biol Cell 24(14):2160–2170. https://doi.org/10.1091/mbc.E12-12-0862

152. Weckbecker D, Longen S, Riemer J et al (2012) Atp23 biogenesis reveals a chaperone-like folding activity of Mia40 in the IMS of mitochondria. EMBO J 31(22):4348–4358. https://doi.org/10.1038/emboj.2012.263

153. Petrungaro C, Zimmermann KM, Küttner V et al (2015) The Ca(2+)-Dependent Release of the Mia40-Induced MICU1-MICU2 Dimer from MCU Regulates Mitochondrial Ca(2+) Uptake. Cell Metab 22(4):721–733. https://doi.org/10.1016/j.cmet.2015.08.019

154. Wurm CA, Neumann D, Lauterbach MA et al (2011) Nanoscale distribution of mitochondrial import receptor Tom20 is adjusted to cellular conditions and exhibits an inner-cellular gradient. Proc Natl Acad Sci USA 108(33):13546–13551. https://doi.org/10.1073/pnas.1107553108

155. Bohnert M, Zerbes RM, Davies KM et al (2015) Central role of Mic10 in the mitochondrial contact site and cristae organizing system. Cell Metab 21(5):747–755. https://doi.org/10.1016/j.cmet.2015.04.007

156. Barbot M, Jans DC, Schulz C et al (2015) Mic10 oligomerizes to bend mitochondrial inner membranes at cristae junctions. Cell Metab 21(5):756–763. https://doi.org/10.1016/j.cmet.2015.04.006

157. Körner C, Barrera M, Dukanovic J et al (2012) The C-terminal domain of Fcj1 is required for formation of crista junctions and interacts with the TOB/SAM complex in mitochondria. Mol Biol Cell 23(11):2143–2155. https://doi.org/10.1091/mbc.E11-10-0831

158. Herrmann JM (2011) MINOS is plus: a Mitofilin complex for mitochondrial membrane contacts. Dev Cell 21(4):599–600. https://doi.org/10.1016/j.devcel.2011.09.013

159. Varabyova A, Topf U, Kwiatkowska P et al (2013) Mia40 and MINOS act in parallel with Ccs1 in the biogenesis of mitochondrial Sod1. FEBS J 280(20):4943–4959. https://doi.org/10.1111/febs.12409

160. Malsburg K von der, Müller JM, Bohnert M et al (2011) Dual role of mitofilin in mitochondrial membrane organization and protein biogenesis. Dev Cell 21(4):694–707. https://doi.org/10.1016/j.devcel.2011.08.026

161. Waegemann K, Popov-Čeleketić D, Neupert W et al (2015) Cooperation of TOM and TIM23 complexes during translocation of proteins into mitochondria. J Mol Biol 427(5):1075–1084. https://doi.org/10.1016/j.jmb.2014.07.015

162. Ellenrieder L, Opaliński Ł, Becker L et al (2016) Separating mitochondrial protein assembly and endoplasmic reticulum tethering by selective coupling of Mdm10. Nat Commun 7:13021. https://doi.org/10.1038/ncomms13021

163. Flinner N, Ellenrieder L, Stiller SB et al (2013) Mdm10 is an ancient eukaryotic porin co-occurring with the ERMES complex. Biochim Biophys Acta 1833(12):3314–3325. https://doi.org/10.1016/j.bbamcr.2013.10.006

164. Wideman JG, Go NE, Klein A et al (2010) Roles of the Mdm10, Tom7, Mdm12, and Mmm1 proteins in the assembly of mitochondrial outer membrane proteins in *Neurospora crassa*. Mol Biol Cell 21(10):1725–1736. https://doi.org/10.1091/mbc.e09-10-0844

165. Elbaz-Alon Y, Eisenberg-Bord M, Shinder V et al (2015) Lam6 regulates the extent of contacts between organelles. Cell Rep 12(1):7–14. https://doi.org/10.1016/j.celrep.2015.06.022

166. González Montoro A, Auffarth K, Hönscher C et al (2018) Vps39 interacts with Tom40 to establish one of two functionally distinct vacuole-mitochondria contact sites. Dev Cell 45(5):621–636.e7. https://doi.org/10.1016/j.devcel.2018.05.011