

# Mechanisms of Sec61/SecY-Mediated Protein Translocation Across Membranes

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## Keywords

endoplasmic reticulum, secretory proteins, membrane proteins, membrane barrier

## Abstract

The Sec61 or SecY channel, a universally conserved protein-conducting channel, translocates proteins across and integrates proteins into the eukaryotic endoplasmic reticulum (ER) membrane and the prokaryotic plasma membrane. Depending on channel-binding partners, polypeptides are moved by different mechanisms. In cotranslational translocation, the ribosome feeds the polypeptide chain directly into the channel. In posttranslational translocation, a ratcheting mechanism is used by the ER-luminal chaperone BiP in eukaryotes, and a pushing mechanism is utilized by the SecA ATPase in bacteria. In prokaryotes, posttranslational translocation is facilitated through the function of the SecD/F protein. Recent structural and biochemical data show how the channel opens during translocation, translocates soluble proteins, releases hydrophobic segments of membrane proteins into the lipid phase, and maintains the barrier for small molecules.

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## INTRODUCTION

Many proteins are transported across or are integrated into the eukaryotic endoplasmic reticulum (ER) membrane or the prokaryotic plasma membrane. Soluble proteins, such as those ultimately secreted from the cell or localized to the ER lumen, cross the membrane completely and usually have amino-terminal, cleavable signal sequences, the major feature of which is a segment of 7 to 12 hydrophobic amino acids. Membrane proteins, such as those in the plasma membrane or in other organelles of the secretory pathway, are integrated into the lipid bilayer by transmembrane (TM) segments composed of about 20 hydrophobic amino acids; hydrophilic segments of a membrane protein either cross the membrane or remain in the cytosol. Both types of proteins are translocated by the same protein-conducting channel, which is formed from a conserved heterotrimeric membrane protein complex called the Sec61 complex in eukaryotes and the SecY complex in bacteria and archaea. The Sec61/SecY channel has two main activities: It allows soluble polypeptides to cross the membrane and hydrophobic TM segments of membrane proteins to exit laterally into the lipid phase.

The Sec61/SecY channel alone is a passive pore; it must associate with partners that provide a driving force for translocation. In cotranslational translocation, the main partner is the ribosome. This pathway exists in all cells and is used for the translocation of secretory proteins as well as for the integration of most membrane proteins. Some proteins are translocated by the Sec61/SecY channel after completion of their synthesis, that is, posttranslationally. This pathway is used mostly by secretory proteins that possess only moderately hydrophobic signal sequences or are too short to be efficiently recognized by the signal recognition particle (SRP) during their synthesis. Posttranslational translocation of secretory proteins occurs by different mechanisms in eukaryotes and bacteria. In eukaryotes, the channel partners with another membrane-protein complex, the Sec62/Sec63 complex, and with the luminal chaperone BiP, a member of the Hsp70 family of ATPases. BiP acts as a molecular ratchet to bias the passive movement of a polypeptide in the Sec61 channel. In bacterial posttranslational translocation, the channel partners with the cytosolic ATPase SecA. SecA uses the energy of ATP hydrolysis to push a polypeptide chain through

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**ER:** endoplasmic reticulum

**TM:** transmembrane

**SRP:** signal recognition particle

**Hsp70 family:**

comprises heat-shock proteins of ~70 kDa that consist of an ATPase domain and a peptide-binding domain

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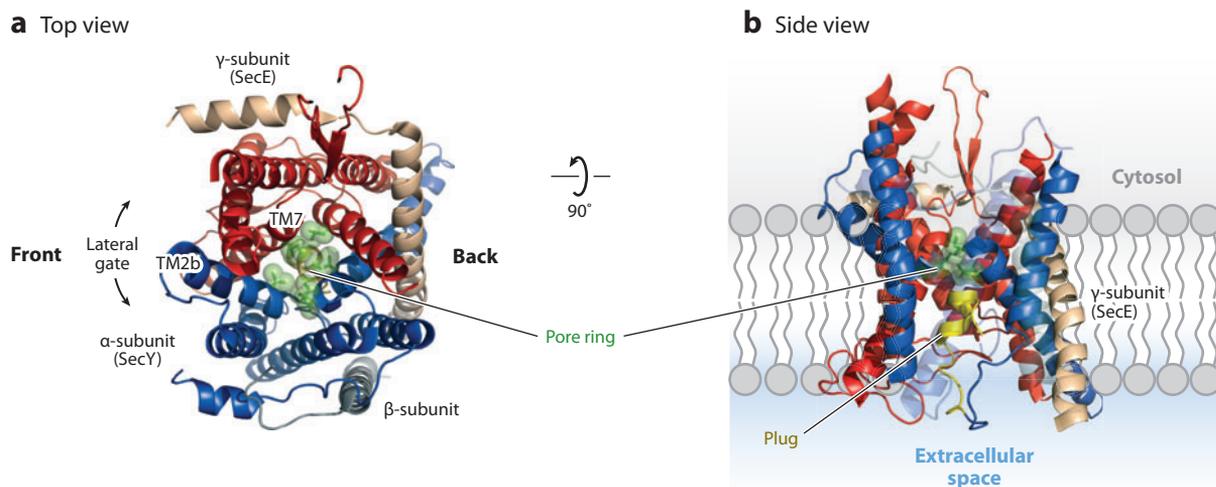
the channel. Translocation in bacteria is also stimulated by an electrochemical gradient across the membrane. Archaea probably use both cotranslational and posttranslational translocation (47, 67), but it is unknown how posttranslational translocation occurs because these organisms lack SecA, the Sec62/Sec63 complex, and BiP.

In this review, we discuss these protein translocation pathways and the mechanisms of membrane integration, with special emphasis on recent developments. The reader is referred to earlier reviews that cover some aspects in more detail (11, 20, 70, 77).

## THE Sec61/SecY CHANNEL

The channel-forming Sec61/SecY complex consists of three subunits,  $\alpha$ ,  $\beta$ , and  $\gamma$ . The  $\alpha$ - and  $\gamma$ -subunits show significant sequence conservation, and both subunits are essential for cell viability. The  $\beta$ -subunits are not universally conserved and are not essential. The  $\alpha$ -subunit forms the pore of the channel, as initially shown by photocross-linking experiments, which demonstrated that the  $\alpha$ -subunit of the Sec61 complex surrounds the polypeptide chain during its passage across the membrane (64). In addition, reconstitution experiments showed that the Sec61/SecY complex is the essential membrane component for protein translocation (1, 8, 31). The channel has an aqueous interior, as indicated by electrophysiology experiments (86) and by measurements of the fluorescence lifetime of probes incorporated into a translocating polypeptide chain (12, 13).

The crystal structure of an archaeal SecY complex (from *Methanococcus jannaschii*) provided important insight into the function of the translocation channel (93). Viewed from the cytosol, the  $\alpha$ -subunit is divided into two halves, TM segments 1–5 and 6–10, which surround a central pore (Figure 1a). The loop between TM segments 5 and 6 at the back of the  $\alpha$ -subunit serves as a hinge, allowing the  $\alpha$ -subunit to open at the front and form the lateral gate. The  $\gamma$ -subunit links the two halves of the  $\alpha$ -subunit at the back by extending a TM segment diagonally across their



**Figure 1**

Crystal structure of the *Methanococcus jannaschii* SecY channel. (a) Channel viewed from the cytosol (top view). The transmembrane (TM) segments 1–5 of the N-terminal domain of the  $\alpha$ -subunit, called SecY in prokaryotes, are blue, and the TM segments 6–10 of the C-terminal domain are red. The  $\beta$ -subunit is gray and the  $\gamma$ -subunit (SecE in prokaryotes) is beige. Pore residues are shown as transparent spheres with side chains in green. The lateral gate containing TM segments 2b and 7 is indicated. (b) Side view of the channel extending through the plasma membrane. The plug helix underneath the pore ring is yellow.

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**Presenilin:**

an intramembrane aspartyl protease in the  $\gamma$ -secretase protein complex, which cleaves various substrates, including amyloid precursor protein

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interface. The  $\beta$ -subunit makes only a few contacts with the  $\alpha$ -subunit, which may explain why it is dispensable and less conserved. Viewed from the side, the channel has an hourglass-shaped pore with a constriction about halfway across the membrane (**Figure 1b**). Whereas the cytoplasmic funnel is empty, the external funnel is filled with a short helix—the plug. The constriction of the channel is formed by a ring of six hydrophobic residues that project their side chains radially inward. The residues forming this pore ring are amino acids with bulky, hydrophobic side chains. In *Escherichia coli* all six pore residues are isoleucines.

The structure of bacterial SecY complexes is similar to that of the archaeal complex, as shown initially by the similarities to a lower-resolution structure of the *E. coli* SecY complex, determined by electron microscopy from two-dimensional crystals (7). Three-dimensional crystal structures of SecY complexes from *Thermotoga maritima* (102), *Aquifex aeolicus* (102), *Thermus thermophilus* (92), and *Pyrococcus furiosus* (23) all show the same architecture as the *M. jannaschii* complex, except that the bacterial  $\beta$ -subunit has an additional TM segment preceding the one that is common to all SecY complexes. In addition, in several bacterial structures the lateral gate is opened in response to interaction with a binding partner. No crystal structure of a eukaryotic Sec61 complex is available, but sequence conservation and electron microscopy structures suggest a similar architecture (3, 57).

## CHANNEL OPENING FOR SECRETORY PROTEIN TRANSLOCATION

The mechanism of protein translocation is best understood for secretory proteins. The process begins with the loop insertion of the polypeptide substrate into the channel: The signal sequence is intercalated into the walls of the channel and the following segment is located in the pore (84). Opening of the channel for loop insertion probably occurs in two steps. The first is the binding of a channel partner—the ribosome, the Sec62/Sec63 complex, or SecA. Crystal structures of SecY complexes with bound SecA show that the lateral gate is partially opened and the plug is displaced, although it still seals the pore (102). Lateral gate opening might be induced by an interaction between SecA and the loop between TM8 and TM9, as an antibody bound to this loop has a similar effect (92).

The second step is the intercalation of the hydrophobic part of a signal sequence into the opened lateral gate. Photocross-linking experiments show that the bound signal sequence forms a helix of about two turns, which is intercalated between TM segments 2b and 7 of the lateral gate (74). The signal sequence can also be cross-linked to phospholipid molecules, indicating that it sits at the interface between channel and lipid. The binding of the signal sequence would further separate TM segments 2b and 7 and destabilize plug interactions, causing the plug to move out of the way. Finally, the open state of the channel would be fixed by the insertion of the polypeptide chain distal to the signal sequence into the pore proper. Consistent with this model for channel opening, many mutations that allow the translocation of proteins with defective or even missing signal sequences (signal sequence suppressor mutations) are expected to destabilize the closed channel (16, 87, 90).

Once the channel is open, the signal sequence remains stationary during subsequent translocation, whereas the rest of the polypeptide moves through the pore. Interestingly, a synthetic signal peptide can act in *trans*, allowing a polypeptide without a signal sequence to move through the channel (32). The plug can return to the center of Sec61/SecY only when the polypeptide chain has left the pore. At some point during translocation, the signal sequence is cleaved by signal peptidase. In eukaryotes, the signal peptide is then further degraded by signal peptide peptidase, a presenilin-like enzyme that cleaves the hydrophobic segment within the membrane (97).

## A SINGLE COPY OF THE Sec61/SecY COMPLEX FORMS THE PORE OF THE CHANNEL

The crystal structures indicate that a single copy of the Sec61/SecY complex forms the pore; a polypeptide moves from the cytoplasmic funnel, through the pore ring, and into the external funnel. This model is supported by disulfide cross-linking experiments with a SecA-dependent translocation substrate: Both the signal sequence and the following polypeptide segment could be cross-linked to the same SecY molecule (69). Moreover, cysteines placed in a translocation substrate could efficiently form a disulfide bridge with cysteines placed at the constriction of the hourglass-shaped SecY channel, indicating that the polypeptide chain moves through the center of a single SecY molecule (9). This model likely applies to cotranslational translocation as well. Electron-microscopy structures show that a single copy of the Sec61/SecY complex is bound to a nontranslating ribosome, with the cytoplasmic funnel of the translocation channel located underneath the ribosome tunnel (60, 61). A similar architecture is seen with translating ribosomes (3, 27). Disulfide cross-linking experiments show that, in an intact *E. coli* cell, more than 70% of SecY can be occupied with a defined ribosome-associated polypeptide chain (72). All these data support the idea that a single Sec61/SecY molecule forms the translocation pore.

A consequence of this conclusion is that the pore is relatively narrow. In fact, the diameter of the pore ring, as observed in the crystal structure, is barely large enough to allow the passage of an extended polypeptide chain. The pore ring can widen by movements of the helices to which the pore ring residues are attached, as indicated by molecular dynamics simulations and electrophysiology experiments (34, 35, 79, 89). However, the structures indicate that the pore could not be larger than ~15 to 20 Å in diameter, much smaller than suggested by fluorescence-quenching experiments (40–60 Å) (37). It is currently unclear why the latter experiments led to an overestimate of the pore size.

The small pore size means that a translocating polypeptide is in an extended conformation or perhaps forms an  $\alpha$ -helix in the channel, but not tertiary structure, in agreement with experimental data (2, 51). The aqueous interior of the channel, its hourglass shape, and the lack of interactions between the hydrophobic pore residues and the hydrophilic polypeptide backbone all help to minimize the energy required to move a translocation substrate through the membrane.

## OLIGOMERIC STATE OF THE TRANSLOCATION CHANNEL

Although the pore is formed by only one Sec61/SecY molecule, this does not necessarily mean that protein translocation can occur with just one copy. For example, it is conceivable that additional Sec61/SecY molecules stabilize the ribosome-channel junction.

Oligomeric Sec61/SecY channels have indeed been detected in intact membranes by cross-linking (18, 81, 95), fluorescence energy transfer (63, 88), and freeze-fracture electron microscopy experiments (39, 82). A back-to-back orientation of two SecY molecules is suggested by a two-dimensional structure of the *E. coli* SecY complex in a lipid bilayer and by cross-linking data (6, 7, 18, 95). However, other orientations of the Sec61/SecY molecules in the oligomers have not been excluded. After solubilization of membranes in detergent, the Sec61/SecY oligomers dissociate into monomers, although under gentle conditions, oligomers can be detected by native gel electrophoresis (5).

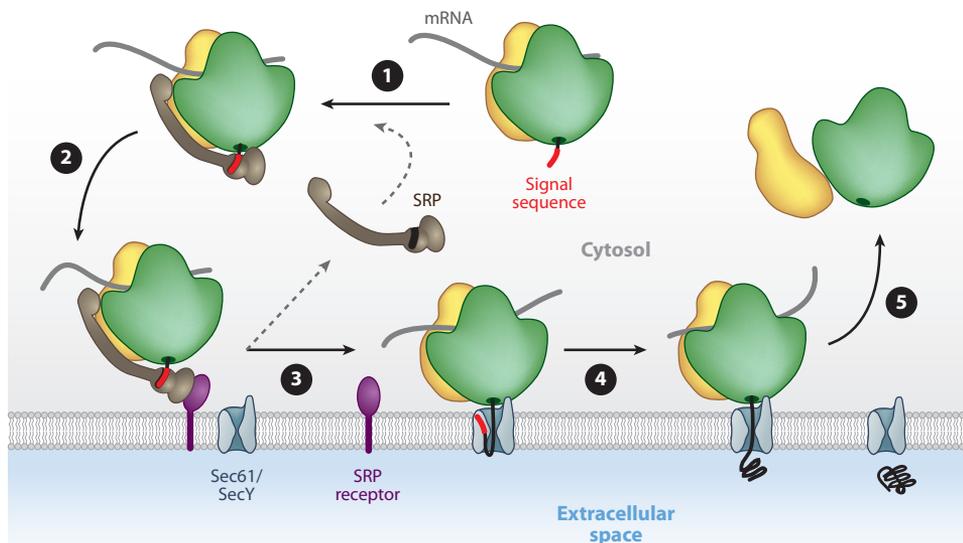
Functionally, oligomers of SecY complexes have been implicated in SecA-mediated translocation, based on the observation that a SecY molecule defective in translocation can be rescued by linking it covalently with a wild-type SecY copy (69). The crystal structures of SecA-SecY complexes show only one SecA molecule bound to one SecY molecule (102), but disulfide bridge cross-linking experiments indicate interactions between SecA and a cytosolic loop of SecY

(69, 92), which cannot be explained by the crystal structure. It was proposed that SecA binds through one of its domains to a nontranslocating SecY copy and pushes the polypeptide chain through a neighboring SecY copy (69). The interaction with the nontranslocating copy could prevent complete detachment of SecA during the nucleotide hydrolysis cycle and thus ensure processivity during polypeptide translocation. This view is supported by single-molecule experiments (18), but a recent study using similar techniques reported that a single copy of SecY is sufficient for translocation (50). This discussion demonstrates that many issues about the oligomeric state of Sec61/SecY complexes remain unresolved.

## MECHANISM OF COTRANSLATIONAL TRANSLOCATION

Cotranslational translocation begins with the signal or TM sequence of a growing polypeptide chain being recognized by the SRP (**Figure 2**). Next, the ribosome–nascent chain–SRP complex binds to the membrane, first by an interaction between SRP and its membrane receptor and then by an interaction between the ribosome and the Sec61/SecY channel (for review of the targeting phase, see References 33, 36, 80). Subsequently, the elongating polypeptide chain moves directly from the tunnel inside the ribosome into the associated membrane channel. GTP hydrolysis is required for chain elongation by the ribosome, but polypeptide movement through the channel is independent of nucleotide hydrolysis (10).

Electron microscopy structures of ribosome–channel complexes show that the exit site of the nascent chain from the ribosome is aligned with the pore of the channel, supporting the notion that a nascent chain emerging from the ribosome tunnel could be transferred directly



**Figure 2**

Model of cotranslational translocation. The scheme shows different steps in the cotranslational translocation of a secretory protein. Step 1: Binding of the signal recognition particle (SRP) to a ribosome carrying a nascent chain with exposed signal sequence. Step 2: Binding of the ribosome–nascent chain–SRP complex to the SRP receptor. Step 3: Release of SRP, binding of the ribosome to the Sec61/SecY channel, and transfer of the nascent chain into the channel. Step 4: Translocation of the polypeptide chain, signal sequence cleavage, and folding of the polypeptide on the other side of the membrane. Step 5: Termination of translocation and dissociation of the ribosome into its two subunits.

into the Sec61/SecY channel (3, 27, 60, 61). Surprisingly, two recent structures obtained with ribosome–nascent chain–channel complexes in detergent show no significant conformational change of the channel compared with its resting state (3). However, in the most recent structure, obtained with the SecY complex incorporated into lipid nanodiscs, the lateral gate of the channel was partially opened (27). Electron density for the TM segment of the nascent chain was seen adjacent to the channel, probably corresponding to a state after its lateral release from the channel interior. Future work must be directed toward a higher-resolution structure of ribosome–nascent chain–channel complexes in which the presence of a nascent chain inside the channel is verified by biochemical experiments.

**Nanodisc:**

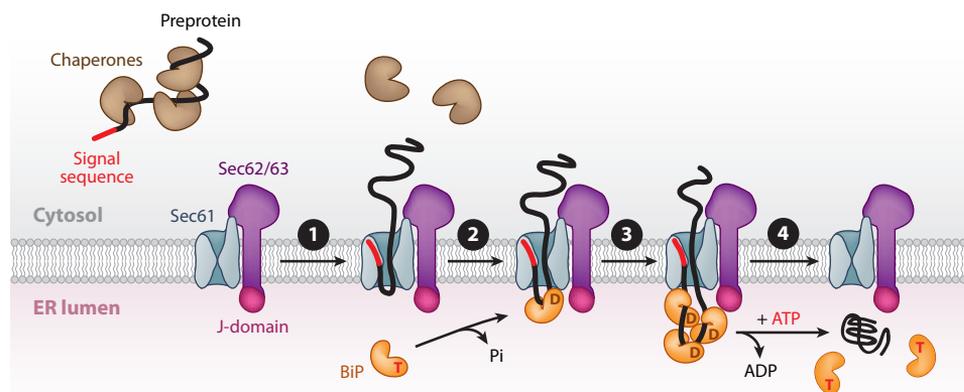
a small (~10- to 15-nm-diameter) lipid bilayer disc stabilized by an engineered apolipoprotein fragment

**TPR motif:**

tetratricopeptide repeat motif

**MECHANISM OF BiP-DEPENDENT POSTTRANSLATIONAL TRANSLOCATION**

The mechanism of BiP-mediated posttranslational translocation has been elucidated in yeast but is probably the same in all eukaryotes (Figure 3). Translocation begins with the binding of a translocation substrate to the Sec complex, consisting of the trimeric Sec61 complex and the Sec62/Sec63 complex (in *Saccharomyces cerevisiae* composed of Sec62p, Sec63p, Sec71p, and Sec72p). During this step, all cytosolic chaperones bound to the substrate are released (75). Several different chaperones appear to cycle on and off the completed polypeptide chain, but once the substrate is bound to the Sec complex, their rebinding is prevented. Sec72p contains TPR motifs, which might interact with cytosolic Hsp70 and Hsp90 proteins, perhaps facilitating their release from the translocation substrate. However, neither Sec71p nor Sec72p are essential (25, 26), and the mammalian complex lacks both proteins. The function of the essential large cytosolic domains of Sec62p and Sec63p also remains unclear.



**Figure 3**

Model of posttranslational translocation in eukaryotes. The scheme shows different steps in the posttranslational translocation of a eukaryotic secretory protein. Step 1: Binding of a completed polypeptide chain to the Sec complex, consisting of the Sec61 channel and the Sec62/Sec63 complex. Chaperones associated with a completed polypeptide chain are released during its insertion into the channel. Step 2: BiP in its ATP-bound state (T) interacts with the J-domain in Sec63. Following ATP hydrolysis, BiP binds to the polypeptide substrate in its ADP state (D), preventing the polypeptide from sliding back into the cytosol. Step 3: When the polypeptide chain has moved a sufficient distance into the endoplasmic reticulum (ER) lumen, the next BiP molecule binds. This process is repeated until the polypeptide has completely traversed the channel. Step 4: Nucleotide exchange releases BiP from the polypeptide chain.

**J-domain:** a domain found in all J-proteins, which stimulates the ATPase activity of the Hsp70 protein partners

Once the polypeptide is inserted into the channel, its translocation occurs by a ratcheting mechanism. The polypeptide chain in the channel can slide in either direction by Brownian motion, but its binding to BiP inside the lumen of the ER results in net forward translocation. A Brownian ratcheting mechanism is supported by the observation that ATP-independent translocation can occur when BiP is replaced by antibodies to the substrate (59).

BiP starts out in its ATP state with an open peptide-binding pocket (**Figure 3**). After interaction with the J-domain of Sec63p, ATP is hydrolyzed, and the peptide-binding pocket closes around the translocating polypeptide chain. The location of the J-domain ensures that BiP activation only occurs close to the channel. Although BiP preferentially binds hydrophobic peptides under equilibrium conditions, it shows little sequence specificity when activated by the J-domain of Sec63p (62). Because BiP is too large to move through the channel, it prevents the bound polypeptide chain from sliding back into the cytosol. When the polypeptide has moved a sufficient distance in the forward direction, the next BiP molecule can bind. This process is repeated until the polypeptide chain has completely traversed the channel. Finally, exchange of ADP for ATP opens the peptide-binding pocket and releases BiP.

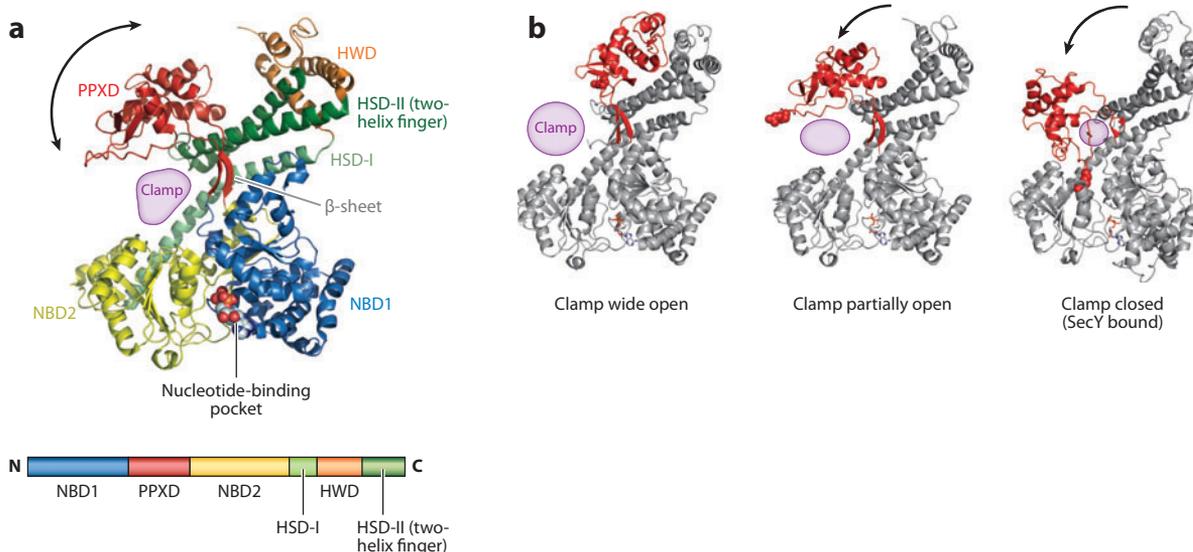
## MECHANISM OF SecA-MEDIATED TRANSLOCATION

SecA is a cytosolic ATPase of the RecA family. It consists of several domains: two nucleotide-binding domains (NBD1 and NBD2) with the nucleotide bound at their interface; a helical scaffold domain (HSD), consisting of a long helix (HSD-I) and two shorter helices (HSD-II), dubbed the “two-helix finger”; a polypeptide-cross-linking domain (PPXD); and a helical wing domain (HWD) (**Figure 4a**). Crystal structures of SecA from different species were all obtained in ADP or without nucleotide and differ greatly in the position of the PPXD relative to the HWD (**Figure 4b**); the PPXD is either packed against the HWD (46, 94, 101) or rotated away from it toward the NBD2 (68, 71). The groove between the PPXD, NBD2, and parts of the HSD is referred to as the clamp, which can thus be in open or closed states (**Figure 4a,b**).

Several different dimers of SecA have been observed in crystal structures and some of these dimers coexist in solution (19, 98). Although some experiments suggest that SecA functions as a dimer during translocation (48, 52), it seems more likely that it acts as a monomer (66, 102); the role of the dimer may be to maintain a low basal rate of ATPase activity in the resting state (30).

Crystal structures of a complex of SecA and the SecY channel were obtained in the presence of ADP plus beryllium fluoride ( $\text{BeF}_3^-$ ), mimicking a state similar to the ATP-bound state (102). One SecA is bound to one copy of SecY (**Figure 5**). The flat SecA molecule is oriented approximately parallel to the plane of the membrane. Most of the interactions with SecY are mediated by the PPXD. Compared with the conformation of SecA, in which the clamp is wide open, the PPXD has rotated by  $80^\circ$ , inserting a loop between the NBDs (**Figure 4b**). The movement of the PPXD allows the clamp to capture a translocation substrate. The clamp is located above the SecY pore (**Figure 5a**), enabling a polypeptide chain to move through the clamp into the channel, a postulate confirmed by systematic disulfide cross-linking experiments (2).

The long helix of the HSD also makes contact with the SecY channel. It lies across SecY and might serve as a lever arm that transmits and amplifies nucleotide-dependent movements between the NBDs to the other domains of SecA. The two-helix finger of SecA is inserted into the cytoplasmic funnel of SecY, with the loop between the two helices right above the pore entrance (**Figure 5**). It was postulated that, upon ATP binding by SecA, the finger would move toward the channel and drag the polypeptide chain with it (**Figure 6**). Upon ATP hydrolysis, the finger would reset. This process would be repeated until the entire polypeptide chain is translocated. Resetting of the finger could be coordinated with clamp tightening and holding the polypeptide. Both



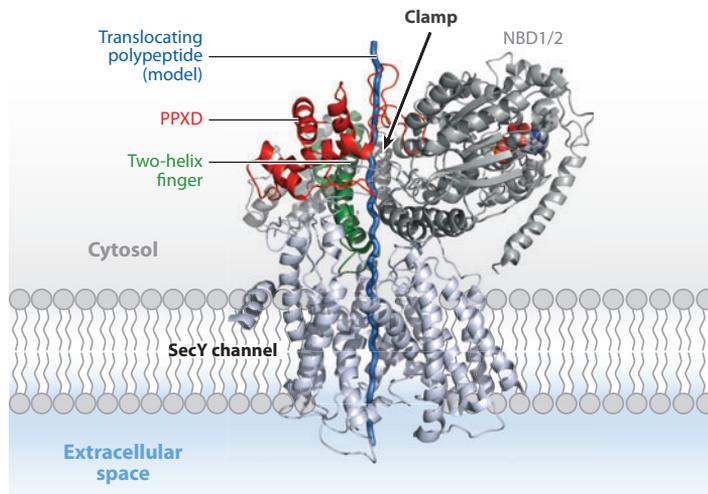
**Figure 4**

Structure of the bacterial SecA ATPase. (*a*) Domain organization of SecA. The arrow indicates the movement of the PPXD (see structures in panel *b*). A polypeptide substrate moves perpendicular to the plane through the clamp (*purple*). The polypeptide probably forms a transient  $\beta$ -strand of three to four residues with the indicated  $\beta$ -sheet at the back of the clamp. (*b*) Different crystal structures of SecA corresponding to states in which the clamp is either wide open (PDB:1M74), partially open (PDB:1TF2), or closed (SecY bound; PDB:3DIN). The movement of the PPXD is proposed to capture the polypeptide substrate in the clamp. An amino acid at the tip of a loop of the PPDX inserts between the two NBDs in the SecY-bound state (shown in ball presentation). Abbreviations: PPXD, polypeptide-cross-linking domain; HWD, helical wing domain; NBD, nucleotide-binding domain; HSD, helical scaffold domain.

the movement of the two-helix finger and the tightening of the clamp are speculative. Alternative models, such as the polypeptide is pushed into the channel by the clamp tilting toward the channel, although perhaps less likely, cannot be excluded.

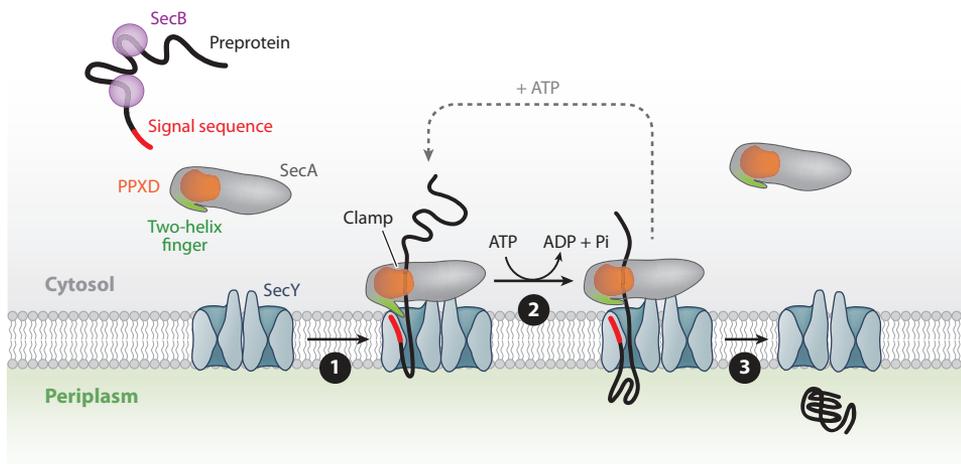
Alanine scanning mutagenesis of the two-helix finger has shown that a tyrosine in the loop between the two helices is important for translocation (24). The tyrosine can be replaced by other bulky, hydrophobic amino acids, but not by small or charged residues. Most SecA proteins in the database indeed have a tyrosine at the critical position, but other bulky, hydrophobic residues are occasionally observed. In the crystal structure, the tyrosine does not contact SecY, suggesting that its essential role is to interact with the translocating polypeptide chain. Indeed, disulfide cross-linking experiments show that a polypeptide chain passes by the fingertip before entering the SecY pore (2, 24). Interestingly, peptide-translocating hexameric ATPases, such as ClpX, ClpA, HslU, p97, and FtsH, may use an analogous mechanism: Each subunit has a loop with an aromatic residue at its tip, which contacts the polypeptide chain and transports it through the central pore (15, 44, 73, 85, 100). How the aromatic residue interacts with the polypeptide chain is not understood in any of the cases.

How can SecA transport a large range of substrates that differ widely in their sequence? One answer is probably that the clamp embraces a polypeptide chain, similar to how some chaperones bind their diverse substrates in a deep groove. However, it appears that SecA also interacts with translocating polypeptides in a sequence-independent manner by inducing a short  $\beta$ -strand in the substrate that extends the  $\beta$ -sheet at the back of the clamp (**Figure 4a**). Such a  $\beta$ -strand augmentation mechanism is suggested by a crystal structure of *Bacillus subtilis* SecA with a synthetic peptide (103), as well as by two structures of *B. subtilis* SecA. In one structure, a C-terminal domain



**Figure 5**

Structure of the *Thermotoga maritima* SecA-SecY complex. A hypothetical translocating polypeptide chain is shown in blue. The clamp formed by rotation of the PPXD (red) positions the polypeptide over the SecY pore. The two-helix finger (green) contacts the polypeptide with its tip. The nucleotide bound between the NBDs is shown in ball presentation. Abbreviations: PPXD, polypeptide-cross-linking domain; NBD, nucleotide-binding domain.



**Figure 6**

Model of SecA-mediated posttranslational translocation in bacteria. The scheme shows the postulated steps in the posttranslational translocation of a secretory protein. Step 1: SecA binds to a completed polypeptide chain and inserts it into the SecY channel. The cytosolic chaperone SecB is released during this process. Step 2: During repeated ATP hydrolysis cycles, movements of the two-helix finger push the polypeptide into the channel. The clamp might hold the polypeptide chain while the two-helix finger resets to grab the next segment of the substrate. Step 3: After translocation is terminated, SecA is released from SecY. Abbreviation: PPXD, polypeptide-cross-linking domain.

of SecA interacts in a  $\beta$ -strand conformation with the clamp (46), and in the other, two SecA molecules interact in the crystal such that a  $\beta$ -strand is generated in one copy that is bound to the clamp of the other copy (101). In all these cases, the curvature of the  $\beta$ -sheet would direct the additional  $\beta$ -strand into the clamp. The induced  $\beta$ -strand involves only three to four residues, so the interaction could be transient and allow polypeptide movement during translocation.

Although the main function of SecA may be in the translocation of secretory proteins, it is involved in the biosynthesis of membrane proteins with large periplasmic domains (14). Ribosome- and SecA-mediated translocation modes might alternate during the synthesis of these proteins, a possibility that may be related to the surprising finding of SecA binding to ribosomes (45).

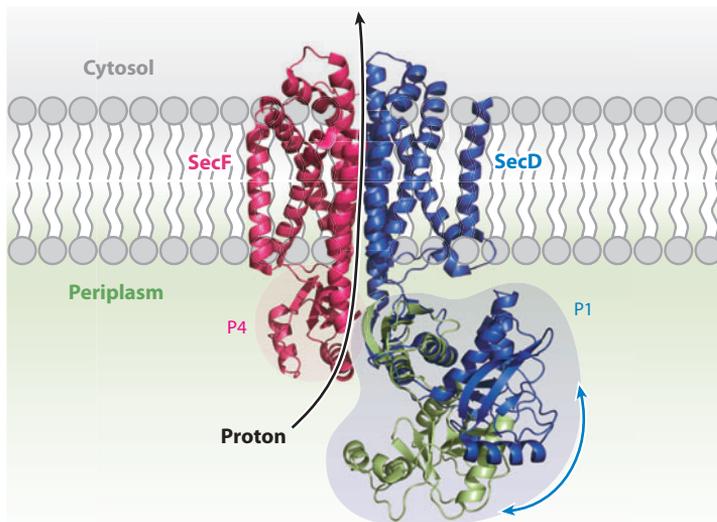
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**RND family:** comprises the resistance-nodulation-division proteins in gram-negative bacteria, which couple ion flux with the export of compounds from the cell

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## THE ROLE OF SecD/F AND OF A MEMBRANE POTENTIAL IN TRANSLOCATION

SecA-mediated protein translocation is stimulated by the multispanning membrane protein complex SecD/F, which associates with the SecY channel (21, 76). A recent crystal structure shows that *T. thermophilus* SecD/F contains 12 TM segments, 6 TM segments in both SecD and SecF, arranged in a pseudosymmetrical manner (91) (**Figure 7**). In several species SecD/F consists of a single polypeptide chain. The membrane-embedded domains qualify SecD/F as a member of the RND family of transporters. The protein also contains two periplasmic domains, P1 and P4. P1 can interact with polypeptides and undergo a conformational change. The membrane-embedded part can conduct protons at the SecD-SecF interface, with conserved aspartate and arginine residues lining the pathway across the membrane. The proton-conduction pathway seems to be similar to that of AcrB, another member of the RND family of transporters (65). Interestingly, some halophilic *Vibrio* bacteria contain two SecD/F genes; one apparently conducts protons and the



**Figure 7**

Structure of the *Thermus thermophilus* SecD/F complex. SecD/F consists of 12 transmembrane segments and two large periplasmic domains (P1 and P4). The membrane-embedded parts of SecD (blue) and SecF (magenta) conduct protons. This may be coupled to a conformational change in the periplasmic domain P1 (blue and green conformations; the arced arrow indicates the transition between them), by which a translocating polypeptide chain is bound and released, thereby pulling it into the periplasm.

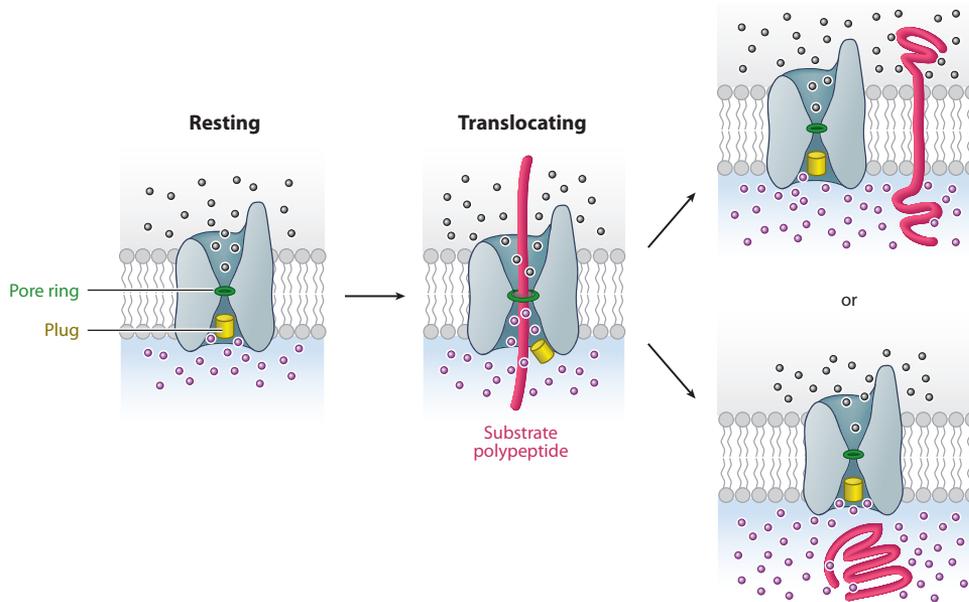
other sodium ions (91). It has been proposed that the movement of the ions through the membrane is coupled to the conformational change of the P1 domain, resulting in SecD/F pulling on a translocating polypeptide on the periplasmic side of the membrane. Indeed, late stages of SecA-mediated protein translocation can occur without ATP in a SecD/F- and proton-gradient-dependent manner (91).

## MAINTAINING THE MEMBRANE BARRIER FOR SMALL MOLECULES DURING TRANSLOCATION

The Sec61/SecY channel must prevent the free movement of small molecules, such as ions or metabolites, both in its resting state and when translocating a polypeptide. Maintaining the membrane barrier is particularly important for prokaryotes, because ion gradients across the membrane are their main energy source. The mammalian ER membrane is somewhat permeable to small molecules (53), but it must also prevent the free flow of  $\text{Ca}^{2+}$  ions.

The mechanism by which the permeability barrier is maintained has been controversial. Results from fluorescence-quenching experiments with ER membranes suggest that the channel itself is not a barrier for small molecules; it would have a pore size of 9 to 15 Å in the resting state and widen to 40 to 60 Å during translocation (37, 38). The seal would be provided by BiP binding to the luminal end of the channel in the resting state and by the translating ribosome binding to the cytoplasmic side of the channel during translocation. BiP would also close the channel when a cytosolic domain of a membrane protein is synthesized (38). This would be triggered when the TM segment of the nascent chain is still inside the ribosome tunnel (55–57), but it is difficult to see how a long hydrophobic sequence can be recognized inside the hydrophilic ribosome tunnel. A tight seal between the ribosome and channel is also at odds with electron microscopy structures that reveal a gap of 12 to 15 Å between them (4, 27, 60, 61). Finally, this model does not explain how the membrane barrier is maintained in the absence of a ribosome in posttranslational translocation or in the absence of BiP in prokaryotes.

Structural and biochemical studies suggest a different model, in which the membrane barrier is formed by the channel itself (**Figure 8**). In the resting state, the seal would be provided by both the plug and the pore ring. This conclusion is supported by electrophysiology experiments, which indicate that the resting SecY channel, reconstituted into a planar membrane, is indeed impermeable to ions and water and opens on plug displacement (79). Recent experiments with intact *E. coli* cells also show that the resting wild-type SecY channel is impermeable to small molecules but becomes permeable when the plug is deleted or when pore residues are replaced by amino acids with small side chains (72). The plug interacts with the pore ring, which explains why both features are required to seal the resting channel (54, 93). These experiments also clarify how the membrane barrier is maintained during translocation (72). In the active channel, the plug is displaced and the pore ring forms a gasket-like seal around the translocating polypeptide chain. The translocating polypeptide chain itself serves as the major obstacle for small molecules; without it, the open channel allows many small molecules to pass. The model implies that whenever the polypeptide leaves the channel, either toward the extracellular side after termination of translocation or sideways into the lipid after the arrival of a hydrophobic TM segment, the plug returns and reseals the channel. This mechanism would apply to both cotranslational and posttranslational translocation. Given the sequence conservation of the SecY and Sec61 channels, the proposed model may be universal. However, in prokaryotes, a tight seal is essential for cell viability, whereas in eukaryotes, the intracellular ER membrane may tolerate some leakiness. This may explain why mutations of Sec61p pore residues in *S. cerevisiae* cause only minor growth defects, whereas the equivalent mutations in *E. coli* are lethal (49, 72).



**Figure 8**

Model for maintaining the membrane barrier by the SecY/Sec61 channel. (*Left*) In the resting state of the channel, small molecules on either side of the membrane (*black and purple spheres*) are prevented from moving through the channel by both the pore ring (*green*) and the plug domain (*yellow*). (*Middle*) During translocation the plug is displaced. The pore ring forms a gasket-like seal around the translocating polypeptide chain to prevent the free flow of small molecules. When the polypeptide leaves the channel, the plug returns and reseals the channel. This occurs either after translocation is terminated, when the polypeptide has moved completely toward the extracellular side (*right, lower panel*), or after the arrival of a hydrophobic transmembrane segment of a membrane protein, when the transmembrane segment exits sideways into the lipid bilayer (*right, upper panel*).

## MEMBRANE PROTEIN INTEGRATION BY THE Sec61/SecY CHANNEL

Most membrane proteins are integrated cotranslationally into the lipid bilayer. In the simplest model, TM segments insert sequentially into the lipid phase; hydrophilic segments between the TM segments move alternately from the ribosome, through the aqueous channel, to the external side of the membrane, or they emerge between the ribosome and channel into the cytosol via a gap, which can be visualized in electron microscopy structures (4, 27, 60, 61). Each TM segment exiting the ribosome enters the Sec61/SecY channel and then leaves the channel through the lateral gate into the lipid phase. The size of the channel, as seen in crystal structures, indicates that TM segments exit laterally one by one or in pairs.

The lateral gate is formed from short segments of four TM segments at the front of Sec61/SecY. This seam in the channel wall is probably weak, as indicated by structures of the SecY channel in which it is partially open (23, 92, 102). The lateral gate might be either permanently open once a channel partner is bound or continuously open and close. In either case, a polypeptide segment located in the aqueous channel would be exposed to the surrounding hydrophobic lipid phase, allowing it to partition between the hydrophilic and hydrophobic environments. If hydrophobic enough, the segment would exit through the lateral gate into the lipid phase. This model is supported by photocross-linking experiments that examined the lateral exit of a TM

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**Translocating chain-associating membrane (TRAM):**

a mammalian protein that interacts with weak signal sequences and TM segments containing charges

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segment in different translocation intermediates (42), as well as by the agreement between a hydrophobicity scale derived from peptide partitioning into an organic solvent and the tendency of a peptide to span the membrane (43). However, there may also be a kinetic component to the partitioning process, because mutations in the pore ring of *S. cerevisiae* Sec61p affect the efficiency of hydrophobic sequence integration into the lipid phase (49), and moderately hydrophobic sequences integrate more efficiently into the lipid phase when the rate of translocation is reduced (22).

According to the sequential insertion model, the first TM segment in a multispanning membrane protein determines the orientation of the subsequent segments. The first TM segment has its N terminus in the cytosol if the segment preceding the hydrophobic region is long or positively charged (positive-inside rule; 40, 41). In one model, the N terminus is retained in the cytosol by being folded or by the interaction between its positive charges and negatively charged lipid head groups. An alternative model has been suggested for some proteins: The N terminus first moves through the channel to the other side of the membrane and then returns to the cytosol (17, 29). Immediate translocation of the N terminus occurs at least with proteins, which have their N terminus ultimately on the extracellular side. Translocation of the N terminus occurs if the N terminus lacks positive charges, the hydrophobic sequence is long, and the preceding polypeptide segment is not tightly folded (for review, see Reference 78).

The sequential insertion model does not apply to all membrane proteins, as there are cases in which internal TM segments have a preferred orientation regardless of the behavior of preceding TM segments (28, 58, 96). In the most striking example, mutation of C-terminal amino acids in the EmrE protein changed the topology not only of the last TM segment but also of the preceding three TM segments (83). This raises the possibility that TM segments previously integrated into the lipid phase can invert their topology at late stages during synthesis of the membrane protein.

It is possible that intermediates in the synthesis of multispanning membrane proteins need to be stabilized by membrane chaperones, for example, if the membrane-inserted part of the protein contains charges that are only compensated for by parts of the protein that have yet to be synthesized. In bacteria, a candidate for a membrane chaperone is the SecY-associated YidC protein (for review, see Reference 99). An analogous function has been proposed for the translocating chain-associating membrane (TRAM) protein in higher eukaryotes (for review, see Reference 78).

### SUMMARY POINTS

1. A universally conserved protein-conducting channel, referred to as the Sec61 channel in eukaryotes and as the SecY channel in prokaryotes, is responsible for the translocation of proteins across and the integration of proteins into cellular membranes.
2. Crystal structures of the SecY channel and biochemical data show that a single copy of the heterotrimeric Sec61 or SecY complex forms the channel, resulting in a relatively narrow pore through which a polypeptide moves in an extended conformation.
3. The channel opens for secretory protein translocation by insertion of a polypeptide as a loop, with the signal sequence intercalated into the lateral gate and the following segment in the actual pore. This results in displacement of the plug domain of the channel.

4. Depending on binding partners, the channel translocates polypeptides by different mechanisms. In cotranslational translocation, the ribosome feeds the polypeptide chain directly into the channel. In posttranslational translocation, a ratcheting mechanism is used by the ER luminal chaperone BiP in eukaryotes and a pushing mechanism is utilized by the SecA ATPase in bacteria. In prokaryotes, posttranslational translocation is facilitated through the function of the SecD/F protein.
5. The channel itself is responsible for maintaining the membrane barrier for small molecules, such as ions or metabolites. In the resting state, the channel is sealed by the pore ring amino acids as well as the plug domain. When in the active state, the pore ring forms a gasket-like seal around the translocating polypeptide chain.
6. The channel integrates membrane proteins into the lipid bilayer by allowing TM segments to partition from the aqueous interior of the channel, through the lateral gate, into the lipid phase.

### FUTURE ISSUES

1. What is the oligomeric state of the Sec61/SecY channel during translocation? Is an oligomer required for translocation?
2. How exactly does SecA push a polypeptide through the channel?
3. How does the Sec62/Sec63 complex activate the Sec61 channel for posttranslational translocation in eukaryotes? What is the structure of the eukaryotic Sec proteins?
4. How exactly does SecD/F associate with the SecY channel and facilitate protein translocation?
5. What is the function of YidC in bacteria and of the TRAM protein in mammals? Are they membrane chaperones that associate with insertion intermediates of membrane proteins?
6. An important research focus should be the determination at high resolution of a structure of an active channel translocating a polypeptide.

### DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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32. Shows that a synthetic signal peptide allows the SecA-mediated translocation of a polypeptide lacking a signal sequence.

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102. On the basis of crystal structures of SecA-SecY channel complexes, proposes a model for SecA-mediated protein translocation.

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