

## REVIEW

# A clearer picture of the ER translocon complex

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## ABSTRACT

The endoplasmic reticulum (ER) translocon complex is the main gate into the secretory pathway, facilitating the translocation of nascent peptides into the ER lumen or their integration into the lipid membrane. Protein biogenesis in the ER involves additional processes, many of them occurring co-translationally while the nascent protein resides at the translocon complex, including recruitment of ER-targeted ribosome–nascent-chain complexes, glycosylation, signal peptide cleavage, membrane protein topogenesis and folding. To perform such varied functions on a broad range of substrates, the ER translocon complex has different accessory components that associate with it either stably or transiently. Here, we review recent structural and functional insights into this dynamically constituted central hub in the ER and its components. Recent cryo-electron microscopy (EM) studies have dissected the molecular organization of the co-translational ER translocon complex, comprising the Sec61 protein-conducting channel, the translocon-associated protein complex and the oligosaccharyl transferase complex. Complemented by structural characterization of the post-translational import machinery, key molecular principles emerge that distinguish co- and post-translational protein import and biogenesis. Further cryo-EM structures promise to expand our mechanistic understanding of the various biochemical functions involving protein biogenesis and quality control in the ER.

**KEY WORDS:** Cryo-EM, Endoplasmic reticulum, N-glycosylation, Protein folding, Translocon

## Introduction

The endoplasmic reticulum (ER) is the starting point of the secretory pathway (Johnson and van Waes, 1999). Freshly synthesized proteins are translocated into the lumen of the ER or integrated into the ER membrane, in the case of membrane proteins, prior to their subsequent transport to the plasma membrane or to organelles of the endocytic and exocytic pathways. Approximately 30% of all eukaryotic proteins utilize the secretory pathway. Synthesis of secretory pathway proteins primarily occurs at the surface of the ER, where ER-bound ribosomes give rise to the ‘rough’ morphology of large parts of the ER (Palade, 1975).

Many secretory pathway proteins are targeted to the ER via a hydrophobic N-terminal signal peptide (SP) (Blobel and Dobberstein, 1975). As the nascent SP emerges from the ribosome, it binds the soluble signal recognition particle (SRP), which mediates recruitment of the ribosome–nascent-chain (RNC) complex to the ER via the ER-membrane residing SRP receptor (SR) (Egea et al., 2005). The ER-resident signal peptidase complex (SPC) eventually cleaves off the SP from the nascent peptide (Evans

et al., 1986). SP-equivalent N-terminal transmembrane helices that are not cleaved off can also target proteins to the ER through the same mechanism. In this SRP-dependent co-translational ER-targeting mode, ribosomes associate with the ER membrane via ER translocon complexes. These membrane protein complexes translocate nascent soluble proteins into the ER, integrate nascent membrane proteins into the ER membrane, mediate protein folding and membrane protein topogenesis, and modify them chemically. In addition to co-translational protein import and translocation, distinct ER translocon complexes enable post-translational translocation and membrane integration. This post-translational pathway is widespread in yeast (Panzner et al., 1995), whereas higher eukaryotes primarily use it for relatively short peptides (Schlenstedt and Zimmermann, 1987; Shao and Hegde, 2011).

ER translocon complexes are dynamic entities, organized around an invariant core, the Sec61 protein-conducting channel. Sec61 is a trimeric membrane protein complex that is structurally and functionally highly conserved throughout all domains of life, known as SecYEG in bacteria and SecYEβ in archaea (Rapoport et al., 2017). In the co-translational mode, the ribosome binds to the Sec61 complex, enabling the nascent unfolded peptide to enter the Sec61 channel. In higher eukaryotes, the translocon-associated protein (TRAP) complex binds constitutively to Sec61 and a ribosome (Menetret et al., 2008; Pfeffer et al., 2014, 2017), possibly to support the recruitment of specific SPs (Nguyen et al., 2018) and membrane topogenesis of some substrates (Sommer et al., 2013). The oligosaccharyl transferase complex (OST), which is responsible for glycosylation of specific asparagine residues (N-glycosylation), binds to the ribosome–Sec61–TRAP complex in near stoichiometric ratios (Pfeffer et al., 2015, 2014), whereas other accessory components appear to rather bind transiently to the co-translational ER translocon in specific states in the biogenesis of specific proteins. These accessory factors include the SPC, ER-luminal chaperones and also members of the Oxa1/Alb3/YidC insertase family (Anghel et al., 2017), which cooperates with SecYEG or SecYEβ in the insertion of multi-transmembrane helix proteins into prokaryotic membranes (du Plessis et al., 2011).

In the post-translational mode, Sec61 forms a stable complex with the dimeric Sec62–Sec63 complex, and in fungi, additionally with Sec71 and Sec72 (Deshaies et al., 1991). These accessory proteins facilitate the transient binding of chaperones, in particular heat-shock 70 (Hsp70) family proteins, to the cytosolic and luminal side of the ER post-translocon complex (Sanders et al., 1992). Hsp70 family proteins prevent misfolding of translocated substrates in the ER lumen.

Besides ER protein biogenesis, the ER translocon is also directly implicated in the unfolded protein response (UPR), the cellular mechanism to counteract abnormally high amounts of unfolded proteins in the ER. Inositol-requiring enzyme 1 (IRE1, also known as ERN1), which initiates one of the three UPR branches, has recently been found to bind to Sec61 and the ribosome (Plumb et al., 2015).

Collectively, the ER translocon complex with its Sec61 core appears to be analogous to a ‘Swiss army knife’ that can adapt to

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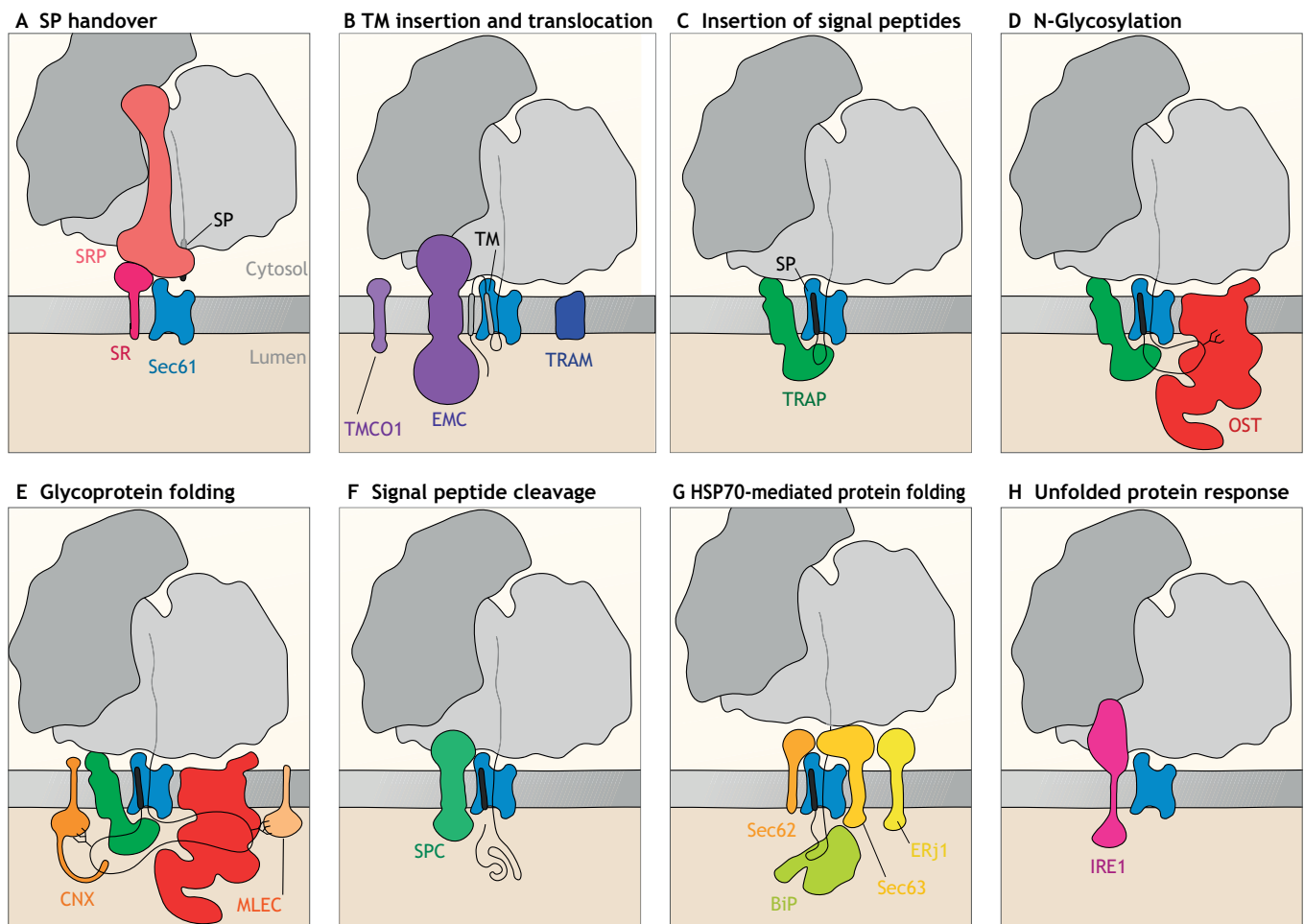
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different requirements with regard to substrates and cellular state (Fig. 1). The membrane-associated nature of the ER translocon complex has traditionally made it difficult to obtain structural insights into its functional and regulatory mechanisms. Advances in cryo-electron microscopy (cryo-EM) modalities have profoundly changed this situation (Callaway, 2015). Cryo-EM single-particle analysis (SPA) has provided numerous insights at near-atomic resolution into purified ER translocon complexes and their components. However, isolation-based approaches have their limits because the required solubilization tends to disrupt transient interactions and those involving lipids. Cryo-electron tomography (ET) complements studies of isolated components because it can image the ER translocon in its native ER environment – in the form of ER-derived vesicles or even in unperturbed cells (Beck and Baumeister, 2016). Here, we review recent structural and mechanistic insights into the co- and post-translational ER translocon complex and the molecular principles that distinguish these modes.

### Components and overall structure of the ER co-translocon complex

The development of cell-free assays allowed Blobel and co-workers to prove the ‘signal hypothesis’ (Blobel and Dobberstein, 1975),

that is that the targeting of many proteins to the ER through an N-terminal SP. The combination of rabbit reticulocyte lysate with ER-derived microsomes from dog pancreas enabled the reconstitution of co-translational protein import and SP cleavage. Isolation of ribosome-associated membrane proteins (RAMPs) from solubilized pancreatic microsomes provided clues about the molecular composition of the ER translocon complex. Depending on the choice of detergent and salt concentration, different proteins remained in these RAMP fractions. The most-detergent- and salt-resistant proteins are those belonging to the Sec61 complex, the translocating chain-associated membrane protein (TRAM1), the SPC and the protein RAMP4, later coined stress-associated endoplasmic reticulum protein 1 (SERP1) (Gorlich and Rapoport, 1993). Additional components, such as the oligosaccharyl transferase complex (specifically its ribophorin subunits; Kreibich et al., 1978a,b), the TRAP complex (Hartmann et al., 1993), the lectin calnexin (Chevet et al., 1999), and the J-domain protein ERj1 (also known as DNAJC1) (Dudek et al., 2005), were observed with the use of milder detergents or lower salt concentrations. Cross-linking prior to isolation revealed additional components such as p180 (also known as ribosome-binding protein 1, RRBP1) (Collins and Gilmore, 1991), and specific substrates such as the prion protein



**Fig. 1. Sec61 ‘Swiss army knife’ and cofactors.** (A) The SR recruits the RNC–SRP complex to the ER translocon and hands over the SP to Sec61. (B) Insertion of TMs is aided by insertases, such as EMC or possibly TMCO1, whereas TRAM helps to overcome pauses during translocation (Hegde et al., 1998). (C) Insertion of some SPs requires TRAP. (D) As translocation continues, the OST N-glycosylates translocating peptides. (E) Processed glycans associate with lectin chaperones maleic acid (MLEC) and calnexin (CNX), which are transiently recruited for glycoprotein folding. (F) Eventually, the SP is cleaved off by the SPC. (G) Primarily in post-translational import, but also for some co-translational substrates, the Hsp70 BiP is recruited by the Sec62–Sec63 or ERj1. (H) The constitutive interaction of IRE1 with Sec61 directly links ER translocation and the UPR.

(PrP) indicated an association between the Sec62–Sec63 complex and the co-translational ER translocon complex (Conti et al., 2015). However, these isolation studies did not address which of these components are indeed stoichiometric components, and the structural arrangement also remained unresolved.

Cryo-electron ET is uniquely suited to study the structures of macromolecular complexes under close-to-native conditions (Beck and Baumeister, 2016). In particular, this approach is also applicable to transient interactions, which are inherently difficult to address by purification-based approaches. In combination with image processing methods to enhance the low signal of cryo-ET raw data by averaging approaches (Briggs, 2013; Förster and Hegerl, 2007) and ‘classify’ distinct molecular configurations of assemblies (Chen et al., 2014; Förster et al., 2008), cryo-ET can reveal the structures of assemblies and relative abundances of complex types in native settings with sub-nanometer resolution. While the integral membrane proteins TRAM1 and SERP1 are difficult to detect by this approach because they can only be distinguished from the lipid membrane at resolutions notably better than 1 nm, all the other potential translocon components possess sufficiently large luminal or cytosolic domains to be detected. For instance, application of cryo-ET to ER-derived vesicles defined the mammalian core ER co-translocon complex; its main stoichiometric components are Sec61 and the TRAP complex, as determined by comparison of the wild-type translocon complex to that from knockdown cells (Pfeffer et al., 2014) (Fig. 2A).

In mammals, the octameric OST complex is found in near-stoichiometric ratios associated with the ER co-translocon (Fig. 2A). For instance, in canine pancreatic ER-derived microsomes, ~70% of all ribosome–translocon complexes had OST bound (Pfeffer et al., 2014), while in fibroblasts this proportion was 60–70% (Pfeffer et al., 2017) and in HeLa (Pfeffer et al., 2014) and HEK cells (Braunger et al., 2018) ~40–45%. By contrast, in the algae *Chlamydomonas reinhardtii*, only ~15% of all ribosome–translocon complexes contain OST (Pfeffer et al., 2017). Thus, OST occupancy varies strongly depending on species and cell type, possibly reflecting different degrees of N-glycosylation.

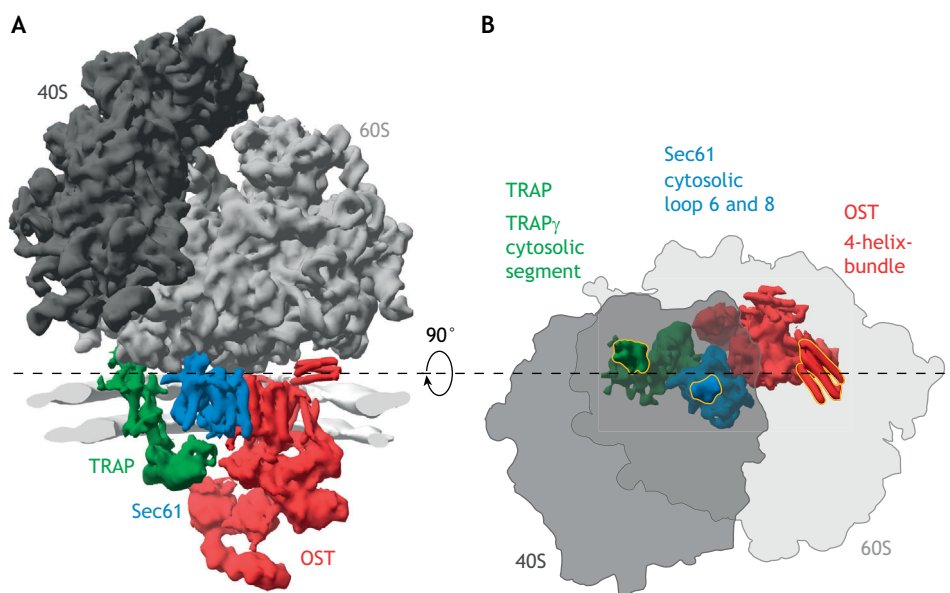
In the subnanometer-resolution reconstruction of the *in situ* ribosome–Sec61–TRAP–OST complex (Braunger et al., 2018; Pfeffer et al., 2015), Sec61 binds to ribosomal proteins (uL23 and

eL29) at the end of the ribosomal exit tunnel, as also observed in previous cryo-EM SPA of solubilized samples (Becker et al., 2009; Gogala et al., 2014; Voorhees et al., 2014). The transmembrane portion of TRAP is positioned near the C-terminal domain of Sec61, and its location is stabilized by associations with the ribosome through a cytosolic domain and to Sec61 through its luminal portion (Pfeffer et al., 2015). The transmembrane (TM) portion of OST binds to the N-terminal half of Sec61, and only its cytosolic domain binds the ribosome. Together, the ribosome-binding sites of TRAP, Sec61 and OST effectively form a line, stabilizing this giant molecular assembly (Fig. 2B).

### SRP receptor

Co-translational translocation through the ER translocon complex requires the SRP, which is a complex of SRP RNA and six proteins (SRP9, SRP14, SRP19, SRP54, SRP68 and SRP72; Walter and Blobel, 1982). SRP together with the heterodimeric SRP receptor (SR $\alpha$  and SR $\beta$ , encoded by *SRPRA* and *SRPRB*, hereafter SR $\alpha\beta$ ) are responsible for targeting ribosome–nascent-chain (RNC) complexes to the ER membrane and inserting the peptide chain into the Sec61 protein-conducting channel (Fig. 1A).

SRP and SR $\alpha\beta$  have structurally related ‘NG’ GTPase domains in SRP54 and SR $\alpha$ , respectively (Freymann et al., 1997). Crystallographic structures indicate that the GTPase activity of these NG domains must be activated by a conformational switch in the SRP–SR complex (Freymann et al., 1997; Padmanabhan and Freymann, 2001). This activation occurs concurrently with SP handover from the RNC–SRP–SR $\alpha\beta$  to the ER translocon complex (Shen et al., 2012). Thus, RNC–SRP–SR $\alpha\beta$  exists in two ER-translocon-bound states: a pre-handover complex, where the RNC–SRP–SR $\alpha\beta$  associates with the ER translocon, with the SP bound to the SRP, and an activated post-handover complex, where the SP is inserted into Sec61. Solubilized bacterial RNC–SRP–SR $\alpha\beta$ –SecYEG complexes that were locked in a post-hydrolysis form with GDP–AlFx, representing a transient intermediate between the targeting and translocation states, have been analyzed by cryo-EM SPA (Jomaa et al., 2017). The molecular interpretation of the obtained cryo-EM map suggests that a major structural remodeling of the ribosome–Sec61 complex occurs: Sec61 and the ribosome undergo a relative rotation of 180° in plane when transitioning from



**Fig. 2. Overview of the ribosome-bound ER translocon complex.** (A) The overall structure of the native complex as determined by cryo-ET (EMDB 4315; PDB 6FTG) shows the molecular organization of Sec61 embedded in the ER membrane and associated to the ribosome and accessory factors TRAP and OST. (B) Schematic view of the ribosome ER translocon complex contacts (yellow contours) formed by TRAP $\gamma$ , Sec61 $\alpha$  and the OST subunit ribophorin 1 as seen from the top.



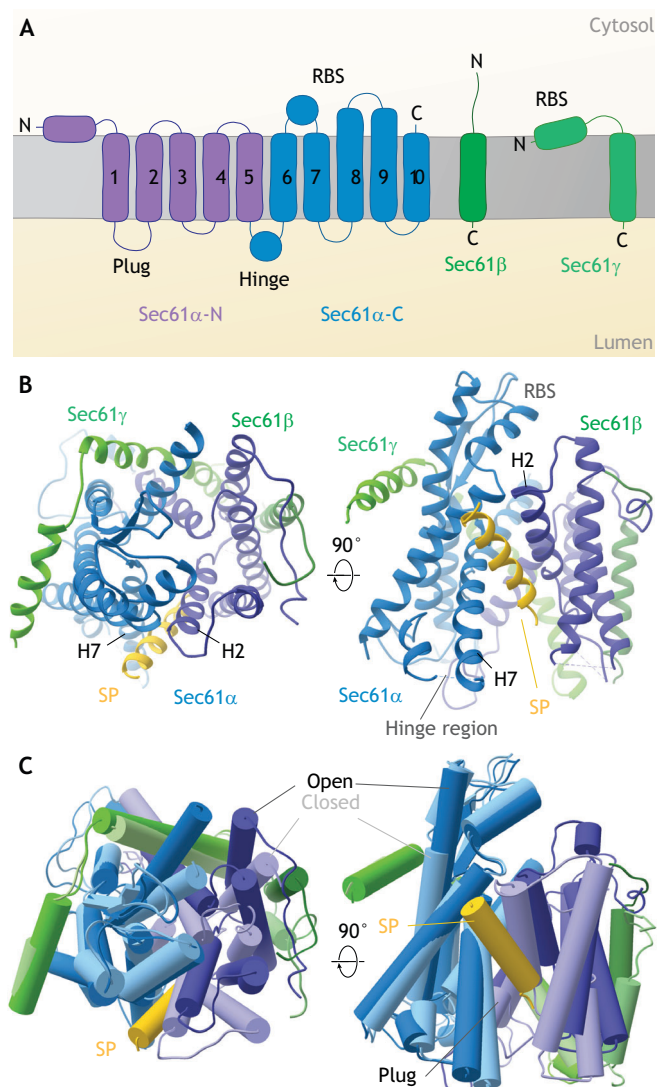
targeting to translocation (Jomaa et al., 2017) (Fig. S1). However, such dramatic conformational changes have to be confirmed in native settings.

Biochemical studies have also provided some glimpses into the molecular mechanisms underlying the switching of ER translocon complexes from their SRP-dependent co-translational mode to their post-translational one (Jadhav et al., 2015). Both SR $\alpha\beta$  and the ribosome individually induce the dissociation of Sec62 from Sec61, suggesting that the binding of SR $\alpha\beta$  and ribosome sterically interferes with the binding of Sec62 to Sec61. Thus, the recruitment of RNC–SRP–SR $\alpha\beta$  to Sec61 releases Sec62–Sec63 complexes from Sec61, suggesting that Sec62–Sec63 may only be involved in co-translational translocation at later stages, if at all.

### Protein-conducting channel Sec61

Sec61 consists of the three transmembrane proteins Sec61 $\alpha$  (note there are Sec61 $\alpha$ 1 and Sec61 $\alpha$ 2 forms in mammals), Sec61 $\beta$  and Sec61 $\gamma$  (Fig. 3A), and the prokaryotic homologs of Sec61 $\alpha$ , Sec61 $\beta$  and Sec61 $\gamma$  are SecY, SecE and SecE (bacteria), and SecY, Secb and SecE (archaea), respectively. Crystallographic analysis of the archaeal SecY (Sec61 $\alpha$  homolog) revealed that the two pseudo-symmetrical transmembrane domains of Sec61 $\alpha$  form a narrow channel (Van den Berg et al., 2004). Contrary to previous hypotheses proposing a functional oligomerization of Sec61 complexes (Beckmann et al., 2001), the structure suggested that Sec61 may function as a single complex and conduct peptides through its central channel (Van den Berg et al., 2004). Furthermore, the structure also indicates that a lateral window to the lipid membrane might form at the interface of the two TM domains of Sec61 $\alpha$  (Fig. 3B), possibly to facilitate insertion of transmembrane helices from the substrate into the ER membrane. Subsequent crystallographic structures of prokaryotic Sec61 homologs revealed a distinct conformation in which the repositioning of their two TM domains opens the lateral window (Egea and Stroud, 2010; Zimmer et al., 2008). In both ‘open’ structures, the cytosolic face of the Sec61 $\alpha$  homolog SecY is in contact with another molecule, either the bacterial ATPase SecA (Zimmer et al., 2008) or a neighboring SecY molecule through crystal contacts (Egea and Stroud, 2010). A third observation was that a short helix forms a ‘plug’ in the ER lumen, thereby closing the translocation channel, which was hypothesized to open in the translocation process.

Cryo-EM SPA studies of solubilized RNC–Sec61 complexes confirmed that a single Sec61 complex acts as a translocation channel (Gogala et al., 2014; Voorhees et al., 2014). Notably, the conformational states of Sec61 appear heterogeneous in these studies. Whereas the C-terminal domain of Sec61 $\alpha$  binds to the ribosome in a well-defined manner, the positioning of the N-terminal domain is variable. In those cryo-EM studies, analysis of minor, structurally well-defined classes that display defined translational states reveal better-resolved closed Sec61 conformers with the plug remaining in place. The finding of a closed Sec61 conformation is in contrast to that observed in the native non-solubilized ribosome-bound translocon; here, it is effectively exclusively observed in an open conformation (Pfeffer et al., 2015) (Fig. 3C). Interestingly, the map of the native translocon complex displays a rod-like density at the lateral gate (Pfeffer et al., 2015). This initially unassigned density co-localizes with the SP, as determined later on in the structure of solubilized RNC–Sec61 complexes with a non-cleaved SP (Voorhees and Hegde, 2016), as well as a crystal structure of the prokaryotic homolog (Li et al., 2016). In these high-resolution structures, the SPs (the SP of pre-prolactin and OmpA, respectively) bind almost identically to helix 2



**Fig. 3. Sec61 conformations and SP-binding.** (A) Subunit composition and topology of the Sec61 complex. Ribosome-binding sites (RBS) are annotated. (B) The cryo-EM SPA structure of the ‘open’ SP-bound Sec61 conformation (PDB 3JC2) shows the central pore (left, top view) and the Sec61 $\alpha$  helices H2 and H7, which delimit the lateral gate, accommodating the SP (right, side view). (C) Comparison of ‘open’ SP-bound conformation with the closed conformation observed in detergent-solubilized, idle ribosome–Sec61 complexes (PDB 3J7Q). The short ‘plug’ helix of Sec61 $\alpha$  closes the protein-conducting channel formed by Sec61 $\alpha$ .

of the open Sec61 $\alpha$ /SecY. In the RNC–Sec61–SP structure, the plug is not resolved, suggesting that it becomes unstructured (Voorhees and Hegde, 2016), whereas, in the SecY–SP crystal structure, it undergoes a secondary structure change, which makes way for the SP (Li et al., 2016). Remarkably, a narrow pore ring in the protein-conducting channel encloses the nascent peptide and maintains a permeability barrier for ions (Li et al., 2016).

It is somewhat puzzling that the predominant form of ribosome-bound Sec61 complexes in the native membrane appears to be an open conformation with an SP bound (Pfeffer et al., 2015), whereas major biochemical efforts are required to stabilize this conformation in isolated, solubilized complexes (Voorhees and Hegde, 2016). Thus, the SP appears prone to being released from its binding site in the lateral gate upon treatment with detergent. The cryo-ET experiments, at this point, cannot conclusively address whether

the SPs bound to the native Sec61 are still predominantly bound to a nascent chain, or whether SPC cleavage has readily occurred, because the resolution is insufficient to distinguish nascent chains.

From prokaryotes, it is well established that the insertion of many multi-transmembrane helix proteins into membranes requires cooperation of SecYEG/SecYEF with YidC (du Plessis et al., 2011). This protein is part of the YidC/Alb3/Oxa1 membrane protein family, which is conserved in all kingdoms of life (Hennon et al., 2015). Its members function as insertases in bacterial (YidC), thylakoid (Alb3) and mitochondrial membranes (Oxa1). A unique structural feature of these proteins is a hydrophilic, positively charged groove open to the membrane interior, which may transport acidic portions of proteins across the membrane, while inserting TM segments into the membrane (Kumazaki et al., 2014). The structure of domain of unknown function 106 (DUF106) recently expanded the YidC/Alb3/Oxa1 protein family (Borowska et al., 2015). Based on the presence of DUF106, which interestingly binds to RNCs *in vitro*, three ER-residing eukaryotic members YidC/Alb3/Oxa1 family could be identified (Fig. 1B): TMCO1, the Get1 subunit of the Get1–Get2 complex and the subunit EMC3 of the ER membrane complex (EMC) (Anghel et al., 2017; Guna et al., 2018). Whereas the Get complex is thought to function independently of the ER translocon complex in the insertion of tail-anchored proteins, the EMC is considered to cooperate co-translationally with the ER translocon complex for the integration of multi-transmembrane helix proteins (Shurtleff et al., 2018). However, the precise function of TMCO1 and its possible interactions with the ER translocon complex remain to be elucidated.

#### Translocon-associated protein complex

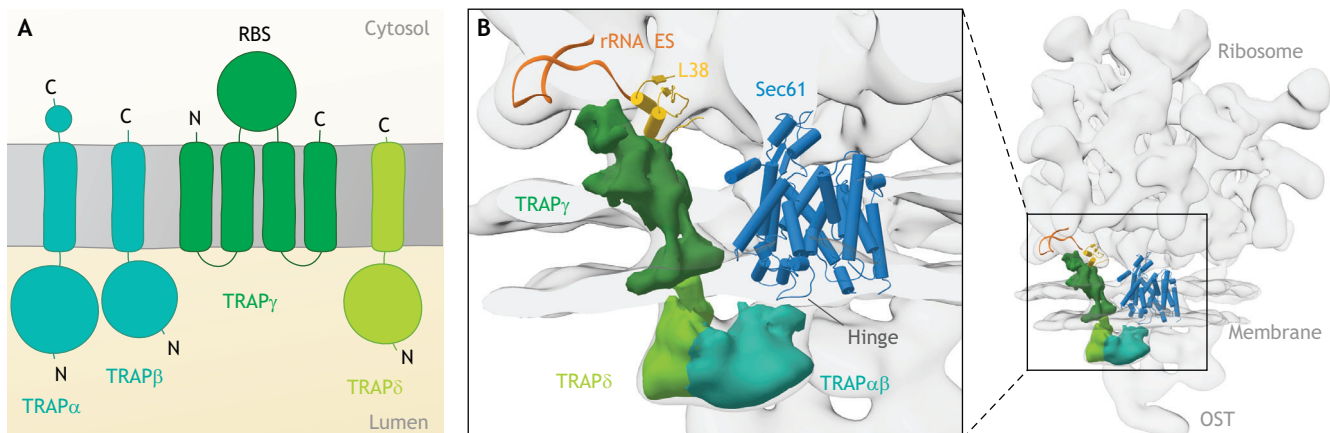
The ER translocon complex of metazoans comprises the hetero-oligomeric TRAP complex as a constitutive subunit (Figs 1C and 4A). In animals, it is a hetero-tetramer assembled by the transmembrane proteins TRAP $\alpha$ , TRAP $\beta$ , TRAP $\gamma$  and TRAP $\delta$  (also known as SSR1, SSR2, SSR3 and SSR4, respectively) (Hartmann et al., 1993), whereas in plants it is a dimer, TRAP $\alpha$ –TRAP $\beta$  (Pfeffer et al., 2017). The TRAP complex is required by specific substrates for the initiation of translocation (Fons et al., 2003). Proteomics analysis suggests that TRAP might be essential for the translocation of substrates that have SPs with low helical propensity due to high glycine and proline content (Nguyen et al., 2018). Furthermore, mutations of the TRAP complex in

glycosylation-deficient patients suggest its involvement in the N-glycosylation of specific substrates (Losfeld et al., 2014).

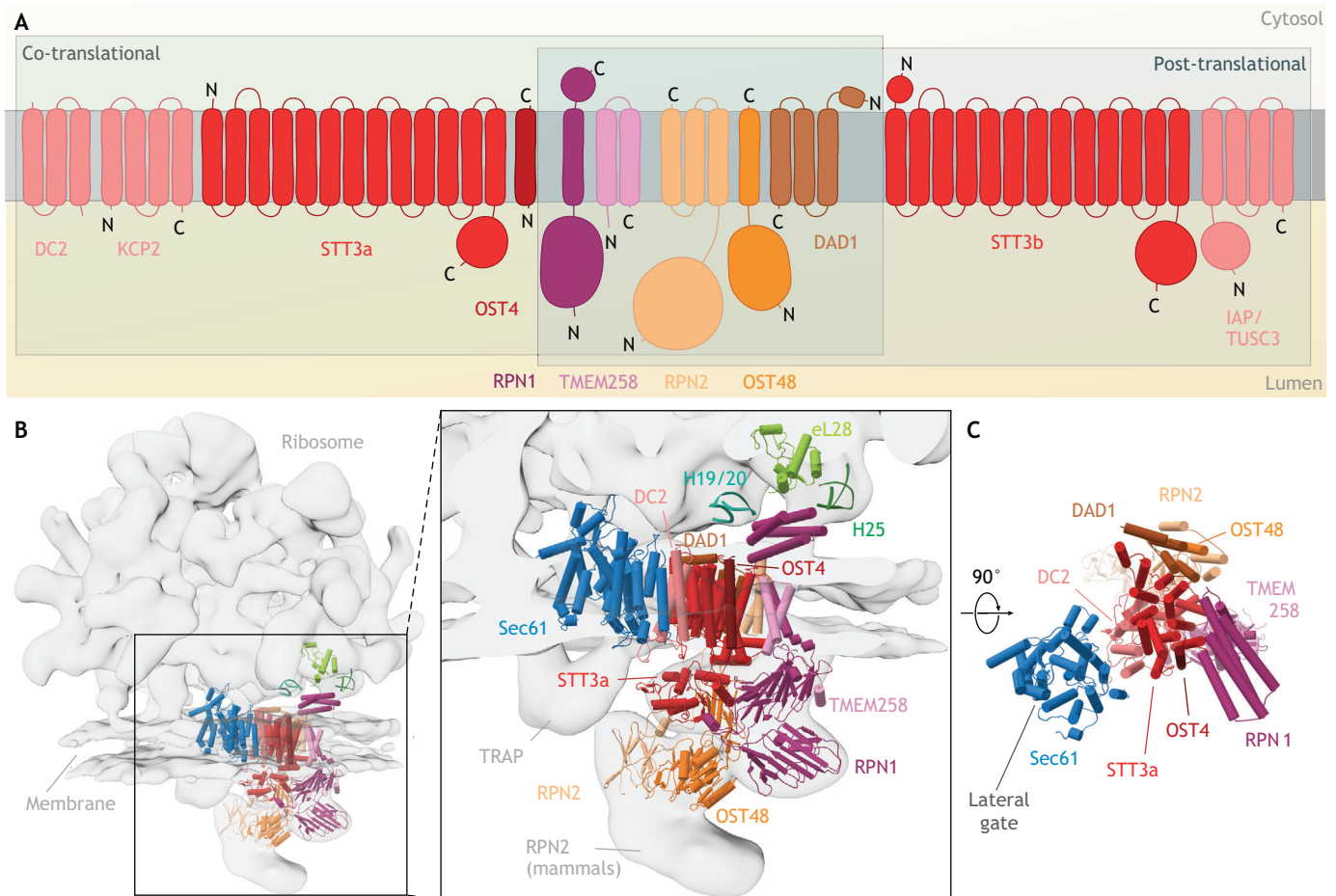
Comparative cryo-ET studies of mammalian cells and plants show that the animal-specific cytosolic TRAP $\gamma$  domain associates with the large ribosome subunit via the protein L38 and a rRNA expansion segment (ES) (Pfeffer et al., 2017) (Fig. 4B). The evolutionarily conserved luminal domains in TRAP $\alpha$  and TRAP $\beta$  bind to each other to form a heterodimer, which associates with the hinge region of Sec61 (Pfeffer et al., 2017). The cryo-ET structure of the ER translocon complex from cells of a TRAP $\delta$ -deficient patient, who suffers from congenital glycosylation defects, shows that the luminal domain of TRAP $\delta$  is positioned in proximity with OST subunit ribophorin 2 (Pfeffer et al., 2017). However, higher-resolution structures are required to mechanistically explain the function of TRAP in SP integration (possibly through TRAP $\alpha$ –TRAP $\beta$ ) and its involvement in N-glycosylation, which might be mediated through TRAP $\delta$ .

#### Oligosaccharyltransferase complex

For many nascent proteins of the secretory pathway, glycosylation of asparagine residues (N-glycosylation) is essential. The initially transferred glycan is identical for all N-glycosylations and subsequent action of glycosidases and glycosyltransferases in ER and Golgi yields great chemical variation. The hetero-oligomeric OST complex catalyzes the transfer of Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub> glycans from a pre-formed lipid-linked oligosaccharides (LLOs) to specific asparagine residues of proteins in the lumen of the ER (Kelleher and Gilmore, 2006) (Fig. 1D). Whereas OST is a monomeric enzyme in prokaryotes, its eukaryotic homolog STT3 is integrated into a larger oligomeric complex (Fig. 5A). Higher eukaryotes have two distinct catalytic paralogs, STT3a and STT3b, while simpler eukaryotes such as yeast only have a single ortholog (STT3). The modular composition and assembly of the yeast OST complex and the STT3b-containing mammalian complex are analogous; subcomplex 1 contains OST1 (ribophorin 1 in mammals) and OST5 (transmembrane protein 258, TMEM258), subcomplex 2 comprises STT3 (STT3b), OST3 or OST6 (TUSC3 or IAP) and OST4 (OST4), whereas subcomplex 3 involves OST2 (DAD1), WBP1 (OST48) and SWP1 (ribophorin 2) (Mueller et al., 2015). The STT3a-containing OST complex has the additional subunits DC2 (OSTC) and KCP2, while it lacks TUSC3 and IAP.



**Fig. 4. Architecture of the TRAP complex.** (A) Mammalian TRAP subunits and their membrane topology with the ribosome-binding site (RBS) indicated. (B) Molecular arrangement of TRAP within the native ER translocon complex. The cytosolic TRAP $\gamma$  domain mediates binding to the ribosome, whereas TRAP $\alpha$ –TRAP $\beta$  contacts the hinge region of Sec61 (EMDB 4315).



**Fig. 5. Composite atomic model of yeast and mammalian OST.** (A) Mammalian co- and post-translational OST paralogs, consisting of a common (RPN1, TMEM258, RPN2, OST48 and DAD1) and paralog-specific set of subunits (STT3a and DC2 and KCP2; STT3b and IAP or TUSC3). The STT3a-containing complex associates with the ER co-translocon. (B) A composite atomic model of the mammalian co-translational OST (PDB 6FTG) fragment complemented with luminal domains of the yeast OST complex (PDB 6EZ) is positioned into the filtered density map of the ribosome-associated translocon (EMDB 4315). The central panel shows a magnified view of the complex and its interacting sites in the ribosome and translocon. (C) Top view from the cytosol where the ER membrane resides in the paper plane.

Gene silencing experiments indicate that there is a preference of the STT3a-containing OST for co-translational N-glycosylation, compared to the prevalent post-translational glycosylation mediated by STT3b (Ruiz-Canada et al., 2009). Cryo-ET studies of cells in which STT3a and STT3b had been knocked out, showed that there is indeed a strict separation of co- and post-translational N-glycosylation, in that only the STT3a-containing OST associates with the ribosome-bound ER translocon (Braunger et al., 2018).

Two independent cryo-EM SPA studies provided the atomic structure of the yeast OST (Bai et al., 2018; Wild et al., 2018). The structure of the active subunit STT3 is similar to its prokaryotic counterparts (Lizak et al., 2011; Matsumoto et al., 2013) with conservation of the residues involved in substrate binding and oligosaccharyl transfer (Bai et al., 2018; Wild et al., 2018). Thus far, specific enzymatic functions have only been assigned to the orthologs OST3 (TUSC3) and OST6 (IAP), which function as oxidoreductases in the (post-translational) OST to facilitate the breakage of disulfide bridges in folded domains, which might prevent N-glycosylation (Mohorko et al., 2014). In the SPA structures, only the TM domain of OST3 is resolved, which localizes in immediate proximity to the LLO, as inferred from the prokaryotic STT3 homolog (Napiorkowska et al., 2017). The large

luminal domains of OST1 (ribophorin 1), WBP1 (OST48) and SWP1 (ribophorin 2) mostly form immunoglobulin G-like  $\beta$ -sandwich folds that are thought to serve as docking platforms for accessory proteins and possibly have chaperone function (Bai et al., 2018; Wild et al., 2018). Of note, ribophorin 2 has an additional N-terminal domain located further distally from the membrane, which is not present in yeast and algae (Pfeffer et al., 2017).

Complementary studies of the native mammalian ribosome-bound co-translational OST by cryo-ET and solubilized RNC–Sec61–OST by SPA have provided insights into the structure and function of the cytosolic domains and some of its TM regions (Braunger et al., 2018) (Fig. 5B). A four-helix bundle at the C-terminus of Rpn1 mediates the interaction with the large ribosomal subunit by contacting ribosomal RNA helices H19, H20 and H25, and ribosomal protein eL28. Compared to its paralog STT3a, STT3b has an additional cytosolic N-terminal domain, which would be positioned in such a way that it likely interferes with ribosome binding.

The OST subunit DC2, which has three TM regions, positions between STT3a and Sec61, and associates with the latter via its amphipathic N-terminus that projects near the Sec61 hinge (Braunger et al., 2018). Remarkably, the SPA maps reveal two distinct relative orientations of OST and Sec61, while the native



conformation seen by cryo-ET differs from both. Therefore, the integration of OST into the ER translocon through only two major contact sites provides specificity, while at the same time accommodating for flexibility with regard to how Sec61 and OST are arranged relative to each other, which might be important for OST function and its regulation.

In ER protein biogenesis, N-glycans play an essential role in glycoprotein folding and quality control in the ER. N-glycans are sequentially trimmed by processing  $\alpha$ -glucosidase I and processed glycans are bound by lectin chaperones malectin (MLEC) and calnexin (CNX) (Fig. 1E). Both, MLEC and CNX, associate with the ER co-translocon (Lakkaraju et al., 2012; Qin et al., 2012). Recruitment of CNX is dependent on TRAP, whereas MLEC associates with OST subunit Rpn1 (Lakkaraju et al., 2012; Takeda et al., 2014). The interplay between MLEC and Rpn1 regulates the subcellular distribution of MLEC and attenuates secretion of misfolded proteins (Takeda et al., 2014; Yang et al., 2018), highlighting its importance in the early steps of the glycoprotein folding pathway. Further structural studies are important to understand how the chaperones are organized at the ER translocon.

### Signal peptidase complex

The SPC is responsible for cleavage of the SP. The SPC acts on a broad range of substrates, but also has to distinguish signal peptides from TM segments. The highly specific binding mode of the SP to Sec61 (Li et al., 2016; Voorhees and Hegde, 2016) may be key to substrate selectivity.

The SPC evolved from a monomeric membrane-bound signal peptidase in prokaryotes (SPase I). The structure of the soluble domain of *E. coli* SPase I reveals that it is a serine protease with an unusual Ser-His dyad instead of the conventional Ser-His-Asp triad found in this class of enzymes (Paetzel et al., 1998). This active site is embedded into a large hydrophobic surface, which is thought to be proximal to the membrane to facilitate cleavage of the SP. Mammals possess two SPase I paralogs, SPC18 (also known as SEC11A) and SPC21 (also known as SEC11C), which are complemented by three non-catalytic subunits SPC12 (SPCS1), SPC22 or SPC23 (both encoded by *SPCS3*), and SPC25 (Evans et al., 1986). Yeast only has the single ortholog Sec11 (van Dijk et al., 1992), suggesting that mammals may have two distinct SPCs, either containing SPC18 or SPC21, analogous to the single OST complex in yeast and the two variants in mammals.

Although mammalian SPC co-purifies with the ER translocon complex (Gorlich and Rapoport, 1993), it was not found to be a (near) stoichiometric component (Pfeffer et al., 2014). Thus, SPC likely associates only transiently with the translocon in order to cleave off Sec61-bound SP. In line with this, cell-free assays demonstrated that the SPC has access to the nascent chain later than the OST (1) (Nilsson and von Heijne, 1993). Furthermore, pre-prolactin constructs have to translate at least for a ~80 residues further after their successful incorporation into Sec61 in order for their SP to be cleaved from the nascent chain (Mothes et al., 1994). The exact mechanisms underlying SPC association and cleavage are still poorly understood, and structures of both SPC alone and with the ER translocon complex will be insightful.

### ER post-translocon complex

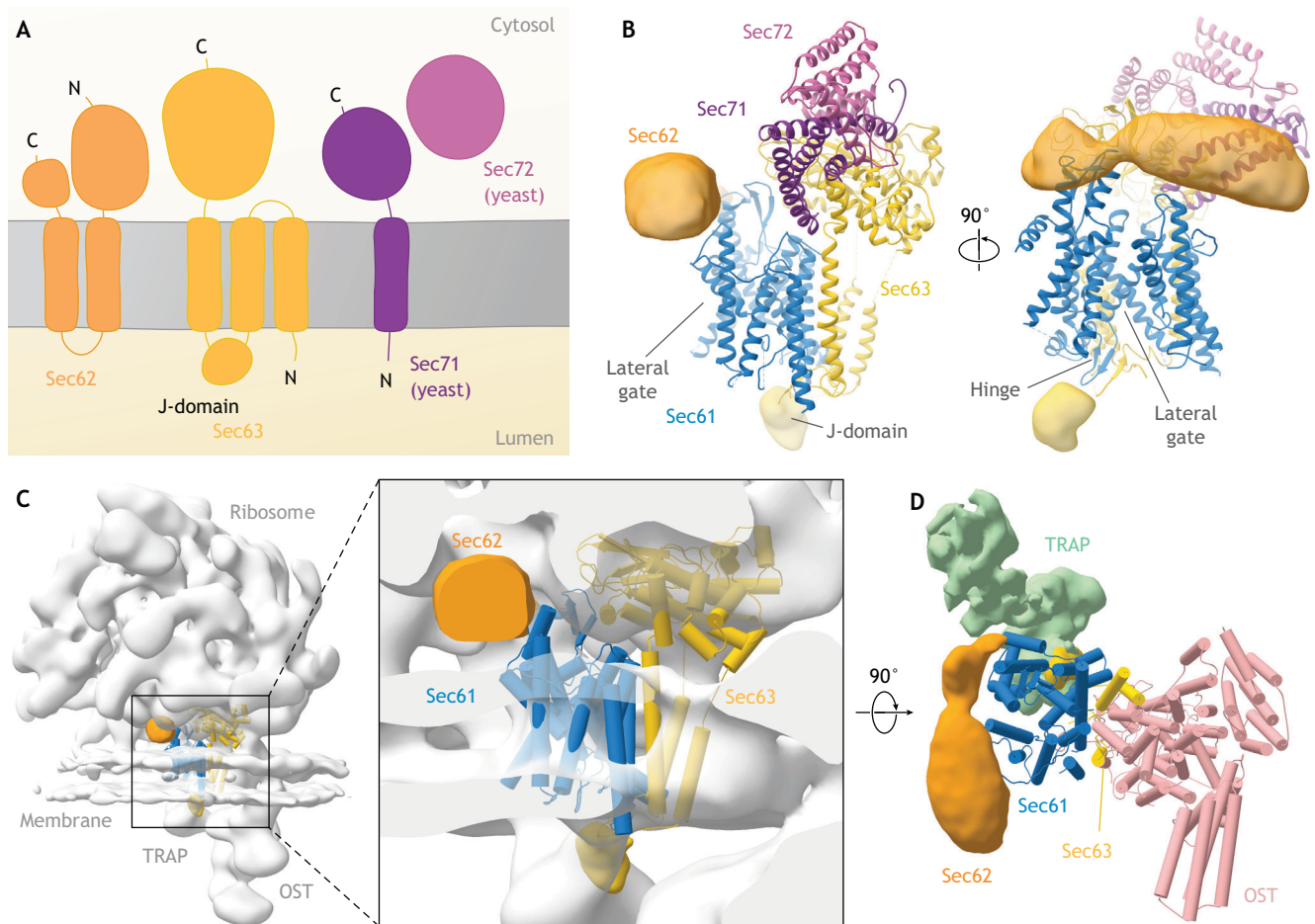
Sec61 also facilitates SRP-independent protein translocation. Whereas co-translational translocation is powered by GTP hydrolysis of the ribosome, the ATP-dependent Hsp70-type

chaperone binding immunoglobulin protein (BiP, also known as HSPA5, Kar2 in yeast), which is the most abundant ER-luminal protein, assists in post-translational translocation. BiP is thought to catalyze the directional translocation by binding the polypeptide chain that is translocated through Sec61, which prevents its backward movement into the cytosol. To acquire its full ATPase activity, the N-terminal ATPase domain of Hsp70 proteins associate with J-domains of their Hsp40-type co-chaperones, termed ERj proteins (Lang et al., 2017). While yeast has only a single membrane-bound ERj (Sec63), humans have seven; ERj3–ERj6 (DNAJB11, DNAJB9, DNAJC10, DNAJC3) are soluble proteins, whereas ERj1 (DNAJC1), Sec63 (ERj2; SEC63) and ERj7 (DNAJC25) are membrane-resident.

In yeast, the Sec61–Sec62–Sec63–Sec71–Sec72 (Sec) complex (Fig. 6A) facilitates post-translational import together with the transiently bound BiP (Deshaies et al., 1991; Sanders et al., 1992). Recently, the cryo-EM SPA structure of the Sec complex has been solved (Itskanov and Park, 2019; Wu et al., 2018) (Fig. 6B). Sec63 localizes opposite of the lateral gate and is tightly associated with Sec61, which involves cytosolic, transmembrane and luminal segments of all three Sec61 subunits (Itskanov and Park, 2019; Wu et al., 2018). In contrast to what is found in cryo-EM studies of the solubilized, idle ER translocon complex (Voorhees et al., 2014), Sec61 adopts its open conformation in the idle Sec complex, likely owing the extensive contacts with Sec63. Although the resolution of Sec62 was insufficient to build an atomic model, the localization of the cytosolic domain of Sec62 in proximity to the lateral gate of Sec61 in the low-resolution nevertheless supports its involvement in membrane protein insertion and topogenesis (Jung et al., 2014; Reithinger et al., 2013).

Superimposing the yeast Sec complex and the mammalian ribosome-associated translocon complex indicates that the entire cytosolic domain of Sec63 clashes with the large ribosomal subunit. Furthermore, the TM helices of Sec63 and OST overlap in their Sec61-binding site (Fig. 6C). In addition, the luminal domain of Sec63 interferes with the luminal domain of TRAP $\alpha$ –TRAP $\beta$ , which binds to the hinge region of Sec61 (Fig. 6C,D). Collectively, these steric considerations suggest that there is a strict separation of SRP-dependent co- and post-translational translocation using distinct complexes, the ER co-translocon and Sec complex, respectively. This notion is in agreement with earlier biochemical studies (Jadhav et al., 2015).

Mammals have the simpler Sec61–Sec62–Sec63 complex (Meyer et al., 2000). Interestingly, in mammalian systems, the Sec62–Sec63 dimer has been implicated in co-translational translocation of specific substrates, such as the prion protein (Conti et al., 2015; Lang et al., 2012). One possibility to reconcile these findings with the steric clashes between the ribosome and Sec62–Sec63, as indicated above, is by assuming that the prion protein already folds in the cytosol, thereby causing a partial release of the ribosome from Sec61, together with recruitment of Sec62–Sec63 and possible dissociation of TRAP (Conti et al., 2015). Recently, it has been suggested that the yeast Sec complex is also involved in co-translational translocation through a cytosolic Hsp70, which might also mediate the unfolding of the nascent chain after its release from the ribosomal exit tunnel and prior to insertion into Sec61 (Tripathi et al., 2017). Further structural studies will be required to investigate the precise mechanism of Sec62–Sec63-mediated co-translational translocation of specific substrates, as well as the structural basis of BiP-dependent regulation of translation by ERj1 (Benedix et al., 2010; Blau et al., 2005; Dudek et al., 2005, 2002).



**Fig. 6. Structure of the yeast Sec complex.** (A). In addition to Sec61, the Sec complex comprises the essential subunits Sec62 and Sec63, which recruit the Hsp70 BiP through its J-domain. The yeast Sec complex furthermore contains the non-essential subunits Sec71 and Sec72. (B) The cryo-EM SPA structure of the yeast Sec complex (EMDB 0336; PDB 6N3Q) reveals the atomic model of Sec63, Sec71 and Sec72 located opposite to the lateral gate of Sec61, as well as poorly resolved density of Sec62. (C) Superposition of the cryo-ET map of native mammalian ribosome-bound ER-translocon complex (gray, EMDB 4315) and the cryo-EM SPA yeast Sec translocon structure (colored, PDB 6FTG) illustrating the steric clashes of Sec63 with the ribosome and TRAP. (D) A top view of the ER translocon complex subunits (TRAP and OST) surrounding Sec61 compared with Sec translocon subunits Sec62 and Sec63 indicating that recruitment of Sec63 and the OST complex to Sec61 are mutually exclusive. Ribosome, membrane and cytosolic Sec63 densities are clipped to provide a better overview.

### Unfolded protein response machinery

The unfolded protein response (UPR) reduces the load of translated ER proteins and increases the ER-folding capacity upon stress through the initiation of a transcription program that increases the protein-folding capacity and decreases further protein influx (Hetzel, 2012; Walter and Ron, 2011). In yeast, the ER-resident kinase inositol-requiring protein 1 (IRE1) initiates this signaling cascade, whereas mammalian UPR exhibits two additional kinase branches [PERK (also known as EIF2AK3) and ATF6]. The mammalian protein IRE1 $\alpha$  controls translation of the transcription factor X box-binding protein 1 (XBP1). IRE1 $\alpha$  forms constitutive, but highly sub-stoichiometric complexes with Sec61, to which its substrate, *XBP1* mRNA, is recruited by a pseudo-SP of the XBP nascent chain (Plumb et al., 2015). At moderate stress levels, IRE1 $\alpha$  is bound to Sec61 and active, while at higher levels of stress, it is released from Sec61 and forms high-order oligomers (Sundaram et al., 2017). Consistent with these findings, IRE1 $\alpha$  has also been found to directly associate with the ER-bound ribosome (Acosta-Alvear et al., 2018). Thus, the co-translational translocation machinery and the IRE1 $\alpha$  branch of the UPR are intimately linked, although this remains to be structurally elucidated in detail. In this endeavor, the intrinsically low abundance of IRE1 $\alpha$  compared to other ER

translocon complex constituents is a major challenge for cryo-EM approaches that are essentially statistics-based and thus rely on the analysis of a large number of complexes.

### ER-associated protein degradation

The ER does not possess its own proteases for the degradation of (misfolded) proteins. Instead, the ER makes use of the cytosolic ubiquitin-proteasome system (UPS); this so-called ER-associated degradation (ERAD) process requires the retro-translocation of substrates into the cytosol, where they are ubiquitinated and eventually degraded by the 26S proteasome (Hiller et al., 1996). Some viruses, such as cytomegalovirus (CMV), hijack ERAD to evade the immune system as they encode for viral factors that target MHC-I complexes, which are involved in triggering an immune response, for degradation (Wiertz et al., 1996). Affinity purification experiments that made use of the CMV genes US2 and US11 demonstrated that Sec61 interacts with ERAD substrates (Wiertz et al., 1996). Moreover, in yeast, a physical interaction between Sec61 and ERAD components has also been demonstrated (Schafer and Wolf, 2009). Although an earlier hypothesis that Sec61 serves as a retrotranslocation channel is no longer favored (Carvalho et al., 2010), the relevance of the physical interaction between the ER



translocon and ERAD complexes remains to be elucidated, possibly with the help of structural exploration.

## Conclusions and outlook

Recent structural studies using different cryo-EM modalities have advanced the mechanistic understanding of ER translocation and protein biogenesis in the ER. Central to these processes is the Sec61 protein-conducting channel, which appears to function almost like a Swiss army knife; with the help of a large number of accessory components associated with it, its core translocation function can be complemented by a variety of co-translational tasks. Structural analysis revealed that binding of many of these accessory ER translocon complex components is mutually exclusive. For example, the formation of the Sec translocon is incompatible with the association of Sec61 with TRAP and OST.

Future research will reveal the precise interaction mode of further Sec61 interactors, such as YidC-like integrases complexes, SR, SPC and IRE1 $\alpha$ , as well as the functional regulation of their association. Cryo-EM SPA in combination with affinity purification of native complexes from yeast has been the basis of many structural studies in the past (e.g. OST, ER post-translocon) and gene editing will extend this success to other organisms. Nevertheless, a fundamental limitation in the analysis of membrane-associated complexes by SPA is that their purification requires solubilization. While the effect of solubilization on protein–protein interactions within single protein complexes such as the OST may be moderate, interactions between complexes involving lipids become significantly distorted as illustrated for the RNC–translocon super-complex. Thus, cryo-ET may be the most efficient route to get a high-resolution structure of the ER translocon complex, including its currently poorly resolved transmembrane components, such as TRAP or TRAM. Ongoing rapid developments to increase the data throughput of cryo-ET (Chreifi et al., 2019) together with improved processing (Himes and Zhang, 2018) will be key to substantially increase resolution. Similarly, less-abundant super-complexes involved in ERAD and the UPR will likely only be resolved in their native membranes. Efficient structural characterization may involve enrichment in (functional) membrane fractions or ultimately imaging in the cell, combined with the identification of specific events by correlative super-resolution light microscopy (Tuijtel et al., 2019).

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## Competing interests

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## Supplementary information

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## References

Acosta-Alvear, D., Karagoz, G. E., Frohlich, F., Li, H., Walther, T. C. and Walter, P. (2018). The unfolded protein response and endoplasmic reticulum protein targeting machineries converge on the stress sensor IRE1. *eLife* **7**, e43036. doi:10.7554/eLife.43036

Anghel, S. A., McGilvray, P. T., Hegde, R. S. and Keenan, R. J. (2017). Identification of Oxa1 homologs operating in the eukaryotic endoplasmic reticulum. *Cell Rep.* **21**, 3708–3716. doi:10.1016/j.celrep.2017.12.006

Bai, L., Wang, T., Zhao, G., Kovach, A. and Li, H. (2018). The atomic structure of a eukaryotic oligosaccharyltransferase complex. *Nature* **555**, 328–333. doi:10.1038/nature25755

Beck, M. and Baumeister, W. (2016). Cryo-electron tomography: can it reveal the molecular sociology of cells in atomic detail? *Trends Cell Biol.* **26**, 825–837. doi:10.1016/j.tcb.2016.08.006

Becker, T., Bhushan, S., Jarasch, A., Armache, J.-P., Funes, S., Jossinet, F., Gumbart, J., Mielke, T., Berninghausen, O., Schulten, K. et al. (2009). Structure of monomeric yeast and mammalian Sec61 complexes interacting with the translating ribosome. *Science* **326**, 1369–1373. doi:10.1126/science.1178535

Beckmann, R., Spahn, C. M. T., Eswar, N., Helmers, J., Penczek, P. A., Sali, A., Frank, J. and Blobel, G. (2001). Architecture of the protein-conducting channel associated with the translating 80S ribosome. *Cell* **107**, 361–372. doi:10.1016/S0092-8674(01)00541-4

Benedix, J., Lajoie, P., Jaiswal, H., Burgard, C., Greiner, M., Zimmermann, R., Rospert, S., Snapp, E. L. and Dudek, J. (2010). BiP modulates the affinity of its co-chaperone ERJ1 for ribosomes. *J. Biol. Chem.* **285**, 36427–36433. doi:10.1074/jbc.M110.143263

Blau, M., Mullanpudi, S., Becker, T., Dudek, J., Zimmermann, R., Penczek, P. A. and Beckmann, R. (2005). ERJ1p uses a universal ribosomal adaptor site to coordinate the 80S ribosome at the membrane. *Nat. Struct. Mol. Biol.* **12**, 1015–1016. doi:10.1038/nsmb998

Blobel, G. and Dobberstein, B. (1975). Transfer of proteins across membranes. I. presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* **67**, 835–851. doi:10.1083/jcb.67.3.835

Borowska, M. T., Dominik, P. K., Anghel, S. A., Kosiakoff, A. A. and Keenan, R. J. (2015). A YidC-like protein in the archaeal plasma membrane. *Structure* **23**, 1715–1724. doi:10.1016/j.str.2015.06.025

Braunger, K., Pfeffer, S., Shimal, S., Gilmore, R., Berninghausen, O., Mandon, E. C., Becker, T., Förster, F. and Beckmann, R. (2018). Structural basis for coupling protein transport and N-glycosylation at the mammalian endoplasmic reticulum. *Science* **360**, 215–219. doi:10.1126/science.aar7899

Briggs, J. A. G. (2013). Structural biology in situ — the potential of subtomogram averaging. *Curr. Opin. Struct. Biol.* **23**, 261–267. doi:10.1016/j.sbi.2013.02.003

Callaway, E. (2015). The revolution will not be crystallized: a new method sweeps through structural biology. *Nature* **525**, 172–174. doi:10.1038/525172a

Carvalho, P., Stanley, A. M. and Rapoport, T. A. (2010). Retrotranslocation of a misfolded luminal ER protein by the ubiquitin-ligase Hrd1p. *Cell* **143**, 579–591. doi:10.1016/j.cell.2010.10.028

Chen, Y., Pfeffer, S., Fernández, J. J., Sorzano, C. O. S. and Förster, F. (2014). Autofocused 3D classification of cryoelectron subtomograms. *Structure* **22**, 1528–1537. doi:10.1016/j.str.2014.08.007

Chevet, E., Wong, H. N., Gerber, D., Cochet, C., Fazal, A., Cameron, P. H., Gushue, J. N., Thomas, D. Y. and Bergeron, J. J. M. (1999). Phosphorylation by CK2 and MAPK enhances calnexin association with ribosomes. *EMBO J.* **18**, 3655–3666. doi:10.1093/emboj/18.13.3655

Chreifi, G., Chen, S., Metskas, L. A., Kaplan, M. and Jensen, G. J. (2019). Rapid tilt-series acquisition for electron cryotomography. *J. Struct. Biol.* **205**, 163–169. doi:10.1016/j.jsb.2018.12.008

Collins, P. G. and Gilmore, R. (1991). Ribosome binding to the endoplasmic reticulum: a 180-kD protein identified by crosslinking to membrane-bound ribosomes is not required for ribosome binding activity. *J. Cell Biol.* **114**, 639–649. doi:10.1083/jcb.114.4.639

Conti, B. J., Devaraneni, P. K., Yang, Z., David, L. L. and Skach, W. R. (2015). Cotranslational stabilization of Sec62/63 within the ER Sec61 translocon is controlled by distinct substrate-driven translocation events. *Mol. Cell* **58**, 269–283. doi:10.1016/j.molcel.2015.02.018

Deshaies, R. J., Sanders, S. L., Feldheim, D. A. and Schekman, R. (1991). Assembly of yeast Sec proteins involved in translocation into the endoplasmic reticulum into a membrane-bound multisubunit complex. *Nature* **349**, 806–808. doi:10.1038/349806a0

du Plessis, D. J. F., Nouwen, N. and Driessen, A. J. M. (2011). The Sec translocase. *Biochim. Biophys. Acta* **1808**, 851–865. doi:10.1016/j.bbamem.2010.08.016

Dudek, J., Volkmer, J., Bies, C., Guth, S., Müller, A., Lerner, M., Feick, P., Schäfer, K. H., Morgenstern, E., Hennessy, F. et al. (2002). A novel type of co-chaperone mediates transmembrane recruitment of DnaK-like chaperones to ribosomes. *EMBO J.* **21**, 2958–2967. doi:10.1093/emboj/cdf315

Dudek, J., Greiner, M., Müller, A., Hendershot, L. M., Kopsch, K., Nastainczyk, W. and Zimmermann, R. (2005). ERJ1p has a basic role in protein biogenesis at the endoplasmic reticulum. *Nat. Struct. Mol. Biol.* **12**, 1008–1014. doi:10.1038/nsmb1007

Egea, P. F. and Stroud, R. M. (2010). Lateral opening of a translocon upon entry of protein suggests the mechanism of insertion into membranes. *Proc. Natl. Acad. Sci. USA* **107**, 17182–17187. doi:10.1073/pnas.1012556107

Egea, P. F., Stroud, R. M. and Walter, P. (2005). Targeting proteins to membranes: structure of the signal recognition particle. *Curr. Opin. Struct. Biol.* **15**, 213–220. doi:10.1016/j.sbi.2005.03.007

- Evans, E. A., Gilmore, R. and Blobel, G. (1986). Purification of microsomal signal peptidase as a complex. *Proc. Natl. Acad. Sci. USA* **83**, 581-585. doi:10.1073/pnas.83.3.581
- Fons, R. D., Bogert, B. A. and Hegde, R. S. (2003). Substrate-specific function of the translocon-associated protein complex during translocation across the ER membrane. *J. Cell Biol.* **160**, 529-539. doi:10.1083/jcb.200210095
- Förster, F. and Hegerl, R. (2007). Structure determination in situ by averaging of tomograms. *Methods Cell Biol.* **79**, 741-767. doi:10.1016/S0091-679X(06)79029-X
- Förster, F., Pruggnaller, S., Seybert, A. and Frangakis, A. S. (2008). Classification of cryo-electron sub-tomograms using constrained correlation. *J. Struct. Biol.* **161**, 276-286. doi:10.1016/j.jsb.2007.07.006
- Freyman, D. M., Keenan, R. J., Stroud, R. M. and Walter, P. (1997). Structure of the conserved GTPase domain of the signal recognition particle. *Nature* **385**, 361-364. doi:10.1038/385361a0
- Gogala, M., Becker, T., Beatrix, B., Armache, J.-P., Barrio-Garcia, C., Berninghausen, O. and Beckmann, R. (2014). Structures of the Sec61 complex engaged in nascent peptide translocation or membrane insertion. *Nature* **506**, 107-110. doi:10.1038/nature12950
- Görlach, D. and Rapoport, T. A. (1993). Protein translocation into proteoliposomes reconstituted from purified components of the endoplasmic reticulum membrane. *Cell* **75**, 615-630. doi:10.1016/0092-8674(93)90483-7
- Guna, A., Volkmar, N., Christianson, J. C. and Hegde, R. S. (2018). The ER membrane protein complex is a transmembrane domain insertase. *Science* **359**, 470-473. doi:10.1126/science.aao3099
- Hartmann, E., Görlach, D., Kostka, S., Otto, A., Kraft, R., Knespel, S., Burger, E., Rapoport, T. A. and Prehn, S. (1993). A tetrameric complex of membrane proteins in the endoplasmic reticulum. *Eur. J. Biochem.* **214**, 375-381. doi:10.1111/j.1432-1033.1993.tb17933.x
- Hegde, R. S., Voigt, S., Rapoport, T. A. and Lingappa, V. R. (1998). TRAM regulates the exposure of nascent secretory proteins to the cytosol during translocation into the endoplasmic reticulum. *Cell* **92**, 621-631. doi:10.1016/S0092-8674(00)81130-7
- Hennon, S. W., Soman, R., Zhu, L. and Dalbey, R. E. (2015). YidC/Alb3/Oxa1 family of insertases. *J. Biol. Chem.* **290**, 14866-14874. doi:10.1074/jbc.R115.638171
- Hetz, C. (2012). The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat. Rev. Mol. Cell Biol.* **13**, 89-102. doi:10.1038/nrm3270
- Hiller, M. M., Finger, A., Schweiger, M. and Wolf, D. H. (1996). ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. *Science* **273**, 1725-1728. doi:10.1126/science.273.5282.1725
- Himes, B. A. and Zhang, P. (2018). emClarity: software for high-resolution cryo-electron tomography and subtomogram averaging. *Nat. Methods* **15**, 955-961. doi:10.1038/s41592-018-0167-z
- Itskanov, S. and Park, E. (2019). Structure of the posttranslational Sec protein-translocation channel complex from yeast. *Science* **363**, 84-87. doi:10.1126/science.aav6740
- Jadhav, B., McKenna, M., Johnson, N., High, S., Sinning, I. and Pool, M. R. (2015). Mammalian SRP receptor switches the Sec61 translocase from Sec62 to SRP-dependent translocation. *Nat. Commun.* **6**, 10133. doi:10.1038/ncomms10133
- Johnson, A. E. and van Waes, M. A. (1999). The translocon: a dynamic gateway at the ER membrane. *Annu. Rev. Cell Dev. Biol.* **15**, 799-842. doi:10.1146/annurev.cellbio.15.1.799
- Jomaa, A., Fu, Y.-H. H., Boehringer, D., Leibundgut, M., Shan, S.-O. and Ban, N. (2017). Structure of the quaternary complex between SRP, SR, and translocon bound to the translating ribosome. *Nat. Commun.* **8**, 15470. doi:10.1038/ncomms15470
- Jung, S.-J., Kim, J. E. H., Reithinger, J. H. and Kim, H. (2014). The Sec62-Sec63 translocon facilitates translocation of the C-terminus of membrane proteins. *J. Cell Sci.* **127**, 4270-4278. doi:10.1242/jcs.153650
- Kelleher, D. J. and Gilmore, R. (2006). An evolving view of the eukaryotic oligosaccharyltransferase. *Glycobiology* **16**, 47R-62R. doi:10.1093/glycob/cwj066
- Kreibich, G., Czako-Graham, M., Grebenau, R., Mok, W., Rodriguez-Boulant, E. and Sabatini, D. D. (1978a). Characterization of the ribosomal binding site in rat liver rough microsomes: ribophorins I and II, two integral membrane proteins related to ribosome binding. *J. Supramol. Struct.* **8**, 279-302. doi:10.1002/jss.400080307
- Kreibich, G., Ulrich, B. L. and Sabatini, D. D. (1978b). Proteins of rough microsomal membranes related to ribosome binding. I. Identification of ribophorins I and II, membrane proteins characteristics of rough microsomes. *J. Cell Biol.* **77**, 464-487. doi:10.1083/jcb.77.2.464
- Kumazaki, K., Chiba, S., Takemoto, M., Furukawa, A., Nishiyama, K.-I., Sugano, Y., Mori, T., Dohmae, N., Hirata, K., Nakada-Nakura, Y. et al. (2014). Structural basis of Sec-independent membrane protein insertion by YidC. *Nature* **509**, 516-520. doi:10.1038/nature13167
- Lakkaraju, A. K. K., Abrami, L., Lemmin, T., Blaskovic, S., Kunz, B., Kihara, A., Dal Peraro, M. and van der Goot, F. G. (2012). Palmitoylated calnexin is a key component of the ribosome-translocon complex. *EMBO J.* **31**, 1823-1835. doi:10.1038/embj.2012.15
- Lang, S., Benedix, J., Fedeles, S. V., Schorr, S., Schirra, C., Schauble, N., Jalal, C., Greiner, M., Hassdenteufel, S., Tatzelt, J. et al. (2012). Different effects of Sec61alpha, Sec62 and Sec63 depletion on transport of polypeptides into the endoplasmic reticulum of mammalian cells. *J. Cell Sci.* **125**, 1958-1969. doi:10.1242/jcs.096727
- Lang, S., Pfeffer, S., Lee, P.-H., Cavalié, A., Helms, V., Förster, F. and Zimmermann, R. (2017). An update on Sec61 channel functions, mechanisms, and related diseases. *Front. Physiol.* **8**, 887. doi:10.3389/fphys.2017.00887
- Li, L., Park, E., Ling, J. J., Ingram, J., Ploegh, H. and Rapoport, T. A. (2016). Crystal structure of a substrate-engaged SecY protein-translocation channel. *Nature* **531**, 395-399. doi:10.1038/nature17163
- Lizak, C., Gerber, S., Numao, S., Aebi, M. and Locher, K. P. (2011). X-ray structure of a bacterial oligosaccharyltransferase. *Nature* **474**, 350-355. doi:10.1038/nature10151
- Losfeld, M. E., Ng, B. G., Kircher, M., Buckingham, K. J., Turner, E. H., Eroshkin, A., Smith, J. D., Shendure, J., Nickerson, D. A., Bamshad, M. J. et al. (2014). A new congenital disorder of glycosylation caused by a mutation in SSR4, the signal sequence receptor 4 protein of the TRAP complex. *Hum. Mol. Genet.* **23**, 1602-1605. doi:10.1093/hmg/ddt550
- Matsumoto, S., Shimada, A., Nyirenda, J., Igura, M., Kawano, Y. and Kohda, D. (2013). Crystal structures of an archaeal oligosaccharyltransferase provide insights into the catalytic cycle of N-linked protein glycosylation. *Proc. Natl. Acad. Sci. USA* **110**, 17868-17873. doi:10.1073/pnas.1309777110
- Ménétret, J. F., Hegde, R. S., Aguiar, M., Gygi, S. P., Park, E., Rapoport, T. A. and Akey, C. W. (2008). Single copies of Sec61 and TRAP associate with a nontranslating mammalian ribosome. *Structure* **16**, 1126-1137. doi:10.1016/j.str.2008.05.003
- Meyer, H.-A., Grau, H., Kraft, R., Kostka, S., Prehn, S., Kalies, K.-U. and Hartmann, E. (2000). Mammalian Sec61 is associated with Sec62 and Sec63. *J. Biol. Chem.* **275**, 14550-14557. doi:10.1074/jbc.275.19.14550
- Mohorko, E., Owen, R. L., Malojčić, G., Brozzo, M. S., Aebi, M. and Glockshuber, R. (2014). Structural basis of substrate specificity of human oligosaccharyl transferase subunit n33/tusc3 and its role in regulating protein N-glycosylation. *Structure* **22**, 590-601. doi:10.1016/j.str.2014.02.013
- Mothes, W., Prehn, S. and Rapoport, T. A. (1994). Systematic probing of the environment of a translocating secretory protein during translocation through the ER membrane. *EMBO J.* **13**, 3973-3982. doi:10.1002/j.1460-2075.1994.tb06713.x
- Mueller, S., Wahlander, A., Selevsek, N., Otto, C., Ngwa, E. M., Poljak, K., Frey, A. D., Aebi, M. and Gauss, R. (2015). Protein degradation corrects for imbalanced subunit stoichiometry in OST complex assembly. *Mol. Biol. Cell* **26**, 2596-2608. doi:10.1091/mbc.E15-03-0168
- Napiórkowska, M., Boilevin, J., Sovdat, T., Darbre, T., Reymond, J.-L., Aebi, M. and Locher, K. P. (2017). Molecular basis of lipid-linked oligosaccharide recognition and processing by bacterial oligosaccharyltransferase. *Nat. Struct. Mol. Biol.* **24**, 1100-1106. doi:10.1038/nsmb.3491
- Nguyen, D., Stutz, R., Schorr, S., Lang, S., Pfeffer, S., Freeze, H. H., Förster, F., Helms, V., Dudek, J. and Zimmermann, R. (2018). Proteomics reveals signal peptide features determining the client specificity in human TRAP-dependent ER protein import. *Nat. Commun.* **9**, 3765. doi:10.1038/s41467-018-06188-z
- Nilsson, I. M. and von Heijne, G. (1993). Determination of the distance between the oligosaccharyltransferase active site and the endoplasmic reticulum membrane. *J. Biol. Chem.* **268**, 5798-5801.
- Padmanabhan, S. and Freymann, D. M. (2001). The conformation of bound GMPNP suggests a mechanism for gating the active site of the SRP GTPase. *Structure* **9**, 859-867. doi:10.1016/S0969-2126(01)00641-4
- Paetz, M., Dalbey, R. E. and Strynadka, N. C. J. (1998). Crystal structure of a bacterial signal peptidase in complex with a beta-lactam inhibitor. *Nature* **396**, 186-190. doi:10.1038/24196
- Palade, G. (1975). Intracellular aspects of the process of protein synthesis. *Science* **189**, 347-358. doi:10.1126/science.1096303
- Panzner, S., Dreier, L., Hartmann, E., Kostka, S. and Rapoport, T. A. (1995). Posttranslational protein transport in yeast reconstituted with a purified complex of Sec proteins and Kar2p. *Cell* **81**, 561-570. doi:10.1016/0092-8674(95)90077-2
- Pfeffer, S., Dudek, J., Gogala, M., Schorr, S., Linxweiler, J., Lang, S., Becker, T., Beckmann, R., Zimmermann, R. and Förster, F. (2014). Structure of the mammalian oligosaccharyl-transferase complex in the native ER protein translocon. *Nat. Commun.* **5**, 3072. doi:10.1038/ncomms4072
- Pfeffer, S., Burbaum, L., Unverdorben, P., Pech, M., Chen, Y., Zimmermann, R., Beckmann, R. and Förster, F. (2015). Structure of the native Sec61 protein-conducting channel. *Nat. Commun.* **6**, 8403. doi:10.1038/ncomms9403
- Pfeffer, S., Dudek, J., Schaffer, M., Ng, B. G., Albert, S., Plitzko, J. M., Baumeister, W., Zimmermann, R., Freeze, H. H., Engel, B. D. et al. (2017). Dissecting the molecular organization of the translocon-associated protein complex. *Nat. Commun.* **8**, 14516. doi:10.1038/ncomms14516
- Plumb, R., Zhang, Z. R., Appathurai, S. and Mariappan, M. (2015). A functional link between the co-translational protein translocation pathway and the UPR. *eLife* **4**, e07426. doi:10.7554/eLife.07426
- Qin, S.-Y., Hu, D., Matsumoto, K., Takeda, K., Matsumoto, N., Yamaguchi, Y. and Yamamoto, K. (2012). Malectin forms a complex with ribophorin I for

- enhanced association with misfolded glycoproteins. *J. Biol. Chem.* **287**, 38080-38089. doi:10.1074/jbc.M112.394288
- Rapoport, T. A., Li, L. and Park, E.** (2017). Structural and mechanistic insights into protein translocation. *Annu. Rev. Cell Dev. Biol.* **33**, 369-390. doi:10.1146/annurev-cellbio-100616-060439
- Reithinger, J. H., Kim, J. E. H. and Kim, H.** (2013). Sec62 protein mediates membrane insertion and orientation of moderately hydrophobic signal anchor proteins in the endoplasmic reticulum (ER). *J. Biol. Chem.* **288**, 18058-18067. doi:10.1074/jbc.M113.473009
- Ruiz-Canada, C., Kelleher, D. J. and Gilmore, R.** (2009). Cotranslational and posttranslational N-glycosylation of polypeptides by distinct mammalian OST isoforms. *Cell* **136**, 272-283. doi:10.1016/j.cell.2008.11.047
- Sanders, S. L., Whitfield, K. M., Vogel, J. P., Rose, M. D. and Schekman, R. W.** (1992). Sec61p and BiP directly facilitate polypeptide translocation into the ER. *Cell* **69**, 353-365. doi:10.1016/0092-8674(92)90415-9
- Schafer, A. and Wolf, D. H.** (2009). Sec61p is part of the endoplasmic reticulum-associated degradation machinery. *EMBO J.* **28**, 2874-2884. doi:10.1038/emboj.2009.231
- Schlenstedt, G. and Zimmermann, R.** (1987). Import of frog prepropeptide GLA into microsomes requires ATP but does not involve docking protein or ribosomes. *EMBO J.* **6**, 699-703. doi:10.1002/j.1460-2075.1987.tb04810.x
- Shao, S. and Hegde, R. S.** (2011). A calmodulin-dependent translocation pathway for small secretory proteins. *Cell* **147**, 1576-1588. doi:10.1016/j.cell.2011.11.048
- Shen, K., Arslan, S., Akopian, D., Ha, T. and Shan, S.-O.** (2012). Activated GTPase movement on an RNA scaffold drives co-translational protein targeting. *Nature* **492**, 271-275. doi:10.1038/nature11726
- Shurtleff, M. J., Itzhak, D. N., Hussmann, J. A., Schirle Oakdale, N. T., Costa, E. A., Jonikas, M., Weibezahn, J., Popova, K. D., Jan, C. H., Sinitcyn, P. et al.** (2018). The ER membrane protein complex interacts cotranslationally to enable biogenesis of multipass membrane proteins. *eLife* **7**, e37018. doi:10.7554/eLife.37018
- Sommer, N., Junne, T., Kalies, K.-U., Spiess, M. and Hartmann, E.** (2013). TRAP assists membrane protein topogenesis at the mammalian ER membrane. *Biochim. Biophys. Acta* **1833**, 3104-3111. doi:10.1016/j.bbamcr.2013.08.018
- Sundaram, A., Plumb, R., Appathurai, S. and Mariappan, M.** (2017). The Sec61 translocon limits IRE1alpha signaling during the unfolded protein response. *eLife* **6**, e27187. doi:10.7554/eLife.27187
- Takeda, K., Qin, S.-Y., Matsumoto, N. and Yamamoto, K.** (2014). Association of malectin with ribophorin I is crucial for attenuation of misfolded glycoprotein secretion. *Biochem. Biophys. Res. Commun.* **454**, 436-440. doi:10.1016/j.bbrc.2014.10.102
- Tripathi, A., Mandon, E. C., Gilmore, R. and Rapoport, T. A.** (2017). Two alternative binding mechanisms connect the protein translocation Sec71-Sec72 complex with heat shock proteins. *J. Biol. Chem.* **292**, 8007-8018. doi:10.1074/jbc.M116.761122
- Tuijtel, M. W., Koster, A. J., Jakobs, S., Faas, F. G. A. and Sharp, T. H.** (2019). Correlative cryo super-resolution light and electron microscopy on mammalian cells using fluorescent proteins. *Sci. Rep.* **9**, 1369. doi:10.1038/s41598-018-37728-8
- Van den Berg, B., Clemons, W. M., Jr., Collinson, I., Modis, Y., Hartmann, E., Harrison, S. C. and Rapoport, T. A.** (2004). X-ray structure of a protein-conducting channel. *Nature* **427**, 36-44. doi:10.1038/nature02218
- van Dijk, J. M., de Jong, A., Vehmaanperä, J., Venema, G. and Bron, S.** (1992). Signal peptidase I of *Bacillus subtilis*: patterns of conserved amino acids in prokaryotic and eukaryotic type I signal peptidases. *EMBO J.* **11**, 2819-2828. doi:10.1002/j.1460-2075.1992.tb05349.x
- Voorhees, R. M. and Hegde, R. S.** (2016). Structure of the Sec61 channel opened by a signal sequence. *Science* **351**, 88-91. doi:10.1126/science.aad4992
- Voorhees, R. M., Fernández, I. S., Scheres, S. H. W. and Hegde, R. S.** (2014). Structure of the Mammalian ribosome-sec61 complex to 3.4 Å resolution. *Cell* **157**, 1632-1643. doi:10.1016/j.cell.2014.05.024
- Walter, P. and Blobel, G.** (1982). Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature* **299**, 691-698. doi:10.1038/299691a0
- Walter, P. and Ron, D.** (2011). The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334**, 1081-1086. doi:10.1126/science.1209038
- Wiertz, E. J. H. J., Tortorella, D., Bogoy, M., Yu, J., Mothes, W., Jones, T. R., Rapoport, T. A. and Ploegh, H. L.** (1996). Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. *Nature* **384**, 432-438. doi:10.1038/384432a0
- Wild, R., Kowal, J., Eyring, J., Ngwa, E. M., Aebi, M. and Locher, K. P.** (2018). Structure of the yeast oligosaccharyltransferase complex gives insight into eukaryotic N-glycosylation. *Science* **359**, 545-550. doi:10.1126/science.aar5140
- Wu, X., Cabanos, C. and Rapoport, T. A.** (2018). Structure of the post-translational protein translocation machinery of the ER membrane. *Nature* **566**, 136-139. doi:10.1038/s41586-018-0856-x
- Yang, Q.-P., Fu, M.-F., Gao, H., Yamamoto, K., Hu, D. and Qin, S.-Y.** (2018). Subcellular distribution of endogenous malectin under rest and stress conditions is regulated by ribophorin I. *Glycobiology* **28**, 374-381. doi:10.1093/glycob/cwy034
- Zimmer, J., Nam, Y. and Rapoport, T. A.** (2008). Structure of a complex of the ATPase SecA and the protein-translocation channel. *Nature* **455**, 936-943. doi:10.1038/nature07335