Published Online on 13 December 2017

Proc Indian Natn Sci Acad **83** No. 4 December 2017 pp. 877-891

DOI: 10.16943/ptinsa/2017/49225

Review Article

The complexities of human mitochondrial inner-membrane protein translocases in maintenance of *organeller* function

DEVANJAN SINHA*

Cytogenetics Laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221 005, India

(Received on 31 August 2017; Revised on 11 October 2017; Accepted on 10 November 2017)

Human mitochondria require ~1500 proteins for its constitutive functions. However, the mitochondrial genome is gene deficient and most of the mitochondrial proteome is nuclear encoded and transported into the *organelle*. Import of majority of mitochondrial proteome is mediated by presequence pathway and requires functional interplay between a subset of molecular chaperones, along with accessory factors. Although detailed analysis has been carried out in yeast system, our understanding of human mitochondrial proteome maintenance has been severely limited by the absence of significant structural and functional information on the organization TIM23 complex in humans. Recent studies have revealed the presence of multiple presequence translocases in human mitochondria that show considerable diversity in their substrate and physiological specificity. The core organization and inter-molecular interactions between these machineries seems to be evolutionary conserved. These translocases have principally diversified due to divergence of Hsp70 co-chaperones and channel component Tim17. Another important feature associated with human mitochondrial translocases, which in most cases are absent in lower eukaryotes, is its multifunctionality. Other than housekeeping protein import, human mitochondrial translocases have gained secondary functions such asinvolvment in ROS sensing and regulation of redox balance, modulation of cellular sensitivity to xenobiotic drugs, maintenance of mitochondrial DNA, assembly of respiratory complexes' and import of non-canonical mitochondrial substrates. The origin of multifunctionality is indicative towards the possibility of functional connections between mitochondrial protein import and regulation of cellular pathways; hence, opening up new avenues of future research.

Keywords: Mitochondria; Protein Transport; Protein Folding; Hsp70; J-Proteins; Cancer; Neurodegeneration Metabolic Disorders

Introduction

Mitochondria are endosymbiotic cellular *organelle* enclosed in double membrane and form an integral part of eukaryotic cells. The word mitochondrion comes from the Greek ìßôïò, mitos, i.e. "thread", and ÷ïíäñßïí, chondrion, i.e. "granule". The term "mitochondria" was coined by Carl Benda in 1898 (cited in Margulis, 1970). Benjamin F. Kingsbury (1912) first related these *organelles* with cell respiration (cited in Sagan, 1967), followed by Otto Heinrich Warburg (Ernster and Schatz, 1981; Gray *et al.*, 1999). In addition to supplying cellular energy, mitochondria are involved in other tasks such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth (Gray *et al.*, 1999). Mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction, and may play a role in the aging process (Fulda *et al.*, 2010; Gulbins *et al.*, 2003; Hayashi *et al.*, 2009; Kroemer, 2006; Lesnefsky *et al.*, 2001; McBride *et al.*, 2006).

Mitochondrial genomes are very small and show a great deal of variation as a result of divergent evolution (Ernster and Schatz, 1981; Henze and Martin, 2003; Peng *et al.*, 2005). The human mitochondrial genome is a circular DNA molecule of about 16 kilobases. It encodes 37 genes of which 13 code for subunits of respiratory complexes I, III, IV

*Author for Correspondence: E-mail: devanjan@bhu.ac.in

and V, 22 for mitochondrial tRNA (for the 20 standard amino acids, plus an extra gene for leucine and serine), and 2 for rRNA. One mitochondrion can contain two to ten copies of its DNA (Anderson *et al.*, 1981; Chan, 2006; Ekstrand *et al.*, 2004; Emelyanov, 2003; Ernster and Schatz, 1981; Gray *et al.*, 1999; Iacovino *et al.*, 2009; Lang *et al.*, 1999; Leung and Halestrap, 2008; Wiesner *et al.*, 1992). It is quite evident from the mitochondrial genome organization that mitochondrial DNA is gene-deficient. However, the diverse functions of the *organelle* require a vast repertoire of proteins. For example, in humans, normal mitochondria require around 1500 proteins for its functions. Majority of these proteins are encoded by the nuclear genome and translated in the cytosolic compartment. Only 1% is encoded by the mitochondrial genome and synthesized on ribosomes in the mitochondrial matrix (Neupert *et al.*, 1990; Neupert and Herrmann, 2007; Rehling *et al.*, 2004; Rehling *et al.*, 2001). Hence, transport of proteins across the cellular membranes is a process that is instrumental for the biogenesis of the cell and its *organelles*. In *Saccharomyces cerevisiae* almost half of the proteins synthesized are transported into or across at least one cellular membrane to reach their functional destination (Neupert *et al.*, 1990; Neupert and Herrmann, 2007; Schnell and Hebert, 2003; Wickner and Schekman, 2005). This raises several fundamental questions such as how the precursor proteins are directed to the correct target *organelle*, how they are translocated across the hydrophobic membranes, and how they are sorted and assembled into their functional forms.

The Associated Machineries with Mitochondrial Protein Translocation

It was an initial belief that all mitochondrial precursor proteins are imported via one main pathway and the mechanism was called as the presequence pathway. This pathway involved an outer and inner membrane translocase, with amino-terminal presequences in precursor proteins acting as classical targeting signals directing proteins into mitochondria. However, the demonstration that the precursors of metabolite carrier proteins of the mitochondrial inner membrane use different signals and a different sorting route suggested that there were two routes for mitochondrial import: the presequence pathway for translocation of the matrix directed preproteins and single transmembrane helix containing proteins; and the carrier pathway for membrane integration of multi-channel inner membrane associated preproteins (Neupert *et al.*, 1990; Neupert and Herrmann, 2007). Further investigations lead to the identification of newer principles of import such as redox-regulated import and two-membrane coupling of translocases (A, 2012; Banci *et al.*, 2009; Stojanovski *et al.*, 2008). Also it became evident that the precursor protein translocases are composed of modular units that cooperate with each other in a highly dynamic manner. Therefore, mitochondria consist of four major routes of directing proteins into their intramitochondrial destinations.

a. *The β-barrel pathway* – incorporation of outer membrane proteins through the outer mitochondrial membrane associated 'Sorting and Assembly Machinery of the Outer Mitochondrial Membrane' or SAM complex (Chan and Lithgow, 2008; Stojanovski *et al.*, 2007).

b. *The redox-regulated import pathway* – for delivering precursor proteins into the intermembrane space via the Mitochondrial Intermembrane Space Import and Assembly or MIA machinery (Banci *et al.*, 2009; Chacinska *et al.*, 2004; Grumbt *et al.*, 2007; Stojanovski *et al.*, 2008).

c. *The carrier protein pathway* – integration of complex proteins containing multiple transmembrane helices into inner mitochondrial membrane. This pathway involves the carrier translocase of the Inner Mitochondrial Membrane or the Tim22 machinery (Chan and Lithgow, 2008; Curran *et al.*, 2002a; Curran *et al.*, 2002b; Jensen and Dunn, 2002; Rehling *et al.*, 2004).

d. *The presequence pathway* – constitutes the major pathway of mitochondrial import. It involves import of matrix proteins and those associated with the inner membrane by a single transmembrane helix. The pathway involves the TIM23 complex or the presequence translocase (Baker *et al.*, 2007; Elsner *et al.*, 2009; Moro *et al.*, 2002; Neupert and Brunner, 2002; Rehling *et al.*, 2001).

However, the initial translocation of all the precursor proteins, destined to different import pathways across the mitochondrial outer membrane,

occurs through the multisubunit machinery in the outer membrane – the TOM complex (Ahting *et al.*, 2001; Becker *et al.*, 2008; Chacinska *et al.*, 2005; Wu and Sha, 2006; Yamano *et al.*, 2008; Young *et al.*, 2003).

The newly synthesized proteins that have their final destination into the mitochondria contain an N-terminal recognition sequence that act as an identification "flag" and distinguishes them as mitochondrial proteins (Rehling *et al.*, 2001; Voisine *et al.*, 1999). Such proteins are present in the cytosol as complexes with factors that are thought to stabilize them, as these proteins are not in their final conformation and therefore, are prone to degradation and aggregation (Stuart *et al.*, 1990; Young *et al.*, 2003). There are two kinds of targeting sequences – matrix targeting sequence, and internal targeting sequence. The N-terminal targeting sequences are also called as matrix targeting sequences (MTSs) because they bring the N-terminus of the precursor protein across the inner membrane into the matrix. In the absence of any further sorting information like presence of hydrophobic intermembrane regions, the MTS signal directs proteins into the matrix. They form amphipathic á-helices with one hydrophobic and one positively charged face. There is no consensus in the primary structure, which often differs considerably even between closely related orthologs. Post-translocation, the MTS is cleaved by matrix proteases to generate the mature protein. A large number of mitochondrial proteins are not synthesized with cleavable presequences but contain targeting information within regions of the mature protein. The members of this family of proteins include all mitochondrial outer membrane proteins, the majority of intermembrane space proteins, numerous multispanning inner membrane proteins, and a few matrix proteins (Neupert and Brunner, 2002; Neupert *et al.*, 1990; Neupert and Herrmann, 2007; Pfanner and Geissler, 2001; Rehling *et al.*, 2001).

The Presequence Pathway

The presequence pathway is responsible for translocation of about 60% of the mitochondrial proteins and arguably the major translocation machinery of the mitochondria. This pathway and machineries involved in the processes constitute the core focus of this review. Our initial understanding of this pathway is derived from studies in the yeast system.

The TIM23 complex is the major preprotein translocase in the inner membrane of mitochondria. In cells that are highly active in oxidative metabolism, such as fungal cells or metazoan muscle cells, its client proteins may make up some 20% of total cellular protein. The TIM23 complex translocates all precursors of matrix proteins, most inner membrane proteins, and many proteins of the intermembrane space region (Popov-Celeketic *et al.*, 2008). The TIM23 complex forms the most complicated translocase and sorting machinery of mitochondria. The reaction cycle of the TIM23 complex involves cooperation with the TOM complex of the outer membrane, the respiratory chain of the inner membrane, and the motor of the matrix (Alder *et al.*, 2008; Chacinska *et al.*, 2005; Gevorkyan-Airapetov *et al.*, 2009; Mokranjac *et al.*, 2005b; Tamura *et al.*, 2009). This translocase involves the utilization of two different energy sources, the electrochemical membrane potential and ATP. The components of the TIM23 complex can be subdivided into two groups, which operate in a sequential and cooperative manner: (*a*) the protein-conducting channel forming the membrane sector; also called as the TIM23 core channel and (*b*) components that drive the translocation into the matrix space; also called as the import motor (Chacinska *et al.*, 2009; Neupert and Herrmann, 2007; Okamoto *et al.*, 2002).

The membrane sector is composed of the three essential subunits, Tim50, Tim23, and Tim17; and two proteins which are dispensable for TIM23 function, Tim21 and a recently indentified protein Tam41 (Alder *et al.*, 2008; Elsner *et al.*, 2009; Geissler *et al.*, 2002; Gevorkyan-Airapetov *et al.*, 2009; Jensen and Dunn, 2002; Martinez-Caballero *et al.*, 2007; Meinecke *et al.*, 2006; Mokranjac *et al.*, 2005b; Mokranjac *et al.*, 2009; Popov-Celeketic *et al.*, 2008; Tamura *et al.*, 2006; van der Laan *et al.*, 2006). Tim23 and Tim17 form the membrane embedded core of the TIM23 complex. Both proteins share a phylogenetically related membrane domain consisting of four transmembrane segments (Matta *et al.*, 2017; Pareek *et al.*, 2013). Tim23 forms the transmembrane channel responsible for the entry and translocation of the precursors across the inner membrane. Tim17 was proposed to play a critical role in gating of the TIM23 pore (Martinez-Caballero *et al.*, 2007). Additionally,

Tim17 is also involved in motor recruitment and lateral sorting of preproteins (Demishtein-Zohary *et al.*, 2017). The other component, Tim50 is anchored into the inner membrane by an N-terminal transmembrane domain and exposes a large domain into the IMS. Tim50 interacts with incoming polypeptide chains as they reach the *trans* site of the TOM complex and presumably passes them on to Tim23. Tim50 not only functions as a passive import receptor but also seems to play a critical role in the regulation of import channel's permeability and thus, might coordinate the translocation process of preproteins by the TIM23 complex. Hence, Tim50 has a receptor function in the intermembrane space where it binds to the incoming polypeptide chain released from the TOM complex (Geissler *et al.*, 2002; Gevorkyan-Airapetov *et al.*, 2009; Meinecke *et al.*, 2006; Mokranjac *et al.*, 2009; Tamura *et al.*, 2009). Tim21 on the other hand, was observed to interact with the IMS domain of outer membrane protein, Tom22. This suggested a role of Tim21 in the interaction of the TIM23 complex with the TOM complex (Chacinska *et al.*, 2005; van der Laan *et al.*, 2006). Tim21 was also proposed to play a role in regulating the association of the import motor with the membrane sector of the TIM23 complex (Chacinska *et al.*, 2005). Based on this idea it was suggested that the TIM23 complex exists in two forms, one with both the membrane sector and import motor, and the other one without the import motor. The first form that is free of Tim21 may be specialized in translocation of precursor proteins into the matrix, whereas the second one may be specialized for translocation of precursors which are subjected to stop transfer (Chacinska *et al.*, 2009; Mokranjac *et al.*, 2005a). Tim50, Tim23 and Tim21 expose domains into the intermembrane space that are involved in the transient interaction of TIM23 complex with the TOM complex and thus facilitate preprotein translocation from the outer to inner membrane. Recent reports have shown that Tim21 also participates in the transient coupling of TIM23 complex with the respiratory chain complexes III and IV, and thus supports the membrane potential (Äø)-driven import step (Mick *et al.*, 2012; Murcha *et al.*, 2014). Liposomes based reconstitution of the purified translocase revealed that the minimal unit for preprotein integration into the inner membrane is the four-subunit TIM23 complex, a cardiolipin-rich membrane, and a membrane potential. Cardiolipin is the characteristic dimeric phospholipid of mitochondrial inner membrane (Acehan *et al.*, 2011; Bihlmaier *et al.*, 2008; Corcelli *et al.*, 2010; Tamura *et al.*, 2013). Preproteins following this insertion pathway into the inner membrane contain a hydrophobic sorting signal behind the matrix-targeting signal. The sorting signal arrests translocation in the inner membrane and causes a lateral release into the lipid phase of the membrane. This mechanism is also called as the Stop transfer mechanism. Although the details of the mechanism are not understood, the release requires a lateral opening of the Tim23 channel and involves the activity of Tim17 (Baker *et al.*, 2007; Bauer *et al.*, 2002; Bolender *et al.*, 2008; Chacinska *et al.*, 2009; Frazier *et al.*, 2003; Herrmann and Neupert, 2003; Neupert and Herrmann, 2007).

This import motor consists of three essential components – A peripheral inner membrane protein Tim44, mitochondrial Hsp70 (mtHsp70), matrix localized Mge1 and the membrane associated J-proteins Pam18 and Pam16 (Blom *et al.*, 1993; Bolliger *et al.*, 1994; Bracher and Verghese, 2015; D'Silva *et al.*, 2004; D'Silva *et al.*, 2005; Frazier *et al.*, 2004; Kang *et al.*, 1990; Laloraya *et al.*, 1995; Mokranjac *et al.*, 2003; Schneider *et al.*, 1994; Truscott *et al.*, 2003; Ungermann *et al.*, 1996). Tim44 is a peripheral membrane protein at the inner face of the inner mitochondrial membrane, where it interacts with the transmembrane components of the translocase (D'Silva *et al.*, 2004). It is thought that Tim44 sits at the outlet of the translocation channel and is responsible for recruiting mtHsp70 to the import site. Tim44 is also involved in interactions with Pam16 and forms a scaffold on which all the import reactions take place. Mge1 serves as the nucleotide exchange factor that replenishes the ADP bound state of mtHsp70 with that of ATP bound state (D'Silva *et al.*, 2004; D'Silva *et al.*, 2008; Hutu *et al.*, 2008; Moro *et al.*, 2002; Schiller, 2009; Schiller *et al.*, 2008).

MtHsp70 forms the central subunit of the matrix-exposed import motor or the PAM complex (Kang *et al.*, 1990). The mtHsp70 chaperone binds to the translocating polypeptide chain and drives its movement into the matrix in a reaction cycle that utilizes energy coming from hydrolysis of ATP (Liu *et al.*, 2003; Matouschek *et al.*, 2000; Mayer and Bukau, 2005). The nucleotide exchange factor Mge1, a homolog of bacterial GrpE, promotes the release of

ADP from mtHsp70 and thus stimulates a new round of ATP binding and precursor protein translocation. Other than Mge1, the import motor consists of four membrane bound co-chaperones that direct and regulate the activity of mtHsp70 at the exit site of the Tim23 channel. Tim44 provides a dynamic ATP-sensitive binding site for mtHsp70 close to the channel (Bolliger *et al.*, 1994; Laloraya *et al.*, 1995; Schiller *et al.*, 2008; Schneider *et al.*, 1994; Voos *et al.*, 1994). Moreover, Tim44 itself also binds to the preprotein in transit and is the first motor subunit that contacts the preprotein emerging from the matrix side of the Tim23 channel (Schiller *et al.*, 2008; Ungermann *et al.*, 1996). The J-domain of Pam18 stimulates the ATPase activity of mtHsp70 (D'Silva *et al.*, 2003). Pam16, which is a J-like protein, forms a stable complex with Pam18 and is responsible for recruiting Pam18 to the import channel and controlling its activity (D'Silva *et al.*, 2008; D'Silva *et al.*, 2005). The fourth co-chaperone, Pam17 is involved in the organization of the TIM23-PAM interaction. Pam17 exerts its function before the preprotein is translocated through the channel and is then released from the motor (Hutu *et al.*, 2008; Schiller, 2009; van der Laan *et al.*, 2005). The import motor subunits interact with several sites on the TIM23 core complex (Chacinska *et al.*, 2009). For example, the N-terminal IMS region of Pam18 interacts with the C-terminal of Tim17, though this interaction is dispensable for normal cell growth; Pam17 binds to Tim23 and is involved in maintenance of Pam16-Pam18 subcomplex. Deletion of Pam17 causes dissociation of both Pam16 and Pam18 from the translocase (Hutu *et al.*, 2008; Matta *et al.*, 2017; Schiller, 2009). Hence, the mitochondrial import motor is one of the most complex Hsp70 systems known.

The proteins that are destined to the mitochondrial matrix possess an N-terminal signal sequence (presequence) that is recognized by TOM receptors present on the outer membrane (Chacinska *et al.*, 2005; van der Laan *et al.*, 2006). These proteins are then internalized through the outer membrane and presorted to the TIM23 complex. The incoming polypeptide is captured by the Tim23-Tim50 complex (Geissler *et al.*, 2002; Gevorkyan-Airapetov *et al.*, 2009; Meinecke *et al.*, 2006; Mokranjac *et al.*, 2009). The initial translocation across the Tim23 channel is membrane potential dependent. The membrane potential difference across the inner membrane exerts an electrophoretic force on the positively charged presequence (Mick *et al.*, 2012; Murcha *et al.*, 2014). The final step of import is an energy dependent process governed by mtHsp70 which through repeated cycles of ATP binding and hydrolysis pull the polypeptide into the matrix (Liu *et al.*, 2003; Matouschek *et al.*, 2000; Mayer and Bukau, 2005; Schiller, 2009; Schiller *et al.*, 2008). The MPP protease in the matrix cleaves the presequence to generate the mature protein (Neupert and Herrmann, 2007).

Human Mitochondrial Protein Transport – An Enigma

It is now evident that most of our understanding of the mitochondrial protein import process has been derived from studies in the yeast system and there has been considerable advance in the understanding of how nuclear-encoded precursor proteins are translocated across and into the membranes of mitochondria (Schulz *et al.*, 2015; Sokol *et al.*, 2014). Human mitochondria translocation machineries were being thought to be similar to that of yeast (Bauer *et al.*, 1999). However, it had been very difficult to contemplate the existence of similar machinery in human mitochondria which is a much more complex *organelle* with diversified functions. Human mitochondria apart from regulating the metabolic pathways are involved in progression of cancer, neurodegenerative disorders, responses to xenobiotic stress and induction of apoptosis (Bernardi *et al.*, 1999; Chalah and Khosravi-Far, 2008; Chan, 2006; Formentini *et al.*, 2010). Numerous reports have shown that mutations and overexpression of human orthologs of translocase components are associated with various cancer subtypes (Bonora *et al.*, 2006; Davey *et al.*, 2006; Jubinsky *et al.*, 2005; Tagliati *et al.*, 2010). Such disease conditions also involve targeting of specific proteins that reprograms *organellar* functions and alters the cellular phenotype (Bernardi *et al.*, 1999; Gough *et al.*, 2009; Khan *et al.*, 2013; Nithipongvanitch *et al.*, 2007).

From the inferences derived from yeast system, it has been perceived that the presequence pathway constitutes major pathway of mitochondrial protein transport in mammalian system (Bauer *et al.*, 1999). Hence, initial studies in mammalian systems have focused on presequence pathway and led to the identification of proteins that are homologous to components of yeast TIM23 complex (Ishihara and

Mihara, 1998; Wada and Kanwar, 1998). The first component of human transport machinery identified was the Tim17 homologue. In contrast to yeast, two TIM17 genes were found to be expressed in humans and were named as hTim17a and hTim17b paralogs (Bauer *et al.*, 1999). Bioinformatics analysis supported by cellular data have suggested presence of two isoforms of Tim17b namely, Tim17b1 and Tim17b2 (Sinha *et al.*, 2014). Subsequently, the cDNAs corresponding to hTim17, hTim23 and hTim44 were isolated, as constituents of the human mitochondrial inner membrane translocase (Bauer *et al.*, 1999; Ishihara and Mihara, 1998).

Further studies have shown an intrinsic connection between translocation machinery and pathophysiological conditions of the cells, where components of the transport machinery were associated with mutations and overexpression. Tim50 overexpression was associated with enhanced tumour growth in breast cancer (Sankala *et al.*, 2011). Tim44 mutations led to increased predisposition to thyroid carcinomas (Bonora *et al.*, 2006). An Hsp40 class of protein, Magmas, was overexpressed in neoplastic prostrate and pituitary adenomas (Jubinsky *et al.*, 2005; Tagliati *et al.*, 2010). Increased Tim17a expression was associated with breast cancer patients (Salhab *et al.*, 2012). Loss of J-protein, JC15 expression has been found to be associated with development of chemoresistance upon chemotherapeutic treatment of certain cancer subtypes (Lindsey *et al.*, 2006; Shridhar *et al.*, 2001; Strathdee *et al.*, 2005). Point mutations in the J-domain of J-protein JC19 were associated with disease progression in dilated cardiomyopathy patients (Davey *et al.*, 2006; Ojala *et al.*, 2012). In addition, human mitochondria also show a unique phenomenon of harboring several oncoproteins and cell signaling molecules that lack mitochondrial targeting signal sequence and predominantly localize in cytosol or nucleus. For example, p53's mitochondrial localization in normal cells led to induction of apoptosis. In contrast, in cancer cells, the sequestration of p53 in the mitochondria promoted increased cell proliferation (Nithipongvanitch *et al.*, 2007; Park *et al.*, 2009; Saleem and Hood, 2013; Sankala *et al.*, 2011; Wadhwa *et al.*, 2002). Ras localization in mitochondria triggered apoptosis (Gough *et al.*, 2009; Kocher *et al.*, 2005). ERK mitochondrial distribution led to oncogenic proliferation and Ras mediated transformation (Rasola *et al.*, 2010; Zhu *et al.*, 2003). Sirtulins, also regarded as the anti-aging molecules of the cell, have been shown to promote carcinogenesis through their localization into the *organelle* and modulation of electron transport chain complexes (Hafner *et al.*, 2010; Verdin *et al.*, 2010). STAT family of proteins has been very recently shown to localize into the mitochondria where they aid in normal mitochondrial function and promote tumor growth in cancer cells (Gough *et al.*, 2009; Khan *et al.*, 2013; Mantel *et al.*, 2012; Wegrzyn *et al.*, 2009). Other than those mentioned above, there are numerous cell signaling molecules and transcription factors that localize into the mitochondria with unknown physiological function (Chan, 2006).

Thus, taking a note on the complexity of human mitochondrial function, its functional diversity, the vast repertoire of protein transported into the *organelle* to maintain its proteome and the established notion of parallel structure-function evolution resulted in a more favourable perception on the existence of a more complex machinery in humans.

The Organization of Human Inner Membrane Protein Translocation Machinery

The mammalian system involves a complex array of trafficking proteins that range from a series of receptors, import pores, and diverse molecular machines. Hence, transportation of these proteins into the *organelle* requires highly evolved and complex import machinery. In contrast to yeast which is characterized by the presence of a single inner membrane presequence translocase, human presequence translocases exist in multiple numbers to accommodate the diverse repertoire of proteins targeted into the mitochondria (Sinha *et al.*, 2014).

Although Tim17 was the first channel component to be identified, a J-like protein Magmas was the first characterized component of import motor (Jubinsky *et al.*, 2001; Sinha *et al.*, 2010). Magmas was previously considered as a granulocyte-macrophage colony stimulating factor that was overexpressed in prostate cancer (Jubinsky *et al.*, 2005; Jubinsky *et al.*, 2003). However, Magmas primarily functioned in regulating the function of import motor by inhibiting the mtHsp70 ATPase stimulatory activity of J-proteins. Magmas is the functional ortholog of yeast Pam16,

with similar primary structure and domain organization, and is an essential part of mammalian import motor. Magmas could complement Pam16's function in yeast and was able to maintain viability of yeast cells deleted for *PAM16*. The overlapping function of Magmas and Pam16 showed that the functional aspects of import motor regulation are conserved across species (Sinha *et al.*, 2010).

However, there exists a considerable degree of complexity at the level of J-protein organization at the import channel. The protein translocation activity in mitochondria is dependent on efficient chaperoning activity of mtHsp70 in order to vectorially pull the incoming polypeptide chain into the matrix compartment (Goswami *et al.*, 2010; Liu *et al.*, 2003). Since, the protein translocation function is an ATP-dependent process; it requires an optimum ATPase activity of mtHsp70. The ATPase activity of mtHsp70 is stimulated by the J-protein counterpart Pam18 in the case of yeast (D'Silva *et al.*, 2003). In contrast to yeast, which consists of a single J-protein Pam18 as a part of the TIM23 complex (Truscott *et al.*, 2003), human mitochondria is quite unique in expressing two J-proteins, namely JC19 and JC15, as a part of the inner membrane translocation machineries. Both the J-protein paralogs are involved in formation of two mutually distinct and functionally independent sub-complexes with their common J-like protein counterpart Magmas, in comparison to a single Pam16-Pam18 sub complex in yeast (Sinha *et al.*, 2016; Sinha *et al.*, 2014) (Fig. 1). Interestingly, JC15

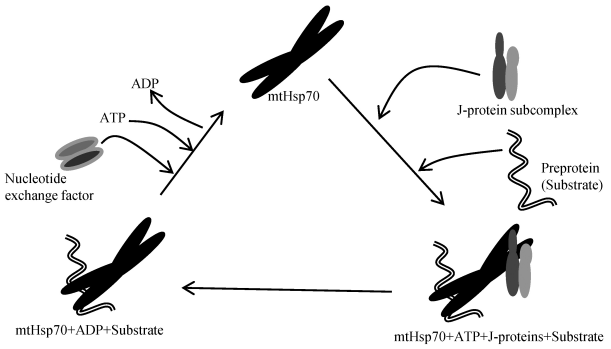

Fig. 1: The Hsp70 chaperone cycle at the import channel. At human import channel, the Hsp70 substrate binding-release cycle is regulated independently by functional interplay of two J-protein sub complexes-Magmas-JC19/Magmas-JC15 and a heterodimer of nucleotide exchange factors GrpEL1 and GrpEL2

possesses the ability to complement Pam18 function in yeast, as suggested by its ability to rescue the viability of "*pam18* yeast cells. JC15 could also efficiently import the precursor proteins in yeast mitochondria in absence of Pam18. The overlapping function of JC15 and Pam18 is probably due to their higher sequence complementarily and similar domain organization (Sinha *et al.*, 2016). This indicates that JC15 is evolutionary more closely related to Pam18. In contrast, JC19 lacks the ability to rescue the viability "*pam18* yeast cells which can probably be due to the absence of the region corresponding to Pam18's N-terminal region, in JC19 (Sinha *et al.*, 2016). Since, J-protein function is primarily implicated to a functional J-domain; it is intriguing why JC19 is unable to functionally complement Pam18.

The channel component of the mammalian translocases have principally diversified based on the presence of Tim17 and J-protein paralogs as constituting subunits; and contain Tim50, Tim23, Tim44, and Magmas as the common channel components. Humans show existence of three different presequence translocases. Translocase A is composed of Tim17a together with J-protein JC15, whereas translocase B1 and B2 are constituted by JC19 and Tim17b1 and Timb2 isoforms respectively (Sinha *et al.*, 2014). Further analysis of the complexes formed by these complexes showed translocase A to be an ancient translocase which corresponded to the transport machineries present in lower eukaryotes. Translocase B1 and B2 in turn, form mammalian specific translocases (Sinha *et al.*, 2014). Moreover, translocase B1 and B2 form the major machinery for transport of proteins into human mitochondria and translocase A mainly possess a supportive role but is responsible for piggy-backed import of non-canonical substrates into mitochondria (Sinha *et al.*, 2014) (Fig. 2). However, it will be intriguing to know whether translocase A have gained additional unknown function which has enabled it to be retained during evolution.

Diversified functions of human presequence translocases

In addition to the primary function of transporting precursor proteins into the *organelle*, the components of human mitochondrial translocase have gained secondary functions in maintenance of cellular homeostasis. Magmas, other than regulating the

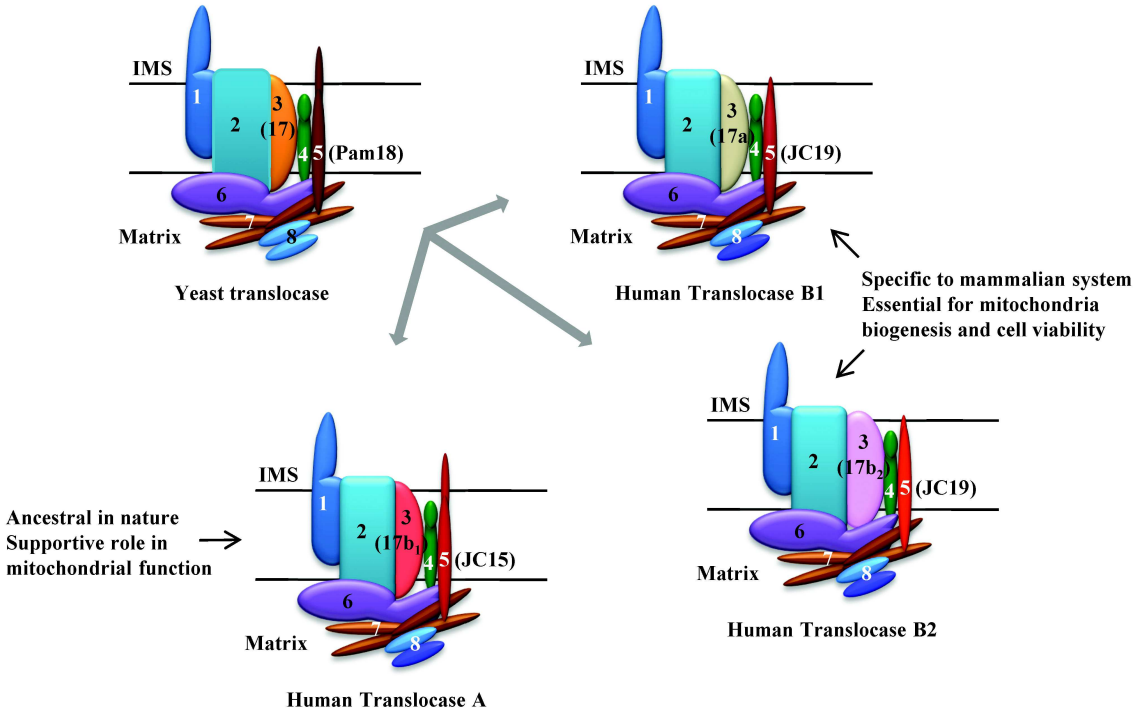

Fig. 2: Diversification of transport machineries. Lower eukaryotes such as yeast are characterized by presence of a single presequence translocase. The different subunits of the machinery and their corresponding orthologs in human are similarly numbered. In case of yeast translocase: (1) Tim50; (2) Tim23; (3) Tim17 (4) Pam16 (5) Pam18 (6) Tim44 (7) mitochondrial Hsp70 –Ssc1 (8) Mge1 homodimer. The core components of human translocase: (1) hTim50; (2) hTim23; (4) Magmas (6) hTim44 (7) mitochondrial Hsp70 –Mortalin (8) GrpEL1/2 heterodimer. The translocase A is characterized by (3) Tim17a and (5) DnaJC15, translocase B1 is characterized by (3) Tim17b1 and (5) DnaJC19 and translocase B2 is characterized by (3) Tim17b2 and (5) DnaJC19

activity of import motor, also plays an essential role in maintenance of cellular redox balance (Srivastava *et al.*, 2014). Overexpression of Magmas has been shown to reduce the oxidative stress load in the cell and protect the cellular biomolecules from oxidative damage. Downregulation of the protein increases the accumulation of reactive oxygen species and lower their survivability in the presence of external stress (Srivastava *et al.*, 2014). Interestingly, such dual functions have also been reported for the nucleotide exchange factor (NEF), Mge1 at yeast import motor. Initially, Mge1 was shown to act as a thermosensor. During heat stress, Mge1 undergoes conformational changes that result in lower efficiency of mtHsp70 ATPase cycle (Marada *et al.*, 2016). Later studies revealed Mge1's role as a sensor of oxidative stress. Presence of prooxidants led to dissociation of Mge1 from mtHsp70, resulting in reduced import rate. This phenotype probably, is due to oxidation of a near N-terminal methionine that might be leading to conformational instability; hence causing dissociation of active dimer to inactive monomer (Allu *et al.*, 2015; Marada *et al.*, 2013). Interestingly, the reactive oxygen species (ROS) sensing function of NEF remained conserved during evolution. GrpEL1, the human ortholog of Mge1, acts as a ROS sensor and is associated with reduced expression and better cell survivability under high ROS conditions (Srivastava *et al.*, 2017). However, Mge1 has diverged into a second paralog GrpEL2, specifically in chordates, which is resistant to oxidative stress and probably responsible for maintaining baseline mitochondrial activity under stress conditions (Naylor *et al.*, 1998; Srivastava *et al.*, 2017). In contrast to NEF homodimer of lower eukaryotes, human NEFs GrpEL1 and GrpEL2 function as heterodimer under normal conditions (Srivastava *et al.*, 2017) (Fi. 1).

However, such dual functionality is not true for all yeast import motor proteins. The yeast J-protein, Pam18's role resides only in import of proteins. The human ortholog of Pam18, JC15 shows bifunctionality.

Loss of JC15's expression is associated with the development of chemoresistance in clinical samples of breast and ovarian cancer. JC15 also acted as an inhibitor of respiratory complexes (Hatle *et al.,* 2013; Lindsey *et al.*, 2006; Shridhar *et al.*, 2001; Strathdee *et al.*, 2004). This property of JC15 could also be replicated *in vitro* culture systems. Mechanistically, JC15 as a part of translocase A was shown to release cyclophilin D from the inhibitory complexes present in mitochondrial matrix. JC15 thereby, couples cyclophilin D to mitochondrial permeability transition pore complex, triggering permeability transition and induction of cell death (Sinha and D'Silva, 2014). Such property is not shown by yeast J-proteins. In certain cases, as in Tim17, there is further fine tuning of secondary function from lower eukaryotes to humans. Expression of Tim17 in yeast protects against loss of mitochondrial genome in absence of genes responsible of mitochondrial DNA maintenance (Iacovino *et al.*, 2009). In humans, overexpression of Tim17a, the human ortholog closely related to yeast, has been found to be responsible for high mitochondrial DNA copy number in cancer cells which in turn, promote higher cellular proliferative rates (Sinha *et al.*, 2014). The translocase A has also been shown to be responsible for mitochondrial import of non-canonical substrates such p53, STAT3 and Erk family of proteins which lack mitochondrial targeting sequence (Sinha *et al.*, 2014). This generates a possibility that translocase A might be broadly responsible for the import of piggy-backed proteins into the *organelle*. Moreover, the mammalian Tim21 secondarily functions in the shuttling of inner membrane proteins from the translocase to the assembly intermediates of respiratory chain complexes, in addition to transport function (Mick *et al.*, 2012). Cross talk between the presequence pathway and other mitochondrial import pathways in sequestration of p53, in various cancer subtypes, is being considered as a possible step toward more effective cancer therapies (Lu *et al.*, 2011; Sankala *et al.*, 2011). Additionally, other mitochondrial import pathways has being depicted in progression of genetically inherited mitochondrial syndromes and various neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease and Amyotrophic lateral sclerosis (Schon and Przedborski, 2011). The role of mitochondrial import pathways in physiological well-being has been elaborately discussed in a recent review (Rogovin *et al.*, 2014).

Concluding Remarks

The past decade has introduced the field of mitochondrial protein transport with a vast repertoire of import components, primarily derived from studies in yeast system. In seems from initial analysis that transport system in case of higher vertebrates is far more complex. Presequence transport machinery, though major, constitutes only a fraction of the pathways responsible for transporting proteins into the mitochondria. This throws up an open field where there is a need for identifying and characterizing the different transport machines of mammalian mitochondria for better understanding of *organeller* functions. The complexity within the system is further enhanced through gain of additional roles by transport machinery components. It is quite possible that multifunctionality of import proteins might result in further hardwiring of the transport process with other cellular pathways; and hence, present new challenges for future research.

Acknowledgement

The author wishes to thank Dr. Shubhi Srivastava, IISc Bangalore, for her helpful comments during preparation of the manuscript. This work is supported by grants from University Grant Commission, India and CAS-Zoology, Banaras Hindu University.

References

A Anitha (2012) Brain region-specific altered expression and association of mitochondria-related genes in autism *Molecular Autism* **3**

Acehan D, Malhotra A, Xu Y, Ren M, Stokes D L, *et al.* (2011) Cardiolipin affects the supramolecular organization of ATP synthase in mitochondria *Biophys J* **100** 2184-2192

Ahting U, Thieffry M, Engelhardt H, Hegerl R, Neupert W, *et al.* (2001) Tom40, the pore-forming component of the protein-conducting TOM channel in the outer membrane of mitochondria *J Cell Biol* **153**:1151-1160

Alder N N, Jensen R E and Johnson A E (2008) Fluorescence mapping of mitochondrial TIM23 complex reveals a water-facing, substrate-interacting helix surface *Cell* **134** 439-450

Allu P K, Marada A, Boggula Y, Karri S, Krishnamoorthy T, *et al.* (2015) Methionine sulfoxide reductase 2 reversibly regulates Mge1, a cochaperone of mitochondrial Hsp70, during oxidative stress *Mol Biol Cell* **26** 406-419

Anderson S, Bankier A T, Barrell B G, de Bruijn M H, Coulson A R, *et al.* (1981) Sequence and organization of the human mitochondrial genome *Nature* **290** 457-465

Baker M J, Frazier A E, Gulbis J M and Ryan M T (2007) Mitochondrial protein-import machinery: correlating structure with function *Trends Cell Biol* **17** 456-464

Banci L, Bertini I, Cefaro C, Ciofi-Baffoni S, Gallo A, *et al.* (2009) MIA40 is an oxidoreductase that catalyzes oxidative protein folding in mitochondria *Nat Struct Mol Biol* **16** 198-206

Bauer M F, Gempel K, Reichert A S, Rappold G A, Lichtner P, *et al.* (1999) Genetic and structural characterization of the human mitochondrial inner membrane translocase *J Mol Biol* **289** 69-82

Bauer M F, Hofmann S and Neupert W (2002) Import of mitochondrial proteins *Int Rev Neurobiol* **53** 57-90

Becker T, Pfannschmidt S, Guiard B, Stojanovski D, Milenkovic D, *et al.* (2008) Biogenesis of the mitochondrial TOM complex: Mim1 promotes insertion and assembly of signal-anchored receptors *J Biol Chem* **283** 120-127

Bernardi P, Scorrano L, Colonna R, Petronilli V and Di Lisa F (1999) Mitochondria and cell death. Mechanistic aspects and methodological issues *Eur J Biochem* **264** 687-701

Bihlmaier K, Bien M and Herrmann J M (2008) In vitro import of proteins into isolated mitochondria *Methods Mol Biol* **457** 85-94

Blom J, Kubrich M, Rassow J, Voos W, Dekker P J, *et al.* (1993) The essential yeast protein MIM44 (encoded by MPI1) is involved in an early step of preprotein translocation across the mitochondrial inner membrane *Mol Cell Biol* **13**:7364-7371

Bolender N, Sickmann A, Wagner R, Meisinger C and Pfanner N (2008) Multiple pathways for sorting mitochondrial precursor proteins *EMBO Rep* **9** 42-49

Bolliger L, Deloche O, Glick B S, Georgopoulos C, Jeno P, *et al.* (1994) A mitochondrial homolog of bacterial GrpE interacts with mitochondrial hsp70 and is essential for viability *EMBO J* **13** 1998-2006

Bonora E, Evangelisti C, Bonichon F, Tallini G and Romeo G (2006) Novel germline variants identified in the inner mitochondrial membrane transporter TIMM44 and their role in predisposition to oncocytic thyroid carcinomas *Br J Cancer* **95** 1529-1536

Bracher A and Verghese J (2015) The nucleotide exchange factors of Hsp70 molecular chaperones *Front Mol Biosci* **2** 10

Chacinska A, Koehler C M, Milenkovic D, Lithgow T and Pfanner N (2009) Importing mitochondrial proteins: machineries and mechanisms *Cell* **138** 628-644

Chacinska A, Lind M, Frazier A E, Dudek J, Meisinger C, *et al.* (2005) Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17 *Cell* **120** 817-829

Chacinska A, Pfannschmidt S, Wiedemann N, Kozjak V, Sanjuan Szklarz L K, *et al.* (2004) Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins *EMBO J* **23** 3735-3746

Chalah A and Khosravi-Far R (2008) The mitochondrial death pathway *Adv Exp Med Biol* **615** 25-45

Chan D C (2006) Mitochondria: dynamic organelles in disease, aging, and development *Cell* **125** 1241-1252

Chan N C and Lithgow T (2008) The peripheral membrane subunits of the SAM complex function codependently in mitochondrial outer membrane biogenesis *Mol Biol Cell* **19** 126-136

Corcelli A, Saponetti M S, Zaccagnino P, Lopalco P, Mastrodonato M, *et al.* (2010) Mitochondria isolated in nearly isotonic KCl buffer: focus on cardiolipin and organelle morphology *Biochim Biophys Acta* **1798** 681-687

Curran S P, Leuenberger D, Oppliger W and Koehler C M (2002a) The Tim9p-Tim10p complex binds to the transmembrane domains of the ADP/ATP carrier *EMBO J* **21** 942-953

Curran S P, Leuenberger D, Schmidt E and Koehler C M (2002b) The role of the Tim8p-Tim13p complex in a conserved import pathway for mitochondrial polytopic inner membrane proteins *J Cell Biol* **158** 1017-1027

D'Silva P, Liu Q, Walter W and Craig E A (2004) Regulated interactions of mtHsp70 with Tim44 at the translocon in the mitochondrial inner membrane *Nat Struct Mol Biol* **11** 1084-1091

D'Silva P D, Schilke B, Walter W, Andrew A and Craig E A (2003) J protein cochaperone of the mitochondrial inner membrane required for protein import into the mitochondrial matrix *Proc Natl Acad Sci U S A* **100** 13839-13844

D'Silva P R, Schilke B, Hayashi M and Craig E A (2008) Interaction of the J-protein heterodimer Pam18/Pam16 of the mitochondrial import motor with the translocon of the inner membrane *Mol Biol Cell* **19** 424-432

D'Silva P R, Schilke B, Walter W and Craig E A (2005) Role of Pam16's degenerate J domain in protein import across the mitochondrial inner membrane *Proc Natl Acad Sci U S A* **102** 12419-12424

Davey K M, Parboosingh J S, McLeod D R, Chan A, Casey R, *et al.* (2006) Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition *J Med Genet* **43** 385-393

Demishtein-Zohary K, Gunsel U, Marom M, Banerjee R, Neupert W, *et al.* (2017) Role of Tim17 in coupling the import motor to the translocation channel of the mitochondrial presequence translocase *Elife* **6**

Ekstrand M I, Falkenberg M, Rantanen A, Park C B, Gaspari M, *et al.* (2004) Mitochondrial transcription factor A regulates mtDNA copy number in mammals *Hum Mol Genet* **13** 935-944

Elsner S, Simian D, Iosefson O, Marom M and Azem A (2009) The Mitochondrial Protein Translocation Motor: Structural Conservation between the Human and Yeast Tim14/Pam18-Tim16/Pam16 co-Chaperones *Int J Mol Sci* **10** 2041-2053

Emelyanov V V (2003) Mitochondrial connection to the origin of the eukaryotic cell *Eur J Biochem* **270** 1599-1618

Ernster L and Schatz G (1981) Mitochondria: a historical review *J Cell Biol* **91** 227s-255s

Formentini L, Martinez-Reyes I and Cuezva J M (2010) The mitochondrial bioenergetic capacity of carcinomas *IUBMB Life* **62** 554-560

Frazier A E, Chacinska A, Truscott K N, Guiard B, Pfanner N, *et al.* (2003) Mitochondria use different mechanisms for transport of multispanning membrane proteins through the intermembrane space *Mol Cell Biol* **23** 7818-7828

Frazier A E, Dudek J, Guiard B, Voos W, Li Y, *et al.* (2004) Pam16 has an essential role in the mitochondrial protein import motor *Nat Struct Mol Biol* **11** 226-233

Fulda S, Galluzzi L and Kroemer G (2010) Targeting mitochondria for cancer therapy *Nat Rev Drug Discov* **9** 447-464

Geissler A, Chacinska A, Truscott K N, Wiedemann N, Brandner K, *et al.* (2002) The mitochondrial presequence translocase: An essential role of Tim50 in directing preproteins to the import channel *Cell* **111** 507-518

Gevorkyan-Airapetov L, Zohary K, Popov-Celeketic D, Mapa K, Hell K, *et al.* (2009) Interaction of Tim23 with Tim50 Is essential for protein translocation by the mitochondrial TIM23 complex *J Biol Chem* **284** 4865-4872

Goswami A V, Chittoor B and D'Silva P (2010) Understanding the functional interplay between mammalian mitochondrial Hsp70 chaperone machine components *J Biol Chem* **285** 19472-19482

Gough D J, Corlett A, Schlessinger K, Wegrzyn J, Larner A C, *et al.* (2009) Mitochondrial STAT3 supports Ras-dependent oncogenic transformation *Science* **324** 1713-1716

Gray M W, Burger G and Lang B F (1999) Mitochondrial evolution *Science* **283** 1476-1481

Grumbt B, Stroobant V, Terziyska N, Israel L and Hell K (2007) Functional characterization of Mia40p, the central component of the disulfide relay system of the mitochondrial intermembrane space *J Biol Chem* **282** 37461-37470

Gulbins E, Dreschers S and Bock J (2003) Role of mitochondria in apoptosis *Exp Physiol* **88** 85-90

Hafner A V, Dai J, Gomes A P, Xiao C Y, Palmeira C M, *et al.* (2010) Regulation of the mPTP by SIRT3-mediated deacetylation of CypD at lysine 166 suppresses age-related cardiac hypertrophy *Aging (Albany NY)* **2** 914-923

Hatle K M, Gummadidala P, Navasa N, Bernardo E, Dodge J, *et al.* (2013) MCJ/DnaJC15, an endogenous mitochondrial repressor of the respiratory chain that controls metabolic alterations *Mol Cell Biol* **33** 2302-2314

Hayashi T, Rizzuto R, Hajnoczky G and Su T P (2009) MAM: more than just a housekeeper *Trends Cell Biol* **19** 81-88

Henze K and Martin W (2003) Evolutionary biology: essence of mitochondria *Nature* **426** 127-128

Herrmann J M and Neupert W (2003) Protein insertion into the inner membrane of mitochondria *IUBMB Life* **55** 219-225

Hutu D P, Guiard B, Chacinska A, Becker D, Pfanner N, *et al.* (2008) Mitochondrial protein import motor: differential role of Tim44 in the recruitment of Pam17 and J-complex to the presequence translocase *Mol Biol Cell* **19** 2642-2649

Iacovino M, Granycome C, Sembongi H, Bokori-Brown M, Butow R A, *et al.* (2009) The conserved translocase Tim17 prevents mitochondrial DNA loss *Hum Mol Genet* **18** 65-74

Ishihara N and Mihara K (1998) Identification of the protein import components of the rat mitochondrial inner membrane, rTIM17, rTIM23, and rTIM44 *J Biochem* **123** 722-732

Jensen R E and Dunn C D (2002) Protein import into and across the mitochondrial inner membrane: role of the TIM23 and TIM22 translocons *Biochim Biophys Acta* **1592** 25-34

Jubinsky P T, Messer A, Bender J, Morris R E, Ciraolo G M, *et al.* (2001) Identification and characterization of Magmas, a novel mitochondria-associated protein involved in granulocyte-macrophage colony-stimulating factor signal transduction *Exp Hematol* **29** 1392-1402

Jubinsky P T, Short M K, Mutema G, Morris R E, Ciraolo G M, *et al.* (2005) Magmas expression in neoplastic human prostate *J Mol Histol* **36** 69-75

Jubinsky P T, Short M K, Mutema G and Witte D P (2003) Developmental expression of Magmas in murine tissues and its co-expression with the GM-CSF receptor *J Histochem Cytochem* **51** 585-596

Kang P J, Ostermann J, Shilling J, Neupert W, Craig E A, *et al.* (1990) Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins *Nature* **348** 137-143

Khan R, Lee J E, Yang Y M, Liang F X and Sehgal P B (2013) Live-cell imaging of the association of STAT6-GFP with mitochondria *PLoS One* **8** e55426

Kocher H M, Senkus R, Al-Nawab M and Hendry B M (2005) Subcellular distribution of Ras GTPase isoforms in normal human kidney *Nephrol Dial Transplant* **20** 886-891

Kroemer G (2006) Mitochondria in cancer *Oncogene* **25** 4630-4632

Laloraya S, Dekker P J, Voos W, Craig E A and Pfanner N (1995) Mitochondrial GrpE modulates the function of matrix Hsp70 in translocation and maturation of preproteins *Mol Cell Biol* **15** 7098-7105

Lang B F, Gray M W and Burger G (1999) Mitochondrial genome evolution and the origin of eukaryotes *Annu Rev Genet* **33** 351-397

Lesnefsky E J, Moghaddas S, Tandler B, Kerner J and Hoppel C L (2001) Mitochondrial dysfunction in cardiac disease: ischemia—reperfusion, aging, and heart failure *J Mol Cell Cardiol* **33** 1065-1089

Leung A W and Halestrap A P (2008) Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore *Biochim Biophys Acta* **1777** 946-952

Lindsey J C, Lusher M E, Strathdee G, Brown R, Gilbertson R J, *et al.* (2006) Epigenetic inactivation of MCJ (DNAJD1) in malignant paediatric brain tumours *Int J Cancer* **118** 346-352

Liu Q, D'Silva P, Walter W, Marszalek J and Craig E A (2003) Regulated cycling of mitochondrial Hsp70 at the protein import channel *Science* **300** 139-141

Lu W J, Lee N P, Kaul S C, Lan F, Poon R T, *et al.* (2011) Mortalin-p53 interaction in cancer cells is stress dependent and constitutes a selective target for cancer therapy *Cell Death Differ* **18** 1046-1056

Mantel C, Messina-Graham S, Moh A, Cooper S, Hangoc G, *et al.* (2012) Mouse hematopoietic cell-targeted STAT3 deletion: stem/progenitor cell defects, mitochondrial dysfunction, ROS overproduction, and a rapid aging-like phenotype *Blood* **120** 2589-2599

Marada A, Allu P K, Murari A, PullaReddy B, Tammineni P, *et al.* (2013) Mge1, a nucleotide exchange factor of Hsp70, acts as an oxidative sensor to regulate mitochondrial Hsp70 function *Mol Biol Cell* **24** 692-703

Marada A, Karri S, Singh S, Allu P K, Boggula Y, *et al.* (2016) A Single Point Mutation in Mitochondrial Hsp70 Cochaperone Mge1 Gains Thermal Stability and Resistance *Biochemistry* **55** 7065-7072

Margulis L (1970) Origin of Eukaryotic Cells *Yale Univ Press* 349 pp

Martinez-Caballero S, Grigoriev S M, Herrmann J M, Campo M L and Kinnally K W (2007) Tim17p regulates the twin pore structure and voltage gating of the mitochondrial protein import complex TIM23 *J Biol Chem* **282** 3584-3593

Matouschek A, Pfanner N and Voos W (2000) Protein unfolding by mitochondria. The Hsp70 import motor *EMBO Rep* **1** 404-410

Matta S K, Pareek G, Bankapalli K, Oblesha A and D'Silva P (2017) Role of Tim17 Transmembrane Regions in Regulating the Architecture of Presequence Translocase and Mitochondrial DNA Stability *Mol Cell Biol* **37**

Mayer M P and Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism *Cell Mol Life Sci* **62** 670-684

McBride H M, Neuspiel M and Wasiak S (2006) Mitochondria: more than just a powerhouse *Curr Biol* **16** R551-560

Meinecke M, Wagner R, Kovermann P, Guiard B, Mick D U, *et al.* (2006) Tim50 maintains the permeability barrier of the mitochondrial inner membrane *Science* **312** 1523-1526

Mick D U, Dennerlein S, Wiese H, Reinhold R, Pacheu-Grau D, *et al*. (2012) MITRAC links mitochondrial protein translocation to respiratory-chain assembly and translational regulation *Cell* **151** 1528-1541

Mokranjac D, Popov-Celeketic D, Hell K and Neupert W (2005a) Role of Tim21 in mitochondrial translocation contact sites *J Biol Chem* **280** 23437-23440

Mokranjac D, Sichting M, Neupert W and Hell K (2003) Tim14, a novel key component of the import motor of the TIM23 protein translocase of mitochondria *EMBO J* **22** 4945-4956

Mokranjac D, Sichting M, Popov-Celeketic D, Berg A, Hell K, *et al.* (2005b) The import motor of the yeast mitochondrial TIM23 preprotein translocase contains two different J proteins, Tim14 and Mdj2 *J Biol Chem* **280** 31608-31614

Mokranjac D, Sichting M, Popov-Celeketic D, Mapa K, Gevorkyan-Airapetov L, *et al.* (2009) Role of Tim50 in the transfer of precursor proteins from the outer to the inner membrane of mitochondria *Mol Biol Cell* **20** 1400-1407

Moro F, Okamoto K, Donzeau M, Neupert W and Brunner M (2002) Mitochondrial protein import: molecular basis of the ATP-dependent interaction of MtHsp70 with Tim44 *J Biol Chem* **277** 6874-6880

Murcha M W, Kubiszewski-Jakubiak S, Wang Y and Whelan J (2014) Evidence for interactions between the mitochondrial import apparatus and respiratory chain complexes via Tim21-like proteins in Arabidopsis *Front Plant Sci* **5** 82

Naylor D J, Stines A P, Hoogenraad N J and Hoj P B (1998) Evidence for the existence of distinct mammalian cytosolic, microsomal, and two mitochondrial GrpE-like proteins, the Co-chaperones of specific Hsp70 members *J Biol Chem* **273** 21169-21177

Neupert W and Brunner M (2002) The protein import motor of mitochondria *Nat Rev Mol Cell Biol* **3** 555-565

Neupert W, Hartl F U, Craig E A and Pfanner N (1990) How do polypeptides cross the mitochondrial membranes? *Cell* **63** 447-450

Neupert W and Herrmann J M (2007) Translocation of proteins into mitochondria *Annu Rev Biochem* **76** 723-749

Nithipongvanitch R, Ittarat W, Cole M P, Tangpong J, Clair D K, *et al.* (2007) Mitochondrial and nuclear p53 localization in cardiomyocytes: redox modulation by doxorubicin (Adriamycin)? *Antioxid Redox Signal* **9** 1001-1008

Ojala T, Polinati P, Manninen T, Hiippala A, Rajantie J, *et al.* (2012) New mutation of mitochondrial DNAJC19 causing dilated and noncompaction cardiomyopathy, anemia, ataxia, and male genital anomalies *Pediatr Res* **72** 432-437

Okamoto K, Brinker A, Paschen S A, Moarefi I, Hayer-Hartl M, *et al.* (2002) The protein import motor of mitochondria: a targeted molecular ratchet driving unfolding and translocation *EMBO J* **21** 3659-3671

Pareek G, Krishnamoorthy V and D'Silva P (2013) Molecular insights revealing interaction of Tim23 and channel subunits of presequence translocase *Mol Cell Biol* **33** 4641-4659

Park J Y, Wang P Y, Matsumoto T, Sung H J, Ma W, *et al.* (2009) p53 improves aerobic exercise capacity and augments skeletal muscle mitochondrial DNA content *Circ Res* **105** 705-712, 711 p following 712

Peng J, Huang C H, Short M K and Jubinsky P T (2005) Magmas gene structure and evolution *In Silico Biol* **5** 251-263

Pfanner N and Geissler A (2001) Versatility of the mitochondrial protein import machinery *Nat Rev Mol Cell Biol* **2** 339-349

Popov-Celeketic D, Mapa K, Neupert W and Mokranjac D (2008) Active remodelling of the TIM23 complex during translocation of preproteins into mitochondria *EMBO J* **27** 1469-1480

Rasola A, Sciacovelli M, Chiara F, Pantic B, Brusilow W S, *et al.* (2010) Activation of mitochondrial ERK protects cancer cells from death through inhibition of the permeability transition *Proc Natl Acad Sci U S A* **107** 726-731

Rehling P, Brandner K and Pfanner N (2004) Mitochondrial import and the twin-pore translocase *Nat Rev Mol Cell Biol* **5** 519-530

Rehling P, Wiedemann N, Pfanner N and Truscott K N (2001) The mitochondrial import machinery for preproteins *Crit Rev Biochem Mol Biol* **36** 291-336

Rogovin K A, Khrushcheva A M, Shekarova O N, Ushakova M V, Manskikh V N, *et al.* (2014) Effects of mitochondria-targeted plastoquinone derivative antioxidant (SkQ1) on demography of free-breeding Campbell dwarf hamsters (Phodopus campbelli) kept in outdoor conditions. reproduction and lifespan: explanation in the framework of ultimate loads *Biochemistry (Mosc)* **79** 1117-1129

Sagan L (1967) On the origin of mitosing cells *J Theor Biol* **14** 225-274

Saleem A and Hood D A (2013) Acute exercise induces tumour suppressor protein p53 translocation to the mitochondria and promotes a p53-Tfam-mitochondrial DNA complex in skeletal muscle *J Physiol* **591** 3625-3636

Salhab M, Patani N, Jiang W and Mokbel K (2012) High TIMM17A expression is associated with adverse pathological and clinical outcomes in human breast cancer *Breast Cancer* **19** 153-160

Sankala H, Vaughan C, Wang J, Deb S and Graves P R (2011) Upregulation of the mitochondrial transport protein, Tim50, by mutant p53 contributes to cell growth and chemoresistance *Arch Biochem Biophys* **512** 52-60

Schiller D (2009) Pam17 and Tim44 act sequentially in protein import into the mitochondrial matrix *Int J Biochem Cell Biol* **41** 2343-2349

Schiller D, Cheng Y C, Liu Q, Walter W and Craig E A (2008) Residues of Tim44 involved in both association with the translocon of the inner mitochondrial membrane and regulation of mitochondrial Hsp70 tethering *Mol Cell Biol* **28** 4424-4433

Schneider H C, Berthold J, Bauer M F, Dietmeier K, Guiard B, *et al.* (1994) Mitochondrial Hsp70/MIM44 complex facilitates protein import *Nature* **371** 768-774

Schnell D J and Hebert D N (2003) Protein translocons: multifunctional mediators of protein translocation across membranes *Cell* **112** 491-505

Schon E A and Przedborski S (2011) Mitochondria: the next (neurode)generation *Neuron* **70** 1033-1053

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

Shridhar V, Bible K C, Staub J, Avula R, Lee Y K, *et al.* (2001) Loss of expression of a new member of the DNAJ protein family confers resistance to chemotherapeutic agents used in the treatment of ovarian cancer *Cancer Res* **61** 4258-4265

Sinha D and D'Silva P (2014) Chaperoning mitochondrial permeability transition: regulation of transition pore complex by a J-protein, DnaJC15 *Cell Death Dis* **5** e1101

Sinha D, Joshi N, Chittoor B, Samji P and D'Silva P (2010) Role of Magmas in protein transport and human mitochondria biogenesis *Hum Mol Genet* **19** 1248-1262

Sinha D, Srivastava S and D'Silva P (2016) Functional diversity of human mitochondrial J-proteins is independent of their association with the inner membrane presequence translocase *J Biol Chem* **291** 17345-17359

Sinha D, Srivastava S, Krishna L and D'Silva P (2014) Unraveling the intricate organization of mammalian mitochondrial presequence translocases: existence of multiple translocases for maintenance of mitochondrial function *Mol Cell Biol* **34** 1757-1775

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

Srivastava S, Savanur M A, Sinha D, Birje A R V, *et al.* (2017) Regulation of mitochondrial protein import by the nucleotide exchange factors GrpEL1 and GrpEL2 in human cells *J Biol Chem-in press*

Srivastava S, Sinha D, Saha P P, Marthala H and D'Silva P (2014) Magmas functions as a ROS regulator and provides cytoprotection against oxidative stress-mediated damages *Cell Death Dis* **5** e1394

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

Stojanovski D, Muller J M, Milenkovic D, Guiard B, Pfanner N, *et al.* (2008) The MIA system for protein import into the mitochondrial intermembrane space *Biochim Biophys Acta* **1783** 610-617

Strathdee G, Davies B R, Vass J K, Siddiqui N and Brown R (2004) Cell type-specific methylation of an intronic CpG island controls expression of the MCJ gene *Carcinogenesis* **25** 693-701

Strathdee G, Vass J K, Oien K A, Siddiqui N, Curto-Garcia J, *et al.* (2005) Demethylation of the MCJ gene in stage III/IV epithelial ovarian cancer and response to chemotherapy *Gynecol Oncol* **97** 898-903

Stuart R A, Nicholson D W and Neupert W (1990) Early steps in mitochondrial protein import: receptor functions can be substituted by the membrane insertion activity of apocytochrome c *Cell* **60** 31-43

Tagliati F, Gentilin E, Buratto M, Mole D, degli Uberti E C, *et al.* (2010) Magmas, a gene newly identified as overexpressed in human and mouse ACTH-secreting pituitary adenomas, protects pituitary cells from apoptotic stimuli *Endocrinology* **151** 4635-4642

Tamura Y, Harada Y, Nishikawa S, Yamano K, Kamiya M, *et al.* (2013) Tam41 is a CDP-diacylglycerol synthase required for cardiolipin biosynthesis in mitochondria *Cell Metab* **17** 709-718

Tamura Y, Harada Y, Shiota T, Yamano K, Watanabe K, *et al.* (2009) Tim23-Tim50 pair coordinates functions of translocators and motor proteins in mitochondrial protein import *J Cell Biol* **184** 129-141

Tamura Y, Harada Y, Yamano K, Watanabe K, Ishikawa D, *et al.* (2006) Identification of Tam41 maintaining integrity of the TIM23 protein translocator complex in mitochondria *J Cell Biol* **174** 631-637

Truscott K N, Voos W, Frazier A E, Lind M, Li Y, *et al.* (2003) A J-protein is an essential subunit of the presequence translocase-associated protein import motor of mitochondria *J Cell Biol* **163** 707-713

Ungermann C, Guiard B, Neupert W and Cyr D M (1996) The delta psi- and Hsp70/MIM44-dependent reaction cycle driving early steps of protein import into mitochondria *EMBO J* **15** 735-744

van der Laan M, Chacinska A, Lind M, Perschil I, Sickmann A, *et al*. (2005) Pam17 is required for architecture and translocation activity of the mitochondrial protein import motor *Mol Cell Biol* **25** 7449-7458

van der Laan M, Wiedemann N, Mick D U, Guiard B, Rehling P, *et al.* (2006) A role for Tim21 in membrane-potential-dependent preprotein sorting in mitochondria *Curr Biol* **16** 2271-2276

Verdin E, Hirschey M D, Finley L W and Haigis M C (2010) Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling *Trends Biochem Sci* **35** 669-675

Voisine C, Craig E A, Zufall N, von Ahsen O, Pfanner N, *et al.* (1999) The protein import motor of mitochondria: unfolding and trapping of preproteins are distinct and separable functions of matrix Hsp70 *Cell* **97** 565-574

Voos W, Gambill B D, Laloraya S, Ang D, Craig E A, *et al.* (1994) Mitochondrial GrpE is present in a complex with hsp70

and preproteins in transit across membranes *Mol Cell Biol* **14** 6627-6634

Wada J and Kanwar Y S (1998) Characterization of mammalian translocase of inner mitochondrial membrane (Tim44) isolated from diabetic newborn mouse kidney *Proc Natl Acad Sci U S A* **95** 144-149

Wadhwa R, Yaguchi T, Hasan M K, Mitsui Y, Reddel R R, *et al.* (2002) Hsp70 family member, mot-2/mthsp70/GRP75, binds to the cytoplasmic sequestration domain of the p53 protein *Exp Cell Res* **274** 246-253

Wegrzyn J, Potla R, Chwae Y J, Sepuri N B, Zhang Q, *et al.* (2009) Function of mitochondrial Stat3 in cellular respiration *Science* **323** 793-797

Wickner W and Schekman R (2005) Protein translocation across biological membranes *Science* **310** 1452-1456

Wiesner R J, Ruegg J C and Morano I (1992) Counting target molecules by exponential polymerase chain reaction: copy number of mitochondrial DNA in rat tissues *Biochem Biophys Res Commun* **183** 553-559

Wu Y and Sha B (2006) Crystal structure of yeast mitochondrial outer membrane translocon member Tom70p *Nat Struct Mol Biol* **13** 589-593

Yamano K, Yatsukawa Y, Esaki M, Hobbs A E, Jensen R E, *et al.* (2008) Tom20 and Tom22 share the common signal recognition pathway in mitochondrial protein import *J Biol Chem* **283** 3799-3807

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 J H, Guo F, Shelburne J, Watkins S and Chu C T (2003) Localization of phosphorylated ERK/MAP kinases to mitochondria and autophagosomes in Lewy body diseases *Brain Pathol* **13** 473-481.