

# Fates of Sec, Tat, and YidC Translocases in Mitochondria and Other Eukaryotic Compartments

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Associate editor: Yoko Satta

## Abstract

Formation of mitochondria by the conversion of a bacterial endosymbiont was a key moment in the evolution of eukaryotes. It was made possible by outsourcing the endosymbiont's genetic control to the host nucleus, while developing the import machinery for proteins synthesized on cytosolic ribosomes. The original protein export machines of the nascent organelle remained to be repurposed or were completely abandoned. This review follows the evolutionary fates of three prokaryotic inner membrane translocases Sec, Tat, and YidC. Homologs of all three translocases can still be found in current mitochondria, but with different importance for mitochondrial function. Although the mitochondrial YidC homolog, Oxa1, became an omnipresent independent insertase, the other two remained only sporadically present in mitochondria. Only a single substrate is known for the mitochondrial Tat and no function has yet been assigned for the mitochondrial Sec. Finally, this review compares these ancestral mitochondrial proteins with their paralogs operating in the plastids and the endomembrane system.

**Key words:** eukaryogenesis, membrane trafficking, protein targeting, neofunctionalization.

## Introduction

The mitochondrion evolved from the endosymbiont (Portier 1918; Sagan 1967; Yang et al. 1985) of  $\alpha$ -proteobacterial (Lang et al. 1997; Andersson et al. 1998) or other ancestry (Martijn et al. 2018). The transformation of the endosymbiont into a core eukaryotic organelle involved a multitude of steps, reshaping its genome and proteome and integrating the mitochondrion into eukaryote-specific pathways (Gray 2015). From the organellés perspective, the evolutionary path from the endosymbiont has been paved by gene losses deepening the mitochondria's dependence on the host cell (McCutcheon 2016; Husnik and Keeling 2019). This gradual process has been slow and, rightfully, mitochondria can be regarded as the oldest endosymbionts existing now (McCutcheon 2016). Yet, the consequences were bidirectional and the host cell had to invest in the integration of the new compartment (Gabaldón and Huynen 2007). The key step was the evolution of mitochondrial protein import pathways (translocases) that enabled the nucleus-encoded proteins to cross the two organellar membranes (Ernster and Schatz 1981; Cavalier-Smith and Lee 1985).

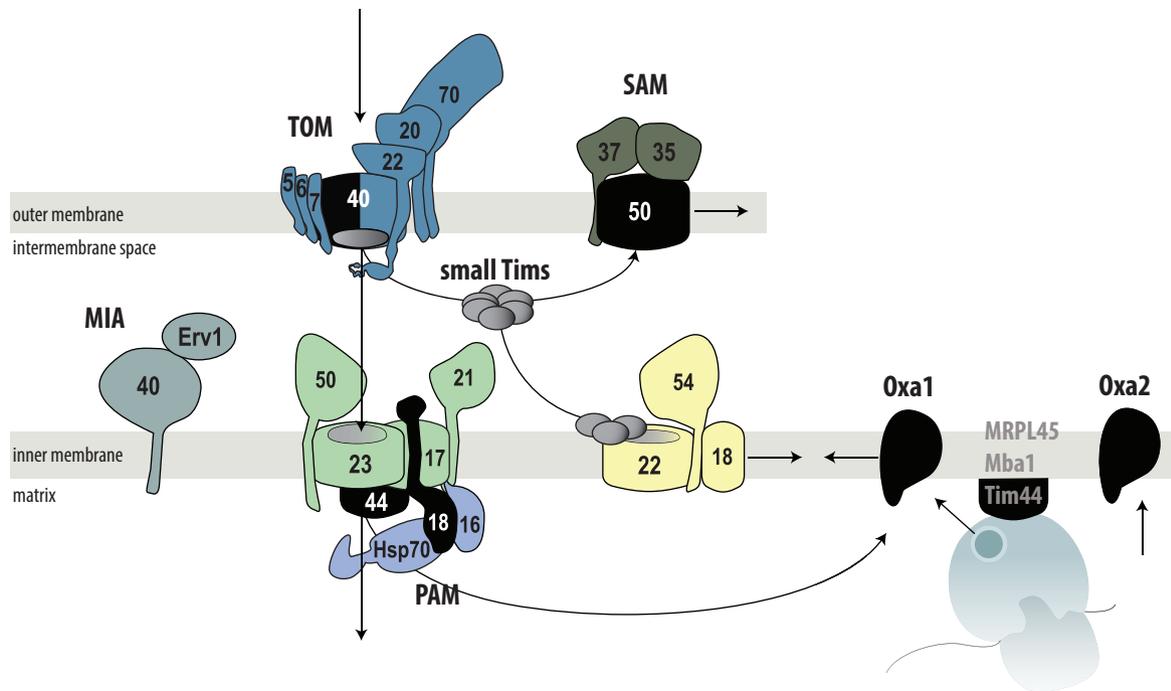
The formation of mitochondria was accompanied by the loss of genes from the endosymbiont/mitochondrial genome, fixation of some them in the host nucleus, and the installation of new mitochondrial functions driven by the host. This all led to the loss of importance of endosymbiont's/mitochondrial translation and the secretion of the proteins. Our knowledge of protein secretion in current free-living and symbiotic bacteria (Hempel et al. 2009; Poueymiro and Genin 2009;

Gillespie et al. 2015) suggests that the endosymbiont transported over a thousand proteins across or into its inner (cytoplasmic) membrane using three main protein translocases, Sec, YidC, and Tat. Other tens to hundreds of proteins were exported further across the outer membrane into the host cell cytoplasm via a number of other secretion systems (Costa et al. 2015).

Only a fraction of proteins are still encoded in the mitochondrial genome (Gray et al. 2004), some of which follow the "outward" transport pathways, whereas over a thousand proteins (Panigrahi et al. 2009; Calvo et al. 2016; Morgenstern et al. 2017) arrive from the cytosol at the receptors of the outer mitochondrial membrane for their "inward" import into the organelle (Wiedemann and Pfanner 2017).

Currently available genomic and functional data suggest that eukaryotes have been rather inventive when redesigning the endosymbiont's membranes for protein translocation. Hence, mitochondrial protein import relies on molecular machines mostly unknown to prokaryotes (fig. 1).

The main entry gate to mitochondria is formed by the translocase of the outer membrane (TOM) complex, built around Tom40, a  $\beta$ -barrel protein of the eukaryotic porin family. Tom40 allows unfolded polypeptides to enter the intermembrane space (IMS) (Hill et al. 1998), whereas the surrounding receptors determine its specificity toward mitochondrial proteins (Perry et al. 2006; Endo and Yamano 2010; Mani et al. 2016; Fukasawa et al. 2017; Doležal et al. 2019; Makki et al. 2019; Rout et al. 2021). All membrane  $\beta$ -barrel proteins occupy the outer mitochondrial membrane and require the specialized sorting



**Fig. 1.** Mitochondrial protein import and the evolutionary links to the bacterial components. (The best characterized system of *Saccharomyces cerevisiae* mitochondria is shown). TOM, SAM, MIA, and TIM complexes in the mitochondrial membranes are molecular machines of mostly eukaryotic origin. Only several components were found to have clear bacterial homologs (in black) such as Sam50, Pam18, Tim44, and Oxa1 and Oxa2. Despite the  $\beta$ -barrel structure, typical for bacterial outer membrane proteins, no clear bacterial homolog of Tom40 has been found yet. The function of Tim44 paralog (MRPL45, Mba1) in binding the mitoribosome to the inner mitochondrial membrane is shown.

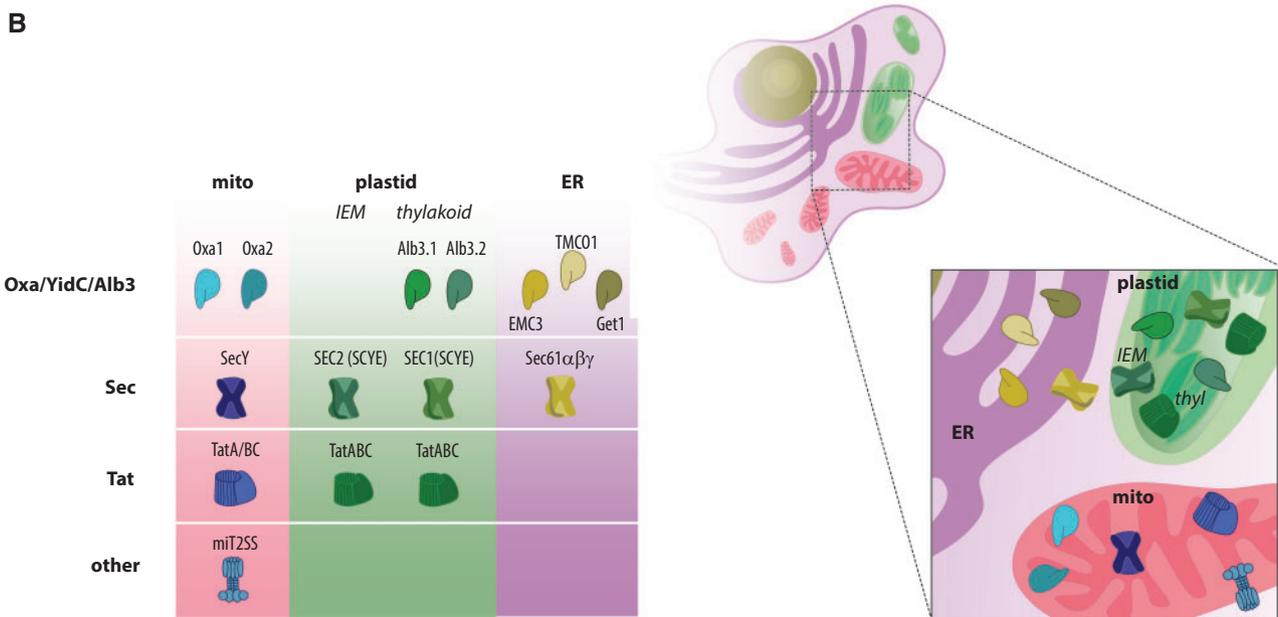
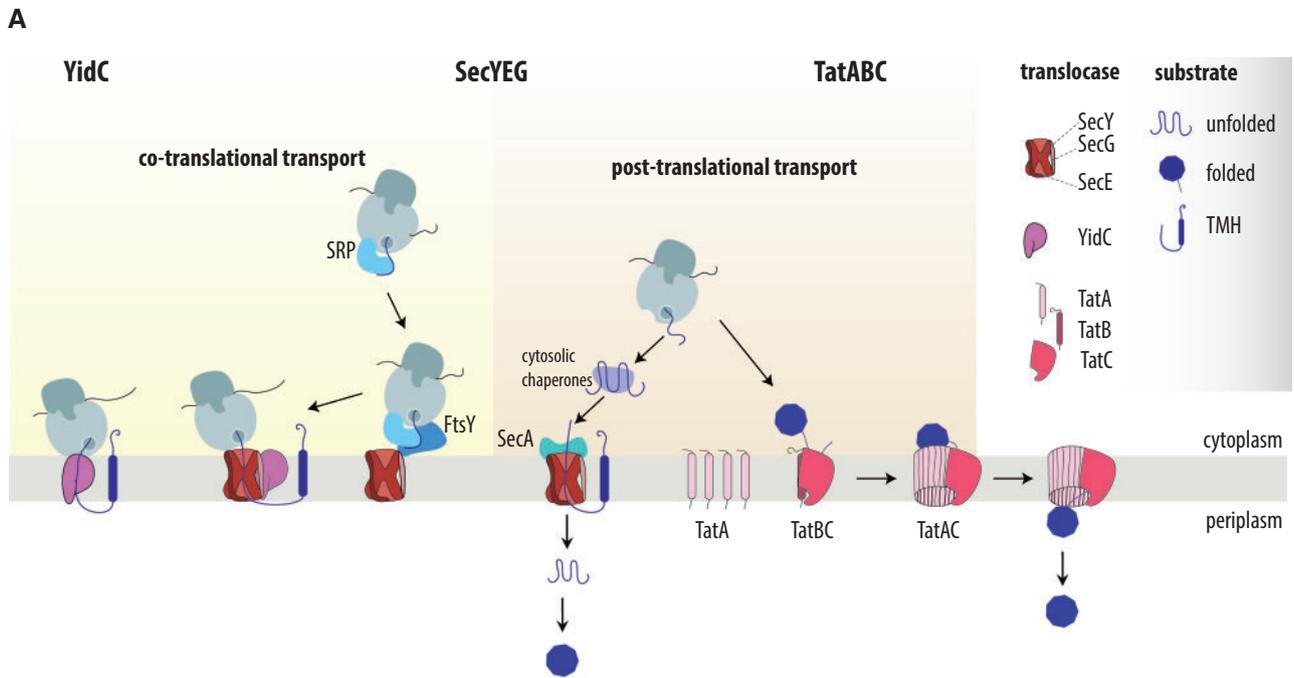
and assembly machine (SAM) complex for their proper placement and assembly (Kozjak et al. 2003). At the center of the SAM complex is Sam50, a protein orthologous to bacterial BamA that mediates the identical function in the outer membranes of Gram-negative bacteria as part of the BAM complex (Gentle et al. 2004). Both proteins share the same topology and hence the directionality of protein insertion from the IMS/periplasm (fig. 1) (Dolezal et al. 2006).

Most proteins occupying the IMS require Mia40 and Erv1, which constitute a disulfide relay system that traps translocated proteins via the formation of intramolecular disulfide bonds (Chacinska et al. 2004; Rissler et al. 2005). These include the small Tims, a group of paralogous proteins chaperoning hydrophobic proteins through the aqueous environment of the IMS either to the SAM complex or to the inner membrane translocase (Wiedemann and Pfanner 2017). The highly impermeable inner membrane posed the greatest membrane barrier for the incoming proteins. Eukaryotes overcame this by developing the TIM22 and TIM23 translocases specific for inner membrane and matrix proteins, respectively, which are built around proteins of the Tim17 protein family (Meinecke et al. 2006; Alder et al. 2008; Žárský and Doležal 2016; Mokranjac 2020). The activity of both translocases requires membrane potential, and in the case of TIM23, ATP hydrolysis by the associated motor complex (PAM) is also required to fully import polypeptides into the matrix (Wiedemann and Pfanner 2017).

Neither of the TIM complexes installed in the inner mitochondrial membrane took advantage of pre-existing bacterial translocases, as evidenced by the absence of TIM component orthologs in prokaryotes (Žárský and Doležal 2016). Yet, on the other hand, the subunits of all three core bacterial translocases Sec, YidC, and Tat remain preserved in eukaryotes (Burger et al. 2013). This review highlights the fate of these three protein transport machines upon the formation of mitochondria. It summarizes their role in extant mitochondria and discusses the reasons behind the successful integration of YidC and highly limited roles of mitochondrial Tat and Sec. Finally, it compares the integration of these prokaryotic translocases in mitochondria with the situation in plastids and the endoplasmic reticulum (ER).

## Export of Proteins from Bacteria

About one third of the bacterial proteome is exported from the cytoplasm to be either integrated into the bacterial membranes or released to the periplasm and the extracellular space (Tsirigotaki et al. 2017). Three molecular machines (Sec, YidC, and Tat) facilitate such immense protein translocation in bacteria (fig. 2A). They are functionally specialized to effect the transport of substrate proteins ranging from unfolded polypeptides of polytopic membrane transporters (Driessen and Nouwen 2008) to folded holoproteins of secreted enzymes (Palmer and Berks 2012).



**Fig. 2.** Prokaryotic protein translocases in the cytoplasmic membrane and their eukaryotic homologs. (A) Schematic representation of the translocation pathways across the cytoplasmic (inner) membrane of bacteria. Three protein translocases Sec, YidC, and Tat mediate transport across or into the membrane. The cotranslational pathway serves for the insertion of the inner membrane proteins. It is initiated by the ribosome-bound SRP, which binds to the Sec complex-bound SRP receptor (FtsY). When the preprotein-loaded ribosome docks onto SecYEG, SRP–FtsY complex dissociates and the transport through SecY channel occurs. Membrane proteins are released to the membrane with or without the contribution of YidC translocase. YidC translocase can also work independently. The posttranslational pathway is responsible for transporting the proteins to the periplasm. The unfolded substrate is transferred to SecA, which drives the passage through the SecYEG channel by ATP hydrolysis. Cytosolic chaperones (e.g., SecB, Trigger Factor) contribute to keep the preprotein in a translocation-competent state. The folded or multisubunit substrates are recognized by TatBC receptor complex. The conformational change induced by the signal peptide binding displaces TatB for TatA which oligomerizes to form a transient translocation pore. TMH, transmembrane helix. (B) Eukaryotic homologs of prokaryotic translocases can be found in the membranes of mitochondria, plastids, and the ER. Mito, mitochondrion; ER, endoplasmic reticulum; IEM, inner envelope membrane; thyl, thylakoid membrane.

## Sec—The Primary Bacterial Protein Transport Machine

Sec is by far the most fundamental bacterial protein translocase as it secures the export of most of the exported polypeptides, for example, 96% of such proteins in *Escherichia coli* (Orfanoudaki and Economou 2014). These polypeptides are marked either by N-terminal cleavable signal peptides (Blobel and Dobberstein 1975) or by noncleavable internal sequences (Smets et al. 2019). The Sec pathway is centered around the SecYEG protein-conducting channel, which allows unfolded polypeptides to traverse the inner membrane or to remain anchored in it (Natale et al. 2008) (fig. 2A). The three membrane subunits SecY, SecE, and SecG (fig. 2A) were identified as *prl* (protein localization) or *sec* (secretion) genes in genetic screens in *E. coli* (Bieker et al. 1990; Schatz and Beckwith 1990), and soon after, the SecYEG complex was shown to constitute the actual translocase in vitro (Akimaru et al. 1991). The hourglass-shaped channel of SecYEG is formed by ten transmembrane helices of SecY, where two funnel-like cavities create an aqueous environment and connect the cytoplasmic and the external space (van den Berg et al. 2004; Cannon et al. 2005). Importantly, SecY also forms a lateral gate through which hydrophobic helices of translocated transmembrane polypeptides can be released into the membrane (van den Berg et al. 2004). The SecE subunit embraces and stabilizes the channel on a site opposite to the lateral gate, whereas the function of SecG remains uncertain (Belin et al. 2015). Correspondingly, the orthologs of SecY and SecE show high sequence similarity among prokaryotes and they are always essential (Denks et al. 2014), whereas SecG proteins are more variable and dispensable (Nishiyama et al. 1994).

Moreover, structural analyses suggest that the heterotrimeric complex itself is sufficient to maintain translocation, even within larger oligomeric assemblies (van den Berg et al. 2004; Ménétret et al. 2007; Park and Rapoport 2012). When the channel is inactive, a plug created by the second transmembrane domain of SecY prevents the passage of small molecules through the central pore ring (van den Berg et al. 2004). The translocon operates in a cotranslational mode for proteins destined for the inner membrane, whereas secreted proteins are transported posttranslationally.

The cotranslational pathway requires the action of the soluble ribonucleoprotein of the signal recognition particle (SRP) that binds the N-terminal signal peptide on a nascent polypeptide cargo and recruits the entire polypeptide-ribosome complex to Sec's membrane receptor FtsY (Angelini et al. 2005). In this case, translocation is propelled entirely by continued proteosynthesis. In the posttranslational pathway, cytosolic chaperones like SecB and Trigger Factor bind to a completed polypeptide chain and pass it to the SecA ATPase. With the help of proton-motive force SecA drives the passage of the polypeptide through the channel (Karamanou et al. 2007).

The properties of the N-terminal signal peptides are well conserved and they usually contain three distinct parts; an amino-terminal positively charged region (n-region), a central hydrophobic part (h-region), and a more polar carboxy-

terminal part (c-region) (von Heijne 1990). The signal peptide is cleaved off by signal peptidase following protein cargo delivery through the membrane. There is a universal AXA motif present downstream of the cleavage site (Owji et al. 2018).

## YidC—The Specialized Membrane Protein Insertase

YidC is a single-subunit translocase spanning the inner membrane with six transmembrane helices (Sääf et al. 1998). Strictly speaking, YidC is an insertase mediating the insertion of a substrate polypeptide into the membrane (fig. 2A). YidC is also capable of translocating small protein segments (soluble domains) into the periplasm (Kiefer and Kuhn 2018). The core of the protein is formed by five helices which remain closely packed except for a positively charged hydrophilic groove open from the cytosolic side. It has been proposed that the hydrophilic groove provides the initial binding surface for the hydrophilic N-terminal region of the substrate. The substrate is first translocated to the periplasmic side of the membrane, which allows the following hydrophobic segment to slide along the transmembrane helices of YidC into the lipid bilayer (Kumazaki et al. 2014; Shimokawa-Chiba et al. 2015).

YidC operates strictly in a cotranslational mode, binding the nascent polypeptide emerging from the ribosome and facilitating its insertion (fig. 2A) (Kohler et al. 2009). Only few substrates are known to be inserted into the membrane directly by YidC of *E. coli*, such as ATP synthase subunit C, MscL (Facey et al. 2007), and Tssl (Aschtgen et al. 2012) inner membrane proteins plus Pf3 and M13 bacteriophage coat proteins (Samuelson et al. 2000). The main role of YidC is its coordinated action with SecYEG (Shanmugam and Dalbey 2019) during the insertion of membrane components of the mitochondrial respiratory chain complexes (Yi et al. 2004; Du Plessis et al. 2006; Kol et al. 2009; Price and Driessen 2010) and TatC subunit of Tat translocase (Zhu et al. 2012).

## Tat—The Translocase for Folded Substrates

The extraordinary feature of the Tat translocase is its ability to transport folded proteins or multisubunit complexes across the inner membrane (Frain et al. 2019) (fig. 2A). The prototypical translocase of *E. coli* is built of two TatA-type proteins (TatA, TatB) and a single TatC subunit. TatA-type proteins have one N-terminal transmembrane helix and a C-terminal amphipathic helix exposed to cytoplasm, whereas TatC is a polytopic membrane protein spanning the membrane six times (Sargent et al. 1998; Palmer and Berks 2012).

Despite the similarity between TatA and TatB, the proteins have distinct roles within the translocase. TatB forms a 1:1 complex with TatC (Bolhuis et al. 2001; Orriss et al. 2007; Tarry et al. 2009) and together they act as the receptor complex binding the signal peptide of substrate proteins (de Leeuw et al. 2002; Alami et al. 2003).

According to the current mechanistic model, the binding of the substrate's signal peptide induces a conformational change in the complex thereby releasing TatB from its primary binding site on TatC. The same site of TatC is now available for TatA binding, and further TatA subunits

subsequently oligomerize around the substrate, creating a transient pore of variable diameter (Alcock et al. 2016). In the alternative model, the accumulation of TatA subunits may just destabilize the cytoplasmic membrane due to their short transmembrane helices, hereby allowing the passage of the substrate (Hou et al. 2018). The passenger domain of the substrate protein crosses the membrane while the signal peptide remains bound to TatC. The signal peptide is then cleaved by signal peptidase at the periplasmic side of the membrane and TatAs are dispersed into monomers. Finally, the original TatBC complex is restored awaiting another cargo protein.

The translocase requires the proton-motive force at an unknown stage of the transport (Hamsanathan and Musser 2018). In contrast to the constitutively active Sec translocase or YidC insertase, the essentiality of Tat often depends on environmental conditions and can be dispensable in laboratory strains (Palmer and Stansfeld 2020). Tat substrates are involved in diverse cellular processes such as respiration and cell division and their number is highly lineage-specific, ranging from just a few proteins to one-fifth of the entire secretome in some species (Dilks et al. 2003).

In general, Tat signal peptides are similar to those recognized by the Sec translocase, except that their N-terminal positively charged region is longer and contains a Tat consensus motif with twin arginine residue (S/T-R-R-X-F-L-K) (Berks 1996; Palmer and Berks 2012); hence their name, Twin-arginine translocase (Tat). In addition, the h-region of Tat signal peptides is less hydrophobic and the c-region contains extra positively charged residues also called a “Sec-avoidance motif,” which minimizes the mistargeting among the translocases. However, the same signal peptidase seems to be involved in cleaving off the Sec and Tat signal peptides (Yahr and Wickner 2001).

## Endosymbiont Protein Translocases in the Inner Mitochondrial Membrane

Despite the formation of TOM and TIM complexes, mitochondria of some extant eukaryotes carry homologs of Sec, YidC, and Tat translocases in their membranes. Although the reconstructions of phylogenetic relationships among the components of the translocases typically do not allow us to pinpoint their ancestral bacterial lineage, the most parsimonious explanation for their presence is their arrival with the original endosymbiotic bacterium. The fates of Sec, Tat, and YidC translocases in mitochondria and their importance for mitochondrial biology have been markedly different. Our current understanding of the mechanistic details and structural properties of the bacterial translocases has allowed us to picture the possible pressures behind their assignments to mitochondrial roles.

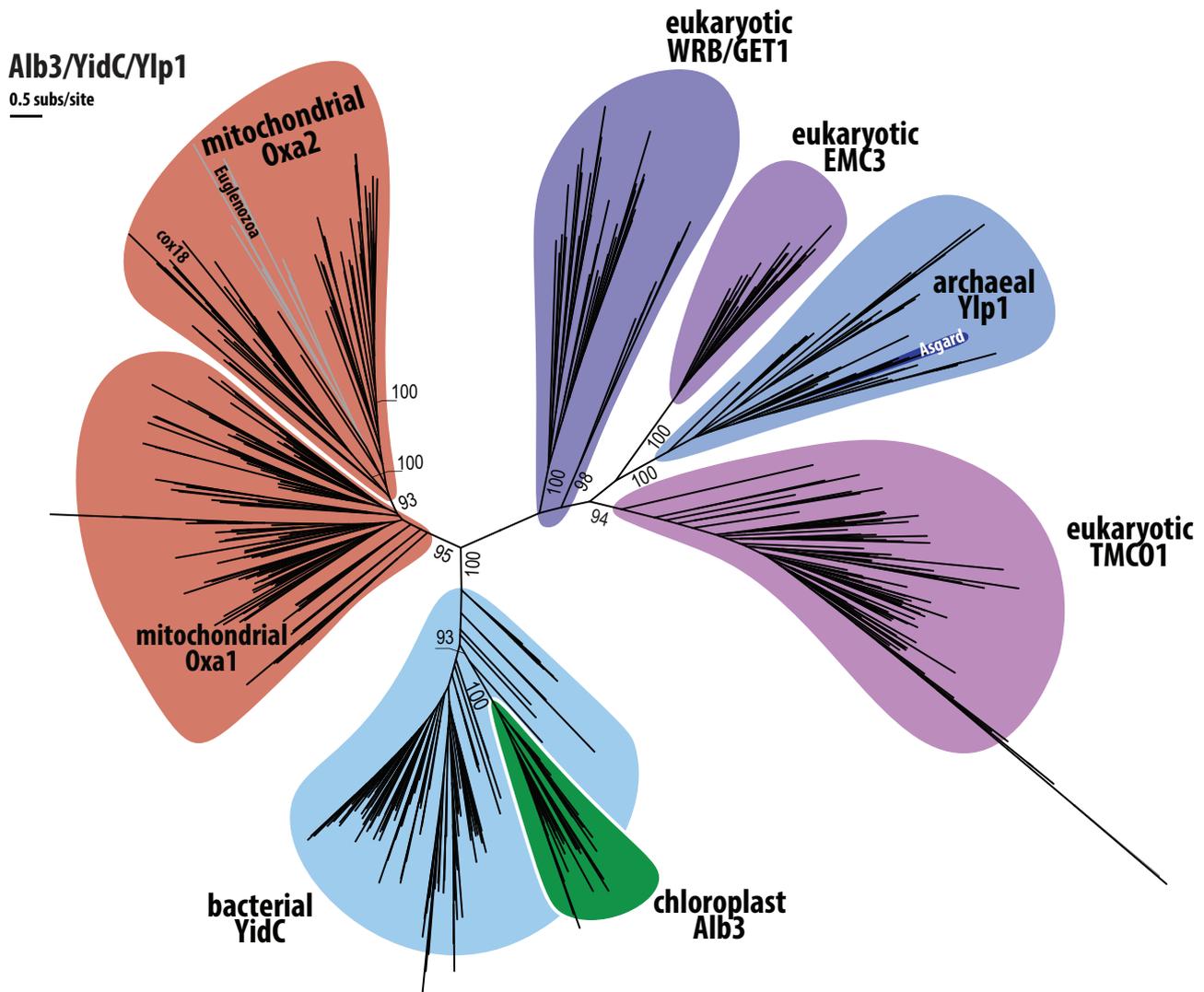
### Oxa1—A Ubiquitous Mitochondrial Insertase

Oxa1 is the mitochondrial homolog of YidC and its mutations in yeast were originally found to induce respiration-deficient cells (cytochrome OXidase Activity) (Bonney et al. 1994).

Oxa1 was later characterized as a key machinery for the insertion of mitochondrially encoded proteins (Hell et al. 2001) (fig. 1). The substrate repertoire of Oxa1 has been recently enlarged with a number of nuclear-encoded inner membrane proteins (one of them is Oxa1 itself), including components of the TIM22 complex (Stiller et al. 2016). Hence, the deficiency in Oxa1 activity can also manifest as a defect in the import of the mitochondrial carrier proteins (Stiller et al. 2016). The structure of Oxa1 differs from *E. coli* YidC by lacking the very N-terminal transmembrane domain and the domain exposed to the periplasm/IMS (Kumazaki et al. 2014). These parts of the protein are, however, also absent in multiple bacterial lineages and were even shown to be dispensable for *E. coli* in culture (Jiang et al. 2003). Instead, Oxa1 carries a specific C-terminal extension that binds the mitoribosome and it does so either directly (Haque et al. 2010) or via interaction with membrane-associated receptors (Ott et al. 2006). In addition to Oxa1, the vast majority of eukaryotes contain another mitochondrial YidC homolog termed Oxa2 or Cox18. Oxa2 is much less abundant in mitochondria and lacks the C-terminal ribosome-binding region (Ghaemmaghami et al. 2003). In accordance with this missing region, Oxa2 was found only to mediate the posttranslational translocation of the C-terminal domain of mitochondrion-encoded Cox2 (Saracco and Fox 2002; Funes et al. 2004). The size of the C-terminal domain likely exceeds the capacity of the single Oxa insertase and hence an additional Oxa protein comes to action (Saracco and Fox 2002; Lewis and Hegde 2021). Interestingly, Cox2 can be experimentally nucleus-encoded and assembled in the inner mitochondrial membrane when carrying a mitochondrial targeting sequence and a point mutation in the N-terminal region (Supekova et al. 2010). In such situation, Oxa2 is dispensable for protein translocation (Elliott et al. 2012).

The presence of two Oxa proteins across the eukaryotic tree of life (fig. 3) suggests that Oxa1 and Cox2-specialized Oxa2 proteins were already present in the last eukaryotic common ancestor (LECA) (Bonney et al. 2009; Zhang et al. 2009). Several adaptations to this set up have been recorded in different lineages of eukaryotes. The split of the *cox2* gene into two halves in dinoflagellates and apicomplexans likely eased the translocation and assembly of the protein. Both groups lost Oxa2 and transferred the split *cox2* genes from mitochondria into the nucleus (Gardner et al. 2002; Waller and Keeling 2006). Recent transfer of the full-length *cox2* gene from the mitochondrion to the nucleus of legumes was accompanied by reduced Cox2 hydrophobicity and perhaps no involvement of Oxa2 in translocation (Daley et al. 2002).

A unique situation occurred in kinetoplastids which possess two or three unusual Oxa homologs that do not robustly branch with either Oxa1 or Oxa2 and probably originate in a duplication event specific to this lineage (fig. 3) (Bonney et al. 2009; Zhang et al. 2009). Whether kinetoplastids lost one of the original Oxa proteins or separated from the rest of eukaryotes before the formation of Oxa1 and Oxa2 remains to be elucidated. The evolutionary relationship between Oxa and YidC proteins also remains unclear as the phylogenetic



**Fig. 3.** Evolutionary relationships of Oxa superfamily of proteins. The maximum likelihood tree shows the eukaryotic Oxa superfamily proteins and their affinity to bacterial and archaeal components. Two mitochondrial paralogs Oxa1 and Oxa2 are present in all major eukaryotic groups except Euglenozoa, which carry only proteins derived from Oxa2. The origin of the mitochondrial proteins remains unresolved. The three ER members of the superfamily (EMC3, Get1, and TMC01) were likely derived from a single archaeal protein. The tree was constructed as described previously (Škodová-Sveráková et al. 2020) using LG4M model+4xGAMMA.

reconstructions failed to accurately determine the prokaryotic origin of the mitochondrial protein (fig. 3) (Bonney et al. 2009; Zhang et al. 2009).

Importantly, the evolutionary adaptation of mitochondria in some parasitic eukaryotes and those living in anoxic environments led to the identical situation concerning the function of Oxa protein(s). These eukaryotes carrying so called mitochondrion-related organelles (MROs) such as hydrogenosomes and mitosomes, lost their organellar genome and translation machinery due to the overall reduction of mitochondrial functions including the respiratory chain (Roger et al. 2017). In all cases, the loss of mitochondrion/MRO-encoded proteins was accompanied by the loss of Oxa protein(s) (fig. 5), which consistent with the function of the insertase.

### Mitochondrial Tat—A Single Substrate Translocase

Components of Tat can be found in the mitochondria of multiple eukaryotic lineages ranging from protists to plants and some Metazoa (i.e., sponges) (Burger et al. 2013; Pett and Lavrov 2013; Carrie et al. 2016) (fig. 2B). However, the vast majority of these organisms only encode the TatC subunit. In contrast to Oxa, TatC is always encoded in the mitochondrial genome, which is likely because of its high hydrophobicity (Petrů et al. 2018). The mitochondrial genomes of several protist lineages such as jakobids, cryptophytes, and ochrophytes also encode TatA, which is, together with TatC, able to functionally complement the *E. coli* Tat translocase in the transport of a synthetic substrate (Petrů et al. 2018). Yet, their natural substrate has not been identified to date.

Recently, a eukaryote-specific mitochondrial TatB was discovered in the nuclear genome of plants (Carrie et al. 2016) and later also those ochrophytes and cryptophytes that carry a mitochondrial-encoded TatC (Petrů et al. 2018). This TatBC complex was shown to translocate the folded iron–sulfur cluster-containing domain of the Rieske protein into the IMS (Schäfer et al. 2020), a process mediated by Bcs1 chaperone in Tat-lacking eukaryotes (Wagener et al. 2011). However, the presence of these two machines is not mutually exclusive, suggesting that they likely perform additional roles. The TatC phylogeny, in agreement with the presence of the gene in the mitochondrial genome, shows that mitochondrial Tat is inherited from the  $\alpha$ -proteobacterial ancestor (Petrů et al. 2018). The origin of the nucleus-encoded mitochondrial TatB is, however, difficult to identify due to its small size.

### Mitochondrial Sec—The Last Few Remaining Proteins without a Known Function

Similar to the Tat translocase, the discovery of the mitochondrial Sec translocase came with characterization of the mitochondrial genome from the jakobid *Reclinomonas americana*. This genome encodes 67 proteins, the maximum known thus far

(Lang et al. 1997; fig. 2B). Among eukaryotes, the mitochondrial genomes of jakobids are the most gene-rich and retain several bacteria-like features including similarities in gene orders and the presence of the original bacterial RNA polymerase gene (Gray et al. 2004). Comparison of mitochondrial genomes across the eukaryotic tree of life revealed that it is only jakobids which carry a *secY* gene in their mitochondrial genomes (Lang et al. 1997; Burger et al. 2013). All identified jakobid SecY homologs represent the most divergent SecY proteins known (Tong et al. 2011). In addition, due to small available data set of mitochondrial SecY sequences, their evolutionary origin is currently difficult to deduce (fig. 4A). Yet, homology modeling of *R. americana* SecY by Swiss-Model (Biasini et al. 2014), using the *Thermus thermophilus* protein as a template, shows that despite sharing only 25 percent identity with its bacterial counterpart, the *R. americana* protein is capable of accommodating the conserved SecY structure including its plug domain (fig. 4B). Remarkably though, neither of the smaller Sec subunits, SecE and SecG, have been identified in the mitochondrial genome or in available nuclear genome sequences (Gray 2015; Horváthová et al. 2021). Whether the jakobid SecY translocase actually transports proteins from the mitochondrial matrix to the IMS thus needs further experimental examination. A comparative analysis of mitochondrial genomes showed that Cox11 could be the putative substrate of mtSecY (Tong et al. 2011). Unlike in other eukaryotes, the jakobid Cox11 is still encoded by the mitochondrial genome and carries a signal peptide on its N-terminus. Cox11 is N-terminally anchored in the inner membrane and exposes its copper-binding domain into the IMS (Timón-Gómez et al. 2018). Hence, mtSecY may be responsible for the translocation of Cox11 domain into the IMS, where Cox11 fulfils its function in cytochrome oxidase complex assembly (Tong et al. 2011).

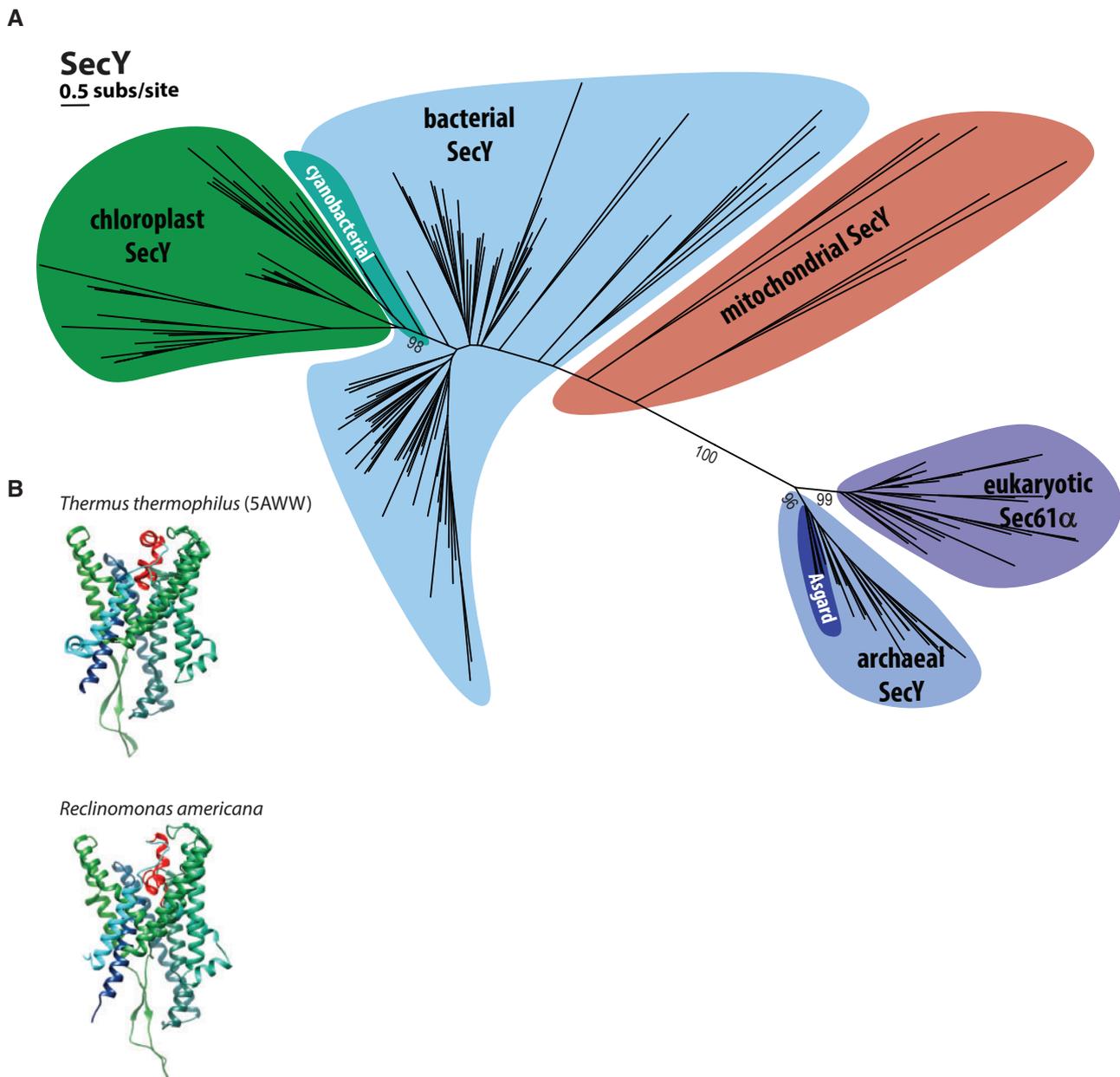
The limited presence of Sec in mitochondria raises a question: why was the central bacterial Sec translocase lost from mitochondria when Oxa remained preserved in all mt genome-carrying eukaryotes? As recently proposed, Oxa might functionally replace SecY for the membrane insertion of membrane proteins that do not require translocation of large soluble domains across the membrane. The components of the mitochondrial respiratory complexes which remain encoded by the mitochondrial genome entirely fulfill this criterion and, hence, might have facilitated the loss of the Sec translocase (Lewis and Hegde 2021).

### Molecular Evolutionary Context of SecY, YidC, and Tat Translocases in Mitochondria

The protein translocases, like other types of membrane transporters, can operate as independent molecular machines (Dolezal et al. 2006). Yet, usually there are receptors, chaperones, proteases, and downstream factors associated with the translocases as part of the transport pathways. Some of these additional components were preserved and found their role in mitochondria. Analogously to signal peptide processing in the bacterial periplasmic space by Signal peptidase I (SPase I), two peptidases, Imp1 and Imp2, face the IMS anchored in the inner mitochondrial membrane (Schneider et al. 1991). Their substrates are nucleus- or mitochondrion-encoded and hence arrive from both directions to function in the IMS (Esser et al. 2004). Some mitochondria were found to contain signal recognition particle protein (Ffh) and its receptor (FtsY) of alphaproteobacterial origin, perhaps recruiting the active mitoribosome during the cotranslational insertion of mitochondrially encoded proteins (Pyrih et al. 2021). Interestingly, mitochondria of several protist lineages were found to contain multiple components of the type 2 secretion pathway (T2SS), which allows bacteria to export folded proteins from the periplasm across the outer membrane to the extracellular environment (figs. 2B and 5) (Horváthová et al. 2021). Although the function of this pathway has not been demonstrated, it is likely that the mitochondria of these organisms are able to transport proteins to the cytosol. Broad phylogenetic distribution of the mitochondrial T2SS components strongly suggests that the protein secretion from mitochondria was also occurring in the LECA (Horváthová et al. 2021).

### Successful Integration of Prokaryotic Translocases into Plastids and the ER

Comparable to mitochondria plastids underwent an analogous transformation from an endosymbiotic cyanobacterium and became fully integrated into metabolic and protein transport pathways (Sibbald and Archibald 2020). Yet, the evolutionary fate of the prokaryotic translocases in plastids seems much more like a story of the conservation of original functions (fig. 2) (Celedon and Cline 2013). Similarly to mitochondria, hundreds to thousands of nucleus-encoded proteins reach the plastid stroma after passing through the plastid envelope membranes, which contain plastid translocase complexes composed of subunits of both bacterial and eukaryotic origin (Richardson et al. 2017). Importantly, over three



**FIG. 4.** Sec translocase functions in all domains of life. (A) The phylogenetic reconstruction of eukaryotic and prokaryotic SecY/Sec61 $\alpha$  homologs. The maximum likelihood tree shows that the plastidial proteins, in contrast to their mitochondrial counterparts, show clear affinity to bacterial homologs (cyanobacteria). However, there are only few highly divergent mitochondrial SecY proteins, those in the mitochondria of jakobids. The endoplasmic reticulum Sec61 $\alpha$  shows a close relationship to archaeal proteins, likely from Asgard archaea. The tree was constructed as described previously (Škodová-Sveráková et al. 2020) using LG4M model+4xGAMMA. (B) Structural homology modeling of *Reclinomonas americana* mitochondrial SecY using *Thermus thermophilus* structure (5AWW). The model shows that the mitochondrial SecY contains all necessary motifs of the translocase, including ten transmembrane helices (in blue to green gradient) and the plug domain (in red), which seals the channel in the resting state.

hundred proteins, including those encoded and translated in the plastids, are transported from the stroma to the envelope or lumen of thylakoids (Peltier et al. 2002; Albiñak et al. 2012). Plastids of the green lineage (Viridiplantae) carry two nucleus-encoded SecYE translocases that are localized in the thylakoid membrane (SecY1E1) or the inner plastid membrane (SecY2E2) (Skalitzky et al. 2011); the SecG subunit is missing (Celedon and Cline 2013). Both SecYE complexes are oriented to mediate the transport of proteins from the plastid stroma to or across either of the membranes. Although the former

transports soluble proteins into the thylakoid lumen or membrane proteins with large luminal domains (Cline and Dabney-Smith 2008), the latter integrates a small subset of proteins into the inner plastid membrane (Li et al. 2017). Interestingly, both complexes retained specific SecA motor proteins which are absent from mitochondria. In contrast, the majority of red lineage (Rhodophyta and rhodophyte-derived secondary plastids) carry just one SecY translocase and the SecA motor, both encoded in the organellar genome (Valentin 1993).

Unlike mitochondria, plastids carry a fully functional and well-characterized Tat translocase. The translocase itself was in fact discovered first in plastids before its characterization in bacteria (Settles et al. 1997). TatA and TatB exhibit dual localization, in thylakoids as well as the inner plastid membrane, however, only assembly intermediates of the translocase are likely contained in the latter (Celedon and Cline 2013). Plastid Tat is believed to transport folded proteins into the lumen of thylakoids and the size range of the predicted substrates is 4–60 kDa (Peltier et al. 2002; Albinia et al. 2012). Additionally, the insertion of two membrane proteins requires the function of Tat: a thylakoid Rieske protein and FtsH protease, the former also requiring the involvement of the thylakoid Sec complex (Summer et al. 2000; Molik et al. 2001).

The plastid homolog of Oxa, Alb3, is exclusively localized in the thylakoid membrane (Gerdes et al. 2006) and is involved in the biogenesis of photosynthetic complexes in both SecY-dependent and independent protein insertion (Moore et al. 2000; Yen et al. 2001). Almost all plastid-containing eukaryotes possess two homologs of Alb3, and other duplication events occurred lineage-specifically, which may have enabled Alb3 paralogs to specialize toward distinct substrates (Gerdes et al. 2006; Benz et al. 2009).

From the above, it is clear that Sec, Tat, and YidC translocases remained fully functional to maintain the biogenesis and the function of thylakoids inherited from the cyanobacterial endosymbiont (Vothknecht and Westhoff 2001). The import of several hundreds of proteins, some with complicated topologies and large soluble luminal domains, demands the action of all three ancestral translocases. This is in striking contrast to just a handful of proteins that require their insertion/translocation from the mitochondrial matrix.

Aside from mitochondria and plastids, several prokaryotic protein transport machines have been successfully integrated into the eukaryotic endomembrane system (fig. 5). Analogously to mitochondria, the origin of the endomembrane system has been linked to the very formation of the eukaryotic cell from the archaeal host cell (Martin et al. 2015; Vosseberg et al. 2021). During the course of evolution, the endomembrane system has developed into a remarkably dynamic structure with eukaryote-specific transport systems (Gabaldón et al. 2006; Wentz and Rout 2010; Schlacht et al. 2014). Yet, protein translocases of archaeal origin remained at the center of protein transport across or into the membranes of the endomembrane system.

The proteinaceous channel into the lumen of the ER, Sec61 translocase, is composed of Sec61 $\alpha$ ,  $\beta$  and  $\gamma$  subunits. The translocase is similar to the bacterial SecYEG complex but its  $\alpha$ ,  $\beta$  and  $\gamma$  subunits are derived from the archaeal Y,  $\beta$ /G and E proteins (fig. 2B), respectively (Cao and Saier 2003; Rapoport 2008). Similar to prokaryotic translocases, Sec61 operates in both co- and posttranslational modes, the latter of which involves a SRP derived from the archaeal ancestor (Eichler and Moll 2001; Cao and Saier 2003). The phylogenetic reconstruction further suggests that it was the ancient relative of an Asgard archaeon that provided the Sec translocase to the LECA (fig. 4A).

The recent identification of the archaeal YidC-like protein (Ylp1) (Borowska et al. 2015) led to the subsequent discovery

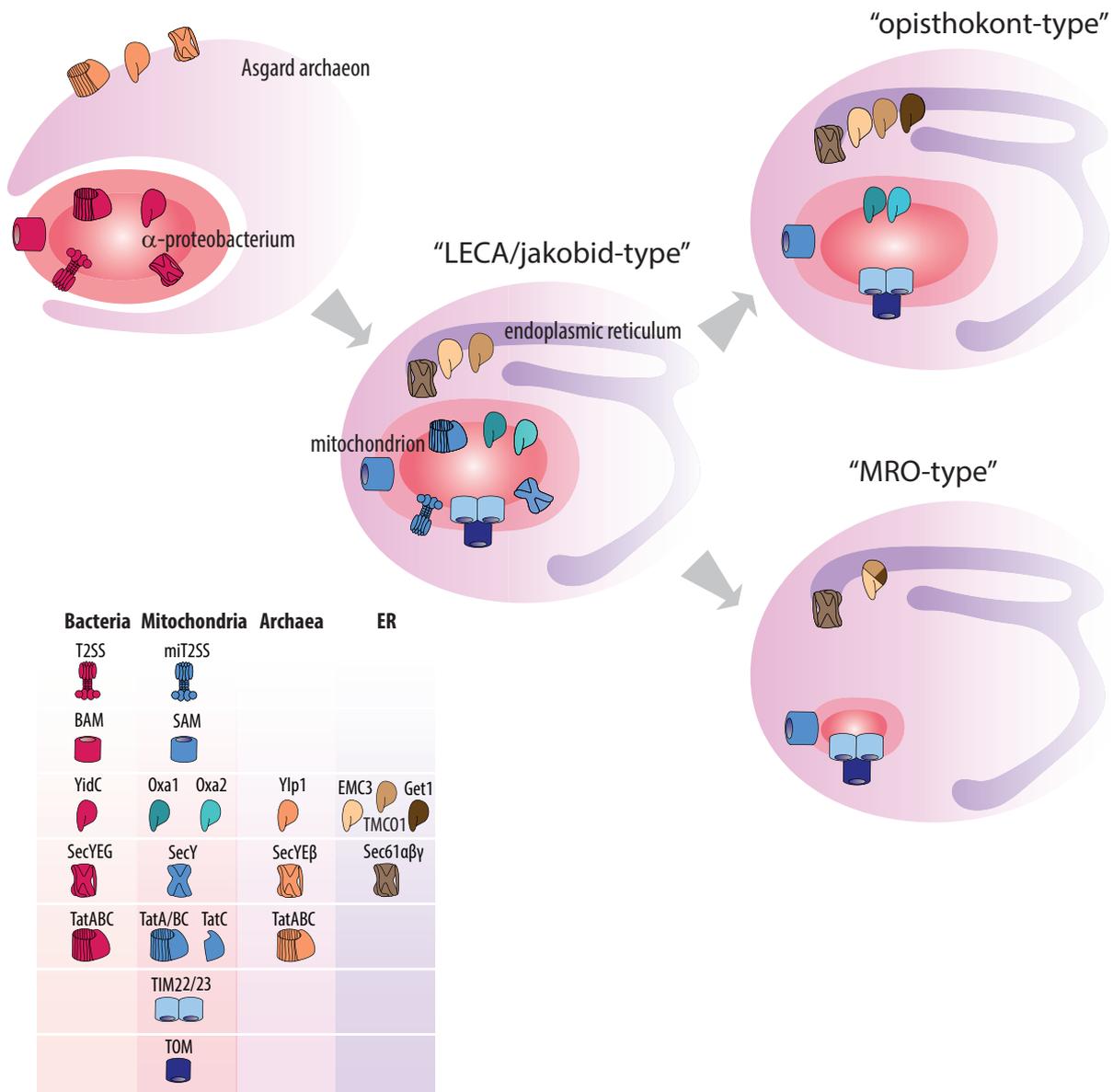
of its three eukaryotic homologs in the ER, that is, Get1, EMC3, and TMCO1, all of which fall into the Oxa superfamily (fig. 2B) (Anghel et al. 2017; Chen and Dalbey 2018). These proteins were originally described as components of different molecular complexes, yet they all likely perform analogous roles in membrane protein insertion. Get1 is a component of a membrane module of the GET (guided entry of tail-anchored proteins) pathway, which is responsible for the posttranslational membrane insertion of tail-anchored (TA) proteins like SNAREs (Schuldiner et al. 2008). EMC3 is a subunit of the ER membrane protein complex (EMC), which mediates the insertion of TA proteins carrying less hydrophobic transmembrane domains (Guna et al. 2018). Lastly, TMCO1 was found to be part of a novel translocon that also contains a Sec61 channel and which associates with ribosomes to insert polytopic membrane proteins (McGilvray et al. 2020).

The phylogenetic reconstruction of these newly discovered Oxa superfamily members shows, similarly to Sec61 $\alpha$ , their clear affinity to archaeal proteins (fig. 3). Get1, EMC3, and TMCO1 form distinct clades, with EMC3 separating by a longer basal branch suggestive of an early divergence of this paralog, whereas Get1 seems to represent a later eukaryotic invention. Still, this topology suggests a single evolutionary source of the endomembrane protein translocases in archaeal Ylp1.

## Membrane Topology Matters

These recent findings illustrate nicely the concept of evolutionary tinkering when proteinaceous components gain new functionalities by integration into novel and often more complex molecular machines (Jacob 1977) (fig. 5). Yet, in the case of protein translocases, the obvious constraint for repurposing the ancestral prokaryotic translocases was their membrane orientation in respect to the particular compartment where they are encoded and synthesized and thus the direction of substrate protein transport.

In all known eukaryotic homologs of Sec, YidC, and Tat translocases, their ancestral orientation remained preserved across the eukaryotic compartments, always transporting proteins from the equivalent of prokaryotic cytosol (mitochondrial matrix, plastid stroma, or eukaryotic cytosol) to the *trans*-compartment (IMS, thylakoid lumen, or ER lumen). The transport by protein translocases is always unidirectional, dictated by the translocase structure and the occurrence of the motor entity. Hence, the hypothetical reversal of the direction of protein transport is probably only possible when the membrane topology of the translocase inverts, too. Although the topology of the membrane protein can change during evolution (von Heijne 2006), it is difficult to envision that an entire membrane protein complex undergoes such change. This is especially complicated in the case of protein translocases, which require themselves for membrane insertion, and adaptive processes toward their inverted topology would ultimately end in loss of function. As a consequence of reduced mitochondrial gene expression and the absence of additional internal compartment like thylakoids,



**Fig. 5.** Schematic evolution of the ancestral prokaryotic protein transport machines in eukaryotes. The eukaryotic cell originated from the symbiosis of an archaeal and an  $\alpha$ -proteobacterial cell. One of the key steps following this endosymbiosis was to rebuild the protein transport pathways across the two mitochondrial bounding membranes. Some of the original bacterial transporters were lost or retained lineage-specifically (SecY, T2SS), whereas others were integrated into the newly evolving protein transport machines (YidC, Tat, BAM). Importantly, entirely new pathways evolved for the protein delivery from the host cytoplasm (TOM and TIM complexes, MIA pathway). Archaeal homologs of here described bacterial protein translocases are shown as well. Some of them were repurposed in the newly evolving ER (SecYE $\beta$ , Ylp1). A conceptual composition of the LECA protein transport machines is depicted that corresponds to the arrangement found in some jakobids. The best experimentally characterized cellular systems of opisthokonts lack Sec and Tat translocases in their mitochondria but carry an extra Ylp1 homolog, Get1, in the ER membrane. Eukaryotes living in anoxic environments reduced their mitochondria to so-called MROs (mitochondria-related organelles), which mostly lack the organellar genome. Consequently, Oxa proteins involved in respiratory chain subunit insertion were lost from the MROs. Additional reductions in the cellular metabolism often led to the loss of Ylp1 homologs in the ER in these organisms.

mitochondria have gradually ceased to use their ancestral protein translocases such Sec and Tat. Thus, Oxa-mediated translocation remained the only widely conserved protein transport pathway homologous to the protein secretion of the original bacterial ancestor of mitochondria. It is the remaining set of membrane proteins devoid of bulky soluble domains, which is responsible for “the evolutionary success” of YidC/Oxa translocase in mitochondria.

## Acknowledgments

We thank Felicity Alcock for her helpful comments. This work was supported by the project “Centre for Research of Pathogenicity and Virulence of Parasites” (No. CZ.02.1.01/0.0/0.0/16\_019/0000759) funded by European Regional Development Fund and by Moore-Simons Project on the Origin of the Eukaryotic Cell to P.D. (<https://doi.org/10.37807/GBMF9738>).

## Data Availability

The data sets were derived from sources in the public domain <https://www.ncbi.nlm.nih.gov/>. The alignments and the tree files will be shared on request to the corresponding author.

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