

Review

Open Access

Sebastian P. Straub, Sebastian B. Stiller, Nils Wiedemann and Nikolaus Pfanner^{a,*}

Dynamic organization of the mitochondrial protein import machinery

DOI 10.1515/hsz-2016-0145

Received February 27, 2016; accepted May 17, 2016; previously published online June 10, 2016

Abstract: Mitochondria contain elaborate machineries for the import of precursor proteins from the cytosol. The translocase of the outer mitochondrial membrane (TOM) performs the initial import of precursor proteins and transfers the precursors to downstream translocases, including the presequence translocase and the carrier translocase of the inner membrane, the mitochondrial import and assembly machinery of the intermembrane space, and the sorting and assembly machinery of the outer membrane. Although the protein translocases can function as separate entities *in vitro*, recent studies revealed a close and dynamic cooperation of the protein import machineries to facilitate efficient transfer of precursor proteins *in vivo*. In addition, protein translocases were found to transiently interact with distinct machineries that function in the respiratory chain or in the maintenance of mitochondrial membrane architecture. Mitochondrial protein import is embedded in a regulatory network that ensures protein biogenesis, membrane dynamics, bioenergetic activity and quality control.

Keywords: MIA; MICOS; protein sorting; protein translocase; SAM; TIM; TOM.

Introduction

Mitochondria are essential cell organelles in virtually all eukaryotic cells with a large variety of functions from bioenergetics to metabolism, signaling and apoptosis (Newmeyer and Ferguson-Miller, 2003; McBride et al., 2006; Neupert and Herrmann, 2007; Lill, 2009; Galluzzi et al., 2012; Nunnari and Suomalainen, 2012; Harbauer et al., 2014b; Raimundo, 2014; Strich, 2015). The mitochondrial proteome comprises more than 1,000 different proteins (Sickmann et al., 2003; Prokisch et al., 2004; Reinders et al., 2006; Pagliarini et al., 2008; Calvo and Mootha, 2010; Schmidt et al., 2010). Mitochondria are derived from a prokaryotic ancestor related to α -proteobacteria and have retained their own genome and protein synthesis machinery in the matrix (Margulis, 1970; Gray et al., 1999; Dolezal et al., 2006; Kutik et al., 2009). The mitochondrial genome typically encodes a few hydrophobic subunits of the oxidative phosphorylation machinery (Foury et al., 1998; Gray et al., 1999; Allen, 2003; Ott and Herrmann, 2010). Thus, in most organisms only ~1% of mitochondrial proteins are synthesized in the matrix and are exported into the inner membrane (Figure 1).

The majority of mitochondrial proteins are encoded by nuclear genes (Sickmann et al., 2003; Reinders et al., 2006; Neupert and Herrmann, 2007; Pagliarini et al., 2008; Chacinska et al., 2009). The genes were either transferred from the primordial mitochondrial genome to the nucleus or new genes coding for mitochondrial proteins evolved in the eukaryotic cell. Thus, most mitochondrial proteins are synthesized in the cytosol as precursor forms. The precursor proteins are endowed with targeting information to direct the proteins to mitochondria and into the mitochondrial subcompartments (Neupert and Herrmann, 2007; Chacinska et al., 2009). The most frequent targeting signal is an amino-terminal cleavable presequence, found in about 60% of all mitochondrial proteins (Schatz and Dobberstein, 1996; Vögtle et al., 2009; Schulz et al., 2015). Non-cleavable precursor proteins contain different kinds of internal targeting signals located in the mature

^aNikolaus Pfanner is the recipient of the 2015 Otto Warburg Medal.

***Corresponding author: Nikolaus Pfanner**, Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany; and BIOS Centre for Biological Signalling Studies, University of Freiburg, 79104 Freiburg, Germany, e-mail: nikolaus.pfanner@biochemie.uni-freiburg.de

Sebastian P. Straub and Sebastian B. Stiller: Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany; and Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany

Nils Wiedemann: Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany; and BIOS Centre for Biological Signalling Studies, University of Freiburg, 79104 Freiburg, Germany

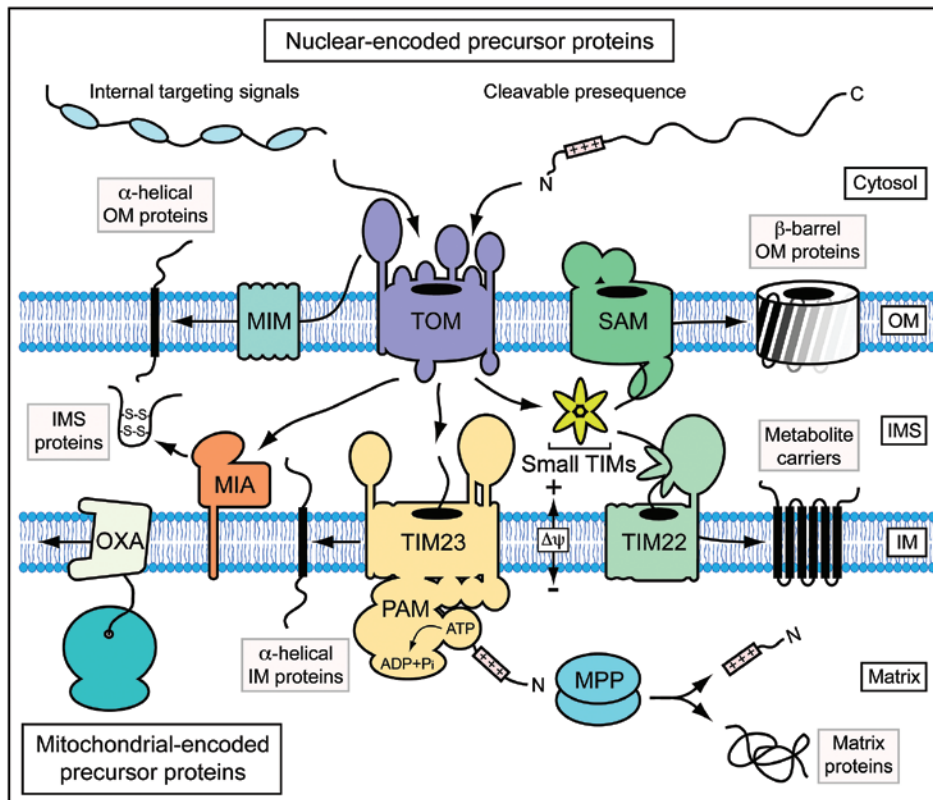


Figure 1: Protein biogenesis pathways of mitochondria.

The large majority of mitochondrial proteins are synthesized in the cytosol and imported by the translocase of the outer membrane (TOM). Downstream machineries are responsible for sorting of the precursor proteins to the intramitochondrial compartments outer membrane (OM), intermembrane space (IMS), inner membrane (IM) and matrix. The mitochondrial IMS import and assembly (MIA) machinery promotes import and oxidation of proteins by insertion of disulfide bonds. The presequence translocase of the IM (TIM23) can sort precursor proteins into the inner membrane or, in cooperation with the presequence translocase-associated motor (PAM), into the matrix. The mitochondrial processing peptidase (MPP) cleaves off the amino-terminal presequences. The small TIM chaperones in the IMS deliver β-barrel precursors to the sorting and assembly machinery (SAM) of the OM and non-cleavable precursors of multi-spanning inner membrane proteins to the carrier translocase of the IM (TIM22). The membrane potential ($\Delta\psi$) drives protein translocation by TIM23 as well as TIM22. Some α-helical OM proteins can bypass the TOM channel and are inserted into the OM by the mitochondrial import (MIM) complex. Mitochondrial-encoded proteins are exported into the IM by the oxidase assembly (OXA) translocase.

portion of the proteins (Chacinska et al., 2009). Here we first provide a short overview of the five major pathways of protein translocation into mitochondria and then discuss the emerging principle that the protein translocases are embedded in a network of different machineries controlling mitochondrial bioenergetics, architecture and dynamics.

Principles of mitochondrial protein import

The mitochondrial outer membrane forms the barrier between mitochondria and the cytosol. All nuclear-encoded precursors of mitochondrial proteins have to

be translocated into or across the outer membrane. The translocase of the outer mitochondrial membrane (TOM) is responsible for importing most proteins into mitochondria (Ryan et al., 2000; Dolezal et al., 2006; Chacinska et al., 2009; Mokranjac and Neupert, 2009; Schmidt et al., 2010; Endo et al., 2011; Stojanovski et al., 2012a; Wenz et al., 2015b). The TOM receptors specifically recognize the precursor proteins via their targeting signals (Figure 1). The protein-conducting channel Tom40 is subsequently used by the vast majority of precursor proteins.

Tom40 translocates precursor proteins that are destined for at least four different protein import pathways (Figure 1). (i) More than half of all mitochondrial proteins are imported by the presequence pathway (Vögtle et al., 2009). The presequences direct the proteins through

the TOM complex to the presequence translocase of the inner mitochondrial membrane (TIM23) (Endo and Yamano, 2009; Mokranjac and Neupert, 2010; van der Laan et al., 2010; Schulz et al., 2015). The mitochondrial processing peptidase (MPP) removes the presequences (Hawlitsek et al., 1988; Taylor et al., 2001; Mossmann et al., 2012; Teixeira and Glaser, 2013) and the proteins are imported into the matrix with the help of the presequence translocase-associated motor (PAM) (Kang et al., 1990; Matouschek et al., 1997; D'Silva et al., 2003; Mokranjac et al., 2003; Truscott et al., 2003; Schulz et al., 2015). Two energy sources drive protein translocation. The membrane potential ($\Delta\psi$) across the inner membrane activates the channel protein Tim23 and moves the positively charged presequences in an electrophoretic manner (Martin et al., 1991; Truscott et al., 2001; Shariff et al., 2004; Krayl et al., 2007; van der Laan et al., 2007; Malhotra et al., 2013). PAM with the matrix heat shock protein of ~70 kDa (mtHsp70) is driven by ATP (Kang et al., 1990; Wickner and Schekman, 2005). Some precursor proteins that contain hydrophobic stop transfer sequences are arrested in the inner membrane and are laterally released into the inner membrane (Glick et al., 1992; Bömer et al., 1997; Ieva et al., 2014). (ii) The large carrier family of the inner membrane includes numerous metabolite carriers that contain six transmembrane segments. The non-cleavable precursors of metabolite carriers are also imported by TOM, yet are then transferred to hexameric chaperones in the intermembrane space, the so-called small TIM chaperones (Neupert and Herrmann, 2007; Chacinska et al., 2009). Insertion of the precursors into the inner membrane is mediated by the carrier translocase of the inner membrane (TIM22) (Rehling et al., 2003). The TIM22 complex is driven by $\Delta\psi$, yet does not require ATP-dependent chaperones. (iii) Many intermembrane space proteins contain characteristic cysteine motifs (Koehler, 2004; Gabriel et al., 2007). Upon passage through the Tom40 channel, these precursor proteins interact with the mitochondrial intermembrane space import and assembly (MIA) machinery (Stojanovski et al., 2012b; Ceh-Pavia et al., 2013). The core component Mia40 recognizes the precursor proteins and mediates the formation of disulfide bonds in the imported proteins. (iv) The precursors of β -barrel proteins of the outer membrane are translocated through the TOM channel to the small TIM chaperones and are inserted into the outer membrane by the sorting and assembly machinery (SAM, also termed TOB complex) (Walther and Rapaport, 2009; Höhr et al., 2015).

In addition to β -barrel proteins, the mitochondrial outer membrane contains several types of membrane

proteins with α -helical transmembrane segments (Walther and Rapaport, 2009). The mechanisms of insertion of these proteins into the outer membrane are only understood in part. The mitochondrial import (MIM) complex has been found to support membrane insertion of a number of outer membrane proteins, in particular proteins with amino-terminal or multiple transmembrane segments (Becker et al., 2008, 2011a; Popov-Čeleketić et al., 2008b; Hulett et al., 2008; Papić et al., 2011; Dimmer et al., 2012; Wenz et al., 2014). TOM receptors can help in the recognition of α -helical precursor proteins. The lipid composition of the outer membrane is important for the biogenesis of outer membrane proteins (Gebert et al., 2009; Becker et al., 2013; Horvath and Daum, 2013; Vögtle et al., 2015). It has been proposed that some precursor proteins, in particular proteins with a carboxy-terminal membrane anchor (tail-anchored proteins), do not use proteinaceous machineries but just depend on the proper lipid composition for insertion into the outer membrane (Setoguchi et al., 2006; Kemper et al., 2008; Krumpke et al., 2012; Merklinger et al., 2012).

The TOM complex interacts with downstream translocases

The TOM complex forms the major entry site for mitochondrial proteins. The receptors Tom20 and Tom22 mainly recognize precursor proteins with presequences (Moczko et al., 1997; Komiya et al., 1998; van Wilpe et al., 1999; Abe et al., 2000; Saitoh et al., 2007; Yamano et al., 2008; Shiota et al., 2011). Tom70 shows a preference for precursor with hydrophobic segments such as the metabolite carriers (Wiedemann et al., 2001; Wu and Sha, 2006; Yamamoto et al., 2009). The receptors deliver the precursor proteins to Tom40 that forms a β -barrel channel for translocation of different kinds of proteins into mitochondria (Hill et al., 1998; Ahting et al., 2001; Suzuki et al., 2004; Becker et al., 2005). The inside of the channel provides acidic binding sites for positively charged presequences and hydrophobic regions for hydrophobic precursors. Thus, although hydrophilic and hydrophobic precursor proteins use the same channel, they interact with different residues inside the channel pore (Shiota et al., 2015a).

The TOM complex is crucial for transferring the precursor proteins in a competent state to downstream translocases. Recent studies revealed that TOM directly interacts with proteinaceous machineries of at least three different import pathways in a dynamic manner. (i) The

amino-terminal segment of Tom40 that is located in front of its β -barrel domain shows a remarkable topology. The segment passes from the cytosolic side through the β -barrel channel interior to the intermembrane space side where it transiently interacts with the small TIM chaperones (Figure 2A). The direct interaction between Tom40 and the chaperone complex of the intermembrane space promotes efficient transfer of hydrophobic precursor segments of metabolite carriers from the channel interior to the protective chaperone environment (Shiota et al., 2015a). The small TIM chaperones deliver the carrier precursors to the TIM22 complex of the inner membrane (Rehling et al., 2003). Thus, a misfolding or aggregation of metabolite carriers is prevented. The dynamic TOM-small TIM interaction likely also helps in the transfer of β -barrel precursors, yet this has not been shown experimentally so far. (ii) For the transfer of β -barrel precursors, a further TOM interaction was identified. The TOM complex forms a transient supercomplex with the SAM complex (Figure 2A). The receptor Tom22 and the peripheral SAM subunit Sam37 interact on the cytosolic side of the outer

membrane and recruit a fraction of TOM and SAM complexes into a TOM-SAM supercomplex that promotes the efficient transfer of β -barrel precursors (Qiu et al., 2013; Wenz et al., 2015a). The TOM complex thus undergoes interactions on each side of the outer membrane in order to position the small TIM chaperones and SAM in its direct vicinity. (iii) The TOM complex also forms a supercomplex with the TIM23 complex. This two-membrane-spanning supercomplex is stabilized by a precursor protein in transit (Figure 2B) (Dekker et al., 1997; Sirrenberg et al., 1997; Geissler et al., 2002; Chacinska et al., 2003, 2010; Frazier et al., 2003; Popov-Čeleketić et al., 2008a) and involves the intermembrane space domain of Tom22 as well as several Tim proteins that expose domains to the intermembrane space: Tim50, Tim23 and Tim21 (Moczko et al., 1997; Geissler et al., 2002; Yamamoto et al., 2002; Chacinska et al., 2005; Mokranjac et al., 2005, 2009; Meinecke et al., 2006; Alder et al., 2008; Tamura et al., 2009; de la Cruz et al., 2010; Bajaj et al., 2014; Waegemann et al., 2015). Tim50 functions as a receptor in the intermembrane space and accepts precursor proteins after

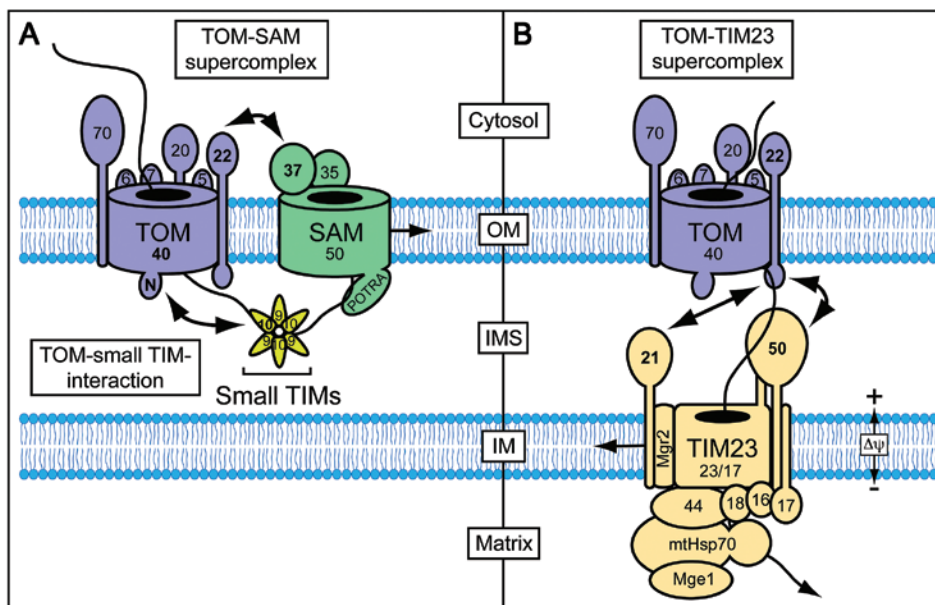


Figure 2: TOM cooperates with downstream transport machineries.

(A) The protein-conducting channel Tom40 of the outer membrane (OM) possesses an amino-terminal α -helical segment that passes through the interior of the Tom40 β -barrel channel to the intermembrane space (IMS) side and recruits small TIM chaperones. Thus, the precursors of membrane proteins, β -barrel proteins of the OM and metabolite carriers of the inner membrane (IM), can be directly transferred from the Tom40 channel to chaperones in the IMS to prevent misfolding and aggregation of hydrophobic precursor regions. In addition, TOM and SAM complexes form a transient supercomplex via the interaction of the receptor Tom22 and the peripheral membrane protein Sam37 to promote efficient transfer of precursor proteins. POTRA, polypeptide transport-associated domain of Sam50; SAM, sorting and assembly machinery; TOM, translocase of the outer membrane. (B) During translocation of preproteins, TOM and TIM23 form a two membrane-spanning supercomplex. The intermembrane space domain of Tom22 and Tim proteins with domains in the IMS, in particular Tim50, Tim21 and Tim23, participate in the formation, regulation and/or dissociation of the TOM-TIM23 supercomplex. $\Delta\psi$, membrane potential; TIM23, presequence translocase of the inner membrane.

their translocation through Tom40 and binding to the Tom22-intermembrane space domain (Schulz et al., 2011; Shiota et al., 2011; Waegemann et al., 2015). Tim21 plays a regulatory role and helps in displacing precursor proteins from Tom22 (Chacinska et al., 2005; Albrecht et al., 2006; Bolender et al., 2008). The amino-terminal domain of Tim23 cooperates with Tim50 and helps in precursor transfer to the protein import channel formed by the carboxy-terminal domain of Tim23 (Truscott et al., 2001; Tamura et al., 2009; Qian et al., 2011; Schulz et al., 2011; Li and Sha, 2015).

The TOM complex is more abundant than other protein translocases such as TIM23 or SAM. Thus only a fraction of TOM complexes are recruited into the individual supercomplexes (Dekker et al., 1997; Sirrenberg et al., 1997; Ghaemmaghani et al., 2003). Additionally, all supercomplexes involving protein translocases are of transient, dynamic nature. We propose that the translocase supercomplexes do not represent rigid entities but their formation and dissociation are part of the reaction cycle in transferring precursor proteins.

The TIM23 complex cooperates with the import motor and respiratory chain complexes

The TIM23 complex cooperates with machineries located in three different mitochondrial subcompartments: the TOM complex of the outer membrane (Figure 2B), respiratory chain complexes of the inner membrane, and the import motor PAM on the matrix side (Figure 3). In each case, dynamic supercomplexes are formed.

(i) The TOM-TIM23 supercomplex has been discussed in the previous chapter. (ii) The interaction of TIM23 with the respiratory chain complexes III (bc_1 complex) and IV (cytochrome *c* oxidase) is of particular importance for precursor proteins that are laterally sorted into the inner membrane. These precursors contain hydrophobic sorting signals that stop translocation in the inner membrane and induce a lateral opening of the TIM23 complex (Glick et al., 1992; Geissler et al., 2000; Neupert and Herrmann, 2007; Botelho et al., 2011; Park et al., 2013).

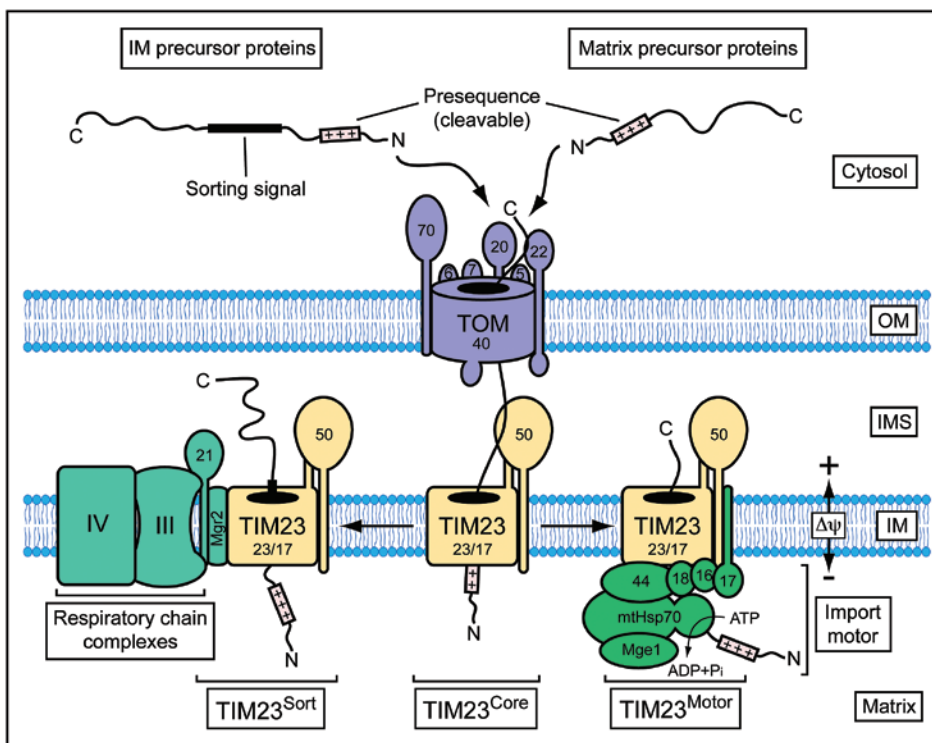


Figure 3: The presequence translocase interacts with different partner complexes.

The TIM23 core machinery interacts dynamically with the TOM complex, the respiratory chain complexes III and IV, and with modules of the import motor. Inner membrane (IM) precursor proteins with a hydrophobic stop transfer (sorting) signal are arrested in the TIM23 complex and are laterally released involving the gatekeeper Mgr2. Tim21 couples proton-pumping respiratory chain complexes to TIM23. Protein translocation into the matrix involves TIM23 and the import motor with the central chaperone mtHsp70 that is driven by ATP. Organization and mechanisms of the TIM23-PAM machinery are only understood in part. $\Delta\psi$, membrane potential; IMS, intermembrane space; OM, outer mitochondrial membrane; TIM23, presequence translocase of the inner membrane; TOM, translocase of the outer membrane.

The lateral opening involves Tim17, a partner protein of Tim23, and the lateral gatekeeper protein Mgr2 (Chacinska et al., 2005; van der Laan et al., 2007, 2013; Malhotra et al., 2013; Ieva et al., 2014; Steffen and Koehler, 2014). Tim21, which is associated with Mgr2, binds to the respiratory chain complexes and positions them in close vicinity of the TIM23 translocation channel (Figure 3) (van der Laan et al., 2006; Wiedemann et al., 2007; Gebert et al., 2012). Laterally sorted precursors depend on the membrane potential as energy source for driving translocation. The close vicinity to respiratory chain complexes is of particular importance when mitochondria have a lower energetic activity (lower membrane potential), e.g. under conditions of limited food supply. The exact mechanism has not been identified, but it is conceivable that translocases in direct vicinity of proton-pumping respiratory complexes experience a higher proton-motive force. A further function of the TIM23-respiratory chain link has been found in human mitochondria. Here Tim21 is part of an intermediate complex for assembly of respiratory complexes, termed MITRAC (mitochondrial translation regulation assembly intermediate of cytochrome *c* oxidase) (Mick et al., 2012). The MITRAC complex provides a link between protein sorting via the TIM23 complex, respiratory chain assembly and regulation of protein synthesis in the mitochondrial matrix. Additionally, the ADP/ATP carrier of the inner membrane associates with the TIM23 complex independently of the respiratory chain (Dienhart and Stuart, 2008; Mehnert et al., 2014). This association may also promote protein import under conditions of low respiratory activity. (iii) The TIM23 complex tightly cooperates with the import motor PAM. PAM is required for the translocation of polypeptide segments into the matrix. The central component mtHsp70 is driven by ATP binding and hydrolysis. mtHsp70 binds the polypeptides in transit and generates an import-driving activity by a combination of pulling and trapping of the polypeptides (Kang et al., 1990; Ungermann et al., 1996; Voos et al., 1996; Voisine et al., 1999; Liu et al., 2003). Four membrane-bound partner proteins/co-chaperones coordinate the activity of mtHsp70 at the presequence translocase. Tim44 functions as an adaptor molecule that links mtHsp70 to the translocation channel (Kronidou et al., 1994; Rassow et al., 1994; Schneider et al., 1994). The J-protein Pam18 (Tim14) stimulates the ATPase activity of mtHsp70 and the J-like protein Pam16 (Tim16) controls the stimulatory function of Pam18 (D'Silva et al., 2003; Truscott et al., 2003; Frazier et al., 2004; Kozany et al., 2004; Li et al., 2004; Mokranjac et al., 2006). Pam17 plays a regulatory role in the recruitment of motor modules to the presequence translocase (van der Laan et al., 2005; Hutu et al., 2008;

Popov-Čeleketić et al., 2008a). In addition, the soluble nucleotide exchange factor Mge1 stimulates the release of ADP from mtHsp70 and thus promotes the initiation of a new mtHsp70 reaction cycle (Laloraya et al., 1994; Westermann et al., 1995). The import motor is a highly dynamic machine and its exact molecular mechanism is still the subject of an ongoing debate.

The current evidence suggests that the TIM23 complex does not interact with all partner proteins simultaneously, but undergoes a reaction cycle where the association with partner complexes changes. In particular, the TIM23 complex either binds to the TOM complex (early phase of preprotein translocation from the outer membrane to the inner membrane) or to respiratory chain complexes (lateral sorting of preproteins) (Chacinska et al., 2005, 2010; van der Laan et al., 2006, 2007; Wiedemann et al., 2007; Dienhart and Stuart, 2008). The situation is more complex for the TIM23-PAM interaction where different models about the stability of the interaction are discussed. The models range from a permanent association of TIM23 and PAM to a dynamic interaction of different PAM modules with TIM23 (Chacinska et al., 2005, 2010; Popov-Čeleketić et al., 2008a; Ting et al., 2014). It is as yet unknown whether the three states of the TIM23 complex depicted in Figure 3 represent different snapshots during the TIM23 reaction cycle or if the TIM23 and PAM subunits remain largely associated and just undergo conformational changes. It is generally accepted, however, that Tim21 and Pam17 bind to the TIM23 complex in an antagonistic manner (van der Laan et al., 2005; Wiedemann et al., 2007; Popov-Čeleketić et al., 2008a). Tim21 is of particular importance for the recruitment of respiratory chain complexes during lateral sorting of precursors by TIM23 (van der Laan et al., 2006; Wiedemann et al., 2007), whereas Pam17 promotes early stages of motor recruitment (van der Laan et al., 2005; Hutu et al., 2008; Popov-Čeleketić et al., 2008a; Schiller, 2009), indicating that the TIM23-PAM machinery undergoes substantial dynamic rearrangements during the translocation and sorting of precursor proteins.

Protein translocases and mitochondrial architecture

The surface of the mitochondrial inner membrane is considerably larger than that of the outer membrane. The inner membrane forms invaginations, termed cristae, which are the major site for oxidative phosphorylation (Gilkerson et al., 2003). The respiratory chain complexes and the F_1F_o -ATP synthase are mainly located in the cristae

membranes, whereas protein translocases are enriched in the inner boundary membrane that is adjacent to the outer membrane (Figure 4) (Werner and Neupert, 1972; Vogel et al., 2006; Wurm and Jakobs, 2006; Dudkina et al., 2010; Davies et al., 2011, 2012; Stoldt et al., 2012; Daum et al., 2013). Crista junctions are narrow tubular openings that connect the cristae membranes to the inner boundary membrane (Palade, 1953; Perkins et al., 1997; Frey and Mannella, 2000; Frey et al., 2002; Mannella, 2006a,b).

Recent studies led to the identification of a large protein complex that is enriched at crista junctions and termed the mitochondrial contact site and cristae organizing system (MICOS) (Harner et al., 2011, 2014; Hoppins et al., 2011; von der Malsburg et al., 2011; Alkhaja et al., 2012; Pfanner et al., 2014; Friedman et al., 2015; Wideman and Muñoz-Gómez, 2016). The MICOS complex is crucial for maintaining the architecture of the inner membrane. The smallest subunit, Mic10, forms large oligomers in the inner membrane that likely constitute the basis of crista junctions (Barbot et al., 2015; Bohnert et al., 2015). In addition, the MICOS complex forms contact sites with the

mitochondrial outer membrane. Here the largest subunit, Mic60 (previously termed mitofilin), is of particular importance. MICOS interacts with several major protein complexes of the outer membrane, including the metabolite channel Porin (VDAC) and the protein translocases TOM and SAM (Figure 4) (Xie et al., 2007; Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011; Körner et al., 2012; Ott et al., 2012; Zerbes et al., 2012). Mic60 of the inner membrane thus stimulates the biogenesis of β -barrel proteins of the outer membrane (Bohnert et al., 2012). The exact molecular mechanism has not been identified, yet it was found that Mic60 promotes the early stage of translocation of β -barrel precursors through TOM to the intermembrane space side.

Mic60 also transiently interacts with Mia40 of the intermembrane space import and assembly machinery. Mia40 binds to intermembrane space precursors containing cysteine motifs upon their translocation through the Tom40 channel (Milenkovic et al., 2007, 2009; Sideris and Tokatlidis, 2007; Sideris et al., 2009). Mia40 forms a transient mixed disulfide bond between an own cysteine

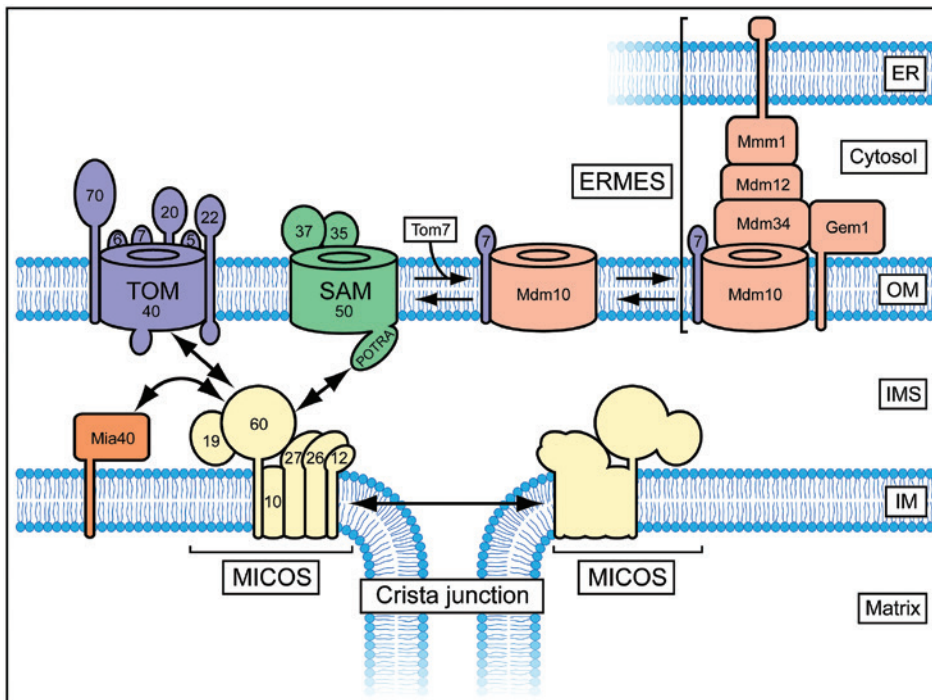


Figure 4: ERMIONE, an ER-mitochondria organizing network.

The mitochondrial contact site and cristae organizing network (MICOS) is enriched at crista junctions of the inner membrane (IM) and interacts with the translocases TOM and SAM of the outer membrane (OM) and Mia40 of the intermembrane space (IMS) import machinery. The mitochondrial distribution and morphology protein Mdm10 has a dual localization in SAM and the ER-mitochondria encounter structure (ERMES). Tom7 also has a dual localization, as subunit of TOM and as binding partner of Mdm10. These machineries form the core of a large dynamic organizing center, termed ERMIONE, that spans three membranes from the ER via OM to IM and is important for mitochondrial membrane architecture, protein biogenesis and lipid transfer. ER, endoplasmic reticulum; POTRA, polypeptide transport-associated domain of Sam50; SAM, sorting and assembly machinery; TOM, translocase of the outer membrane.

residue and a cysteine residue of the precursor (Banci et al., 2009; Stojanovski et al., 2012b). As Mic60 transiently interacts with both Mia40 and the TOM complex (Figure 4), it helps to position Mia40 at the exit of the TOM channel such that Mia40 can immediately grab the incoming precursor protein (von der Malsburg et al., 2011). In a disulfide relay involving the sulfhydryl oxidase Erv1, Mia40 inserts disulfide bonds into the imported precursors (Mesecke et al., 2005; Rissler et al., 2005; Müller et al., 2008; Böttinger et al., 2012). The intramolecular disulfide bonds stabilize the folded state of the imported proteins (Figure 1), leading to trapping of the proteins in the intermembrane space.

MICOS thus forms a dynamic network of interactions between the inner and outer membranes of mitochondria. This network also involves endoplasmic reticulum (ER)-mitochondria contact sites. The ER-mitochondria encounter structure (ERMES) connects ER and the mitochondrial outer membrane and plays a role in maintaining the shape of mitochondria and promoting phospholipid transfer between both organelles (Burgess et al., 1994; Sogo and Yaffe, 1994; Berger et al., 1997; Kornmann et al., 2009; Kopec et al., 2010; Voss et al., 2012; Elbaz-Alon et al., 2014; Schauder et al., 2014; Wideman and Muñoz-Gómez, 2016). Figure 4 depicts a remarkable connection between the SAM complex and ERMES. The mitochondrial distribution and morphology protein Mdm10 has a dual localization (Boldogh et al., 2003; Meisinger et al., 2004, 2007; Kornmann et al., 2009; Thornton et al., 2010; Wideman et al., 2010; Yamano et al., 2010a; Becker et al., 2011b). It is a subunit of both SAM and ERMES. Mdm10 shuttles between both complexes, providing a link between protein biogenesis and organellar contact sites. Tom7, a small subunit of the TOM complex, plays a regulatory role in Mdm10 shuttling. While Tom7 is mainly located in the TOM complex, a fraction of Tom7 molecules bind to Mdm10 that has been released from SAM and stimulate its transfer to ERMES (Meisinger et al., 2006; Yamano et al., 2010b; Becker et al., 2011b). In addition, genetic interactions have been reported for ERMES and MICOS subunits (Hoppins et al., 2011). These findings reveal that *in vivo* protein translocases and morphology machineries do not function as independent entities, but they are embedded into a large regulatory network that controls mitochondrial architecture and biogenesis. We termed this dynamic network spanning across three membranes the ER-mitochondria organizing network (ERMIONE) (van der Laan et al., 2012; Wideman and Muñoz-Gómez, 2016).

Contact sites between the mitochondrial outer and inner membranes are not only formed via the MICOS complex, but several more dynamic contact structures

have been reported (summarized in Reichert and Neupert, 2002; van der Laan et al., 2012; Horvath et al., 2015). With regard to protein translocases, the TOM-TIM23 supercomplex forms an important contact site. While MICOS is enriched directly at crista junctions, the TOM-TIM23 import sites are on average 30–60 nm away from crista junctions (Gold et al., 2014). TOM complexes thus exist in several pools that contribute to TOM-TIM23 and TOM/SAM-MICOS organizing centers of mitochondria. Considering the dynamic behavior of the contact sites, we envisage that the TOM pools are in exchange with each other.

Unconventional translocation mechanisms

The dynamic organization of the mitochondrial protein import machineries is underscored by an increasing number of unusual protein translocation routes that cannot be assigned to any of the known five major protein import pathways (Figure 1). A striking example is the biogenesis of the abundant outer membrane protein Om45. This protein contains an amino-terminal membrane anchor and a large hydrophilic domain that is exposed to the intermembrane space. The precursor of Om45 is first imported by the presequence pathway using TOM and TIM23 including the membrane potential $\Delta\psi$, yet then escapes from this pathway (Song et al., 2014; Wenz et al., 2014). Om45 is exported and assembled into the outer membrane in a process involving the MIM complex that so far had only been known to import proteins delivered from the cytosolic side (Becker et al., 2008, 2011a; Thornton et al., 2010). The Om45 route reveals that elements from different pathways, which were previously considered to be completely independent, can be combined into new mitochondrial protein sorting routes.

Reverse translocation of proteins will receive increasing attention as a means of regulating protein distribution. The sorting route of fumarate, which is located in both the cytosol and mitochondria, is an elegant example (Sass et al., 2003). For both forms, mitochondrial and cytosolic fumarate, the protein is synthesized as precursor with cleavable amino-terminal presequence in the cytosol. The precursor is imported via the TOM-TIM23 complexes such that the presequence can be cleaved off by MPP in the matrix, whereas the mature portion is still located in the TOM-TIM23 import site and on the mitochondrial surface (Figure 5A). The final destination of the fumarate molecules depends on the kinetics of translocation into mitochondria and folding in the matrix versus

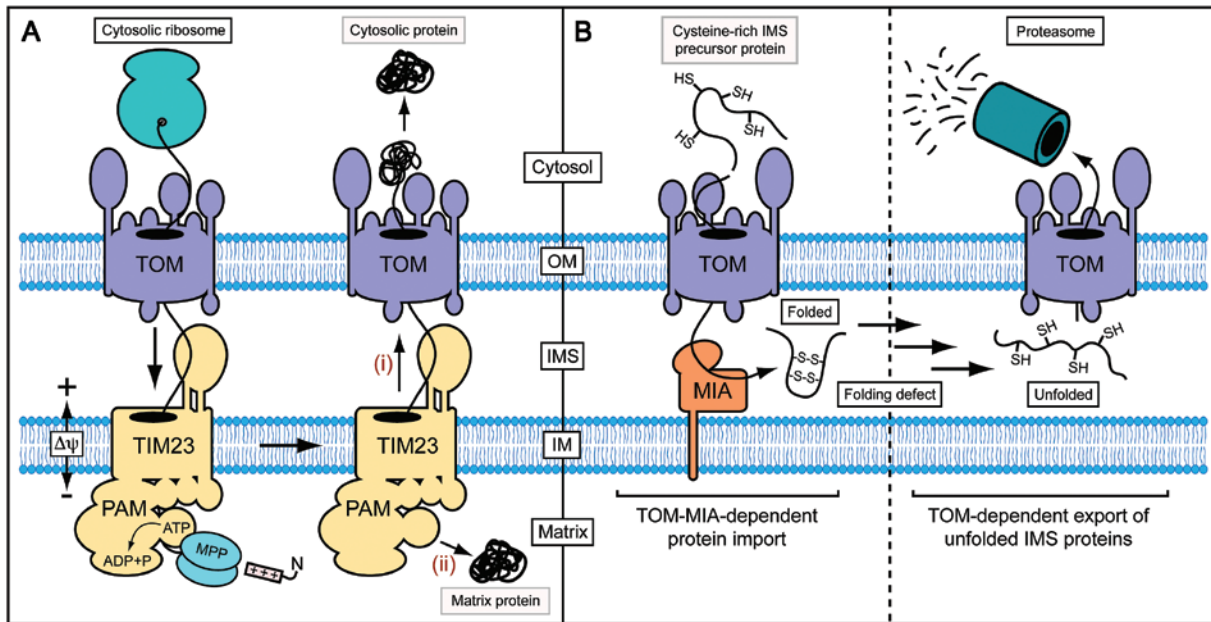


Figure 5: Unconventional protein translocation routes of mitochondria.

(A) The precursor of fumarase is synthesized with a presequence. Upon synthesis on cytosolic ribosomes, the precursor is translocated into the TOM-TIM23 supercomplex such that the presequence reaches the matrix and is cleaved off by the mitochondrial processing peptidase (MPP). More than half of the cleaved fumarase molecules are translocated in a reverse manner into the cytosol (route i), yielding mature cytosolic fumarase. The other fumarase molecules are fully imported into the matrix (route ii), yielding mitochondrial fumarase. $\Delta\psi$, membrane potential; IM, inner mitochondrial membrane; OM, outer mitochondrial membrane; PAM, presequence translocase-associated motor; TIM23, presequence translocase of the inner membrane; TOM, translocase of the outer membrane. (B) Intermembrane space (IMS) proteins that are imported via the MIA machinery can be exported by the TOM complex when their stable folding in the IMS (oxidation with formation of intramolecular disulfides) is impaired. The exported proteins, which are not properly folded, become substrates of the proteasome in the cytosol. MIA, Mitochondrial intermembrane space import and assembly machinery.

the kinetics of reverse translocation and folding on the cytosolic surface of mitochondria (Sass et al., 2003). For more than half of the molecules, folding of the mature protein occurs outside mitochondria and thus they will not be fully imported, but are released from the mitochondrial import sites and become located in the cytosol. The remaining fumarase molecules are fully imported into mitochondria and attain the final location in the matrix. Thus, a presequence-carrying precursor protein can lead to processed mature proteins located in two different cellular compartments.

Retro-translocation is a further recently identified mechanism for regulating the mitochondrial protein content. Whereas in reverse translocation proteins in transit can move back and forth in the translocation channels, retro-translocation means that fully imported proteins are subsequently exported from mitochondria (Kalderon and Pines, 2014). Bragoszewski et al. (2015) showed that the content of the mitochondrial intermembrane space can be regulated by retro-translocation. Many intermembrane space proteins are imported via TOM and the MIA system. The insertion of disulfide bonds into

the imported proteins by oxidative folding stabilizes the mature proteins. When proteins do not fold properly, e.g. caused by a mutational replacement of a cysteine residue or by a reduction of their disulfide bonds, they can be retro-translocated into the cytosol (Figure 5B). Retro-translocation will contribute to the quality control of mitochondrial intermembrane space proteins. Misfolded or damaged proteins are exported to the cytosol where they become substrates of the proteasomal degradation machinery (Bragoszewski et al., 2015).

Conclusions and open questions

The mitochondrial protein translocases do not function as independent machines but are embedded into a network of machineries that operate in protein biogenesis, bioenergetics, membrane architecture and organellar dynamics. This network involves dynamic physical contacts between protein translocases and various protein complexes such as respiratory chain complexes and morphology

machineries. The network is not limited to mitochondria but includes contact sites with the ER, leading to a three membrane-spanning network termed ERMIONE (van der Laan et al., 2012; Wideman and Muñoz-Gómez, 2016). We propose that ERMIONE serves as an organizing center for coordinating a broad variety of tasks from protein and lipid biogenesis to mitochondrial architecture and membrane remodeling.

Important questions for future research concern the regulation of the translocase network under different metabolic states and stress conditions. Recent studies revealed that the main protein entry gate, the TOM complex, is the subject of intensive regulation by cytosolic signaling cascades, including modification of Tom proteins by protein kinase A, casein kinases 1 and 2, and cyclin-dependent kinase (Schmidt et al., 2011; Harbauer et al., 2014a,b). This is just one example of regulatory processes. It is likely that most, if not all, protein translocases will be regulated by cellular signaling processes.

The role of cytosolic factors in the regulation of mitochondrial protein biogenesis will receive major attention. Whereas numerous studies on the protein transport processes occurring inside mitochondria have been reported, only limited information is available on the early phase of mitochondrial protein biogenesis in the cytosol and its regulation (Harbauer et al., 2014b; Haynes, 2015; Kalderon et al., 2015; Shiota et al., 2015b; Wang and Chen, 2015; Wrobel et al., 2015). Mitochondrial precursor proteins can be bound to cytosolic chaperones and possibly further factors. The specific interaction of chaperones or putative targeting factors with the membrane-bound protein import machinery of mitochondria will be an attractive area of research (Young et al., 2003; Schmidt et al., 2011; Papić et al., 2013). The majority of mitochondrial proteins can be imported in a post-translational manner, i.e. after completion of their synthesis on cytosolic ribosomes. However, the relevance of co-translational translocation is the subject of a long-standing debate and mRNA targeting and/or nascent chain-ribosome targeting to mitochondria represent attractive means of regulating mitochondrial biogenesis (Kellems et al., 1975; Marc et al., 2002; Yogeve et al., 2007; Eliyahu et al., 2010; Quenault et al., 2011; Williams et al., 2014).

The dynamics of the mitochondrial protein import machinery and its connection to cytosolic processes will be of increasing importance for our understanding of cellular stress response and quality control. The activity of the mitochondrial protein import machinery is influenced by the energetic state of mitochondria and the folding behavior of proteins and thus will be an important sensor of mitochondrial fitness and quality (Harbauer et al.,

2014b). Important topics are the mitochondrial stress response (Ryan and Hoogenraad, 2007), the differential localization of the activating transcription factor associated with stress-1 (ATFS-1) (Nargund et al., 2012; Haynes et al., 2013) and of the mitochondrial kinase PINK1 with important implications for the pathogenesis of familial cases of Parkinson's disease (Lazarou et al., 2012; Yamano and Youle, 2013), and proteostatic responses in the cytosol caused by the accumulation of mistargeted mitochondrial proteins (Haynes, 2015; Wang and Chen, 2015; Wrobel et al., 2015). Research on mitochondrial biogenesis thus spans a broad spectrum from basic studies on molecular mechanisms to cellular regulation, quality control and the involvement of mitochondrial functions in the pathogenesis of human diseases.

Acknowledgments: This work was supported by the Deutsche Forschungsgemeinschaft (PF 202/8-1), the Sonderforschungsbereiche 746 and 1140, the Excellence Initiative of the German federal and state governments (EXC 294 BIOS; GSC-4 Spemann Graduate School), the European Research Council (ERC) Consolidator Grant No. 648235, and the Hector Fellow Academy.

References

- Abe, Y., Shodai, T., Muto, T., Mihara, K., Torii, H., Nishikawa, S., Endo, T., and Kohda, D. (2000). Structural basis of presequence recognition by the mitochondrial protein import receptor Tom20. *Cell* 100, 551–560.
- Ahting, U., Thieffry, M., Engelhardt, H., Hegerl, R., Neupert, W., and Nussberger, S. (2001). Tom40, the pore-forming component of the protein-conducting TOM channel in the outer membrane of mitochondria. *J. Cell Biol.* 153, 1151–1160.
- Albrecht, R., Rehling, P., Chacinska, A., Brix, J., Cadamuro, S.A., Volkmer, R., Guiard, B., Pfanner, N., and Zeth, K. (2006). The Tim21 binding domain connects the preprotein translocases of both mitochondrial membranes. *EMBO Rep.* 7, 1233–1238.
- Alder, N.N., Jensen, R.E., and Johnson, A.E. (2008). Fluorescence mapping of mitochondrial TIM23 complex reveals a water-facing, substrate-interacting helix surface. *Cell* 134, 439–450.
- Alkhaja, A.K., Jans, D.C., Nikolov, M., Vukotic, M., Lytovchenko, O., Ludewig, F., Schliebs, W., Riedel, D., Urlaub, H., Jakobs, S., et al. (2012). MINOS1 is a conserved component of mitofilin complexes and required for mitochondrial function and cristae organization. *Mol. Biol. Cell* 23, 247–257.
- Allen, J.F. (2003). The function of genomes in bioenergetic organelles. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 358, 19–38.
- Bajaj, R., Munari, F., Becker, S., and Zweckstetter, M. (2014). Interaction of the intermembrane space domain of Tim23 protein with mitochondrial membranes. *J. Biol. Chem.* 289, 34620–34626.

- Banci, L., Bertini, I., Cefaro, C., Ciofi-Baffoni, S., Gallo, A., Martinelli, M., Sideris, D.P., Katrakili, N., and Tokatlidis, K. (2009). MIA40 is an oxidoreductase that catalyzes oxidative protein folding in mitochondria. *Nat. Struct. Mol. Biol.* 16, 198–206.
- Barbot, M., Jans, D.C., Schulz, C., Denkert, N., Kroppen, B., Hoppert, M., Jakobs, S., and Meinecke, M. (2015). Mic10 oligomerizes to bend mitochondrial inner membranes at cristae junctions. *Cell Metab.* 21, 756–763.
- Becker, L., Bannwarth, M., Meisinger, C., Hill, K., Model, K., Krimmer, T., Casadio, R., Truscott, K.N., Schulz, G.E., Pfanner, N., et al. (2005). Preprotein translocase of the outer mitochondrial membrane: reconstituted Tom40 forms a characteristic TOM pore. *J. Mol. Biol.* 353, 1011–1020.
- Becker, T., Pfannschmidt, S., Guiard, B., Stojanovski, D., Milenkovic, D., Kutik, S., Pfanner, N., Meisinger, C., and Wiedemann, N. (2008). Biogenesis of the mitochondrial TOM complex: Mim1 promotes insertion and assembly of signal-anchored receptors. *J. Biol. Chem.* 283, 120–127.
- Becker, T., Wenz, L.-S., Krüger, V., Lehmann, W., Müller, J.M., Goroncy, L., Zufall, N., Lithgow, T., Guiard, B., Chacinska, A., et al. (2011a). The mitochondrial import protein Mim1 promotes biogenesis of multispanning outer membrane proteins. *J. Cell Biol.* 194, 387–395.
- Becker, T., Wenz, L.-S., Thornton, N., Stroud, D., Meisinger, C., Wiedemann, N., and Pfanner, N. (2011b). Biogenesis of mitochondria: dual role of Tom7 in modulating assembly of the preprotein translocase of the outer membrane. *J. Mol. Biol.* 405, 113–124.
- Becker, T., Horvath, S.E., Böttlinger, L., Gebert, N., Daum, G., and Pfanner, N. (2013). Role of phosphatidylethanolamine in the biogenesis of mitochondrial outer membrane proteins. *J. Biol. Chem.* 288, 16451–16459.
- Berger, K.H., Sogo, L.F., and Yaffe, M.P. (1997). Mdm12p, a component required for mitochondrial inheritance that is conserved between budding and fission yeast. *J. Cell Biol.* 136, 545–553.
- Bohnert, M., Wenz, L.-S., Zerbes, R.M., Horvath, S.E., Stroud, D.A., von der Malsburg, K., Müller, J.M., Oeljeklaus, S., Perschil, I., Warscheid, B., et al. (2012). Role of mitochondrial inner membrane organizing system in protein biogenesis of the mitochondrial outer membrane. *Mol. Biol. Cell* 23, 3948–3956.
- Bohnert, M., Zerbes, R.M., Davies, K.M., Mühleip, A.W., Rampelt, H., Horvath, S.E., Boenke, T., Kram, A., Perschil, I., Veenhuis, M., et al. (2015). Central role of Mic10 in the mitochondrial contact site and cristae organizing system. *Cell Metab.* 21, 747–755.
- Boldogh, I.R., Nowakowski, D.W., Yang, H.-C., Chung, H., Karmon, S., Royes P., and Pon, L.A. (2003). A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. *Mol. Biol. Cell* 14, 4618–4627.
- Bolender, N., Sickmann, A., Wagner, R., Meisinger, C., and Pfanner, N. (2008). Multiple pathways for sorting mitochondrial precursor proteins. *EMBO Rep.* 9, 42–49.
- Bömer, U., Meijer, M., Guiard, B., Dietmeier, K., Pfanner, N., and Rassow, J. (1997). The sorting route of cytochrome b_2 branches from the general mitochondrial import pathway at the preprotein translocase of the inner membrane. *J. Biol. Chem.* 272, 30439–30446.
- Botelho, S.C., Österberg, M., Reichert, A.S., Yamano, K., Björkholm, P., Endo, T., von Heijne, G., and Kim, H. (2011). TIM23-mediated insertion of transmembrane α -helices into the mitochondrial inner membrane. *EMBO J.* 30, 1003–1011.
- Böttlinger, L., Gornicka, A., Czerwik, T., Bragoszewski, P., Loniewska-Lwowska, A., Schulze-Specking, A., Truscott, K., Guiard, B., Milenkovic, D., and Chacinska, A. (2012). In vivo evidence for cooperation of Mia40 and Erv1 in the oxidation of mitochondrial proteins. *Mol. Biol. Cell* 23, 3957–3969.
- Bragoszewski, P., Wasilewski, M., Sakowska, P., Gornicka, A., Böttlinger, L., Qiu, J., Wiedemann, N., and Chacinska, A. (2015). Retro-translocation of mitochondrial intermembrane space proteins. *Proc. Natl. Acad. Sci. USA* 112, 7713–7718.
- Burgess, S.M., Delannoy, M., and Jensen, R.E. (1994). *MMM1* encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. *J. Cell Biol.* 126, 1375–1391.
- Calvo, S.E. and Mootha, V.K. (2010). The mitochondrial proteome and human disease. *Annu. Rev. Genomics Hum. Genet.* 11, 25–44.
- Ceh-Pavia, E., Spiller, M.P., and Lu, H. (2013). Folding and biogenesis of mitochondrial small Tim proteins. *Int. J. Mol. Sci.* 14, 16685–16705.
- Chacinska, A., Rehling, P., Guiard, B., Frazier, A.E., Schulze-Specking, A., Pfanner, N., Voos, W., and Meisinger, C. (2003). Mitochondrial translocation contact sites: separation of dynamic and stabilizing elements in formation of a TOM-TIM-preprotein supercomplex. *EMBO J.* 22, 5370–5381.
- Chacinska, A., Lind, M., Frazier, A.E., Dudek, J., Meisinger, C., Geissler, A., Sickmann, A., Meyer, H.E., Truscott, K.N., Guiard, B., et al. (2005). Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17. *Cell* 120, 817–829.
- Chacinska, A., Koehler, C.M., Milenkovic, D., Lithgow, T., and Pfanner, N. (2009). Importing mitochondrial proteins: machineries and mechanisms. *Cell* 138, 628–644.
- Chacinska, A., van der Laan, M., Mehnert, C.S., Guiard, B., Mick, D.U., Hutu, D.P., Truscott, K.N., Wiedemann, N., Meisinger, C., Pfanner, et al. (2010). Distinct forms of mitochondrial TOM-TIM supercomplexes define signal-dependent states of preprotein sorting. *Mol. Cell Biol.* 30, 307–318.
- Daum, B., Walter, A., Horst, A., Osiewacz, H.D., and Kühlbrandt, W. (2013). Age-dependent dissociation of ATP synthase dimers and loss of inner-membrane cristae in mitochondria. *Proc. Natl. Acad. Sci. USA* 110, 15301–15306.
- Davies, K.M., Strauss, M., Daum, B., Kief, J.H., Osiewacz, H.D., Rycovska, A., Zickermann, V., and Kühlbrandt, W. (2011). Macromolecular organization of ATP synthase and complex I in whole mitochondria. *Proc. Natl. Acad. Sci. USA* 108, 14121–14126.
- Davies, K.M., Anselmi, C., Wittig, I., Faraldo-Gómez, J.D., and Kühlbrandt, W. (2012). Structure of the yeast F_1F_0 -ATP synthase dimer and its role in shaping the mitochondrial cristae. *Proc. Natl. Acad. Sci. USA* 109, 13602–13607.
- Dekker, P.J.T., Martin, F., Maarse, A.C., Bömer, U., Müller, H., Guiard, B., Meijer, M., Rassow, J., and Pfanner, N. (1997). The Tim core complex defines the number of mitochondrial translocation contact sites and can hold arrested preproteins in the absence of matrix Hsp70-Tim44. *EMBO J.* 16, 5408–5419.

- de la Cruz, L., Bajaj, R., Becker, S., and Zweckstetter, M. (2010). The intermembrane space domain of Tim23 is intrinsically disordered with a distinct binding region for presequences. *Protein Sci.* 19, 2045–2054.
- Dienhart, M.K. and Stuart, R.A. (2008). The yeast Aac2 protein exists in physical association with the cytochrome *bc₁*-COX supercomplex and the TIM23 machinery. *Mol. Biol. Cell* 19, 3934–3943.
- Dimmer, K.S., Papić, D., Schumann, B., Sperl, D., Krumpe, K., Walther, D.M., and Rapaport, D. (2012). A crucial role for Mim2 in the biogenesis of mitochondrial outer membrane proteins. *J. Cell Sci.* 125, 3464–3473.
- Dolezal, P., Likic, V., Tachezy, J., and Lithgow, T. (2006). Evolution of the molecular machines for protein import into mitochondria. *Science* 313, 314–318.
- D'Silva, P.D., Schilke, B., Walter, W., Andrew, A., and Craig, E.A. (2003). J protein cochaperone of the mitochondrial inner membrane required for protein import into the mitochondrial matrix. *Proc. Natl. Acad. Sci. USA* 100, 13839–13844.
- Dudkina, N.V., Oostergetel, G.T., Lewejohann, D., Braun, H.P., and Boekema, E.J. (2010). Row-like organization of ATP synthase in intact mitochondria determined by cryo-electron tomography. *Biochim. Biophys. Acta* 1797, 272–277.
- Elbaz-Alon, Y., Rosenfeld-Gur, E., Shinder, V., Futerman, A.H., Geiger, T., and Schuldiner, M. (2014). A dynamic interface between vacuoles and mitochondria in yeast. *Dev. Cell* 30, 95–102.
- Eliyah, E., Pnueli, L., Melamed, D., Scherrer, T., Gerber, A.P., Pines, O., Rapaport, D., and Arava, Y. (2010). Tom20 mediates localization of mRNAs to mitochondria in a translation-dependent manner. *Mol. Cell. Biol.* 30, 284–294.
- Endo, T. and Yamano, K. (2009). Multiple pathways for mitochondrial protein traffic. *Biol. Chem.* 390, 723–730.
- Endo, T., Yamano, K., and Kawano, S. (2011). Structural insight into the mitochondrial protein import system. *Biochim. Biophys. Acta* 1808, 955–970.
- Foury, F., Roganti, T., Lecrenier, N., and Purnelle, B. (1998). The complete sequence of the mitochondrial genome of *Saccharomyces cerevisiae*. *FEBS Lett.* 440, 325–331.
- Frazier, A.E., Chacinska, A., Truscott, K.N., Guiard, B., Pfanner, N., and Rehling, P. (2003). Mitochondria use different mechanisms for transport of multispanning membrane proteins through the intermembrane space. *Mol. Cell. Biol.* 23, 7818–7828.
- Frazier, A.E., Dudek, J., Guiard, B., Voos, W., Li, Y., Lind, M., Meisinger, C., Geissler, A., Sickmann, A., Meyer, H.E., et al. (2004). Pam16 has an essential role in the mitochondrial protein import motor. *Nat. Struct. Mol. Biol.* 11, 226–233.
- Frey, T.G. and Mannella, C.A. (2000). The internal structure of mitochondria. *Trends Biochem. Sci.* 25, 319–324.
- Frey, T.G., Renken, C.W., and Perkins, G.A. (2002). Insight into mitochondrial structure and function from electron tomography. *Biochim. Biophys. Acta* 1555, 196–203.
- Friedman, J.R., Mourier, A., Yamada, J., McCaffery, J.M., and Nunnari, J. (2015). MICOS coordinates with respiratory complexes and lipids to establish mitochondrial inner membrane architecture. *eLife* 4, e07739.
- Gabriel, K., Milenkovic, D., Chacinska, A., Müller, J., Guiard, B., Pfanner, N., and Meisinger, C. (2007). Novel mitochondrial intermembrane space proteins as substrates of the MIA import pathway. *J. Mol. Biol.* 365, 612–620.
- Galluzzi, L., Kepp, O., and Kroemer, G. (2012). Mitochondria: master regulators of danger signalling. *Nat. Rev. Mol. Cell Biol.* 13, 780–788.
- Gebert, N., Joshi, A.S., Kutik, S., Becker, T., McKenzie, M., Guan, X.L., Mooga, V.P., Stroud, D.A., Kulkarni, G., Wenk, M.R., et al. (2009). Mitochondrial cardiolipin involved in outer-membrane protein biogenesis: implications for Barth syndrome. *Curr. Biol.* 19, 2133–2139.
- Gebert, M., Schrempp, S.G., Mehnert, C.S., Heißwolf, A.K., Oeljeklaus, S., Ieva, R., Bohnert, M., von der Malsburg, K., Wiese, S., Kleinschroth, T., et al. (2012). Mgr2 promotes coupling of the mitochondrial presequence translocase to partner complexes. *J. Cell Biol.* 197, 595–604.
- Geissler, A., Krimmer, T., Bömer, U., Guiard, B., Rassow, J., and Pfanner, N. (2000). Membrane potential-driven protein import into mitochondria: the sorting sequence of cytochrome *b₂* modulates the $\Delta\psi$ -dependence of translocation of the matrix-targeting sequence. *Mol. Biol. Cell* 11, 3977–3991.
- Geissler, A., Chacinska, A., Truscott, K.N., Wiedemann, N., Brandner, K., Sickmann, A., Meyer, H.E., Meisinger, C., Pfanner, N., and Rehling, P. (2002). The mitochondrial presequence translocase: an essential role of Tim50 in directing preproteins to the import channel. *Cell* 111, 507–518.
- Ghaemmaghami, S., Huh, W.-K., Bower, K., Howson, R.W., Belle, A., Dephoure, N., O'Shea, E.K., and Weissman, J.S. (2003). Global analysis of protein expression in yeast. *Nature* 425, 737–741.
- Gilkerson, R.W., Selker, J.M., and Capaldi, R.A. (2003). The cristal membrane of mitochondria is the principal site of oxidative phosphorylation. *FEBS Lett.* 546, 355–358.
- Glick, B.S., Brandt, A., Cunningham, K., Müller, S., Hallberg, R.L., and Schatz, G. (1992). Cytochromes *c₁* and *b₂* are sorted to the intermembrane space of yeast mitochondria by a stop-transfer mechanism. *Cell* 69, 809–822.
- Gold, V.A.M., Ieva, R., Walter, A., Pfanner, N., van der Laan, M., and Kühlbrandt, W. (2014). Visualizing active membrane protein complexes by electron cryotomography. *Nat. Commun.* 5, 4129.
- Gray, M.W., Burger, G., and Lang, B.F. (1999). Mitochondrial evolution. *Science* 283, 1476–1481.
- Harbauer, A.B., Opalińska, M., Gerbeth, C., Herman, J.S., Rao, S., Schönfisch, B., Guiard, B., Schmidt, O., Pfanner, N., and Meisinger, C. (2014a). Cell cycle-dependent regulation of mitochondrial preprotein translocase. *Science* 346, 1109–1113.
- Harbauer, A.B., Zahedi, R.P., Sickmann, A., Pfanner, N., and Meisinger, C. (2014b). The protein import machinery of mitochondria – a regulatory hub in metabolism, stress, and disease. *Cell Metab.* 19, 357–372.
- Harner, M., Körner, C., Walther, D., Mokranjac, D., Kaesmacher, J., Welsch, U., Griffith, J., Mann, M., Reggiori, F., and Neupert, W. (2011). The mitochondrial contact site complex, a determinant of mitochondrial architecture. *EMBO J.* 30, 4356–4370.
- Harner, M.E., Unger, A.-K., Izawa, T., Walther, D.M., Özbacı, C., Geimer, S., Reggiori, F., Brügger, B., Mann, M., Westermann, B., et al. (2014). Aim24 and MICOS modulate respiratory function, tafazzin-related cardiolipin modification and mitochondrial architecture. *eLife* 3, e01684.
- Hawltischek, G., Schneider, H., Schmidt, B., Tropschug, M., Hartl, F.U., and Neupert, W. (1988). Mitochondrial protein import: identification of processing peptidase and of PEP, a processing enhancing protein. *Cell* 53, 795–806.

- Haynes, C.M., Fiorese, C.J., and Lin, Y.-F. (2013). Evaluating and responding to mitochondrial dysfunction: the mitochondrial unfolded-protein response and beyond. *Trends Cell Biol.* 23, 311–318.
- Haynes, C.M. (2015). Cell biology: surviving import failure. *Nature*, 524, 419–420.
- Hill, K., Model, K., Ryan, M.T., Dietmeier, K., Martin, F., Wagner, F., and Pfanner, N. (1998). Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins. *Nature* 395, 516–521.
- Höhr, A.I., Straub, S.P., Warscheid, B., Becker, T., and Wiedemann, N. (2015). Assembly of β -barrel proteins in the mitochondrial outer membrane. *Biochim. Biophys. Acta* 1853, 74–88.
- Hoppins, S., Collins, S.R., Cassidy-Stone, A., Hummel, E., Devay, R.M., Lackner, L.L., Westermann, B., Schuldiner, M., Weissman, J.S., and Nunnari, J. (2011). A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane organization in mitochondria. *J. Cell Biol.* 195, 323–340.
- Horvath, S.E. and Daum, G. (2013). Lipids of mitochondria. *Prog. Lipid Res.* 52, 590–614.
- Horvath, S.E., Rampelt, H., Oeljeklaus, S., Warscheid, B., van der Laan, M., and Pfanner, N. (2015). Role of membrane contact sites in protein import into mitochondria. *Protein Sci.* 24, 277–297.
- Hulett, J.M., Lueder, F., Chan, N.C., Perry, A.J., Wolyneć, P., Likić, V.A., Gooley, P.R., and Lithgow, T. (2008). The transmembrane segment of Tom20 is recognized by Mim1 for docking to the mitochondrial TOM complex. *J. Mol. Biol.* 376, 694–704.
- Hutu, D.P., Guiard, B., Chacinska, A., Becker, D., Pfanner, N., Rehling, P., and van der Laan, M. (2008). Mitochondrial protein import motor: differential role of Tim44 in the recruitment of Pam17 and J-complex to the presequence translocase. *Mol. Biol. Cell* 19, 2642–2649.
- Ieva, R., Schrempp, S.G., Opaliński, Ł., Wollweber, F., Höß, P., Heißwolf, A.K., Gebert, M., Zhang, Y., Guiard, B., Rospert, S., et al. (2014). Mgr2 functions as lateral gatekeeper for preprotein sorting in the mitochondrial inner membrane. *Mol. Cell* 56, 641–652.
- Kalderon, B. and Pines, O. (2014). Protein folding as a driving force for dual protein targeting in eukaryotes. *Front. Mol. Biosci.* 1, 23.
- Kalderon, B., Kogan, G., Bubis, E., and Pines, O. (2015). Cytosolic Hsp60 can modulate proteasome activity in yeast. *J. Biol. Chem.* 290, 3542–3551.
- Kang, P.J., Ostermann, J., Shilling, J., Neupert, W., Craig, E.A., and Pfanner, N. (1990). Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. *Nature* 348, 137–143.
- Kellems, R.E., Allison, V.F., and Butow, R.A. (1975). Cytoplasmic type 80S ribosomes associated with yeast mitochondria. IV. Attachment of ribosomes to the outer membrane of isolated mitochondria. *J. Cell Biol.* 65, 1–14.
- Kemper, C., Habib, S.J., Engl, G., Heckmeyer, P., Dimmer, K.S., and Rapaport, D. (2008). Integration of tail-anchored proteins into the mitochondrial outer membrane does not require any known import components. *J. Cell Sci.* 121, 1990–1998.
- Koehler, C.M. (2004). The small Tim proteins and the twin Cx₃C motif. *Trends Biochem. Sci.* 29, 1–4.
- Komiya, T., Rospert, S., Koehler, C., Looser, R., Schatz, G., and Mihara, K. (1998). Interaction of mitochondrial targeting signals with acidic receptor domains along the protein import pathway: evidence for the ‘acid chain’ hypothesis. *EMBO J.* 17, 3886–3898.
- Kopeck, K.O., Alva, V., and Lupas A.N. (2010). Homology of SMP domains to the TULIP superfamily of lipid-binding proteins provides a structural basis for lipid exchange between ER and mitochondria. *Bioinformatics* 26, 1927–1931.
- Körner, C., Barrera, M., Dukanovic, J., Eydt, K., Harner, M., Rabl, R., Vogel, F., Rapaport, D., Neupert, W., and Reichert, A.S. (2012). The C-terminal domain of Fc11 is required for formation of crista junctions and interacts with the TOB/SAM complex in mitochondria. *Mol. Biol. Cell* 23, 2143–2155.
- Kornmann, B., Currie, E., Collins, S.R., Schuldiner, M., Nunnari, J., Weissman, J.S., and Walter, P. (2009). An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* 325, 477–481.
- Kozany, C., Mokranjac, D., Sichting, M., Neupert, W., and Hell, K. (2004). The J domain-related cochaperone Tim16 is a constituent of the mitochondrial TIM23 preprotein translocase. *Nat. Struct. Mol. Biol.* 11, 234–241.
- Krayl, M., Lim, J.H., Martin, F., Guiard, B., and Voos W. (2007). A cooperative action of the ATP-dependent import motor complex and the inner membrane potential drives mitochondrial preprotein import. *Mol. Cell Biol.* 27, 411–425.
- Kronidou, N.G., Oppliger, W., Bolliger, L., Hannavy, K., Glick, B.S., Schatz, G., and Horst, M. (1994). Dynamic interaction between Isp45 and mitochondrial hsp70 in the protein import system of the yeast mitochondrial inner membrane. *Proc. Natl. Acad. Sci. USA* 91, 12818–12822.
- Krumpe, K., Frumkin, I., Herzig, Y., Rimon, N., Özbacı, C., Brügger, B., Rapaport, D., and Schuldiner, M. (2012). Ergosterol content specifies targeting of tail-anchored proteins to mitochondrial outer membranes. *Mol. Biol. Cell* 23, 3927–3935.
- Kutik, S., Stroud, D.A., Wiedemann, N., and Pfanner, N. (2009). Evolution of mitochondrial protein biogenesis. *Biochim. Biophys. Acta* 1790, 409–415.
- Laloraya, S., Gambill, B.D., and Craig, E.A. (1994). A role for a eukaryotic GrpE-related protein, Mge1p, in protein translocation. *Proc. Natl. Acad. Sci. USA* 91, 6481–6485.
- Lazarou, M., Jin, S.M., Kane, L.A., and Youle, R.J. (2012). Role of PINK1 binding to the TOM complex and alternate intracellular membranes in recruitment and activation of the E3 ligase Parkin. *Dev. Cell* 22, 320–333.
- Li, J. and Sha, B. (2015). The structure of Tim50(164–361) suggests the mechanism by which Tim50 receives mitochondrial presequences. *Acta Crystallogr. F Struct. Biol. Commun.* 71, 1146–1151.
- Li, Y., Dudek, J., Guiard, B., Pfanner, N., Rehling, P., and Voos, W. (2004). The presequence translocase-associated protein import motor of mitochondria: Pam16 functions in an antagonistic manner to Pam18. *J. Biol. Chem.* 279, 38047–38054.
- Lill, R. (2009). Function and biogenesis of iron-sulphur proteins. *Nature* 460, 831–838.
- Liu, Q., D’Silva, P., Walter, W., Marszałek, J., and Craig, E.A. (2003). Regulated cycling of mitochondrial Hsp70 at the protein import channel. *Science* 300, 139–141.

- Malhotra, K., Sathappa, M., Landin, J.S., Johnson, A.E., and Alder, N.N. (2013). Structural changes in the mitochondrial Tim23 channel are coupled to the proton-motive force. *Nat. Struct. Mol. Biol.* 20, 965–972.
- Mannella, C.A. (2006a). The relevance of mitochondrial membrane topology to mitochondrial function. *Biochim. Biophys. Acta* 1762, 140–147.
- Mannella, C.A. (2006b). Structure and dynamics of the mitochondrial inner membrane cristae. *Biochim. Biophys. Acta* 1763, 542–548.
- Marc, P., Margeot, A., Devaux, F., Blugeon, C., Corral-Debrinski, M., and Jacq, C. (2002). Genome-wide analysis of mRNAs targeted to yeast mitochondria. *EMBO Rep.* 3, 159–164.
- Margulis, L. (1970). Aerobiosis and the mitochondrion. In *Origin of Eukaryotic Cells; Evidence and Research Implications for a Theory of the Origin and Evolution of Microbial, Plant, and Animal Cells on the Precambrian Earth* (New Haven, CT, USA: Yale University Press), pp. 178–207.
- Martin, J., Mahlke, K., and Pfanner, N. (1991). Role of an energized inner membrane in mitochondrial protein import: $\Delta\psi$ drives the movement of presequences. *J. Biol. Chem.* 266, 18051–18057.
- Matouschek, A., Azem, A., Ratliff, K., Glick, B.S., Schmid, K., and Schatz, G. (1997). Active unfolding of precursor proteins during mitochondrial protein import. *EMBO J.* 16, 6727–6736.
- McBride, H.M., Neuspiel, M., and Wasiak, S. (2006). Mitochondria: more than just a powerhouse. *Curr. Biol.* 16, R551–560.
- Mehnert, C.S., Rampelt, H., Gebert, M., Oeljeklaus, S., Schrempf, S.G., Kochbeck, L., Guiard, B., Warscheid, B., and van der Laan, M. (2014). The mitochondrial ADP/ATP carrier associates with the inner membrane presequence translocase in a stoichiometric manner. *J. Biol. Chem.* 289, 27352–27362.
- Meinecke, M., Wagner, R., Kovermann, P., Guiard, B., Mick, D.U., Hutu, D.P., Voos, W., Truscott, K.N., Chacinska, A., Pfanner, N., et al. (2006). Tim50 maintains the permeability barrier of the mitochondrial inner membrane. *Science* 312, 1523–1526.
- Meisinger, C., Rissler, M., Chacinska, A., Sanjuán Szklarz, L.K., Milenkovic, D., Kozjak, V., Schönfisch, B., Lohaus, C., Meyer, H.E., Yaffe, M.P., et al. (2004). The mitochondrial morphology protein Mdm10 functions in assembly of the preprotein translocase of the outer membrane. *Dev. Cell* 7, 61–71.
- Meisinger, C., Wiedemann, N., Rissler, M., Strub, A., Milenkovic, D., Schönfisch, B., Müller, H., Kozjak, V., and Pfanner, N. (2006). Mitochondrial protein sorting: differentiation of β -barrel assembly by Tom7-mediated segregation of Mdm10. *J. Biol. Chem.* 281, 22819–22826.
- Meisinger, C., Pfannschmidt, S., Rissler, M., Milenkovic, D., Becker, T., Stojanovski, D., Youngman, M.J., Jensen, R.E., Chacinska, A., Guiard, B., et al. (2007). The morphology proteins Mdm12/Mmm1 function in the major β -barrel assembly pathway of mitochondria. *EMBO J.* 26, 2229–2239.
- Merklinger, E., Gofman, Y., Kedrov, A., Driessen, A.J.M., Ben-Tal, N., Shai, Y., and Rapaport, D. (2012). Membrane integration of a mitochondrial signal-anchored protein does not require additional proteinaceous factors. *Biochem. J.* 442, 381–389.
- Mesecke, N., Terziyska, N., Kozany, C., Baumann, F., Neupert, W., Hell, K., and Herrmann, J.M. (2005). A disulfide relay system in the intermembrane space of mitochondria that mediates protein import. *Cell* 121, 1059–1069.
- Mick, D.U., Dennerlein, S., Wiese, H., Reinhold, R., Pacheu-Grau, D., Lorenzi, I., Sasarman, F., Weraarpachai, W., Shoubbridge, E.A., Warscheid, B., et al. (2012). MITRAC links mitochondrial protein translocation to respiratory-chain assembly and translational regulation. *Cell* 151, 1528–1541.
- Milenkovic, D., Gabriel, K., Guiard, B., Schulze-Specking, A., Pfanner, N., and Chacinska, A. (2007). Biogenesis of the essential Tim9–Tim10 chaperone complex of mitochondria: site-specific recognition of cysteine residues by the intermembrane space receptor Mia40. *J. Biol. Chem.* 282, 22472–22480.
- Milenkovic, D., Ramming, T., Müller, J.M., Wenz, L.-S., Gebert, N., Schulze-Specking, A., Stojanovski, D., Rospert, S., and Chacinska, A. (2009). Identification of the signal directing Tim9 and Tim10 into the intermembrane space of mitochondria. *Mol. Biol. Cell* 20, 2530–2539.
- Moczko, M., Bömer, U., Kübrich, M., Zufall, N., Hönlinger, A., and Pfanner, N. (1997). The intermembrane space domain of mitochondrial Tom22 functions as a *trans* binding site for preproteins with N-terminal targeting sequences. *Mol. Cell. Biol.* 17, 6574–6584.
- Mokranjac, D. and Neupert, W. (2009). Thirty years of protein translocation into mitochondria: unexpectedly complex and still puzzling. *Biochim. Biophys. Acta* 1793, 33–41.
- Mokranjac, D. and Neupert, W. (2010). The many faces of the mitochondrial TIM23 complex. *Biochim. Biophys. Acta* 1797, 1045–1054.
- Mokranjac, D., Sichtung, M., Neupert, W., and Hell, K. (2003). Tim14, a novel key component of the import motor of the TIM23 protein translocase of mitochondria. *EMBO J.* 22, 4945–4956.
- Mokranjac, D., Popov-Čeleketić, D., Hell, K., and Neupert, W. (2005). Role of Tim21 in mitochondrial translocation contact sites. *J. Biol. Chem.* 280, 23437–23440.
- Mokranjac, D., Bourenkov, G., Hell, K., Neupert, W., and Groll, M. (2006). Structure and function of Tim14 and Tim16, the J and J-like components of the mitochondrial protein import motor. *EMBO J.* 25, 4675–4685.
- Mokranjac, D., Sichtung, M., Popov-Čeleketić, D., Mapa, K., Gevorkyan-Airapetov, L., Zohary, K., Hell, K., Azem, A., and Neupert, W. (2009). Role of Tim50 in the transfer of precursor proteins from the outer to the inner membrane of mitochondria. *Mol. Biol. Cell* 20, 1400–1407.
- Mossmann, D., Meisinger, C., and Vögtle, F.-N. (2012). Processing of mitochondrial presequences. *Biochim. Biophys. Acta* 1819, 1098–1106.
- Müller, J.M., Milenkovic, D., Guiard, B., Pfanner, N., and Chacinska, A. (2008). Precursor oxidation by Mia40 and Erv1 promotes vectorial transport of proteins into the mitochondrial intermembrane space. *Mol. Biol. Cell* 19, 226–236.
- Nargund, A.M., Pellegrino, M.W., Fiorese, C.J., Baker, B.M., and Haynes, C.M. (2012). Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science* 337, 587–590.
- Neupert, W. and Herrmann, J.M. (2007). Translocation of proteins into mitochondria. *Annu. Rev. Biochem.* 76, 723–749.
- Newmeyer, D.D. and Ferguson-Miller, S. (2003). Mitochondria: releasing power for life and unleashing the machineries of death. *Cell* 112, 481–490.
- Nunnari, J. and Suomalainen, A. (2012). Mitochondria: in sickness and in health. *Cell* 148, 1145–1159.

- Ott, M. and Herrmann, J.M. (2010). Co-translational membrane insertion of mitochondrially encoded proteins. *Biochim. Biophys. Acta* 1803, 767–775.
- Ott, C., Ross, K., Straub, S., Thiede, B., Götz, M., Goosmann, C., Krischke, M., Mueller, M.J., Krohne, G., Rudel, T., et al. (2012). Sam50 functions in mitochondrial intermembrane space bridging and biogenesis of respiratory complexes. *Mol. Cell Biol.* 32, 1173–1188.
- Pagliarini, D.J., Calvo, S.E., Chang, B., Sheth, S.A., Vafai, S.B., Ong, S.-E., Walford, G.A., Sugiana, C., Boneh, A., Chen, W.K., et al. (2008). A mitochondrial protein compendium elucidates complex I disease biology. *Cell* 134, 112–123.
- Palade, G.E. (1953). An electron microscope study of the mitochondrial structure. *J. Histochem. Cytochem.* 1, 188–211.
- Papić, D., Krumpe, K., Dukanovic, J., Dimmer, K.S., and Rapaport, D. (2011). Multispan mitochondrial outer membrane protein Ugo1 follows a unique Mim1-dependent import pathway. *J. Cell Biol.* 194, 397–405.
- Papić, D., Elbaz-Alon, Y., Koerdert, S.N., Leopold, K., Worm, D., Jung, M., Schuldiner, M., and Rapaport, D. (2013). The role of Djpl in import of the mitochondrial protein Mim1 demonstrates specificity between a co-chaperone and its substrate protein. *Mol. Cell Biol.* 33, 4083–4094.
- Park, K., Botelho, S.C., Hong, J., Österberg, M., and Kim, H. (2013). Dissecting stop transfer versus conservative sorting pathways for mitochondrial inner membrane proteins *in vivo*. *J. Biol. Chem.* 288, 1521–1532.
- Perkins, G., Renken, C., Martone, M.E., Young, S.J., Ellisman, M., and Frey, T. (1997). Electron tomography of neuronal mitochondria: three-dimensional structure and organization of cristae and membrane contacts. *J. Struct. Biol.* 119, 260–272.
- Pfanner, N., van der Laan, M., Amati, P., Capaldi, R.A., Caudy, A.A., Chacinska, A., Darshi, M., Deckers, M., Hoppins, S., Icho, T., et al. (2014). Uniform nomenclature for the mitochondrial contact site and cristae organizing system. *J. Cell Biol.* 204, 1083–1086.
- Popov-Čeleketić, D., Mapa, K., Neupert, W., and Mokranjac, D. (2008a). Active remodelling of the TIM23 complex during translocation of preproteins into mitochondria. *EMBO J.* 27, 1469–1480.
- Popov-Čeleketić, J., Waizenegger, T., and Rapaport, D. (2008b). Mim1 functions in an oligomeric form to facilitate the integration of Tom20 into the mitochondrial outer membrane. *J. Mol. Biol.* 376, 671–680.
- Prokisch, H., Scharfe, C., Camp, D.G. 2nd, Xiao, W., David, L., Andreoli, C., Monroe, M.E., Moore, R.J., Gritsenko, M.A., Kozany, C., et al. (2004). Integrative analysis of the mitochondrial proteome in yeast. *PLoS Biol.* 2, e160.
- Qian, X., Gebert, M., Höpker, J., Yan, M., Li, J., Wiedemann, N., van der Laan, M., Pfanner, N., and Sha, B. (2011). Structural basis for the function of Tim50 in the mitochondrial presequence translocase. *J. Mol. Biol.* 411, 513–519.
- Qiu, J., Wenz, L.-S., Zerbes, R.M., Oeljeklaus, S., Bohnert, M., Stroud, D.A., Wirth, C., Ellenrieder, L., Thornton, N., Kutik, S., et al. (2013). Coupling of mitochondrial import and export translocases by receptor-mediated supercomplex formation. *Cell* 154, 596–608.
- Quenault, T., Lithgow, T., and Traven, A. (2011). PUF proteins: repression, activation and mRNA localization. *Trends Cell Biol.* 21, 104–112.
- Raimundo, N. (2014). Mitochondrial pathology – stress signals from the energy factory. *Trends Mol. Med.* 20, 282–292.
- Rassow, J., Maarse, A.C., Krainer, E., Kübrich, M., Müller, H., Meijer, M., Craig, E.A., and Pfanner, N. (1994). Mitochondrial protein import: biochemical and genetic evidence for interaction of matrix hsp70 and the inner membrane protein MIM44. *J. Cell Biol.* 127, 1547–1556.
- Rehling, P., Model, K., Brandner, K., Kovermann, P., Sickmann, A., Meyer, H.E., Kühlbrandt, W., Wagner, R., Truscott, K.N., and Pfanner, N. (2003). Protein insertion into the mitochondrial inner membrane by a twin-pore translocase. *Science* 299, 1747–1751.
- Reichert, A.S., and Neupert, W. (2002). Contact sites between the outer and inner membrane of mitochondria – role in protein transport. *Biochim Biophys Acta* 1592, 41–49.
- Reinders, J., Zahedi, R.P., Pfanner, N., Meisinger, C., and Sickmann, A. (2006). Toward the complete yeast mitochondrial proteome: multidimensional separation techniques for mitochondrial proteomics. *J. Proteome Res.* 5, 1543–1554.
- Rissler, M., Wiedemann, N., Pfannschmidt, S., Gabriel, K., Guiard, B., Pfanner, N., and Chacinska, A. (2005). The essential mitochondrial protein Erv1 cooperates with Mia40 in biogenesis of intermembrane space proteins. *J. Mol. Biol.* 353, 485–492.
- Ryan, M.T. and Hoogenraad, N.J. (2007). Mitochondrial-nuclear communications. *Annu. Rev. Biochem.* 76, 701–722.
- Ryan, M.T., Wagner, R., and Pfanner, N. (2000). The transport machinery for the import of preproteins across the outer mitochondrial membrane. *Int. J. Biochem. Cell Biol.* 32, 13–21.
- Saitoh, T., Igura, M., Obita, T., Ose, T., Kojima, R., Maenaka, K., Endo, T., and Kohda, D. (2007). Tom20 recognizes mitochondrial presequences through dynamic equilibrium among multiple bound states. *EMBO J.* 26, 4777–4787.
- Sass, E., Karniely, S., and Pines, O. (2003). Folding of fumarase during mitochondrial import determines its dual targeting in yeast. *J. Biol. Chem.* 278, 45109–45116.
- Schatz, G. and Dobberstein, B. (1996). Common principles of protein translocation across membranes. *Science* 271, 1519–1526.
- Schauder, C.M., Wu, X., Saheki, Y., Narayanaswamy, P., Torta, F., Wenk M.R., De Camilli, P., and Reinisch, K.M. (2014). Structure of a lipid-bound extended synaptotagmin indicates a role in lipid transfer. *Nature* 510, 552–555.
- Schiller, D. (2009). Pam17 and Tim44 act sequentially in protein import into the mitochondrial matrix. *Int. J. Biochem. Cell Biol.* 41, 2343–2349.
- Schmidt, O., Pfanner, N., and Meisinger, C. (2010). Mitochondrial protein import: from proteomics to functional mechanisms. *Nat. Rev. Mol. Cell Biol.* 11, 655–667.
- Schmidt, O., Harbauer, A.B., Rao, S., Eyrich, B., Zahedi, R.P., Stojanovski, D., Schönfisch, B., Guiard, B., Sickmann, A., Pfanner, N., et al. (2011). Regulation of mitochondrial protein import by cytosolic kinases. *Cell* 144, 227–239.
- Schneider, H.C., Berthold, J., Bauer, M.F., Dietmeier, K., Guiard, B., Brunner, M., and Neupert, W. (1994). Mitochondrial Hsp70/MIM44 complex facilitates protein import. *Nature* 371, 768–774.
- Schulz, C., Lytovchenko, O., Melin, J., Chacinska, A., Guiard, B., Neumann, P., Ficner, R., Jahn, O., Schmidt, B., and Rehling, P. (2011). Tim50's presequence receptor domain is essential for signal driven transport across the TIM23 complex. *J. Cell Biol.* 195, 643–656.

- Schulz, C., Schendzielorz, A., and Rehling, P. (2015). Unlocking the presequence import pathway. *Trends Cell Biol.* 25, 265–275.
- Setoguchi, K., Otera, H., and Mihara, K. (2006). Cytosolic factor- and TOM-independent import of C-tail-anchored mitochondrial outer membrane proteins. *EMBO J.* 25, 5635–5647.
- Shariff, K., Ghosal, S., and Matouschek, A. (2004). The force exerted by the membrane potential during protein import into the mitochondrial matrix. *Biophys. J.* 86, 3647–3652.
- Shiota, T., Mabuchi, H., Tanaka-Yamano, S., Yamano, K., and Endo, T. (2011). In vivo protein-interaction mapping of a mitochondrial translocator protein Tom22 at work. *Proc. Natl. Acad. Sci. USA* 108, 15179–15183.
- Shiota, T., Imai, K., Qiu, J., Hewitt, V.L., Tan, K., Shen, H.-H., Sakiyama, N., Fukasawa, Y., Hayat, S., Kamiya, M., et al. (2015a). Molecular architecture of the active mitochondrial protein gate. *Science* 349, 1544–1548.
- Shiota, T., Traven, A., and Lithgow, T. (2015b). Mitochondrial biogenesis: cell-cycle-dependent investment in making mitochondria. *Curr Biol.* 25, R78–80.
- Sickmann, A., Reinders, J., Wagner, Y., Joppich, C., Zahedi, R., Meyer, H.E., Schönfisch, B., Perschil, I., Chacinska, A., Guiard, B., et al. (2003). The proteome of *Saccharomyces cerevisiae* mitochondria. *Proc. Natl. Acad. Sci. USA* 100, 13207–13212.
- Sideris, D.P. and Tokatlidis, K. (2007). Oxidative folding of small Tims is mediated by site-specific docking onto Mia40 in the mitochondrial intermembrane space. *Mol. Microbiol.* 65, 1360–1373.
- Sideris, D.P., Petrakis, N., Katrakili, N., Mikropoulou, D., Gallo, A., Ciofi-Baffoni, S., Banci, L., Bertini, I., and Tokatlidis, K. (2009). A novel intermembrane space-targeting signal docks cysteines onto Mia40 during mitochondrial oxidative folding. *J. Cell Biol.* 187, 1007–1022.
- Sirrenberg, C., Endres, M., Becker, K., Bauer, M.F., Walther, E., Neupert, W., and Brunner, M. (1997). Functional cooperation and stoichiometry of protein translocases of the outer and inner membranes of mitochondria. *J. Biol. Chem.* 272, 29963–29966.
- Sogo, L.F. and Yaffe, M.P. (1994). Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J. Cell Biol.* 126, 1361–1373.
- Song, J., Tamura, Y., Yoshihisa, T., and Endo, T. (2014). A novel import route for an N-anchor mitochondrial outer membrane protein aided by the TIM23 complex. *EMBO Rep.* 15, 670–677.
- Steffen, J. and Koehler, C.M. (2014). The great escape: Mgr2 of the mitochondrial TIM23 translocon is a gatekeeper tasked with releasing membrane proteins. *Mol. Cell* 56, 613–614.
- Stojanovski, D., Bohnert, M., Pfanner, N., and van der Laan, M. (2012a). Mechanisms of protein sorting in mitochondria. *Cold Spring Harb. Perspect. Biol.* 4, a011320.
- Stojanovski, D., Bragoszewski, P., and Chacinska, A. (2012b). The MIA pathway: a tight bond between protein transport and oxidative folding in mitochondria. *Biochim. Biophys. Acta* 1823, 1142–1150.
- Stoldt, S., Wenzel, D., Hildenbeutel, M., Wurm, C.A., Herrmann, J.M., and Jakobs, S. (2012). The inner-mitochondrial distribution of Oxa1 depends on the growth conditions and on the availability of substrates. *Mol. Biol. Cell* 23, 2292–2301.
- Strich, R. (2015). Programmed cell death initiation and execution in budding yeast. *Genetics* 200, 1003–1014.
- Suzuki, H., Kadowaki, T., Maeda, M., Sasaki, H., Nabekura, J., Sakaguchi, M., and Mihara, K. (2004). Membrane-embedded C-terminal segment of rat mitochondrial TOM40 constitutes protein-conducting pore with enriched β -structure. *J. Biol. Chem.* 279, 50619–50629.
- Tamura, Y., Harada, Y., Shiota, T., Yamano, K., Watanabe, K., Yokota, M., Yamamoto, H., Sesaki, H., and Endo, T. (2009). Tim23-Tim50 pair coordinates functions of translocators and motor proteins in mitochondrial protein import. *J. Cell Biol.* 184, 129–141.
- Taylor, A.B., Smith, B.S., Kitada, S., Kojima, K., Miyaura, H., Otwinowski, Z., Ito, A., and Deisenhofer, J. (2001). Crystal structures of mitochondrial processing peptidase reveal the mode for specific cleavage of import signal sequences. *Structure* 9, 615–625.
- Teixeira, P.F. and Glaser, E. (2013). Processing peptidases in mitochondria and chloroplasts. *Biochim. Biophys. Acta* 1833, 360–370.
- Thornton, N., Stroud, D.A., Milenkovic, D., Guiard, B., Pfanner, N., and Becker, T. (2010). Two modular forms of the mitochondrial sorting and assembly machinery are involved in biogenesis of α -helical outer membrane proteins. *J. Mol. Biol.* 396, 540–549.
- Ting, S.-Y., Schilke, B.A., Hayashi, M., and Craig, E.A. (2014). Architecture of the TIM23 inner mitochondrial translocon and interactions with the matrix import motor. *J. Biol. Chem.* 289, 28689–28696.
- Truscott, K.N., Kovermann, P., Geissler, A., Merlin, A., Meijer, M., Driessen, A.J., Rassow, J., Pfanner, N., and Wagner, R. (2001). A presequence- and voltage-sensitive channel of the mitochondrial preprotein translocase formed by Tim23. *Nat. Struct. Biol.* 8, 1074–1082.
- Truscott, K.N., Voos, W., Frazier, A.E., Lind, M., Li, Y., Geissler, A., Dudek, J., Müller, H., Sickmann, A., Meyer, H.E., et al. (2003). A J-protein is an essential subunit of the presequence translocase-associated protein import motor of mitochondria. *J. Cell Biol.* 163, 707–713.
- Ungermann, C., Guiard, B., Neupert, W., and Cyr, D.M. (1996). The $\Delta\Psi$ - and Hsp70/MIM44-dependent reaction cycle driving early steps of protein import into mitochondria. *EMBO J.* 15, 735–744.
- van der Laan, M., Chacinska, A., Lind, M., Perschil, I., Sickmann, A., Meyer, H.E., Guiard, B., Meisinger, C., Pfanner, N., and Rehling, P. (2005). Pam17 is required for architecture and translocation activity of the mitochondrial protein import motor. *Mol. Cell Biol.* 25, 7449–7458.
- van der Laan, M., Wiedemann, N., Mick, D.U., Guiard, B., Rehling, P., and Pfanner, N. (2006). A role for Tim21 in membrane-potential-dependent preprotein sorting in mitochondria. *Curr. Biol.* 16, 2271–2276.
- van der Laan, M., Meinecke, M., Dudek, J., Hutu, D.P., Lind, M., Perschil, I., Guiard, B., Wagner, R., Pfanner, N., and Rehling, P. (2007). Motor-free mitochondrial presequence translocase drives membrane integration of preproteins. *Nat. Cell Biol.* 9, 1152–1159.
- van der Laan, M., Hutu, D.P., and Rehling, P. (2010). On the mechanism of preprotein import by the mitochondrial presequence translocase. *Biochim. Biophys. Acta* 1803, 732–739.
- van der Laan, M., Bohnert, M., Wiedemann, N., and Pfanner, N. (2012). Role of MINOS in mitochondrial membrane architecture and biogenesis. *Trends Cell Biol.* 22, 185–192.
- van der Laan, M., Schrempp, S.G., and Pfanner, N. (2013). Voltage-coupled conformational dynamics of mitochondrial protein-import channel. *Nat. Struct. Mol. Biol.* 20, 915–917.

- van Wilpe, S., Ryan, M.T., Hill, K., Maarse, A.C., Meisinger, C., Brix, J., Dekker, P.J.T., Moczek, M., Wagner, R., Pfanner, N., et al. (1999). Tom22 is a multifunctional organizer of the mitochondrial preprotein translocase. *Nature* 401, 485–489.
- Vogel, F., Bornhövd, C., Neupert, W., and Reichert, A.S. (2006). Dynamic subcompartmentalization of the mitochondrial inner membrane. *J. Cell Biol.* 175, 237–247.
- Vögtle, F.-N., Wortelkamp, S., Zahedi, R.P., Becker, D., Leidhold, C., Gevaert, K., Kellermann, J., Voos, W., Sickmann, A., Pfanner, N., et al. (2009). Global analysis of the mitochondrial N-proteome identifies a processing peptidase critical for protein stability. *Cell* 139, 428–439.
- Vögtle, F.-N., Keller, M., Taskin, A.A., Horvath, S.E., Guan, X.L., Prinz, C., Opalińska, M., Zorzin, C., van der Laan, M., Wenk, M.R., et al. (2015). The fusogenic lipid phosphatidic acid promotes the biogenesis of mitochondrial outer membrane protein Ugo1. *J. Cell Biol.* 210, 951–960.
- Voisine, C., Craig, E.A., Zufall, N., von Ahsen, O., Pfanner, N., and Voos, W. (1999). The protein import motor of mitochondria: unfolding and trapping of preproteins are distinct and separable functions of matrix Hsp70. *Cell* 97, 565–574.
- von der Malsburg, K., Müller, J.M., Bohnert, M., Oeljeklaus, S., Kwiatkowska, P., Becker, T., Loniewska-Lwowska, A., Wiese, S., Rao, S., Milenkovic, D., et al. (2011). Dual role of mitofilin in mitochondrial membrane organization and protein biogenesis. *Dev. Cell* 21, 694–707.
- Voos, W., von Ahsen, O., Müller, H., Guiard, B., Rassow, J., and Pfanner, N. (1996). Differential requirement for the mitochondrial Hsp70–Tim44 complex in unfolding and translocation of preproteins. *EMBO J.* 15, 2668–2677.
- Voss, C., Lahiri, S., Young, B.P., Loewen, C.J., and Prinz, W.A. (2012). ER-shaping proteins facilitate lipid exchange between the ER and mitochondria in *S. cerevisiae*. *J. Cell. Sci.* 125, 4791–4799.
- Waegemann, K., Popov-Čeleketić, D., Neupert, W., Azem, A., and Mokranjac, D. (2015). Cooperation of TOM and TIM23 complexes during translocation of proteins into mitochondria. *J. Mol. Biol.* 427, 1075–1084.
- Walther, D.M. and Rapaport, D. (2009). Biogenesis of mitochondrial outer membrane proteins. *Biochim. Biophys. Acta* 1793, 42–51.
- Wang, X. and Chen, X.J. (2015). A cytosolic network suppressing mitochondria-mediated proteostatic stress and cell death. *Nature* 524, 481–484.
- Wenz, L.-S., Opaliński, Ł., Schuler, M.-H., Ellenrieder, L., Ieva, R., Böttlinger, L., Qiu, J., van der Laan, M., Wiedemann, N., Guiard, B., et al. (2014). The presequence pathway is involved in protein sorting to the mitochondrial outer membrane. *EMBO Rep.* 15, 678–685.
- Wenz, L.-S., Ellenrieder, L., Qiu, J., Bohnert, M., Zufall, N., van der Laan, M., Pfanner, N., Wiedemann, N., and Becker, T. (2015a). Sam37 is crucial for formation of the mitochondrial TOM-SAM supercomplex, thereby promoting β -barrel biogenesis. *J. Cell Biol.* 210, 1047–1054.
- Wenz, L.-S., Opaliński, Ł., Wiedemann, N., and Becker, T. (2015b). Cooperation of protein machineries in mitochondrial protein sorting. *Biochim Biophys Acta* 1853, 1119–1129.
- Werner, S. and Neupert, W. (1972). Functional and biogenetical heterogeneity of the inner membrane of rat-liver mitochondria. *Eur. J. Biochem.* 25, 379–396.
- Westermann, B., Prip-Buus, C., Neupert, W., and Schwarz, E. (1995). The role of the GrpE homologue, Mge1p, in mediating protein import and protein folding in mitochondria. *EMBO J.* 14, 3452–3460.
- Wickner, W. and Schekman, R. (2005). Protein translocation across biological membranes. *Science* 310, 1452–1456.
- Wideman, J.G. and Muñoz-Gómez, S.A. (2016). The evolution of ERMIONE in mitochondrial biogenesis and lipid homeostasis: an evolutionary view from comparative cell biology. *Biochim. Biophys. Acta*, doi:10.1016/j.bbali.2016.01.015.
- Wideman, J.G., Go, N.E., Klein, A., Redmond, E., Lackey, S.W.K., Tao, T., Kalbacher, H., Rapaport, D., Neupert, W., and Nargang, F.E. (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, 1725–1736.
- Wiedemann, N., Pfanner, N., and Ryan, M.T. (2001). The three modules of ADP/ATP carrier cooperate in receptor recruitment and translocation into mitochondria. *EMBO J.* 20, 951–960.
- Wiedemann, N., van der Laan, M., Hutu, D.P., Rehling, P., and Pfanner, N. (2007). Sorting switch of mitochondrial presequence translocase involves coupling of motor module to respiratory chain. *J. Cell Biol.* 179, 1115–1122.
- Williams, C.C., Jan, C.H., and Weissman, J.S. (2014). Targeting and plasticity of mitochondrial proteins revealed by proximity-specific ribosome profiling. *Science* 346, 748–751.
- Wrobel, L., Topf, U., Bragoszewski, P., Wiese, S., Sztolsztener, M.E., Oeljeklaus, S., Varabyova, A., Lirski, M., Chroscicki, P., Mroczek, S., et al. (2015). Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. *Nature* 524, 485–488.
- Wu, Y. and Sha, B. (2006). Crystal structure of yeast mitochondrial outer membrane translocase member Tom70p. *Nat. Struct. Mol. Biol.* 13, 589–593.
- Wurm, C.A. and Jakobs, S. (2006). Differential protein distributions define two sub-compartments of the mitochondrial inner membrane in yeast. *FEBS Lett.* 580, 5628–5634.
- Xie, J., Marusich, M.F., Souda, P., Whitelegge, J., and Capaldi, R.A. (2007). The mitochondrial inner membrane protein Mitofilin exists as a complex with SAM50, metaxins 1 and 2, coiled-coil-helix coiled-coil-helix domain-containing protein 3 and 6 and DnaJC11. *FEBS Lett.* 581, 3545–3549.
- Yamamoto, H., Esaki, M., Kanamori, T., Tamura, Y., Nishikawa, S., and Endo, T. (2002). Tim50 is a subunit of the TIM23 complex that links protein translocation across the outer and inner mitochondrial membranes. *Cell* 111, 519–528.
- Yamamoto, H., Fukui, K., Takahashi, H., Kitamura, S., Shiota, T., Terao, K., Uchida, M., Esaki, M., Nishikawa, S., Yoshihisa, T., et al. (2009). Roles of Tom70 in import of presequence-containing mitochondrial proteins. *J. Biol. Chem.* 284, 31635–31646.
- Yamano, K. and Youle, R.J. (2013). PINK1 is degraded through the N-end rule pathway. *Autophagy* 9, 1758–1769.
- Yamano, K., Yatsukawa, Y., Esaki, M., Hobbs, A.E., Jensen, R.E., and Endo, T. (2008). Tom20 and Tom22 share the common signal recognition pathway in mitochondrial protein import. *J. Biol. Chem.* 283, 3799–3807.
- Yamano, K., Tanaka-Yamano, S., and Endo, T. (2010a). Mdm10 as a dynamic constituent of the TOB/SAM complex directs coordinated assembly of Tom40. *EMBO Rep.* 11, 187–193.
- Yamano, K., Tanaka-Yamano, S., and Endo, T. (2010b). Tom7 regulates Mdm10-mediated assembly of the mitochondrial import channel protein Tom40. *J. Biol. Chem.* 285, 41222–41231.

- Yogev, O., Karniely, S., and Pines, O. (2007). Translation-coupled translocation of yeast fumarase into mitochondria *in vivo*. *J. Biol. Chem.* 282, 29222–29229.
- Young, J.C., Hoogenraad, N.J., and Hartl, F.U. (2003). Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70. *Cell* 112, 41–50.
- Zerbes, R.M., Bohnert, M., Stroud, D.A., von der Malsburg, K., Kram, A., Oeljeklaus, S., Warscheid, B., Becker, T., Wiedemann, N., Veenhuis, M., et al. (2012). Role of MINOS in mitochondrial membrane architecture: cristae morphology and outer membrane interactions differentially depend on mitofilin domains. *J. Mol. Biol.* 422, 183–191.