# CHAPTER 5

# Membrane Protein Insertion in Bacteria from a Structural Perspective

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## Abstract

Membrane proteins are inserted into the lipid bilayer in bacteria by two pathways. The Sec machinery is responsible for the insertion of the majority of the membrane proteins after targeting by the SRP/FtsY components. However, there is also a class of membrane proteins that insert independent of the Sec machinery. These proteins require a novel protein called YidC. Recently, the structural details of the Sec machinery have come to light via X-crystallographic analysis. There are now structures of the membrane-embedded Sec protein-conducting channel, the SecA ATPase motor, and the targeting components. The structures give clues to how a polypeptide is translocated across the membrane and how the transmembrane segments of a membrane protein are released from the Sec complex. Additionally, the structure of the targeting components sheds light on how the membrane substrates are selected for transport and delivered to the membrane.

## Introduction

Membrane proteins are ubiquitous in nature and comprise around 30% of the total proteins within the cell. Membrane proteins play vital functions for the cell. They act as receptors where they are involved in transmitting information from the extracellular environment into the interior of the cell. Membrane proteins also function as transporters to move sugars, amino acids and other energy rich molecules and ions into the cell. Other functions of membrane proteins include energy harvesting and energy transduction roles in photosynthesis and oxidative phosphorylation, as well as functions in lipid synthesis and catabolism. Given the wide variety of functions, there is a diversity of membrane protein structures. However, generally almost all bacterial inner-membrane integral membrane proteins have helical transmembrane segments that range from 20 to 30 residues in length, with tryptophan and tyrosine residues being enriched near phospholipid headgroups and the connecting loops between helical transmembrane segments tend to be short.[1] In this review, we will bring the reader up on the latest developments in bacterial membrane protein biogenesis with a focus on structural aspects of the targeting and translocation components that facilitate insertion.

In the field of membrane protein biogenesis, there are at least four main problems. (1) How do membrane proteins with hydrophobic surfaces avoid aggregating in the cytoplasm?

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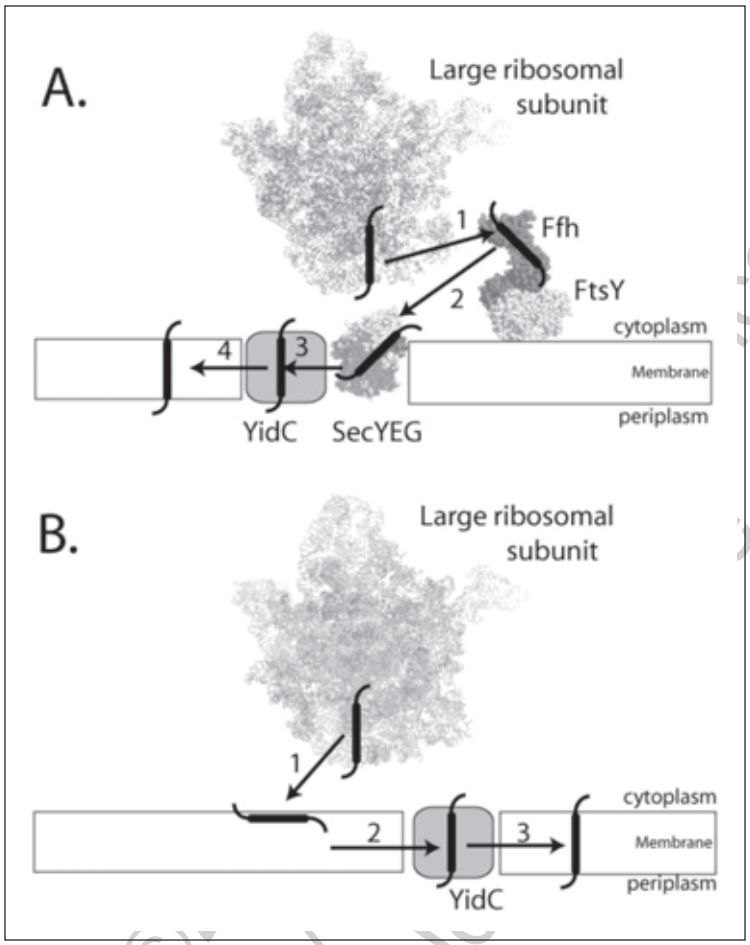


Figure 1. Schematic depiction of the two known membrane protein integration (assembly) pathways. A) The Sec-dependent pathway (the heterotrimer SecDFyajC and the ATPase SecA are not shown). B) The YidC pathway. The PDB coordinates used for the large ribosomal subunit from *Deinococcus radiodurans*[65] were 1NKW, the PDB coordinates used for SecYEβ from *Methanococcus janaschii,*[47] were 1RHZ, The PDB coordinated used for Ffh from *Sulfolobus solfataricus*[16] and FtsY from *Thermus aquaticus*,[21] were 1QZW and 1RJ9, respectively. The program PyMol[66] was used to make this figure.

(2) How are hydrophilic domains translocated across the membrane? (3) How are hydrophobic domains integrated into the membrane? (4) What are the energetics of membrane protein insertion? Not surprising, there are proteins that catalyze the targeting of proteins to the membrane and the insertion into the lipid bilayer. In bacteria, there are two pathways used for membrane protein insertion; the Sec-pathway and YidC pathway. The majority of proteins use the Sec pathway for insertion (Fig. 1A). A subset of proteins insert by a Sec-independent pathway involving YidC (Fig. 1B).

The goal of understanding the molecular events involved in membrane protein assembly is not only of significant scientific interest in the membrane biogenesis area but is essential for the understanding of the disease states that result when these events go wrong.[2,3]

## Insertion by the Sec-Translocase Mediated Pathway

Many membrane proteins inserted by the Sec pathway are targeted to the membrane by the evolutionarily-conserved Signal Recognition Particle (SRP) route. In this pathway, the cytosolic component SRP, comprised of Ffh and the 4.5S RNA[4,5] binds to the membrane protein and targets the protein to the SRP receptor FtsY. SRP binds to the hydrophobic region of the membrane protein as it emerges from the ribosomal tunnel (Fig. 1A). Then, the ribosome/mRNA/nascent membrane protein/Ffh complex is targeted to FtsY that is associated with the membrane.

Insertion of a protein into the membrane is initiated by a cleavable signal peptide or a noncleaved transmembrane segment. The transmembrane segments are integrated into the membrane and the hydrophilic domains are either translocated across the membrane or remain within the cytoplasm. The membrane protein uses the Sec translocase for insertion into the membrane and translocation of hydrophilic domains across the membrane (Fig. 1A). In *E. coli*, the Sec translocase is comprised of the SecYEG protein-conducting channel and the trimeric SecDFYajC complex (for review see ref. 6). The protein YidC interacts with the hydrophobic regions of membrane proteins during the insertion of the protein into the membrane.[7] In some cases, the membrane-associated ATPase SecA is required for the translocation of large hydrophilic domains of membrane proteins.[8-10]

### *Targeting*

The targeting components Ffh and FtsY are important for the insertion of membrane proteins as depletion of Ffh and FtsY within the cell has been shown to inhibit the insertion of a variety of membrane proteins. The SRP component Ffh in *E. coli* is homologous to the 54 KDa subunit of the eukaryotic SRP[11] which is comprised of 6 polypeptides and a 7S RNA component.[5] Ffh exists in complex with a 4.5S RNA instead of the 7S RNA seen in the eukaryotic complex. SRP Ffh has been shown to bind to signal peptides of exported proteins and hydrophobic segments of membrane proteins.[10,12] For membrane proteins containing multiple hydrophobic regions, it may be sufficient for Ffh to bind to the first hydrophobic domain and target the protein to the membrane. Efficient membrane targeting of proteins which have hydrophobic surfaces is important as it prevents aggregation in the aqueous cytoplasm. The SRP receptor in bacteria (FtsY) is simpler than the SRP receptor (SR) in eukaryotes which contain two subunits, SRα and SRβ. The membrane-associated protein FtsY is homologous to the SRα subunit. Both FtsY and Ffh are essential bacterial proteins.[13,14] Ffh has been shown to form a complex with FtsY, in a GTP-dependent manner.[15] Following GTP hydrolysis, the Ffh and FtsY complex disassembles from the targeted nascent protein and the nascent chain can insert into the Sec machinery. Interestingly, it has been found that the GTPase activity of Ffh is stimulated by FtsY[15] and the GTPase activity of FtsY is stimulated by Ffh.

In order to provide insight into the protein targeting mechanism, it is very useful to obtain structural knowledge of the targeting components. Ffh contains three domains, i.e, the amino-terminal N domain, the GTPase G domain and the methionine rich M domain (Fig. 2A).[16,17] The M-domain is connected to the N and G-domains by a flexible linker. The crystal structure of the M domain from *Thermus aquaticus* reveals a hydrophobic groove lined with methionine residues that has been proposed to bind to the signal peptide or the membrane anchor domain of the nascent polypeptide.[17] Interestingly, a crystal structure of the *E. coli* Ffh domain with the domain IV of the 4.5S RNA suggests that the signal sequence recognition domain is comprised of both protein and RNA (SRP)(Table 1A).[18] A structure of the complete SRP54 (Ffh) in complex with helix 8 of the SRP RNA component revealed the overall juxtaposition of the M, G and N domains relative to each other.[16]

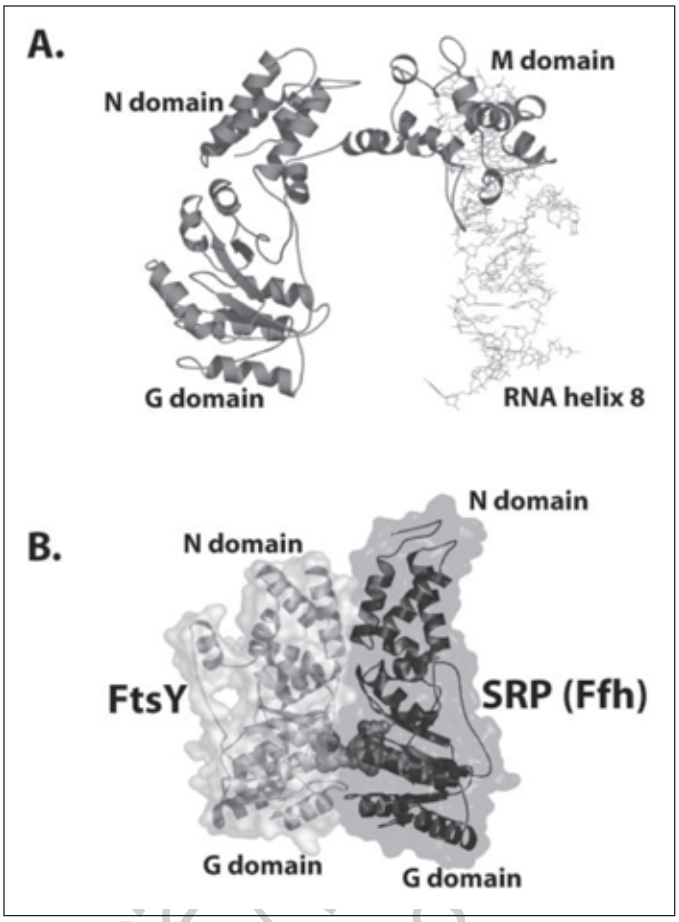


Figure 2. A) A ribbon diagram of the overall structure of the SRP core from the archaeon *Sulfolobus solfataricus*.[16] The structure reveals the interdomain communication between the N domain, the G domain, the M domain and helix 8 of SRP RNA. The RNA is shown in a stick diagram. The PDB coordinates 1QZW and the program PyMol[66] were used to make this figure. B) A ribbon diagram with transparent surface showing the heterodimeric complex of the signal recognition particle protein Ffh and its receptor FtsY from the species *Thermus aquaticus*.[21] Ffh is rendered in a darker shade and FtsY is shown in a lighter shade. The bound GTP analogue molecules are shown in van der Waal's spheres. The N-terminal domains (N domain) and the GTP binding domains (G domain) for each protein are labeled. The PDB coordinates 1RJ9 and the program PyMol[66] were used to make this figure.

Numerous structures are available for the NG domains of the Ffh from archaeal homologs. These structures have been solved both in the presence and absence of GDP or nonhydrolyzable GTP analogs (see Table 1B). The N domain is comprised of a four-helix bundle, which is closely associated with the G domain (Ras-like GTPase) that has a core made up of a five-stranded β-sheet surrounded by α-helices. The G domain also contains an Insertion Box Domain (IBD) which is unique to the SRP GTPases. A similar structural

***Table 1A. SRP Protein/RNA complexe structures***

| PDB ID | Source | Method | Description | R Value | Resolution [Å] | Reference |
|---|---|---|---|---|---|---|
| 1DUL | *E. coli* | X-ray | Domain IV of 4.5 S RNA, domain M of Ffh | 0.199 | 1.8 | Batey et al. 2000[18] |
| 1HQ1 | *E. coli* | X-ray | 4.5S RNA, M-domain of Ffh | 0.151 | 1.5 | Batey et al. 2001 [67] |
| 1QZW | *S. solfataricus* | X-ray | The complete SRP 54 (Ffh) with helix 8 | 0.340 | 4.1 | Rosendal et al. 2003[16] |

arrangement is found in the N and G domains of *E. coli* FtsY (SRα), which has been solved to 2.2Å resolution.[19]

The structure of the catalytic core (N and G domains) formed by the Ffh/FtsY complex from *T. aquaticus* has been solved to 1.9 Å resolution in complex with the nonhydrolyzable GTP analog GMP-PCP.[20,21] The structures show that Ffh and FtsY form a quasi-two-fold symmetrical hetero-dimer having interaction surfaces both in the N-domain and the G-domain but with the majority of the protein-protein interactions occur between the G-domains (Fig. 2B). Comparison with structures of the uncomplexed proteins shows there are major conformational changes that occur upon formation of the heterodimer. Binding of GTP verses GDP results in small structural adjustments in the free proteins.[22] The structures reveal that the 3' OH of the GTPs are essential for Ffh/FtsY association, activation and catalysis. The structures show that there is a shared composite active site containing the two GTPs at the interface, explaining why the reason why binding of Ffh to FtsY is GTP-dependent and why the complex disassembles after GTP hydrolysis. The structural rearrangement upon complex formation results in bringing catalytic residues in the IBD loop into the active site. The only interactions at the active site between the GTPases occur between the nucleotides. The GTP molecules are aligned head to tail such that the γ-phosphate of each GTP is hydrogen-bonded to the other GTP's ribose 3' OH group. Hydrolysis of the GTP releases the γ-phosphate. This essentially breaks the contact between the active sites and the GTP substrate and initiates the Ffh/FtsY dissociation. All the three-dimensional structural information of the bacterial and archaeal SRP targeting components currently available are listed in (Table 1A, B, and C). The Signal Recognition Particle Database (SRPDB) (http://psyche.uthct.edu/dbs/SRPDB/SRPDB.html) provides up to date access to alignments of the SRP and SR sequences and phylogenic analysis of these proteins and RNAs.

The function of the SRP/FtsY domains become more clear upon structural analysis. Not only do the structures shed light on how the SRP Ffh M domain binds to the signal peptide, but they also deepen our understanding into why Ffh and FtsY respectively acts as each other's GTPase activating protein. The structures of the Ffh/FtsY (NG domain) complex reveal that Ffh and FtsY interact via the NG domains with the two GTPs forming a composite active site and explains why the targeting of ribosome nascent chain-bound Ffh to FtsY requires GTP (Fig. 5A). The transfer of the nascent membrane protein to the SecY complex cannot take place until Ffh bound to FtsY dissociates from the nascent chain. This only occurs after GTP has been hydrolyzed from Ffh and FtsY.

*Table 1B. SRP Ffh anf FtsY Protein structures*

| PDB ID | Source | Method | Description | R Value | Resolution [Å] | Reference |
|---|---|---|---|---|---|---|
| 1FFH | *T. aquaticus* | X-ray | N and G domains of Ffh | 0.186 | 2.0 | Freymann et al. 1997[68] |
| 2FFH | *T. aquaticus* | X-ray | M domain of Ffh | 0.257 | 3.2 | Keenan et al. 1998[17] |
| 1NG1 | *T. aquaticus* | X-ray | N and G domains of Ffh with GDP bound | 0.189 | 2.0 | Freymann et al. 1999[69] |
| 2NG1 | *T. aquaticus* | X-ray | N and G domains of Ffh with GDP bound | 0.200 | 2.0 | Freymann et al. 1999[69] |
| 3NG1 | *T. aquaticus* | X-ray | N and G domains of Ffh with no GDP bound | 0.199 | 2.3 | Freymann et al. 1999[69] |
| 1J8M | *A. ambivalens* | X-ray | G domain of Ffh | 0.219 | 2.0 | Montoya et al. 2000[70] |
| 1J8Y | *A. ambivalens* | X-ray | G domain of Ffh, T112A mutant | 0.227 | 2.0 | Montoya et al. 2000[70] |
| 1JPJ | *T. aquaticus* | X-ray | N and G domain of Ffh with the non-hydrolyzable GTP analog GMPPNP (N1 = crystal form 1) | 0.201 | 2.3 | Padmanabhan et al. 2001[71] |
| 1JPN | *T. aquaticus* | X-ray | N and G domain of Ffh with non-hydrolyzable GTP analog GMPPNP (N2 = crystal form 2) | 0.190 | 1.9 | Padmanabhan et al. 2001[71] |
| 1LS1 | *T. aquaticus* | X-ray | Apo Ffh N and G domain | 0.137 | 1.1 | Ramirez et al. 2002[72] |
| 1QZX | *S. solfataricus* | X-ray | Complete Ffh without helix 8 | 0.313 | 4.0 | Rosendal et al. 2003[16] |
| 1O87 | *T. aquaticus* | X-ray | N and G domain of Ffh with MgGDP | 0.197 | 2.1 | Focia et al. 2004[20] |
| 1OKK | *T. aquaticus* | X-ray | N and G domain of Ffh in complex with N and G domain of FtsY | 0.156 | 2.0 | Focia et al. 2004[20] |
| 1RJ9 | *T. aquaticus* | X-ray | N and G domain of Ffh in complex with N and G domain of FtsY | 0.206 | 1.9 | Egea et al. 2004[21] |
| 1FTS | *E. coli* | X-ray | FtsY | 0.222 | 2.2 | Montoya et al. 1997[19] |

***Table 1C. SRP RNA Structures***

| PDB ID | Source | Method | Description | R Value | Resolution [Å] | Reference |
|---|---|---|---|---|---|---|
| 1CQ5 | *E. coli* | NMR | SRP RNA domain IV (43-mer) (*averaged structure*) | n/a | n/a | Schmitz et al. 1999[73] |
| 1CQL | *E. coli* | NMR | SRP RNA domain IV (43-mer) (*ensemble*) | n/a | n/a | Schmitz et al. 1999[73] |
| 1DUH | *E. coli* | X-ray | The conserved domain IV of 4.5S RNA | 0.230 | 2.7 | Jovine et al. 2000[74] |
| 28SP | *E. coli* | NMR | The most conserved RNA motif in SRP RNA (SRP54 Binding Domain) (*ensemble*) | n/a | n/a | Schmitz et al. In press |
| 28SR | *E. coli* | NMR | The most conserved RNA motif in SRP RNA (SRP54 Binding Domain) (*averaged structure*) | n/a | n/a | Schmitz et al. In press |

### *Translocation/Insertion*

After targeting to the membrane, the hydrophobic signal anchor of the nascent membrane protein inserts into the Sec YEG channel. The hydrophilic region of the membrane polypeptide is translocated through the Sec complex to the other side of the membrane and the membrane anchor region leaves the channel laterally. How the ribosome-bound membrane targeted protein is transferred to the SecYEG channel is not known. One possibility is that there is a direct interaction between FtsY and the translocation machinery, which then facilitates the insertion of the targeted protein into the SecYEG channel. The chloroplast FtsY forms a large complex that includes SecY and Alb3, the chloroplast YidC homolog.[23] A very active area of research addresses the mechanism by which membrane proteins enter the channel and translocate their hydrophilic regions across the membrane. What part of the SecYEG complex does the hydrophobic domain of the membrane protein binds to? How is the hydrophobic segment of the inserting membrane protein released from the Sec machinery and integrated into the bilayer? How does the Sec machinery perform these translocation and integration functions while maintaining a tight seal to prevent exchange of ions and solutes across the membrane.

In bacteria, the Sec components SecY and SecE form the minimum translocation machinery.[24] SecYEG is sufficient to insert the membrane protein FtsQ in vitro.[25] Although SecDF is not essential for insertion, it does facilitate translocation.[26] SecG also promotes protein translocation but is not essential for insertion.[26-28] In some cases, SecA uses the energy of ATP hydrolysis to promote translocation of the hydrophilic domain of a membrane protein across the membrane.

The SecA-driven translocation of hydrophilic domains of membrane proteins most likely occurs in steps of 20 to 25 amino acid residues, as shown for the exported protein proOmpA.[29] By this same mechanism, the SecA bound to a membrane protein inserts into the membrane upon ATP binding taking with it a segment (20 to 25 residues) of the polypeptide domain to be translocated. Following ATP hydrolysis, SecA dissociates from the membrane protein and SecA returns to the cytoplasmic side of the membrane. By repeated cycles of SecA insertion and deinsertion, the polypeptide domain of the membrane protein is moved across the membrane.

#### Structure of SecA

SecA is a multifaceted protein. It binds to phospholipids, ATP, SecY, signal peptide, the mature domain of exported proteins, and SecB (for review see ref 30). For membrane proteins, SecA is believed to bind to the hydrophobic domain (analogous to a signal peptide), and a part of the hydrophilic domain to be translocated. SecA belongs to the group of ATPase that show similarity to the DEAD-box helicases.[31,32]

Crystal structures are available for SecA from *Bacillus subtilis* (Fig. 3A) (Table 2)[33] and *Mycobacterium tuberculosis*.[34] These were solved both in the apo-form and in complex with ADP. Both studies were consistent with an antiparallel physiological dimer which was seen in solution by FRET experiments. Interestingly, the packing interactions are different in the two structures. The *B. subtilis* SecA has also been crystallized under conditions that result in a monomeric form of the SecA (Fig. 3B) which adopts a more open conformation than the dimeric form.[35] Previous biochemical studies had shown that interaction with SecY, acidic phospholipids or signal peptides induces SecA into a monomeric form[36] with significant conformational changes. The monomeric crystals also gave improved resolution, diffracting to 2.2 Å resolution and revealing interpretable electron density for most of the molecule (Fig. 3A). The structure of Sec A can be thought of as having two separate regions, the motor region and the translocation region. The motor region is made up of two nucleotide binding fold domains (NBF1 and NBF2) and the translocation region is made up of the preprotein crosslinking domain (PPXD), the helical wing domain (HWD) and the helical scaffold domain (HSD).

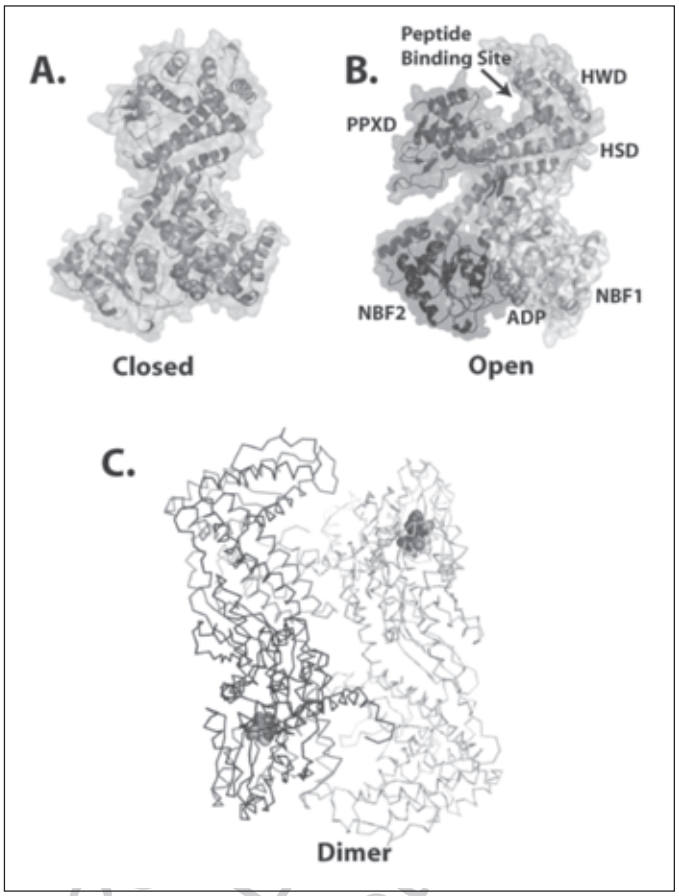


Figure 3. A) A ribbon diagram with transparent surface showing the closed form of SecA from *Bacillus subtilis*.[33] B) A ribbon diagram with transparent surface showing the open monomeric form of SecA from *Bacillus subtilis*.[35] The nucleotide binding fold 1 domain (NBF1), the nucleotide binding fold 2 domain (NBF2), the helical scaffold domain (HSD), the preprotein cross-linking domain (PPXD) and the helical wing domain (HWD) are labeled. The bound ADP is shown in van der Waals spheres. The deep groove proposed to be the signal sequence binding domain is pointed out with an arrow. PDB coordinates 1TF5 and the program PyMol[66] were used to make this figure. C) A Cα trace diagram showing the dimeric closed state of the SecA protein from *Bacillus subtilis*.[33] The bound ADP is shown in van der Waals spheres. PDB coordinates 1M74 and the program PyMol[66] were used to make both Figures 3A and C.

From the crystal structures, the binding of ADP does not appear to change the structures of the NBF domains. The major difference between the dimeric (Fig. 3C) and monomeric (Fig. 3B) forms of SecA is a result of an approximately 60° rotation of the PPXD and a rotation of the HWD and HSD of approximately 15°, resulting in the formation of a large groove between the PPXD, HSD and HWD. This groove is postulated to be the peptide-binding site. In all the crystal structures of the entire SecA protein available so far, there has not been experimental electron density for the C-terminal zinc-binding domain. The structure of this zinc-binding domain alone has been solved in solution by NMR.[37,38] It has also been solved by X-ray crystallography in complex with the targeting protein SecB.[39]

*Table 2. SecA Structures*

| PDB ID | Source | Method | Description | R Value | Resolution [Å] | Reference |
|---|---|---|---|---|---|---|
| 1M6N | *B. subtilis* | X-ray | Nucleotide free | 0.220 | 2.7 | Hunt et al. 2002[33] |
| 1M74 | *B. subtilis* | X-ray | Mg-ADP-bound | 0.217 | 3.0 | Hunt et al. 2002[33]. |
| 1NKT | *M. tuberculosis* | X-ray | Complex with ADP-bS | 0.216 | 2.6 | Sharma et al. 2003[34]. |
| 1NL3 | *M. tuberculosis* | X-ray | Nucleotide free | 0.196 | 2.8 | Sharma et al. 2003[34] |
| 1TF2 | *B. subtilis* | X-ray | Monomeric, open conformation, ADP bound | 0.228 | 2.9 | Osborne et al. 2004[35] |
| 1TF5 | *B. subtilis* | X-ray | Monomeric, open conformation, nucleotide free | 0.241 | 2.2 | Osborne et al. 2004[35] |
| 1OZB | *H. influenzae* | X-ray | SecB complexed with the SecA C-terminus | 0.226 | 2.8 | Zhou and Xu, 2003[39] |
| 1SX0 | *E. coli* | NMR | C-terminal zinc-binding domain of SecA | n/a | n/a | Dempsey et al. 2004[37] |
| 1SX1 | *E. coli* | NMR | C-terminal zinc-binding domain of SecA | n/a | n/a | Dempsey et al. 2004[37] |
| 1TM6 | *E. coli* | NMR | The free zinc binding C-terminal domain of SecA | n/a | n/a | Matousek and Alexandrescu, 2004[38] |

**Structure of the SecYEb Complex**

A central question in the membrane protein biogenesis and protein export field asks what are the structural and mechanistic characteristics of the protein-conducting SecYEG machinery. 2D electron microscopy studies provided the first clues to this question. The oligomeric forms of SecYEG complex from *E. coli*[40] and *B. subtilis*[41] are dimers. However, there are some tetramers that form when SecA is bound.[40] A three-dimensional structure of the *Escherichia coli* SecYEG complex was initially reported from cryo-electron microscopy analysis of 2D crystals.[42] The results suggested that SecYEG was a dimer with a closed cavity at the interface between the two monomers.

Low resolution cryo-EM studies have also been performed on the ER Sec translocon. These studies revealed an oligomeric Sec61 complex[43] and a ribosome-Sec61 complex with the pore of the Sec61 complex aligning with the exit tunnel located within the large ribosomal subunit.[44] It was suggested that the central part of the Sec61 complex represented an aqueous pore because previous studies using fluorescently-labeled polypeptide chain positioned within the translocation channel suggested the chain to be in an aqueous environment.[45] Additionally, the size of the aqueous channel was determined to be 40 to 60 Å.[46]

Then came the big surprise in 2004 with the X-ray crystal structure of the SecY complex (Sec61αβγ) from archaea[47] (Table 3). The crystal structure of the heterotrimeric SecYEβ complex was solved to 3.2 Å resolution in the presence of the detergent diheptanoylphosphatidyl choline (Fig. 4A). The archaeon *Methanococcus janaschii* was chosen as the source of the Sec components, based on the stability and crystallizability of the complex after screening proteins from 10 different species. The structure shows that the SecY (Sec61α-subunit) protein consists of 10 transmembrane segments with the helices packed such that the protein makes two symmetrical halves with both the amino- and carboxy-termini facing the cytoplasm (transmembrane segments 1-5 and 6-10 form the symmetrical halves). The Sec61β and SecE(Sec61γ) subunits each have one transmembrane segment with the amino terminus facing the cytoplasm (Fig. 4A). Surprisingly, the structure suggests that the translocase pore resides at the center of one copy of the heterotrimeric SecYEβ. As mentioned above, previous biochemical and cryo-electron microscopy evidence had suggested that the pore may be assembled from multiple copies of SecYEβ. A cross-section of the channel reveals an overall shape of an hourglass with a ring of isoleucine residues that lines the constriction point (approximately 3 Å in diameter) near the center of the membrane. Interestingly, the structure of the channel, which is presumably in the closed state, reveals a small helix that sits on top of the pore and plugs the channel. The extremely small diameter of the pore suggests that the transmembrane segments of the SecYEβ would go through a significant rearrangement in the open state to accommodate a substrates in the process of translocation or an α-helix which would need to escape the channel and partition into the lipids of the membrane. The authors propose a ribosomal binding surface for the homologous eukaryotic translocon, and a binding site for the Sec A ATPase in eubacteria.[47] There is approximately 50% sequence similarity in the eubacterial and eukaryotic genes SecYE and Sec61αγ, respectively. Sec61β and SecG show no sequence similarity.

A model can be proposed based on the structure of the SecYEβ complex and biochemical data, on how the hydrophobic transmembrane helix of a membrane protein binds to the SecYEβ complex.[47] The helix would bind to the SecYEβ complex in a manner analogous to how binding of a signal peptide of an exported protein was proposed.[47] Binding would cause dissociation of the plug from the pore, thereby allowing initiation of the steps of translocation to proceed (Fig. 4B). In the case of a membrane protein containing one hydrophobic transmembrane helix, binding would allow the hydrophilic flanking region to pass through the channel in a manner that needs to be defined in the future. For a membrane protein that is cotranslationally inserted into the membrane, the ribosome is most likely bound to the SecY complex and the energy driving translocation is derived from protein synthesis (Fig. 5B). For

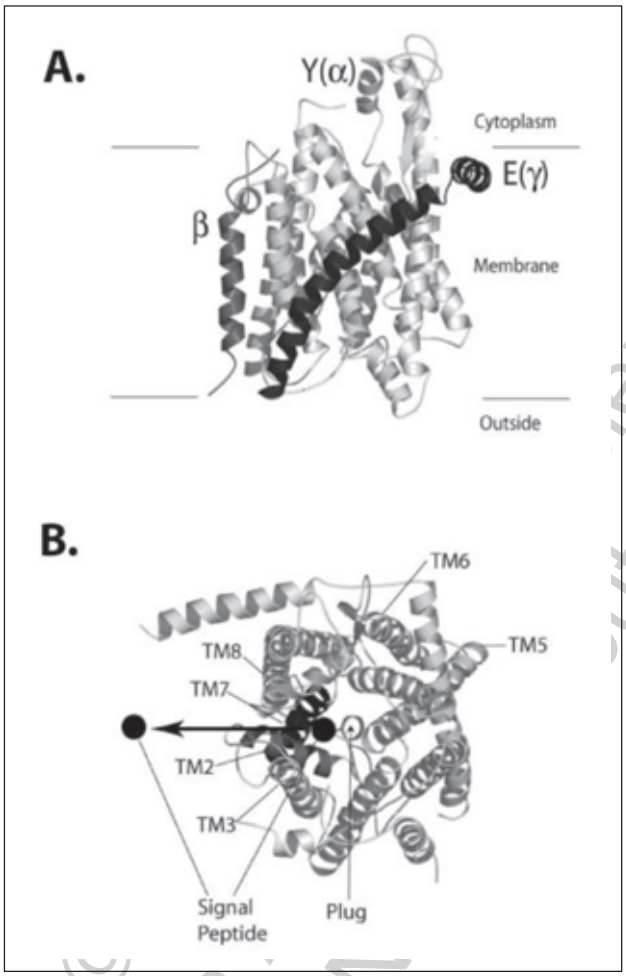


Figure 4. A) A ribbon diagram showing the heterotrimeric complex of SecYEβ from the archaean *Methanococcus jannaschii*.[47] The SecY (or Sec61α-subunit), SecE (or Sec61γ-subunit ) and Sec61β-subunit (no sequence similarity to SecG) are labeled. The orientation of the channel is shown relative to the phospholipid bilayer it resides in. PDB coordinates 1RHZ and the program PyMol[66] were used to make this figure. B) A ribbon diagram showing the top (cytoplasmic) view of the SecYEβ channel (light grey). The transmembrane segments TM2 and TM7, proposed to be part of the exit route for the substrates hydrophobic segments, are rendered in a dark shade and labeled. The short helical plug in the center of the channel is also labeled. A black dot designates the possible position of a signal peptide.

SecA-dependent translocation of the hydrophilic domain, the ribosome of the nascent membrane protein complex would have to detach from the SecYEG in order for SecA to bind to SecYEG and initiate translocation of the hydrophilic region in steps of 20 to 25 residues (Fig. 5C). How this is achieved is not clear. SecA could lead to translocation of the polypeptide chain by a region of SecA itself moving through the channel. However, it is hard to imagine how this could occur with a monomeric SecYEG complex. Or SecA itself does not penetrate

***Table 3. SecYEG structures***

| PDB ID | Source | Method | Description | R Value | Resolution [Å] | Reference |
|---|---|---|---|---|---|---|
| 1RH5 | *M. jannaschii* | X-ray | Double mutant | 0.242 | 3.2 | Van Den Berg et al. 2004[47] |
| 1RHZ | *M. jannaschii* | X-ray | Wild-type | 0.254 | 3.5 | Van Den Berg et al. 2004[47] |
| a | *E. coli* | Cryo-EM | | n/a | 8.0 | Breyton et al, 2002[42] |

a. The electron density file of a SecYEG dimer with the noncrystallographic symmetry imposed is available from the Supplementary Information from the Nature webpage (http://www.nature.com).

the Sec complex. Alternatively, SecA could simply bind to the SecYEG channel, thereby causing a conformational change in the SecY complex that opens the channel to allow translocation of the polypeptide chain.

## *Lateral Integration, Assembly and Folding*

Recent studies have focused on how membrane proteins laterally integrate into the membrane bilayer after inserting into the SecY complex and then assemble into their three-dimensional structure. Van der Berg et al[47] hypothesized that the substrate's hydrophobic transmembrane helices may escape from the channel via the interface between the two symmetrical halves of the SecY protein. The structural information, along with previous photocrosslinking data,[48] suggests that newly assembling transmembrane domains (anchor segment) of membrane proteins may insert between the SecY transmembrane segments TM7 and TM2 (Fig. 4B) which make up a lateral gate (along with TM8 and TM3) through which the newly assembling transmembrane segments may partition into the surrounding lipid. The insertion between TM7 and part of TM2 would also trigger an opening of the channel structure allowed by a proposed ~15° hinge motion between TM5 and TM6 (the connection point between the two pseudo-symmetrically related halves of the SecY molecule). The hinge motion of the structure would allow for a proposed 15-20Å by 10-15Å pore opening for the tranlocation of hydrophilic loops. However, the process may be mediated by YidC specifically recognizing transmembrane regions of membrane proteins in *E. coli*.[7,49] YidC has been suggested to function as an assembly site for hydrophobic regions of Mannitol permease (MtlA). Muller and coworkers showed that hydrophobic domain 3 of a nascent Mtl membrane protein inserts at the SecY/YidC interface while the hydrophobic domain 1 and 2 are still in contact with YidC.[50] Therefore, after the hydrophobic region leaves laterally from the SecYEG complex it may interact with YidC which would stabilize the hydrophobic region until it integrates into the membrane (Fig. 5D).

Even more recently, the best evidence thus far for YidC playing a role in folding of a membrane protein was presented. Nagamori et al showed that Lac permease, which spans the membrane twelve times, inserts quite normally when membranes contain deficient levels of YidC.[51] However, the inserted lac permease under YidC depleted conditions appears to be aberrantly folded as monoclonal antibodies that specifically recognize certain periplasmic loops of lac permease are impaired in their binding.

The role of YidC in the insertion of Sec-dependent proteins varies, depending on the membrane protein being studied. For membrane proteins such as Lep and FtsQ, which have

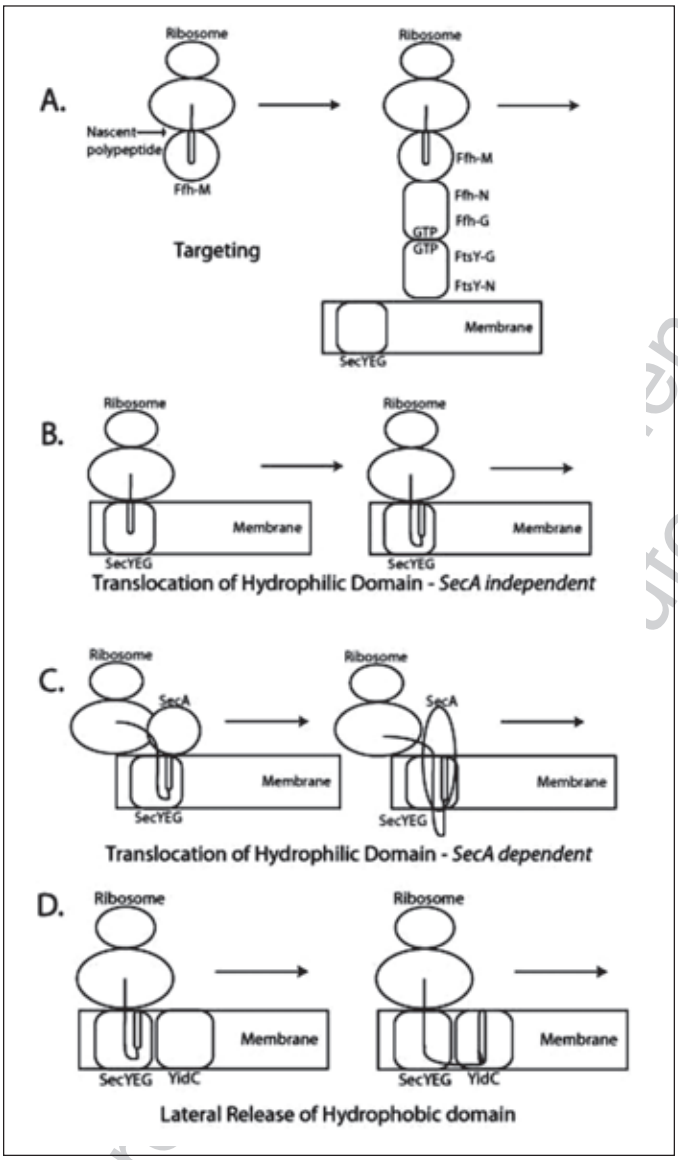


Figure 5. A schematic depiction of the possible individual steps of membrane protein assembly. A) Targeting of the nascent protein to the membrane. The Ffh-bound nascent chain is targeted to the membrane in a GTP-dependent manner by the interaction of the Ffh NG domain with the NG domain of FtsY. B) SecA-independent translocation of hydrophobic domain. Translocation of the chain within the channel is driven by the energy of protein synthesis. C) SecA-dependent translocation of hydrophilic domain. The binding of SecA to the protein chain drives translocation of a loop across the membrane. D) Release of the hydrophobic domain from the SecYEG complex. After release of the hydrophobic segment from the SecYEG channel, the transmembrane segment is stabilized by YidC. See the text for details of the individual steps.

large C-terminal domains, YidC does not play an important translocation role.[52,53] However, YidC is required in vivo for insertion of the Sec-dependent a and b subunits of the $F_1F_0$ATP synthase.[54]

Presently, effort is needed to solve the structure of YidC so as to reveal key features of the protein such as whether YidC has channel or transporter properties. To provide information about the region of YidC important for its membrane insertase function, we have studied a detailed collection of deletion and substitution mutants.[55] YidC is a 60 kDa integral membrane protein with six transmembrane segments. Transmembrane regions two, three and six are important for activity and contain residues that are critical for membrane insertase activity. It will be necessary to determine which parts of YidC constitute the substrate binding region and how the transmembrane segments within the protein interact. In addition, the oligomeric structure of YidC will also need to be determined within intact membranes. The formation of a YidC oligomer with intact membrane would explain why some purified YidC appears as a dimer upon blue native polyacrylamide electrophoresis.[56] It should be noted that Oxa1, the mitochondrial homolog, is a tetramer.[57]

## Insertion by the Novel YidC Pathway

The second route by which proteins can insert into the membrane is by the YidC pathway (for review see ref. 58) (Fig. 1B). Strong evidence for the evolutionarily-conserved nature of this insertion pathway was obtained when it was discovered that the Sec-independent phage M13 procoat and the Pf3 protein require YidC for membrane insertion.[52,59] Previously, the YidC homologs in mitochondria and chloroplasts (Oxa1 and Alb3, respectively) were found to play a role in membrane protein insertion in these membrane organelles (see ref. 60 for review). YidC plays a direct role in the membrane insertion process as it comes into contact with Pf3 coat protein during membrane insertion of the phage protein.[49] Both M13 procoat and Pf3 coat protein do not require the SRP pathway for insertion.

To date, the only endogenous *E. coli* protein that has been discovered to require YidC and insert by a Sec-independent mechanism is subunit c of the $F_1F_0$ATP synthase.[54,61] In vivo and in vitro studies have demonstrated that subunit c inserts independent of the Sec translocase and does not require the SRP targeting components for insertion (see ref. 62 for a differing opinion). Unlike the M13 procoat and Pf3 coat proteins, insertion of subunit c does not require the proton motive force across the membrane. Yet, like the M13 procoat and Pf3 coat proteins, subunit c is small in size and has short translocated regions. What structural features render a protein completely dependent on YidC for its membrane insertion are not known.

It is not clear whether YidC acts alone in intact cells or whether there are other proteins which make membrane insertion more efficient or regulate insertion by this pathway. Interestingly, the mitochondrial homolog Oxa1 has recently been shown to bind to the ribosome,[63,64] specifically to the large ribosomal protein Mrp20 (homologous to the L23 *E. coli* protein).[63] This raises the question of whether YidC also binds to the ribosome in *E. coli*.

## Conclusion and Future Questions

During the last few years, we have seen the first three-dimensional structures of the membrane-localized protein-conducting channel, its ATPase motor, and the SRP targeting components Ffh and FtsY. These structures have provided tremendous insights into the role these proteins play in membrane protein biogenesis. However, there are limitations to the current work because they do not provide information on the dynamic nature of the components during the translocation process. Despite significant advances in this area, we are only now beginning to understand how membrane proteins are assembled within the lipid bilayer.

To understand how the membrane protein inserts into the lipid bilayer and folds into a stable and active conformation, it will be necessary to shed light on how the protein partitions into the membrane, where the the helical transmembrane segments associate to form the transmembrane domain of the protein. This will require a multidisciplinary approach involving biophysical studies, structural analysis as well as cell biology, genetics and molecular biology.

The recent availability of the three-dimensional structures of the proteins and protein complexes involved in membrane assembly now provides the opportunity for detailed structurefunction studies and for molecular dynamics simulation analysis which could provide important insights into the mechanism of this very dynamic molecular machinery. Cryo-electron microscopy experiments with 2D crystals will also be helpful in the understanding of the movements within this system.

To provide a deeper understanding of the membrane insertion mechanism, it will be necessary to examine how membrane proteins interact with the Sec machinery at an atomic level. Thus, future directions include determining the three-dimensional structure of Sec complex intermediates, such as the SecYEG complex with a bound membrane protein, signal peptide or in complex with SecA as well as with the channel in the open state. Elucidating the structures of the eubacterial SecDFyajC complex and YidC may help to provide clues as to their functional roles in membrane protein translocation and membrane protein assembly. Also needed is a high resolution structure of a signal peptide bound to the M-domain of Ffh of the SRP and the complete complex between Ffh containing the M-domain and FtsY (SRα). Other open questions remaining in this field which will require biochemical, biophysical and genetic methodologies to answer are: How are membrane proteins integrated into the lipid bilayer after they leave the Sec complex and what is the role of YidC in this process. Does YidC have a general chaperone-like function to help fold membrane proteins? Answering these questions are essential to obtaining a complete picture of how proteins are assembled into the membrane.



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