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Structural insight into the protein translocation channel

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A structurally conserved protein translocation channel is formed by the heterotrimeric Sec61 complex in eukaryotes, and SecY complex in archaea and bacteria. Electron microscopy studies suggest that the channel may function as an oligomeric assembly of Sec61 or SecY complexes. Remarkably, the recently determined X-ray structure of an archaeal SecY complex indicates that the pore is located at the center of a single molecule of the complex. This structure suggests how the pore opens perpendicular to the plane of the membrane to allow the passage of newly synthesized secretory proteins across the membrane and opens laterally to allow transmembrane segments of nascent membrane proteins to enter the lipid bilayer. The electron microscopy and X-ray results together suggest that only one copy of the SecY or Sec61 complex within an oligomer translocates a polypeptide chain at any given time.

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Abbreviations

EM	electron microscopy
ER	endoplasmic reticulum
OST	oligosaccharyl transferase complex
TM	transmembrane

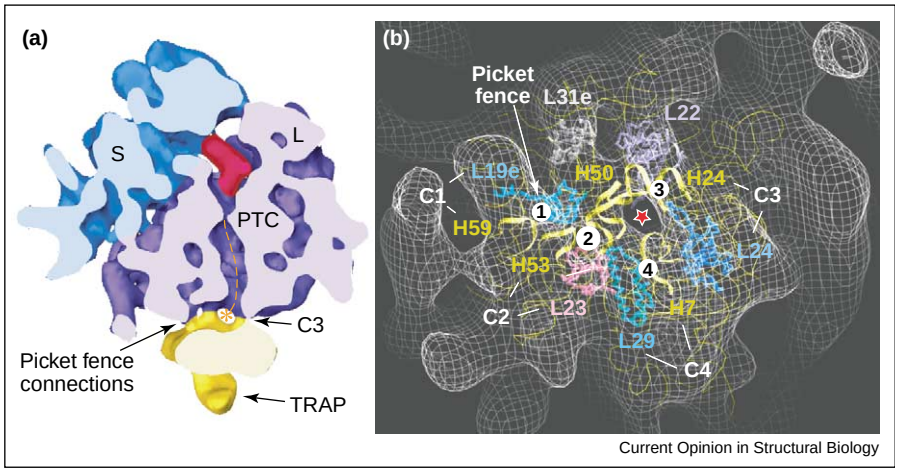
Introduction

Protein translocation across the endoplasmic reticulum (ER) membrane in eukaryotes and the plasma membrane in prokaryotes is a decisive step in the biosynthesis of many proteins. Secretory proteins are targeted to the membrane by cleavable signal sequences and cross the lipid bilayer completely. Membrane proteins are targeted by signal sequences or non-cleaved transmembrane (TM) segments. Their TM segments are integrated into the lipid bilayer, while appropriate hydrophilic regions are left in the cytosol or translocated across the membrane. Both types of proteins use a protein-conducting channel for translocation. This channel is formed from a conserved heterotrimeric membrane protein complex, called the Sec61 complex in eukaryotes and the SecY complex in prokaryotes. It consists of an α-subunit, which spans the membrane ten times, and β- and γ- subunits, which, in most organisms, are single-span proteins. The channel is a passive conduit that associates with partners that provide the driving force for different modes of translocation. In the co-translational pathway, the channel binds the translating ribosome. In the post-translational pathway in eukaryotes, the channel associates with the tetrameric Sec62/63 complex and the lumenal chaperone BiP, whereas in the post-translational pathway in bacteria, it binds to SecA, a cytoplasmic ATPase. In addition to these partners, the channel associates with several other components. Examples in mammals include the signal peptidase complex, the oligosaccharyl transferase complex (OST), the TRAP complex and the TRAM protein. TRAP is a tetrameric membrane protein complex of unknown function that is associated with the mammalian ribosome-bound Sec61 complex (Figure 1a), whereas TRAM is a multispanning protein that is thought to function as a chaperone during early stages of secretory protein synthesis and membrane protein integration.

The Sec61/SecY channel has a hydrophilic interior and its pore is flexible, allowing the passage of even bulky chemical groups attached to the sidechains of amino acids *in vitro* [1] or a disulfide-bonded peptide loop of 13 residues in a secretory protein [2]. Despite this flexibility, the channel maintains a barrier during protein translocation that may prevent the passage of ions and other small molecules. The channel also opens sideways: hydrophobic TM segments of nascent membrane proteins move from the aqueous interior of the channel through a lateral gate into the hydrophobic environment of the lipid bilayer. The ability of the channel to open in two directions, across the membrane and laterally within the membrane, distinguishes it from many other channels.

Significant insight into the function of the Sec61/SecY channel has come from structural studies. Here, we discuss these results and their contribution to current models of protein translocation.

Figure 1



EM structure of a ribosome–channel complex. **(a)** Ribosome–channel complexes derived from native dog pancreatic ER membranes were analyzed by single-particle cryo-EM. A cut-away surface view is shown, with the small ribosomal subunit (S) in cyan, the large ribosomal subunit (L) in purple and a tRNA molecule present in the E-site of the ribosome in red. The channel and its connections to the ribosome are in gold. The location of the peptidyl transferase center (PTC) is indicated. The ribosome tunnel provides a path for a nascent chain (orange dashed line) to the junction between the channel and the ribosome. There is sufficient space in the junction for appropriate regions of a nascent membrane protein to move into the cytosol, either laterally or straight out of the plane of the panel. **(b)** Surface of the mammalian ribosome to which the channel docks shown as a meshwork in 'O'. Features from the atomic map of the *Haloarcula marismortui* large ribosomal subunit are drawn as ribbons. The four major connections between the ribosome and the channel are indicated by numbered white circles, and protein and rRNA components that may contribute to each connection are indicated. The picket fence is formed by connections C1, C2 and C4. The exit tunnel is indicated by a red asterisk.

Freeze-fracture and negative-stain electron microscopy

The earliest views of the channel came from freeze-fracture electron microscopy (EM) of ER membranes containing bound ribosomes. These pictures showed intramembrane particles underneath the ribosomes with a diameter of ~05–110 Å [3,4]. Ring-like structures of similar dimension were also seen when proteoliposomes containing reconstituted, purified Sec61 complex were analyzed by freeze-fracture EM [5]. When visualized by negative-stain EM, purified Sec61 or SecY complexes in detergent appeared as donut-like structures, with stain-filled central cavities [5,6]. The particles were ~85–100 Å wide and ~50–60 Å high, and their size suggested that they are formed from either three or four copies of the Sec61/SecY complex. The assembly of these ring-like oligomers may be regulated, as suggested by freeze-fracture EM of reconstituted proteoliposomes; the number of ring-like particles was higher when ribosomes were bound or when the Sec62/63 complex was co-reconstituted with Sec61 [5]. These data suggested that binding of the channel partner may induce the formation of oligomers during translocation.

Electron microscopy structures of ribosome–channel complexes

The first cryo-EM reconstruction of ribosome–channel complexes was done using purified yeast Sec61 complex bound to the ribosome in detergent [7]. The 26 Å reconstruction showed a 15–20 Å gap between the ribosome and the channel, bridged by a single contact. At the chosen contour level, the channel had an ~25 Å hole underneath the ribosomal exit tunnel, consistent with the idea that a nascent chain could move from the ribosome directly into the membrane channel. Subsequent reconstructions using mammalian and yeast ribosome–channel complexes derived from solubilized native membranes or reconstituted proteoliposomes provided a similar picture. At ~27 Å resolution, these maps indicated few differences in the presence or absence of a nascent polypeptide [8]. Ribosome–channel complexes derived from native ER membranes showed an additional structural feature that may correspond to the TRAP complex ([8]; J-F Ménétret *et al.*, unpublished). The early ribosome–channel EM reconstructions and negative-stain EM pictures led to the assumption that a large pore is formed at the interface of three or four copies of the Sec61 complex. However, in the most recent reconstructions at ~15–17 Å resolution, with an improved definition of the contour level, a pore is no longer visible (Figure 1a). Instead, a central indentation is seen on the lumenal side of the channel [9,10]. In these maps, the ribosome makes multiple connections with the channel, contributed by several candidate segments of rRNA and protein; these connections are similar in yeast and mammals (Figure 1b). As expected, the distribution of the contacts is asymme-

trical and several of the connections (C1, C2 and C4; Figure 1b) form a ridge across the top of the channel. This 'picket fence' would allow a nascent polypeptide chain emerging from the ribosomal exit tunnel to make contact with only one side of the oligomeric channel. Importantly, even at higher resolution, there is still significant space at the ribosome–channel junction (Figure 1a). This gap may provide a path for nascent polypeptides from the ribosomal exit tunnel into the cytosol, as is required when the ribosome synthesizes cytosolic domains of membrane proteins.

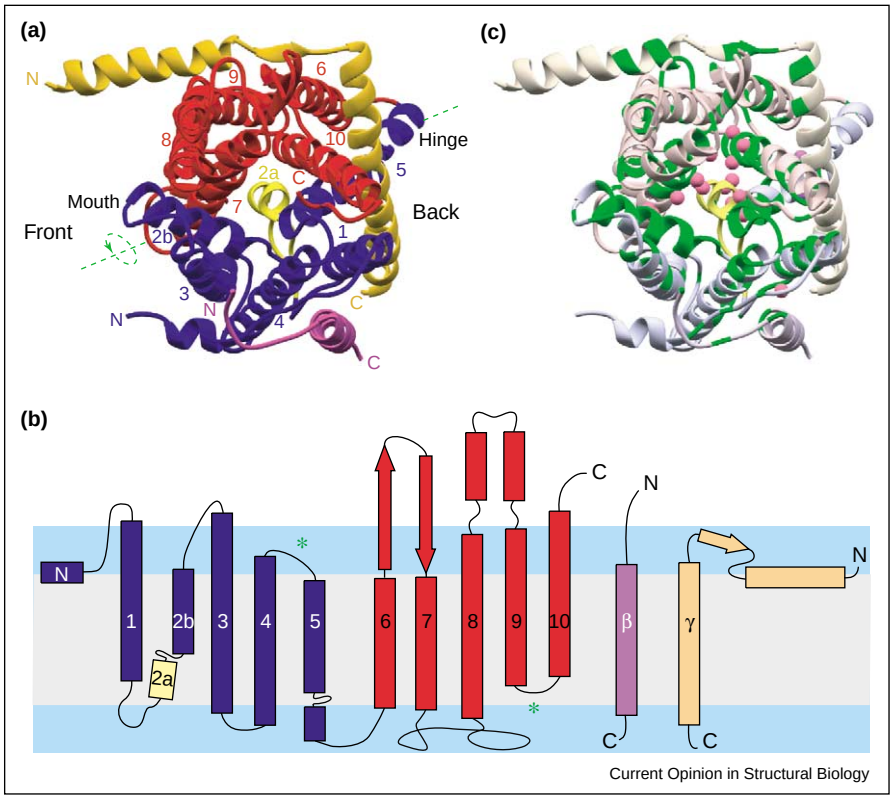
Two-dimensional crystal structure of a SecY complex

Two-dimensional crystals of the *Escherichia coli* SecY complex in a lipid bilayer were analyzed by cryo-EM and three-dimensional reconstruction to provide a map with an in-plane resolution of 8 Å. In this map, all 15 expected TM helices were visible [11•], but the resolution was insufficient for their identification. The cytosolic domains of the SecY complex extend ~15 Å from the plane of the membrane, consistent with the observed gap between the channel and the ribosome in single-particle reconstructions [9,10]. The crystal packing is formed, in part, by an extended array of dimers of the SecY complex, packed in a side-by-side fashion.

X-ray structure of a SecY complex: general description

A high-resolution structure (3.2 Å) was recently obtained for the SecY complex from the archaea *Methanoccocus jannaschii* [12••]. When viewed from the cytosol, the channel has a square shape with an approximate edge length of 50 Å (Figure 2a). The α-subunit consists of two domains, TM1–TM5 and TM6–TM10, which form a 'clam shell' hinged by the loop between TM5 and TM6 (Figure 2a,b). The two halves are related to each other by pseudo-symmetry, with a twofold axis in the plane of the membrane. The domains are clamped together by the essential γ-subunit, which wraps its

Figure 2



Overview of the X-ray structure of the *M. jannaschii* SecY complex. **(a)** Ribbon diagram of the SecY complex viewed from the cytoplasm. The α-subunit is colored with the N-terminal domain in blue (TM1–TM5), the C-terminal domain in red (TM6–TM10) and helix TM2a (the 'plug') in yellow. The different TM helices are numbered. The γ-subunit is colored in gold and the β-subunit in purple. Faces designated as front and back are indicated, together with the positions of the hinge and mouth of the clam shell. Green dashed lines represent the pseudo-twofold axis in the plane of the membrane. **(b)** Topology diagram of the SecY complex. The hydrophilic and hydrophobic regions of the membrane are indicated by blue and gray boxes, respectively. Secondary structures are drawn with positions relative to the location in the membrane, colored as in (a). Two loops that would be related by the pseudo-symmetry are indicated by green asterisks. **(c)** The locations of signal sequence suppressor mutations are represented as pink spheres. Conserved regions are colored in green. Panels (a,c) made using RIBBONS and POV-ray.

TM helix across the interface of the two domains and has an N-terminal amphipathic helix that lies flat on the cytoplasmic surface of the membrane. The β-subunit makes limited contact with the α-subunit, consistent with it being non-essential. Together, the two smaller subunits occupy three sides of the α-subunit, leaving open the front of the clam shell (the mouth).

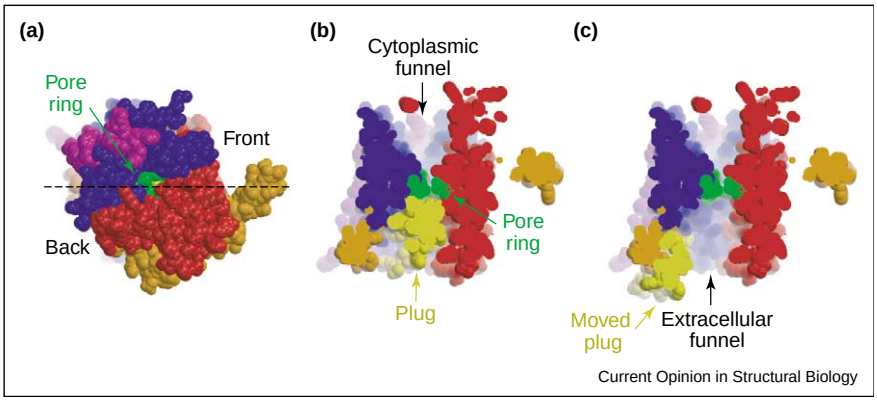
The structures determined by X-ray diffraction from three-dimensional crystals and by EM from two-dimensional crystals are nearly identical. This agreement shows that the structures are the same whether the complex is in detergent or in a lipid bilayer, and whether it is derived from a bacterial or archaeal species. Together with the observed sequence homology across all kingdoms, this allows the large body of experimental data obtained for translocation systems in *E. coli*, yeast and mammals to be interpreted on the basis of the structure of the archaeal SecY complex.

A single SecY complex forms the channel

The X-ray structure shows that there is a hydrophobic belt around the SecY complex that defines the membrane-embedded surface; this is typical of most membrane proteins. Thus, a large hydrophilic pore cannot be generated by a simple interaction between the lateral surfaces of several Sec61/SecY complexes. This raises the possibility that the pore is formed within a single copy of the complex. Indeed, the majority of mutations that allow proteins with defective or missing signal sequences to be transported (prl mutations) are located in the central region of the SecY complex [13,14] (Figure 2c). These mutations are expected to be within the pore, because they may either stabilize the open state of the channel or destabilize its closed state. Conserved residues are also concentrated in the center of the complex (Figure 2c). Many of these residues are located in a cytoplasmic funnel that probably marks the entrance to the pore. The funnel has an ~20 Å diameter on the cytoplasmic side of the complex and tapers to a close in the center of the membrane (Figure 3a,b). The pore probably opens by movement of a short helix, dubbed the 'plug', which is located in the center of the complex and extends from the extracellular side halfway through the membrane (Figure 3c). The plug would move into a cavity on the extracellular side of the channel, bringing it close to the TM helix of the γ-subunit, as has been demonstrated by cross-linking experiments [15]. This would create an hourglass-shaped channel, comprising two aqueous funnels separated by a narrow constriction in the middle of the membrane. The constriction is formed by a ring of six hydrophobic pore residues with branched sidechains, which are often isoleucines.

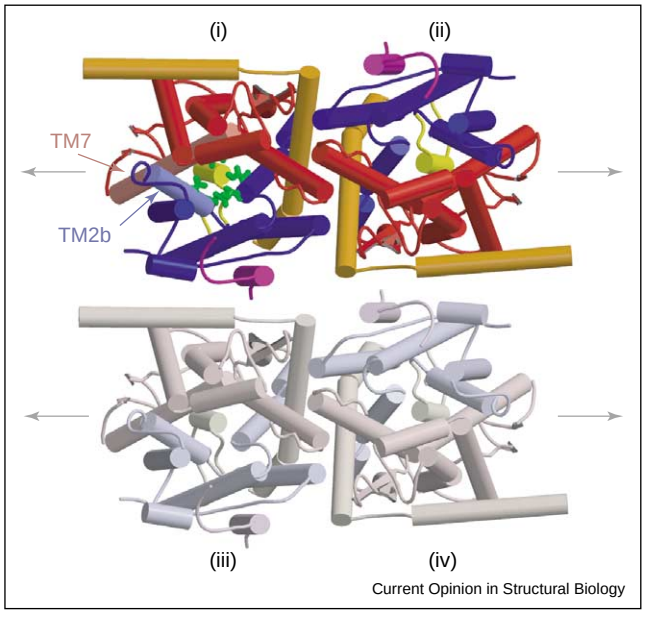
The hourglass shape of the pore may limit the contacts made by a translocating chain to a single layer of hydrophobic pore residues, thus minimizing the energy required for its movement through the membrane (Figure 3c). The pore residues could fit like a gasket around the translocating polypeptide, hindering ions and other small molecules from passing through the channel. This model is different from proposals in which the seal during mammalian co-translational translocation is provided by the binding of either the ribosome to the cytoplasmic side of the channel or BiP to the lumenal side [16,17]. The new model explains how the membrane barrier can be maintained in all modes of translocation, including post-translational translocation, in which a ribosome is not involved, and translocation in prokaryotes, where BiP does not exist. In addition, it explains why the

Figure 3



Proposed mechanism of pore opening. **(a)** Space-filling model of the SecY complex viewed from the cytoplasm, colored as in Figure 2a. The pore ring is indicated in green. A dashed line represents the slice through the molecule shown in (b,c). **(b)** Closed pore viewed from the side [90° rotated with respect to (a)]. The SecY complex was sliced through the middle to reveal the interior of the pore. The plug is colored in yellow and the cytoplasmic funnel is indicated. **(c)** Modeled open pore in the same view as in (b), with the plug (yellow) moved. The resulting extracellular funnel is indicated. Figure made using Molscript and Raster3D.

Figure 4



Proposed oligomeric assembly of the channel: the four Sec61/SecY complexes in the proposed double dimer. The two brightly colored complexes (i and ii) are the dimer seen in the two-dimensional crystal structure. A second dimer (gray colors) may associate with it side by side. The lateral exit sites of each Sec61/SecY complex are indicated with gray arrows. In complex i, TM2b and TM7 are indicated, and the pore ring is shown in stick representation in green. Figure made using Molscript and Raster3D.

gap between the ribosome and channel seen in EM studies does not compromise the membrane barrier [9,10]. Channel opening may be triggered by the binding of both a channel partner (the ribosome, Sec62/63 complex or SecA) and a signal sequence (or a TM segment in the case of a membrane protein). Signal sequences intercalate between TM2b and TM7 at the front of the molecule [18], and this may require the clam shell to open at its front (Figure 4). This in turn may destabilize interactions of the plug helix that keep it in the center of the complex, thus triggering its movement to create an open pore. The front of the clam shell would also provide a lateral gate for hydrophobic TM segments of nascent membrane proteins to exit into the lipid phase. In the case of a multispanning protein, TM segments would exit sequentially as they are being synthesized.

Pore size

The pore ring has dimensions of ~5–8 Å (measured as center-of-atom to center-of-atom distances) and must therefore widen to accommodate a translocating chain. This flexibility could come from the lateral displacement of the helices to which the pore residues are attached. In particular, glycine-rich sequences exist in the symmetry-related loops between TM4 and TM5, and between TM9 and TM10 that could serve as hinges [12••]. Preliminary molecular dynamics modeling shows that a ball of 10–12 Å diameter can be pulled through the pore on a nanosecond timescale (P Tian and I Andricioaei, personal communication), indicating that a polypeptide chain could easily pass through the pore, even as an α-helix.

Fluorescence quenching experiments have suggested that the channel pore is much larger (40–60 Å) than indicated by the X-ray structure. This conclusion was reached by introducing fluorescent probes into nascent polypeptides and demonstrating that even large reagents could pass through the membrane channel to quench them. Surprisingly, these reagents quenched probes that were located inside the ribosomal tunnel, which clearly has a diameter much narrower than 40 Å [10,19]. Thus, the fluorescence quenching results are at odds with both the channel and ribosome structures. Additional experiments are required to clarify these discrepancies. As mentioned before, a central pore seen in early EM reconstructions was due to the choice of contour level. The region of low density formed at the interface of multiple Sec61 complexes may be filled with lipid or detergent.

Oligomerization

Although a single copy of the Sec61/SecY complex may form the pore, there is no question that oligomers are the active species in translocation. A dimer with the back-to-back orientation of two SecY complexes, with the two lateral gates pointing in opposite directions, may represent the basic functional unit (Figure 4). This is the arrangement seen in two-dimensional crystals and its importance during translocation is supported by cross-linking and other experiments [11•,20••,21]. These data argue against the front-to-front association of Sec61/SecY complexes, which is the only possibility for forming a larger pore from several copies of the complex. In addition, the X-ray structure shows that wide opening of the lateral gates and gross re-organization would be required to fuse the pores of several complexes, making this unlikely.

Two dimers may associate to form double dimers (four copies of the SecY complex) during post-translational translocation in bacteria, as suggested by EM data [22]. Likewise, a double dimer may be present beneath the ribosome during co-translational translocation in eukaryotes (J-F Ménétret *et al.*, unpublished). The double dimers may be formed by two dimers that associate in a side-by-side manner (Figure 4), similar to the arrangement observed in the lattice of the two-dimensional crystals. In principle, this mode of interaction could lead to the formation of an extended ribbon of dimers. However, when bound to the ribosome, each Sec61 complex may reside in a different environment beneath the ribosome, as shown by the distinctive pattern of connections.

Thus, interactions with the ribosome may induce the double dimer of the complex, preventing the formation of an extended ribbon. Taking the EM and X-ray data together, it appears that only one copy of the Sec61/SecY complex in the oligomeric assembly translocates a polypeptide at any given time. The single active copy in the oligomer would also be responsible for the lipid integration of all TM segments in a polypeptide chain.

If the pore is formed from one copy of the Sec61/SecY complex, what is the role of oligomerization? The answer is not known, but several possibilities can be envisioned. Oligomerization may create new binding sites for components such as the signal peptidase complex, OST, TRAP or TRAM, which must be recruited to the translocation site. Another, not mutually exclusive, possibility is that oligomerization regulates the binding of the ribosome. Oligomerization would increase the number of linkages, resulting in strong ribosome binding during translocation. Dissociation of the oligomers would be expected to weaken this interaction and thereby facilitate ribosome release upon termination of translocation. Clearly, the role of oligomerization remains an intriguing question for future work.

Conclusions

Structural studies of the protein-conducting channel have resulted in significant advance of our understanding of protein translocation and allow interpretation of a large body of previously obtained experimental data. However, several of the implications of the X-ray structure remain speculative, particularly because the channel was crystallized in its closed state. Some of the hypotheses can be now be tested directly with biochemical methods, for example, by cross-linking with probes attached to specific residues. However, further structural studies are also needed. An immediate goal is EM reconstruction of ribosome–channel complexes of sufficiently high resolution that would allow the docking of the X-ray structure. In addition, high-resolution structures of the channel with associated SecA or with a bound signal peptide would be highly desirable. The ultimate goal must be structures of the actively translocating channel, associated with both a partner and a substrate.

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