Abstract: Mitochondrial precursor proteins with amino-terminal presequences are imported via the presequence pathway, utilizing the TIM23 complex for inner membrane translocation. Initially, the precursors pass the outer membrane through the TOM complex and are handed over to the TIM23 complex where they are sorted into the inner membrane or translocated into the matrix. This handover process depends on the receptor proteins at the inner membrane, Tim50 and Tim23, which are critical for efficient import. In this review, we summarize key findings that shaped the current concepts of protein translocation along the presequence import pathway, with a particular focus on the precursor handover process from TOM to the TIM23 complex. In addition, we discuss functions of the human TIM23 pathway and the recently uncovered pathogenic mutations in TIM50.

Keywords: membrane translocation; mitochondria; presequence pathway; protein translocation; TIM23 complex; TIM50.

Introduction

Mitochondria are integral to cell function, primarily as the site of ATP production, but they also play key roles in metabolic and signaling processes. Over 1000 nuclear-encoded proteins are required for mitochondria to fulfill their functions, all of which are translated on cytosolic ribosomes as mitochondrial precursor proteins and imported into their appropriate mitochondrial subcompartment (Morgenstern et al., 2017; Vogtle et al., 2017). The import process is highly selective and requires specialized machinery that can recognize and sort the different categories of mitochondrial precursor proteins. Approximately two thirds of mitochondrial precursor proteins have an N-terminal, positively charged, presequence that is approximately 15–50 amino acids long (Chacinska et al., 2009). The presequence targets precursor proteins to the translocase of the outer membrane (TOM complex), which is the main entry gate across the outer mitochondrial membrane. From there, presequence-containing precursors are passed onto the translocase of the inner membrane 23 (TIM23 complex) and the presequence is cleaved by the mitochondrial presequence peptidase. Precursors are then directed into either the inner membrane or the matrix. This pathway is known as the presequence pathway, or more commonly, the TIM23 pathway.

The TIM23 complex is the designated inner membrane translocase for translocation of presequence-containing precursors. It cooperates with the TOM complex to coordinate precursor passage across the two mitochondrial membranes. The dynamic nature of the complex enables it to switch composition to enable a precursor to be released laterally into the inner membrane, or driven into the matrix (Chacinska et al., 2005). Unraveling the mechanisms of the TIM23 pathway has spanned several decades, which is testament to the complexity and intricacies of the pathway. In this review, we outline the structural components of the presequence pathway from a chronological perspective and describe the latest model for precursor translocation, with a special focus on precursor handover between the TOM complex and TIM23 complex.

Most of the seminal work on this pathway has been performed using yeast. However, as more information is starting to emerge about the human presequence pathway, it is becoming clear that there are functional differences. An overview of the human TIM23 pathway will be given, with respect to some of the newly acquired functions of translocase components. Mutations in components of the TIM23 machinery have been linked to mitochondrial disease (Kang et al., 2018; Pfanner et al., 2019). Several of...
these have been within the intermembrane space (IMS)-filling component of the TIM23 complex, TIM50, which is critical for precursor handover (Shahrour et al., 2017; Reyes et al., 2018; Tort et al., 2019). The pathogenic effects of these mutations underline the importance of efficient precursor handover for human health.

A short history of mitochondrial presequence translocase components

The earliest accounts of mitochondria at the beginning of the 20th century, described them as intracellular structures that contain proteins and lipids (Regaud, 1908). Several decades later, once mitochondrial isolation methods had been established, it became apparent that mitochondria had the ability to synthesize their own proteins (McLean et al., 1958), a phenomenon that was explained shortly thereafter by the discovery of mitochondrial DNA (Nass and Nass, 1963; Luck and Reich, 1964; Schatz et al., 1964) and mitochondrial ribosomes (Kuntzel and Noll, 1967). However, the complexity of mitochondrial biogenesis was not fully realized until the late 1960s by a series of experiments that used radiolabeling of isolated rat mitochondria to track the fate of mitochondrial translated products. After radiolabeling, mitochondrial membranes were fractionated and remarkably, labeled translation products could only be observed in the inner mitochondrial membrane (Beattie et al., 1967; Neupert et al., 1967; Werner and Neupert, 1972). This implied that the remainder of the mitochondrial proteome originates from nuclear genes, is translated on cytosolic ribosomes and is then imported into mitochondria. These paradigm-shifting findings gave birth to the field of mitochondrial protein import (Figure 1).

The establishment of an in vitro transport system that enabled the monitoring of mitochondrial protein uptake, for example, of in vitro translated proteins, provided a means to assess the mechanism of protein translocation (Hallermaier et al., 1977; Harmey et al., 1977; Maccecchini et al., 1979a,b; Nelson and Schatz, 1979; Gasser et al., 1982). Early import experiments led to the identification of cytosolic precursors for mitochondrial proteins that are imported into mitochondria in a kinetically resolvable manner. Remarkably some of these precursors underwent proteolytic processing. An amphiphatic N-terminal cleavable signal was later described to be the mitochondrial targeting signal which directs proteins of the presequence pathway to specific receptors on the outer mitochondrial membrane (Riezman et al., 1983; Hurt et al., 1984; Roise et al., 1988).

The translocase of the outer membrane

The discovery, that mitochondria selectively take up proteins from the cytosol, initiated a long quest to identify the pathways and machinery that enable protein import. The first component identified was the pore-forming protein in the outer mitochondrial membrane, Tom40 (Vestweber et al., 1989; Baker et al., 1990; Kiebler et al., 1990; Hill et al., 1998). This was closely followed by the purification of a receptor complex from the outer mitochondrial membrane, which resulted in the identification of the presequence receptor subunit, Tom20 (Sollner et al., 1989; Moczko et al., 1992) and a receptor specializing in the recognition of precursors with internal targeting signals, Tom70 (Hines et al., 1990; Sollner et al., 1990; Steger et al., 1990). Another receptor component, Tom22, was later characterized and found to mediate the transfer of precursors from Tom20 and Tom70 to the Tom40 import pore (Lithgow et al., 1994). In addition to the import pore and the main receptors, the TOM complex is made up of
several small molecular weight constituents, Tom5, Tom6 and Tom7 (Kassenbrock et al., 1993; Alconada et al., 1995; Honlinger et al., 1996; Dietmeier et al., 1997). These aid in the assembly and stability of the TOM complex. The TOM complex is broadly regarded as the general entry site for mitochondrial proteins and a detailed review on this translocase is included in this issue (Bausewein et al., 2020).

The presequence translocase

These early studies indicated that different pathways exist for presequence-containing precursors and precursors with internal targeting signals (Zimmerman et al., 1979; Riezman et al., 1983). The subset of mitochondrial proteins that contain presequences are directed to the mitochondrial matrix or the inner membrane – but how do they get there? It was believed that, like their passage through the outer membrane, this process is facilitated by a proteinaceous import channel within the inner membrane. To decipher this translocation machinery, researchers exploited the power of yeast genetics. In one approach, a screen was devised to obtain mutants of Saccharomyces cerevisiae that were unable to import a mitochondrial targeted reporter enzyme (Maarse et al., 1992). Concomitantly, in an alternative approach, another group of researchers analyzed temperature sensitive mutants of S. cerevisiae that accumulate cytosolic precursors at the non-permissive temperature of 37°C (Emtage and Jensen, 1993). Analysis of these mutants uncovered the first translocase components of the inner mitochondrial membrane, Tim44, Tim23 and Tim17. All of these components were found to be not only required for import, but also essential for cell viability (Maarse et al., 1992, 1994; Scherer et al., 1992; Blom et al., 1993; Dekker et al., 1993; Emtage and Jensen, 1993; Ryan et al., 1994; Martinez-Caballero et al., 2007).

The Tim23 and Tim17 proteins display sequence similarity, although they cannot functionally compensate for each other (Maarse et al., 1994; Ryan et al., 1994). Both proteins have four transmembrane spans and possess a topology in which the N- and C-termini face the IMS. Although Tim23 alone has channel activity, it is natively found in complex with Tim17 (Dekker et al., 1997; Ryan et al., 1998; Truscott et al., 2001) and requires Tim17 to maintain pore structure (Meier et al., 2005; Martinez-Caballero et al., 2007). Therefore, it is believed that, together, these two subunits make up the channel unit of the translocase. A unique feature of Tim23 is its hydrophilic N-terminal segment, which was later shown to bind incoming presequences (Bauer et al., 1996; Truscott et al., 2001; de la Cruz et al., 2010).

Tim44 is peripherally associated with the channel at the inner mitochondrial membrane and has a large matrix facing domain. The primary function of Tim44 is to act as a scaffold for the binding of the chaperone mtHsp70 (Kronidou et al., 1994; Schneider et al., 1994). mtHsp70 is located in the mitochondrial matrix and had already been identified several years earlier in an analysis of temperature-sensitive import-deficient yeast mutants. At that time it was shown to have a role in the import and folding of the mature precursor within the matrix (Kang et al., 1990). Some years later, the ATP-activated mtHsp70 was discovered to provide the driving force that moves precursors into the matrix (Ungermann et al., 1994; Berthold et al., 1995).

Despite the identification of translocase constituents, it remained unclear how the TIM23 complex coordinates with the TOM complex to receive cargo, or how it is capable of sorting precursors into their diverse compartments – the inner membrane or the matrix. In 2002, three separate groups identified a sixth core component of the TIM23 machinery (Geissler et al., 2002; Yamamoto et al., 2002; Mokranjac et al., 2003a,b). This 50 kDa protein with a large IMS spanning domain was termed Tim50. Like the Tim23 IMS domain, Tim50 is also capable of binding precursors, but with a greater affinity (de la Cruz et al., 2010; Marom et al., 2011). The Tim50 IMS region is composed of two domains; a C-terminal presequence binding domain (PBD) and a core domain. Both domains are capable of interacting with presequences, but the core domain can additionally associate with Tim23, Tim21 and the PBD region of Tim50 itself (Schulz et al., 2011; Qian et al., 2011; Lytvovenko et al., 2013; Rahman et al., 2014). Therefore, Tim50 represents the main inner membrane receptor for matrix-targeted precursors, guiding them from the TOM complex to the channel insertion site (Geissler et al., 2002; Yamamoto et al., 2002; Mokranjac et al., 2003a,b).

Subsequently, two DnaJ-homologs of 14 and 16 kDa were identified (D’Silva et al., 2003; Mokranjac et al., 2003a,b; Truscott et al., 2003; Kozany et al., 2004). These proteins can both dynamically associate with mtHsp70 and Tim44, and together, they make up the presequence translocase-associated motor (PAM). The 14 and 16 kDa proteins were therefore termed Pam18 (Tim14) and Pam16 (Tim16), respectively. While the membrane anchored Pam18 stimulates the ATPase activity of mtHsp70 at the channel exit, the membrane associated Pam16 inhibits the stimulatory effect of Pam18 when in complex together. In this way, by working in concert, Pam18 and Pam16 control the activity of the import motor. Another new translocase component, termed Pam17, was found some years later (van der Laan et al., 2005). Pam17 is anchored to the membrane and is recruited to the translocase by Tim50 where
it has a specific role in promoting the translocation of precursors that are hypersensitive to membrane potential, possibly by regulating the gating activity of Tim23 (Schendzielorz et al., 2017).

Precursors that are destined for the inner membrane contain a hydrophobic stop transfer (sorting) signal adjacent to the presequence and are imported independently of the mtHsp70 motor in a process driven solely by the membrane potential (Gartner et al., 1995; Gruhler et al., 1997). However, the mechanics by which the translocase discriminates between matrix transport and membrane insertion remained unaddressed. The identification of the inner membrane protein, Tim21 (Chacinska et al., 2005; Mokranjac et al., 2005) led to the discovery of a state of the TIM23 complex that lacks the import motor and is dedicated to lateral release. The purified complex is capable of inserting precursors with sorting signal into the lipid phase of a membrane in a reconstituted system (van der Laan et al., 2007). Accordingly, the TIM23 complex exists in two different modular states; the TIM23SORT, which contains Tim21 but lacks the PAM complex, and the TIM23MOTOR, in which the motor is associated with the translocase but Tim21 is absent (Chacinska et al., 2005). Interestingly, Tim21 also interacts with the IMS domain of Tom22, which has been suggested to act as a trans binding site for presequences at the TOM complex. Binding of Tim21 to Tom22 is likely to facilitate the release of precursors from the TOM complex and direct them to Tim50 (Mokranjac et al., 2005; Albrecht et al., 2006).

The latest component of the TIM23 complex to be discovered was Mgr2, a small hydrophobic protein with two transmembrane spans (Gebert et al., 2012). Mgr2 was suggested to support binding of Tim21 to the TIM23SORT complex and regulates the lateral release of precursors into the inner membrane (Ieva et al., 2014). As Mgr2 can be crosslinked to precursors in transit, it suggests that it may be a component of the channel (Gebert et al., 2012).

The discovery of the components of the presequence machinery spanned over two decades and had provided an architectural map of a complex but fascinating machinery. In the research that follows, many mechanistic aspects of the presequence translocase continue to be dissected. Below we outline the mechanisms of precursor translocation by TIM23 upon handover from the TOM complex.

From TOM to TIM23: the precursor handover

The transport of proteins into mitochondria and across the inner membrane requires the transfer of precursors from the TOM complex to the inner membrane translocation machineries. Upon their exit from the Tom40 pore, pre-sequence containing precursors are passed on to the TIM23 complex in a process that relies on an intricate relay of interactions between the TOM complex and constituents of the TIM23 complex. The precursor exit site on the IMS side of the TOM complex is a composite site, made up of the IMS domain of Tom22, Tom7 and Tom40, all of which are able to bind presequences (Bolliger et al., 1995; Moczko et al., 1997; Rapaport et al., 1997; Komiya et al., 1998; Kanamori et al., 1999; Araiso et al., 2019; Tucker and Park, 2019). Once positioned at this exit site, the precursor is then transferred to Tim50, the primary receptor of the TIM23 complex (Figure 2).

To facilitate the handover process, the two translocases draw into close proximity, thereby forming a TOM-TIM23 supercomplex (Dekker et al., 1997; Chacinska et al., 2010; Gold et al., 2014). The formation of the TOM-TIM23 supercomplex is triggered by the precursor protein. In fact, the supercomplex can only be stabilized for detection using in-vitro mitochondrial import of an artificial precursor with a folded C-terminal domain that cannot pass the Tom40 pore, thereby trapping both complexes together (Rassow et al., 1990; Dekker et al., 1997).

The generation and dynamics of the TOM-TIM23 supercomplex are critical to our understanding of precursor transfer between the translocases. The C-terminal IMS domain of Tom22 is critical for the stabilization of the TOM-TIM23 supercomplex. Ablation of the IMS domain of Tom22 dramatically affected supercomplex stability, resulting in a reduction in presequence import efficiency (Chacinska et al., 2010). As the main presequence receptor, Tim50 also supports supercomplex formation, but once formed, Tim50 is dispensable for maintaining the stability of the supercomplex (Chacinska et al., 2010). Crosslinking studies have shown that the IMS domain of Tim50 can form a direct link with the IMS domain of Tom22, which not only facilitates the formation of the supercomplex, but also positions Tim50 in a primed state to receive the precursor from the Tom22 IMS (Shiota et al., 2011). In the presence of precursor protein, the crosslinks between Tom22 and Tim50 are diminished, suggesting that the precursor competes for the binding site on the Tim50 IMS, which then causes the two subunits to dissociate (Shiota et al., 2011). Another contact between the TOM and TIM23 translocases occurs through the interaction between Tim21 and the IMS domain of Tom22 (Mokranjac et al., 2005; Albrecht et al., 2006; van der Laan et al., 2006). However, the role of Tim21 in supercomplex formation is less clear. The interaction between the Tom22 IMS and Tim21 is transient and once the precursor has been
Figure 2: Mechanism of precursor handover and translocation via TIM23.

(A) After translocation through the TOM complex, the presequence-containing precursor is positioned at the TOM complex exit site. The TOM and TIM23 complexes associate via interactions between the Tom22IMS and Tim21, Tim23IMS and Tim50. The presequence is then recognized and bound by Tim50PBD. (B) After the handover process, Tim50 then shifts the precursor protein from the Tim50PBD to the Tim50CORE. From the Tim50CORE, the precursor is passed onto the Tim23IMS, from where it has access to the channel. The channel is activated by Tim50 and the precursor is then driven through the channel, along the electrochemical gradient (ΔΨ) across the inner membrane and assisted by cation-selective residues lining the pore of the channel. (C) The TIM23 complex exists in two states. TIM23MOTOR is the motor associated form for matrix-destined precursor proteins. ATP-powered mtHsp70 associates with the translocase and, upon hydrolysis of ATP by Pam18 (18), binds to the emerging precursor and dissociates from the translocase. Subsequent mtHsp70 molecules bind to the precursor in a similar manner to draw it further into the matrix in a ratchet-like motion. TIM23SORT is specialized for the lateral release of inner membrane bound precursor proteins that have a hydrophobic “stop-transfer” signal after the presequence. TIM23SORT is defined by the presence of Tim21 and the lateral gatekeeper Mgr2. Both are released from the complex, allowing the “stop-transfer” signal lateral migration into the inner membrane.
transferred to the TIM23 complex, Tim21 dissociates from the translocase (Lytovchenko et al., 2013).

At the inner membrane, Tim50 is set to receive the incoming precursor protein from the TOM complex. The IMS domain of Tim50 has two presequence binding regions; one within its C-terminal presequence binding domain, Tim50\textsc{PBD}, and a second region, which has an even higher presequence affinity, within its core domain, Tim50\textsc{CorE} (Schulz et al., 2011; Qian et al., 2011; Lytovchenko et al., 2013; Rahman et al., 2014). Notably, the binding region within Tim50\textsc{CorE} is also capable of binding the Tim50\textsc{PBD}, suggesting that upon receiving the presequence from Tim50\textsc{CORE} (Schulz et al., 2011; Qian et al., 2011; Lytovchenko et al., 2013; Rahman et al., 2014). As the IMS region of Tim23 is also capable of binding presequences, this arrangement would conveniently position the IMS region of Tim23 at the PBD domain, Tim50 can then transfer it to its higher affinity core domain (Rahman et al., 2014). Crystallization of the core domain of Tim50 enabled visualization of the large presequence binding groove and identified a nearby extended beta loop, which not only assists in the recognition and binding of presequences, but is also required for interaction with the IMS domain of Tim23 (Geissler et al., 2002; Yamamoto et al., 2002; Tamura et al., 2009; Qian et al., 2011; Li and Sha, 2015; Dayan et al., 2019). As the IMS region of Tim23 is also capable of binding presequences, this arrangement would conveniently position Tim23\textsc{IMS} in a primed position to receive the incoming precursor from Tim50\textsc{CORE}.

While Tim50 remains bound to Tim23, the gating activity of the channel remains closed, thereby preventing ion leakage across the inner membrane (Meinecke et al., 2006; Alder et al., 2008). At this point, Tim23 exists as a dimer in a state that is dependent on the electrochemical proton gradient across the inner membrane (Bauer et al., 1996). However, once the precursor has been transferred to the Tim23\textsc{IMS}, Tim50 separates from the translocase, the Tim23 dimer dissociates and the channel is activated (Bauer et al., 1996; Meinecke et al., 2006). Upon opening of the channel, the precursor protein, with its positively charged presequence is drawn through the channel by the membrane potential (Martin et al., 1991). Furthermore, the channel is lined with cation selective amino acids that recognize the positively charged presequence and act to further drive it through the channel (Denkert et al., 2017). Once inside the channel, the precursor can be laterally released or translocated further into the matrix.

Full matrix translocation of a precursor requires additional driving forces. In this case, the emerging precursor binds to Tim44 and is then transported to the matrix, by the ATP-powered presequence translocase-associated motor (PAM) (Marom et al., 2011). The mtHsp70 protein is the central component of the PAM, which is recruited to the translocase by Tim44 and regulated by the Pam18/Pam16 complex (Kronidou et al., 1994; Schneider et al., 1994; Hutu et al., 2008). The function of the import motor in precursor translocation is reviewed in detail elsewhere in this issue (Mokranjac, 2020). The association of Pam18 to the TIM23 complex favors matrix transport of precursors by stimulating mtHsp70. Additionally, Pam18 spans the inner membrane with a single transmembrane span that, when associated with TIM23, physically obstructs the lateral gate (D’Silha et al., 2003; Mokranjac et al., 2003a,b; Truscott et al., 2003). Therefore, in the case of precursors with a stop transfer (sorting) signal, which are destined for the inner membrane, Pam18 must dissociate from the translocase to enable precursor access to the lateral gate (Schendzielorz et al., 2018).

In contrast to matrix-translocated precursors, laterally released proteins do not require any additional forces other than membrane potential. The hydrophobic sorting signal, located downstream of the presequence, stalls the precursor in the channel. The release of the precursors into the lipid bilayer is thought to occur through a lateral gate of the translocase. Mgr2 has been proposed to act as a gatekeeper, partitioning proteins depending on their hydrophobicity of their sorting signals (Ieva et al., 2014; Lee et al., 2019). Mgr2 or Pam18 are mutually exclusively positioned at the lateral gate (Schendzielorz et al., 2018). Tim21 has been shown to be exclusively present in the translocase in the absence of the import motor. However, the role of Tim21 in lateral sorting is still unclear. Interestingly, Tim21 interacts with proton pumping respiratory chain complexes and may therefore position the translocase in regions of higher membrane potential to facilitate sorting (van der Laan et al., 2006).

Conservation of the presequence pathway in mammals and its role in disease

The TIM23 complex is essential for life and as such, the yeast complex remains largely conserved in mammals. In human, the five membrane integrated subunits of the translocase are; the receptor TIM50, the channel forming units TIM23 and TIM17A/B, as well as the TIM23\textsc{CorE} components TIM21 and ROMO1 (human homolog of yeast Mgr2) (Bauer et al., 1999; Guo et al., 2004; Mick et al., 2012; Sinha et al., 2014; Wrobel et al., 2016; Richter et al., 2019). Additionally, the majority of motor subunits also have human counterparts. These include; TIM44, DNAJC15 and DNAJC19 (both homologs of Pam18), MAGMAS (PAM16), Mortalin (mtHSP70) and GrpE (human homolog of yeast Mge1) (Bhattacharyya et al., 1995; Bauer et al., 1999;
Borges et al., 2003; Sinha et al., 2010) (Figure 3). The only yeast TIM23 constituent without a human homolog is Pam17. The lack of Pam17 in mammals possibly reflects a greater resilience to fluctuations in bioenergetic availability, whereby Pam17 is no longer required to support translocation of membrane potential-hypersensitive precursors (Schendzielorz et al., 2017).

Although these components are all conserved, most have undergone at least some degree of functional divergence and this is strongly evidenced by differences in the interaction networks of presequence pathway components between yeast and human (Pfanner et al., 2019). One well-described example is TIM21, which in mammals, has acquired the additional function where it shuttles newly imported components of respiratory complexes I and IV from the TIM23 complex to early assembly intermediates, known as mitochondrial translation regulation assembly intermediate of cytochrome c oxidase, MITRAC (Mick et al., 2012; Richter-Dennerlein et al., 2016).

In doing so, TIM21 coordinates the import of nuclear encoded respiratory complex subunits with the synthesis of mitochondrial encoded subunits. ROMO1 is important for the association of TIM21 with the translocase and consequently also affects the biogenesis of respiratory chain complexes (Richter et al., 2019). ROMO1 has also functionally diverged from its yeast counterpart Mgr2, as it is not essential for the lateral sorting of precursor proteins, but instead has a specific role in the import of the protease YME1L1 and itself displays a very short half-life (Richter et al., 2019). YME1L1 was recently found to mediate the

![Figure 3](image-url)

**Figure 3:** The human TIM23 complex – TIM50 in disease.

(A) The human TIM23 complex is formed by the core components TIM50 (50), TIM23 (23) and TIM17A/B (17A/B). The sorting elements are TIM21 (21) and ROMO1, and the motor elements include TIM44 (44), PAM18 (18; DNAJC15 and DNAJC19), PAM16 (16; MAGMAS), mtHSP70 (Mortalin) and GrpE. During precursor sorting into the inner membrane, TIM21 can associate with an emerging nuclear-encoded polypeptide for a respiratory complex component. It then delivers the polypeptide to the actively translating mitochondrial-encoded subunit, still associated with the mitoribosome, in a process facilitated by respiratory complex intermediates known as MITRAC and the insertase OXA1L. This forms part of sequential OXPHOS complex assembly. (B) The four characterized patient mutations on TIM50 are indicated.
stress-induced proteolysis of proteins essential for mito-
chondrial biogenesis; such as translocase components,
lipid transfer proteins and metabolic enzymes. Proteolysis
by YME1L1 enables rapid adaptation to stress conditions
and the limiting of mitochondrial biogenesis supports cell
growth (MacVicar et al., 2019). Therefore, as a regulator of
YME1L1 levels, ROMO1 is vital for controlling mito-
chondrial proteostasis and quality control.

A substrate of YME1L1 is TIM17A (Rainbolt et al.,
2013). TIM17A and TIM17B exist as translocase com-
ponents in a mutually exclusive manner (Bauer et al., 1999).
Under stress conditions, TIM17A is rapidly degraded and
consequently, protein import is reduced (Rainbolt et al.,
2013). It therefore appears that in mammals, entry via
the presequence pathway is under proteolytic control,
which would enable a rapid mitochondrial response
during stress conditions. Import motor components also
appear to have acquired new functions in mammals.
Although DNAJC15 and DNAJC19 can stimulate the activ-
ity of mtHSP70 (Sinha et al., 2010; Schusdziarra et al.,
2013), only DNAJC15 can complement the activity of yeast
Pam18 (Schusdziarra et al., 2013). In fact, DNAJC19 forms
a complex with prohibitins and is involved in cardiolipin
remodeling, so its role in mitochondrial import is still
unclear (Richter-Dennerlein et al., 2014). The activity of the
PAM relies on the regeneration of mtHSP70. In human,
this is expected to occur via the Mge1 homolog, GrpE, but
although GrpE was shown to bind to mtHSP70 in vitro, its
function, including its exchange factor activity, has not
yet been verified (Choglay et al., 2001).

Human TIM50 is also likely to have functionally diver-
sified. Although both the yeast and human TIM50 possess
a C-terminal CTD-like phosphatase domain, human
TIM50 lacks the C-terminal PBD binding domain that in
yeast is required for precursor handover (Qian et al., 2011),
suggesting that the mechanism of precursor handover in
humans may differ from that in yeast.

Several TIM23 components have been implicated
in various diseases and these have been described in
several recent reviews (Kang et al., 2018; Pfanner et al.,
2019). Intriguingly, three separate reports have recently
described mutations in TIM50, which is critical for pre-
cursor handover. These studies report on four unrelated
patients, all of which experienced severe lactic aci-
dosis and seizures (Shahrour et al., 2017; Reyes et al.,
2018; Tort et al., 2019). Two patients had homozygous
missense mutations (p.Arg217Trp and p.Thr252Met) in
conserved residues within the TIM50 IMS domain and
both patients also exhibited intellectual disability and
3-methylglutaconic aciduria (Shahrour et al., 2017).
Another subject, with compound heterozygous mutations
within the transmembrane region of TIM50 (p.Ser112*
and p.Gly190Ala) had strongly reduced levels of TIM50
and compromised mitochondrial import. Consequently,
biochemical analyses revealed defects in the steady state
levels of complex I, II and IV and a general respiratory
deficiency (Reyes et al., 2018). Finally, a fourth subject
with compound heterozygous mutations in highly con-
served residues within the IMS domain (p.Arg114Gln and
p.Gly269Ser) also exhibited 3-methylglutaconic aciduria,
as well as vision loss, symptoms of Leigh syndrome and
dilated hypercardiomyopathy (Tort et al., 2019). Like
the previous patient, this subject had reduced levels of
TIM50 protein and compromised respiratory capacity.
Furthermore, the patient exhibited a general reduction
in the activity of complexes I–V and defective respiratory
complex assembly (Tort et al., 2019).

Interestingly, loss of TIM50 in both zebra fish and
mice also resulted in increased heart and cardiomyocyte
growth (Guo et al., 2004; Tang et al., 2017), which appears
to be linked to increased ROS production in the absence
of TIM50 (Tang et al., 2017). TIM50 expression has addi-
tionally been implicated in various cancers (Gao et al.,
2016; Zhang et al., 2019). Overall, these studies provide
an insight into the function of human TIM50 but further
studies are needed to explain the diversity in the physio-
logical and biochemical phenotypes.

Conclusions and future perspectives

Mitochondrial function depends on the efficient and regu-
lated import of nuclear-encoded proteins, most of which
are directed through the presequence pathway. In work
that has spanned decades, researchers have developed
specialized tools to unravel the machinery and intricate
details of this highly sophisticated import system. In
this review we focused on the handover of the precursor
protein from the TOM complex to the TIM23 complex; a
process that requires a juxtapositioning of the inner and
outer membranes, precursor recognition by the TIM23
complex, precursor translocation through the channel
and its subsequent release into the inner membrane or
the matrix.

Despite the current model, several key aspects of
precursor handover remain to be resolved; (1) How do
the two membranes come into close contact? Are there
other protein complexes that facilitate this? (2) How do
the TOM complex and TIM23 cooperate during precursor
handover and how is the TOM-TIM23 supercomplex gen-
erated? (3) How does precursor transfer occur in human
mitochondria? These questions require high resolution analysis of the dynamics and proteomic landscape of the inner mitochondrial membrane, as well as structural studies of the TIM23 complex, alone and in complex with TOM. Furthermore, although components of the TIM23 complex are well conserved in mammals, the major functional variations indicate that there are evolutionary differences in the translocation process. Already, the lack of an extended presequence binding domain in the IMS of human TIM50 implies a different precursor handover mechanism. Therefore, detailed mechanistic and structural studies are warranted to elucidate the translocation mechanism of the human presequence translocase. This would not only enable a greater understanding of the role of pathogenic mutations in TIM23 components, but may pave the way for therapeutic modulation of the pathway.

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