

CHAPTER 2

Preprotein Translocation through the Sec Translocon in Bacteria

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Abstract

The Sec translocase or translocon is the essential and ubiquitous system for protein translocation across or into the membrane. The core channel, the SecYE complex, is conserved across biological kingdoms and most of the polypeptide chains which are routed to extracellular or membrane locations in Bacteria use this pathway. Biochemical and genetic approaches have yielded a substantial body of information about functional aspects of Sec-mediated translocation and this information has recently been enriched with structural data at atomic resolution. This chapter reviews previously acquired facts and concepts concerning the Sec translocase of Bacteria in light of recent structural results and considers implications of these findings.

Introduction

In *E. coli*, components of the Sec pathway were identified during the mid-1980s using elegant genetic screens. Conditional-lethal mutations associated with a generalized protein-secretion defect or mutations restoring translocation of proteins with secretion-defective leader peptides allowed the identification of most of the Sec components.¹⁻³ The translocation pathway was then successfully reconstituted in vitro in the early 1990s to allow biochemical dissection of the subreactions of the translocation event.⁴ It was shown that targeting of the protein substrate to the translocase is mediated by the dedicated chaperone SecB or by the signal recognition particle (SRP).⁵⁻⁷ The biochemical analysis showed further that the translocon is comprised of a membrane-embedded SecYE channel complex⁸ and a peripheral SecA ATPase which functions as a motor to drive translocation (Fig. 1).⁹ Genomic analysis revealed that SecYE is highly conserved in Bacteria, Archaea and eukaryotes.^{10,11} Isolation of large amounts of SecYE complex or its eukaryotic homolog, the Sec61 $\alpha\gamma$ complex, allowed for further biochemical, biophysical and structural analysis. Both complexes copurify with a small subunit, SecE and Sec61 β respectively, although these subunits do not share obvious homology.¹¹ Additional components, such as the heterotrimeric complex SecDFyajC¹² and the proton motive force (PMF),¹³ were found to contribute to the Sec pathway but in vitro reconstitution experiments demonstrated that SecA, SecE and SecY are necessary and sufficient for the basal activity

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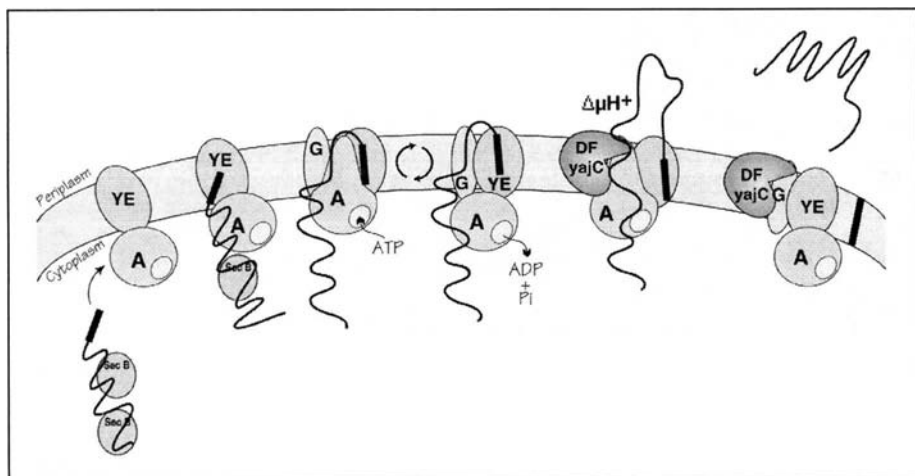


Figure 1. Model for preprotein translocation across the cytoplasmic membrane. See text for details.

of the translocase.^{14,15} The following paragraphs describe in finer detail more recent findings and address current questions on the bacterial translocation system.

The SecYEG Translocon at the Atomic Level

Biochemical, biophysical and electrophysiology studies established early on that the Sec complex serves as the channel through which preproteins traverse the membrane. The recent solution of the two-dimensional^{16,17} and three-dimensional¹⁸ structures of SecYEG (~75 kDa) and SecYEG from the bacterium *E. coli* and the archaeon *M. jannaschii*, respectively, provide structural support and new insight into the translocation mechanism. As a full chapter on the Sec channel structure appears elsewhere in this book (see Chapter 4), only a brief description is given here (Fig. 2). The SecY subunit consists of two sub-domains, the transmembrane segments TM1-TM5 and TM6-TM10, arranged like a clamp and related to each other by a two-fold pseudo-symmetry axis. The essential SecE subunit docks its TM helix across the interface of the two SecY domains, clamping them together. The proposed translocation channel is located in the center of the SecY subunit which is filled by a short distorted helix (TM2a, termed the plug) extending halfway to the center of the membrane. Movement of the plug would yield a continuous aqueous channel through which preproteins would be translocated. Halfway across the membrane plane, the channel is also constricted by a ring of six hydrophobic amino acid residues. This ring is proposed to seal the channel but would also widen just enough, probably by shifts in the helices forming the channel, to allow the passage of a polypeptide chain. The small SecZ subunit (SecG-like subunit) is peripherally attached and makes limited contact with SecY, consistent with its nonessential role in translocation. A groove situated between the TM segments at the edges of the two SecY halves (interface between TM2 and TM7) is accessible to the lipid bilayer. As the other sides of SecY are contacted by the SecE and SecZ subunits, this groove may form a lateral gate for release of TM segment of membrane protein.

Binding and Orientation of the Leader Peptide into the Translocon

Leader peptides consist of a short positively charged N-terminal region followed by a central hydrophobic core and a leader peptidase cleavage site. The physicochemical properties

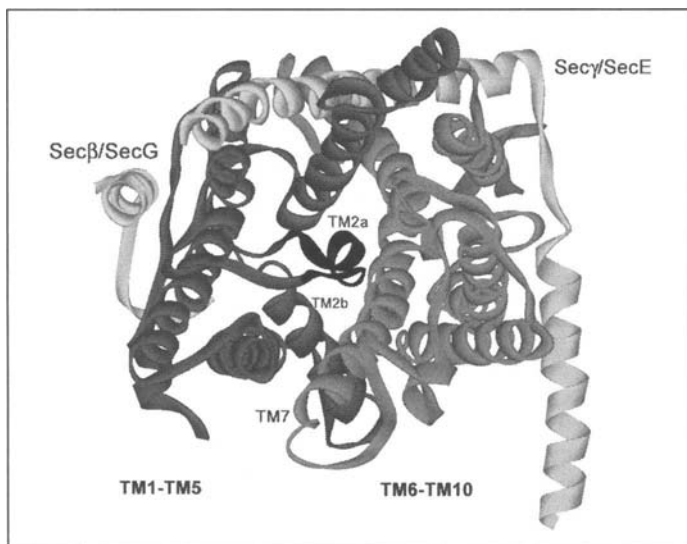


Figure 2. Structure of the SecYEG complex. The structure of the Sec complex from *M. jannaschii* (PDB: 1RHZ) viewed from the periplasmic side. The two halves of Sec α /SecY (TM1-TM5 and TM6-TM10) are shown in dark and pale tone respectively. Both halves form a clamp that might open between TM2b and TM7 to deliver transmembrane proteins. TM2a plugs the channel and may move toward SecE and away from the channel cavity, yielding a passage way for a translocating polypeptide.

of leader peptides are essential for correct interaction with the translocon, such that the N-terminus of the leader peptide stays in the cytosol while the hydrophobic core crosses the membrane.¹⁹⁻²¹ Insertion of the leader peptide into the channel at an early stage of translocation has been thoroughly analyzed by photo-crosslinking technologies. With photoreactive probes positioned at single sites along the leader peptide, it was shown that the opposite sides of the hydrophobic core contact TM segments 2 and 7 of the SecY subunit.²²⁻²⁴ Each residue of the leader peptide could also be cross-linked to phospholipids, suggesting that the binding site is located at the interface of the protein channel and the lipid phase.^{23,25} These earlier experiments are now supported by the atomic structure of SecY which reveals that TM2 and TM7 are located at the interface of the two SecY halves, adjacent to the pore channel and accessible both from the lipid and cytoplasmic side of the membrane.¹⁸ Moreover, the sequences of TM domains 2 and 7 are well-conserved, suggesting a similar mechanism of leader peptide recognition across evolution.¹¹ Binding and orientation of the leader peptide into the translocon also involves specific charged residues in the cytoplasmic and periplasmic loops of SecY. Site-directed charge-reversal mutations indicated that these conserved amino-acyl residues functionally interact with charged residues in the N-terminus of the leader peptide in order to set its correct topology in the channel.^{26,27} The residues immediately after the signal sequence were found in contact with the SecY subunit but not with lipids, supporting a model in which the polypeptide chain inserts in a loop-like configuration into the channel.²³

Opening of the Translocation Channel

Pioneering experiments showed that the addition of synthetic leader peptides to the cytoplasmic side of reconstituted *E. coli* membrane bilayer opens aqueous pores detectable by

conductivity measurements.²⁸ The model derived from the atomic structure predicts that two movements may occur in order for the channel to open and to accommodate the leader and attached polypeptide.¹⁸ First, the plug domain may move away from its blocking position in the protein channel and second, the N- and C-terminal halves of SecY may move apart to create a lateral opening of the translocation pore necessary to embrace the polypeptide chain. Alternatively, a diaphragm-like movement of the TM segments would widen the pore sufficiently to allow insertion of the polypeptide chain. Although these putative movements await experimental support, some earlier experiments are in favor of these models. It was shown that unique cysteines introduced in the domain of SecY forming the plug (TM2a) and at the C-terminal end of SecE can form a disulfide bridge.²⁹ Since these two cysteines are 20 Å apart in the closed channel structure, the observed cross-link is now explained by the movement of the plug away from the center of the channel. Moreover, the disulfide bridge formation had a dominant lethal effect,²⁹ as expected if the channel was locked into a permanently open state by the covalent modification. Other possible experimental support is provided by Prl mutants, a collection of mutations in SecY or SecE which up-regulate the activity of the translocase. Since these mutations allow secretory proteins with defective or even deleted leader peptides to be transported,^{30,31} they may mimic the effect of signal sequence binding. A previous study indicated that the Prl mutations increase the conformational flexibility of the translocon,³² and the atomic structure shows that most of the mutations are located in the center of the channel, particularly on the internal side of TM7 and in the plug.¹⁸ Thus, it is postulated the Prl mutations could increase the dynamics of the plug movement or facilitate widening of the pore during initiation of translocation, and therefore reduce the requirement for a functional leader peptide.

Translocation Pause

Short hydrophobic stretches in the mature domain of preproteins induce a transient pause in the translocation movement which leads to the formation of translocation intermediates across the channel.³³ Deletion or relocation of these hydrophobic segments significantly alters the pattern of intermediates, while increasing the length and hydrophobicity of the stretch can lead to complete translocation arrest.^{34,35} These observations suggest that the mechanism involved during translocation pause and translocation arrest are probably similar. The atomic structure shows that the channel is shaped as an hourglass with a constriction of hydrophobic residues in its center. It is proposed that the hydrophobic ring may form a seal around the translocating polypeptide chain while the hourglass shape may serve to limit the contact of the chain with the channel walls.¹⁸ While this organization may minimize the energy required for polypeptide movement through the membrane, it may also serve in the recognition of hydrophobic stretches. If the length and hydrophobicity of the stretch is sufficient to span the membrane, the protein is eventually released into the lipid phase of the membrane.^{35,36} The TM segment of a nascent membrane protein has been shown to move from the aqueous interior of the channel to the hydrophobic environment of the lipid bilayer.^{25,37} The atomic structure shows that the TM2/TM7 interface is the only possible escape for lateral release of TM segments of membrane proteins in transit through the translocon.

The Quaternary Structure of the SecYEG Translocon

The understanding of the translocation channel is further complicated by the fact that SecYEG exists as dimeric and tetrameric assemblies. Low resolution electron microscopy (EM) images of purified mammalian, yeast and bacterial translocon all revealed the oligomeric state of the Sec complex.³⁸⁻⁴¹ The stoichiometry of these assemblies was then established using various biochemical and biophysical investigations such as crosslinking,⁴² sedimentation analysis¹⁶ and

blue-native gel electrophoresis.⁴³ In the SecYEG dimer, SecE is located at the interface of the protomers, such that the TM2/TM7 interfaces are pointed in opposite directions and toward the lipid bilayer. This is the organization seen in the 2D crystal structure,¹⁷ which is also supported by a cysteine crosslinking study showing that the two SecE subunits are close to one another.⁴⁴ The organization of the Sec protomers within the tetrameric assemblies is unknown. It is argued that two dimers, each with an organization similar to that observed in the lattice of the two-dimensional crystals, may associate in a side-by-side manner to form a tetramer.⁴⁵ Such organization leaves the lateral gates oriented toward the lipid bilayer but other configurations are, nonetheless, possible. Since evidence for the existence of SecYEG as a monomer in the membrane is lacking, it is unclear why the SecYEG complex exists as an oligomer while a single copy of SecYEG seems to form the translocation channel. The central depression seen in the oligomeric ring-like structure was initially postulated to form a translocation pore.³⁸⁻⁴¹ In the 2D crystals, the dimeric translocon presents a funnel-like cavity formed by adjacent protomers and closed on its periplasmic face.¹⁷ It is now proposed that the pore-like structure formed by the tetramer or the cavity observed within the dimer in fact reflect a depression at the interface of the protomers rather than a true translocation channel.

Dynamic Behavior of SecYEG Oligomers

It is also unclear whether the Sec complex undergoes transitions in its oligomeric status as part of the translocation event. Native electrophoresis experiments show that SecYEG dimers reversibly dissociate into monomers in a detergent-dependent manner⁴³ and a protein concentration-dependent equilibrium between Sec tetramers and monomers exists, as detected by analytical centrifugation.¹⁶ However, such dynamic association of Sec protomers takes place in detergent solution and may be different once the SecYEG complex is embedded in the phospholipid bilayer. The reconstitution of membranes containing active SecYEG involves the dilution of detergent and thus prompts the formation of dimers.⁴³ The same is true for the growth of 2D crystals and only dimeric assemblies formed in the crystallized membrane.¹⁶ Moreover, subunit exchange studies⁴⁶ and fluorescence resonance energy transfer experiments⁴⁷ failed to observe any exchange of the protomeric components within the membranous oligomer. Altogether, these observations argue that the translocon may not experience rearrangements in its oligomeric status per se, although translocation-related dynamic changes may occur. Indeed, cysteine-scanning mutagenesis identified enhancement of the interhelical SecE contact at the initiation of translocation,⁴² suggesting that translocation results in a rearrangement of SecE molecules within the SecYEG oligomer. Electron microscopy analysis of proteoliposome-reconstituted, detergent-solubilized dimeric SecYEG revealed that binding of SecA with nucleotides lead to the recruitment of two SecYEG dimers to form the tetrameric SecYEG assembly.⁴¹ Similarly, reconstitution of the mammalian translocon ring structure required the presence of ribosomes for recruitment of individual Sec61 eukaryotic complexes.⁴⁸

Atomic Structure of the SecA Translocation Motor

The SecA ATPase (~100 kDa) interacts with the SecYEG channel to drive translocation. In addition to its high affinity for SecYEG,⁴⁹ SecA also interacts with numerous ligands: leader and mature regions of preproteins, acidic phospholipids, SecB, nucleotides, Mg,²⁺ Zn²⁺ and its own mRNA.⁵⁰ Accordingly, the crystal structure of SecA from *B. subtilis* reveals a complex multidomain protein (Fig. 3).⁵¹ The motor ATPase domain is made up of two RecA-like folds (termed nucleotide binding folds, NBF) similar to those found in superfamily 1 and 2 helicases.⁵² The interface between NBF1 and NBF2 forms the nucleotide binding site. Three other domains are linked to the ATPase domain: the preprotein cross-link domain (PPXD), helical wing domain (HWD), and helical scaffold domain (HSD).⁵¹ The PPXD domain can be

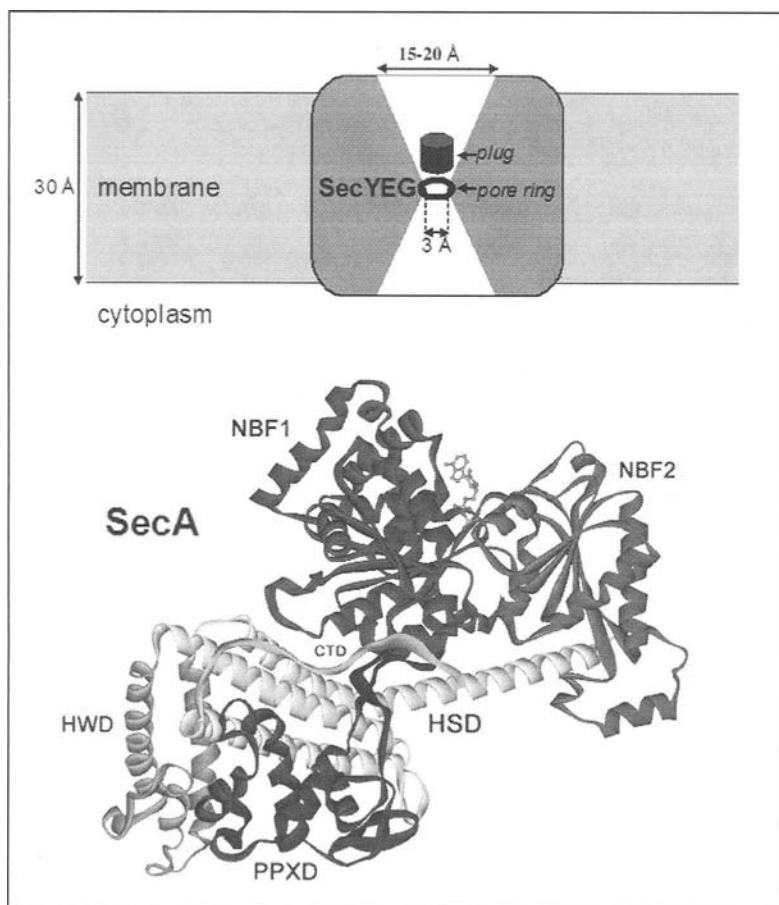


Figure 3. Modular structure of SecA. Ribbon representation of *B. subtilis* SecA (PDB: 1M74). See text for an introduction to NBF1, NBF2, HSD, HWD, PPXD and CTD domains. Above SecA, a fit-to-scale sketch represents the monomeric translocon inside the hydrophobic bilayer of the cytoplasmic membrane. The channel inside the translocon has an hourglass shape with the characteristic dimensions indicated.

cross-linked to the leader and mature domain of preprotein.^{53,54} The HSD domain contains a long α -helix acting as a connection between the motor and translocation domains of SecA. The HWD domain is an insertion in the HSD domain and seems to be flexible and loosely linked with the rest of the molecule. The extreme C-terminal region (CTD) is also flexible and has been shown to bind lipid, Zn^{2+} and SecB.^{55,56} Recently, comparison of the atomic structures of monomeric and dimeric SecA revealed that SecA monomerization generates relative movement of the PPXD, HSD and HWD domains such that a potential preprotein-binding groove forms at the surface of SecA⁵⁷ (see below also).

Binding of the SecA Motor to the SecYEG Channel

The regions of interaction between SecA and SecYEG remain to be characterized. Ligand affinity blotting experiments indicate that SecA binds to the first 107 amino acid residues of SecY⁵⁸ and intergenic suppressor studies suggest that the C-terminal cytoplasmic loop

(between TM8 and TM9) and the C-terminal tail of SecY are important for SecA interaction.⁵⁹⁻⁶¹ Mutations in the C-terminal tail do not abolish the binding of SecA, but prevents its activation.⁵⁹ Similarly, random targeted mutagenesis identified residues in the cytoplasmic loop TM8/TM9 as indispensable for productive SecA-dependent translocation, but their effect on SecA binding is unknown.⁶⁰ The sequences of SecA that interact with SecYEG are also poorly defined. Extragenic suppressors of SecY mutations map all over SecA molecules.⁶² Ligand affinity experiments identified the C-terminal third of SecA as the SecYEG-interacting domain.⁵⁸ In contrast, characterization of the binding constant of truncated SecA derivatives indicate that the N-terminal domain comprising the ATPase motor is responsible for the interaction with SecYEG⁶³ but both domains may contribute to optimal binding of SecA to the SecYEG complex. Moreover, the stoichiometry of the SecA-SecYEG association is largely unknown (see below).

How Does SecA Use ATP to Catalyze Translocation?

The SecYEG-bound SecA ATPase activity is stimulated by a translocation-competent preprotein.⁹ This activity, termed SecA translocation ATPase, is responsible for the preprotein translocation reaction. In vitro reconstitution shows that the initiation step requires ATP binding but not its hydrolysis.⁶⁴ This initial event leads the leader peptide and attached polypeptide to cross the channel in a loop-like configuration such that it can be processed by a signal peptidase at the periplasmic face of the membrane. Continued translocation then requires ATP hydrolysis which causes cycles of binding and the release of the preprotein from SecA.^{64,65} Under appropriate in vitro conditions, it has been shown that translocation is a stepwise process, corresponding to translocation steps of 20-30 amino acid residues of the polypeptidic chain.^{64,67} The mechanism by which the energy of ATP binding hydrolysis at SecA is converted into the movement of preproteins across the membrane has been related to the SecA transmembrane mobility at SecYEG, called the insertion-deinsertion cycle.⁶⁸ This model is based on the observation that SecA becomes protected from added protease under the conditions of active preprotein translocation.^{68,69} Several regions of SecA are indeed accessible for chemical modification from the periplasmic side of the membrane.⁷⁰⁻⁷² The membrane insertion-desinsertion of SecA is regulated by ATP and repeated cycles of these movements has been proposed to drive the stepwise movement of preprotein across the membrane.⁶⁸ Whether SecA truly inserts into the translocon and across the membrane, and how the ATP-derived energy is coupled to the preprotein movement, remains controversial and not yet fully understood. What is clear is that SecA undergoes conformational changes that are coupled to its interaction with ligands and driven by the ATPase cycle. By analogy to the helicase working mechanism,⁵² the two RecA-like domains of SecA may move relative to one another during the ATPase cycle, creating domain movements which may be propagated via the long α -helix (HSD) to the other SecA domains to generate preprotein motion.^{73,74} Steady-state tryptophan fluorescence anisotropy spectroscopy suggests that nucleotide-free SecA is in a domain-dissociated conformation which may have high affinity for SecYEG.⁵¹ In contrast, nucleotide binding would result in the presentation of compact conformations with low affinity. Thus, both SecA conformational changes and variation in SecYEG-affinity would provide the driving force for translocation to occur.

The SecA Monomer-Dimer Equilibrium

An understanding of the SecA mechanism seems further complicated by the fact that SecA exists in solution as a dimer in equilibrium with a small fraction of monomers.^{75,76} The dimeric organization maximizes the buried solvent-accessible surface area and intermolecular protomeric contacts, but the interface between the SecA dimer is not extensive.^{51,57} The equilibrium can be shifted towards the monomeric state by relatively small changes in the SecA

primary sequence or incubation conditions.^{75,77} An early study based on fluorescence resonance energy transfer experiments and involving heterodimers with one ATPase-inactive subunit has suggested that the SecA dimer is the active species in translocation,⁷⁸ but this view has been recently challenged. Acidic lipids, which are essential for SecA activation were found to induce dissociation of the dimer.⁷⁷ Furthermore, synthetic signal peptides can induce monomerization of SecA while a mutant that fails to dimerize retains some translocation activity.^{77,79} Finally, the rotation of the PPXD domain, which generates a large groove probably involved in preprotein binding, occurs upon monomerization of SecA.⁵⁷ Altogether, these recent observations suggest that the active form of SecA may be monomeric or that SecA monomerization may be critical at some stage of the translocation reaction. The oligomeric state of SecA during translocation and when bound at SecYEG remains, however, to be determined. Recent native electrophoresis and analytical centrifugation experiments suggest that detergent-solubilized and stabilized dimeric SecYEG can bind both monomeric and dimeric SecA with a stoichiometry modulated by nucleotides.^{80,81} The variability in the stoichiometry of the SecYEG-SecA complex might carry significant implications. It is now hypothesized that the mechanism by which SecA mediates protein translocation may resemble the mechanism by which helicases mediate unwinding of nucleic acid duplexes.^{82,83} Two distinct mechanisms, called the inch-worm model and the active rolling model, can be envisioned. According to the 'inchworm' mechanism, the SecA monomer is the functionally active species: cycles of ATP binding and hydrolysis would trigger localized conformational changes in the SecYEG-bound SecA monomer, leading to processive feeding of the polypeptide through the channel. According to the 'rolling' model, ATP-driven cycles of SecA monomerization-dimerization would mediate the processive passage of preprotein: a free SecA monomer may bind a new segment of preproteins before reassociating with the SecYEG-bound SecA monomer. In both models, a SecYEG-bound SecA monomer would be maintained in close association with the channel throughout the translocation process.

The Translocase Makes Use of the Proton Motive Force

Preprotein translocation is strongly stimulated by the PMF, both with native membranes and with purified and reconstituted SecYEG translocase.^{84,85} Several subreactions of the translocation process seem to be simultaneously affected by the PMF. Earlier studies have shown that PMF can drive forward movement of preprotein translocation intermediates when SecA is no longer associated with the polypeptidic chain.^{67,86} The $\Delta\psi$ and ΔpH components of the PMF may act on the preprotein itself via some sort of electrophoretic or folding effect on the polypeptide chain in transit.⁸⁷ The binding of leader peptide to the cytoplasmic membrane and its subsequent insertion in the translocation channel may also be optimized by the PMF.⁸⁹ Alternatively, or in addition, the PMF may directly modify the conformation of the translocation channel and its subsequent interaction with the translocation partners. Indeed, the *Prl* mutations which may alter the conformation of the channel render the *in vivo* and *in vitro* translocation less PMF-dependent, suggesting that *Prl* mutations may mimic the effect of the PMF.⁹⁰ Furthermore, the PMF accelerates the conformational changes of SecA that occur during translocation⁹¹ and the stimulatory effect of the PMF is more obvious at low SecA concentrations.⁹² The same *Prl* mutations, which decrease the PMF-dependency of translocation,⁹⁰ also increase the affinity of SecA for SecYEG.⁹³ It is thus possible that the PMF could change the conformation of the channel such that it modifies the dynamics of the SecYEG-SecA association.

Additional Subunits Make the Translocase Holo-Enzyme

In contrast to SecA, SecY and SecE, the SecG subunit is not essential for cell viability and translocation and is not conserved outside the bacterial kingdom.¹¹ SecG is a 12-kDa

protein with two TM segments connected via a short apolar cytosolic segment.^{94,95} This small subunit enhances the translocation rate and this enhancing effect is particularly seen in vitro. SecG is not needed for the high-affinity binding of SecA to SecYE but it readily stimulates SecA activity.^{13,96} The in vivo contribution of SecG is clearly observed only when SecA function is compromised by mutations or when SecA activity may become more critical for translocation such as at low temperatures, in the absence of SecDF, in absence of acidic phospholipids, or at low transmembrane PMF.^{13,97} It has been shown that SecG exists in two inverted topological states in the membrane and interconversion between these states is linked to the SecA membrane insertion reaction.⁹⁸ SecG may enhance translocation and SecA activity by acting on the conformation of the translocation channel. However, the atomic structure shows that SecG is located at the periphery of the SecYE complex¹⁸ and EM pictures apparently indicate that SecG is not required for the formation of the SecYE ring-like structures.⁴⁰

The core SecYE also associates with the SecDFyajC heterotrimeric membrane protein complex.^{13,99} SecD and SecF present sequence similarity, each spanning the membrane six times and possessing a large periplasmic loop between the first and second transmembrane segments.¹⁰⁰ In *B. subtilis*, SecD and SecF are even fused into one large polypeptide.¹⁰¹ YajC, a small single transmembrane protein, exists in tight complex with SecDF^{13,99} and its gene is located in the same operon. Altogether, these observations suggest that the role of these three proteins is somehow linked but their true function remains largely unknown. In vivo, the absence of SecDF, but not YajC, severely affects cell viability and the efficiency of protein translocation.¹² In vitro, the stimulatory effect of SecDFyajC is obvious only when membranes are depleted for SecG, suggesting that the stimulatory function of SecG covers that of SecDFyajC in the reconstituted system.¹⁵ Interestingly, the level of SecG in membranes is decreased upon SecDFyajC depletion and recovered to a normal level when SecDFyajC is expressed,¹⁰² suggesting that a coordinated balance between these stimulatory subunits exist. At low translocation rates, it has been shown SecDFyajC increases the formation and accumulation of preprotein translocation intermediates in transit across the channel.¹⁰³ These translocation intermediates are then propelled forward after energization of the membrane by the PMF. This result suggests that SecDFyajC may serve to coordinate the action of ATP- and PMF-driven translocation.¹⁰³ SecDFyajC has also been shown to modulate the behaviour of SecA toward stabilization of the membrane-inserted conformation.¹⁰⁴ Since Archaea contain SecD and SecF homologues while a SecA homologue is absent,¹⁰¹ this later effect may be indirect and rather caused by the stabilization of the translocation intermediate.¹⁰³ Finally, it has been proposed that SecD plays a role in protein release following the translocation event.¹⁰⁵

Concluding Remarks

Genetic and biochemical studies have provided the first elementary and essential insight into the mechanism of preprotein translocation. Structural studies have resulted in a significant advance of our understanding and allowed further interpretation of previously obtained experimental data. As discussed in several places in this chapter, many aspects of the translocation reaction are accompanied by dynamic conformational changes and transient associations. The combination of advanced biochemical tools together with high-resolution structural approaches will soon allow for an exact description of the relation of structure to function, leading to detailed knowledge about the mechanism of protein translocation across and into the cytoplasmic membrane. Finally, further challenges include the description of translocon function integrated in the larger context of the cell physiology, and its cross-talk with the other translocation systems present in the cell envelope.

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