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Evolution and diversification of mitochondrial protein import systems

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Abstract

More than 95% of mitochondrial proteins are encoded in the nucleus, synthesised in the cytosol and imported into the organelle. The evolution of mitochondrial protein import systems was therefore a prerequisite for the conversion of the α-proteobacterial mitochondrial ancestor into an organelle. Here, I review that the origin of the mitochondrial outer membrane import receptors can best be understood by convergent evolution. Subsequently, I discuss an evolutionary scenario that was proposed to explain the diversification of the inner membrane carrier protein translocases between yeast and mammals. Finally, I illustrate a scenario that can explain how the two specialised inner membrane protein translocase complexes found in most eukaryotes were reduced to a single multifunctional one in trypanosomes.

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Introduction

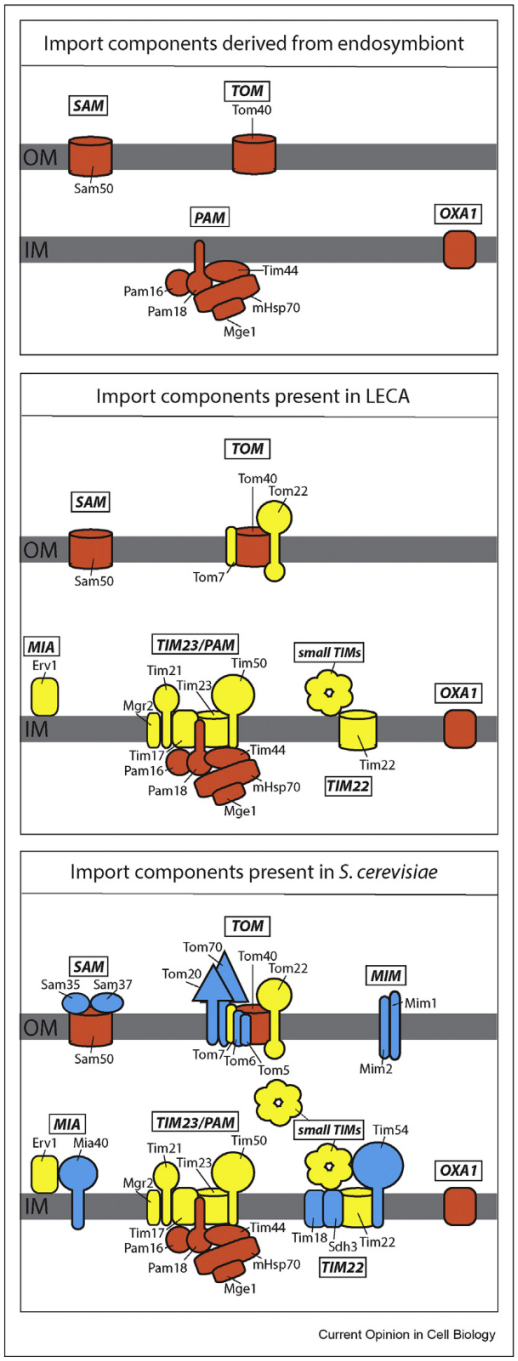
The origin of eukaryotes more than 1.5 billion years ago marks the most important transition in biology, allowing the evolution of complex life. A key event in that transition, and possibly the driving force behind it, was an endosymbiotic event where an α-proteobacterium was taken up by an archaeal host cell and converted into the mitochondrion [1–4]. During this process, much of the endosymbiont's genome was either lost or transferred to the host cell. In order for the symbiont to survive, it had to evolve machineries that allowed it to import proteins [5–7]. The evolution of the mitochondrial protein import systems is therefore central for the understanding of organellogenesis. Figure 1 shows which components of the import systems were likely commandeered from the α-proteobacterial ancestor of the mitochondrion, which of their components were present in the last eukaryotic common ancestor (LECA) as determined by phylogenetic analysis [8] and how these systems are composed in *Saccharomyces cerevisiae* where they have been most extensively studied [9]. Exhaustive reviews on the evolution of mitochondrial protein import systems already exist [8,10–13]. The focus here is on recent advances in the understanding of how these systems diversified in different eukaryotic lineages. I will discuss (i) the origin of mitochondrial protein import receptors of the outer membrane (OM), (ii) the accretion of subunits to the TIM22 complex of yeast and mammals and (iii) the evolution of a single multifunctional TIM complex in trypanosomes.

Convergent evolution of mitochondrial protein import receptors

Surprisingly, the TOM complex in LECA lacked primary protein import receptors (Figure 1), indicating that binding of substrates to the secondary receptor Tom22 and/or directly to the Tom40 import channel provided sufficient specificity for protein import [8,10]. Subsequently, different receptor pairs evolved in different eukaryotic lineages resulting Tom20/Tom70 in yeast, Tom20/OM64 in plants and ATOM46/ATOM69 in trypanosomes, respectively (Figure 2).

Numerous mainly *in vitro* studies established that yeast Tom20 preferentially recognises presequence-containing proteins, whereas typical substrates for Tom70 are mitochondrial carrier proteins (MCPs) [14–20]. However, a recent proteome-wide *in vivo* analysis indicates that import of many more proteins, including some presequence-containing ones, also depends on Tom70 [21*,22]. Moreover, in trypanosomes, a similar study determined to which extent the receptors ATOM46 and ATOM69 [23] contribute to *in vivo* import of which groups of substrates [24*]. The conclusion for both systems is that import of many proteins depends on various extent on both receptors. However, while the receptors are not strictly substrate-specific, they do have substrate preferences. Yeast Tom20 and ATOM46 prefer presequence-containing hydrophilic proteins, whereas yeast Tom70 and ATOM69 prefer presequence-lacking hydrophobic

Figure 1



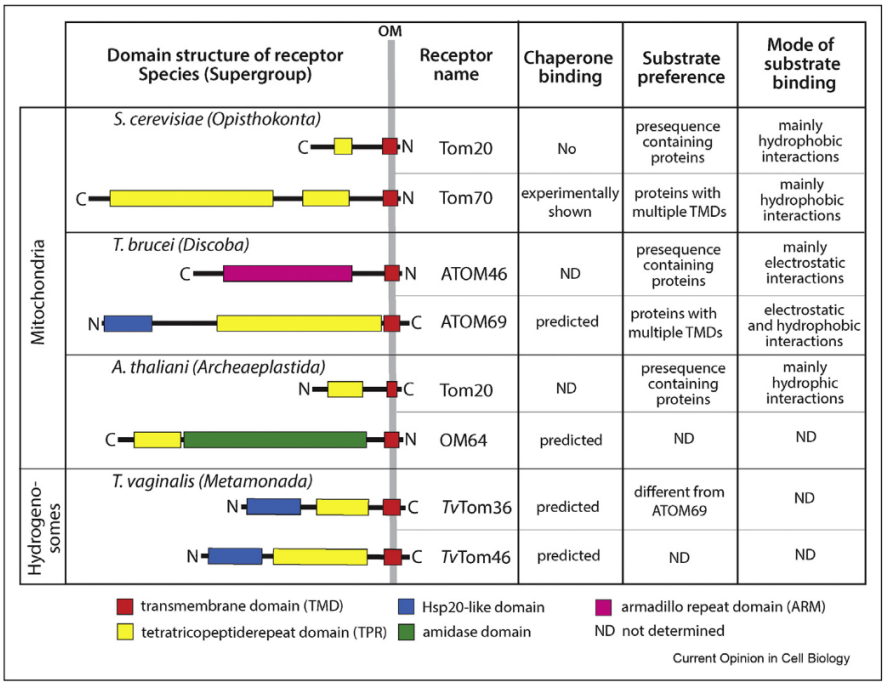
Evolutionary origin of the subunits of mitochondrial protein import systems. The top panel depicts the components of the indicated import systems (red) that have bacterial orthologues and therefore likely were commandeered from the α-proteobacterial ancestor of the mitochondrion. Tom40 is a special case, its β-barrel structure points to a bacterial origin, even though no specific bacterial orthologue could be identified. However, more recently, it has been suggested that Tom40 and related proteins evolved within eukaryotes by multiple duplications from a double ββ hairpin of bacterial origin [52]. For key publications on the evolution of SAM, PAM and OXA complexes, see Refs. [53–55]. The middle panel shows the components of the protein import systems (yellow) that were already present in LECA as determined by phylogenetic analyses [8]. The bottom panel depicts the components of the import systems of *S. cerevisiae*. The subunits that evolved after divergence of eukaryotes into different lineages are shown in blue.

membrane proteins. For the plant receptor pair, Tom20 and OM64, less data are available, but the situation appears to be similar [14,25]. Thus, yeast Tom20, plant Tom20 and ATOM46 likely have similar functions even though they show different domain structures. Yeast Tom20 resembles plant Tom20, but the latter has an inverse topology [26] (Figure 2), and the trypanosomal ATOM46 has multiple armadillo repeat domains (ARMs) not found in the other receptors [23]. The situation is mirrored for Tom70, OM64 and ATOM69 which have different domain structures but appear to have similar substrate preferences. Tom70 and ATOM69 have multiple TPR repeats but inverse topologies [23] (Figure 2). OM64 has the same topology than Tom70 but in addition to TPR repeats also contains an amidase domain [27]. Interestingly, the three receptors appear to be able to bind to cytosolic Hsp70 and/or Hsp90, Tom70 and OM64 through their TPR repeats and ATOM69 with its Hsp20-like domain [22,25,28]. Thus, the receptor pairs are products of convergent evolution as they likely have the same function in all systems (Figure 2).

For Tom20 and ATOM46, substrate binding has been analysed *in vitro*, and it could be shown that while their substrate preference is the same, the way they recognise their substrates is not. Tom20 binds its substrates mainly by hydrophobic interactions [29], whereas in the case of ATOM46, electrostatic interactions appear to be dominant [24*]. Recently, *Tv*Tom36 and *Tv*Tom46 receptors that mediate protein import into the mitochondria-derived hydrogenosomes of *Trichomonas* have been characterised [30**]. Their domain structure resembles ATOM69, as they have an N-terminal Hsp20-like domain that is followed by TPR repeats and a C-terminal transmembrane domain. However, bioinformatic analysis indicates they evolved independently from ATOM69 [30**]. Moreover, complementation experiments in *Trypanosoma brucei* suggest *Tv*Tom36 has a different substrate specificity than ATOM69 [24*].

In summary, it appears that mitochondrial protein import requires two receptors with preferences for more hydrophilic and more hydrophobic proteins, respectively, and that the latter needs the capability to bind cytosolic chaperones (Figure 2). The receptor pairs evolved independently in different eukaryotic groups often using the same toolkit of preexisting TPR repeats or other domains. The repeated evolution of at least four, but probably many more, receptor pairs with the same substrate preferences but different structures strongly suggest they confer an adaptive advantage to

Figure 2



Convergent evolution of protein import receptors. Comparative analysis of the indicated mitochondrial and hydrogenosomal and protein import receptors. The eukaryotic supergroup is indicated in parentheses. Representation of the topology and domain structure of the four receptor pairs is shown drawn to scale. The mitochondrial OM is indicated. All four receptor pairs evolved independently. Tom20 of *S. cerevisiae* and Tom20 of *A. thaliana* are not phylogenetically related even though they share the same name. References: Tom20 [14–16,20]/Tom70 [17–20,28]; ATOM46 [24*]/ATOM69 [24*]; Tom20 [14]/OM64 [25]; *Tv*Tom36 [24*,30**]/*Tv*Tom46 [30**].

their respective systems. However, what this advantage might be is difficult to understand. It is likely not connected to the complexity of the system since receptor pairs are found in both unicellular and multicellular systems. It would be an attractive idea that the necessity for receptor pairs with different substrate preferences correlates with the number of imported proteins. However, this does not fit with the prediction that the number of imported proteins in the ancestral mitochondria of LECA that lacked dedicated receptors (Figure 1) was already very high [31,32].

Diversification of TIM22 complexes by constructive neutral evolution

The TIM22 complex functions in insertion and assembly of MCPs as well as other IM proteins containing multiple transmembrane domains [33,34]. Its core subunit is Tim22 of the Tim17/Tim22/Tim23 protein family [35] which likely functions an insertase. Tim22 and a small Tim hexamer, consisting of the intermembrane space chaperones Tim9 and Tim10a, are highly conserved and likely were present in LECA (Figure 3) [36**].

The TIM22 complexes have been studied in detail in yeast and mammals by biochemical methods, and more recently, Cryo EM analyses have determined their structures at high resolution [37**,38**]. These studies have shown that both TIM22 complexes are tightly associated with a unique small Tim hexamer which contains three copies of Tim9 and two copies of Tim10a and is completed by one copy of Tim12 in yeast and one copy of Tim10b in mammals. Moreover, the structure of mammalian TIM22 in addition contains a second small Tim hexamer of the ancestral type. Tim12 and Tim10b mediate interactions of the hexamers with the other TIM22 subunits in their respective systems. Bioinformatic analyses suggest that Tim12 evolved by duplication of Tim10a at the base of the fungal group and Tim10b by duplication from Tim9 early in the metazoans (Figure 3) [36**].

The yeast TIM22 complex contains Tim54 [33] and the human one acyl glycerol kinase (AGK) [39,40] as further subunits. Initially, it was thought that the two proteins are unrelated; however, sensitive homology searches using Tim54 hidden Markov model profiles (HMMer) retrieved animal AGKs [36**]. Moreover, the structure of Tim54 contains a four-stranded β-sheet sandwiched by four α-helizes [38**]. Such motifs are found in lipid kinases including AGK and indicate that the two