Review

Peeping at TOMs - Diverse entry gates to mitochondria provide insights into the evolution of eukaryotes

Jan Mani[a], Chris Meisinger[b] and André Schneider[a, 1]

[a]Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland

[b]Institut für Biochemie und Molekularbiologie, ZBMZ and BIOSS Centre for Biological Signalling Studies, Universität Freiburg, 79104 Freiburg, Germany

[1] Correspondence to: andre.schneider@dcb.unibe.ch

source: https://doi.org/10.7892/boris.92438 | downloaded: 12.10.2024

Mitochondria are essential for eukaryotic life and more than 95% of their proteins are imported as precursors from the cytosol. The targeting signals for this post-translational import are conserved in all eukaryotes. However, this conservation does not hold true for the protein translocase of the mitochondrial outer membrane that serves as entry gate for essentially all precursor proteins. Only two of its subunits, Tom40 and Tom22, are conserved and thus likely were present in the last eukaryotic common ancestor. Tom7 is found in representatives of all supergroups except the Excavates. This suggests that it was added to the core of the translocase after the Excavates segregated from all other eukaryotes. A comparative analysis of the biochemically and functionally characterized outer membrane translocases of yeast, plants and trypanosomes, which represent three eukaryotic supergroups, shows that the receptors that recognize the conserved import signals differ strongly between the different systems. They present a remarkable example of convergent evolution at the molecular level. The structural diversity of the functionally conserved import receptors therefore provides insight into the early evolutionary history of mitochondria.

Protein import distinguishes mitochondria from its endosymbiontic ancestor

The origin of eukaryotic cells arguably is the most important transition in evolution besides the origin of life itself. It is now widely accepted that the development of the nucleus, the name-giving feature of eukaryotes, required a highly efficient energy metabolism that could only be provided by mitochondria (Lane 2014). The acquirement of a bacterial endosymbiont by the archeal ancestor of eukaryotes (Williams, et al. 2013; Koonin and Yutin 2014; Spang, et al. 2015), that subsequently was converted into the mitochondrion was therefore likely the event that triggered the evolution of eukaryotes. The process of organellogenesis was accompanied by a massive reduction of the endosymbiont's genome. While part of it was lost, some genes were transferred to the host's nucleus, a process designated endosymbiotic gene transfer (EGT) (Thorsness and Weber 1996; Adams and Palmer 2003; Timmis, et al. 2004). Today mitochondrial genomes harbor very few genes encoding between 3 and 65 different proteins (Gray, et al. 1999; Allen 2003), which stands in sharp contrast to the complexity of the mitochondrial proteomes which even in unicellular organisms amounts to more than 1000 proteins (Meisinger, et al. 2008; Niemann, et al. 2013; Huang, et al. 2014). This indicates that essentially all mitochondrial proteins, many of which are encoded by genes that had been transferred to the nucleus by EGT, are imported from the cytosol.

Gaining the capability to import proteins is therefore the defining event that marks the transition of the endosymbiont to a genetically integrated organelle that largely is under the control of the nucleus (Dolezal, et al. 2006; Lithgow and Schneider 2010; Hewitt, et al. 2011; Gray 2012). The question of how mitochondrial protein import evolved is therefore tightly linked to the more general question of the origin of the eukaryotic cell.

Protein import has mainly been studied in yeast

Saccharomyces cerevisiae has been a powerful model to investigate mitochondrial protein import resulting in a wealth of data on the machineries and the mechanism of the process (Chacinska, et al. 2009; Schmidt, et al. 2010; Schulz, et al. 2015). Bioinformatic and experimental evidence shows that protein import and the factors mediating it are highly conserved between yeast and mammals (Dolezal, et al. 2006). Since these two systems are morphologically very different, it is often assumed that the observed conservation may extend to all eukaryotes. However, this is a misconception: according to the latest molecular phylogenetic tree eukaryotes are divided into a small number of supergroups, that diverged very early in evolution (Adl, et al. 2005; Burki 2014). Moreover, metazoans including mammals, and yeast belong to the same supergroup of the Opisthokonts, indicating that on the large scale they are closely related (fig. 1).

Thus, except for plants which define the supergroup of the Archeaplastida and whose protein import system has been investigated for many years (Murcha, et al. 2014), there are few experimental studies on mitochondrial protein import in non-opisthokont organisms. However, since very recently there is a new kid on the block. Studies in the parasitic protozoa *Trypanosoma brucei*, a member of the supergroup of the Excavates, identified and characterized the composition and function of the translocase that mediates protein transport across the mitochondrial outer membrane (OM) (Mani, et al. 2015).

Thus, for the first time we have sufficient data of the mitochondrial protein import machinery of representatives of three different eukaryotic supergroups that allow us to perform a comparative analysis that is not simply based on sequence similarities but on structural and functional features of import components. We deliberately excluded eukaryotes with mitochondria-related organelles that lack an own genome from our analysis as their protein import systems have been subject to reductive evolution (Heinz and Lithgow 2013).

Protein import is mediated by four major heteroligomeric protein complexes in the OM and the inner membrane (IM) (fig. 2). In this review we focus on the translocase of the OM (TOM) the entry gate for essentially all mitochondrial proteins. It is localized at the interface of the organelle and the cytosol and thus the first machinery with which imported proteins need to engage.

Mitochondrial import signals are conserved

The mitochondrial proteome of which more than 95% is imported from the cytosol, is not only of comparable size in yeast, plants and trypanosomes but also contains substrates for all the different types of import pathways. (Meisinger, et al. 2008; Niemann, et al. 2013; Huang, et al. 2014) (table 1).

The largest class which includes approximately 60% of all mitochondrial proteins is targeted to mitochondria by N-terminal presequences, that are rich in basic and hydroxylated amino acids that have the propensity to form amphiphilic α–helices. Presequences are found on most matrix and many IM protein precursors. They mediate import across the TOM and TIM23 complexes (fig. 1) (Habib, et al. 2007; Chacinska, et al. 2009). After import they are generally processed by the heterodimeric mitochondrial processing peptidase (MPP) and in some cases further trimmed by two other proteases, Icp55 and Oct1. Finally, the processed presequences are degraded by Cym1 and Prd1 (Desy, et al. 2012; Mossmann, et al. 2012; Teixeira and Glaser 2013).

All these proteases, as well as the features of the presequences themselves are conserved, although their specific substrates may vary in the different systems (Carrie, et al. 2015). The only discernable differences in the presequences is that the plant ones are on average longer and contain more serine residues than the ones in yeast and trypanosomes. This might be due to the fact that in plant mitochondrial targeting signals must be differentiated from the plastid ones (Murcha, et al. 2014).

A few IM proteins facing the intermembrane space (IMS) have in addition to a presequence also a sorting signal that is removed by the IM protease (IMP) or other proteases (Chacinska, et al. 2009). IMP is highly conserved, it is found in all eukaryotes and shows homology to the bacterial leader peptidase (Schneider, et al. 1991; Teixeira and Glaser 2013).

Presequences are not only highly similar between different species but also functionally conserved. They correctly localize proteins heterologously expressed in different

supergroups, both in vivo and in vitro (table 2) although exceptions exist (van Wilpe, et al. 1999).

Carrier proteins (MCP) define a conserved mitochondrial protein family that is localized in the IM and whose members have six transmembrane helices. Except for a small subset in plants, MCPs lack presequences and instead have internal as yet poorly defined targeting sequences. MCPs cross the OM using the TOM complex before they engage with the TIM22 complex that inserts them into the IM. Yeast, plants and trypanosomes have a comparable number of carrier proteins (Palmieri, et al. 1996; Colasante, et al. 2009; Haferkamp and Schmitz-Esser 2012), all of which must be imported (table 1). Again in vivo and in vitro experiments suggest that carrier proteins are correctly localized when heterologously expressed (table 2).

β-barrel proteins are initially imported into the IMS using the TOM complex and subsequently inserted into the OM by the sorting and assembly machinery (SAM) (Hohr, et al. 2015). β-barrel proteins contain a loosely defined signal after the last β-strand that in yeast is recognized by Sam35 of the SAM (Kutik, et al. 2007). What features of β-barrel proteins are recognized by TOM is unclear, however the proteins are generally correctly localized when expressed in heterologous systems. Interestingly, even some bacterial and chloroplast β-barrel proteins can be targeted to mitochondria when expressed in non-plant eukaryotes (table 2).

Finally, there are a handful of IMS-localized small TIM chaperones which have a cysteine-containing internal targeting signal and which are imported by the mitochondrial intermembrane space assembly (MIA) pathway (Herrmann and Riemer 2012). Both the small TIMs as well as their targeting signals appear to be conserved in essentially all eukaryotes.

In summary, these results strongly suggests that the mitochondrial targeting signals and the machineries that process them were already established in the last common ancestor of all eukaryotes. This is supported by the observation that a sizable fraction of extant bacterial proteins contains N-terminal extensions predisposed for mitochondrial targeting (Lucattini, et al. 2004).

While, for some substrates, e. g. cytochrome c (Babbitt, et al. 2015) and c1 (Priest and Hajduk 2003), organism-specific variations in the import pathway exist, the function of the TOM, mediating import across the OM of approximately 1000 different proteins which contain the same conserved targeting signals, is the same in yeast, plants and trypanosomes. The situation in plants however is complicated by the fact that they also

have plastids (Perry, et al. 2008), whose proteins must be excluded from mitochondria. Moreover, there is a large fraction of plant proteins that are dually localized to both organelles (Carrie and Whelan 2013). It has been shown in these cases that in vitro and in vivo import systems do not always faithfully mirror the physiological situation (Hurt, et al. 1986; Lister, et al. 2001; Fuss, et al. 2013).

Thus, since the translocases in yeast, plant and trypanosomatids were largely shaped by the same functional constraints one might expect their composition and the structures of their subunits to be very similar. Surprisingly, this is not the case and a comparative analysis between the TOM complexes of the three species reveals striking differences (fig. 3).

TOM complex architecture

TOM consists of 6-7 subunits which depending on the affinities to each other can be divided into core and peripheral components (Perry, et al. 2008). The core of TOM includes a β-barrel protein and 3-4 tightly associated subunits, some of which are very small. The remaining proteins are more loosely associated with the complex. Whereas in yeast and trypanosomes the TOM subunits are coded for by single copy genes, we often find multiple genes encoding highly similar isoforms of TOM subunits in plants (Lister, et al. 2004) (table 3). Except for one β-barrel membrane protein, all subunits contain a single membrane-spanning α-helix. The molecular weight of the whole complex ranges from 220 kDa in plants to 1000 kDa in *T. brucei.* It is known that all components are present in more than one copy but the exact subunit stoichiometry has not yet been determined for any system. In the next few paragraphs we discuss the different TOM subunits in order of their degree of conservation.

The β-barrel pore

The protein-conducting pore of TOM is formed by a β-barrel protein of approximately 40 kDa that has been identified in the isolated TOM complexes of fungi, plants and trypanosomes (Moczko, et al. 1992; Sollner, et al. 1992; Jansch, et al. 1998; Werhahn, et al. 2001; Mani, et al. 2015). An orthologue of this protein, termed Tom40, can be found in all eukaryotes (table 4, supplementary table S1, Supplementary Material online) and was shown to be essential for life in all species where it has been tested (Maćasev, et al. 2004; Dolezal, et al. 2006; Perry, et al. 2008; Pusnik, et al. 2009; Hewitt, et al. 2011).

Using bioinformatics it was initially not possible to identify a Tom40 orthologue in trypanosomes (Schneider, et al. 2008; Pusnik, et al. 2009) and the β-barrel protein forming the OM import pore was finally discovered by a biochemical approach and termed ATOM40 for archaic translocase of the OM of 40 kDa. BLAST analyses revealed a limited sequence similarity of ATOM40 to a subgroup of a bacterial Omp85-like proteins (Pusnik, et al. 2011). HHPred analyses (Soding, et al. 2005) on the other hand suggested that ATOM40 might be a highly diverged VDAC-like protein (Zarsky, et al. 2012). The main members of this protein family are the protein import pore Tom40 and the metabolite transporter voltage dependent anion channel (VDAC) (Pusnik, et al. 2009), which are found in all eukaryotes. However, while ATOM40 could be grouped into the VDAC-like protein family, it is too diverged to be categorized into a specific subfamily. (See supplementary fig. S2 in (Schnarwiler, et al. 2014)).

Recombinant yeast Tom40 and trypanosomal ATOM40 have been analyzed by electrophysiology using the planar lipid bilayer technique that allows single channel measurements (Hill, et al. 1998; Künkele, et al. 1998; Harsman, et al. 2012). Consistent with their function as protein-conducting pores both proteins form a wide hydrophilic channel that shows selectivity for cations and that can be blocked by addition of a synthetic presequence. However, a more in depth analysis performed in parallel for yeast Tom40 and ATOM40 revealed that recombinant Tom40 inserted as a monomer whose gating behavior is dominated by fast flickering, whereas recombinant ATOM40 was active as a trimer that showed low frequency gating only (Harsman, et al. 2012). Thus, in this respect ATOM40 behaves more similar to chloroplast and bacterial versions of Omp85-like protein import and export channels, rather than to Tom40 of yeast.

The β-barrel nature of Tom40 and ATOM40 indicate their bacterial origin. The fact that both can be grouped into the VDAC-like protein family points to a single evolutionary origin of the protein. However, with which - if any - specific β-barrel protein in extant bacteria it shares common ancestry is unclear at present.

Tom22-like proteins

All TOM complexes isolated so far have a subunit with a cytosolically exposed N-terminus, whose molecular weights range from 10 kD in plants to 18 kDa in yeast. They share homology within and around their single transmembrane domains, suggesting

that they derive from a single common ancestor (Maćasev, et al. 2004) (supplementary fig. S1, Supplementary Material online).

The yeast subunit, termed Tom22, functions as a secondary receptor. It is tightly bound to Tom40 and interacts with the primary receptors Tom20 and Tom70 (see below). In addition to its transmembrane region Tom22 consists of a N-terminal cytosolic and a C-terminal IMS domain, which both contain clusters of acidic amino acids (Kiebler, et al. 1993; Mayer, et al. 1995). Most importantly, independent of the receptor function of Tom22, its transmembrane domain is required for TOM assembly into a 450 kDa complex (Wilpe, et al. 1999). However, despite its dual function yeast lacking Tom22 can grow, albeit very slowly.

The plant orthologue of Tom22 was first identified in isolated TOM from *A. thaliana* and *S. tuberosum* (Jansch, et al. 1998; Werhahn, et al. 2001). Surprisingly the protein, termed Tom9, has a much shorter cytosolic domain, which is basic. The IMS domain of Tom9, on the other hand, retained an excess of acidic residues (Maćasev, et al. 2004). Studies investigating the specific function of plant Tom9 have not been published and it is not known whether the protein is essential.

The purified ATOM complex of *T. brucei* contains a 14 kD protein whose transmembrane domain including flanking regions show similarity to Tom22 and Tom9 when analyzed by HHPred (Soding, et al. 2005) (supplementary fig. S1, Supplementary Material online), although it lacks the conserved proline residue in the transmembrane domain (fig. 3). The protein was termed ATOM14 and is highly conserved in all trypanosomatids. An alignment with yeast Tom22 and plant Tom9 suggests that the cytosolic domain of ATOM14 essentially lacks acidic amino acids and is even shorter than in plant Tom9 (supplementary fig. S1, Supplementary Material online). In contrast, ATOM14 has an IMS domain that is twice as long as yeast Tom22 or plant Tom9. ATOM14 is tightly associated with ATOM40. The protein is essential under all conditions and, as Tom22 in yeast, plays an important role in (A)TOM assembly. In its absence much less of the ATOM complex was formed and ATOM11 and to some extent ATOM69 became unstable (Mani, et al. 2015).

In summary, there are two types of Tom22-like proteins. The ones typified by the yeast protein which have a cluster of acidic residues in the cytosolically exposed N-terminal domain and the ones exemplified by the plant Tom9 that lack this domain and thus generally are shorter. In yeast the acidic cytosolic domain has been implicated in presequence binding whereas in plant Tom9 the corresponding much shorter, non-acidic domain cannot bind presequences (Rimmer, et al. 2011). However, even in yeast

Tom22 the cytosolic acidic residues can be replaced without significantly affecting protein import or cell growth (Nargang, et al. 1998). This, indicates that in yeast presequences may preferentially be bound by hydrophobic interactions or in the case of plants that another protein may compensate for inability of plant Tom9 to bind presequences. It has been shown that also the IMS domain of yeast Tom22 can bind presequences (Kiebler, et al. 1993; Mayer, et al. 1995; Komiya, et al. 1998). The same likely applies for the IMS domain of plant Tom9 as it is able to functionally replace the corresponding domain of the yeast protein (Maćasev, et al. 2004).

Yeast-type Tom22 orthologues with a cytosolic acidic cluster are largely restricted to Opisthokonts (Maćasev, et al. 2004) (table 4). Moreover, the yeast *Saccharomyces castellii* has a plant-type Tom22 orthologue with a short cytosolic domain lacking an acidic cluster. This lack appears to be compensated for by the gain of an acidic cluster in the cytosolic domain of the primary receptor Tom20 (Hulett, et al. 2007).

The most widespread form of Tom22 is of the plant-type. It is not only found in most Archeaplastida but also in Excavates, e. g. ATOM14 in trypanosomatids, and in at least a few representatives of the Stramenopiles and the Alveolates (Maćasev, et al. 2004) (table. 4).

Tom7

The TOM complex of yeast and plants contains a small protein each consisting of 60 and 75 amino acids respectively. These proteins, termed Tom7, show only low sequence similarity but have a conserved sequence motif in their single atypical membrane-spanning domains (Maćasev, et al. 2004). This suggests that yeast and plant Tom7 derive from the same common ancestor. One of the functions of yeast Tom7 is to destabilize TOM possibly to allow the incorporation of new subunits (Hönlinger, et al. 1996; Becker, et al. 2011). This role is antagonistic to yeast Tom6 described below. The function of plant Tom7 has not been investigated yet (table 3).

Bioinformatic searches identified putative Tom7 orthologues in representatives of all eukaryotic supergroups except the Excavates which include the trypanosomatids (Maćasev, et al. 2004)(table. 4).

Tom5 and Tom6 in yeast and plants

Purified TOM of yeast and plants each contain two proteins, of approximately 50 and 60 amino acids in length, termed Tom5 and Tom6, which have a single transmembrane domain each.

Yeast Tom5 is tightly associated with Tom40 and has its N-terminus exposed to the cytosol. It helps to transfer precursor proteins from the receptors to Tom40 and supports TOM biogenesis. Tom6 stabilizes the large TOM complex and thus has an antagonistic function to Tom7 which promotes its disassembly (Dietmeier, et al. 1997; Model, et al. 2001). Neither Tom5 nor Tom6 is essential for yeast. In plants the specific functions of Tom5 and Tom6 have not been investigated.

Interestingly, yeast Tom5 appears to be similar to plant Tom6 and yeast Tom5 to plant Tom6 (supplementary fig. S2, Supplementary Material online). This suggests that the two proteins in yeast and plants share a common evolutionary origin. However, a bioinformatic analysis did not reveal any candidates for orthologues of Tom5 and Tom6 in other supergroups (table. 4). It should be considered though that very small open reading frames are often missed when genomes are annotated.

Small ATOM subunits in trypanosomatids

ATOM from *T. brucei* contains two small subunits with a single membrane spanning domain, termed ATOM11 (100 aa) and ATOM12 (105 aa), which are conserved but specific for Kinetoplastids (Mani, et al. 2015) (table. 4). Both proteins are essential and RNAi-mediated ablation shows that ATOM11 mediates the interaction of the ATOM core complex with the two peripheral subunits ATOM46 and ATOM69 (see below), whereas ATOM12 has an antagonistic function and prevents this association (Mani, et al. 2015). Thus, despite the lack of sequence similarity with any Tom subunit of yeast and plants the function of trypanosomal ATOM11 and ATOM12 seem at least in part analogous to yeast Tom6 and Tom7, respectively.

TOM receptor subunits

The TOM core complex dynamically interacts with more loosely associated subunits that function as primary import receptors. The best studied ones are yeast and mammalian Tom20 and Tom70 (Endo and Kohda 2002). Tom20 is a N-terminally anchored membrane protein whose cytosolically exposed domain contains a single tetratricopeptide repeat (TPR) domain. TPR folds provide a protein-protein interaction

platform, the specificity of which is determined by the variable residues in the conserved TPR motif (Abe, et al. 2000). Tom20 preferentially recognizes soluble precursor proteins carrying N-terminal targeting signals. It binds to the hydrophobic surface of presequences and subsequently transfers the precursors to Tom22 (Söllner, et al. 1989; Ramage, et al. 1993; Saitoh, et al. 2007).

Tom70 is the primary receptor for mitochondrial carrier proteins that have internal targeting sequences and can also bind hydrophobic precursor proteins that carry presequences (Hines, et al. 1990; Steger, et al. 1990; Hines and Schatz 1993). It is N-terminally anchored in the membrane and contains a large cytosolically exposed segment consisting of 11 TPR motifs. The eight TPR motifs distal to the membrane directly recognize substrate proteins (Chan, et al. 2006; Wu and Sha 2006). The three TPR motifs proximal to the membrane form the clamp domain that interacts with the cytosolic chaperone Hsp70 in yeast as well as Hsp90 in mammals from which Tom70 can receive precursors proteins (Hachiya, et al. 1995; Young, et al. 2003).

While Tom20 and Tom70 have a preference for hydrophilic and hydrophobic substrates, respectively, they have in part redundant functions. Yeast can grow and respire in the absence of Tom70. Loss of Tom20 abolished respiration but was not lethal and respiration could be restored by overexpression of Tom70. Finally, deletion of both receptors caused a severe growth phenotype but did not kill the cells provided that the secondary receptor Tom22 was still present (Ramage, et al. 1993; Harkness, et al. 1994; Lithgow, et al. 1994; Moczko, et al. 1994; Yamamoto, et al. 2009).

Tom20 and Tom70 are found in all Opisthokonts. Whereas Tom20 is restricted to this supergroup, putative orthologues of Tom70 were recently discovered in the Stramenopiles of the SAR supergroup. In *Blastocystis* the protein localizes to the mitochondria-related organelle and its cytosolic domain was functional in the context of the yeast protein (Tsaousis, et al. 2011). However, in the Excavates and the Archeaplastidae no Tom70 orthologues could be found (Chan, et al. 2006) (table 4).

The single protein import receptor associated with plant TOM is also termed Tom20 (Heins and Schmitz 1996). Superficially plant and opisthokont Tom20 are very similar, both have a single transmembrane helix and a cytosolic domain containing TPR motifs - the yeast Tom20 has one and the plant protein two - which recognize presequence-containing proteins. Furthermore, both proteins show the same domain organization and share conserved residues in their transmembrane regions, *but* only if their sequences are aligned in an antiparalell way. In other words, yeast Tom20 is signal-anchored, whereas plant Tom20 is anchored to the membrane via its C-terminus (tail-

anchored). It is difficult to imagine genetic mechanisms that during evolution could lead to the sequence reversal that is observed between the two proteins. Therefore plant and yeast Tom20, while being functional analogues, most likely have different evolutionary origins (Lister and Whelan 2006; Perry, et al. 2006).

Besides Tom20 plants have another protein, termed OM64, that likely acts as a receptor for protein import (Chew, et al. 2004). OM64 is N-terminally anchored in the mitochondrial OM but not associated with isolated TOM. Its large cytosolic segment includes an amidase domain flanked by three C-terminal TPR domains, that similar to Tom70 of yeast can bind the cytosolic chaperones Hsp70 and Hsp90 (Panigrahi, et al. 2014). OM64 plays a role in import of at least some mitochondrial proteins in vivo and was shown to interact with a number of precursor proteins in vitro (Lister, et al. 2007). It is a paralogue of Toc64, a protein associated with the OM protein translocase of plastids, that likely functions as a receptor for plastid protein import.

Inactivation of all three active Tom20 genes in *A. thaliana* results in a moderate reduction in growth but is not lethal. OM64 mutants showed only mild phenotypic abnormalities (Lister, et al. 2007). However, if all three Tom20 isoforms and OM64 are knocked out in the same plant an embryo-lethal phenotype is obtained (Duncan, et al. 2013). C-terminally anchored Tom20 is found in the Archeaplastida and within this supergroup appears to be absent in red algae, which also lack a classical Tom20. The phylogenetic distribution of OM64 is more restricted, it is present in most vascular plants but absent in green and red algae, as well as in other lower plant lineages (Carrie, et al. 2010). Thus it is likely that other as yet undiscovered receptors are present in these clades.

ATOM of trypanosomatids contains two receptors, termed ATOM46 and ATOM69, that have large domains exposed to the cytosol (Mani, et al. 2015). ATOM69 is superficially similar to Tom70. Both have the same molecular weight and multiple TPR-like motifs. However, ATOM69 in addition has an N-terminal CS/Hsp20-like domain, which in other proteins was shown to bind Hsp90. Moreover, analogous to yeast and plant Tom20, ATOM69 is tail-anchored whereas Tom70 has a N-terminal membrane anchor. ATOM46 also has an N-terminal membrane anchor and an armadillo (ARM) repeat domain. The ARM motif functions as a protein-protein interaction module, it is specific for eukaryotes and is found in a number of unrelated proteins including soluble nuclear transport receptors (Tewari, et al. 2010). Thus, except for the TPR domain in ATOM69, the two trypanosomal import receptors do not share any similarity to TOM subunits of other species which illustrates their independent evolutionary history. The cytosolic domains

of ATOM69 and ATOM46 were shown to bind a number of different precursor proteins. In these assays ATOM69 showed a preference for presequence-containing substrates and ATOM46 most efficiently bound the hydrophobic MCPs (Mani, et al. 2015).

Ablation of ATOM46 did not cause any growth or import phenotype under standard conditions. Inducible RNAi of ATOM69, however, caused an accumulation of cytosolic precursor proteins that was accompanied by a growth arrest. If both proteins are ablated simultaneously these phenotypes are strongly exacerbated and occur much earlier, suggesting that ATOM69 and ATOM46 are to some extent redundant mitochondrial protein import receptors with distinct but partially overlapping substrate specificities (Mani, et al. 2015).

ATOM69 and ATOM46 have been found in all kinetoplastids including the free-living relative of trypanosomatids *Bodo saltans*, illustrating that the unique features of ATOM are not an adaptation to the parasitic life style of *T. brucei* (table 4).

Implications for mitochondrial evolution

The comparative analysis of yeast, plants and trypanosomes supports a two step model for the evolution of TOM. It posits that a simple version of TOM evolved in the mitochondrial ancestor (Cavalier-Smith 2006; Dolezal, et al. 2006; Perry, et al. 2006). It consisted of the β-barrel import pore that was comandeered from the endosymbiont, and a tightly associated accessory protein of the Tom22/Tom9-type, that acted as primordial receptor recognizing preexisting targeting signals on the imported substrates. This is a plausible scenario since the function of yeast Tom22 as a secondary receptor is well established and yeast lacking both primary receptors are viable provided that Tom22 is still present (Lithgow, et al. 1994). Alternatively, one or more of the present or as yet to be discovered receptors may have been present in the ancestor of all eukaryotes and later been replaced in at least two probably more of the basic eukaryotic lineages.

All TOMs contain a suite of small proteins whose main function is to regulate the assembly and disassembly of the complex. Tom7 is found in a wide range of eukaryotes but not in the Excavates (Maćasev, et al. 2004), whereas Tom5 and Tom6 appear to be present in Opisthokonts and plants only (table 4) (supplementary fig. S2, Supplementary Material Online). The Excavates lack any of these proteins and the ATOM complex of trypanosomatids instead contains the two unrelated small proteins ATOM11 and ATOM12 that are functionally analogous to Tom6 and Tom7, respectively. In

summary this suggests that Tom5, 6 and 7 evolved after the ancestor of the Excavates diverged from all the other eukaryotes, supporting models that place the root of the eukaryotic evolutionary tree at this position (He, et al. 2014).

All three systems have two primary receptors on the surface of the OM (fig. 4). These receptor pairs are functionally equivalent but evolutionary distinct, which is surprising since the signals they recognize are conserved (table 2). They therefore arose by convergent evolution after the fundamental eukaryotes lineages were already established. The occurence of the distinct receptors overlaps but is not congruent with the eukaryotic supergroups.

Yeast-type Tom20 as well as Tom70 are present in all Opisthokonts and in at least some members of its sistergroup the Amoebozoans. However, orthologues of Tom70 also appear to occur in the Stramenopiles (Tsaousis, et al. 2011) (table 4). This is difficult to explain, however the stramenopile Tom70 might have been acquired by horizontal gene transfer from an Opisthokont. Alternatively it might not be a true Tom70 orthologue. Indeed the observed sequence identity between the stramenopile and the opisthokont Tom70s is only weak, which makes it difficult to exclude that it arose by convergent evolution. Not all Archeaplastidae have both Tom20 and OM64, and the red algae lack both proteins (Carrie, et al. 2010) (table 4). ATOM46 and ATOM69 are specific for Kinetoplastids (table 4). This suggests that they are a deep branching clade within the Excavates or, as has been proposed before, that this supergroup has a polyphyletic origin (Cavalier-Smith 2010). In any case we expect that additional as yet unidentified import receptors are present in this group (fig. 4).

Protein import was already operational in the last common ancestor of eukaryotes. However, it appears that only after the establishment of the major eukaryotic lineages the number of imported proteins became so large, probably driven by ongoing EGT, that it required an increase in the specificity and efficiency of the process, that could only be achieved by a pair of dedicated receptor proteins. Mitochondrial protein import is one of the first - if not the first - mitochondria-specific character. The variations that are seen in functionally identical but evolutionary distinct modules of TOM, such as the receptor subunits, therefore likely mirror the early diversification of eukaryotes. Uncovering the diversity of protein import receptors of the mitochondrial OM might therefore help to reveal the early branches of the eukaryotic evolutionary tree.

Biochemical constraints on receptor function

The comparative analysis presented in this review shows that only the β-barrel import pore and the Tom22/Tom9 component of TOM are universally conserved (table 4). A Tom7 orthologue is present in all but one and Tom5 and Tom6 in two supergroups. The small ATOM subunits, on the other hand, evolved independently. This suggests that small proteins that regulate the assembly state of the complex are a basic requirement for a functional TOM.

All three TOMs dynamically interact with protein import receptors which evolved independently. These receptors represent different solutions to the same biological problem, namely the efficient and specific import of 1000 or more different mitochondrial proteins (table 1).

All systems appear to need a pair of receptors that have distinct substrate preferences and some degree of redundancy. Removal of the receptor, that binds the broader range of substrates including presequence-containing proteins, causes stronger effects on protein import and fitness than if the other receptor with a preference for hydrophobic substrates is ablated. Moreover, in all cases ablation of both receptors causes a stronger effect than their individual removal might suggest.

Based on the three known receptor pairs we can identify the overarching structural features of the individual import receptors. Both require a single transmembrane region and an exposed cytosolic domain, however, whether the protein is anchored in a N_{in} - C_{out} or N_{out} – C_{in} orientation is not important. The cytosolic domain of at least one receptor subunit must have a substrate binding domain consisting of multiple TPR motifs and a binding site for cytosolic chaperones such as Hsp70 or Hsp90, that might be based on specialized TPR motifs or possibly on a CS/Hsp20 domain. However, in which order these modules are arranged is not important. The soluble domain of the second receptor also requires a protein-protein interaction domain, which may include a TPR motif or an ARM domain.

There are still many clades, especially in the Excavates and the SAR supergoup, where mitochondrial protein import receptors have not been identified yet (fig. 4). The general features of import receptors defined above, indicate that TPR motif-containing proteins with a predicted N- or C-terminal transmembrane domain are excellent candidates for such proteins. However, the TPR domain is widespread and also found in other receptor-like proteins such as Pex5 and Sec72 (Schlegel, et al. 2007). Confident identification of novel import receptors therefore requires an experimental approach.

While the genomes of many eukaryotes of interest have been sequenced, they are often not easily accessible to biochemical investigations. However, even in such systems it should in many cases be possible: i) to determine the mitochondrial localization of the candidate receptors as well as ii) to test whether they can bind import signals, since such an experiment requires recombinant proteins only.

Thus, we believe that characterizing novel import receptors across the eukaryotic phylogeny is both feasible and rewarding. We expect that the study of the pattern of receptor variation, will shed light on the basic eukaryotic lineages, whereas identifying the shared traits between them will allow to define the fundamental biochemical features mandatory for their function.

Acknowledgments

Research in the groups of C. M. was funded by the Deutsche Forschungsgemeinschaft and the Excellence Initiative of the German Federal & State Governments (EXC 294 BIOSS Centre for Biological Signalling Studies). Research in the lab of A. S. was supported by grant 138355 of the Swiss National Foundation.

References

Abe Y, Shodai T, Muto T, Mihara K, Torii H, Nishikawa S, Endo T, Kohda D. 2000. Structural basis of presequence recognition by the mitochondrial protein import receptor Tom20. Cell 100:551-560.

Adams KL, Palmer JD. 2003. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. Mol. Phylogenet. Evol. 29:380-395.

Adl SM, Simpson AG, Farmer MA, Andersen RA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, et al. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J. Eukaryot. Microbiol 52:399-451.

Alikhani N, Berglund AK, Engmann T, Spanning E, Vögtle FN, Pavlov P, Meisinger C, Langer T, Glaser E. 2011. Targeting capacity and conservation of PreP homologues localization in mitochondria of different species. J Mol Biol 410:400-410.

Allen JF. 2003. The function of genomes in bioenergetic organelles. Philos. Trans. R Soc. Lond. B Biol. Sci. 358:19-37.

Babbitt SE, Sutherland MC, Francisco BS, Mendez DL, Kranz RG. 2015. Mitochondrial cytochrome c biogenesis: no longer an enigma. Trends Biochem Sci 40:446-455.

Becker T, Wenz LS, Thornton N, Stroud D, Meisinger C, Wiedemann N, Pfanner N. 2011. Biogenesis of mitochondria: dual role of Tom7 in modulating assembly of the preprotein translocase of the outer membrane. J Mol Biol 405:113-124.

Bowler C, Alliotte T, vandenBulcke M, Bauw G, Vandekerckhove J, vanMontagu M, Inzé D. 1989. A plant manganese superoxide dismutase is efficiently imported and correctly processed by yeast mitochondria. Proc. Natl. Acad. Sci. USA 86:3237-3241.

Burki F. 2014. The eukaryotic tree of life from a global phylogenomic perspective. Cold Spring Harb Perspect Biol 6:a016147.

Carrie C, Murcha MW, Whelan J. 2010. An in silico analysis of the mitochondrial protein import apparatus of plants. BMC Plant Biol 10:249.

Carrie C, Venne AS, Zahedi RP, Soll J. 2015. Identification of cleavage sites and substrate proteins for two mitochondrial intermediate peptidases in Arabidopsis thaliana. J Exp Bot 66:2691-2708.

Carrie C, Whelan J. 2013. Widespread dual targeting of proteins in land plants: when, where, how and why. Plant Signal Behav 8.

Cavalier-Smith T. 2010. Kingdoms Protozoa and Chromista and the eozoan root of the eukaryotic tree. Biol. Lett. 6:342-345.

Cavalier-Smith T. 2006. Origin of mitochondria by intracellular enslavement of a photosynthetic purple bacterium. Proc Biol Sci 273:1943-1952.

Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N. 2009. Importing mitochondrial proteins: machineries and mechanisms. Cell 138:628-644.

Chan NC, Likić VA, Waller RF, Mulhern TD, Lithgow T. 2006. The C-terminal TPR domain of Tom70 defines a family of mitochondrial protein import receptors found only in animals and fungi. J. Mol. Biol. 358:1010-1022.

Changmai P, Horakova E, Long S, Cernotikova-Stribrna E, McDonald LM, Bontempi EJ, Lukes J. 2013. Both human ferredoxins equally efficiently rescue ferredoxin deficiency in Trypanosoma brucei. Mol Microbiol 89:135-151.

Chaudhuri M, Nargang FE. 2003. Import and assembly of Neurospora crassa Tom40 into mitochondria of Trypanosoma brucei in vivo. Curr Genet 44:85-94.

Chaumont F, O'Riordan V, Boutry M. 1990. Protein transport into mitochondria is conserved between plant and yeast species. J Biol Chem 265:16856-16862.

Chew O, Lister R, Qbadou S, Heazlewood JL, Soll J, Schleiff E, Millar AH, Whelan J. 2004. A plant outer mitochondrial membrane protein with high amino acid sequence identity to a chloroplast protein import receptor. FEBS Lett 557:109-114.

Colasante C, Diaz PP, Clayton C, Voncken F. 2009. Mitochondrial carrier family inventory of *Trypanosoma brucei brucei*: Identification, expression and subcellular localisation. Mol. Biochem. Parasitol. 167:104-117.

Desy S, Schneider A, Mani J. 2012. Trypanosoma brucei has a canonical mitochondrial processing peptidase. Mol Biochem Parasitol 185:161-164.

Dietmeier K, Hönlinger A, Bomer U, Dekker PJ, Eckerskorn C, Lottspeich F, Kubrich M, Pfanner N. 1997. Tom5 functionally links mitochondrial preprotein receptors to the general import pore. Nature 388:195-200.

Dolezal P, Likic V, Tachezy J, Lithgow T. 2006. Evolution of the molecular machines for protein import into mitochondria. Science 313:314-318.

Dolezal P, Smíd O, Rada P, Zubácová Z, Bursać D, Suták R, Nebesárová J, Lithgow T, Tachezy J. 2005. Giardia mitosomes and trichomonad hydrogenosomes share a common mode of protein targeting. Proc. Natl. Acad. Sci. USA 102:10924-10929.

Duncan O, Murcha MW, Whelan J. 2013. Unique components of the plant mitochondrial protein import apparatus. Biochim. Biophys. Acta 1833:304-313.

Eckers E, Cyrklaff M, Simpson L, Deponte M. 2012. Mitochondrial protein import pathways are functionally conserved among eukaryotes despite compositional diversity of the import machineries. Biol Chem 393:513-524.

Endo T, Kohda D. 2002. Functions of outer membrane receptors in mitochondrial protein import. Biochim. Biophys. Acta 1592:3-14.

Fuss J, Liegmann O, Krause K, Rensing SA. 2013. Green targeting predictor and ambiguous targeting predictor 2: the pitfalls of plant protein targeting prediction and of transient protein expression in heterologous systems. New Phytol 200:1022-1033.

Gray MW. 2012. Mitochondrial evolution. Cold Spring Harb. Perspect. Biol. 4:a011403.

Gray MW, Burger G, Lang BF. 1999. Mitochondrial Evolution. Science 283:1476-1481.

Habib SJ, Neupert W, Rapaport D. 2007. Analysis and prediction of mitochondrial targeting signals. Methods Cell Biol 80:761-781.

Hachiya N, Mihara K, Suda K, Horst M, Schatz G, Lithgow T. 1995. Reconstitution of the initial steps of mitochondrial protein import. Nature 376:705-709.

Haferkamp I, Schmitz-Esser S. 2012. The plant mitochondrial carrier family: functional and evolutionary aspects. Front Plant Sci 3:2.

Harkness TA, Nargang FE, van der Klei I, Neupert W, Lill R. 1994. A crucial role of the mitochondrial protein import receptor MOM19 for the biogenesis of mitochondria. J Cell Biol 124:637-648.

Harsman A, Niemann M, Pusnik M, Schmidt O, Burmann BM, Hiller S, Meisinger C, Schneider A, Wagner R. 2012. Bacterial origin of a mitochondrial outer membrane protein translocase: New perspectives from comparative single channel electrophysiology. J. Biol. Chem. 287:31437-31445.

Hashimi H, McDonald L, Stríbrná E, Lukeš J. 2013. Trypanosome Letm1 protein is essential for mitochondrial potassium homeostasis. J. Biol. Chem. 288:26914-26925.

Hauser R, Pypaert M, Häusler T, Horn EK, Schneider A. 1996. In vitro import of proteins into mitochondria of *Trypanosoma brucei* and *Leishmania tarentolae*. J. Cell Sci. 109:517-523.

Häusler T, Stierhof Y-D, Blattner J, Clayton C. 1997. Conservation of mitochondrial targeting sequence function in mitochondrial and hydrogenosomal proteins from the early-branching eukaryotes *Crithidia*, *Trypanosoma* and *Trichomonas*. Eur. J. Cell Biol. 73:240-251.

He D, Fiz-Palacios O, Fu CJ, Fehling J, Tsai CC, Baldauf SL. 2014. An alternative root for the eukaryote tree of life. Curr Biol 24:465-470.

Heins L, Schmitz UK. 1996. A receptor for protein import into potato mitochondria. Plant J 9:829-839.

Heinz E, Lithgow T. 2013. Back to basics: a revealing secondary reduction of the mitochondrial protein import pathway in diverse intracellular parasites. Biochim. Biophys. Acta 1833:295-303.

Herrmann JM, Riemer J. 2012. Mitochondrial disulfide relay: redox-regulated protein import into the intermembrane space. J Biol Chem 287:4426-4433.

Hewitt V, Alcock F, Lithgow T. 2011. Minor modifications and major adaptations: the evolution of molecular machines driving mitochondrial protein import. Biochim. Biophys. Acta. 1808:947-954.

Hill K, Model K, Ryan MT, Dietmeier K, Martin F, Wagner R, Pfanner N. 1998. Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins. Nature 395:516-521.

Hines V, Brandt A, Griffiths G, Horstmann H, Brutsch H, Schatz G. 1990. Protein import into yeast mitochondria is accelerated by the outer membrane protein Mas70. EMBO J 9:3191-3200.

Hines V, Schatz G. 1993. Precusor binding to yeast mitochondria: a general role for the outer membrane protein Mas70p. J. Biol. Chem. 268:449-454.

Hohr AI, Straub SP, Warscheid B, Becker T, Wiedemann N. 2015. Assembly of beta-barrel proteins in the mitochondrial outer membrane. Biochim Biophys Acta 1853:74-88.

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

Huang S, Jacoby RP, Millar AH, Taylor NL. 2014. Plant mitochondrial proteomics. Methods Mol Biol 1072:499-525.

Hulett JM, Walsh P, Lithgow T. 2007. Domain stealing by receptors in a protein transport complex. Mol Biol Evol 24:1909-1911.

Hurt EC, Soltanifar N, Goldschmidt-Clermont M, Rochaix J-D, Schatz G. 1986. The cleavable pre-sequence of an imported chloroplast protein directs attached polypeptides into yeast mitochondria. EMBO 5:1343-1350.

Jansch L, Kruft V, Schmitz UK, Braun HP. 1998. Unique composition of the preprotein translocase of the outer mitochondrial membrane from plants. J Biol Chem 273:17251-17257.

Jedelsky PL, Dolezal P, Rada P, Pyrih J, Smid O, Hrdy I, Sedinova M, Marcincikova M, Voleman L, Perry AJ, et al. 2011. The minimal proteome in the reduced mitochondrion of the parasitic protist Giardia intestinalis. PLoS One 6:e17285.

Kiebler M, Kiel P, Schneider H, Klei IJvd, Pfanner N, Neupert W. 1993. The mitochondrial receptor complex: a central role of MOM22 in mediating preprotein transfer from receptors to the general insertion pore. Cell 74:483-492.

Komiya T, Rospert S, Koehler C, Looser R, Schatz G, Mihara K. 1998. Interaction of mitochondrial targeting signals with acidic receptor domains along the protein import pathway: evidence for the 'acid chain' hypothesis. EMBO 17: 3886-3898.

Koonin EV, Yutin N. 2014. The dispersed archaeal eukaryome and the complex archaeal ancestor of eukaryotes. Cold Spring Harb Perspect Biol 6:a016188.

Künkele K-P, Heins S, Dembowski M, Nargang FE, Benz R, Thieffry M, Walz J, Lill R, Nussberger S, Neupert W. 1998. The preprotein translocation channel of the outer membrane of mitochondria. Cell 93:1009-1019.

Kutik S, Guiard B, Meyer HE, Wiedemann N, Pfanner N. 2007. Cooperation of translocase complexes in mitochondrial protein import. J. Cell. Biol. 179:585-591.

Lane N. 2014. Bioenergetic constraints on the evolution of complex life. Cold Spring Harb Perspect Biol 6:a015982.

Lee CP, Taylor NL, Millar AH. 2013. Recent advances in the composition and heterogeneity of the Arabidopsis mitochondrial proteome. Front Plant Sci 4:4.

Lister R, Carrie C, Duncan O, Ho LH, Howell KA, Murcha MW, Whelan J. 2007. Functional definition of outer membrane proteins involved in preprotein import into mitochondria. Plant Cell 19:3739-3759.

Lister R, Chew O, Lee MN, Heazlewood JL, Clifton R, Parker KL, Millar AH, Whelan J. 2004. A transcriptomic and proteomic characterization of the Arabidopsis mitochondrial protein import apparatus and its response to mitochondrial dysfunction. Plant Physiol 134:777-789.

Lister R, Chew O, Rudhe C, Lee MN, Whelan J. 2001. Arabidopsis thaliana ferrochelatase-I and -II are not imported into Arabidopsis mitochondria. FEBS Lett 506:291-295.

Lister R, Whelan J. 2006. Mitochondrial protein import: convergent solutions for receptor structure. Curr Biol 16:R197-199.

Lithgow T, Junne T, Wachter C, Schatz G. 1994. Yeast mitochondria lacking the two import receptors Mas20p and Mas70p can efficiently and specifically import precursor proteins. J. Biol. Chem. 269:15325-15330.

Lithgow T, Schneider A. 2010. Evolution of macromolecular import pathways in mitochondria, hydrogenosomes and mitosomes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365:799-817.

Long S, Changmai P, Tsaousis AD, Skalicky T, Verner Z, Wen YZ, Roger AJ, Lukes J. 2011. Stage-specific requirement for Isa1 and Isa2 proteins in the mitochondrion of Trypanosoma brucei and heterologous rescue by human and Blastocystis orthologues. Mol Microbiol 81:1403-1418.

Long S, Jirku M, Ayala FJ, Lukes J. 2008. Mitochondrial localization of human frataxin is necessary but processing is not for rescuing frataxin deficiency in Trypanosoma brucei. Proc Natl Acad Sci U S A 105:13468-13473.

Long S, Vavrova Z, Lukes J. 2008. The import and function of diatom and plant frataxins in the mitochondrion of Trypanosoma brucei. Mol Biochem Parasitol 162:100-104.

Lucattini R, Likic VA, Lithgow T. 2004. Bacterial proteins predisposed for targeting to mitochondria. Mol Biol Evol 21:652-658.

Luzikov VN, Novikova LA, Whelan J, Hugosson M, Glaser E. 1994. Import of the mammalian cytochrome P450 (scc) precursor into plant mitochondria. Biochem Biophys Res Commun 199:33-36.

Maćasev D, Whelan J, Newbigin E, Silva-Filho MC, Mulhern TD, Lithgow T. 2004. Tom22', an 8-kDa trans-site receptor in plants and protozoans, is a conserved feature of the TOM complex that appeared early in the evolution of eukaryotes. Mol. Biol. Evol. 21:1557-1564.

Mani J, Desy S, Niemann M, Chanfon A, Oeljeklaus S, Pusnik M, Schmidt O, Gerbeth C, Meisinger C, Warscheid B, et al. 2015. Novel mitochondrial protein import receptors in Kinetoplastids reveal convergent evolution over large phylogenetic distances. Nature Commun. 6:6646.

Mayer A, Nargang FE, Neupert W, Lill R. 1995. MOM22 is a receptor for mitochondrial targeting sequences and cooperates with MOM19. EMBO J 14:4204-4211.

Meisinger C, Sickmann A, Pfanner N. 2008. The mitochondrial proteome: from inventory to function. Cell 134:22-24.

Moczko M, Dietmeier K, Sollner T, Segui B, Steger HF, Neupert W, Pfanner N. 1992. Identification of the mitochondrial receptor complex in Saccharomyces cerevisiae. FEBS Lett 310:265-268.

Moczko M, Ehmann B, Gärtner F, Hönlinger A, Schafer E, Pfanner N. 1994. Deletion of the receptor MOM19 strongly impairs import of cleavable preproteins into Saccharomyces cerevisiae mitochondria. J Biol Chem 269:9045-9051.

Model K, Meisinger C, Prinz T, Wiedemann N, Truscott KN, Pfanner N, Ryan MT. 2001. Multistep assembly of the protein import channel of the mitochondrial outer membrane. Nat Struct Biol 8:361-370.

Mossmann D, Meisinger C, Vögtle FN. 2012. Processing of mitochondrial presequences. Biochim. Biophys. Acta 1819:1098-1106.

Murcha MW, Kmiec B, Kubiszewski-Jakubiak S, Teixeira PF, Glaser E, Whelan J. 2014. Protein import into plant mitochondria: signals, machinery, processing, and regulation. J Exp Bot 65:6301-6335.

Nargang FE, Rapaport D, Ritzel RG, Neupert W, Lill R. 1998. Role of the negative charges in the cytosolic domain of TOM22 in the import of precursor proteins into mitochondria. Mol Cell Biol 18:3173-3181.

Niemann M, Wiese S, Mani J, Chanfon A, Jackson C, Meisinger C, Warscheid B, Schneider A. 2013. Mitochondrial outer membrane proteome of *Trypanosoma brucei* reveals novel factors required to maintain mitochondrial morphology. Mol. Cell. Proteomics 12:515-528.

Palmieri L, Palmieri F, Runswick MJ, Walker JE. 1996. Identification by bacterial expression and functional reconstitution of the yeast genomic sequence encoding the mitochondrial dicarboxylate carrier protein. FEBS Lett 399:299-302.

Panigrahi R, Whelan J, Vrielink A. 2014. Exploring ligand recognition, selectivity and dynamics of TPR domains of chloroplast Toc64 and mitochondria Om64 from Arabidopsis thaliana. J Mol Recognit 27:402-414.

Perry AJ, Hulett JM, Likić VA, Lithgow T, Gooley PR. 2006. Convergent evolution of receptors for protein import into mitochondria. Curr. Biol. 16:221-229.

Perry AJ, Rimmer KA, Mertens HD, Waller RF, Mulhern TD, Lithgow T, Gooley PR. (A-22 co-authors). 2008. Structure, topology and function of the translocase of the outer membrane of mitochondria. Plant Physiol Biochem 46:265-274.

Priest JW, Hajduk SL. 2003. Trypanosoma brucei cytochrome c1 is imported into mitochondria along an unusual pathway. J Biol Chem 278:15084-15094.

Pusnik M, Charrière F, Mäser P, Waller RF, Dagley MJ, Lithgow T, Schneider A. 2009. The single mitochondrial porin of *Trypanosoma brucei* is the main metabolite transporter in the outer mitochondrial membrane. Mol. Biol. Evol. 26:671-680.

Pusnik M, Schmidt O, Perry AJ, Oeljeklaus S, Niemann M, Warscheid B, Lithgow T, Meisinger C, Schneider A. 2011. Mitochondrial preprotein translocase of trypanosomatids has a bacterial origin. Curr. Biol. 21:1738-1743.

Ramage L, Junne T, Hahne K, Lithgow T, Schatz G. 1993. Functional cooperation of mitochondrial protein import receptors. EMBO J. 12:4115-4123.

Rimmer KA, Foo JH, Ng A, Petrie EJ, Shilling PJ, Perry AJ, Mertens HD, Lithgow T, Mulhern TD, Gooley PR. 2011. Recognition of mitochondrial targeting sequences by the import receptors Tom20 and Tom22. J Mol Biol 405:804-818.

Saitoh T, Igura M, Obita T, Ose T, Kojima R, Maenaka K, Endo T, Kohda D. 2007. Tom20 recognizes mitochondrial presequences through dynamic equilibrium among multiple bound states EMBO J. 26:4777-4788.

Schlegel T, Mirus O, von Haeseler A, Schleiff E. 2007. The tetratricopeptide repeats of receptors involved in protein translocation across membranes. Mol Biol Evol 24:2763-2774.

Schmidt O, Pfanner N, Meisinger C. 2010. Mitochondrial protein import: from proteomics to functional mechanisms. Nat. Rev. Mol. Cell. Biol. 11:655-667.

Schmitz UK, Lonsdale DM. 1989. A yeast mitochondrial presequence functions as a signal for targeting to plant mitochondria in vivo. Plant Cell 1:783-791.

Schnarwiler F, Niemann M, Doiron N, Harsman A, Kaser S, Mani J, Chanfon A, Dewar CE, Oeljeklaus S, Jackson CB, et al. 2014. Trypanosomal TAC40 constitutes a novel subclass of mitochondrial beta-barrel proteins specialized in mitochondrial genome inheritance. Proc Natl Acad Sci U S A 111:7624-7629.

Schneider A, Behrens M, Scherer P, Pratje E, Michaelis G, Schatz G. (340 co-authors). 1991. Inner membrane protease I, an enzyme mediating intramitochondrial protein sorting in yeast. EMBO J. 10:247-254.

Schneider A, Bursać D, Lithgow T. 2008. The direct route: a simplified pathway for protein import into the mitochondrion of trypanosomes. Trends Cell Biol. 18:12-18.

Schulz C, Schendzielorz A, Rehling P. 2015. Unlocking the presequence import pathway. Trends Cell Biol 25:265-275.

Smíd O, Matusková A, Harris SR, Kucera T, Novotný M, Horváthová L, Hrdý I, Kutejová E, Hirt RP, Embley TM, et al. 2008. Reductive evolution of the mitochondrial processing peptidases of the unicellular parasites trichomonas vaginalis and giardia intestinalis. PLoS Pathog. 4:e1000243.

Soding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Res 33:W244-248.

Söllner T, Griffiths G, Pfaller R, Pfanner N, Neupert W. 1989. MOM19, an import receptor for mitochondrial precursor proteins. Cell 59:1061-1070.

Sollner T, Rassow J, Wiedmann M, Schlossmann J, Keil P, Neupert W, Pfanner N. 1992. Mapping of the protein import machinery in the mitochondrial outer membrane by crosslinking of translocation intermediates. Nature 355:84-87.

Spang A, Saw JH, Jorgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, Schleper C, Guy L, Ettema TJ. 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521:173-179.

Steger HF, Sollner T, Kiebler M, Dietmeier KA, Pfaller R, Trulzsch KS, Tropschug M, Neupert W, Pfanner N. 1990. Import of ADP/ATP carrier into mitochondria: two receptors act in parallel. J Cell Biol 111:2353-2363.

Teixeira PF, Glaser E. 2013. Processing peptidases in mitochondria and chloroplasts. Biochim Biophys Acta 1833:360-370.

Tewari R, Bailes E, Bunting KA, Coates JC. 2010. Armadillo-repeat protein functions: questions for little creatures. Trends Cell Biol 20:470-481.

Thorsness PE, Weber ER. 1996. Escape and migration of nucleic acids between chloroplasts, mitochondria, and the nucleus. Int Rev Cytol 165:207-234.

Timmis JN, Ayliffe MA, Huang CY, Martin W. 2004. Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. Nat Rev Genet 5:123-135.

Tsaousis AD, Gaston D, Stechmann A, Walker PB, Lithgow T, Roger AJ. 2011. A functional Tom70 in the human parasite Blastocystis sp.: implications for the evolution of the mitochondrial import apparatus. Mol. Biol. Evol. 28:781-791.

Ulrich T, Gross LE, Sommer MS, Schleiff E, Rapaport D. 2012. Chloroplast beta-barrel proteins are assembled into the mitochondrial outer membrane in a process that depends on the TOM and TOB complexes. J Biol Chem 287:27467-27479.

Ulrich T, Oberhettinger P, Schutz M, Holzer K, Ramms AS, Linke D, Autenrieth IB, Rapaport D. 2014. Evolutionary conservation in biogenesis of beta-barrel proteins allows mitochondria to assemble a functional bacterial trimeric autotransporter protein. J Biol Chem 289:29457-29470.

van Wilpe S, Boumans H, Lobo-Hajdu G, Grivell LA, Berden JA. 1999. Functional complementation analysis of yeast bc1 mutants. A study of the mitochondrial import of heterologous and hybrid proteins. Eur J Biochem 264:825-832.

Vögtle FN, Wortelkamp S, Zahedi RP, Becker D, Leidhold C, Gevaert K, Kellermann J, Voos W, Sickmann A, Pfanner N, et al. 2009. Global analysis of the mitochondrial N-proteome identifies a processing peptidase critical for protein stability. Cell 139:428-439.

Weems E, Singha UK, Hamilton V, Smith JT, Waegemann K, Mokranjac D, Chaudhuri M. 2015. Functional Complementation Analyses Reveal that the Single PRAT-Family Protein of Trypanosoma brucei is a Divergent Homolog of Tim17 in Saccharomyces cerevisiae. Eukaryot Cell 14:286-296.

Werhahn W, Niemeyer A, Jänsch L, Kruft V, Schmitz UK, Braun H. 2001. Purification and characterization of the preprotein translocase of the outer mitochondrial membrane from Arabidopsis. Identification of multiple forms of TOM20. Plant Physiol. 125:943-954.

Williams TA, Foster PG, Cox CJ, Embley TM. 2013. An archaeal origin of eukaryotes supports only two primary domains of life. Nature 504:231-236.

Wilpe Sv, Ryan MT, Hill K, Maarse AC, Meisinger C, Brix J, Dekker PJ, Moczko M, Wagner R, Meijer M, et al. 1999. Tom22 is a multifunctional organizer of the mitochondrial preprotein translocase. Nature 401:485-489.

Wojtkowska M, Buczek D, Stobienia O, Karachitos A, Antoniewicz M, Slocinska M, Makalowski W, Kmita H. 2015. The TOM Complex of Amoebozoans: the Cases of the Amoeba Acanthamoeba castellanii and the Slime Mold Dictyostelium discoideum. Protist 166:349-362.

Wu Y, Sha B. 2006. Crystal structure of yeast mitochondrial outer membrane translocon member Tom70p. Nat Struct Mol Biol 13:589-593.

Yamamoto H, Fukui K, Takahashi H, Kitamura S, Shiota T, Terao K, Uchida M, Esaki M, Nishikawa S, Yoshihisa T, et al. 2009. Roles of Tom70 in import of presequence-containing mitochondrial proteins. J. Biol. Chem. 284:31635-31646.

Young JC, Hoogenraad NJ, Hartl FU. 2003. Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70. Cell 112:41-50.

Zarsky V, Tachezy J, Dolezal P. 2012. Tom40 is likely common to all mitochondria. Curr Biol. 22:R479-R481.

Table 1. Imported proteins and their (predicted) targeting signals

	***Species* (supergroup)**			
	***S. cerevisiae* (O)**	***A. thaliana* (A)**	***T. brucei* (E)**	***Giardia*[a] (E)**
No. of mitochondrial proteins	ca. 1000	ca. 1000	ca. 1000	50-100
No. of presequence-containing proteins	ca. 600[b]	ca. 690[b]	ca. 620[c]	some have presequences, many do not
Average length of presequences (aa)	25-30	ca. 50	25-30 (some are much shorter)[d]	ND
Features of presequences	amphiphilic helix	amphiphilic helix (serine rich)	amphiphilic helix	ND
Presequence processing peptidases	α-MPP/β-MPP Icp55 Oct1 Imp1/Imp2 Cym1 Prd1	α-MPP/β-MPP[e] Icp55 Oct1 Imp1/imp2 Cym1 Prd1	α-MPP/β-MPP Icp55 Oct1 Imp1/Imp2 Cym1 Prd1	β-MPP - - - - -
No. of carrier proteins	35	58	24	0
Import signals of carrier proteins	internal sequences	internal sequences, some have presequences	internal sequences	-
No. of β-barrel proteins	5	7	5	1
Import signals of β-barrel proteins[f]	β-signal	β-signal	β-signal	ND
No. of small TIM chaperones	4	5	5	ND
Import signals of small TIM chaperones	internal Cys-containing peptide	internal Cys-containing peptide	internal Cys-containing peptide	ND

ND, no data

[a] *Giardia* is included in this analysis as an example for an organism with mitosomes which underwent extensive reductive evolution. The data listed in this column are from (Dolezal, et al. 2005; Smíd, et al. 2008; Jedelsky, et al. 2011).
[b] Experimentally determined (Vögtle, et al. 2009; Lee, et al. 2013; Huang, et al. 2014).
[c] Bioinformatic prediction.
[d] See (Häusler, et al. 1997).
[e] The MPP subunits of most plants are identical to the core1 and core 2 subunits of complex III of the respiratory chain (Mossmann, et al. 2012).
[f] The β-signal in yeast is recognized by SAM subunit Sam35 (Kutik, et al. 2007)

Table 2. Mitochondrial proteins are correctly localized in heterologous systems[a)]

Species[b)]	Import substrate[c)]	Species[d)]	Super-group[e)]	Localization[f)]	Import pathway[g)]	Experimental evidence[h)]	Reference
S. cerevisiae	Sam50	*L. tarentolae*	E	OM	β-barrel	vitro	(Eckers, et al. 2012)
	Oep24	*P. sativum*	A-Plas	OM	β-barrel	vitro	(Ulrich, et al. 2012)
	Oep37	*P. sativum*	A-Plas	OM	β-barrel	vivo/vitro	(Ulrich, et al. 2012)
	YadA	*Y. enterocolitica*	Bact	OM	β-barrel	vivo/vitro	(Ulrich, et al. 2014)
	Erv1	*L. tarentolae*	E	IMS	?	vitro	(Eckers, et al. 2012)
	Tim1	*L. tarentolae*	E	IMS	?	vitro	(Eckers, et al. 2012)
	Tim17	*T. brucei*	E	IM	carrier	vivo/vitro	(Eckers, et al. 2012; Weems, et al. 2015)
	F1β	*N. plumbaginifolia*	A	IM	preseq	vitro	(Chaumont, et al. 1990)
	LipDH(1-14)[9)]	*T. brucei*	E	Matrix	preseq	vivo/vitro	(Hauser, et al. 1996; Häusler, et al. 1997)
	Hsp60	*L. tarentolae*	E	Matrix	preseq	vitro	(Eckers, et al. 2012)
	MnSOD	*N. plumbaginifolia*	A	Matrix	preseq	vivo/comp	(Bowler, et al. 1989)
	AtPreP	*A. thaliana*	A	Matrix	preseq	vivo/comp	(Alikhani, et al. 2011)
T. brucei	Tom40	*S. cerevisiae*	O	OM	β-barrel	vivo	(Chaudhuri and Nargang 2003)
	VDAC	*N. crassa*	O	OM	β-barrel	vitro	(Eckers, et al. 2012)
	Tim9	*S. cerevisiae*	O	IMS	MIA	vitro	(Eckers, et al. 2012)
	ACC	*S. cerevisiae*	O	IM	carrier	vitro	(Eckers, et al. 2012)
	Tim17	*S. cerevisiae*	O	IM	carrier	vivo/comp	(Weems, et al. 2015)
	Tim23	*S. cerevisiae*	O	IM	carrier	vivo	(Weems, et al. 2015)
	Cox4	*S. cerevisiae*	O	IM	preseq	vivo	(Häusler, et al. 1997)
	Letm1	*H. sapiens*	O	IM	preseq	vivo/comp	(Hashimi, et al. 2013)
	Frataxin	*A. thaliana*	A	IM	preseq	vivo/comp	(Long, Vavrova, et al. 2008)
	Frataxin	*H. sapiens*	O	IM	preseq	vivo/comp	(Long, Jirku, et al. 2008; Long, Vavrova, et al. 2008)
	Frataxin	*T. pseudonana*	S	IM	preseq	vivo/comp	(Long, Vavrova, et al. 2008)
	Ferredoxin	*H. sapiens*	O	IM	preseq	vivo/comp	(Changmai, et al. 2013)
	Su9(1-69)[i)]	*S. cerevisiae*	O	IM	preseq	vitro	(Eckers, et al. 2012)
	Dld1(1-72)[i)]	*S. cerevisiae*	O	IM	preseq	vitro	(Eckers, et al. 2012)
	Isa1	*H. sapiens*	O	Matrix	preseq	vivo/comp	(Long, et al. 2011)
	Isa2	*H. sapiens*	O	Matrix	preseq	vivo/comp	(Long, et al. 2011)
	Adh3	*S. cerevisiae*	O	Matrix	preseq	vitro	(Hauser, et al. 1996)
Plants	CytP450	*H. sapiens*	O	IM	preseq	vitro	(Luzikov, et al. 1994)
	F1β	*S. cerevisiae*	O	IM	preseq	vitro	(Chaumont, et al. 1990)
	F1β	*S. pombe*	O	IM	preseq	vitro	(Schmitz and Lonsdale 1989; Chaumont, et al. 1990)
	MSW	*S. cerevisiae*	O	Matrix	preseq	vivo	(Schmitz and Lonsdale 1989)

Footnotes Table 2

[a)] The table presents examples for mitochondrial targeting of heterologous substrates. It is not comprehensive.
[b)] System in which the heterologous substrates were imported.
[c)] Import substrates tested in the heterologous systems.
[d)] Origin of heterologous substrates.
[e)] Supergroups to which the system belongs: Opisthokonts (O), Excavata (E), Archaeplastida (A), SAR (S). For *S. cerevisiae* two heterologous plastid (plas) and one bacterial (bact) β-barrel protein are listed as well.
[f)] Localization of substrate in the system of origin.
[g)] Import pathway as indicated in fig. 2 in system of origin. Preseq, presequence.
[h)] Vivo, protein localizes to mitochondria in vivo; vitro, protein can be imported into isolated mitochondria; comp, imported protein complements function.
[i)] Dihydrofolatereductase fusions were tested.

Table 3. Composition of TOM and function of its subunits in yeast. plants and trypanosomes

	S. cerevisiae TOM (450 kDa)[a)]			*A. thaliana* TOM (230 kDa)[b)]			*T. brucei* ATOM (450-1000 kDa)[c)]		
	Subunit	**Function**	**Essen-tial**	**Subunit**	**Function**	**Essen-tial**	**Subunit**	**Function**	**Essen-tial**
core complex	Tom40	-translocation channel	yes	Tom40-1 Tom40-2	-translocation channel	yes	ATOM40	-translocation channel	yes
	Tom22	-secondary receptor -TOM organizer	no	Tom9-1 Tom9-2	ND	ND ND	ATOM14	-stabilizes ATOM complex	yes
small subunits	Tom7	-destabilizes TOM complex	no	Tom7-1 Tom7-2	ND	ND	ATOM12	-destabilizes the association of core with ATOM46/69	yes
	Tom6	-stabilizes the TOM complex	no	Tom6	ND	ND	ATOM11	-promotes assembly of core with ATOM46/69	yes
	Tom5	-promotes TOM complex assembly -substrate transfer for Tom22 to Tom40	no	Tom5	ND	ND			
peripheral subunits	Tom20	-receptor for precursors with a presequence	no	Tom20-1 Tom20-2 Tom20-3	-receptor for precursors with a presequence	no no no	ATOM69	-receptor for all precursor proteins	yes
	Tom70	-receptor for hydrophobic precursor proteins	no	mtOM64	-putative receptor for a subset of proteins	no	ATOM46	-receptor for all precursors (preference for hydrophobic proteins) -mediates interaction of core complex with ATOM69	no
	Tom71	-low abundance Tom70 orthologue	no						

ND, no data

[a)] Data from (Chacinska, et al. 2009)
[b)] Data from (Murcha, et al. 2014)
[c)] Data from (Mani, et al. 2015)

Table 4. TOM subunits in representatives of all eukaryotic supergroups[a)]

				Tom40	Tom22 yeast -type	Tom22 plant -type	Tom5	Tom6	Tom7	Tom70	Tom20 yeast -type	Tom20 plant -type	OM64	ATOM11	ATOM12	ATOM69	ATOM46
Supergroup	Ophistokonta	Fungi	*S. cerevisiae*	+	+	-	+	+	+	+	+	-	-	-	-	-	-
		Metazoa	*H. sapiens*	+	+	-	+	+	+	+	+	-	-	-	-	-	-
		Choanoflagellates	*M. brevicollis*	+	-	+	+	-	-	+	+	-	-	-	-	-	-
	Amoebozoa	Dictyostelia	*D. discoideum*	+	+[b)]	-	-	-	+	+	+[b)]	-	-	-	-	-	-
		Discosea	*A. castellanii*	+	+[b)]	-	-	-	+	+[b)]	+[b)]	-	-	-	-	-	-
	Archaeplastida	Plants	*A. thaliana*	+	-	+	+[c)]	+[d)]	+	-	-	+	+	-	-	-	-
			O. sativa	+	-	+	+[c)]	+[d)]	+	-	-	+	+	-	-	-	-
			P. patens	+	-	+	+[c)]	+[d)]	+	-	-	+	+	-	-	-	-
		Red algae	*C. merolae*	+	-	+	-	-	-	-	-	-	-	-	-	-	-
		Green algae	*C. variabilis*	+	-	+	-	-	+	-	-	+	-	-	-	-	-
			C. reinhardtii	+	-	-	+[c)]	-	+	-	-	+	-	-	-	-	-
	Excavata	Kinetoplastids	*T. brucei*	+	-	+[e)]	-	-	-	-	-	-	-	+	+	+	+
			B. saltans	+	-	-	-	-	-	-	-	-	-	+	+	+	+
		Heterolobosea	*N. gruberi*	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	SAR	Stramenopiles	*E. siliculosus*	+	-	+	-	-	+	+	+[f)]	-		-	-	-	-
			B. hominis[g)]	+	-	+	-	-	+	+	-	-	-	-	-	-	-
			P. tricornutum	+	-	+	-	-	+[h)]	+	-	-	-	-	-	-	-
		Alveolata	*P. falciparum*	+	-	+	-	-	-	+	-	-	-	-	-	-	-
			T. thermophila	+	-	+	-	-	+	-	-	-	-	-	-	-	-
		incertae sedis	*G. theta*	+	-	+	-	-	+	-	-	+	-	-	-	-	-

a) For accession numbers and other details see supplementary table S1, Supplementary Materials Online.
b) (Wojtkowska, et al. 2015) We find no bioinformatic support for these proteins to be orthologues of Tom22, Tom70 or Tom20, respectively.
c) HHPred and hmmscan analysis suggests that annotated plant Tom5 proteins are in fact orthologues of yeast Tom6.
d) HHPred and hmmscan analysis suggests that annotated plant Tom6 proteins are in fact orthologues of yeast Tom5.
e) (Mani, et al. 2015)The Tom22 orthologues in trypanosomes is termed ATOM14.
f) Annotated as Tom20 in UniProt. We find no bioinformatic support for this protein to be a Tom20 orthologue.
g) With the exception of *B. hominis,* which harbors mitochondrion-related organelles (MROs), our analysis was restricted to organisms with *bona fide* mitochondria, capable to perform oxidative phosphorylation.
h) (Maćasev, et al. 2004)

Legends

Figure 1. Eukaryotic phylogeny. Unrooted phylogenetic tree of eukaryotes based on genetic and morphological evidences resolves the five supergroups Opisthokonta, Amoebozoa, Excavata, Archaeplastida and SAR (Stramenopiles, Alveolates and Rhizaria), shown in different colours, that diverged very early during eukaryotic evolution. The grey lines indicate that a number of organisms could not yet be confidently associated with any of the supergroups. Within the different supergroups the clades are indicated that are discussed in this review regardless of their taxonomic rank. Essentially all popular model organisms (human, mouse, fungi, worms, flies, yeast etc.) belong to the Opisthokonts. Branch lengths are arbitrary and the branching pattern does not necessarily represent the inferred relationships between the lineages.

Figure 2. Mitochondrial protein import pathways in yeast. Essentially all mitochondrial proteins are imported across the heterooligomeric translocase of the outer mitochondrial membrane (TOM). After OM translocation the import pathways diverge depending on the class of proteins. Presequence-containing proteins (red) are handed over to the translocase of the mitochondrial inner membrane 23 (TIM23) by which they are either laterally released into to IM, in the case of α-helically anchored IM proteins, or pulled into the matrix by the presequence associated motor (PAM) module for soluble proteins. In both cases the presequence gets processed by the heterodimeric mitochondrial processing peptidase (MPP). The hydrophobic mitochondrial carrier proteins (green) associate with the small TIM chaperones in the intermembrane space (IMS) and subsequently are inserted into the IM by TIM22. Small IMS-localized proteins (blue) with cysteine-rich signals are retained in the IMS by the formation of disulfide bonds catalyzed by the mitochondrial intermembrane space assembly (MIA)-pathway. The hydrophobic β-barrel proteins (orange) interact with the small TIM chaperones in the IMS and are then inserted into the OM by the sorting and assembly machinery (SAM) of the OM.

Figure 3. Translocase of the outer mitochondrial membrane (TOM) and its subunits in yeast, plants and trypanosomes. A, model of TOM in the indicated systems. Conserved subunits are shown in the same colour. Organism-specific components are depicted in grey. The topology of the Tom22-orthologues (Tom22/Tom9/ATOM14) and the two peripheral receptor subunits (light grey) is

indicated. Plant OM64 is not stably associated with TOM but likely has a receptor function. B, to scale representation of the domain structure of the TOM subunits in the indicated systems. Membrane anchors, functional domains and conserved prolines in the transmembrane domains are indicated.

Figure 4. Diversity of mitochondrial protein import receptors mapped on a schematic eukaryotic phylogenetic tree. Only clades that are discussed in this review are indicated regardless of their taxonomic rank. Opisthopkont Tom20 and Tom70 are shown in blue. A number of stramenopiles appear to have a Tom70 orthologue (indicated in blue and by broken lines), although convergent evolution cannot be excluded (see text for discussion). Plant Tom20 and OM64 are indicated in green. At least a few green algae have Tom20 but lack OM64. Kinetoplastid ATOM46 and ATOM69 are indicated in red. Expected novel receptor and/or receptor pairs that have not been identified yet are indicated in grey with a question mark.

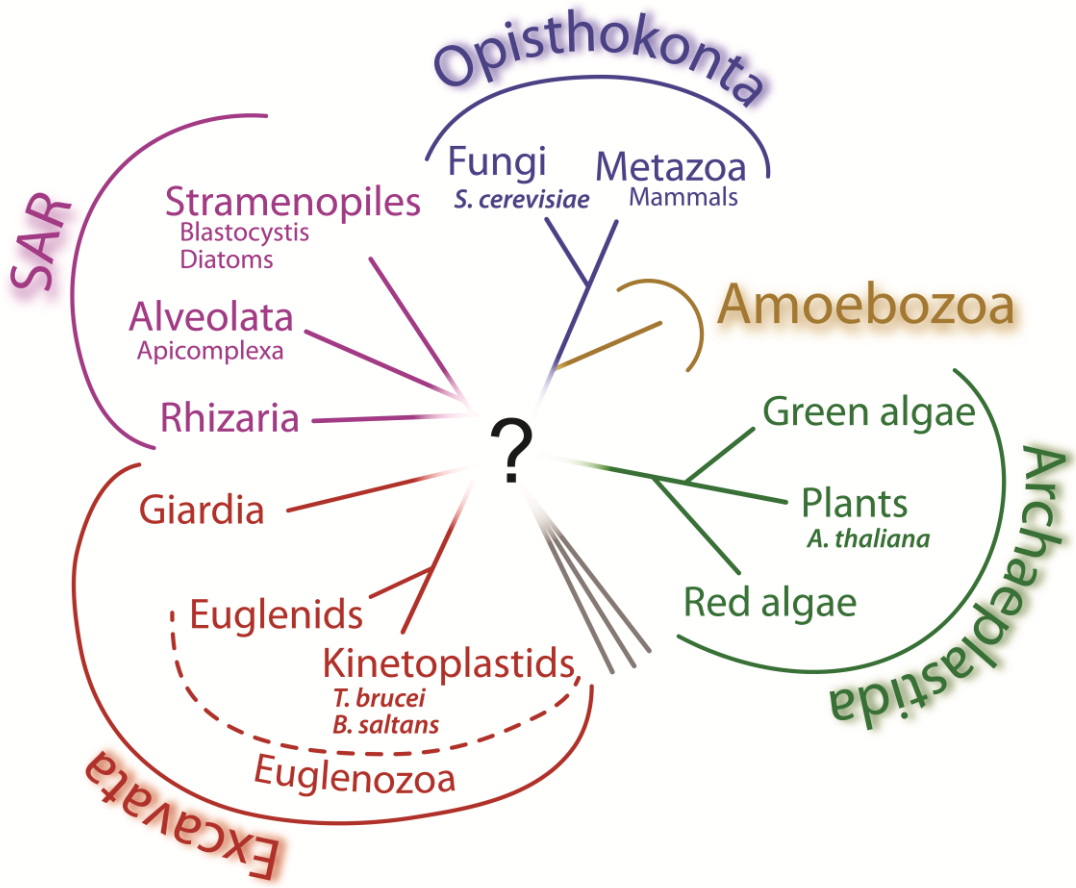
Opisthokonta
Fungi
S. cerevisiae
Metazoa
Mammals
SAR
Stramenopiles
Blastocystis
Diatoms
Alveolata
Apicomplexa
Rhizaria
Amoebozoa
?
Green algae
Plants
A. thaliana
Red algae
Archaeplastida
Giardia
Euglenids
Kinetoplastids
T. brucei
B. saltans
Euglenozoa
Excavata

Figure 1

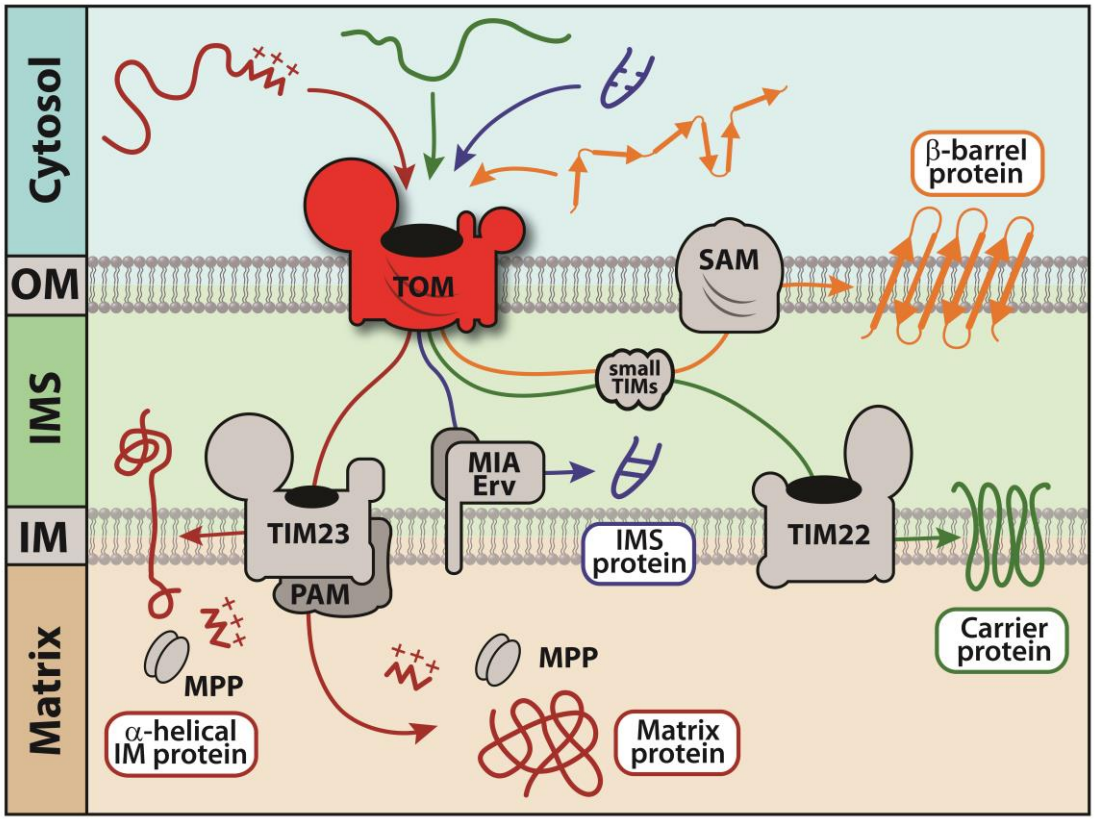
Cytosol
OM
IMS
IM
Matrix
TOM
SAM
β-barrel protein
small TIMs
MIA Erv
IMS protein
TIM23
PAM
TIM22
Carrier protein
MPP
α-helical IM protein
MPP
Matrix protein

Figure 2

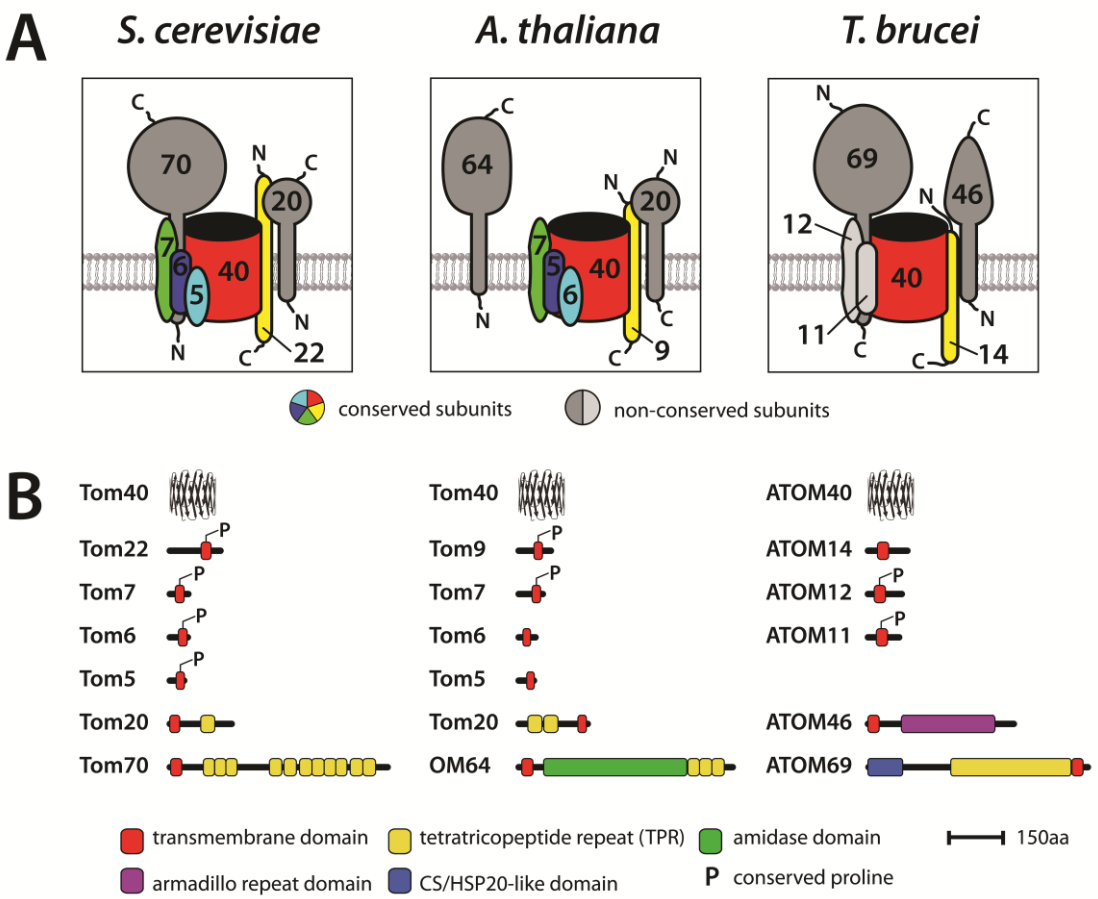
A
S. cerevisiae
A. thaliana
T. brucei
70
20
40
7
6
5
22
64
9
69
46
12
11
14
conserved subunits
non-conserved subunits
B
Tom40
Tom22
Tom7
Tom6
Tom5
Tom20
Tom70
Tom9
OM64
ATOM40
ATOM14
ATOM12
ATOM11
ATOM46
ATOM69
P
transmembrane domain
tetratricopeptide repeat (TPR)
amidase domain
150aa
armadillo repeat domain
CS/HSP20-like domain
conserved proline

Figure 3

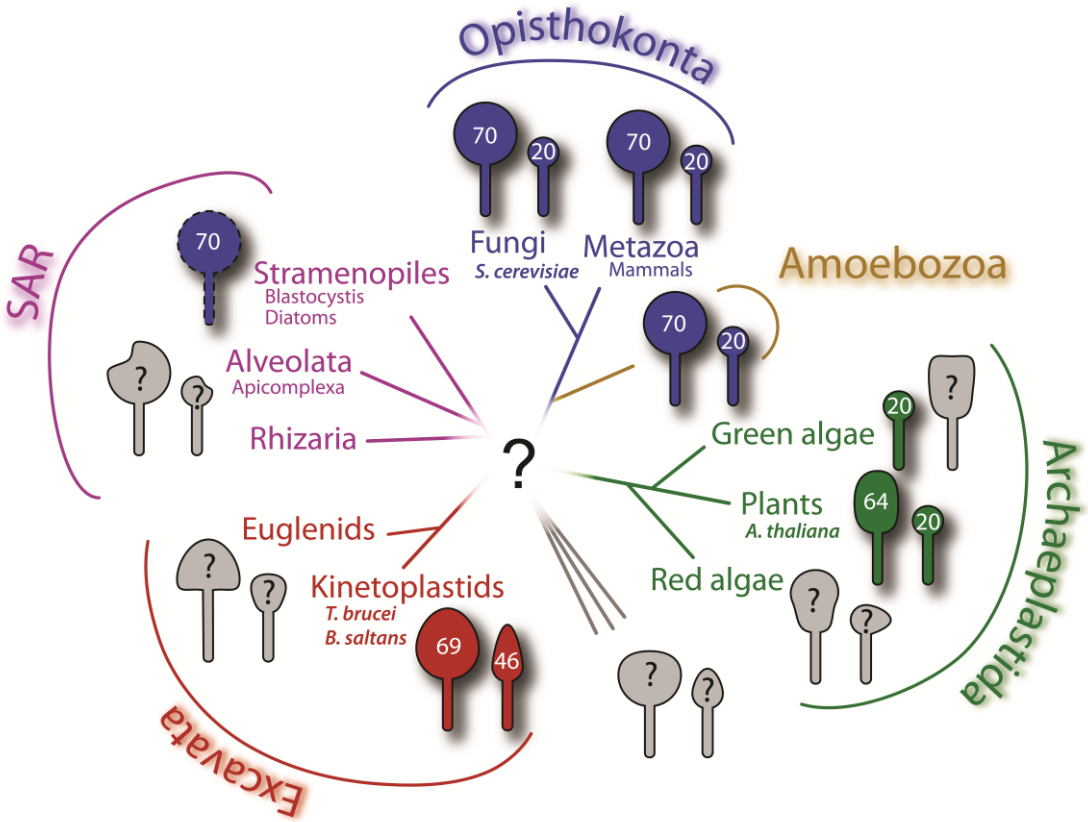
Opisthokonta
70
20
70
20
Fungi
S. cerevisiae
Metazoa
Mammals
Amoebozoa
70
20
SAR
70
Stramenopiles
Blastocystis
Diatoms
Alveolata
Apicomplexa
Rhizaria
?
Green algae
20
Plants
A. thaliana
64
20
Red algae
Archaeplastida
Euglenids
Kinetoplastids
T. brucei
B. saltans
69
46
Excavata

Figure 4