# Protein biosynthesis at the ER: finding the right accessories

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**ABSTRACT** More than 30% of eukaryotic proteins contain domains that must translocate across or integrate into the endoplasmic reticulum (ER) membrane. With few exceptions, protein translocation and transmembrane domain integration at the ER require the conserved Sec61 translocon. Decades of studies have established a clear mechanistic model for how the Sec61 translocon functions. The biosynthesis of distinct subsets of proteins at the ER also involves accessory factors that interact with the Sec61 translocon and translocating nascent proteins. However, assigning specific functions to many translocon accessory factors has been a persistent challenge in the field. This *Perspective* discusses recent insights into mechanisms that promote protein biosynthesis at the ER through accessory factors that directly regulate the Sec61 translocon or chaperone nascent proteins within the ER membrane. These translocon accessory factor functions, and more still to be discovered, are essential for producing a diverse and high-fidelity proteome at the ER.

## THE SEC TRANSLOCON FACILITATES PROTEIN BIOSYNTHESIS AT THE ER

Protein translocation across or integration into a lipid bilayer are fundamental cellular processes facilitated by the conserved "Sec translocon" at the eukaryotic endoplasmic reticulum (ER) or the prokaryotic periplasmic membrane (Komarudin and Driessen, 2019; Itskanov and Park, 2022). The Sec translocon provides proteins access to the exterior of the cell, either directly in prokaryotes or through vesicular trafficking in eukaryotes. The core translocon contains a central subunit, Sec61α (SecY in prokaryotes), in complex with Sec61β and Sec61γ (SecG and SecE, respectively, in prokaryotes). Sec61α forms a membrane-spanning channel that allows for the translocation of unfolded hydrophilic polypeptides from the cytosol into the ER lumen (van den Berg et al., 2004). The Sec61α channel also has a lateral gate that provides polypeptides access from the channel to the lipid bilayer (Figure 1A). The lateral gate engages and is opened by hydrophobic α-helices in the form of cleavable N-terminal signal sequences that initiate protein translocation or transmembrane helices that partition into the membrane.

The Sec translocon functions with several partners. The most common translocon partners are ribosomes, which bind the cytosolic side of Sec61α and drive cotranslational translocation of nascent polypeptides through the channel. While cotranslational protein import into the ER is dominant in metazoans, posttranslational translocation is common in yeast and prokaryotes. In yeast, posttranslational translocation requires the Sec61 translocon in complex with Sec62/Sec63/Sec71/Sec72, which recruits the ER-resident Hsp70 protein BiP through a J domain on Sec63. BiP binds translocating nascent proteins in the ER lumen, thereby preventing the proteins from sliding back into the cytosol. In prokaryotes, the cytosolic ATPase SecA binds and facilitates nascent protein movement through the translocon through cycles of ATP hydrolysis (Komarudin and Driessen, 2019). Extensive biochemical and structural studies on the mechanism of the Sec translocon in cooperation with these partners are well-reviewed elsewhere (Komarudin and Driessen, 2019; Hegde and Keenan, 2022; Itskanov and Park, 2022). This *Perspective* discusses recent mechanistic insights into eukaryotic translocon accessory factors.

## TRANSLOCON ACCESSORY FACTORS MEDIATE DISTINCT ASPECTS OF PROTEIN BIOSYNTHESIS

Protein import into the ER has been reconstituted with purified components comprising the Sec61 complex, a functional partner, and targeting factors (Görlich and Rapoport, 1993; Panzner et al., 1995; Matlack et al., 1999). However, these components are insufficient for the successful biogenesis of many proteins at the
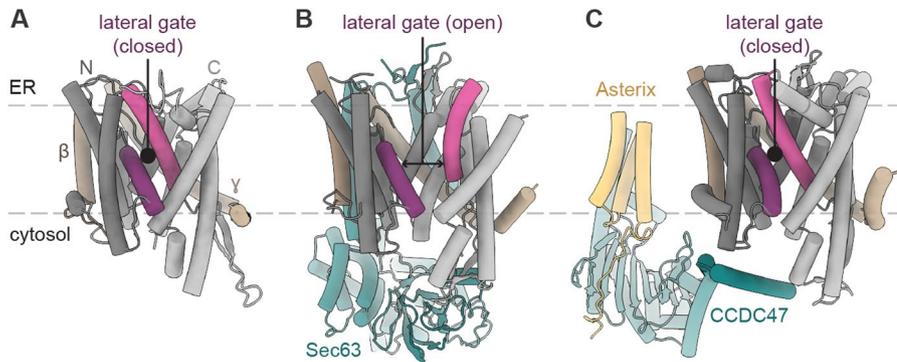
**FIGURE 1:** Accessory factors modulate translocon conformations. (A) The isolated Sec translocon (Protein Data Bank [PDB] 1RH5). Lateral gate helices (purple) and the N-terminal (dark gray) and the C-terminal (light gray) halves of the Sec61α homologue are indicated along with the Sec61β and Sec61γ homologues. (B) The yeast posttranslational translocon (PDB 6N3Q). Sec63 (teal) acts as a brace holding the Sec61α lateral gate open; Sec62 and the yeast-specific Sec72 and Sec73 subunits are not shown. (C) The mammalian translocon associated with the PAT complex (PDB 7TM3) comprising Asterix (yellow) and CCDC47 (teal). The translocon-bound ribosome and the GEL and BOS complexes are not shown.

ER. Particularly in metazoan cells, various "accessory" factors identified through interactions with Sec61 and/or translocating nascent proteins are thought to aid protein biogenesis at the ER (Wiedmann *et al.*, 1987; Görlich *et al.*, 1992; Görlich and Rapoport, 1993; McGilvray *et al.*, 2020; Hegde and Keenan, 2022; Itskanov and Park, 2022). Unlike the translocon partners described above, accessory factors are not required for the basic process of protein translocation through Sec61 but instead facilitate biosynthetic processes specific to distinct subsets of substrates. A few translocon accessory factors, such as the OST (oligosaccharyltransferase) complex, which mediates N-linked glycosylation in the ER, or the signal peptidase complex, which cleaves off signal sequences, have clear functions (Itskanov and Park, 2022). Other accessory factors, such as TRAP (translocon-associated protein complex; also known as the signal sequence receptor, SSR) and TRAM (translocating chain-associating membrane protein), interact with and enhance the biogenesis of numerous substrates (Wiedmann *et al.*, 1987; Görlich *et al.*, 1992; Görlich and Rapoport, 1993; Voigt *et al.*, 1996; Hegde *et al.*, 1998; Meacock *et al.*, 2002; Fons *et al.*, 2003; Sommer *et al.*, 2013; Nguyen *et al.*, 2018; Kriegler *et al.*, 2020; Huang *et al.*, 2021) but have poorly defined mechanisms. Only recently have more precise mechanistic insights into certain translocon accessory factors emerged.

## SEC62/SEC63: A TRANSLOCON MODIFIER
Discovered more than three decades ago (Rothblatt *et al.*, 1989), Sec62/Sec63 is now well appreciated to be physically and functionally linked with the Sec61 translocon. In yeast, Sec62/Sec63/Sec71/Sec72 mediates the posttranslational translocation of proteins that generally have less hydrophobic signal sequences than cotranslationally translocated proteins (Ng *et al.*, 1996). Although posttranslational translocation is uncommon in mammals, Sec62/Sec63 (but not Sec71 or Sec72) is conserved and implicated in an accessory role during the cotranslational biogenesis of certain substrates (Conti *et al.*, 2015; Schorr *et al.*, 2020). Mammalian substrates that rely on Sec62/Sec63 are reported to have less hydrophobic signal sequences and may be especially dependent on BiP recruitment to fold (Conti *et al.*, 2015; Schorr *et al.*, 2020).

A significant advance in understanding how Sec62/Sec63 regulates translocon activity came from recent cryogenic electron microscopy (cryo-EM) structures of the yeast posttranslational translocon complex (Itskanov and Park, 2019; Wu *et al.*, 2019; Itskanov *et al.*, 2021; Weng *et al.*, 2021). In the structures, Sec62/Sec63 acts as a dynamic brace capable of fully opening the lateral gate of the channel (Figure 1B). The effect of Sec62/Sec63 on the conformation of Sec61α is distinct from how translocon binding partners slightly crack the lateral gate to "prime" the translocon for signal sequence engagement (Itskanov and Park, 2022). Full lateral gate opening by Sec62/Sec63 may lower the energetic barrier for nonoptimal signal sequences to initiate protein translocation. Once engaged at the lateral gate, signal sequences may be further stabilized by interaction with Sec62 (Lyman and Schekman, 1997; Plath *et al.*, 1998; Itskanov *et al.*, 2021; Weng *et al.*, 2021). Thus, these structures establish Sec62/Sec63 as an accessory complex that directly regulates the conformation and function of the translocon in addition to its role in recruiting BiP. How Sec62/Sec63 operates cotranslationally, when a ribosome bound to Sec61α is predicted to clash with cytosolic portions of the complex (Itskanov and Park, 2019; Wu *et al.*, 2019), remains to be determined.

## PAT-10/ASTERIX: AN INTRAMEMBRANE PROTEIN CHAPERONE
Many translocon accessory factors probably act directly on nascent proteins. A recently clarified example is the eukaryotic protein Asterix. Early site-specific cross-linking studies identified an interaction between PAT-10 (protein associated with the ER translocon of 10 kDa) and the first transmembrane helix of the multispanning membrane protein rhodopsin (Meacock *et al.*, 2002). This interaction was specific, as PAT-10 cross-links were not observed with other transmembrane helices of the protein (Meacock *et al.*, 2002; Ismail *et al.*, 2006). PAT-10 continues to interact with the first transmembrane helix even after that helix ceases to interact with Sec61α and dissociates only after complete synthesis of the entire protein. These findings implicated PAT-10, whose sequence was unknown at the time, in the biogenesis of multispanning membrane proteins. The probable identity of PAT-10 would not be revealed until nearly 20 years later. With improved mass spectrometry approaches facilitating protein identification, affinity purifications identified the ~10 kDa nascent protein interactor as Asterix, which forms the obligate PAT complex with CCDC47 (Chitwood and Hegde, 2020). Asterix interacts more strongly with transmembrane helices containing hydrophilic amino acids, which may be critical for membrane protein folding and function but are energetically unfavorable within the lipid bilayer. Depleting either CCDC47 or Asterix destabilizes the other PAT complex partner and impairs the biosynthesis of numerous mutispanning membrane proteins (Chitwood and Hegde, 2020; Sundaram *et al.*, 2022), thereby functionally linking the interaction between Asterix and nonoptimal transmembrane helices to protein biosynthesis.

Assignment of Asterix and CCDC47 as translocon accessory factors is further validated by cryo-EM structures (McGilvray *et al.*, 2020; Smalinskaitė *et al.*, 2022). The structures showed ribosome–Sec61

complexes associated not only with 1) the PAT complex but also with 2) the GEL (Get and EMC-like) complex comprising the putative protein insertase TMCO1 (Anghel *et al.*, 2017) and OPTI (obligate partner of TMCO1 insertase; also known as C20orf24 or RAB5IF) and 3) the BOS (back of Sec61) complex comprising TMEM147, Nicalin, and NOMO. The positions of these complexes sterically exclude the OST glycosylation complex from associating with the translocon. The structures additionally revealed that CCDC47 makes multiple interactions with the translocon-bound ribosome (McGilvray *et al.*, 2020) and with the cytosolic side of the N-terminal half of Sec61α, which may keep the Sec61α lateral gate closed (Figure 1C) (Smalinskaitė *et al.*, 2022). The effect of CCDC47 on Sec61α, the relatively large distance between Asterix and Sec61 (Figure 1C), and the observation of a nascent transmembrane helix outside of Sec61α on the side opposite the lateral gate raised the intriguing hypothesis that some transmembrane helices of multispanning proteins insert into the ER membrane independently of the Sec61α lateral gate (Smalinskaitė *et al.*, 2022). How this may occur awaits future investigations. As PAT complex subunits are present in most eukaryotes, the chaperoning function of Asterix and the potential regulation of Sec61 by CCDC47 appear to be broadly used. In contrast, GEL and BOS components occur primarily in metazoans, and their specific roles in protein biosynthesis at the ER remain to be defined.

Collective recruitment of the PAT, GEL, and BOS complexes to ribosome–Sec61 complexes correlates with two nascent protein features: a hydrophilic transmembrane helix that favors Asterix interaction and the presence of multiple transmembrane helices indicative of a multipass membrane protein (Smalinskaitė *et al.*, 2022; Sundaram *et al.*, 2022). These features were identified through pull downs of stable mimics of cotranslational translocation intermediates assembled in a cell-free system using nonstop mRNAs truncated at defined points to stall ribosomes after the synthesis of specific lengths of model membrane proteins. Such pull downs also indicated that the PAT, GEL, and BOS complexes can be exchanged with the OST complex based on changing substrate requirements (Sundaram *et al.*, 2022). Whether accessory factor exchanges occur at similar points during ongoing protein synthesis occurring at ~5–10 amino acids per second remains unclear. Altogether, these studies establish that Asterix acts within a larger translocon accessory complex to chaperone early hydrophilic transmembrane helices during multispanning membrane protein biogenesis at the ER (Meacock *et al.*, 2002; Chitwood and Hegde, 2020; Smalinskaitė *et al.*, 2022; Sundaram *et al.*, 2022).

## PROSPECTS FOR STUDYING TRANSLOCON ACCESSORY FACTORS

The many years needed to gain coherent mechanistic insights into Sec62/Sec63 and Asterix highlight several general lessons and challenges in studying translocon accessory factors. First, knowing precise substrate features that confer reliance on an accessory factor is essential. Current insights into such substrate features are primarily limited to the N-terminal regions of ER-targeted substrates. Identifying specific elements in other parts of multispanning membrane proteins that may require multiple accessory factor functions for biosynthesis becomes exponentially more complicated. Failure to correctly generate any part of such proteins often leads to the degradation of the entire protein. Loss of accessory factor functions also may induce cellular responses that indirectly affect the nascent proteome. Thus, endpoint and steady-state assays, even on a proteome-wide scale, may be insufficient to assign specific substrate elements to a given accessory factor. Moreover, cataloguing substrate features is critical but often still insufficient to generate a clear mechanistic model. For example, both TRAP and TRAM are linked to the successful production of certain substrates, but how they facilitate the biosynthesis of those proteins is still ambiguous. Thus, much work remains to identify and uncouple translocon accessory functions on different nascent protein elements.

Second, although the growing accessibility of cryo-EM will generate more structures of translocon accessory factors in the coming years, gleaning a clear mechanistic understanding from such structures requires sufficient context. Prior knowledge of substrates that use Sec62/Sec63 and of how other binding factors alter the translocon was critical to interpreting how the conformational changes that Sec62/Sec63 induce on Sec61 confer a specific function. By comparison, structures of TRAP have revealed previously unknown ribosomal interactions and the organization of an ER lumenal domain that probably interacts with translocating proteins but are nonetheless linked to various mechanistic interpretations (Pfeffer *et al.*, 2017; Jaskolowski *et al.*, 2022; Karki *et al.*, 2022; Pauwels *et al.*, 2022). Similarly, the structures of the PAT, GEL, and BOS complexes (McGilvray *et al.*, 2020; Smalinskaitė *et al.*, 2022) have generated many new and unexpected questions about translocon mechanisms. Future structural breakthroughs will rely on new approaches for identifying and isolating functional translocon intermediates to visualize how accessory factors engage and handle specific substrate elements.

Finally, the context for understanding translocon accessory factors continually evolves with new knowledge of mechanisms that influence protein biosynthesis at the ER. Notable examples include the identification of putative protein insertases, such as the EMC (ER membrane protein complex) (Guna *et al.*, 2018) and TMCO1 (Anghel *et al.*, 2017), that may carry out functions historically assumed to be handled by Sec61. In addition, a recently reported protein dislocation activity mediated by the ER-resident P5A-ATPase is required for the correct topogenesis of some Sec61 substrates (McKenna *et al.*, 2020; McKenna *et al.*, 2022). These factors are proposed to act on various cotranslational translocation substrates (Chitwood *et al.*, 2018; Feng *et al.*, 2020; McGilvray *et al.*, 2020; McKenna *et al.*, 2020, 2022; Li *et al.*, 2021) and must therefore coordinate their activities with the translocon through currently unknown mechanisms. Some translocon accessory factors may help manage these different activities and facilitate accurate substrate transfer between Sec61 and other insertases or protein quality control factors. Hence, further understanding of translocon accessory factors awaits the discovery of more tractable model substrates and technical innovations to assay specific steps of complex protein biosynthesis pathways.

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