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A unifying model for membrane protein biogenesis

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α-Helical integral membrane proteins comprise approximately 25% of the proteome in all organisms. The membrane proteome is highly diverse, varying in the number, topology, spacing and properties of transmembrane domains. This diversity imposes different constraints on the insertion of different regions of a membrane protein into the lipid bilayer. Here, we present a cohesive framework to explain membrane protein biogenesis, in which different parts of a nascent substrate are triaged between Oxa1 and SecY family members for insertion. In this model, Oxa1 family proteins insert transmembrane domains flanked by short translocated segments, whereas the SecY channel is required for insertion of transmembrane domains flanked by long translocated segments. Our unifying model rationalizes evolutionary, genetic, biochemical and structural data across organisms and provides a foundation for future mechanistic studies of membrane protein biogenesis.

The transfer of molecules and information between the inside and outside of a cell relies on integral membrane proteins exhibiting diverse topologies and characteristics (Box 1). The core processes involved in membrane insertion would have existed at the plasma membrane of the last universal common ancestor[1]. The plasma membrane remains the site of membrane protein insertion in bacteria and archaea. The endoplasmic reticulum (ER), which likely evolved from the archaeal plasma membrane[2], is the major site of membrane protein insertion in eukaryotes. Mitochondria and plastids evolved from bacteria[3], so their inner membranes descended from the ancestral bacterial plasma membrane. All of these evolutionarily related membranes contain members of the Oxa1 family[4,5] or SecY family[6] (Box 2), the only known membrane protein insertion factors that trace back to the last universal common ancestor[7].

The evolutionary path of membrane protein insertion presumably progressed from an unassisted insertion reaction to a process facilitated by insertion factors. This transition broadened the range of proteins that can be inserted into membranes, which in turn allowed the evolution of more elaborate and diversified insertion machinery. By considering how membrane insertion and its accompanying machinery arose, we arrive at a unifying model for membrane protein biogenesis that accommodates the current diversity of the membrane proteome across all organisms. In this model, Oxa1 facilitates insertion of transmembrane domains (TMDs) that are flanked by a short translocated domain, whereas SecY is required for insertion of TMDs that are followed by a long translocated domain. Proteins displaying diverse topologies and properties can be accommodated by dynamically toggling between SecY and different Oxa1 family members during their cotranslational insertion.

The evolution of membrane insertion

Theoretical and experimental studies show that the energetically favorable reaction of partitioning a hydrophobic TMD into the lipid bilayer is sufficient to compensate for the energy cost of translocating a short segment of a flanking hydrophilic polypeptide across the membrane[8–11]. This insertion can be achieved with a single TMD flanked by a short 'tail' at the N or C terminus (Fig. 1, left), or two TMDs with a short intervening loop. This would have been the ancestral mechanism of membrane protein insertion. The substrate range of 'unassisted' insertion was likely very limited, with insertion being strongly hindered by the competing reactions of insolubility and aggregation, especially for proteins with several TMDs.

The substrate range for the unassisted mechanism might have expanded following the evolution of a ribosome receptor that allows synthesis to occur close to the membrane. Such a receptor could simply have been a ribosome-binding peripheral or single-TMD protein that engages near the polypeptide exit tunnel. Membrane-proximal protein synthesis would have facilitated cotranslational insertion of multi-TMD

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BOX 1

Membrane protein topology

α-Helical membrane proteins contain one or more TMDs. They are typically helices composed of ~22 predominantly non-polar amino acids, but can be as short as 13 amino acids or as long as 30 amino acids. They can also have substantially hydrophilic, or even charged, properties[20,127]. The number, location and distribution of TMDs are essentially unrestricted. Membrane protein topology can be N_{cyt} or N_{exo}, depending on whether the N terminus is located on the cytosolic or exoplasmic side of the membrane, respectively. Proteins that initiate membrane insertion via their first TMD in the N_{cyt} topology can have a preceding N-terminal cytosolic domain of any length. Proteins that initiate membrane insertion via their first TMD in the N_{exo} topology typically contain a preceding N-terminal translocated domain shorter than ~50 amino acids. Some proteins initiate membrane insertion in the N_{cyt} topology before proteolytic removal of the first hydrophobic domain occurs, leaving the downstream protein in the N_{exo} topology. The processed hydrophobic domain in these cases is termed a cleavable signal peptide, whose characteristically short hydrophobic segment (~7–9 amino acids) allows selective recognition by signal peptidase[27]. N_{exo} membrane proteins produced in this manner typically contain a translocated N-terminal domain longer than ~100 amino acids between the signal peptide and the first TMD. The topology of the first hydrophobic domain is determined by a combination of flanking domain charge and length and hydrophobic domain length and hydrophobicity[128]. The topologies of downstream TMDs alternate in orientation and are opposite to the orientation of the preceding TMD. The loops between TMDs are typically short (around 15 amino acids, on average), but can be of any length regardless of the side of the membrane that they reside on. The C-terminal hydrophilic domain following the last TMD can be of any length and can be located in the cytosolic or exoplasmic side of the membrane.

proteins by successive insertion of TMD pairs as they emerge from the ribosome. Each newly emerging TMD would rapidly bind to the adjacent membrane surface[12], reducing its exposure to the bulk cytosol. By having only two membrane-associated TMDs exposed at any time, the possibility of substrate aggregation into translocation-incompetent states is reduced.

Although the substrate range remains limited to short translocated tails and loops, the mechanism is compatible with either topology: the first TMD could insert by itself, concomitant with N-tail translocation, or insert as a pair with the next TMD, concomitant with translocation of the intervening loop. Experiments using liposomes or nanodiscs have shown that various multipass membrane proteins can indeed be inserted without any insertion factors, albeit with low efficiency owing to competition from aggregation[13–16]. Unsurprisingly, insertion must occur cotranslationally[17], is favored by a high concentration of membrane[18] and is compatible only with substrates containing short translocated loops and tails.

A membrane protein that reduces the energetic barrier for hydrophilic-segment translocation would relax the constraint on the length of the translocated domain, allowing for translocation of longer tails and loops (Fig. 1, middle). The Oxa1 family is thought to achieve this by providing a hydrophilic vestibule that penetrates part of the way into the membrane[4,19,20]. The vestibule thins the membrane locally[21], reducing the energetic barrier to translocation of a hydrophilic segment from this site. Importantly, the universally conserved core Oxa1 fold, a simple three-TMD bundle with short translocated hydrophilic segments[5,7,20,22], could have evolved at a time when only the ancestral insertion mechanism existed.

Once an Oxa1-like protein had evolved, the substrate range expanded in two ways. First, a lower energetic barrier to flanking-domain translocation allowed insertion of TMDs with lower hydrophobicity. Second, flanking domains could be longer. These changes allowed the evolution of increasingly complex and diverse membrane proteins. Oxa1 family members have been shown to translocate hydrophilic segments of up to ~50 amino acids, depending on composition and folding propensity of the translocated domain, hydrophobicity of the flanking TMD(s) and features of the specific family member[4,20]. Diversification of the Oxa1 family has allowed some members to accommodate longer translocated domains for certain substrates[23].

From membrane insertion to secretion

Translocation of hydrophilic segments longer than ~100 amino acids must generally be done using a membrane-spanning channel. This role is filled by members of the SecY family, pseudosymmetric proteins that have two homologous halves with a channel between them[24]. Structural and sequence analyses have suggested that the core of each SecY half arose from an Oxa1-like ancestor[7]. Through duplication, fusion and anti-parallel interaction, this ancestor would have evolved into a protein that brought together two hydrophilic vestibules on opposite sides of the membrane. These adjacent vestibules would have further evolved to form a transmembrane channel that could facilitate the translocation of long hydrophilic domains across the membrane.

Like the Oxa1 family, from which it might have evolved, SecY facilitates hydrophilic-domain translocation in a reaction that is coupled to the membrane insertion of an adjacent hydrophobic domain. Rather than the hydrophilic segment passing through a locally distorted membrane, it is pulled into the SecY membrane-spanning aqueous channel[25,26]. The hydrophobic domain achieves this by accessing the membrane through a lateral gate in SecY, such that its downstream flanking domain enters the central channel in a looped configuration (Fig. 1, right). Because hydrophobic domain binding at the lateral gate is coupled to channel opening, the initiation of translocation is coupled to membrane insertion.

Once the SecY channel has been opened and the initial downstream segment of hydrophilic polypeptide has been threaded through, there is no limit to the length of protein that can move across the membrane[24]. Translocation ends, and the channel reverts to its inactive closed state in one of two ways: termination of translation or emergence of a downstream TMD. Each of these is described in turn.

Translation termination allows translocation of the C terminus of the polypeptide through SecY. This translocated C-terminal domain remains anchored to the membrane by the preceding hydrophobic domain. The evolution of a membrane-bound protease that liberates the membrane-embedded hydrophobic domain led to the invention of protein secretion. The enzyme that carries out this reaction, called signal peptidase, is specific for particularly short hydrophobic domains known as signal peptides[27,28].

Emergence of a downstream TMD from the ribosome allows the TMD to enter SecY and pass through its lateral gate into the membrane[29,30]. In this way, a long loop of polypeptide between the translocation-initiating hydrophobic domain and translocation-terminating TMD is translocated to the non-cytosolic side of the membrane. If the translocation-initiating hydrophobic domain is a signal peptide, it is proteolytically cleaved to liberate the new N terminus on the *trans* site of the membrane. Thus, a long segment of hydrophilic polypeptide can be translocated through SecY, as long as it is preceded by a hydrophobic domain that engages the lateral gate of SecY.

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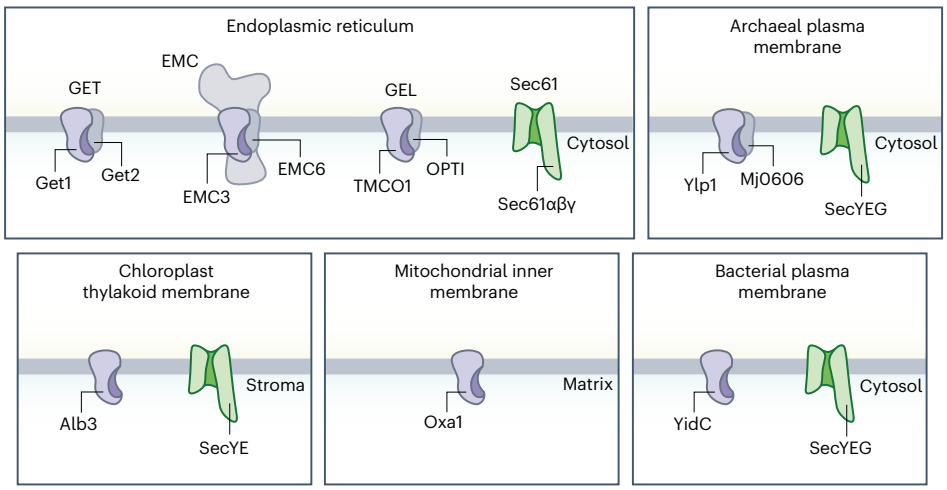
BOX 2

Membrane protein translocation machinery

The prokaryotic plasma membrane, eukaryotic ER and inner membranes of endosymbiont-derived organelles (mitochondria and plastids) derive from a common ancestor that already had membrane insertion factors in the Oxa1 and SecY families[7,107], depicted below in blue and green, respectively. The nomenclature for these families across species is heterogeneous (see figure) because of the manner and order of their discoveries[124]. SecY family members in prokaryotes and endosymbiont-derived organelles are still called SecY proteins, whereas those in the ER are termed Sec61 in *Saccharomyces cerevisiae* and Sec61α in other organisms. These channel-forming subunits are typically associated with two small membrane proteins that have different names in different organisms (Sec61β and Sec61γ in mammals; SecE and SecG in bacteria). Oxa1 family members in the mitochondrial inner membrane are called Oxa1, those in the inner chloroplast membrane are called Alb3 and those in the bacterial plasma membrane are called YidC. Oxa1 family members in the ER are called GET1, EMC3 and TMCO1, each of which is associated with a partner (GET2, EMC6 and OPTI, respectively) as part of the GET, EMC and GEL complexes. GET and EMC are found in most eukaryotes, whereas GEL is less common. Archaeal Oxa1 most closely resembles the ER counterparts and likely associates with a partner similar to GET2, EMC6 and OPTI, consistent with an archaeal origin for the ER. A small number of α-helical membrane proteins are inserted into the outer membrane of mitochondria and plastids, and possibly the peroxisomal membrane, by more recently evolved and unrelated machinery[121–126]. β-Barrel membrane proteins, found only in the outer membranes of bacteria, mitochondria and plastids, are inserted by a different specialized machinery[129,130].



A general model for membrane protein biogenesis

With mechanisms for translocating short hydrophilic tails and loops through Oxa1 and long hydrophilic domains through SecY, one can rationalize how the two together can mediate insertion of the full topologic range of membrane proteins (Box 1). To better convey the overall concept, we describe this framework using the general terms Oxa1 and SecY, rather than species-specific nomenclature (which is summarized in Box 2). The initial step is targeting of the nascent membrane protein to the lipid bilayer[31]. This typically occurs cotranslationally and is mediated by the first hydrophobic segment, either a signal peptide or TMD. This element is engaged by the signal-recognition particle (SRP) and is delivered to a receptor at the membrane, where the remainder of the protein is synthesized. When the only hydrophobic element or elements are within ~70 amino acids of the C terminus, targeting occurs post-translationally, aided by cytosolic factors that keep the substrate soluble until its arrival at the membrane[32–36]. After targeting, insertion of the TMD(s) is accompanied by flanking-domain translocation.

Membrane proteins without any long translocated domains can be inserted by Oxa1 family member(s) and do not require the SecY lateral gate or central channel[37–42]. This typically occurs cotranslationally for all TMDs, except those near the C terminus, which are inserted post-translationally[34,39,43]. Because TMDs flanked by short translocated domains can also insert without assistance[44,45], albeit with lower efficiency and increased risk of aggregation, the Oxa1 requirement for many substrates is not absolute. Proteins with one or more long translocated domains require SecY, with the preceding hydrophobic domain initiating translocation by engaging the SecY lateral gate[46].

Membrane proteins with multiple TMDs and translocated domains of different lengths use both Oxa1 and SecY for different regions, as dictated by translocated domain length. TMDs close to the N or C terminus with a short translocated tail use an Oxa1 family member operating co- or post-translationally, respectively[40,43]. All other TMDs are inserted cotranslationally by a membrane-bound ribosome using Oxa1 for short translocated loops and SecY for long translocated loops and termini[46,47]. During the cotranslational phase of biogenesis, the ribosome provides a binding platform for both Oxa1 and SecY family members[46,48], allowing the nascent chain to access the appropriate factor suited for each segment of polypeptide[46] (Fig. 2).

What emerges is a unified model in which biogenesis of a membrane proteome involves Oxa1 and SecY for the translocation of short and long segments of hydrophilic polypeptide, respectively (Fig. 3). Given that the activity of Oxa1 can be replaced by an unassisted mechanism (albeit with lower efficiency), particularly in the context of a

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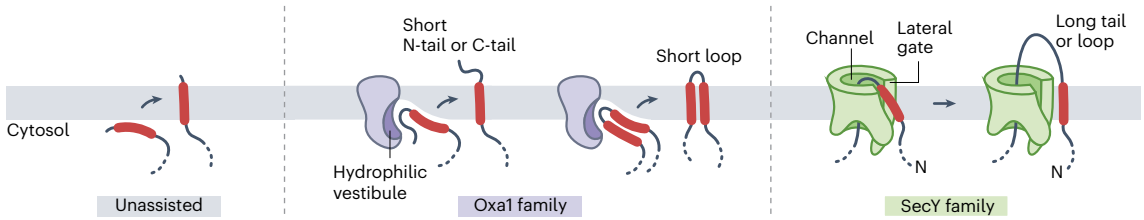

Fig. 1 | Mechanisms for insertion of α-helical membrane proteins. Left, unassisted membrane protein insertion can occur if the energetically favored reaction of TMD (red) partitioning into the hydrophobic membrane offsets the energetic cost of translocation of a short flanking segment of hydrophilic polypeptide. Middle, Oxa1 family members use a hydrophilic vestibule to facilitate translocation of short tails and loops during insertion of one or two TMDs. Right, SecY family members use a central channel to initiate the translocation of long tails and loops, concomitant with TMD insertion through a lateral gate.

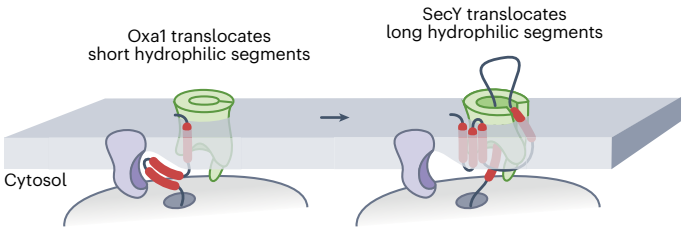

Fig. 2 | Oxa1 and SecY collaborate during multipass protein translocation. The ribosome serves as a binding platform for Oxa1 and SecY modules during multipass protein biogenesis. A nascent chain emerging from the ribosome can toggle between Oxa1 for translocation of short hydrophilic segments and SecY for translocation of long hydrophilic segments.

membrane-bound ribosome, its loss can be tolerated for some substrates and in certain cellular contexts. The translocation activity of SecY cannot be compensated, so it is dispensable only for substrates whose translocated hydrophilic domains are all short. Thus, the SecY family is needed for translocation of long hydrophilic domains, whereas Oxa1 family members carry out the majority of insertion reactions, given that the median length of the translocated domains of membrane proteins is ~20 amino acids in all organisms. This explains why bacteria, yeast, mammalian cells and endosymbiont organelles are each severely compromised or inviable following elimination of Oxa1 family member(s)[4,41,49,50]. The essentiality of secretion provides an explanation for why SecY deletion is lethal in all organisms[24].

Experimental support for the unifying model

A wide range of genetic, biochemical, structural and evolutionary data across experimental systems can be rationalized by the unifying model of membrane biogenesis proposed here. The experimental and predicted structures of diverse Oxa1 family members in bacteria, archaea, endosymbiont organelle inner membranes and the ER have a cytosol-facing hydrophilic vestibule that lowers the barrier for translocation of short domains, but contain no channel to support translocation of long domains[5,7,19,20,22,46,48,51–56]. By contrast, structures of prokaryotic and eukaryotic SecY family members show a reversibly plugged translocation channel and an adjacent lateral gate, where a hydrophobic signal has been observed in bacterial and mammalian systems[24–26,57].

Translocation of long hydrophilic domains coupled to membrane insertion of a preceding hydrophobic domain is strictly dependent on SecY. This finding is supported by immunodepletion experiments in vitro[40,58], SecY-inactivation and SecY-mutation experiments in cells[59–64] and sensitivity of translocation to inhibitors[46,65–72] that bind to and occlude the SecY lateral gate[73,74]. Membrane insertion of a few such substrates can be reconstituted with purified SecY in proteoliposomes[58,69,75–80], a reaction that cannot proceed if the lateral gate is covalently locked by a disulfide bond[81]. Although many of these studies used a cleavable signal peptide as the hydrophobic domain, the extrapolation to a TMD engaging the lateral gate in the same topology is compelling. Thus, SecY is both necessary and sufficient for translocation of long domains, initiated by lateral-gate-mediated membrane insertion of a preceding hydrophobic domain.

By sharp contrast, SecY lateral-gate inhibitors do not impact membrane proteins with short translocated domains[40,43,46,65,66,70–72,82]. Immunodepletion of SecY from mammalian ER microsomes had little or no effect on cotranslational insertion of N-terminal TMDs preceded by a short translocated tail[40] or post-translational insertion of C-terminal TMDs followed by a short translocated tail[34,40,44,83]. Sec-independent post-translational insertion of a C-terminal TMD has also been shown to occur in *Saccharomyces cerevisiae*[84]. The insertion of a number of membrane proteins, each with only short translocated domains, is unaffected upon acute SecY depletion in *Escherichia coli*[41,85–89]. Although other such proteins are affected by SecY depletion[76,85,90–92], interpretation of this result warrants caution because the SecY requirement could reflect its ribosome-binding function, not the use of the channel or lateral gate in insertion[46]. Despite this caveat, TMD insertion coupled to translocation of a short flanking domain generally occurs through a route or routes that do not depend on the SecY channel or lateral gate.

Conversely, depletion of Oxa1 family members in bacteria[41,62–64,87–89,91–94], inner endosymbiont organelle membranes[42,95–99] and the ER[39,40,43,46,47,100] impairs biogenesis of membrane proteins containing short translocated domains. This applies to N-tails, internal translocated loops and C-tails. Extending these elements either precludes translocation or makes translocation dependent on SecY through the preceding hydrophobic domain[43,46,101–103]. N- and C-tails can be physically crosslinked to the hydrophilic vestibule of both bacterial and eukaryotic Oxa1 family members before tail translocation[43,104,105]. SecY-mediated initiation of translocation by a preceding hydrophobic domain and Oxa1-mediated translocation of short domains by the adjacent TMD(s) has been reconstituted with purified prokaryotic and eukaryotic family members[37,39,40,58,76,77,79,80,106].

All membrane systems derived from the plasma membrane of the last universal common ancestor would have originally contained Oxa1 and SecY[7,107] (Box 2). At the inner mitochondrial membrane, across which long domains are no longer translocated from the matrix, SecY (but not Oxa1) is almost always absent in modern organisms. By contrast, SecY and Oxa1 are both retained in the inner membranes of plastids in which translocation of long domains from the stroma is still required. The selective retention of Oxa1 upon loss of long-domain translocation implies that SecY cannot effectively fulfill membrane-insertion reactions typically carried out by Oxa1. One reason could be that opening a closed SecY seems to be slow in native membranes[108,109], so the risk of losing translocation competence would be high when several TMDs are separated by short intervening segments. By contrast, the simple architecture of Oxa1 allows insertion to occur rapidly enough to keep up with translation. It is also possible that

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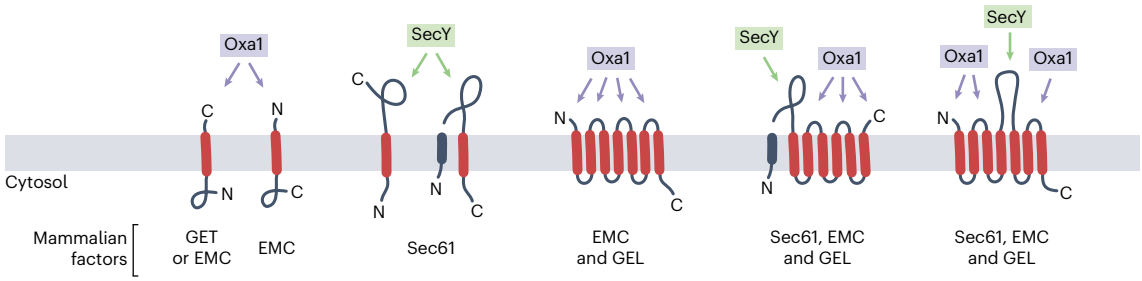

Fig. 3 | Division of labor between Oxa1 and SecY accommodates a diverse membrane proteome. SecY and Oxa1 family members not only work together on different parts of many multipass proteins, but can also work separately on other types of proteins. The translocated tails and loops of topologically diverse membrane proteins are labeled by which type of protein (Oxa1 or SecY family) mediates their translocation. The specific factors thought to be responsible for the different types of translocation in the mammalian ER are indicated (GET, EMC and GEL are Oxa1 family members, and Sec61 is a SecY family member).

the close proximity of the TMDs requires the second TMD to be pulled into the hydrophilic channel of SecY, an energetically unfavorable reaction that impedes lateral-gate engagement by the preceding TMD. By inserting both TMDs together, Oxa1 would bypass this problem.

The paradigm of the mammalian ER

The division of labor between Oxa1 and SecY for the range of substrates comprising the membrane proteome has been demonstrated in bacteria, yeast and mammals. The mechanistic basis of such division, and the cooperation between Oxa1 and SecY during multipass membrane protein biogenesis, is best understood in mammals. The mammalian ER contains three Oxa1 family members in larger complexes called GET, EMC and GEL[5,7,22,46,47]. The core of each complex contains an Oxa1 protein (GET1, EMC3 and TMCO1, respectively) associated with an obligate partner (GET2, EMC6 and OPTI, which are evolutionarily related to each other). The Oxa1 members and their partners derive from archaeal ancestors[7,52]. The SecY family members in eukaryotes are known as Sec61 (ref. 24).

GET, EMC and GEL collectively mediate insertion of TMDs flanked by short translocated domains, whereas Sec61 mediates insertion of TMDs followed by long translocated domains. The choice between GET, EMC and GEL for TMD insertion depends on context and hydrophobicity. GET is used for tail-anchored proteins with a high-hydrophobicity TMD[33,34,39,110–113]. This specificity is imposed by GET3, the targeting factor that delivers tail-anchored proteins to the GET1–GET2 complex. Tail-anchored proteins with lower hydrophobicity use EMC for insertion[39]. The overlap between GET and EMC usage among Tail-anchored protein substrates is substantial, so only the most and least hydrophobic TMDs are strongly reliant on GET and EMC, respectively. This redundancy, together with at least some capacity for unassisted insertion of tail-anchored proteins[44,45], explains why neither GET nor EMC is strictly essential at the single-cell level but the absence of both results in strong synthetic fitness costs[49].

The remaining single-pass membrane proteins target the membrane cotranslationally using SRP, then use either EMC or Sec61 for TMDs flanked by short or long translocated domains, respectively. EMC mediates the cotranslational insertion of TMDs preceded by a translocated N-tail composed of ~50 or fewer amino acids[40,82,104], whereas Sec61 mediates insertion of all other non-TA single-pass membrane proteins[58,70,71]. This clear segregation of pathways is supported by experiments that used Sec61 lateral-gate inhibitors, which inhibit only the latter class of proteins. Triage between these two routes occurs shortly after targeting[104], when nascent substrates first sample EMC for potential insertion before ribosome docking at Sec61. Thus, all single-pass proteins with a short translocated domain use an Oxa1 family member (either GET or EMC), whereas those with a long translocated domain use the Sec61 channel. This same segregation likely applies to *S. cerevisiae* (which has both GET and EMC) and *E. coli* (whose sole Oxa1 family member is YidC).

The insertion machinery used by multipass membrane proteins is also dictated by the length of the translocated domain(s). For proteins containing both short and long translocated domains, more than one factor is used during insertion. If the first translocated domain is short, EMC is used for insertion of the first (or first two) TMD(s) before ribosome docking at Sec61. Once docked on Sec61, subsequent insertion of pairs of TMDs proceeds by one of two routes. If the loop between them is short, the TMD pair is inserted by the ribosome-associating GEL complex[46,47], which is part of a larger multipass translocon[20,46–48,114]. If the loop between them is long, the first TMD engages the Sec61 lateral gate, the loop is translocated through the Sec61 channel and the second TMD inserts through the lateral gate. Although many eukaryotes, such as *S. cerevisiae*, do not contain the GEL complex, EMC could potentially fulfill this role.

The mechanism of toggling between Sec61 and GEL is not clear but seems to involve the PAT complex, a ribosome-binding chaperone conserved widely across eukaryotes[46,47,115]. Part of the ribosome-binding domain of the PAT complex is positioned between Sec61 and the ribosome to preclude opening of the Sec61 lateral gate[46]. In this configuration, the substrate is directed for insertion to GEL, which is adjacent to PAT. Emergence of a long loop downstream of a TMD might displace PAT to allow the TMD to engage the Sec61 lateral gate[46]. The key helix of PAT that blocks Sec61 opening also protrudes into the ribosome exit tunnel, suggestive of a potential mechanism by which accumulation of a non-translocated loop of substrate can trigger PAT displacement.

Insertion of pairs of TMDs through either GEL or Sec61 continues until termination. If there remains a final TMD whose C-terminal flanking domain needs to be translocated, the path used depends on C-tail length[43]. Those longer than ~50 amino acids use the lateral gate of Sec61 (as shown by their sensitivity to a Sec61 inhibitor), whereas shorter tails are translocated by EMC using a mechanism similar to tail-anchored protein insertion. A multipass protein can therefore toggle back and forth between different Oxa1 family members and Sec61, depending on the context of the TMD. For example, a protein could begin insertion using EMC, then a combination of GEL and Sec61 and return to EMC for the final TMD. Despite this complexity, the principle remains straightforward: the SecY family is needed when translocated domains are long, and Oxa1 family members are used when translocated domains are short.

We propose that this unifying principle applies across all forms of life and reflects the evolutionary origins and conservation of the Oxa1 and SecY families. Both the Oxa1 and SecY families are essential at the cell level across organisms. This is readily seen in organisms such as *E. coli*, in which only one of each family member is present, but is only now emerging in the eukaryotic ER, in which multiple Oxa1 family members afford some degree of redundancy and robustness. Although nuances in this paradigm will undoubtedly emerge, interpretations of past results and future studies will benefit from the guiding principle of a division of function between the Oxa1 and SecY families on the basis of length of the flanking translocated domain.

Future challenges

The body of evidence assembled to support our unifying framework for membrane protein insertion derives from many experimental systems using a range of model substrates. Although such diverse sources of data afford a degree of robustness to our model, it will be important to test each part of the model in a systematic manner in key prokaryotic and eukaryotic systems. Molecular dissection is best done in well-controlled and precisely manipulable biochemical systems, with proteome-wide analyses being used to generalize the findings. This is analogous to how the core principles and mechanisms of SRP-mediated targeting were derived from detailed analysis of model substrates[116], followed much later by proteome-wide validation[117–119]. The challenge now is to use fully reconstituted systems of membrane insertion to define the activities and limitations of each key factor, then use global in vivo analyses to corroborate the findings and reveal gaps in our understanding.

A related challenge is reconstruction of the hypothesized evolutionary path of membrane insertion using a series of increasingly complex membrane-protein insertion factors. This would begin with an empty liposome system capable of simple membrane insertion at low efficiency[44,45] and progressively build up the membrane-embedded factors to reach a minimal machinery for efficient biogenesis of complex membrane proteins thought to exist in the last universal common ancestor[7]. The ability to design membrane proteins with desired characteristics de novo[120] makes this goal feasible. Such a bottom-up reconstruction would define the core design principles and minimal requirements for translocation factors that facilitate membrane protein insertion. These insights might also help us understand the mechanisms of other types of insertion factors, such as those in endosymbiont organelle membranes[121–125] or peroxisomes[126], that emerged after the evolution of eukaryotes.

References

1. Weiss, M. C. et al. The physiology and habitat of the last universal common ancestor. *Nat. Microbiol.* **1**, 16116 (2016).
2. Baum, D. A. & Baum, B. An inside-out origin for the eukaryotic cell. *BMC Biol.* **12**, 76 (2014).
3. Zimorski, V., Ku, C., Martin, W. F. & Gould, S. B. Endosymbiotic theory for organelle origins. *Curr. Opin. Microbiol.* **22**, 38–48 (2014).
4. Hennon, S. W., Soman, R., Zhu, L. & Dalbey, R. E. YidC/Alb3/Oxa1 family of insertases. *J. Biol. Chem.* **290**, 14866–14874 (2015).
5. Anghel, S. A., McGilvray, P. T., Hegde, R. S. & Keenan, R. J. Identification of Oxa1 homologs operating in the eukaryotic endoplasmic reticulum. *Cell Rep.* **21**, 3708–3716 (2017).
6. Rapoport, T. A. Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes. *Nature* **450**, 663–669 (2007).
7. Lewis, A. J. O. & Hegde, R. S. A unified evolutionary origin for the ubiquitous protein transporters SecY and YidC. *BMC Biol.* **19**, 266 (2021).
8. White, S. H. & Wimley, W. C. Membrane protein folding and stability: physical principles. *Annu. Rev. Biophys. Biomol. Struct.* **28**, 319–365 (1999).
9. Engelman, D. M. & Steitz, T. A. The spontaneous insertion of proteins into and across membranes: the helical hairpin hypothesis. *Cell* **23**, 411–422 (1981).
10. Hunt, J. F., Rath, P., Rothschild, K. J. & Engelman, D. M. Spontaneous, pH-dependent membrane insertion of a transbilayer α-helix. *Biochemistry* **36**, 15177–15192 (1997).
11. Wimley, W. C. & White, S. H. Designing transmembrane α-helices that insert spontaneously. *Biochemistry* **39**, 4432–4442 (2000).
12. White, S. H. & Wimley, W. C. Hydrophobic interactions of peptides with membrane interfaces. *Biochim. Biophys. Acta* **1376**, 339–352 (1998).
13. Harris, N. J. et al. Methods to study folding of α-helical membrane proteins in lipids. *Open Biol.* **12**, 220054 (2022).
14. Harris, N. J. et al. Structure formation during translocon-unassisted co-translational membrane protein folding. *Sci. Rep.* **7**, 8021 (2017).
15. Rues, R. B., Dötsch, V. & Bernhard, F. Co-translational formation and pharmacological characterization of β1-adrenergic receptor/nanodisc complexes with different lipid environments. *Biochim. Biophys. Acta* **1858**, 1306–1316 (2016).
16. Roos, C. et al. High-level cell-free production of membrane proteins with nanodiscs. *Methods Mol. Biol.* **1118**, 109–130 (2014).
17. Long, A. R., O'Brien, C. C. & Alder, N. N. The cell-free integration of a polytopic mitochondrial membrane protein into liposomes occurs cotranslationally and in a lipid-dependent manner. *PLoS ONE* **7**, e46332 (2012).
18. Soga, H. et al. In vitro membrane protein synthesis inside cell-sized vesicles reveals the dependence of membrane protein integration on vesicle volume. *ACS Synth. Biol.* **3**, 372–379 (2014).
19. Kumazaki, K. et al. Structural basis of Sec-independent membrane protein insertion by YidC. *Nature* **509**, 516–520 (2014).
20. Hegde, R. S. & Keenan, R. J. The mechanisms of integral membrane protein biogenesis. *Nat. Rev. Mol. Cell Biol.* **23**, 107–124 (2022).
21. Wu, X. & Rapoport, T. A. Translocation of proteins through a distorted lipid bilayer. *Trends Cell Biol.* **31**, 473–484 (2021).
22. McDowell, M. A., Heimes, M. & Sinning, I. Structural and molecular mechanisms for membrane protein biogenesis by the Oxa1 superfamily. *Nat. Struct. Mol. Biol.* **28**, 234–239 (2021).
23. Saracco, S. A. & Fox, T. D. Cox18p is required for export of the mitochondrially encoded *Saccharomyces cerevisiae* Cox2p C-tail and interacts with Pnt1p and Mss2p in the inner membrane. *Mol. Biol. Cell* **13**, 1122–1131 (2002).
24. Rapoport, T. A., Li, L. & Park, E. Structural and mechanistic insights into protein translocation. *Annu. Rev. Cell Dev. Biol.* **33**, 369–390 (2017).
25. Voorhees, R. M. & Hegde, R. S. Structure of the Sec61 channel opened by a signal sequence. *Science* **351**, 88–91 (2016).
26. Li, L. et al. Crystal structure of a substrate-engaged SecY protein-translocation channel. *Nature* **531**, 395–399 (2016).
27. Liaci, A. M. et al. Structure of the human signal peptidase complex reveals the determinants for signal peptide cleavage. *Mol. Cell* **81**, 3934–3948(2021).
28. Auclair, S. M., Bhanu, M. K. & Kendall, D. A. Signal peptidase I: cleaving the way to mature proteins. *Protein Sci.* **21**, 13–25 (2012).
29. Do, H., Falcone, D., Lin, J., Andrews, D. W. & Johnson, A. E. The cotranslational integration of membrane proteins into the phospholipid bilayer is a multistep process. *Cell* **85**, 369–378 (1996).
30. Spencer Yost, C., Hedgpeth, J. & Lingappa, V. R. A stop transfer sequence confers predictable transmembrane orientation to a previously secreted protein in cell-free systems. *Cell* **34**, 759–766 (1983).
31. Akopian, D., Shen, K., Zhang, X. & Shan, S. O. Signal recognition particle: an essential protein-targeting machine. *Annu. Rev. Biochem.* **82**, 693–721 (2013).
32. Shan, Sou Guiding tail-anchored membrane proteins to the endoplasmic reticulum in a chaperone cascade. *J. Biol. Chem.* **294**, 16577–16586 (2019).
33. Wang, F., Brown, E. C., Mak, G., Zhuang, J. & Denic, V. A chaperone cascade sorts proteins for posttranslational membrane insertion into the endoplasmic reticulum. *Mol. Cell* **40**, 159–171 (2010).
34. Stefanovic, S. & Hegde, R. S. Identification of a targeting factor for posttranslational membrane protein insertion into the ER. *Cell* **128**, 1147–1159 (2007).

35. Mariappan, M. et al. A ribosome-associating factor chaperones tail-anchored membrane proteins. *Nature* **466**, 1120–1124 (2010).
36. Cho, H. & Shan, S. Substrate relay in an Hsp70-cochaperone cascade safeguards tail-anchored membrane protein targeting. *EMBO J.* **37**, e99264 (2018).
37. Mariappan, M. et al. The mechanism of membrane-associated steps in tail-anchored protein insertion. *Nature* **477**, 61–66 (2011).
38. Wang, F., Chan, C., Weir, N. R. & Denic, V. The Get1/2 transmembrane complex is an endoplasmic-reticulum membrane protein insertase. *Nature* **512**, 441–444 (2014).
39. Guna, A., Volkmar, N., Christianson, J. C. & Hegde, R. S. The ER membrane protein complex is a transmembrane domain insertase. *Science* **359**, 470–473 (2018).
40. Chitwood, P. J., Juszkiewicz, S., Guna, A., Shao, S. & Hegde, R. S. EMC is required to initiate accurate membrane protein topogenesis. *Cell* **175**, 1507–1519(2018).
41. Samuelson, J. C. et al. YidC mediates membrane protein insertion in bacteria. *Nature* **406**, 637–641 (2000).
42. Hell, K., Neupert, W. & Stuart, R. A. Oxa1p acts as a general membrane insertion machinery for proteins encoded by mitochondrial DNA. *EMBO J.* **20**, 1281–1288 (2001).
43. Wu, H., Smalinskaitė, L. & Hegde, R. S. EMC rectifies the topology of multipass membrane proteins. *Nat. Struct. Mol. Biol.* **31**, 32–41(2024).
44. Brambillasca, S. et al. Transmembrane topogenesis of a tail-anchored protein is modulated by membrane lipid composition. *EMBO J.* **24**, 2533–2542 (2005).
45. Brambillasca, S., Yabal, M., Makarow, M. & Borgese, N. Unassisted translocation of large polypeptide domains across phospholipid bilayers. *J. Cell Biol.* **175**, 767–777 (2006).
46. Smalinskaitė, L., Kim, M. K., Lewis, A. J. O., Keenan, R. J. & Hegde, R. S. Mechanism of an intramembrane chaperone for multipass membrane proteins. *Nature* **611**, 161–166 (2022).
47. Sundaram, A. et al. Substrate-driven assembly of a translocon for multipass membrane proteins. *Nature* **611**, 167–172 (2022).
48. McGilvray, P. T. et al. An ER translocon for multi-pass membrane protein biogenesis. *eLife* **9**, e56889 (2020).
49. Guna, A. et al. A dual sgRNA library design to probe genetic modifiers using genome-wide CRISPRi screens. *BMC Genomics* **24**, 651 (2023).
50. Jonikas, M. C. et al. Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum. *Science* **323**, 1693–1697 (2009).
51. McDowell, M. A. et al. Structural basis of tail-anchored membrane protein biogenesis by the GET insertase complex. *Mol. Cell* **80**, 72–86 (2020).
52. Borowska, M. T., Dominik, P. K., Anghel, S. A., Kossiakoff, A. A. & Keenan, R. J. A YidC-like protein in the archaeal plasma membrane. *Structure* **23**, 1715–1724 (2015).
53. Bai, L., You, Q., Feng, X., Kovach, A. & Li, H. Structure of the ER membrane complex, a transmembrane-domain insertase. *Nature* **584**, 475–478 (2020).
54. O'Donnell, J. P. et al. The architecture of EMC reveals a path for membrane protein insertion. *eLife* **9**, e57887 (2020).
55. Pleiner, T. et al. Structural basis for membrane insertion by the human ER membrane protein complex. *Science* **369**, 433–436 (2020).
56. Miller-Vedam, L. E. et al. Structural and mechanistic basis of the EMC-dependent biogenesis of distinct transmembrane clients. *eLife* **9**, e62611 (2020).
57. Van den Berg, B. et al. X-ray structure of a protein-conducting channel. *Nature* **427**, 36–44 (2004).
58. Görlich, D. & Rapoport, T. A. Protein translocation into proteoliposomes reconstituted from purified components of the endoplasmic reticulum membrane. *Cell* **75**, 615–630 (1993).
59. Stirling, C. J., Rothblatt, J., Hosobuchi, M., Deshaies, R. & Schekman, R. Protein translocation mutants defective in the insertion of integral membrane proteins into the endoplasmic reticulum. *Mol. Biol. Cell* **3**, 129–142 (1992).
60. Reithinger, J. H., Yim, C., Kim, S., Lee, H. & Kim, H. Structural and functional profiling of the lateral gate of the sec61 translocon. *J. Biol. Chem.* **289**, 15845–15855 (2014).
61. Kuhn, A. Alterations in the extracellular domain of M13 procoat protein make its membrane insertion dependent on secA and secY. *Eur. J. Biochem.* **177**, 267–271 (1988).
62. Van Bloois, E., Haan, G. J., De Gier, J. W., Oudega, B. & Luirink, J. Distinct requirements for translocation of the N-tail and C-tail of the *Escherichia coli* inner membrane protein CyoA. *J. Biol. Chem.* **281**, 10002–10009 (2006).
63. Celebi, N., Yi, L., Facey, S. J., Kuhn, A. & Dalbey, R. E. Membrane biogenesis of subunit II of cytochrome bo oxidase: contrasting requirements for insertion of N-terminal and C-terminal domains. *J. Mol. Biol.* **357**, 1428–1436 (2006).
64. Du Plessis, D. J. F., Nouwen, N. & Driessen, A. J. M. Subunit a of cytochrome o oxidase requires both YidC and SecYEG for membrane insertion. *J. Biol. Chem.* **281**, 12248–12252 (2006).
65. Tranter, D. et al. Coibamide A targets Sec61 to prevent biogenesis of secretory and membrane proteins. *ACS Chem. Biol.* **15**, 2125–2136 (2020).
66. Paatero, A. O. et al. Apratoxin kills cells by direct blockade of the Sec61 protein translocation channel. *Cell. Chem. Biol.* **23**, 561–566 (2016).
67. Zong, G. et al. Ipomoeassin F binds Sec61α to inhibit protein translocation. *J. Am. Chem. Soc.* **141**, 8450–8461 (2019).
68. Maifeld, S. V. et al. Secretory protein profiling reveals TNF-α inactivation by selective and promiscuous Sec61 modulators. *Chem. Biol.* **18**, 1082–1088 (2011).
69. Garrison, J. L., Kunkel, E. J., Hegde, R. S. & Taunton, J. A substrate-specific inhibitor of protein translocation into the endoplasmic reticulum. *Nature* **436**, 285–289 (2005).
70. McKenna, M., Simmonds, R. E. & High, S. Mycolactone reveals the substrate-driven complexity of Sec61-dependent transmembrane protein biogenesis. *J. Cell Sci.* **130**, 1307–1320 (2017).
71. Morel, J.-D. et al. Proteomics reveals scope of mycolactone-mediated Sec61 blockade and distinctive stress signature. *Mol. Cell. Proteomics* **17**, 1750–1765 (2018).
72. Junne, T. et al. Decatransin, a new natural product inhibiting protein translocation at the Sec61/SecYEG translocon. *J. Cell Sci.* **128**, 1217–1229 (2015).
73. Itskanov, S. et al. A common mechanism of Sec61 translocon inhibition by small molecules. *Nat. Chem. Biol.* **19**, 1063–1071 (2023).
74. Rehan, S. et al. Signal peptide mimicry primes Sec61 for client-selective inhibition. *Nat. Chem. Biol.* **19**, 1054–1062 (2023).
75. Hegde, R. S., Voigt, S. & Lingappa, V. R. Regulation of protein topology by *trans*-acting factors at the endoplasmic reticulum. *Mol. Cell* **2**, 85–91 (1998).
76. Welte, T. et al. Promiscuous targeting of polytopic membrane proteins to SecYEG or YidC by the *Escherichia coli* signal recognition particle. *Mol. Biol. Cell* **23**, 464–479 (2012).
77. Van Der Laan, M., Houben, E. N. G., Nouwen, N., Luirink, J. & Driessen, A. J. M. Reconstitution of Sec-dependent membrane protein insertion: nascent FtsQ interacts with YidC in a Sec YEG-dependent manner. *EMBO Rep.* **2**, 519–523 (2001).
78. Bariya, P. & Randall, L. L. Coassembly of SecyEG and SecA fully restores the properties of the native translocon. *J. Bacteriol.* **201**, e00493-18 (2019).
79. Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J. M. & Wickner, W. The purified *E. coli* integral membrane protein SecY E is sufficient for reconstitution of SecA-dependent precursor protein translocation. *Cell* **62**, 649–657 (1990).

80. Nishiyama, K., Mizushima, S. & Tokuda, H. A novel membrane protein involved in protein translocation across the cytoplasmic membrane of *Escherichia coli*. *EMBO J.* **12**, 3409–3415 (1993).
81. du Plessis, D. J. F., Berrelkamp, G., Nouwen, N. & Driessen, A. J. M. The lateral gate of SecYEG opens during protein translocation. *J. Biol. Chem.* **284**, 15805–15814 (2009).
82. O'Keefe, S. et al. An alternative pathway for membrane protein biogenesis at the endoplasmic reticulum. *Commun. Biol.* **4**, 828 (2021).
83. Kutay, U., Ahnert-Hilger, G., Hartmann, E., Wiedenmann, B. & Rapoport, T. A. Transport route for synaptobrevin via a novel pathway of insertion into the endoplasmic reticulum membrane. *EMBO J.* **14**, 217–223 (1995).
84. Yabal, M. et al. Translocation of the C terminus of a tail-anchored protein across the endoplasmic reticulum membrane in yeast mutants defective in signal peptide-driven translocation. *J. Biol. Chem.* **278**, 3489–3496 (2003).
85. Yi, L., Celebi, N., Chen, M. & Dalbey, R. E. Sec/SRP requirements and energetics of membrane insertion of subunits a, b, and c of the *Escherichia coli* F1F0 ATP synthase. *J. Biol. Chem.* **279**, 39260–39267 (2004).
86. Van Bloois, E., Jan Haan, G., De Gier, J. W., Oudega, B. & Luirink, J. F1F0 ATP synthase subunit c is targeted by the SRP to YidC in the *E. coli* inner membrane. *FEBS Lett.* **576**, 97–100 (2004).
87. Van Der Laan, M., Bechduft, P., Kol, S., Nouwen, N. & Driessen, A. J. M. F1F0 ATP synthase subunit c is a substrate of the novel YidC pathway for membrane protein biogenesis. *J. Cell Biol.* **165**, 213–222 (2004).
88. Facey, S. J., Neugebauer, S. A., Krauss, S. & Kuhn, A. The mechanosensitive channel protein MscL is targeted by the SRP to the novel YidC membrane insertion pathway of *Escherichia coli*. *J. Mol. Biol.* **365**, 995–1004 (2007).
89. Chen, M. et al. Direct interaction of YidC with the Sec-independent Pf3 coat protein during its membrane protein insertion. *J. Biol. Chem.* **277**, 7670–7675 (2002).
90. Shimokawa-Chiba, N. et al. Hydrophilic microenvironment required for the channel-independent insertase function of YidC protein. *Proc. Natl Acad. Sci. USA* **112**, 5063–5068 (2015).
91. Price, C. E. & Driessen, A. J. M. Conserved negative charges in the transmembrane segments of subunit K of the NADH:ubiquinone oxidoreductase determine its dependence on YidC for membrane insertion. *J. Biol. Chem.* **285**, 3575–3581 (2010).
92. Kol, S. et al. Subunit a of the F1F0 ATP synthase requires YidC and SecYEG for membrane insertion. *J. Mol. Biol.* **390**, 893–901 (2009).
93. Yi, L. et al. YidC is strictly required for membrane insertion of subunits a and c of the F1F0ATP synthase and SecE of the SecYEG translocase. *Biochemistry* **42**, 10537–10544 (2003).
94. Dalbey, R. E., Kuhn, A., Zhu, L. & Kiefer, D. The membrane insertase YidC. *Biochim. Biophys. Acta* **1843**, 1489–1496 (2014).
95. Stuart, R. A. Insertion of proteins into the inner membrane of mitochondria: the role of the Oxa1 complex. *Biochim. Biophys. Acta* **1592**, 79–87 (2002).
96. Moore, M., Harrison, M. S., Peterson, E. C. & Henry, R. Chloroplast Oxa1p homolog albino3 is required for post-translational integration of the light harvesting chlorophyll-binding protein into thylakoid membranes. *J. Biol. Chem.* **275**, 1529–1532 (2000).
97. Bellafiore, S., Ferris, P., Naver, H., Göhre, V. & Rochaix, J. D. Loss of Albino3 leads to the specific depletion of the light-harvesting system. *Plant Cell* **14**, 2303–2314 (2002).
98. Göhre, V., Ossenbühl, F., Crèvecoeur, M., Eichacker, L. A. & Rochaix, J. D. One of two Alb3 proteins is essential for the assembly of the photosystems and for cell survival in *Chlamydomonas*. *Plant Cell* **18**, 1454–1466 (2006).
99. Króliczewski, J., Piskozub, M., Bartoszewski, R. & Króliczewska, B. ALB3 insertase mediates cytochrome b_6 co-translational import into the thylakoid membrane. *Sci. Rep.* **6**, 34557 (2016).
100. Schuldiner, M. et al. The GET complex mediates insertion of tail-anchored proteins into the ER membrane. *Cell* **134**, 634–645 (2008).
101. Shanmugam, S. K. et al. New insights into amino-terminal translocation as revealed by the use of YidC and Sec depletion strains. *J. Mol. Biol.* **431**, 1025–1037 (2019).
102. Soman, R., Yuan, J., Kuhn, A. & Dalbey, R. E. Polarity and charge of the periplasmic loop determine the YidC and sec translocase requirement for the M13 procoat lep protein. *J. Biol. Chem.* **289**, 1023–1032 (2014).
103. Hariharan, B. et al. Polarity/charge as a determinant of translocase requirements for membrane protein insertion. *Biochim. Biophys. Acta* **1863**, 183502 (2021).
104. Wu, H. & Hegde, R. S. Mechanism of signal-anchor triage during early steps of membrane protein insertion. *Mol. Cell* **83**, 961–973(2023).
105. He, H., Kuhn, A. & Dalbey, R. E. Tracking the stepwise movement of a membrane-inserting protein in vivo. *J. Mol. Biol.* **432**, 484–496 (2020).
106. Wang, F., Whynot, A., Tung, M. & Denic, V. The mechanism of tail-anchored protein insertion into the ER membrane. *Mol. Cell* **43**, 738–750 (2011).
107. Petrů, M., Dohnálek, V., Füssy, Z. & Pavel, D. Fates of Sec, Tat, and YidC translocases in mitochondria and other eukaryotic compartments. *Mol. Biol. Evol.* **38**, 5241–5254 (2021).
108. Kim, S. J., Mitra, D., Salerno, J. R. & Hegde, R. S. Signal sequences control gating of the protein translocation channel in a substrate-specific manner. *Dev. Cell* **2**, 207–217 (2002).
109. Trueman, S. F., Mandon, E. C. & Gilmore, R. Translocation channel gating kinetics balances protein translocation efficiency with signal sequence recognition fidelity. *Mol. Biol. Cell* **22**, 2983–2993 (2011).
110. Rao, M. et al. Multiple selection filters ensure accurate tail-anchored membrane protein targeting. *eLife* **5**, e21301 (2016).
111. Mateja, A. et al. Structure of the Get3 targeting factor in complex with its membrane protein cargo. *Science* **347**, 1152–1155 (2015).
112. Shao, S., Rodrigo-Brenni, M. C., Kivlen, M. H. & Hegde, R. S. Mechanistic basis for a molecular triage reaction. *Science* **355**, 298–302 (2017).
113. Fry, M. Y. et al. Structurally derived universal mechanism for the catalytic cycle of the tail-anchored targeting factor Get3. *Nat. Struct. Mol. Biol.* **29**, 820–830 (2022).
114. Smalinskaitė, L. & Hegde, R. S. The biogenesis of multipass membrane proteins. *Cold Spring Harb. Perspect. Biol.* **15**, a041251 (2023).
115. Chitwood, P. J. & Hegde, R. S. An intramembrane chaperone complex facilitates membrane protein biogenesis. *Nature* **584**, 630–634 (2020).
116. Keenan, R. J., Freymann, D. M., Stroud, R. M. & Walter, P. The signal recognition particle. *Annu. Rev. Biochem.* **70**, 755–775 (2001).
117. Schibich, D. et al. Global profiling of SRP interaction with nascent polypeptides. *Nature* **536**, 219–223 (2016).
118. Chartron, J. W., Hunt, K. C. L. & Frydman, J. Cotranslational signal-independent SRP preloading during membrane targeting. *Nature* **536**, 224–228 (2016).
119. Costa, E. A., Subramanian, K., Nunnari, J. & Weissman, J. S. Defining the physiological role of SRP in protein-targeting efficiency and specificity. *Science* **359**, 689–692 (2018).
120. Lu, P. et al. Accurate computational design of multipass transmembrane proteins. *Science* **359**, 1042–1046 (2018).
121. Wiedemann, N. & Pfanner, N. Mitochondrial machineries for protein import and assembly. *Annu. Rev. Biochem.* **86**, 685–714 (2017).

122. Shi, L.-X. & Theg, S. M. The chloroplast protein import system: from algae to trees. *Biochim. Biophys. Acta* **1833**, 314–331 (2013).
123. Guna, A. et al. MTCH2 is a mitochondrial outer membrane protein insertase. *Science* **378**, 317–322 (2022).
124. Kizmaz, B. & Herrmann, J. M. Membrane insertases at a glance. *J. Cell Sci.* **136**, jcs261219 (2023).
125. Gupta, A. & Becker, T. Mechanisms and pathways of mitochondrial outer membrane protein biogenesis. *Biochim. Biophys. Acta* **1862**, 148323 (2021).
126. Mayerhofer, P. U. Targeting and insertion of peroxisomal membrane proteins: ER trafficking versus direct delivery to peroxisomes. *Biochim. Biophys. Acta* **1863**, 870–880 (2016).
127. von Heijne, G. The membrane protein universe: what's out there and why bother? *J. Intern. Med.* **261**, 543–557 (2007).
128. Higy, M., Junne, T. & Spiess, M. Topogenesis of Membrane Proteins at the Endoplasmic Reticulum. *Biochemistry* **43**, 12716–12722 (2004).
129. Tomasek, D. & Kahne, D. The assembly of β-barrel outer membrane proteins. *Curr. Opin. Microbiol.* **60**, 16–23 (2021).
130. Höhr, A. I. C., Straub, S. P., Warscheid, B., Becker, T. & Wiedemann, N. Assembly of β-barrel proteins in the mitochondrial outer membrane. *Biochim. Biophys. Acta* **1853**, 74–88 (2015).

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Competing interests

The authors declare no competing interests.

Additional information

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