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Review

The bacterial translocase: a dynamic protein channel complex

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Abstract. The major route of protein translocation in bacteria is the so-called general secretion pathway (Sec-pathway). This route has been extensively studied in *Escherichia coli* and other bacteria. The movement of preproteins across the cytoplasmic membrane is mediated by a multimeric membrane protein complex called translocase. The core of the translocase consists of a proteinaceous channel formed by an oligomeric assembly of the heterotrimeric membrane protein complex SecYEG and the peripheral adenosine triphosphatase (ATPase) SecA as molecular motor. Many secretory proteins utilize the molecular chaperone SecB for targeting and stabilization of the unfolded state prior to translocation, while most nascent inner membrane proteins are targeted to the translocase by the signal recognition particle and its membrane receptor. Translocation is driven by ATP hydrolysis and the proton motive force. In the last decade, genetic and biochemical studies have provided detailed insights into the mechanism of preprotein translocation. Recent crystallographic studies on SecA, SecB and the SecYEG complex now provide knowledge about the structural features of the translocation process. Here, we will discuss the mechanistic and structural basis of the translocation of proteins across and the integration of membrane proteins into the cytoplasmic membrane.

Key words. *E. coli*; preprotein; translocation; SecYEG; SecA; SecDFYajC; SRP.

Introduction

The cytoplasm of a cell is separated from the external environment by a membrane that ensures the maintenance of a unique internal ion and proton composition. The same membrane imposes a barrier for proteins that are synthesized in the cytosol but function outside the cell. Various transport mechanisms have evolved to allow proteins to cross membranes without compromising the barrier function.

In bacteria, the major route of protein translocation across the cytoplasmic membrane is the so-called general secretion pathway (Sec-pathway). Most of the components that function in bacterial protein translocation were originally identified by genetic studies in *Escherichia coli* [1, 2]. The pathway involves a membrane embedded enzyme complex called translocase (fig. 1) that consists of a peripheral adenosine triphosphatase (ATPase) SecA [3] that acts as a molecular motor, and a large integral membrane domain that constitutes the protein-conducting channel. The core of this channel is formed by the SecY [4] and SecE [5] proteins that can associate with the additional integral membrane subunit SecG [6–8], or with another heterotrimeric complex composed of the SecD, SecF and YajC proteins [9–11]. The SecYEG complex is highly conserved, and homologs have been found

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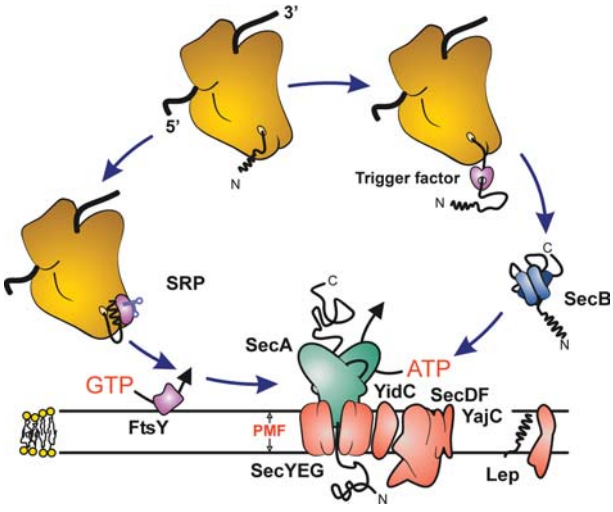

Figure 1. Schematic overview of the components of bacterial translocase. After a preprotein has emerged from the ribosome, it can be targeted to the translocase via two distinct pathways that involve either the molecular chaperone SecB or the signal recognition particle (SRP) and FtsY. Trigger factor competes with SRP for the binding of the nascent polypeptide. Proteins are translocated across the membrane through a membrane-integrated pore complex that consists of the SecY, SecE and SecG proteins. Protein translocation is driven by the hydrolysis of ATP by SecA, and once translocation has been initiated at the expense of ATP, the proton motive force can drive further translocation. During translocation the signal sequence can be removed by signal peptidase (Lep). YidC and the SecDFYajC complex are other components involved in protein translocation, but their exact function is not yet known.

in the cytoplasmic membrane of archaea, the chloroplast thylakoid membrane and the eukaryotic endoplasmic reticulum (reviewed in [12–15]).

Mechanistic and structural insight in protein targeting

A first step in the translocation of preproteins across or integration of inner membrane proteins into the cytoplasmic membrane is their targeting to the translocase. *E. coli* contains two major targeting routes mediated by the molecular chaperone SecB and the signal recognition particle (SRP). Secretory proteins are synthesized as precursor proteins (preproteins) with N-terminal signal sequences (for a review see [16]) that generally comprise 18 up to 30 amino acids. The signal sequence contains a positively charged N-terminal domain (N-domain), a nonpolar hydrophobic core (H-domain) and a more polar C-domain. The C-domain contains the cleavage site for signal peptidase, a membrane-bound protease that removes the signal peptide from the preprotein during the translocation reaction. Inner membrane proteins often do not have a signal peptide, and instead their hydrophobic transmembrane domains function as an internal signal. After translation, preproteins and inner membrane proteins are recognized by the targeting factors that direct them to the translocation site. Secretory proteins are preferentially targeted via the SecB pathway, while some preproteins with a very hydrophobic signal peptide and most inner membrane proteins (IMPs) are targeted by SRP. The divergence between the two targeting pathways occurs when preproteins emerge from the ribosome [17]. The strength of the interaction with SRP is dependent on the hydrophobicity of the H-region [18]. An increased hydrophobicity of the signal sequence enhances the SRP dependency of preprotein translocation, while the requirement for SecB is reduced [19]. SRP binds the signal sequence of short nascent preproteins [18, 20], whereas SecB seems to bind only to long nascent chains (>200 residues) [21]. SRP is proposed to compete with trigger factor for binding of the signal sequence domain of the nascent chain [22]. Trigger factor is a cis-trans proline isomerase that scans the newly synthesized proteins while bound at the ribosome [23]. Interestingly, deletion of the gene-encoding trigger factor (*tig*) results in acceleration of protein export, and preproteins appear no longer dependent on SecB for targeting [24]. This suggests a model in which trigger factor prevents secretory proteins from entering the SRP-dependent cotranslational pathway [22]. Binding of trigger factor to a large portion of the nascent presecretory chain may obstruct early recognition by the translocase, whereupon molecular chaperones such as SecB are needed for stabilization and targeting [24].

SecB-mediated protein targeting

SecB is protein of 17 kDa [25] (for recent reviews see [26, 27]) that is a homotetramer, organized as a dimer of dimers [28, 29]. Biochemical evidence has shown that the SecB tetramer contains two types of preprotein binding sites [30]: one that interacts with polypeptides with extended β-sheet stretches [31, 32] and another that interacts with hydrophobic polypeptide regions [30, 33–36]. In the crystal structure of the *Haemophilus influenza* SecB (fig. 2), the putative peptide-binding groove consists of a 70-Å-long surface-exposed channel at each side of the tetramer [28]. Consistent with the biochemical data, each peptide-binding groove seems to contain two subsites that are involved in recognition of distinct features of the preprotein [28]. One subsite is a deep cleft lined with mostly conserved aromatic residues that might play a role in binding of hydrophobic and aromatic regions of the polypeptides [28], while the other subsite forms a shallow open groove with a hydrophobic surface that might be involved in the binding of β-plated sheets [28]. Since only a single preprotein is bound by the SecB tetramer, the polypeptide is presumably wrapped around SecB to occupy the peptide-binding groove on both sides of the tetramer. As SecB has been crystallized without a peptide substrate in its binding

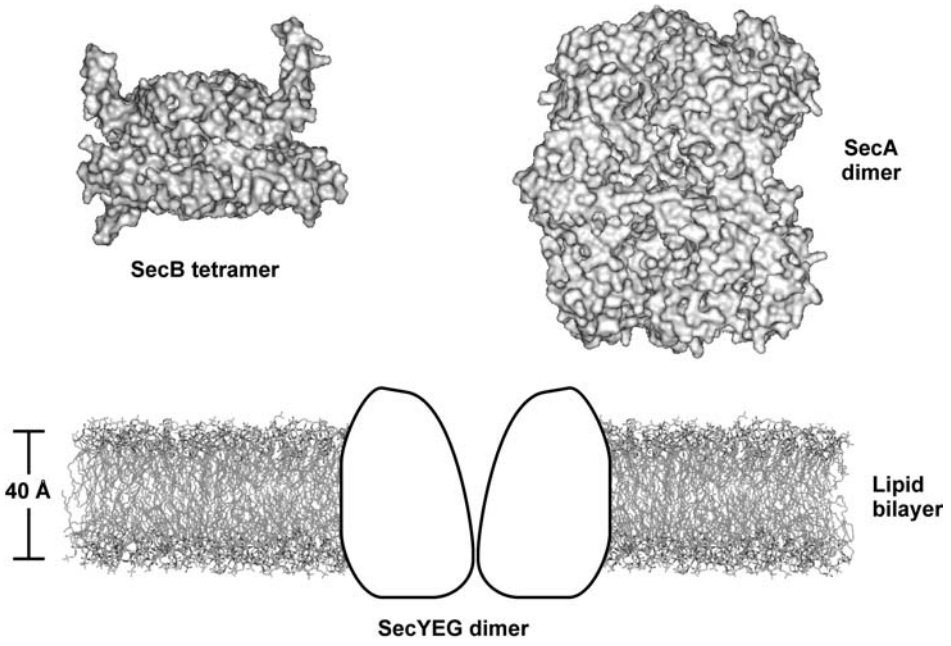

Figure 2. Relative sizes of key components of the bacterial translocase. Solvent accessible surfaces are shown of the *H. influenza* SecB tetramer (pdb entry: 1FX3) [28], *B. subtilis* SecA dimer (pdb entry: 1M74) [83] and a schematic representation of the dimensions of the membrane-integrated SecYEG dimer [126]. A membrane composed of 1-palmitoyl 2-oleoyl phosphatidyl choline.

groove, the exact location of the preprotein binding sites still remains to be established.

The mechanism by which SecB differentiates between secretory and nonsecretory proteins is not well understood. It is generally believed that the signal sequence does not contribute directly to the interaction of preproteins with SecB and that SecB mainly recognizes the mature part of a preprotein (see [36] and references therein). In a model where binding selectivity is based on kinetic partitioning between folding and association with SecB [37], the signal sequence could indirectly affect the SecB binding through retarding the folding of the mature domain of the preprotein [38]. This hypothesis is supported by the negative correlation between the rate of folding of the precursor of maltose-binding protein (MBP) and its ability to associate with SecB [39]. Other studies, however, have shown that the rate of association of SecB with polypeptide substrates is much faster than polypeptide folding and limited only by the rate of collision [33, 35, 40]. Therefore, interaction between SecB and the ribosome emerging preprotein may be limited by the rate of chain elongation rather than the rate of folding [33]. Since in vivo translocation becomes more SecB dependent when the signal sequence of the preprotein is omitted [41], the signal sequence and the retardation of folding are not necessary for the SecB interaction per se. Various methods have been used to identify SecB binding sites in preproteins (reviewed in [26]). A screen of a peptide library for SecB binding activity revealed that peptides with a length of about nine residues are preferentially bound when enriched in aromatic and basic residues, whereas acidic residues were strongly disfavored [42]. These peptide sequences occur regularly but are found with a similar frequency in both cytoplasmic and secretory proteins. Selectivity might be provided by the interaction of SecB and the signal sequence with the other components of the translocase, in particular SecA [42].

SecB targets preproteins to the translocase via a high-affinity interaction with SecYEG-bound SecA (K_d 10–30 nM) [43]. The SecB binding domain in SecA consists of a highly conserved region comprising only the C-terminal 22 amino acyl residues [44]. This region is rich in glycine residues and basic amino acids and contains three cysteines and a histidine residue that together coordinate a zinc atom [45]. These cysteines are not essential to support preprotein translocation [46], but are needed for an interaction between SecA and SecB [45]. Zinc presumably stabilizes the fold of the highly positively charged C-tails of SecA, which are in close proximity within the SecA dimer [47] and are both needed for the high-affinity binding of SecB [44]. Site-directed mutagenesis of SecB indicated that the SecB-SecA interaction is affected by mutations in the well-conserved amino acids Asp20, Glu24, Leu75 and Glu77 [31, 48]. These residues are clustered on a solvent-exposed region on both sides of the SecB tetramer [28], and provide a negatively charged surface that may interact electrostatically with the positively charged SecB-binding C-terminus of SecA [44]. While the mature domain of the secretory protein is bound by SecB, the signal sequence is exposed for down-

stream interactions. Association of the signal sequence with the SecYEG-bound SecA causes an increased SecA-SecB binding affinity [44]. This in turn elicits the dissociation of the preprotein from SecB, while SecB release from the membrane occurs upon the binding of ATP to SecA. This process completes the targeting reaction and allows SecB to re-bind a newly synthesized nascent secretory protein.

SRP-mediated protein targeting

SRP-mediated protein targeting has been extensively studied in the endoplasmic reticulum (ER) of mammals (for a detailed description see [49]). The mammalian SRP consists of a complex of six proteins that are assembled on a 7S RNA scaffold. Interaction with the hydrophobic signal sequence of nascent polypeptide chains occurs via the 54-kDa guanosine triphosphatase (GTPase) subunit. After membrane targeting, the ribosome-nascent chain (RNC) complex binds in a GTP-dependent fashion to the SRP receptor that is composed of the peripheral GTPase SRα and the transmembrane GTPase SRβ. SRP-dependent targeting in prokaryotes appears to follow a similar mechanism as in eukaryotes (for a review see [50]), but the components involved have a less complex subunit composition. *E. coli* SRP is composed of a complex of a 4.5 S RNA and a 48-kDa GTPase called P48 or Ffh (for fifty-four-homolog) [51] that interacts specifically with the signal sequence of nascent preproteins and hydrophobic regions of nascent membrane proteins [20, 52]. In eukaryotes, this interaction retards synthesis of the nascent chain [53], but translational arrest has not been observed in bacteria. SRP from Gram-negative bacteria lacks the 5′ and 3′ RNA domains [54] and the interacting eukaryotic SRP9 and SRP14 subunits that are essential for translational arrest [55]. It has been suggested that the shorter traffic distances and the faster translocation rates in bacteria overcome the need for translation arrest [56]. However, translocation arrest may occur in Gram-positive bacteria. *Bacillus subtilis* contains the 5′ and 3′ RNA domains that are essential for translation arrest in eukaryotes, and also a possible homolog of the eukaryotic SRP9/14 called HBsu has been identified [54].

RNC-bound SRP interacts with FtsY [57], a prokaryotic homolog of the α subunit of the SRP receptor. FtsY can bind to membranes via interaction with phospholipids [58]. It has also been argued that this association involves a yet unidentified membrane protein that may function as a receptor [59], but a bacterial homolog of SRβ has not been found in bacteria. This has led to the suggestion that FtsY fulfills the functions of both the SRα and SRβ subunits [60]. The interaction between FtsY and Ffh changes the nucleotide binding affinity of both proteins and allows them to bind GTP. Upon GTP hydrolysis by both SRP and its receptor, the RNC-SRP-FtsY complex dissociates, and the released RNC complex is transferred to the translocase. Cross-linking data have shown that the release of SRP from the RNC complex and association of the RNC complex with the translocase are linked [61]. It is essential that the RNC complex is released close to the translocase, but the exact regulation of this event and the role of GTP hydrolysis have not been clearly defined.

Ffh is composed of a so-called NG domain (N, amino terminal; G, GTPase) that contains the GTP binding site and a methionine-rich C-terminal M-domain that is implicated in the binding of signal sequences and SRP RNA [62]. The central G-domain is closely related to the p21Ras GTPase family [57]. Nucleotide occupancy of the G-domain is sensed or controlled by the closely associated N-domain via an interdomain contact, and this event could be part of the regulatory steps that control GTP binding and hydrolysis [63]. FtsY contains a similar NG-domain and a strongly acidic N-terminal domain that is involved in its membrane targeting [64]. In both proteins, the N-domain is closely associated with the G-domain, and the interface between both domains is highly conserved [65, 66]. The interaction between Ffh and FtsY appears to occur mainly via their structurally related NG-domains [65, 67]. The NG-domain of FtsY is fully functional in the targeting of Ffh to the membrane when it is fused to an unrelated membrane protein [60]. The 4.5 S RNA and the M-domain of Ffh are not required for the stimulation of GTPase activity in vitro [68, 69]. However, the binding of 4.5 S RNA to Ffh seems to control the association and dissociation of SRP and FtsY [68].

Cross-linking and functional studies have demonstrated that the signal sequence binding site of Ffh is localized in the M-domain [70–72]. This domain consists of a deep groove that is lined almost exclusively with the side chains of conserved hydrophobic residues [73]. The dimensions and the hydrophobic character of the groove suggest that it forms the signal sequence-binding pocket of SRP [73]. A conserved feature of the M-domain is an unusually high content of methionine residues. These methionines were proposed to be arranged in so-called methionine bristles that are involved in signal sequence binding [57]. The position of these methionines in the crystal structure of *Thermus aquaticus* showed that these residues indeed line the putative hydrophobic signal sequence binding groove [73]. Also, the M-domain recognizes the conserved RNA domain IV. As part of the RNA lies adjacent to the groove that has been implicated in signal sequence binding [74], the functional signal sequence binding site may consist of both protein and RNA. Determination of the precise localization of the signal sequence binding site may require a cocrystal of the M-domain-RNA complex and a bound signal peptide.

Mechanism and structural insights in preprotein translocation

The SRP and SecB-mediated targeting routes converge at the translocase [61]. The core of the translocase consists of the motor protein SecA and the integral membrane subunits SecY and SecE. Together they suffice for the reconstitution of preprotein translocation in vitro [75, 76]. In the cell, the SecYE complex is associated with a small integral membrane protein named SecG that although not essential for protein translocation per se, enhances the translocation rate in particular in vitro. SecD, SecF and YajC are integral membrane proteins that form another heterotrimeric complex that can associate with the Sec YEG complex [77]. The exact role of these proteins is not known, but in their absence, pleiotropic translocation defects occur. The following section discusses the structure and function of the translocase and its subunits.

SecA, an ATP-dependent motor protein

The ATPase SecA functions as the ATP-dependent motor that drives the translocation reaction. SecA is a homodimeric protein with 102-kDa subunits [47, 78].

Biochemical evidence has indicated that SecA contains two nucleotide binding sites (NBSs) [79] that are responsible for the high (NBS-I, K_d = 0.13 μM) and low-affinity (NBS-II, K_d = 340 μM) binding of ATP [79]. Both NBSs contain a Walker A (GXXXXGKT) and a Walker B (hXhhD) motif [80]. NBS-I is present in the N-terminal domain of SecA [79]. Its Walker B domain shows homology to the DEAD-box sequence that is found in a subclass of SF-II helicases [81], and is atypical as it is present as a tandem repeat [82]. Recently, the crystal structures of *B. subtilis* SecA and *Mycobacterium tuberculosis* SecA1 were solved [83, 84]. In the *B. subtilis* SecA structure, Mg^{2+}-ADP is bound at the interface between two F_1-type nucleotide binding folds (NBF-I and II) that show resemblance to the tandem motor domains in superfamily I and II helicases [83]. The Walker A motif of NBF-I forms the P-loop which is part of the binding pocket for the α- and β-phosphate of the bound nucleotide [83]. The NBF-I Walker B domain forms a hydrophobic β strand that terminates at Asp207, and this residue contacts the Mg^{2+} cofactor [83]. Mutational analysis indicates that the aspartate of the second Walker B motif of this NBF-I is also involved in coordination of the Mg^{2+} [85]. Experiments suggested that the conserved Asp133 plays a role as a catalytic carboxylate to activate a water molecule to attack the γ-phosphate [86]. However, in the crystal structure of *B. subtilis* SecA Glu208 appears to be the catalytic base in the ATP hydrolysis reaction, as it makes a H-bond to a water molecule that is positioned to make a hydrophilic attack on the γ-phosphate of ATP [83]. Other direct contacts to the nucleotide are made by helicase motifs V and VI, which are located in a different region of NBF-II than the topological equivalents of the Walker A and B motifs [83].

NBS-II was mapped at a more C-terminal position than NBS-I. The use of bifunctional photo-activatable ATP analogs indicates that NBS-II is located at or near the subunit interface of the SecA dimer [87]. The structures of Mg^{2+}-ADP-bound *B. subtilis* SecA and Mg^{2+}-ADP-β-S-bound *M. tuberculosis* SecA1, however, lack indications for the binding of a second nucleotide [83, 84].

Biochemical evidence indicates that both NBS-I and II are needed for the preprotein-stimulated SecA ATPase activity and preprotein translocation [79, 82, 86, 88]. Since in vitro translocation can be driven by ATP concentrations far below the K_d of NBS-II [89], a regulatory role of NBS-II in protein translocation appears more apparent. A recent study suggests that NBS-II and a conserved C-domain sequence called the intramolecular regulator of ATP hydrolysis (IRA-1) regulate the ATP hydrolysis at NBS-I [90]. In this mechanism, NBS-II does not function as a true ATP hydrolytic site. IRA-1 is proposed to downregulate the ATPase activity of cytoplasmic SecA and to permit ATP hydrolysis by SecYEG-bound SecA upon translocation [90]. NBS-II is located within a second IRA domain (IRA-2) in the N-terminal 65-kDa domain that is proposed to control ADP release and optimal ATP catalysis by binding to NBS-I [91].

SecA localizes both to the cytosol and the cytoplasmic membrane [92]. The association with the membrane occurs via a low-affinity interaction with negatively charged phospholipids [93] and a high-affinity interaction with the SecYEG complex [43]. The C-terminus of SecA appears to be involved in the low-affinity electrostatic interaction with anionic lipids [94]. The SecA domains that interact with the major channel subunit SecY are unclear. Ligand affinity blotting identified the C-terminal third of SecA as the interacting domain [95]. In contrast, studies with isolated domains of SecA indicated that the N-terminal domain is responsible for the interaction with the SecYEG complex, while the C-terminal domain interacts with phospholipids [96]. Ligand affinity blotting indicated that SecA binds to the first 107 amino acid residues of SecY [95]. This region covers the first three transmembrane segments (TMSs) of SecY and can also be cross-linked to SecA in vivo [97]. In addition, mutational studies suggest that cytoplasmic loops 5 and 6 (i.e. C-5 and C-6) of SecY are important for a functional SecY-SecA interaction [98]. SecG is not needed for the high-affinity interaction between SecA and SecYE [77, 99], but SecA can be cross-linked to SecG when actively involved in the translocation reaction [100]. During the catalytic cycle of SecA-mediated protein translocation, the two transmembrane domains of SecG undergo a remarkable topology inversion (see below) [101]. A partially functional SecA truncate that lacks the first eight N-

terminal amino acid residues is defective in the SecG topology inversion [102]. These studies suggest that SecA and SecG interact at a later stage of the translocation reaction, but that SecG is not needed for the initial binding of SecA to the SecYE complex.

The structure of the SecA dimer that was proposed to be the active form of *M. tuberculosis*, SecA1, shows a central elliptical pore with a size of 10 × 35 Å [84]. This SecA pore was suggested to align with the integral membrane channel that is formed by the SecYEG complex (see below) [84]. A recent electron micorscopy study proposes that also *E. coli* SecA forms ringlike structures [103]. The ringlike *E. coli* SecA structures are, however, only observed after prolonged (more than 6 h) incubation in the presence of negatively charged phospholipids [103], and their physiological role remains to be established.

When SecA is bound to the SecYEG complex, it is primed for the high-affinity interaction with SecB-preprotein complexes [43]. As outlined before, this interaction is enhanced by binding of the signal sequence to SecA [48], which in turn causes the release of the mature preprotein domain from SecB. SecA can interact both with the signal sequence and mature domain of the preprotein [93]. Cross-linking studies have shown that the N-terminal residues 219–244 of SecA are essential for binding of a synthetic signal peptide [104]. Mutations that allow the translocation of preproteins with a defective signal sequence (*prlD* suppressor mutations) are scattered throughout the entire SecA structure [83]. Many of these *prlD* mutations coincide with *azi* mutations that render SecA resistant to azide, an inhibitor of the translocation ATPase [105, 106]. *prlD* or *azi* mutations alter the conformation of SecA, lower the affinity for ADP and increase the membrane ATPase activity [107]. This suggests that signal sequence suppression is caused by an altered turnover of SecA rather than a restoration of the defective signal sequence recognition event. Cross-linking studies indicate that the preprotein binding region is located just adjacent to NBS-I between amino acid residues 267 and 340 [108]. Mutagenesis of Tyr-326 results in strong translocation defects and abolishes SecA ATPase activity, possibly as a result of a defect in preprotein release [109]. In the *B. subtilis* SecA structure, the putative preprotein binding region is composed of two closely associated subdomains that are inserted in the first nucleotide-binding fold [83]. A similar domain was identified in the *M. tuberculosis* SecA1 structure [84]. In addition to these so-called preprotein cross-linking domains (PPXDs), two other sites in *B. subtilis* SecA were proposed that could be involved in substrate binding [83]. One region forms a weakly packed, methionine-rich, conserved hydrophobic interface between NBF-II and the N-terminus of a C-terminal α-helical domain termed the α-helical scaffold domain (HSD). This region has been assigned as 'methionine canyon' and possibly functions as a binding site for phospholipids or preproteins. The other putative preprotein binding site is a groove with a conserved hydrophobic surface at the interface of NBF-I and the N-terminal PPXD. The surface of this site is surrounded by a conserved acidic environment that may electrostatically attract the positively charged N-terminus of the signal sequence. Since both *B. subtilis* and *M. tuberculosis* SecA were crystallized without the preprotein bound, the exact location of the preprotein binding site remains to be determined.

SecYEG complex, the protein-conducting channel

The protein-conducting pore of the translocase is formed by a complex of the SecY, SecE and SecG proteins. The heterotrimeric organization of the translocation pore is conserved throughout the three kingdoms of life. The translocon of the eukaryotic ER membrane, the Sec61p complex, consists of three subunits, i.e. Sec61α, Sec61β and Sec61γ, of which Sec61α and Sec61γ (Sss1p in *Saccharomyces cerevisiae*) are homologous to SecY and SecE, respectively. Sec61β, which is called Sbh1 in yeast [110], has no sequence similarity to SecG. Archaeal Sec61α, Sec61γ and Sec61β are more closely related to the eukaryotic counterparts than to the bacterial proteins [111, 112].

SecY is the largest subunit of the translocation channel and interacts with SecA, SecE and SecG. SecY is a polytopic membrane protein with a mass of 48 kDa. Hydropathy analysis predicts 10 TMS [113]. Large domains of the primary sequence of SecY are highly conserved. All conditional lethal mutants identified so far map in the conserved regions. Dominant loss-of-function mutations in SecY have only been identified in the C-5 loop, which has been implicated in SecA binding [98, 114]. SecY forms a stable complex with SecE that does not dissociate in vivo [115]. The association with SecE protects SecY from degradation by the membrane-bound protease FtsH [116] that is involved in the degradation of unassembled membrane protein complexes. *E. coli* SecE is a 14-kDa protein with three TMSs [117], of which only the C-terminal part including the third TMS appears to be essential for protein translocation [118]. Genetic and biochemical studies have identified several domains that are involved in the SecY-SecE interaction. Many *prl* mutations that suppress secretion defects of signal sequence-defective mutants map to SecY and SecE. Interestingly, specific combinations of *prl* mutations in SecY and SecE result in synthetic lethality, and it has been proposed that these represent sites of interaction. According to this hypothesis, the first periplasmic loop (P1) of SecY interacts with P2 of SecE, and TMS3 of SecE interacts with TMS7 and TMS10 of SecY [119]. Cysteine-scanning mutagenesis studies have confirmed the close proximity of the

periplasmic domains [120], and identified contacts between SecE TMS3 and SecY TMS 2, 7 and 10 [121–123]. In addition, mutations in C-4 of SecY [124] and the C-2 loop and TMS3 of SecE [125] destabilize the SecY-SecE interaction. Recently, an 8-Å resolution, three-dimensional (3D) map of the SecYEG complex was reconstructed from two-dimensional (2D) SecYEG crystals [126]. In this crystallographic dimer, a highly tilted helix is present at the contact interface. These data are consistent with cysteine-scanning mutagenesis studies that identified the third TMS of SecE at the contact interface. Cysteine-scanning mutagenesis indicated that TMS3 of SecE must be highly tilted while contacting TMS 2, 7, 10 and possibly TMS 5 of SecY [123]. Remarkably, most *prl* mutations in SecY and SecE cluster in this set of TMSs. The *prlA* mutations in SecY point away from the sites of contact with TMS 3 of SecE, and thus likely affect the overall SecYE conformation.

Although SecG is not essential for protein translocation, it stimulates protein translocation in vitro, in particular at lower temperatures [127] and when the proton motive force (PMF) is low or absent [128]. SecG is a 12-kDa protein with two TMSs that are connected via an apolar cytosolic segment [101]. Co-immunoprecipitation studies suggest that SecG interacts only with the SecY subunit [129]. Cysteine-scanning mutagenesis demonstrated that SecG is in close proximity to the C-2 and C-3 domains of SecY [130].

Recent biochemical and structural analysis of the bacterial SecYEG complex and the eukaryotic Sec61p complex has provided insight on how these proteins form a translocation pore. Electron microscopic studies on the eukaryotic Sec61p complex [131] show the formation of ringlike structures upon the addition of ribosomes or after coreconstitution with the yeast Sec62-63 complex. These structures are quasi-pentagonal oligomers composed of three to six Sec61p complexes [131]. A 3D reconstruction of the structure of the mammalian Sec61p complex bound to a nontranslating ribosome suggests that the central cavity or pore of Sec61p aligns with the protein-conducting channel of the large ribosomal subunit [132]. Quasi-pentagonal structures were also observed with the purified *B. subtilis* SecYE complex, but these appeared only as a small fraction [133]. The *E. coli* SecYEG complex was also visualized by electron microscopy. In the absence of translocation ligands, Sec YEG exists as particles with a length of 8.5 nm and a width of 6.5 nm, and a weakly stained central indentation [134]. From mass analysis, these particles were assigned to represent dimeric forms of SecYEG, and their size corresponds with the structure of the SecYEG dimer as analyzed from 2D crystals. Upon addition of SecA in the presence of a nonhydrolysable ATP analog AMP-PNP, or ATP and a preprotein trapped in the translocation channel, the repurified SecYEG complex appeared to be assembled into a much larger structure with a width and length of 10.5 nm and a central stain-filled depression of about 5 nm [134]. Size and mass measurements suggest that this large structure corresponds to a SecYEG tetramer. The putative pore size corresponds to the values determined for the active conformation of the eukaryotic translocon [135].

A chemical cross-linking approach failed to detect SecYEG oligomers [136], and it was suggested that SecYEG functions as a monomer, while oligomers were attributed to an artifact due to the overexpression and purification of the SecYEG complex. Recent data demonstrates that chemical cross-linking interferes with SecYEG oligomerization [137], while cysteine-scanning mutagenesis has provided compelling evidence that the SecYEG complex is at least organized as a dimer [121] even when SecYEG is present at wild-type levels [122]. The exact number of SecYEG heterotrimers within the active translocase complex is still a topic of debate. Sedimentation analysis supported the notion that SecYEG can assemble into tetramers [138], but Blue Native Page analysis indicated that after SecA has been removed, a trapped preprotein translocation intermediate associates only stably with SecYEG dimers and not with tetramers [137]. The 3D structure reconstituted from 2D SecYEG crystals indicates the presence of a deep cavity at the SecYEG dimer interface [126]. Since this cavity is closed at the periplasmic side of the membrane, it was proposed the structure represents the ‘closed state’ of the translocation channel that would be converted into the ‘open state’ when the complex is actively involved in translocation [126]. According to this scenario, the SecYEG dimer would represent the functional form of the SecYEG complex. The cavity is closed at the periplasmic side of the membrane by the tightly interacting TMS3s of two neighboring SecE molecules. Cysteine-scanning mutagenesis indicates that the contact persists during protein translocation or when a preprotein translocation intermediate is trapped in the channel [121, 122]. This strongly suggests that the cavity does not ‘open’ to form a translocation channel. An alternative hypothesis is that the cavity forms a binding site for SecA, thus allowing domains or loops of SecA to deeply penetrate the membrane as suggested by biochemical studies. According to this hypothesis, the channel will be localized at the contact interface of two SecYEG dimers that are recruited by the SecA dimer to form a SecYEG tetramer.

The SecYEG dimer structure indicates per monomer the presence of 13 tightly packed TMSs and two TMSs that are localized more distal [126]. Since SecG is not required for translocation, the more distant TMSs were suggested to represent SecG [126]. Alternatively, the distal TMSs might represent the nonessential transmembrane segments of SecE.

The heterotrimeric SecDFyajC membrane protein complex optimizes secretion

The SecYE complex can also associate with another heterotrimeric membrane protein complex consisting of the SecD, SecF and YajC proteins [77]. In *E. coli* SecD and SecF are integral membrane proteins each with 6 TMSs and a large periplasmic domain [139], while in *B. subtilis* and some other bacteria, SecD and SecF are fused into one large polypeptide with 12 putative TMS domains [140]. YajC is a membrane protein with a single TMS and a large cytosolic domain. Except for the observation that YajC associates with SecDF, it is not needed for protein translocation nor for viability. In contrast, cells lacking SecD and SecF are cold sensitive for growth and display severe protein translocation defects [141]. Coreconstitution studies of SecY and SecE with the SecDF complex did not reveal any catalytic activity of SecD and SecF in vitro [142], but membranes depleted from SecDF or containing SecD and SecF mutants are severely compromised in in vitro protein translocation [N. Nouwen, unpublished results]. The exact function of the SecDFYajC complex remains unresolved. SecDFYajC has been proposed to regulate the catalytic cycle of SecA, thereby controlling the movement of the translocating polypeptide (see later section). It is unclear whether this is a direct effect of this complex, as Archaea contain SecD and SecF homologs while a SecA homolog is absent [14, 143]. It has also been suggested that SecD and SecF are required to maintain the PMF [144], but later studies showed that the lack of a PMF was due to an artifact caused by a reduced expression of succinate dehydrogenase under the growth conditions used for the SecDF depletion [145]. The SecDFYajC complex might be involved at later stages in translocation, as none of the known *prl* mutants map in these *secD* and *secF* genes. SecD has been suggested to be involved in the release of proteins once they have been translocated across the cytoplasmic membrane [146]. SecD and SecF show similarity to resistance-nodulation-cell division (RND)-type multidrug resistance pumps with 12 TMSs (i.e. AcrB) [147, 148]. The large periplasmic domains of the RND-type transporters interact with the outer membrane pore TolC to form a continuous transport pathway from the cytosol to the external medium. However, such a function has not yet been demonstrated for the periplasmic domains of SecDF. Other possible functions might relate to the removal of the signal peptide or phospholipids from the aqueous protein-conducting pore formed by the translocase, or regulation of pore formation.

Mechanism of protein translocation

The driving force for protein translocation is provided by ATP hydrolysis at SecA [149] and the PMF [150–154]. These energy sources function at different stages [155–157]. ATP is essential for the initiation of preprotein translocation [154], and the cycle of events during ATP-dependent translocation has been partially resolved. SecYEG-bound SecA has a low endogenous ATPase activity that is stimulated by the presence of a translocation-competent preprotein. This activity is termed SecA translocation ATPase as it is associated with the preprotein translocation reaction. The binding of the preprotein initiates a series of events that result in complete translocation (fig. 3). At the initiation stage, SecA is presumably in an ADP-bound form as binding of the preprotein stimulates SecA for ADP/ATP exchange [158, 159]. ATP binding to SecA

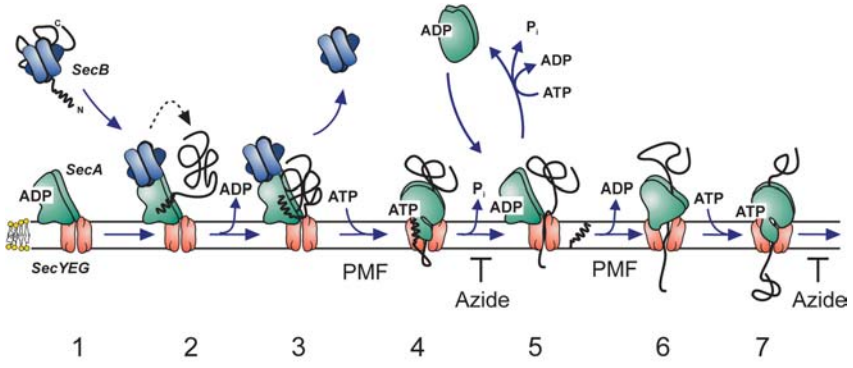

Figure 3. Model for ATP-driven preprotein translocation. At the initiation stage, SecA is presumably in an ADP-bound form (1). The preprotein is targeted to the translocase via the molecular chaperone SecB that binds SecYEG bound SecA with high affinity. Binding of the SecB-preprotein complex is accompanied with the transfer of the signal sequence to SecA. This causes an increase in the SecA-SecB binding affinity and results in dissociation of preprotein from SecB (2). Preprotein binding stimulates SecA for the exchange of ADP for ATP. Binding of ATP results in the release of SecB (3) and causes a conformational change in SecA that allows translocation of approximately 2.5 kDa of precursor protein (4). ATP hydrolysis causes the release of bound preprotein from SecA, and this step is inhibited by azide. Subsequently, SecA can either dissociate from the membrane (5) or rebind to the partially translocated preprotein. Rebinding results in the translocation of another 2–2.5 kDa of preprotein (6) and allows a new round of ATP binding and hydrolysis (7). In this way, SecA can drive the translocation of preproteins in a stepwise manner.

is thought to result in translocation of the signal sequence to the extent that it can be processed by signal peptidase at the periplasmic face of the membrane [160]. The initiation step can be stimulated by the PMF, which affects binding and insertion of signal sequences into the cytoplasmic membrane ([161] and references therein). ATP hydrolysis [162, 163] subsequently causes the release of bound preprotein from SecA [160], after which SecA can either dissociate from the membrane or rebind to the partially translocated preprotein. Rebinding results in the translocation of another 2–2.5 kDa of polypeptide mass [160, 164, 165] and allows a new round of ATP binding and hydrolysis. In this way, SecA can drive the translocation of a preprotein in a stepwise manner, where each catalytic cycle drives the translocation of roughly 5 kDa in two consecutive steps [164]. The exact step size is, however, not known, nor is it clear how the step size is influenced by the secondary structure of the translocatable polypeptide segment. Under conditions where the SecA function becomes limiting, stepwise translocation is accompanied by the accumulation of distinct translocation intermediates [160]. For the translocation of the precursor of outer membrane protein A (proOmpA), the major translocation intermediates are determined by short hydrophobic stretches in its mature domain [166]. Deletion or relocation of these hydrophobic segments significantly alters the pattern of stable intermediates that accumulate in time [166]. The intermediate stages of translocation are reversible [160], and reverse movement can occur in the absence of SecA, ATP and the PMF [167].

Once preprotein translocation has been initiated, the PMF can further drive the reaction and in the late stages even complete translocation in the absence of ATP [156, 160, 168]. Studies with translocation intermediates indicate that the PMF drives preprotein translocation only efficiently when SecA is no longer associated with the translocating preprotein [160, 164]. High SecA concentrations shift the translocation reaction into an ATP-dependent cycle and reduce the requirement for the PMF [169, 170]. Even though PMF driven translocation seems SecA independent, the two modes of translocation are interrelated. The PMF affects several aspects of the ATP-driven reaction, e. g. it optimizes the SecA reaction cycle by stimulating the rate-limiting release of ADP from SecA [170] and accelerates the conformational changes of SecA during translocation [171] (see below). The PMF has been speculated to modulate the opening or even the formation of the translocation channel, as it is required for the translocation of a protein containing a disulfide bridge stabilized intramolecular loop [155, 156]. Interestingly, protein translocation is less PMF dependent when SecY carries a *prlA* mutation [172, 173]. *PrlA* mutants no longer require the PMF for the translocation of proOmpA with a stabilized loop structure [172]. It has been suggested that in *prlA* and *prlG* strains, the translocation pore is in an open state due to a looser association between the SecY and SecE subunits [174]. In such a model, *prl* mutations could reduce PMF dependency via an altered gating of the translocase. Some *prlA* mutations have been shown to cause an increased affinity for SecA [175, 176]. ATP-dependent translocation may be optimized by the tight association of SecA to the SecYEG complex, thereby reducing the requirement for a PMF. Strikingly, the translocation of normal preproteins is much more efficient in *prlA* strains. This has, at least in vitro, been shown to result from reduced rejection of the preprotein by the translocase at the stage of initiation of translocation which has been attributed to the tighter association of the ATP-bound state of SecA with the translocation site [175]. The possible alteration of the gating thus may relate to increased SecA binding affinity, which likely stabilizes the actual translocation pore. In this respect, the PrlA4 mutant shows a different oligomerization behavior in detergent solution with a shift of the monomer-dimer equilibrium towards the dimer [138].

The mechanism by which the energy of ATP hydrolysis at SecA is converted into the movement of preproteins across the membrane is still largely unclear. SecA is a highly dynamic protein. Each SecA monomer contains two domains, which fold independently and whose interactions are influenced by nucleotide binding [162]. Thermal titration of *E. coli* SecA monitored by steady-state tryptophan fluorescence anisotropy spectroscopy suggested that the endothermic conformational transition of soluble SecA [162, 177] is accompanied by a domain dissociation within each SecA protomer [83]. A C-terminal domain called the α-helical wing domain (HWD) was proposed to dissociate from the core of SecA, and this event was proposed to be coupled to a reduction in the nucleotide binding affinity [83]. In addition, a tryptophan in the HSD becomes more solvent exposed during the endothermic transition, and similar conformational changes occur upon interaction with phospholipids or signal peptides [178]. Hunt et al. hypothesized that the endothermic transition of SecA gates the interaction with the SecYEG complex [83]. According to this model, nucleotide-free SecA is in a domain-dissociated conformation with high affinity for the SecYEG complex, while ADP and ATP binding result in similar compact conformations that have a low affinity for SecYEG [83]. ATP-bound SecA was speculated to have a higher thermodynamic stability than ADP-bound SecA, and as a consequence, the complex would be weakened upon ATP hydrolysis [83]. This event would facilitate nucleotide release and shift the equilibrium to the domain-dissociated conformation that has a high affinity for SecYEG [83]. Withdrawal of SecA from the SecYEG complex after completion of translocation could then be driven by ATP binding [83].

Biochemical studies showed that the preprotein-stimulated exchange of SecA-bound ADP for ATP [158, 159]

renders a C-terminal 30-kDa domain and a N-terminal 65-kDa domain of SecA resistant to high concentrations of trypsin and proteinase K [179–181]. The 30-kDa fragment corresponds to segments of the HSD domain and the HWD [83]. Mutations in SecY can reduce the degree of protease protection [171, 182, 183], and the stability of the fragments is influenced by the SecDFyajC complex and the PMF [171, 184, 185]. Since protease resistance is lost upon membrane disruption by freeze-thaw, sonication or detergent solubilization and is susceptible to a chase by an excess of cold SecA [179, 181], the fragments were proposed to represent domains of SecA that insert into the membrane during the translocation cycle [179, 181]. This led to a model where preprotein translocation across the membrane is mediated by coinsertion with SecA [179]. Although SecA protease protection and protein translocation appear to be correlated, the hypothesis that the 65- and 30-kDa fragments represent full membrane insertion with nearly the entire mass of SecA is highly unlikely. Certain domains of SecYEG-bound SecA are accessible for proteases and chemical reagents added from the periplasm side of the membrane [46, 186], but this accessibility does not require SecA to be actively engaged in translocation [187]. These reactive agents possibly gain access to SecA via the translocation pore. The protease-protected fragments of SecA are formed in a similar mode in the presence of detergent-solubilized SecYEG [188, 189], whereas under these conditions SecY is proteolyzed to fragments smaller than 6 kDa [188]. The SecA monomer has a size of about 10 by 6 by 4 nm [83]. Both the dimensions of the tetrameric (or dimeric) SecYEG complex [134] and the thickness of the lipid bilayer are insufficient to accommodate the entire SecA (dimer) molecule (see also fig. 2). In this respect, the observed cavity in the SecYEG dimer [126] may form a membrane-embedded binding site for SecA, which would explain the increased protease-resistant conformation of SecA when associated with SecYEG, and observations that show that inserted preprotein segments can be cross-linked to SecA and SecY simultaneously [190].

Based on the structure of *M. tuberculosis* SecA1, Sharma et al. proposed a macromechanical translocation model that does not require the membrane insertion cycle of SecA [84]. According to this model, ATP binding would result in a movement of the putative preprotein binding region away from the central pore of the *M. tuberculosis* SecA dimer [84]. This movement would be caused by the translation of the long α helix that forms a connection between the motor and translocation domains of SecA [84]. Upon ATP hydrolysis the preprotein binding region would return to its original position. Such movements of the preprotein binding region likely are directed towards the SecYEG pore, thus allowing polypeptide translocation to occur.

The nucleotide binding folds of *B. subtilis* and *M. tuberculosis* SecA show a strong resemblance to the tandem motor domains in superfamily I and II helicases [83, 84]. It has been hypothesized that the mechanism by which SecA mediates protein translocation resembles the mechanism by which these helicases mediate unwinding of nucleic acid duplexes [191, 192]. For helicases two distinct mechanisms, called the inch-worm model and the active rolling model, have been proposed. In the inch-worm model, a monomeric helicase translocates unidirectionally along the DNA (reviewed in [193, 194]). During translocation the helicase contacts the polymer via two distinct domains, which bind the DNA with high and low affinity. ATP binding within the cleft between the two domains results in closure of the cleft and changes the binding affinities of the subdomains. Concomitantly, the helicase translocates along the nucleic acid strand. Helicases function as monomers, while SecA has previously been demonstrated to exist [78] and function [47] as a dimer. Specifically, studies using fluorescence resonance energy transfer (FRET) suggest that the SecA dimer bound to the membrane vesicle-embedded SecYEG complex does not dissociate during translocation, while SecA heterodimers composed of an inactivated and active subunit are inactive with protein translocation [47]. The SecA dimeric structure in the absence of translocation ligand appears conformationally dynamic, and extensive exchange of subunits has been demonstrated in solution [195]. Using chemical cross-linking, Or et al. found that in the presence of acidic phospholipids, detergents and synthetic signal peptides, SecA could no longer be cross-linked as a dimer [192]. This study was conducted with SecYEG proteoliposomes, but under the conditions employed the majority of the SecA is lipid bound and not associated with SecYEG [J. Swaving, unpublished]. Lipid-bound SecA is thermolabile and readily inactivates [93]. Based on the observation that a mutant of SecA that is defective in dimerization retains a minor translocation activity, it was suggested that SecA functions as a monomer that undergoes similar conformational changes as a monomeric helicase, driving translocation by an inch-worm-like mechanism [192]. However, it is impossible to rule out that the low residual activity of the mutant resides from a small fraction of remaining dimeric SecA that has escaped detection. The mutations in the C-terminal domain of SecA likely shifted the dimer-monomer equilibrium towards the inactive SecA monomer [191]. In addition, monomerization of SecA induced by long-chain phospholipids and certain detergents was also observed by tryptophan fluorescence anisotropy and sedimentation velocity experiments [191], but unlike the observation of Or et al. [192] these experiments did not reveal a change in the oligomeric state of dimeric SecA upon addition of synthetic signal peptides. In contrast, signal peptides caused redimerization of lipid-induced SecA monomers [191]. Furthermore, signal pep-

tides seemed to induce polymerization of SecA [191] when added to SecA at a temperature above the endothermic transition [191]. At this temperature, SecA is in the domain-dissociated conformation that was previously proposed to interact with SecYEG [83]. Based on these results it was suggested that a preprotein-bound SecA recruits additional SecA molecules to mediate translocation of the C-terminal segments of the preprotein [191]. This mechanism resembles the rolling model for helicases, where a DNA-bound helicase protomer recruits another protomer to bind an upstream DNA segment and where translocation of the helicase along the nucleic acid strand is achieved by the alternating binding of helicase subunits to the DNA [196]. Concomitant with the recruitment of a new SecA protomer, the bound preprotein segment could be donated to the SecYEG complex. The release and recycling of SecA subunits could be mediated by the lipid-induced monomerization of SecA [191], but this remains to be demonstrated in an authentic protein translocation reaction.

The membrane-embedded subunits of the translocase also seem to undergo extensive reorganization during the translocation reaction. Electron microscopy indicates that activation of the translocase results in assembly of a tetrameric translocation pore from monomeric or dimeric SecYEG complexes [134]. Certain flexibility between and within the SecYEG complexes of the oligomeric channel appears to be essential for the functionality of the translocase, since translocation is inhibited by the formation of thiol-stabilized SecY-SecE and SecE-SecE contacts [121, 122]. Cysteine-scanning mutagenesis identified enhancement of the interhelical SecE contact at the initiation of translocation, suggesting that translocation results in a rearrangement of SecE molecules within the SecYEG oligomer [121]. In the yeast Sec61p complex, initiation of translocation allowed cross-linking of the signal sequence of a preprotein to TMS2 and TMS7 of the SecY homolog Sec61α [197]. As this region partially overlaps with the region of Sec61α that was implicated in the contact with the SecE homolog Sss1p [198], Sss1p was proposed to function as a mock signal sequence that is displaced by the signal sequence of an inserting preprotein at the initiation of translocation [197]. In the bacterial SecYEG complex, however, initiation of translocation does not affect the contact between TMS3 of SecE and TMS2 and TMS7 of SecY [121, 122]. Current evidence argues against a complete displacement of SecE TMS3 by the signal sequence at the stage of the initiation of translocation.

SecG has been proposed to undergo a remarkable topology inversion that is coupled to the SecA membrane cycle [100, 101]. Based on the observation that the cold-sensitive growth defect of a *ΔsecG* strain can be suppressed by mutations in genes involved in phospholipid biosynthesis [199–201], it has been suggested that this topology inversion facilitates membrane insertion of SecA, in particular at lower temperature when the microviscosity of the membrane is high. Several observations point at a correlation between the conformational changes in SecG and SecA. The SecA36 mutant [182], which is independent of SecG for membrane insertion and efficient translocation activity, suppresses the cold-sensitive phenotype of a *secG* null strain [183]. In contrast, a cold-sensitive SecA mutant that appears defective in preprotein binding (CsSecA) strictly requires SecG for membrane insertion and translocation [202]. Furthermore, SecG function is required for expression of azide-resistant and signal sequence suppressor activities of *azi* and *prlD* alleles of *secA* [203]. Azide resistance is a cold-sensitive property [203]. In turn, the *azi* and *prlD* alleles suppress cold sensitivity and export-defective phenotypes of a *secG* null mutant [203]. Tryptophan fluorescence indicates that *azi* and *prlD* mutations shift the first endothermic transition of SecA to a lower temperature, suggesting that these mutations confer a more relaxed conformation to SecA [107]. Unfolding into the relaxed conformation has been proposed to gate the binding of SecA to the SecYEG complex [83]. In such a model, *azi* and *prlD* mutations could facilitate the functional binding of SecA at the SecYE complex without assistance of SecG [107]. In the presence of SecG, this may result in increased translocation activity and suppression of signal sequence mutations. The structural basis for this phenomenon is, however, unresolved.

Integration of IMPs

Initially, IMPs were assumed to insert into the cytoplasmic membrane of *E. coli* independent of the translocase, which was only thought to facilitate the translocation of large periplasmic loops. The phage M13 procoat and Pf3 coat proteins indeed seem to insert into the membrane in the absence of SRP and translocon [204, 205]. On the other hand, cross-linking studies in the ER system demonstrated that transmembrane regions of certain IMPs are in the vicinity of components of the eukaryotic translocase [206–208]. After the improvement of conditional translocase mutant strains and the development of in vitro assays that access the initial stages of IMP insertion, it became apparent that also *E. coli* possesses a Sec-dependent membrane integration pathway. The proper insertion of the polytopic membrane proteins MalF, MtlA and leader peptidase is affected by depletion of SecE or SecY [209–211]. Also, mutations in SecY can affect the topology [212] and insertion [213] of some membrane proteins. Furthermore, the inner membrane proteins MtlA, FtsQ and YidC could be cross-linked to SecY during the initial stage of membrane integration [22, 214, 215].

The requirement for the composition of the insertion site seems to vary with different classes of IMPs. Many IMPs integrate independent of SecA [210, 216–218], while SecA seems to be essential for the proper insertion of other membrane proteins such as AcrB [219]. Nascent FtsQ can be cross-linked not only to membranous components of the translocase, SecY and SecG, but also to SecA [61]. Also, the assembly of MalF was reported to require SecA [209, 216], although SecA dependency varies with the reporter protein used to analyze membrane translocation of the large periplasmic loop of MalF [220].

Sec-dependent membrane insertion may be assisted by other components that do not play a role in protein translocation. Recently, a homolog of the mitochondrial inner membrane protein Oxa1p and the chloroplast thylakoid membrane protein Alb1 (see [221] for a review) was identified in *E. coli*. In mitochondria, Oxa1p is proposed to be involved in the assembly of a variety of both nuclear and mitochondrial encoded IMPs [222, 223]. Likewise, recent studies indicate that this novel component YidC plays a role in IMP membrane insertion. Cross-linking studies with nascent IMPs indicate that YidC is at least temporarily localized in the vicinity of the SecYEG complex. The association seems to occur via the SecD and SecF, with which YidC interacts to form a heterotetrameric YidC-SecDFYajC complex [224]. Site-specific photo-cross-linking studies have demonstrated that YidC interacts with transmembrane segments of nascent inner membrane proteins [225–228], and the initial stages of YidC-dependent FtsQ insertion via the SecYEG complex can be reconstituted in proteoliposomes containing purified SecYEG and YidC [229]. In vivo, however, YidC depletion has little effect on the membrane insertion of these Sec-dependent IMPs [227], while it strongly affects the membrane integration of the Sec-independent phage M13 and Pf3 coat protein [230–232]. The transmembrane region of Pf3 coat protein could be cross-linked to YidC and Ffh [232]. Together, these results suggest that YidC is also involved in the membrane integration of Sec-independent proteins without associating with the translocation channel. Its exact role with Sec-dependent membrane proteins remains to be determined.

How hydrophobic transmembrane domains partition from the translocation pore into the membrane is still largely unresolved. It has been suggested that this is a kinetically controlled phenomenon in which slow translocation of hydrophobic sequences is critical for partitioning. Moderate hydrophobic regions may escape membrane insertion as a result of rapid translocation [233]. Hydrophobic sequences in preproteins serve as stop-transfer sequences that result in a translocation arrest to allow integration into the lipid bilayer [233, 234]. The stop-transfer function of these sequences is correlated to their mean hydrophobicity [234]. In addition, the presence of positive charges around a hydrophobic domain may cause such a translocation arrest [235]. In *E. coli* synthetic stop-transfer regions introduced within proOmpA induce the release (or deinsertion) of SecA from the membrane, after which the hydrophobic domain may partition into the membrane [236]. The movement of hydrophobic segments into the lipid bilayer could be a passive process, but an active process that requires additional enzymes is also conceivable. Studies on the membrane integration of eukaryotic membrane proteins suggest that transmembrane segments may laterally leave the translocation pore formed by the Sec61p complex [237]. Photo-cross-linking indicates that this movement occurs via a mechanism that involves the sequential passage of Sec61α and the translocating chain-associating membrane protein (TRAM) [206, 208], although the involvement of the latter seems to be dependent on the hydrophobicity of the transmembrane segment [208]. TRAM has therefore been suggested to allow retention of TMSs that are not sufficiently hydrophobic to partition into the lipid bilayer [208]. In *E. coli*, the movement of the transmembrane segments from the translocation channel into the lipid bilayer may be assisted by YidC, for example by forming an assembly site for polytopic membrane proteins [225]. Another possibility is that *E. coli* transmembrane segments are released into the lipid bilayer after dissociation of the oligomeric SecYEG pore complex once SecA is deinserted. It is possible that the oligomeric structure of the SecYEG complex may differ for secretory proteins and IMPs. Possibly, the SecYEG dimer suffices to form a site for membrane insertion, while protein translocation would require the formation of a water-filled pore formed upon the SecA-mediated recruitment of two SecYEG dimers.

Conclusions

The last decade has seen a major advance in the study of the bacterial translocase, a multimeric membrane protein complex whose protein-conducting channel is conserved throughout all kingdoms of life. Genetic and biochemical studies have provided detailed insight in the mechanism of preprotein translocation, and the insight in the structure of the translocase is increasing. Recently, the atomic structures of SecB [28] and SecA [83, 84] have been solved, and a medium-resolution structure has been obtained for the SecYEG dimer [126]. In the next years it will be possible to relate function to structure permitting a detailed knowledge about the way in which proteins cross and integrate into the cytoplasmic membrane. However, many interesting questions remain for future research. For instance, How SecA does generate a macromechanical force to drive proteins across the membrane? How do inner membrane proteins integrate into the mem-

brane and assemble into multisubunit complexes? And how is this process linked to nonprotein cofactor insertion?

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