Review

Alexander Grevel, Nikolaus Pfanner* and Thomas Becker*

Coupling of import and assembly pathways in mitochondrial protein biogenesis

https://doi.org/10.1515/hsz-2019-0310
Received July 6, 2019; accepted August 13, 2019; previously published online September 11, 2019

Abstract: Biogenesis and function of mitochondria depend on the import of about 1000 precursor proteins that are produced on cytosolic ribosomes. The translocase of the outer membrane (TOM) forms the entry gate for most proteins. After passage through the TOM channel, dedicated preprotein translocases sort the precursor proteins into the mitochondrial subcompartments. Many proteins have to be assembled into oligomeric membrane-integrated complexes in order to perform their functions. In this review, we discuss a dual role of mitochondrial preprotein translocases in protein translocation and oligomeric assembly, focusing on the biogenesis of the TOM complex and the respiratory chain. The sorting and assembly machinery (SAM) of the outer mitochondrial membrane forms a dynamic platform for coupling transport and assembly of TOM subunits. The biogenesis of the cytochrome *c* oxidase of the inner membrane involves a molecular circuit to adjust translation of mitochondrial-encoded core subunits to the availability of nuclear-encoded partner proteins. Thus, mitochondrial protein translocases not only import precursor proteins but can also support their assembly into functional complexes.

Keywords: mitochondria; protein assembly; protein import; respiratory chain; TOM complex.

***Corresponding authors: Nikolaus Pfanner and Thomas Becker,** Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, D-79104 Freiburg, Germany; and CIBSS Centre for Integrative Biological Signalling Studies, University of Freiburg, D-79104 Freiburg, Germany,
e-mail: Nikolaus.Pfanner@biochemie.uni-freiburg.de (N. Pfanner); thomas.becker@biochemie.uni-freiburg.de (T. Becker)
Alexander Grevel: Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, D-79104 Freiburg, Germany; and Faculty of Biology, University of Freiburg, D-79104 Freiburg, Germany

Introduction

Mitochondria were originated by an endosymbiotic event, in which a eukaryotic ancestor cell incorporated a prokaryote similar to α-proteobacteria more than 1.5 billion years ago (Zimorski et al., 2014; Archibald, 2015). During the course of evolution, the vast majority of the genomic information of the symbiont was transferred to the host nuclear genome. Nowadays mitochondria are known as the powerhouses of the cell as they synthesize the bulk of cellular ATP via oxidative phosphorylation. In addition, biosynthesis of heme, lipids and amino acids as well as the formation of iron-sulfur clusters occur within this cell organelle. The surface of mitochondria forms a signaling platform for apoptosis and inflammation. All these functions reflect that the former endosymbiont became an essential organelle for cell metabolism and development.

Mitochondria contain about 1000 different proteins in baker's yeast and up to 1400 different proteins in human cells (Pagliarini et al., 2008; Morgenstern et al., 2017). The mitochondrial genome encodes eight proteins in yeast and 13 proteins in humans that are mainly part of the oxidative phosphorylation machinery. About 99% of the mitochondrial proteins are nuclear encoded, produced on cytosolic ribosomes as precursors and guided to the mitochondrial surface by cytosolic chaperones (Becker et al., 2019; Hansen and Herrmann, 2019). The outer and inner mitochondrial membranes are equipped with dedicated protein machineries, termed protein translocases, which transport the precursor proteins into the mitochondrial subcompartments: the outer and inner membranes, the intermembrane space and the mitochondrial matrix (Endo et al., 2011; Neupert, 2015; Schulz et al., 2015; Hansen and Herrmann, 2019; Pfanner et al., 2019). Studies of recent years revealed that protein translocases are integrated into large protein networks to coordinate protein import with mitochondrial metabolism, architecture and quality control (Hansen and Herrmann, 2019; Pfanner et al., 2019). Defects in mitochondrial protein sorting lead to cellular stress and can cause severe diseases (Sokol et al., 2014; Kang et al., 2018; Pfanner et al., 2019). It is thus

essential to ensure a full protein import capacity of mitochondria. The ubiquitin-proteasome system is involved in the removal of precursor proteins stalled in mitochondrial import sites (Mårtensson et al., 2019). Impaired protein import triggers programs that lead to increased activity of the proteasome, induction of molecular chaperones, attenuation of cytosolic protein biosynthesis and the removal of non-imported proteins from the mitochondrial surface (Nargund et al., 2012; Wang and Chen, 2015; Wrobel et al., 2015; Weidberg and Amon, 2018; Boos et al., 2019).

Protein complexes carry out essential functions in bioenergetics, biogenesis and the architecture of mitochondria. Examples for such protein machineries are the respiratory chain complexes and the F_1F_o-ATP synthase of the oxidative phosphorylation system of the inner membrane, the membrane-bound preprotein translocases, chaperone systems and mitochondrial ribosomes, the mitochondrial contact site and cristae organizing system (MICOS), and the endoplasmic reticulum (ER)-mitochondria-encounter structure (ERMES) that links the ER and the mitochondrial outer membrane (Eisenberg-Bord et al., 2016; Murley and Nunnari, 2016; Ott et al., 2016; Timón-Gómez et al., 2018; Pfanner et al., 2019). Several mitochondrial protein complexes possess specific features that are not shared by other protein machineries in the cell. The preprotein translocase of the outer membrane (TOM) contains the central β-barrel forming protein Tom40 that assembles with several α-helical membrane proteins, forming an α/β-membrane-integrated protein complex (Shiota et al., 2015; Bausewein et al., 2017). The formation of respiratory chain complexes and the F_1F_o-ATP synthase requires the assembly of mitochondrial-encoded subunits synthesized in the matrix with nuclear-encoded proteins that are synthesized in the cytosol and imported (Smith et al., 2012; Dennerlein et al., 2017; Priesnitz and Becker, 2018; Timón-Gómez et al., 2018).

A particular challenge in the formation of protein complexes is to prevent the accumulation of orphaned subunits that are potentially deleterious for the cell (Juszkiewicz and Hegde, 2018). Mitochondria employ a number of mechanisms to minimize the amount of free unassembled proteins. The import and assembly of subunits into mitochondria are tightly coordinated. The synthesis of mitochondrial-encoded proteins can be stalled until an imported subunit can assemble (Dennerlein et al., 2017; Priesnitz and Becker, 2018; Timón-Gómez et al., 2018). Unassembled subunits can be integrated into assembly intermediates that function as building blocks for the subsequent formation of protein machineries. Eventually, mitochondria are equipped with a set of proteases that can degrade unassembled subunits (Baker and Haynes, 2011; Rugarli and Langer, 2012; Voos, 2013). In this review, we focus on the role of protein translocases in the coordination of protein transport with the assembly of mitochondrial protein complexes.

Overview of mitochondrial protein import pathways

The entry gate for mitochondrial preproteins

The TOM complex functions as entry gate for >90% of mitochondrial proteins (Figure 1). The TOM complex is composed of seven different subunits. Tom20 is the initial receptor for precursors with a cleavable presequence, while Tom70 recognizes precursors of hydrophobic inner and outer membrane proteins (Brix et al., 1997; Abe et al., 2000; Young et al., 2003; Yamano et al., 2008; Yamamoto et al., 2009; Becker et al., 2011a; Papić et al., 2011; Backes et al., 2018). Both receptors show a partially overlapping substrate specificity. In yeast, a paralogue of Tom70, Tom71, exists, however, its function in protein transport remains poorly understood (Bömer et al., 1996; Schlossmann et al., 1996). The central receptor Tom22 recruits Tom20 and Tom70 to the TOM core complex and mediates transfer of the preproteins from the initial receptor proteins to the translocation pore (van Wilpe et al., 1999; Yamano et al., 2008; Shiota et al., 2011). The TOM complex contains two to three translocation pores that are formed by the β-barrel of Tom40 (Hill et al., 1998; Künkele et al., 1998; Ahting et al., 1999; Model et al., 2002; Suzuki et al., 2004; Becker et al., 2005; Shiota et al., 2015; Bausewein et al., 2017; Makki et al., 2019). The transmembrane segment of Tom22 links the Tom40 pores and is thus critical for the assembly of the TOM complex (van Wilpe et al., 1999; Shiota et al., 2015; Bausewein et al., 2017). The three small TOM subunits Tom5, Tom6 and Tom7 support preprotein transport and assembly of the TOM machinery (Alconada et al., 1995; Hönlinger et al., 1996; Dietmeier et al., 1997; Esaki et al., 2004; Schmitt et al., 2005; Sherman et al., 2005; Becker et al., 2010). After passage through the TOM complex, the preproteins are sorted into the different mitochondrial subcompartments. The intermembrane space domains of Tom22, Tom7 and Tom40 initiate sorting to downstream protein translocases (Gabriel et al., 2003; Esaki et al., 2004; Shiota et al., 2015).

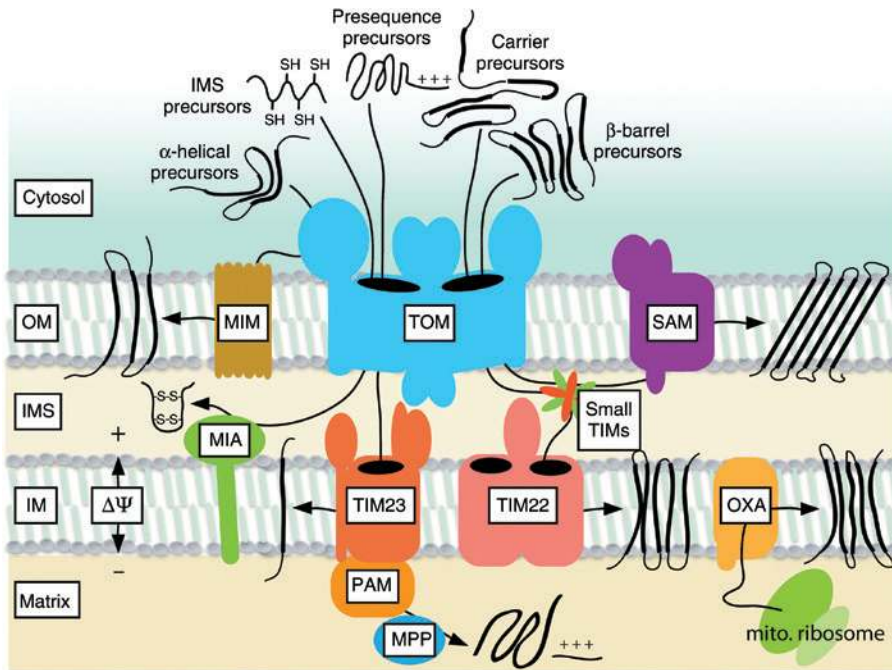

Figure 1: Overview about protein import pathways into mitochondria.
Most mitochondrial precursor proteins are transported through the channel of the TOM complex. After translocation across the outer membrane (OM), four different pathways sort these precursor proteins into the mitochondrial subcompartments. First, the presequence TIM23 complex transports precursors with a cleavable presequence into the inner membrane (IM) or mitochondrial matrix. Transport into the matrix additionally depends on the ATP-consuming activity of the PAM. The MPP removes the presequences from the imported precursors. Second, precursors of the hydrophobic carrier proteins are guided by the small TIM chaperones through the intermembrane space (IMS) to the carrier translocase of the inner membrane (TIM22 complex), which integrates them into the inner membrane. The membrane potential ($\Delta\psi$) across the inner membrane drives the transport via both presequence translocase and carrier translocase. Third, the MIA pathway promotes import and oxidative folding of cysteine-rich proteins in the intermembrane space. Fourth, the small TIM chaperones stabilize the transfer of β-barrel precursors from the TOM complex toward the SAM complex for insertion into the outer membrane. Precursors of outer membrane proteins with several α-helical transmembrane segments are recognized by TOM receptors, but typically bypass the TOM channel and are inserted into the outer membrane by the mitochondrial import (MIM) complex. The OXA insertase of the inner membrane promotes co-translational insertion of mitochondrial-encoded proteins.

Protein sorting into inner mitochondrial subcompartments

Different protein import pathways to the intermembrane space, inner membrane and matrix have been described (Figure 1) (Endo et al., 2011; Neupert, 2015; Schulz et al., 2015; Hansen and Herrmann, 2019; Pfanner et al., 2019). The mitochondrial intermembrane space import and assembly (MIA) machinery mediates import and oxidative folding of cysteine-rich proteins in the intermembrane space. The majority of precursors of inner membrane and matrix proteins are synthesized with a cleavable presequence (Vögtle et al., 2009). The presequence translocase of the inner membrane (TIM23 complex) mediates their lateral release into the inner membrane or together with the presequence translocase-associated motor (PAM) their translocation into the mitochondrial matrix. The activity of the respiratory chain complexes generates a membrane potential ($\Delta\psi$) across the inner membrane that drives the translocation of the positively charged presequences through the pore of the TIM23 complex. Transport into the mitochondrial matrix additionally requires the ATP-dependent activity of the mitochondrial heat shock protein 70 (mtHsp70), which is the central subunit of the motor PAM. The mitochondrial processing peptidase (MPP) removes the presequences from imported proteins. The import of carrier precursors that lack a cleavable presequence occurs via a different mechanism. The small TIM chaperones of the intermembrane space guide these hydrophobic precursors from the TOM complex to the carrier translocase of the inner membrane (TIM22 complex). $\Delta\psi$ promotes the membrane integration of precursor proteins via the carrier translocase. Moreover, the oxidase assembly (OXA) insertase promotes the co-translational insertion of mitochondrial-encoded proteins into the inner membrane (Fox, 2012; Ott et al., 2016; Pfanner et al., 2019).

Protein sorting into the outer mitochondrial membrane

The outer membrane of mitochondria harbors two different types of integral membrane proteins. Proteins with a transmembrane β-barrel are important for protein transport, organelle contact sites and metabolite transport. Proteins embedded in the outer membrane by a single or multiple α-helical transmembrane segments are involved in mitochondrial protein transport, quality control, membrane dynamics and apoptosis. Different import pathways exist for the integration of these proteins into the outer membrane (Dukanovic and Rapaport, 2011; Endo et al., 2011; Ellenrieder et al., 2015; Neupert, 2015; Pfanner et al., 2019). Precursors of β-barrel proteins are first imported via the TOM channel and are subsequently transferred to the sorting and assembly machinery (SAM complex) (Figure 1). The central subunit of the SAM complex, Sam50/Tob55, forms a β-barrel that promotes the lateral insertion of the precursor proteins into the outer membrane (Kozjak et al., 2003; Paschen et al., 2003; Gentle et al., 2004; Klein et al., 2012; Höhr et al., 2018). TOM and SAM complexes form a supercomplex to channel preproteins between the translocases (Qiu et al., 2013; Wenz et al., 2015). The small TIM chaperones of the intermembrane space stabilize the precursor proteins at this transfer step, probably by shielding hydrophobic segments (Wiedemann et al., 2004; Hoppins and Nargang, 2004; Qiu et al., 2013; Weinhäupl et al., 2018).

Precursors of outer membrane proteins with a single or multiple α-helical membrane anchors follow different import pathways. Tom70 recognizes incoming preproteins of multi-spanning outer membrane proteins. Subsequently, the mitochondrial import machinery (MIM complex) inserts these proteins into the outer membrane (Otera et al., 2007; Becker et al., 2011a; Papić et al., 2011; Dimmer et al., 2012; Sinzel et al., 2016) (Figure 1). The MIM complex consists of several copies of Mim1 and one or two copies Mim2 (Dimmer et al., 2012). The MIM subunits are anchored in the outer membrane by single α-helical membrane anchors. The mechanism of protein import via the MIM complex is poorly understood. Mim1 oligomers form a channel in reconstituted membranes that could be involved in the integration of the precursor proteins into the outer membrane (Krüger et al., 2017). Supporting this view, the oligomerization of Mim1 is required to maintain full import capacity (Popov-Celeketić et al., 2008). The MIM complex also inserts several precursor proteins with a single transmembrane α-helix at the N-terminus (Becker et al., 2008; Hulett et al., 2008; Popov-Celeketić et al., 2008; Wenz et al., 2014). It remains to be investigated if the MIM complex plays a general role for the import of single-spanning outer membrane proteins.

Assembly of the TOM complex

A specific feature of key protein complexes of the mitochondrial outer membrane is the presence of a central β-barrel protein that associates either with integral α-helical membrane proteins (in case of the TOM complex) or with peripheral membrane proteins (in the case of the SAM and ERMES complexes). The assembly of the TOM complex in yeast mitochondria represents the best-studied example for the formation of outer membrane protein complexes. It involves the association of six different TOM subunits containing single α-helical membrane anchors with the β-barrel of Tom40. To promote efficient formation of the protein translocase, the assembly process is tightly coupled to the import of the individual subunits (Figure 2).

Formation of the Tom40-small Toms module

The SAM complex plays a central role in the assembly of the TOM complex. The core subunit Tom40 is imported via the TOM-SAM supercomplex like other β-barrel proteins (Wiedemann et al., 2003; Paschen et al., 2003; Qiu et al., 2013; Wenz et al., 2015). However, the Tom40 precursor is much more slowly released from the SAM complex than other β-barrel proteins (Wiedemann et al., 2003; Paschen et al., 2003; Becker et al., 2010; Thornton et al., 2010). The prolonged retention of Tom40 at the SAM complex likely facilitates the assembly with small Tom subunits. Tom5 is the first Tom subunit that associates with the precursor of Tom40 at the SAM complex and thereby promotes the formation of the mature Tom40 β-barrel (Wiedemann et al., 2003; Becker et al., 2010; Qiu et al., 2013). In addition, Tom5 facilitates the assembly of Tom6 with Tom40 (Becker et al., 2010; Thornton et al., 2010) and thus initiates the assembly of further Tom components (Figure 2). How Tom7 is fed into the assembly line is currently unclear, but it associates with the Tom40-small Tom module (Stojanovski et al., 2007).

These observations indicate that the SAM complex functions as an assembly platform for Tom40 and small Tom proteins. Supporting this view, the biogenesis of small Tom proteins depends on a functional SAM complex (Stojanovski et al., 2007; Thornton et al., 2010). Thus, the SAM complex not only inserts Tom40 into the outer membrane, but also cooperates with the small Tom proteins in the assembly of a TOM core complex (Dukanovic et al., 2009; Becker et al., 2010). How the small Tom proteins are delivered to the SAM-bound Tom40 is not entirely clear. The MIM complex promotes the biogenesis of the small Tom proteins and could

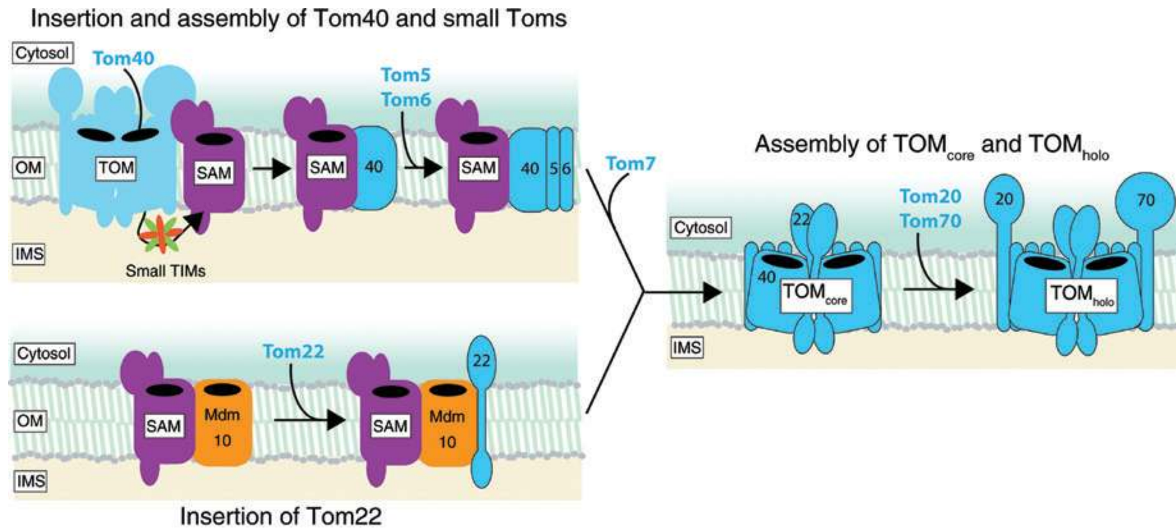

Figure 2: A dynamic SAM platform promotes assembly of the TOM complex in yeast mitochondria.
The TOM complex initially transports the Tom40 precursor across the outer membrane. The SAM complex inserts the Tom40 precursor from the IMS side into the OM and promotes its assembly with the small Tom proteins (upper left). The SAM-Mdm10 complex facilitates the membrane integration of the precursor of Tom22 (lower left). How Tom22 is transferred onto the Tom40-small Toms module is unknown. Tom22 links two Tom40-small Toms modules to form the TOM core complex (right). Finally, the precursors of Tom20 and Tom70 are integrated into the outer membrane in a MIM-dependent manner and associate with the TOM core to form the mature TOM complex, also termed TOM holo complex. IMS, intermembrane space; OM, outer membrane.

contribute to their assembly with Tom40 (Becker et al., 2008; Thornton et al., 2010).

The SAM-Mdm10 complex promotes assembly of Tom22 with TOM subunits

Structural and biochemical studies of the TOM core complex revealed that Tom22 links two Tom40-small Toms modules (van Wilpe et al., 1999; Shiota et al., 2015; Bausewein et al., 2017). Consequently, the assembly of Tom22 with Tom40-small Toms is a critical step in the formation of the TOM complex. The Tom22 precursor is delivered to TOM receptors by cytosolic chaperones (Keil and Pfanner, 1993; Opaliński et al., 2018). Subsequently, the precursor is transferred by an unknown mechanism to a specific subpopulation of the SAM complex that associates with the β-barrel protein Mdm10 (Thornton et al., 2010; Becker et al., 2011b; Ellenrieder et al., 2016). Disruption of the SAM-Mdm10 complex impairs membrane integration of the Tom22 precursor (Ellenrieder et al., 2016).

Thus, two distinct SAM populations are required to build up the TOM complex (Figure 2). In one assembly line, the SAM complex assembles Tom40 with the small Toms. In a second line, the SAM-Mdm10 complex inserts the Tom22 precursor into the outer membrane. How Tom22 is transferred onto the Tom40-small Toms module has not been fully clarified. Mdm10 could play a critical role in this step (Meisinger et al., 2004; Yamano et al., 2010a; Ellenrieder et al., 2016). Mdm10 promotes the release of the Tom40 precursor from the SAM complex (Yamano et al., 2010a) and thereby may coordinate the activity of both SAM populations. Finally, Tom20 and Tom70 associate in a Tom22-dependent manner with the TOM core complex to form the mature TOM complex, also termed the TOM holo complex (van Wilpe et al., 1999; Yamano et al., 2008; Shiota et al., 2011). The MIM complex also promotes the membrane insertion of the precursors of Tom20 and Tom70 into the outer membrane (Becker et al., 2008; Hulett et al., 2008; Popov-Celeketić et al., 2008). Whether or not the MIM complex coordinates the import of Tom20 and Tom70 with their assembly into the TOM complex is unknown.

Regulation of TOM complex assembly

The TOM complex plays a pivotal role in mitochondrial protein biogenesis and its formation has to be fine-tuned to meet cellular needs. Signaling pathways leading to phosphorylation of TOM subunits modulate the formation of the mitochondrial entry gate under different metabolic and growth conditions (Schmidt et al., 2011). Tom22 and Tom6 are critical subunits for the formation and stability of the TOM complex and are therefore

important targets for regulatory processes (Alconada et al., 1995; van Wilpe et al., 1999). Phosphorylation of the cytosolic domain of Tom22 stabilizes the association of Tom20 with the TOM complex (Schmidt et al., 2011; Gerbeth et al., 2013) and cell cycle-dependent phosphorylation of Tom6 promotes the formation of the TOM complex (Harbauer et al., 2014; Sakaue et al., 2019). Interestingly, Tom7 can act as a negative regulator of the formation of the TOM complex. An excess amount of free Tom7 impairs the binding of Tom5 to Tom40 as well as the formation of the SAM-Mdm10 complex (Meisinger et al., 2006; Yamano et al., 2010b; Becker et al., 2011b), indicating that control switches exist to regulate the biogenesis of the TOM complex. Furthermore, quality control systems are important to prevent the formation of malfunctional protein translocases. Recently, it was shown that binding of the major metabolite channel of the outer membrane, termed porin or voltage-dependent anion channel (VDAC), to unassembled Tom22 regulates the formation of the TOM complex (Ellenrieder et al., 2019; Sakaue et al., 2019). In the absence of Tom6, the binding of Tom22 to the TOM complex is destabilized. However, a parallel loss of porin/VDAC and Tom6 restores assembly of Tom22 into the TOM complex. Strikingly, this alternative TOM complex is not fully active (Sakaue et al., 2019), indicating that the interplay between porin/VDAC and Tom6 is an important mechanism to prevent improper integration of Tom22 into the TOM complex.

Biogenesis of respiratory chain complexes

The mitochondrial respiratory chain consists of four multi-subunit complexes: the NADH dehydrogenase (complex I), the succinate dehydrogenase (complex II), the cytochrome bc_1 complex (complex III) and the cytochrome *c* oxidase (COX, complex IV). Formation of the complexes I, III and IV as well as of the F_1F_o-ATP synthase are particularly complicated processes as proteins of two distinct genetic origins have to be assembled into functional protein complexes (Fox, 2012; Dennerlein et al., 2017; Formosa et al., 2018; Priesnitz and Becker, 2018; Timón-Gómez et al., 2018). These oxidative phosphorylation complexes are preferentially located in the folded cristae of the inner membrane, whereas preprotein translocases are enriched in the inner boundary membrane that is positioned adjacent to the outer membrane (Vogel et al., 2006; Stoldt et al., 2018). The formation of oxidative phosphorylation complexes requires the coordination of biosynthesis of mitochondrial-encoded proteins with the import of nuclear-encoded proteins to allow an efficient assembly process in the inner membrane.

Coupling of import and assembly of respiratory chain subunits

The TIM23 complex imports the majority of nuclear-encoded respiratory chain subunits. Super-resolution microscopy revealed that the early, but not the late assembly steps of complex III and complex IV occur predominantly at the inner boundary membrane (Stoldt et al., 2018), pointing to a spatial organization of protein import and assembly steps. Interestingly, a fraction of respiratory chain supercomplexes containing complexes III and IV directly associate with the TIM23 complex in yeast mitochondria (van der Laan et al., 2006; Wiedemann et al., 2007). This translocase-respiratory chain coupling supports the import of precursor proteins that are particularly sensitive toward perturbations of the inner membrane potential (van der Laan et al., 2006; Schendzielorz et al., 2017). The exact molecular mechanism has not been solved, yet TIM23 complexes bound to respiratory chain complexes possibly experience an increased proton motive force or may benefit from a direct transfer of protons. These studies suggest that the respiratory chain is closely linked to preprotein import via the presequence translocase to ensure an efficient supply of nuclear-encoded subunits of the respiratory chain.

A further link between protein import and formation of the respiratory chain was found for the central PAM subunit, mtHsp70. The chaperone associates with the yeast translational activator Mss51 and thus plays a role in the biogenesis of mitochondrial-encoded Cox1 (Fontanesi et al., 2010, 2011). Furthermore, mtHsp70 binds stably to an unassembled nuclear-encoded subunit of complex IV and delivers it to the assembly line when needed (Böttinger et al., 2013). The mtHsp70-bound precursor pool is important to warrant efficient formation of complex IV (Böttinger et al., 2013). Assembly intermediates of complex III and complex IV were detected under several physiological conditions (Mick et al., 2010; Hildenbeutel et al., 2014). The presence of such assembly intermediates could serve two functions. First, the binding of assembly factors stabilizes the unassembled respiratory chain subunits (preventing their premature degradation). Second, the assembly intermediates act as a platform for the assembly of further subunits to allow a rapid formation of the mature complexes.

Translational activators and plasticity in mitochondria

The mitochondrial genome encodes central subunits of the respiratory chain, including the multi-spanning core subunits of complexes III and IV. Studies in yeast demonstrated that the OXA insertase and partner proteins associate with mitochondrial ribosomes to promote co-translational insertion of the nascent proteins from the matrix side into the inner mitochondrial membrane (Figure 1) (Hell et al., 2001; Jia et al., 2003; Szyrach et al., 2003; Ott et al., 2006; Pfeffer et al., 2015).

Depending on the organism studied, different mechanisms have been observed of how translation of mitochondrial-encoded transcripts is regulated. In the model organism yeast, mitochondrial transcripts contain 5′ untranslated regions (5′ UTR) that are frequent targets of translational activators, which are nuclear-encoded and thus have to be imported into mitochondria (Fox, 2012; Smith et al., 2012; Herrmann et al., 2013; Dennerlein et al., 2017; Priesnitz and Becker, 2018; Timón-Gómez et al., 2018). The translational activators Cbp3-Cbp6 and Mss51 coordinate translation and assembly of the mitochondrial-encoded cytochrome *b* of complex III and Cox1 of complex IV, respectively (Decoster et al., 1990; Pérez-Martínez et al., 2003; Gruschke et al., 2011). These translational activators function as crucial elements of molecular circuits that couple the maturation of the mitochondrial-encoded subunits to the assembly with nuclear-encoded subunits and the availability of cofactors like heme (Barrientos et al., 2004; Pérez-Martínez et al., 2009; Fontanesi et al., 2010; Khalimonchuk et al., 2010; Mick et al., 2010; Gruschke et al., 2012; Soto et al., 2012; Hildenbeutel et al., 2014). Mitochondria of baker's yeast do not possess a complex I of dual genetic origin, but contain nuclear-encoded alternative NADH dehydrogenases that transfer electrons from NADH to the respiratory chain (Marres et al., 1991; Luttik et al., 1998; Small and McAlister-Henn, 1998).

In contrast, human mitochondrial mRNAs either lack a 5′ UTR or contain only a short 5′ UTR. Therefore, it remains enigmatic how translational activators like TACO1 stimulate protein biosynthesis in human mitochondria (Herrmann et al., 2013; Dennerlein et al., 2017; Priesnitz and Becker, 2018). A detailed analysis of the biogenesis of human COX1 revealed a different principle how translation of mitochondrial transcripts is linked to the import of nuclear-encoded subunits (Figure 3) (Richter-Dennerlein et al., 2016). The assembly factors COX14 (C12ORF62) and MITRAC12 (COA3) bind to the nascent chain of COX1 while the protein is synthesized on mitochondrial ribosomes. This early form of the mitochondrial translation regulation assembly intermediate of cytochrome *c* oxidase, termed MITRAC, arrests translation of *COX1* mRNA. The *COX1* translation is paused until the first nuclear-encoded subunit, COX4-1, binds to the MITRAC complex (Richter-Dennerlein et al., 2016). Upon binding of COX4-1, *COX1* translation resumes and further complex IV subunits as well as auxiliary factors can associate (Mick et al., 2012; Richter-Dennerlein et al., 2016). Thus, the translation of *COX1* is adjusted to the availability of nuclear-encoded partner proteins, a mechanism termed translational plasticity (Richter-Dennerlein et al., 2016). Mutations of MITRAC subunits lead to complex IV deficiency and have been linked to several diseases, reflecting the central role of this assembly platform (Table 1) (Shoubridge, 2001; Dennerlein and Rehling, 2015; Ghezzi and Zeviani, 2018).

TIM21 of the presequence translocase likely plays a direct role in this assembly process (Mick et al., 2012). The absence of TIM21 impairs the assembly of complex IV subunits like COX4-1 (Mick et al., 2012). Thus, TIM21 may deliver COX4-1 to MITRAC in order to complete the synthesis of COX1 and to promote the assembly of complex IV (Mick et al., 2012; Richter-Dennerlein et al., 2016). In support of this view, TIM21 is a subunit of the late form of the MITRAC complex and couples import of nuclear-encoded subunits to their assembly into complex IV (Figure 3) (Mick et al., 2012).

The studies in yeast and humans thus reveal that, depending on the presence or absence of 5′ UTRs in mitochondrial transcripts, remarkably different mechanisms have evolved in order to coordinate the synthesis of mitochondrial-encoded subunits and their assembly with nuclear-encoded subunits.

Conclusions and perspectives

Protein complexes are essential for mitochondrial biogenesis and function. Several mitochondrial membrane protein complexes display features that are distinct from other protein assemblies of the cell. In the outer membrane, the TOM complex contains the central β-barrel protein Tom40 that associates with subunits possessing single α-helical membrane anchors. In the inner membrane, proteins of dual genetic origin form the NADH dehydrogenase, cytochrome bc_1 complex, cytochrome *c* oxidase and the F_1F_o-ATP synthase. These characteristics impose specific challenges on the formation of these protein complexes. One central mechanism is the coupling of translocation to the assembly of the core subunits of the complex. In the outer membrane, the SAM

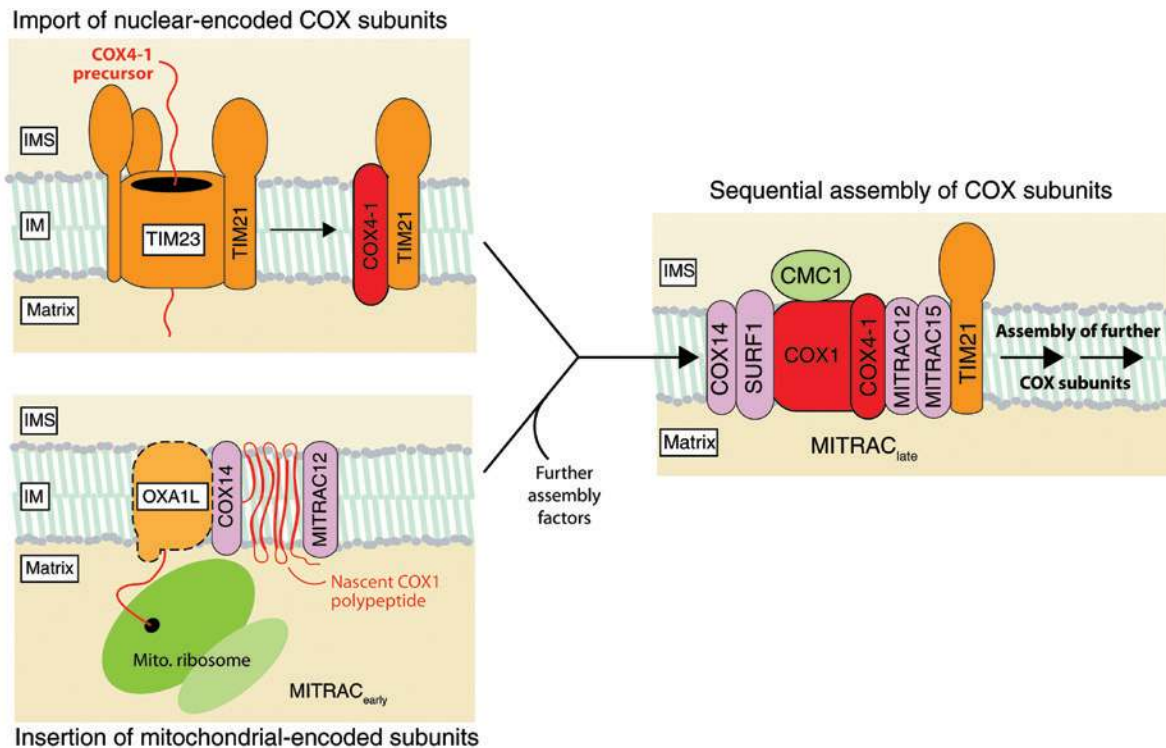

Figure 3: Translational plasticity controls early assembly steps of the human cytochrome *c* oxidase.
The COX4-1 precursor is imported into mitochondria via the presequence pathway (upper left). TIM21 from the TIM23 complex transfers the membrane-inserted COX4-1 precursor from the presequence translocase to the assembly line of the cytochrome *c* oxidase. The insertase OXA1L may promote the insertion of the mitochondrial-encoded COX1 into the IM (lower left). In the early MITRAC form (mitochondrial translation regulation assembly intermediate of cytochrome *c* oxidase), COX14 and MITRAC12 associate with the mitochondrial ribosome-nascent COX1 polypeptide complex. The translation of COX1 is arrested (translational plasticity) until the first nuclear-encoded subunit, COX4-1, has been delivered by TIM21 to the late MITRAC intermediate (right). The late MITRAC intermediate contains further assembly factors (MITRAC15, SURF1 and CMC1) and provides a platform for the assembly of COX4-1 and further COX subunits. This translational plasticity fine-tunes mitochondrial protein biosynthesis to the import of nuclear-encoded subunits. IM, inner membrane; IMS, intermembrane space.

Table 1: MITRAC components and their implications for human disorders.

Human protein	Yeast protein	Proposed function	Human pathology	Reference
COX14/C12ORF62	Cox14	Stabilization of newly translated COX1	Neonatal lactic acidosis	Barrientos et al., 2004; Mick et al., 2010; Fontanesi et al., 2011; Weraarpachai et al., 2012; Richter-Dennerlein et al., 2016
MITRAC12/COA3	Coa3	Stabilization of newly translated COX1	Neuropathy, exercise intolerance, obesity	Mick et al., 2010; Fontanesi et al., 2011; Ostergaard et al., 2015; Richter-Dennerlein et al., 2016
SURF1	Shy1	Insertion or stabilization of heme A in COX1	Leigh syndrome	Tiranti et al., 1998; Zhu et al., 1998; Barrientos et al., 2002; Mick et al., 2007; Reinhold et al., 2011
MITRAC15/COA1	Coa1	Assembly of COX1		Pierrel et al. 2007; Mick et al., 2012
CMC1	Cmc1	Stabilizes COX1/COX14/COA3 intermediate		Horn et al., 2008; Bourens and Barrientos, 2017

complex provides a dynamic platform to allow binding of the imported Tom40 to other Tom subunits. In the inner membrane, the co-translational insertion of mitochondrial-encoded proteins like COX1 is coupled via assembly factors to the binding of nuclear-encoded subunits. The subunit TIM21 of the human TIM23 complex transfers respiratory chain components into the assembly line. Thus, the protein translocases SAM and TIM23 not only

translocate precursor proteins but also facilitate their assembly with partner proteins. Such coupling mechanisms minimize the presence of orphaned proteins, which could be potentially harmful for mitochondrial functions.

There is growing evidence that the mitochondrial membranes contain a reservoir of assembly intermediates to allow the rapid and efficient formation of mature complexes (Thornton et al., 2010; Mick et al., 2010; Böttinger et al., 2013; Hildenbeutel et al., 2014). Shifting cells from fermentative to respiratory growth conditions requires a massive increase of the copy numbers of proteins involved in energy metabolism (Morgenstern et al., 2017). Under these conditions, the presence of assembly intermediates will facilitate the formation of respiratory chain complexes to rapidly adjust mitochondrial metabolism to cellular needs. Future work has to reveal if coupling of import and assembly plays a general role in the formation of mitochondrial protein complexes and how these processes are modulated in response to cellular signaling.

Acknowledgments: Work included in this article has been performed in partial fulfillment of the requirements for the doctoral thesis of A.G. This work was supported by the Deutsche Forschungsgemeinschaft (BE 4679/2-2 and PF 202/9-1) and the Excellence Strategy of the German Federal and State Governments (EXC 2189 CIBSS Project ID 390939984).

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.

Archibald, J.M. (2015). Endosymbiosis and eukaryotic cell evolution. Curr. Biol. *25*, 911–921.

Ahting, U., Thun, C., Hegerl, R., Typke, D., Nargang, F.E., Neupert, W., and Nussberger, S. (1999). The TOM core complex: the general protein import pore of the outer membrane of mitochondria. J. Cell Biol. *147*, 959–968.

Alconada, A., Kübrich, M., Moczko, M., Hönlinger, A., and Pfanner, N. (1995). The mitochondrial receptor complex: the small subunit Mom8b/Isp6 supports association of receptors with the general insertion pore and transfer of preproteins. Mol. Cell. Biol. *15*, 6196–6205.

Backes, S., Hess, S., Boos, F., Woellhaf, M.W., Gödel, S., Jung, M., Mühlhaus, T., and Herrmann, J.M. (2018). Tom70 enhances mitochondrial preprotein import efficiency by binding to internal targeting sequences. J. Cell Biol. *217*, 1369–1382.

Baker, B.M. and Haynes, C.M. (2011). Mitochondrial protein quality control during biogenesis and aging. Trends Biochem. Sci. *36*, 254–261.

Barrientos, A., Korr, D., and Tzagoloff, A. (2002). Shy1p is necessary for full expression of mitochondrial COX1 in the yeast model of Leigh's syndrome. EMBO J. *21*, 43–52.

Barrientos, A., Zambrano, A., and Tzagoloff, A. (2004). Mss51p and Cox14p jointly regulate mitochondrial Cox1p expression in *Saccharomyces cerevisiae*. EMBO J. *23*, 3472–3482.

Bausewein, T., Mills, D.J., Langer, J.D., Nitschke, B., Nussberger, S., and Kühlbrandt, W. (2017). Cryo-EM structure of the TOM core complex from *Neurospora crassa*. Cell *170*, 693–700.

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., Guiard, B., Thornton, N., Zufall, N., Stroud, D.A., Wiedemann, N., and Pfanner, N. (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*, 3106–3113.

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., Song, J., and Pfanner, N. (2019). Versatility of preprotein transfer from the cytosol to mitochondria. Trends Cell Biol. *29*, 534–548.

Bömer, U., Pfanner, K., and Dietmeier, K. (1996). Identification of a third Tom protein with tetratrico peptide repeats. FEBS Lett. *382*, 153–158.

Boos, F., Krämer, L., Groh, C., Jung, F., Haberkant, P., Stein, F., Wollweber, F., Gackstatter, A., Zöller, E., van der Laan, M., et al. (2019). Mitochondrial protein-induced stress triggers a global adaptive transcriptional programme. Nat. Cell Biol. *21*, 442–451.

Böttinger, L., Guiard, B., Oeljeklaus, S., Kulawiak, B., Zufall, N., Wiedemann, N., Warscheid, B., van der Laan, M., and Becker, T. (2013). A complex of Cox4 and mitochondrial Hsp70 plays an important role in the assembly of the cytochrome *c* oxidase. Mol. Biol. Cell *24*, 2609–2619.

Bourens, M. and Barrientos, A. (2017). A CMC1-knockout reveals translation-independent control of human mitochondrial complex IV biogenesis. EMBO Rep. *18*, 477–494.

Brix, J., Dietmeier, K., and Pfanner, N. (1997). Differential recognition of preproteins by the purified cytosolic domains of the mitochondrial import receptors Tom20, Tom22, and Tom70. J. Biol. Chem. *272*, 20730–20735.

Decoster, E., Simon, M., Hatat, D., and Faye, G. (1990). The *MSS51* gene product is required for the translation of the *COX1* mRNA in yeast mitochondria. Mol. Gen. Genet. *224*, 111–118.

Dennerlein, S. and Rehling, P. (2015). Human mitochondrial COX1 assembly into cytochrome *c* oxidase at a glance. J. Cell Sci. *128*, 833–837.

Dennerlein, S., Wang, C., and Rehling, P. (2017). Plasticity of mitochondrial translation. Trends Cell Biol. *27*, 712–721.

Dietmeier, K., Hönlinger, A., Bömer, U., Dekker, P.J.T., Eckerskorn, C., Lottspeich, F., Kübrich, M., and Pfanner, N. (1997). Tom5 functionally links mitochondrial preprotein receptors to the general import pore. Nature *388*, 195–200.

Dimmer, K.S., Papic, 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.

Dukanovic, J., Dimmer, K.S., Bonnefoy, N., Krumpe, K., and Rapaport, D. (2009). Genetic and functional interactions between the mitochondrial outer membrane proteins Tom6 and Sam37. Mol. Cell. Biol. *29*, 5975–5988.

Dukanovic, J. and Rapaport, D. (2011). Multiple pathways in the integration of proteins into the mitochondrial outer membrane. Biochim. Biophys. Acta *1808*, 971–980.

Eisenberg-Bord, M., Shai, N., Schuldiner, M., and Bohnert, M. (2016). A tether is a tether is a tether: tethering at membrane contact sites. Dev. Cell *39*, 395–409.

Ellenrieder, L., Mårtensson, C.U., and Becker, T. (2015). Biogenesis of mitochondrial outer membrane proteins, problems and diseases. Biol. Chem. *396*, 1199–1213.

Ellenrieder, L., Opaliński, Ł., Becker, L., Krüger, V., Mirus, O., Straub, S.P., Ebell, K., Flinner, N., Stiller, S.B., Guiard, B., et al. (2016). Separating mitochondrial protein assembly and endoplasmic reticulum tethering by selective coupling of Mdm10. Nat. Commun. *7*, 13021.

Ellenrieder, L., Dieterle, M.P., Doan, K.N., Mårtensson, C.U., Floerchinger, A., Campo, M.L., Pfanner, N., and Becker, T. (2019). Dual role of mitochondrial porin in metabolite transport across the outer membrane and protein transfer to the inner membrane. Mol. Cell *73*, 1056–1065.

Endo, T., Yamano, K., and Kawano, S. (2011). Structural insight into the mitochondrial protein import system. Biochim. Biophys. Acta *1808*, 955–970.

Esaki, M., Shimizu, H., Ono, T., Yamamoto, H., Kanamori, T., Nishikawa, S.I., and Endo, T. (2004). Mitochondrial protein import: requirement of presequence elements and TOM components for precursor binding to the TOM complex. J. Biol. Chem. *279*, 45701–45707.

Fontanesi, F., Soto, I.C., Horn, D., and Barrientos, A. (2010). Mss51 and Ssc1 facilitate translational regulation of cytochrome *c* oxidase biogenesis. Mol. Cell Biol. *30*, 245–259.

Fontanesi, F., Clemente, P., and Barrientos, A. (2011). Cox25 teams up with Mss51, Ssc1, and Cox14 to regulate mitochondrial cytochrome *c* oxidase subunit 1 expression and assembly in *Saccharomyces cerevisiae*. J. Biol. Chem. *286*, 555–566.

Formosa, L.E., Dibley, M.G., Stroud, D.A., and Ryan, M.T. (2018). Building a complex complex: assembly of mitochondrial respiratory chain complex I. Semin. Cell Dev. Biol. *76*, 154–162.

Fox, T.D. (2012). Mitochondrial protein synthesis, import and assembly. Genetics *192*, 1203–1234.

Gabriel, K., Egan, B., and Lithgow, T. (2003). Tom40, the import channel of the mitochondrial outer membrane, plays an active role in sorting imported proteins. EMBO J. *22*, 2380–2386.

Gentle, I., Gabriel, K., Beech, P., Waller, R., and Lithgow, T. (2004). The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria. J. Cell Biol. *164*, 19–24.

Ghezzi, D. and Zeviani, M. (2018). Human diseases associated with defects in assembly of OXPHOS complexes. Essays Biochem. *62*, 271–286.

Gerbeth, C., Schmidt, O., Rao, S., Harbauer, A.B., Mikropoulou, D., Opalińska, M., Guiard, B., Pfanner, N., and Meisinger, C. (2013). Glucose-induced regulation of protein import receptor Tom22 by cytosolic and mitochondria-bound kinases. Cell Metab. *18*, 578–587.

Gruschke, S., Kehrein, K., Römpler, K., Gröne, K., Israel, L., Imhof, A., Herrmann, J.M., and Ott, M. (2011). Cbp3-Cbp6 interacts with the yeast mitochondrial ribosomal tunnel exit and promotes cytochrome *b* synthesis and assembly. J. Cell Biol. *193*, 1101–1114.

Gruschke, S., Römpler, K., Hildenbeutel, M., Kehrein, K., Kühl, I., Bonnefoy, N., and Ott, M. (2012). The Cbp3-Cbp6 complex coordinates cytochrome *b* synthesis with *bc1* complex assembly in yeast mitochondria. J. Cell Biol. *199*, 137–150.

Hansen, K.G. and Herrmann, J.M. (2019). Transport of proteins into mitochondria. Protein J. *38*, 330–342.

Harbauer, A.B., Opalińska, M., Gerbeth, C., Herman, J.S., Rao, S., Schönfisch, B., Guiard, B., Schmidt, O., Pfanner, N., and Meisinger, C. (2014). Cell cycle-dependent regulation of mitochondrial preprotein translocase. Science *346*, 1109–1113.

Hell, K., Neupert, W., and Stuart, R.A. (2001). Oxa1p acts as a general membrane insertion machinery for proteins encoded by mitochondrial DNA. EMBO J. *20*, 1281–1288.

Herrmann, J.M., Woellhaf, M.W., and Bonnefoy, N. (2013). Control of protein synthesis in yeast mitochondria: the concept of translational activators. Biochim. Biophys. Acta *1833*, 286–294.

Hildenbeutel, M., Hegg, E.L., Stephan, K., Gruschke, S., Meunier, B., and Ott, M. (2014). Assembly factors monitor sequential hemylation of cytochrome *b* to regulate mitochondrial translation. J. Cell Biol. *205*, 511–524.

Hill, K., Model, K., Ryan, M.T., Dietmeier, K., Martin, F., Wagner, R., and Pfanner, N. (1998). Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins. Nature *395*, 516–521.

Höhr, A.I.C., Lindau, C., Wirth, C., Qiu, J., Stroud, D.A., Kutik, S., Guiard, B., Hunte, C., Becker, T., Pfanner, N., and Wiedemann, N. (2018). Membrane protein insertion through a mitochondrial β-barrel gate. Science *359*, eaah6834.

Hoppins, S.C. and Nargang, F.E. (2004). The Tim8-Tim13 complex of *Neurospora crassa* functions in the assembly of proteins into both mitochondrial membranes. J. Biol. Chem. *279*, 12396–12405.

Hönlinger, A., Bömer, U., Alconada, A., Eckerskorn, C., Lottspeich, F., Dietmeier, K., and Pfanner, N. (1996). Tom7 modulates the dynamics of the mitochondrial outer membrane translocase and plays a pathway-related role in protein import. EMBO J. *15*, 2125–2137.

Horn, D., Al-Ali, H., and Barrientos, A. (2008). Cmc1p is a conserved mitochondrial CX_9C protein involved in cytochrome *c* oxidase biogenesis. Mol. Cell Biol. *28*, 4354–4364.

Hulett, J.M., Lueder, F., Chan, N.C., Perry, A.J., Wolynec, 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.

Jia, L., Dienhart, M., Schramp, M., McCauley, M., Hell, K., and Stuart, R.A. (2003). Yeast Oxa1 interacts with mitochondrial ribosomes: the importance of the C-terminal region of Oxa1. EMBO J. *22*, 6438–6447.

Juszkiewicz, S. and Hegde, R.S. (2018). Quality control of orphaned proteins. Mol. Cell *71*, 443–457.

Kang, Y., Fielden, L.F., and Stojanovski, D. (2018). Mitochondrial protein transport in health and disease. Semin. Cell Dev. Biol. *76*, 142–153.

Khalimonchuk, O., Bestwick, M., Meunier, B., Watts, T.C., and Winge, D.R. (2010). Formation of the redox cofactor centers during Cox1 maturation in yeast cytochrome oxidase. Mol. Cell Biol. *30*, 1004–1007.

Keil, P. and Pfanner, N. (1993). Insertion of MOM22 into the mitochondrial outer membrane strictly depends on surface receptors. FEBS Lett. *321*, 197–200.

Klein, A., Israel, L., Lackey, S.W.K., Nargang, F.E., Imhof, A., Baumeister, W., Neupert, W., and Thomas, D.R. (2012). Characterization of the insertase for β-barrel proteins of the outer mitochondrial membrane. J. Cell Biol. *199*, 599–611.

Kozjak, V., Wiedemann, N., Milenkovic, D., Lohaus, C., Meyer, H.E., Guiard, B., Meisinger, C., and Pfanner, N. (2003). An essential role of Sam50 in the protein sorting and assembly machinery of the mitochondrial outer membrane. J. Biol. Chem. *278*, 48520–48523.

Krüger, V., Becker, T., Becker, L., Montilla-Martinez, M., Ellenrieder, L., Vögtle, F.N., Meyer, H.E., Ryan, M.T., Wiedemann, N., Warscheid, B., et al. (2017). Identification of new channels by systematic analysis of the mitochondrial outer membrane. J. Cell Biol. *216*, 3485–3495.

Künkele, K.P., Heins, S., Dembowski, M., Nargang, F.E., Benz, R., Thieffry, M., Walz, J., Lill, R., Nussberger, S., and Neupert, W. (1998). The preprotein translocation channel of the outer membrane of mitochondria. Cell *93*, 1009–1019.

Luttik, M.A., Overkamp, K.M., Kötter, P., de Vries, S., van Dijken, J.P., and Pronk, J.T. (1998). The *Saccharomyces cerevisiae NDE1* and *NDE2* genes encode separate mitochondrial NADH dehydrogenases catalyzing the oxidation of cytosolic NADH. J. Biol. Chem. *273*, 24529–24534.

Makki, A., Rada, P., Žarski, V., Kereïche, S., Kováčik, L., Novotný, M., Jores, T., Rapaport, D., and Tachezy, J. (2019). Triplet-pore structure of a highly divergent TOM complex of hydrogenosomes in *Trichomonas vaginalis*. PLoS Biol. *17*, e3000098.

Marres, C.A., de Vries, S., and Grivell, L.A. (1991). Isolation and inactivation of the nuclear gene encoding the rotenone-insensitive internal NADH:ubiquinone oxidoreductase of mitochondria from *Saccharomyces cerevisiae*. Eur. J. Biochem. *195*, 857–862.

Mårtensson, C.U., Priesnitz, C., Song, J., Ellenrieder, L., Doan, K.N., Boos, F., Floerchinger, A., Zufall, N., Oeljeklaus, S., Warscheid, B., et al. (2019). Mitochondrial protein translocation-associated degradation. Nature *569*, 679–683.

Meisinger, C., Rissler, M., Chacinska, A., Sanjuán Szklarz, L.K.S., 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.

Mick, D.U., Wagner, K., van der Laan, M., Frazier, A.E., Perschil, I., Pawlas, M., Meyer, H.E., Warscheid, B., and Rehling, P. (2007). Shy1 couples Cox1 translation regulation to cytochrome *c* oxidase assembly. EMBO J. *26*, 4347–4358.

Mick, D.U., Vukotic, M., Piechura, H., Meyer, H.E., Warscheid, B., Deckers, M., and Rehling, P. (2010). Coa3 and Cox14 are essential for negative feedback regulation of *COX1* translation in mitochondria. J. Cell Biol. *191*, 141–154.

Mick, D.U., Dennerlein, S., Wiese, H., Reinhold, R., Pacheu-Grau, D., Lorenzi, I., Sasaran, F., Weraarpachai, W., Shoubridge, E.A., Warscheid, B., et al. (2012). MITRAC links mitochondrial protein translocation to respiratory-chain assembly and translational regulation. Cell *151*, 1528–1541.

Model, K., Prinz, T., Ruiz, T., Rademacher, M., Krimmer, T., Kühlbrandt, W., Pfanner, N., and Meisinger, C. (2002). Protein translocase of the outer mitochondrial membrane: role of import receptors in the structural organization of the TOM complex. J. Mol. Biol. *316*, 657–666.

Morgenstern, M., Stiller, S.B., Lübbert, P., Peikert, C.D., Dannenmaier, S., Drepper, F., Weil, U., Höß, P., Feuerstein, R., Gebert, M., et al. (2017). Definition of a high-confidence mitochondrial proteome at quantitative scale. Cell Rep. *19*, 2836–2852.

Murley, A. and Nunnari, J. (2016). The emerging network of mitochondria-organelle contacts. Mol. Cell *61*, 648–653.

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. (2015). A perspective on transport of proteins into mitochondria: a myriad of open questions. J. Mol. Biol. 4*27*, 1135–1158.

Opaliński, Ł., Song, J., Priesnitz, C., Wenz, L.S., Oeljeklaus, S., Warscheid, B., Pfanner, N., and Becker, T. (2018). Recruitment of cytosolic J-proteins by TOM receptors promotes mitochondrial protein biogenesis. Cell Rep. *25*, 2026–2043.

Ostergaard, E., Weraarpachai, W., Ravn, K., Born, A.P., Jønson, L., Duno, Wibrand, F., Shoubridge, E.A., and Vissing, J. (2015). Mutations in COA3 cause isolated complex IV deficiency associated with neuropathy, exercise intolerance, obesity and short stature. J. Med. Genet. *52*, 203–207.

Otera, H., Taira, Y., Horie, C., Suzuki, Y., Suzuki, H., Setoguchi, K., Kato, H., Oka, T., and Mihara, K. (2007). A novel insertion pathway of mitochondrial outer membrane proteins with multiple transmembrane segments. J. Cell Biol. *179*, 1355–1363.

Ott, M., Prestele, M., Bauerschmitt, H., Funes, S., Bonnefoy, N., and Herrmann, J.M. (2006). Mba1, a membrane-associated ribosome receptor in mitochondria. EMBO J. *25*, 1603–1610.

Ott, M., Amunts, A., and Brown, A. (2016). Organization and regulation of mitochondrial protein synthesis. Annu. Rev. Biochem. *85*, 77–101.

Pagliarini, D.J., Calvo, S.E., Chang, B., Sheth, S.A., Vafai, S.B., Ong, S.-E., Walford, G.A., Sugiana, C., Boneh, A., and Chen, W.K. (2008). A mitochondrial protein compendium elucidates complex I disease biology. Cell *134*, 112–123.

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.

Paschen, S.A., Waizenegger, T., Stan, T., Preuss, M., Cyrklaff, M., Hell, K., Rapaport, D., and Neupert, W. (2003). Evolutionary conservation of biogenesis of β-barrel membrane proteins. Nature *426*, 862-866.

Pérez-Martínez, X., Broadley, S.A., and Fox, T.D. (2003). Mss51p promotes mitochondrial Cox1p synthesis and interacts with newly synthesized Cox1p. EMBO J. *22*, 5951–5961.

Pérez-Martínez, X., Butler, C.A., Shingu-Vazquez, M., and Fox, T.D. (2009). Dual functions of Mss51 couple synthesis of Cox1 to assembly of cytochrome *c* oxidase in *Saccharomyces cerevisiae* mitochondria. Mol. Biol. Cell *20*, 4371–4380.

Pfanner, N., Warscheid, B., and Wiedemann, N. (2019). Mitochondrial proteins: from biogenesis to functional networks. Nat. Rev. Mol. Cell Biol. *20*, 267–284.

Pfeffer, S., Woellhaf, M.W., Herrmann, J.M., and Förster, F. (2015). Organization of the mitochondrial translation machinery studied in situ by cryoelectron tomography. Nat. Commun. *6*, 6019.

Pierrel, F., Bestwick, M.L., Cobine, P.A., Khalimonchuk, O., Cricco, J.A., and Winge, D.R. (2007). Coa1 links the Mss51 post-translational function to Cox1 cofactor insertion in cytochrome *c* oxidase assembly. EMBO J. *26*, 4335–4346.

Popov-Celeketić, J., Waizenegger, T., and Rapaport, D. (2008). Mim1 functions in an oligomeric form to facilitate the integration of Tom20 into the mitochondrial outer membrane. J. Mol. Biol. *376*, 671–680.

Priesnitz, C. and Becker, T. (2018). Pathways to balance mitochondrial translation and protein import. Genes Dev. *32*, 1285–1296.

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.

Reinhold, R., Bareth, B., Balleininger, M., Wissel, M., Rehling, P., and Mick, D.U. (2011). Mimicking a SURF1 allele reveals uncoupling of cytochrome *c* oxidase assembly from translational regulation in yeast. Hum. Mol. Genet. *20*, 2379–2393.

Richter-Dennerlein, R., Oeljeklaus, S., Lorenzi, I., Ronsör, C., Bareth, B., Schendzielorz, A.B., Wang, C., Warscheid, B., Rehling, P., and Dennerlein, S. (2016). Mitochondrial protein synthesis adapts to influx of nuclear-encoded protein. Cell *167*, 471–483.

Rugarli, E.I. and Langer, T. (2012). Mitochondrial quality control: a matter of life and death for neurons. EMBO J. *31*, 1336–1349.

Sakaue, H., Shiota, T., Ishizaka, N., Kawano, S., Tamura, Y., Tan, K.S., Imai, K., Motono, C., Hirokawa, T., Taki, K., et al. (2019). Porin associates with Tom22 to regulate the mitochondrial protein gate assembly. Mol. Cell *73*, 1044–1055.

Schendzielorz, A.B., Schulz, C., Lytovshenko, O., Clancy, A., Guiard, B., Ieva, R., van der Laan, M., and Rehling, P. (2017). Two distinct membrane potential-dependent steps drive mitochondrial matrix protein translocation. J. Cell Biol. *216*, 83–92.

Schlossmann, J., Lill, R., Neupert, W., and Court, D.A. (1996). Tom71, a novel homologue of the mitochondrial preprotein receptor Tom70. J. Biol. Chem. *271*, 17890–17895.

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.

Schmitt, S., Ahting, U., Eichacker, L., Granvogl, B., Go, N.E., Nargang, F.E., Neupert, W., and Nussberger, S. (2005). Role of Tom5 in maintaining the structural stability of the TOM complex of mitochondria. J. Biol. Chem. *280*, 14499–14506.

Schulz, C., Schendzielorz, A., and Rehling, P. (2015). Unlocking the presequence import pathway. Trends Cell Biol. *25*, 265–275.

Sherman, E.L., Go, N.E., and Nargang, F.E. (2005). Functions of the small proteins in the TOM complex of *Neurospora crassa*. Mol. Biol. Cell *16*, 4172–4182.

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. U.S.A. *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. (2015). Molecular architecture of the active mitochondrial protein gate. Science *349*, 1544–1548.

Shoubridge, E.A. (2001). Nuclear genetic defects of oxidative phosphorylation. Human. Mol. Gen. *10*, 2277–2284.

Sinzel, M., Tan, T., Wendling, P., Özbalci, C., Chelius, X., Westermann, B., Brügger, B., Rapaport, D., and Dimmer, K.S. (2016). Mcp3 is a novel mitochondrial outer membrane protein that follows a unique IMP-dependent biogenesis pathway. EMBO Rep. *17*, 965–981.

Small, W.C. and McAlister-Henn, L. (1998). Identification of a cytosolically directed NADH dehydrogenase in mitochondria of *Saccharomyces cerevisiae*. J. Bacteriol. *180*, 4051–4055.

Smith, P.M., Fox, J.L., and Winge, D.R. (2012). Biogenesis of the cytochrome bc_1 complex and role of assembly factors. Biochim. Biophys. Acta *1817*, 276–286.

Sokol, A.M., Sztolsztener, M.E., Wasilewski, M., Heinz, E., and Chacinska, A. (2014). Mitochondrial protein translocases for survival and wellbeing. FEBS Lett. *588*, 2484–2495.

Soto, I.C., Fontanesi, F., Myers, R.S., Hamel, O., and Barrientos, A. (2012). A heme-sensing mechanism in the translational regulation of mitochondrial cytochrome *c* oxidase. Cell Metab. *16*, 801–813.

Stojanovski, D., Guiard, B., Kozjak-Pavlovic, V., Pfanner, N., and Meisinger, C. (2007). Alternative function for the mitochondrial SAM complex in biogenesis of α-helical TOM proteins. J. Cell Biol. *179*, 881–893.

Stoldt, S., Wenzel, D., Kehrein, K., Riedel, D., Ott, M., and Jakobs, S. (2018). Spatial orchestration of mitochondrial translation and OXPHOS complex assembly. Nat. Cell Biol. *20*, 528–534.

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.

Szyrach, G., Ott, M., Bonnefoy, N., Neupert, W., and Herrmann, J.M. (2003). Ribosome binding to the Oxa1 complex facilitates co-translational protein insertion in mitochondria. EMBO J. *22*, 6448–6457.

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.

Timón-Gómez, A., Nývltova, E., Abriata, L.A., Hosler, J., and Barrientos, A. (2018). Mitochondrial cytochrome *c* oxidase biogenesis: recent developments. Semin. Cell Dev. Biol. *76*, 163–178.

Tiranti, V., Hoertnagel, K., Carrozzo, R., Galimberti, C., Munaro, M., Granatiero, M., Zelante, L., Gasparini, P., Marzella, R.,

Rocchi, M., et al. (1998). Mutations of SURF-1 in Leigh disease associated with cytochrome *c* oxidase deficiency. Am J. Hum. Genet. *63*, 1609–1621.

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 Wilpe, S., Ryan, M.T., Hill, K., Maarse, A.C., Meisinger, C., Brix, J., Dekker, P.J.T., Moczko, M., Wagner, R., Meijer, M., et al. (1999). Tom22 is a multifunctional organizer of the mitochondrial preprotein translocase. Nature *401*, 485–489.

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.

Vogel, F., Bornhövd, C., Neupert, W., and Reichert, A.S. (2006). Dynamic subcompartmentalization of the mitochondrial inner membrane. J. Cell Biol. *175*, 237–247.

Voos, W. (2013). Chaperone-protease networks in mitochondrial protein homeostasis. Biochim. Biophys. Acta *1833*, 388–399.

Wang, X. and Chen, X.J. (2015). A cytosolic network suppressing mitochondria-mediated proteostatic stress and cell death. Nature *524*, 481–484.

Weidberg, H. and Amon, A. (2018). MitoCPR – a surveillance pathway that protects mitochondria in response to protein import stress. Science *360*, eaan4146.

Weinhäupl, K., Lindau, C., Hessel, A., Wang, Y., Schütze, C., Jores, T., Melchionda, L., Schönfisch, B., Kalbacher, H., Bersch, B., et al. (2018). Structural basis of membrane protein chaperoning through the mitochondrial intermembrane space. Cell *175*, 1365–1379.

Wenz, L.S., Opalinski, L., Schuler, M.H., Ellenrieder, L., Ieva, R., Böttinger, 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. (2015). Sam37 is crucial for formation of the mitochondrial TOM-SAM supercomplex, thereby promoting β-barrel biogenesis. J. Cell. Biol. *210*, 1047–1054.

Weraarpachai, W., Sasarman, F., Nishimura, T., Antonicka, H., Auré, K., Rötig, A., Lombès, A., and Shourbridge, E.A. (2012). Mutations in C12orf62, a factor that couples COX1 synthesis with cytochrome *c* oxidase assembly, cause fatal neonatal lactic acidosis. Am J. Hum. Genet. *90*, 142–151.

Wiedemann, N., Kozjak, V., Chacinska, A., Schönfisch, B., Rospert, S., Ryan, M.T., Pfanner, N., and Meisinger, C. (2003). Machinery for protein sorting and assembly in the mitochondrial outer membrane. Nature *424*, 565–571.

Wiedemann, N., Truscott, K.N., Pfannschmidt, S., Guiard, B., Meisinger, C., and Pfanner, N. (2004). Biogenesis of the protein import channel Tom40 of the mitochondrial outer membrane: intermembrane space components are involved in an early stage of the assembly pathway. J. Biol. Chem. *279*, 18188–18194.

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*, 115–1122.

Wrobel, L., Topf, U., Bragoszewski, P., Wiese, S., Sztolsztener, M.E., Oeljeklaus, S., Varabyova, M., Lirski, M., Chroscicki, P., Mroszek, S., et al. (2015). Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. Nature *524*, 485–488.

Yamamoto, H., Fukui, K., Takahasi, H., Kitamura, S., Shiota, T., Terao, K., Esaki, M., Nishikawa, S., Yoshihisa, T., Yamano, K., et al. (2009). Roles of Tom70 in import of presequence-containing mitochondrial proteins. J. Biol. Chem. *284*, 31635–31646.

Yamano, K., Yatsukawa, Y.I., Esaki, M., Hobbs, A.E.A., 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.

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.

Zhu, Z., Yao, J., Johns, T., Fu, K., De Bie, I., Macmillan, C., Cuthbert, A.P., Newbold, R.F., Wang, J., Chevrette, M., et al. (1998). SURF1, encoding a factor involved in the biogenesis of cytochrome *c* oxidase, is mutated in Leigh syndrome. Nat. Genet. *20*, 337–343.

Zimorski, V., Ku, C., Martin, W.F., and Gould, S.B. (2014). Endosymbiotic theory for organelle origins. Curr. Opin. Microbiol. *22*, 38–48.