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Review

The bacterial Sec-translocase: structure and mechanism

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Most bacterial secretory proteins pass across the cytoplasmic membrane via the translocase, which consists of a protein-conducting channel SecYEG and an ATP-dependent motor protein SecA. The ancillary SecDF membrane protein complex promotes the final stages of translocation. Recent years have seen a major advance in our understanding of the structural and biochemical basis of protein translocation, and this has led to a detailed model of the translocation mechanism.

Keywords: protein translocation; membrane protein insertion; SecY; translocon; SecA

1. INTRODUCTION

After their synthesis on the ribosome until their arrival at their functional location, proteins are faced with a maturation path that is filled with obstacles. In prokaryotes, one such a barrier is the inner membrane, where most proteins either are directed across or into the lipid bilayer. The majority of secretory proteins pass across the inner membrane via the Sec pathway, which comprises a set of cytosolic and membrane proteins that work together to facilitate protein translocation. This pathway also provides an entry for membrane proteins to be inserted into the inner membrane. Proteins are targeted to their final location, i.e. the inner membrane or the periplasm, by their respective hydrophobic transmembrane segments (TMSs) or signal sequences (for a review, see von Heijne [1]).

At an early stage during translation, when the N-terminal signal sequence emerges from the ribosome, signal recognition particle (SRP) and the trigger factor (TF) compete for binding to the nascent chain [2,3]. Targeting sequences (stop–transfer sequences) from inner membrane proteins correspond to TMSs that exhibit high hydrophobicity and that are bound tightly by SRP. This association slows or temporarily halts elongation of the nascent chain, giving SRP time to interact with its membrane receptor FtsY [4,5]. After binding to FtsY, the ribosome nascent chain complex is transferred to the protein-conducting channel SecYEG, where translation continues providing the driving force for insertion of the membrane protein.

The post-translational pathway for protein secretion (figure 1) involves less hydrophobic signal sequences of nascent secretory proteins that are bound by TF, but this reaction does not result in a slowdown of translation. Following elongation, the chaperone activity of TF is taken over by SecB, which keeps the preprotein in an unfolded conformation and directs it to the motor protein SecA [6,7]. Subsequent binding of SecA to SecYEG and binding of ATP to SecA initiate translocation of the preprotein across the inner membrane. SecA is a motor protein that uses ATP as energy source and threads the unfolded polypeptide through the channel. The adjoining SecDF complex is involved in later stages of protein translocation and presumably pulls translocating proteins from the channel at the periplasmic side of the membrane.

Here, we will discuss the post-translational translocation event at the bacterial inner membrane. Recent structural and biochemical analyses have provided detailed insights into the conformational changes in the SecYEG channel during translocation, and single-molecule analyses methods have been used to unravel the oligomeric state of the channel and its binding partner SecA during protein translocation. We will also discuss the role of the SecDF complex, where the recently resolved crystal structure puts previous biochemical data in a new perspective.

2. THE CHANNEL COMPLEX SecYEG

The heterotrimeric protein complex SecYEG is the central player in protein translocation and functions as the membrane channel where cytosolic binding partners dock and provide the energy to translocate unfolded polypeptides through its aqueous interior. Reconstitution studies with the purified SecYEG complex have demonstrated that the minimal translocase consists of the SecYE complex and the motor protein SecA [8]. The crystal structure from *Methanocaldococcus jannaschii* SecYEβ provided the first high-resolution insight into the organization and structure of the translocation channel [9] (figure 2*a*). The SecY protein constitutes the actual channel and is composed of ten α-helical TMSs, where TMSs

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One contribution of 11 to a Theme Issue 'Bacterial protein secretion comes of age'.

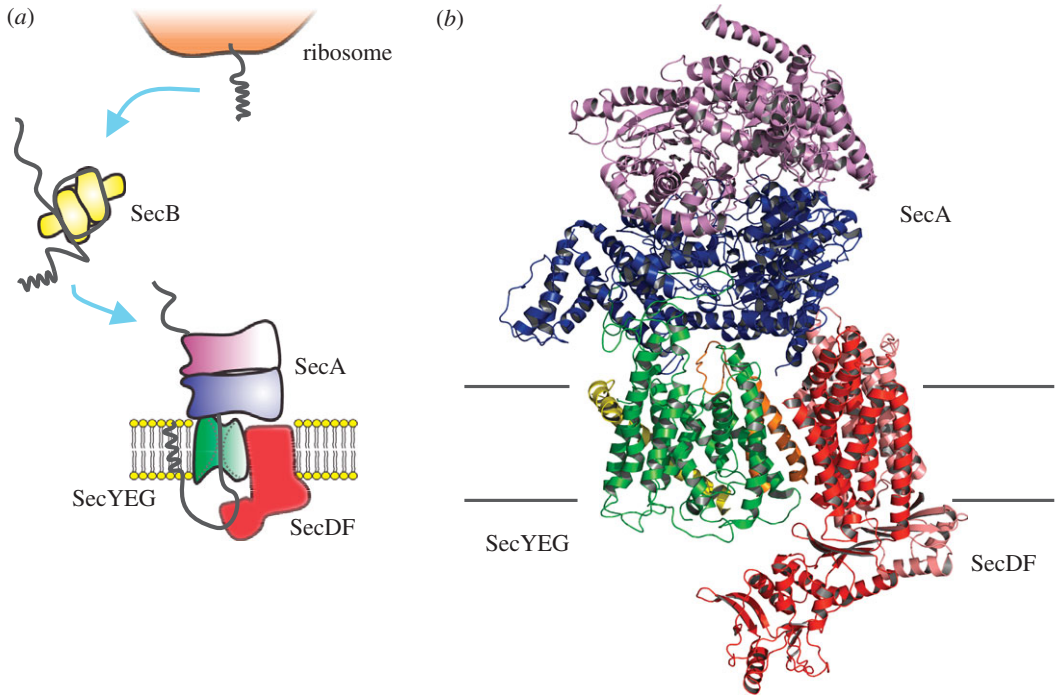

Figure 1. Schematic of the post-translational protein secretion pathway in *Escherichia coli*. (*a*) Preproteins synthesized at the ribosome (red) are captured in an unfolded state by the chaperone SecB (yellow) and targeted to the dimer SecA (purple/blue) protein that is bound to the SecYEG translocation channel (green). SecA functions as an ATP-dependent motor protein that drives the stepwise translocation of preproteins across the SecYEG channel. Late stages of protein translocation are supported by the heterodimer SecDF (red/pink) complex, which uses the PMF to pull preproteins into the periplasm. (*b*) Structural model of the Sec-translocase. A second copy of *Thermotoga maritima* SecA (purple) is docked onto the monomeric protomer (blue) associated with SecYEG (green/yellow/orange; 3DIN). The *Thermus thermophilus* SecDF (red/pink; 3AQP) is placed next to SecG.

1–5 and TMSs 6–10 are pseudo-symmetrically aligned resembling a bivalve shell. SecE enwraps the SecY channel in a V-shaped manner, and contacts the two SecY 'shells' with a tilted helix and an amphipatic helix, respectively. These two helices are connected via a hinge region, providing flexibility to the structure. The Secβ subunit, which presumably is functional homologous to the bacterial SecG, is more peripherally located in the structure.

The SecG protein of *Escherichia coli* possesses two TMSs with the N- and C-terminus in the periplasm. SecG is not essential for translocation or cell viability, but it increases the efficiency of translocation [10–12]. *In vitro*, SecG increases the efficiency of translocation at low temperature or in the absence of a proton motive force (PMF) [13]. *In vivo*, protein translocation in the absence of SecG is cold-sensitive, but the severity of the export defect was shown to be strain-dependent [14–16]. SecG interacts with SecY independently of SecE [17] and cross-linking studies show that it resides beside the N-terminal half of SecY, which was confirmed by the *M. jannaschii* crystal structure [18–20]. It was also found to contact the accessory protein complex SecDF, possibly committing to the formation of the holotranslocon, i.e. the SecYEGDF complex [21,22]. While the exact functioning of SecG is unknown, several studies imply that SecG inverses its topology to assist SecA cycling [23–26]. On the other hand, when SecG was topologically fixed, the translocation mechanism was still fully functional [27]. Various other studies also provide a link between SecG and SecA [28,29]. The structure of SecA bound to SecYEG in an ATP hydrolysis intermediate state [30] confirms the vicinity of the SecG cytoplasmic loop with SecA. A photo-cross-linking study supports this interaction [31], which appears to involve an ionic pair in SecA and affects the coupling of the SecA ATPase activity with protein translocation [32]. SecG association would destabilize this ionic pair and promote conformational changes in SecA, thereby promoting SecA cycling.

While SecG is located peripherally, the SecE–SecY interaction is much more extensive. The *E. coli* SecE subunit has three TMSs, where the two N-terminal TMSs are connected to the third via an amphipathic helix. Although essential for cell viability and protein translocation [33], cells remain viable when the two N-terminal TMSs plus a large part of the amphipathic helix are deleted [34,35]. Furthermore, most SecE homologues consist of only one TMS and the amphipathic helix, so this portion of SecE might have a more specialized function [36]. The most conserved part of SecE concerns the hinge region bridging the two helices enveloping the SecY protein [37]. This region together with the third TMS is essential for the stability of the SecY–SecE complex [38]. In the case of complex dissociation, the SecY unit is rapidly degraded *in vivo* by the membrane protease FtsH [39]; hence the importance of SecE for cell viability.

SecY is the central subunit and forms the actual protein-conducting channel. It features several domains important for proper functioning in protein

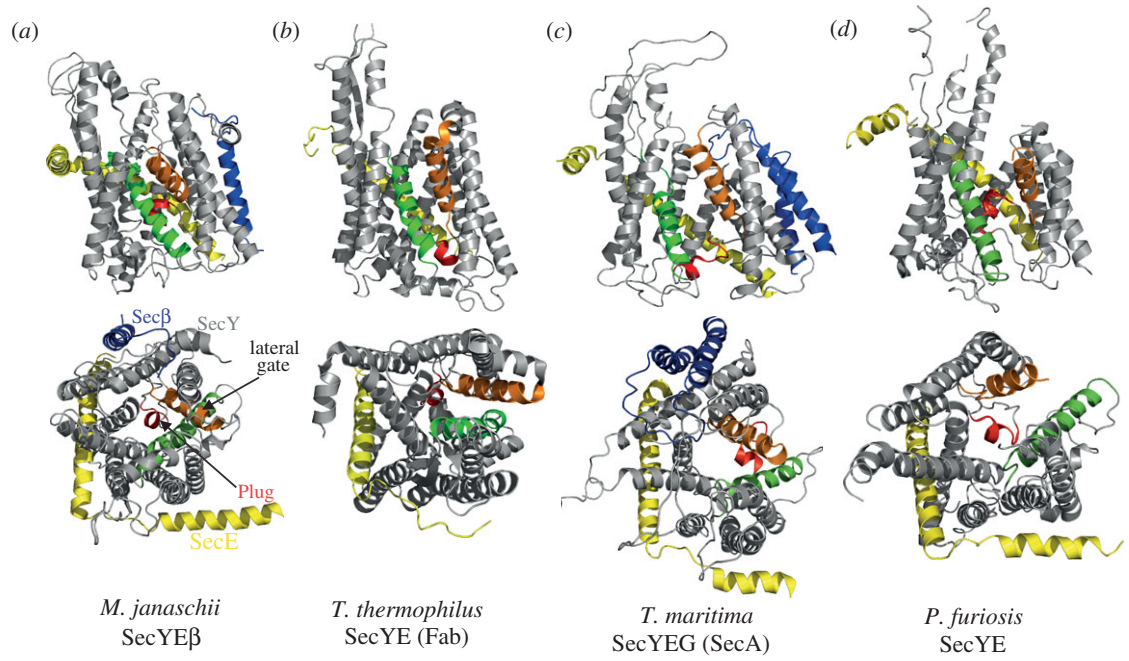

Figure 2. Crystal structures of different SecYE(G/β) complexes shown from a side and top view (from the cytoplasm). SecY (grey) is indicated with the plug (red), the lateral gate helices TMS 2b (orange) and TMS 7 (green), SecE (yellow) and SecG/β (blue). (*a*) SecYEβ from *Methanocaldococcus jannaschii* (PDB code: 1RH5); (*b*) SecYE from *Thermus thermophilus* (2ZJS). The Fab fragment is not shown. (*c*) SecYEG from *T. maritima* co-crystallized with SecA (not shown; 3DIN); (*d*) SecYE from *Pyrococcus furiosis* (3MP7).

translocation and insertion. The hourglass shape of the central channel is constricted, with six hydrophobic residues in the middle of the membrane presumably forming a seal to prevent leakage of water and ions [40,41]. The constriction ring may form a hydrophobic gasket around the translocating polypeptide, maintaining the integrity of the membrane [42]. Below the constriction ring, a small α-helix forms a plug domain, contributing to the barrier in the middle of the channel. The two halves of SecY form a bivalvic shell connected with a hinge on one side and the other side proposed to be a lateral gate for release of polypeptides in the membrane. Signal sequences bind in this lateral gate formed by TMSs 2 and 7. By analogy, nascent TMSs may bind at the lateral gate as well, whereupon they are released in the lipid bilayer [43,44].

At the cytoplasmic face of the channel, several loops protrude from the membrane where they bind to cytosolic binding partners. Structural data show that loops between TMS 6/7 and TMS 8/9 form extensive contacts with the ribosome and SecA [30,45,46]. These observations are supported by cross-linking and mutagenesis studies where residues in these loops were shown to be important in translocation and/or binding of the cytoplasmic partner [46–49]. The interaction of SecA and/or ribosomes with the SecYEG complex likely results in specific conformational changes of the channel, possibly even a partial opening as suggested by the SecA–SecYEG crystallographic structure. Indeed, during the past decade, several crystal structures of SecYEG homologues have been reported that suggest specific ligand-induced structural changes of the channel.

Figure 2 shows four different crystal structures of SecYE(G/β) complexes in different conformational states. The *M. jannaschii* SecYEβ structure seems to correspond to the closed state of the channel [9] (figure 2*a*). The overall conformation is compact, and when viewed from the cytoplasm a vectorial path is clearly obstructed by the plug domain. The lateral gate is closed and TMSs 2 and 7 are in close proximity. The crystal structure of *Thermus thermophilus* SecYE with a Fab fragment bound at the cytoplasmic loop that interacts with SecA [47] appears in a partially opened state (figure 2*b*). Comparison with the 'closed' channel shows several differences which are explained by the binding of the Fab molecule. This 'primed' structure displays a hydrophobic crack at the cytoplasmic side of the lateral gate and it has been speculated to provide a point for signal sequences to enter the presumed binding pocket between TMS 2b and TMS 7. In the middle of the membrane, these two helices are still in close proximity, comparable with the closed structure. When the Fab fragment is removed in molecular dynamics (MD) simulations the channel closes again, indicating that the 'closed' state is energetically most favourable. Physiologically more relevant is the SecYEG crystal structure of *Thermotoga maritima* with SecA bound in an ATP hydrolysis intermediate state [30] (figure 2*c*). Even though SecA is absent in archaea, the overall structure of the archaeal and bacterial SecY channels is very similar. The clear difference with the 'closed' conformation is the expansion of the channel, where the C-terminal half of SecY is shifted outwards. This results in a separation of TMS 2b and TMS 7 forming a gap of about 5 Å between side chains, which only needs a slight increase to accommodate an α-helical signal peptide. Furthermore, the displacement of the plug domain to the periplasmic side of the lateral

gate clears a path for preproteins to be translocated through the centre of the channel. This structure is also referred to as a 'pre-open' state. Recently, the crystal structure of *Pyrococcus furiosis* SecYE was resolved [50] (figure 2*d*). While there is no substrate present, the crystal packing of the SecYE molecules interacted in a way that seems to promote a major conformational change. TMS 10 from SecY was partially inserted in an opposing channel, acting as a nascent chain-mimicking polypeptide. Similar expansion of the lateral gate is observed as in the *T. maritima* structure with SecA bound, although the crevice is now considerably larger and would fit a signal sequence without further increase in size. Another notable difference is that the plug domain still occupies the same position as in the 'closed' conformation and is not shifted as in the SecA-bound structure.

The crystal structures seem to display a different degree of opening of the channel and, in particular, there are clear deviations in the widening of the lateral gate, the opening of the constriction ring and the position of the plug domain. MD simulation studies show that two steps are involved in opening the lateral gate, the first step opens the lateral gate slightly to 2–5 Å and the subsequent step involves the relocation of the plug domain and allows further opening to 6–9 Å [51]. The requirement for an opening for translocation was shown in cross-linking studies in which the lateral gate was immobilized in the middle of the membrane by cross-linkers of different lengths [52]. Short cross-links of 2 Å abolished translocation, whereas cross-linkers longer than 5 Å restored translocation fully. The spacer length introduced had a similar effect on the ability of SecA to hydrolyse ATP, suggesting that lateral gate opening and Sec ATPase catalysis are allosterically linked. Thus, it appears that the lateral gate can switch between an open and closed state as recently demonstrated experimentally by the introduction of an optical switch in the TMS 2/7 contact interface [53]. Likely, the lateral gate can assume various-sized openings to accommodate a range of polypeptides that differ in amino acid composition. Because two of the six hydrophobic residues of the constriction ring are located on the TMSs forming the lateral gate, the opening is accompanied by an increase of the constriction ring dimensions. The *P. furiosis* SecYE crystal structure shows the widest expansion of the lateral gate and constriction ring when compared to other structures. While these events are proposed to loosen the plug interactions, the crystal structure shows that the plug domain still occludes the channel.

When the plug domain is deleted from SecY, cells are still viable with few consequences for protein translocation [54–56]. Although the plug does not seem to be required for the SecY function, electrophysiology experiments show that plug deletion allows passage of ions and causes fluctuation of channel between an open and closed state [41]. Also, MD simulations show that the channel without the original plug is not tightly sealed and allows water molecules to permeate it [40]. When investigated more closely, crystal structures of the SecY channel with the plug domain deleted show that neighbouring loops substitute for the original plug domain [55]. The new plug domains do not interact as strongly with the periplasmic funnel opening of SecY as the original plug domain, but these observations explain the lack of a strong phenotype in the previously mentioned deletion studies. Cross-linking studies have shown that the plug domain has the ability to move to the C-terminal end of SecE during protein translocation [57,58]. However, cross-linking studies indicate that the plug domain stays inside the SecY channel during protein translocation [59]. MD simulations suggest that the plug domain indeed remains near its original position and can sense the hydrophobicity of the incoming polypeptide, thereby clearing the path for hydrophilic secretory polypeptides and blocking the path for hydrophobic polypeptides, guiding them into the lipid bilayer [60]. This phenomenon can be envisioned in the *P. furiosis* crystal structure (figure 2*d*), where the lateral gate is opened and the plug domain still blocks a vectorial path. Biochemical evidence using an environment-sensitive fluorophore on the plug domain supports this model, where no displacement of the plug is observed upon interaction with an inserting TMS domain [61].

Most of the proteins involved in the Sec pathway have been identified by genetic screens, resulting in the identification of the *secY*, *secA*, *secE*, *secD* and *secF* genes. In contrast, SecG was identified biochemically as a protein that stimulates the translocation activity of the SecYE complex, and was found to be associated with the SecYE complex purified from wild-type *E. coli* cells [12]. The *sec* mutations exhibited a conditional lethal phenotype caused by a severe protein-export defect [62–64]. Another genetic approach involved the isolation of suppressor mutations that compensate for a secretion defect of preproteins with a defective or even missing signal sequence [65–67]. The protein localization (*prl*) mutations in *secY* (*prlA*) are primarily localized on the plug domain and the surrounding interior of the translocation channel [68]. Initial models suggested that these mutations restore the ability of SecY to recognize the signal sequence of preproteins. However, the *prlA* mutants are also able to translocate preprotein with an atypical signal sequence and preproteins that even completely lack the signal sequences [65,69,70]. This led to the proposition of a proofreading model that proposes that binding and recognition of the signal sequence by the SecYEG complex results in an opening of the channel [71]. On the other hand, *prl* mutations cause a bypass of the proofreading mechanism by destabilizing the channel, allowing it to open even in the absence of signal sequence. Indeed, MD simulations suggest that the *prlA* mutations have a significant effect on the overall structure of the channel, in particular, in the region where the signal sequence has been proposed to bind [72]. Interestingly, the *prlA* mutations also suppress the PMF-dependence of preprotein translocation [73] and even allow a PMF-independent translocation of preproteins derivatized with large organic molecules [74]. This suggests that both the PMF and the *prlA* mutations affect channel stability, and possibly the pore size.

3. THE MOTOR PROTEIN SecA

The cytoplasmic protein SecA delivers preproteins to the membrane channel SecYEG and facilitates translocation. In the post-translational targeting pathway, SecA interacts with SecB, resulting in a transfer of the preprotein. The interaction with SecA both involves SecB and the signal sequence of the preprotein, and also the mature part of the preprotein plays a role in binding [75,76]. At the membrane, SecA not only binds with high affinity to SecYEG but also shows low-affinity binding with anionic phospholipids [77,78]. SecA belongs to the superfamily of two DExH/D proteins and it contains several conserved motifs, such as the Walker A and Walker B motifs that are involved in nucleotide binding. The structural arrangement shows different domains allocated to binding of the various substrates (figure 3*a*). The interface of the two nucleotide binding domains (NBD1 and NBD2), which contain the Walker A and B motifs, is the position where ATP is hydrolysed to induce a large conformational change in the SecA protein [79–81]. The preprotein cross-linking domain (PPXD) is located on the other side of SecA and is involved in binding of the preprotein [82–84]. The chaperone SecB interacts with SecA at the C-terminal linker domain, which contains a zinc finger [85–87]. This same region was shown to interact with phospholipids [88]. The helical scaffold domain (HSD) is located centrally and bridges NBF1 and the α-helical wing domain (HWD). Regulation of the ATP hydrolysis cycle seems to be dependent on various interdomain interactions. A regulatory domain with two α-helices, called IRA1, which connects the C-terminal linker to the HWD, acts as an inhibitor of ATP hydrolysis. Its deletion results in an increased ATPase activity uncoupled from translocation [89]. A further element that is part of the regulation network is a conserved salt bridge between NBF1 and NBF2, which allows cross talk between the two DEAD motor domains. This electrostatic bridge called gate 1 controls the conformation of NBF2 and thereby ADP release, which is thought to be a rate-limiting step in translocation [90,91]. Additionally, the PPXD domain seems to control the affinity and hydrolysis of nucleotides in NBF2 via gate 1. The co-crystallization of SecA with a bound peptide shows a rotation of the PPXD domain towards NBF2 [81], thereby clamping the peptide, which possibly occurs at the initial stage of preprotein capturing in the cytosol (figure 3*b*). The structure of SecA bound to SecYEG [30] shows an even further rotation of the PPXD domain towards NBF2, closing the clamp even tighter, and aligns the preprotein with the SecY lateral gate (figure 3*c*). The details of how binding of preproteins to SecA and the subsequent binding to SecYEG regulate the ATP hydrolysis cycle remains to be investigated.

The concentration of SecA in the cytosol greatly exceeds the subnanomolar dimer dissociation constant, indicating that most SecA molecules are dimeric in the cell [92,93]. Although binding of chaperones, preproteins and nucleotides might influence the monomer–dimer equilibrium, it is widely believed that SecA is present in the cell as a homodimer [94–96]. Several crystal structures of SecA have been published and these show a number of different dimeric interfaces [97–101]. However, SecA is subject to a range of treatments before crystallization, including temperature shifts and high salt treatment, and these will have a significant effect on the dimer dissociation [102], thereby promoting the possibilities of aberrant dimer formation. Several studies have been reported in search of the SecA dimer interface in solution, and C-terminal intermolecular cross-linking [103] and Förster resonance energy transfer (FRET) [104] are in favour of the antiparallel orientation found in three of the published crystal structures.

The SecA dimer in solution does not necessarily reflect the state of SecA during protein translocation. SecA has been suggested to be either a monomer or a dimer when associated with SecYEG. Early FRET studies show that SecA is dimeric during translocation [96], whereas biochemical investigations show that the SecA dimer dissociates upon binding to lipid vesicles or high concentration of a signal peptide [105–107]. Apparently, the SecA dimer–monomer equilibrium is influenced by various ligands and conditions. However, a recent single-molecule study suggested that the dimeric organization of SecA is maintained when actively engaged in preprotein translocation [93]. Covalent stabilization of the SecA dimer by cross-linking did not interfere with the protein translocation activity [93,103,108,109]. In this respect, the *T. maritima* SecYEG–SecA crystal structure shows a monomer of SecA bound to SecYEG, but this structure may not reflect the physiological oligomeric state of SecA as the crystallization process was accompanied with detergents and high salt, both of which have been shown to negate interactions in a SecA dimer interface [93,106]. While the binding interface of SecYEG and SecA buries some residues that were shown to be important in several SecA dimers that were observed in crystal structures, it is reasonable to believe that they do not play a role when the SecA dimer is bound to SecYEG. The exposed area of the SecYEG-bound SecA displays several highly conserved residues, and interestingly these form the dimer interface interactions in the crystal structure of *E. coli* SecA [98]. This has led to the idea that a SecA protomer binds antiparallel to the SecYEG-bound SecA. This was supported by a recent single-molecule spectroscopic study showing that the binding of a monomer to SecYEG is salt-resistant, whereas the binding of a second SecA is salt-sensitive [93]. Interestingly, the same study showed that although nanomolar concentrations of SecA are enough to saturate the SecYEG binding site, an increase in SecA concentrations to a more than 100-fold excess promotes the translocation activity of the SecYEG complex. This lends strong support for the notion that the SecA dimer is functionally active and cycling occurs between SecYEG-bound and cytosolic SecA. The structural model derived from this study is shown in figure 1*b*.

4. THE ACCESSORY COMPLEX SecDF

The heterodimeric membrane complex SecDF fulfils an accessory role in Sec-mediated protein translocation.

The genes encoding these two proteins were identified by a mutant screen using a reporter assay for translocation [110,111]. Sequence analysis and topological determination showed that both proteins are integral membrane proteins with large periplasmic loops. This led to the suggestion that the complex acts at later stages in protein translocation, where the periplasmic loops would interact with the secreted protein. Studies supporting this idea involved time course experiments in spheroplasts where SecD was rendered dysfunctional by the binding of an antibody. The secretion defect indeed implied a role of SecDF in later steps in translocation [112]. While the deletion of SecD and SecF results in a severe defect in protein export *in vivo*, SecDF barely increases the translocation activity when co-reconstituted with SecYE *in vitro* [113,114]. However, such *in vitro* assays primarily assess the translocation activity and not later steps in translocation, such as folding or protein release. The secretion defect is similar only when SecF or SecD is deleted, suggesting they act in concert. Further research implicated a role of the SecDF complex in the membrane cycling of SecA, where overexpression of SecDF led to increased membrane association of SecA [115]. Deletion of SecDF resulted in a decrease of what was believed to be a membrane-inserted SecA [116], as signified by the appearance of a protease-protected SecA fragment [117,118]. However, this protease resistance seems to arise from a more densely packed SecA conformation, possibly corresponding to a conformational state that is specific for the SecYEG-bound form of SecA. The presence of SecDF also slows down the back and forward preprotein movement inside the SecYEG channel [119]. An interaction of the large periplasmic domains of SecDF [120] with the preprotein at the periplasmic side of the membrane may be the cause of slowed movement.

A major breakthrough was the recent crystal structure of *T. thermophilus* SecDF, which provided functional insight on its role [121]. This organism shows the SecD and SecF in one open reading frame, which has been observed in more species [122]. The overall structure confirms previous TMS topology predictions, showing 12 TMSs and two large periplasmic domains (figure 4). Additionally, the crystal structure of the isolated periplasmic domain corresponding to SecD (P1) was resolved. Interestingly, the conformation of P1 in solution showed a rotation of its head domain compared with P1 in the full-length structure, thereby implying a functional change (figure 4). Which part of the head domain interacts with the emerging polypeptide is still unknown, but hypothesized preproteins may interact with the β-strands located at the tip of the head domain. A swivel induced by the PMF would rotate the head domain over 120° and the tip would travel over 75 Å, corresponding to a movement of about 25 amino acids of unfolded polypeptide. Cross-linking experiments confirm the existence of the two states of the P1 domain, and immobilization of the head domain leads to severe inhibition of translocation. Additionally, the P1 domain was shown to interact with an unfolded polypeptide, thus supporting the idea that SecDF interacts with an emerging secretory protein preventing backward movement. The conformational change of the head domain would promote the forward movement and result in increased translocation efficiency. Because of the absence of ATP in the periplasm, the energy for the rotation of the head domain is derived from a different source. The structural arrangement of the TMSs showed homology to AcrB [123], which belongs to the same RND superfamily as SecDF [124]. AcrB is thought to transport protons in an antiport reaction with drugs. The charged residues important for this process are conserved in the SecDF proteins. Patch clamp experiments using spheroplasts containing SecDF indeed showed ion conduction, and the mutation of the charged residues as well as removal of the head domain disabled these ion channel characteristics. On the other hand, binding of an unfolded polypeptide and the imposition of a pH gradient increased the ion conductivity. Possibly, the P1 domain captures an emerging polypeptide from the SecYEG channel, preventing its backward movement and furthermore promoting translocation by employing the PMF to rotate the head domain. It is tempting to speculate that SecDF is involved in stepwise translocation observed previously [125,126]. Because the preprotein translocation intermediates could be released by solely the PMF, it could well be that this step is mediated by SecDF. The presumed swivel of the head domain would pull out 25 amino acids, corresponding with the step of 2–2.5 kDa observed. The PMF has also other stimulatory effects apart from SecDF functioning, which is evident from studies with SecYE proteoliposomes lacking SecDF that show a PMF-dependent translocation [127,128]. Also, in *secDF*-depleted cells or membrane vesicles, protein translocation remains PMF-dependent. Future studies should point out the exact role of SecDF in translocation.

The above mechanism can take place only when the SecDF complex is closely associated with the SecYEG translocation channel. Co-immunoprecipitation studies show that SecDF and SecYEG interact [129]. A small single membrane-spanning protein YajC, encoded on the *secD* operon, was found to be associated with SecF and also with SecYEG [22,130]. YajC is not essential for cell viability or protein translocation [114] and, more recently, the protein was found to co-crystallize with the multidrug efflux pump AcrB [131]. The role of YajC is unclear [132]. SecDF was also found to interact with the membrane protein insertase YidC (for review, see Kol *et al.* [133]) and was proposed to form a scaffold to bring YidC and SecYEG together [130]. While the exact interface between SecYEG and SecDF is not known, depletion studies led to the suggestion that SecG and SecDF interact with each other [21] as indicated in figure 1*b*.

5. MECHANISTIC MODELS FOR PROTEIN TRANSLOCATION

One of the early models proposed for the protein translocation mechanism was based on the suggestion that SecA inserts deeply into the SecYEG

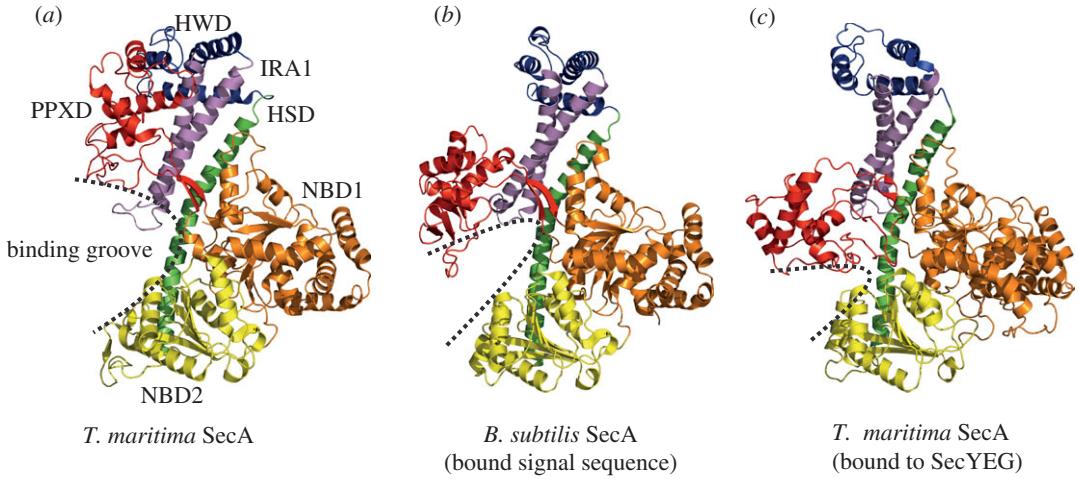

Figure 3. Solution structures of the SecA ATPase. Nucleotide binding domains 1 and 2 (NBD1 and NBD2) are shown in orange and yellow, respectively. The helical scaffold domain (HSD) is shown in green, the intramolecular region of ATP hydrolysis 1 (IRA1) in purple and the helical wing domain (HWD) in blue. The preprotein cross-linking domain (PPXD) is shown in red and the proposed binding groove for preproteins is indicated with a dashed line. (*a*) ADP-bound *Thermotoga maritima* SecA, (*b*) preprotein bound *Bacillus subtilis* SecA (preprotein not shown) and (*c*) SecYEG-bound SecA (SecYEG not shown) were based on coordinates obtained from the protein data bank as 3JUX, 3JV2 and 3DIN, respectively. Where relevant, only one of the protomers of the SecA dimer is shown.

translocation channel [116,134,135]. While nowadays, this experimental observation is thought to be the result of a conformational change, the basic mechanistic implication of the model is that SecA inserts the polypeptide into the channel using its ATP hydrolytic force. Additionally, various results reveal a stepwise translocation, where approximately 20–25 amino acids are translocated each step [125,126,136]. Taken together, this resulted in the *power stroke model*, where insertion or a conformational change of SecA would account for the translocation of a defined stretch of amino acid residues. The structure of SecA bound to SecYEG indicates that a two-helix finger of SecA is partly inserted into the SecYEG channel. This region was proposed to couple the conformational change of SecA to preprotein translocation [137]. Owing to spatial restrictions, it is difficult to image how the movement of the two-helix finger can account for the translocation of a polypeptide stretch corresponding to 20–25 amino acids [138]. However, the association of the two-helix finger with the preprotein and clamping of a SecA domain around the translocating polypeptide provide a possible mechanism that may prevent backsliding of the preprotein. This reaction may also favour forward preprotein movements as SecA may act as a ratchet that is bound and released in an ATP-dependent manner, while the actual translocation may occur by a random Brownian motion [139]. However, this *Brownian ratchet model* does not explain the stepwise translocation with a near to uniform step size.

A further refined model, partly based on the observation that the SecYEG complex associates asymmetrically with the dimeric SecA is the *reciprocating piston model* [140]. This model makes a clear distinction between two stages in the translocation process, i.e. preprotein translocation induced by SecA binding to the preprotein, and subsequent ATP-dependent translocation events [125,126]. During the initial stages of translocation, SecB delivers the unfolded preprotein to the SecYEG-bound dimeric SecA protein [141,142]. SecYEG-bound SecA is primed for ATP binding and hydrolysis, and this is augmented by the interaction of the preprotein with SecA which results in activation of the SecA ATPase activity [77,143]. Binding of ATP to SecA allows an initial insertion of a loop-like structure comprising the signal sequence and the early mature domain of the preprotein into the SecYEG channel, concomitantly with a dissociation of SecB from SecA [144,145]. Likely, the conformational changes within SecA are induced by the binding of ATP, and the preprotein is transferred to the SecYEG complex resulting in a partial opening of the SecYEG channel [146]. Indeed, the SecA ATPase activity and SecY channel opening appear allosterically linked [52]. Next, hydrolysis of ATP by SecA leads to a release of one of the SecA protomers from the complex, whereas the SecYEG interacting SecA protomer remains bound to SecYEG. Subsequent rebinding of SecA from the cytosolic pool and thus the restoration of the SecA dimer may be responsible for the translocation step that does not involve nucleotide binding. Next, the binding of ATP to SecA would drive an additional translocation step [116,134], while repeated cycles of SecA dissociation, rebinding and ATP binding and hydrolysis will result in a stepwise translocation progress of the preprotein. It should be emphasized that it is still uncertain as to whether the SecA dimer completely dissociates in the catalytic cycle, but the observation that the catalytic activity of SecA is needed at concentrations that are far in excess of the concentration needed to saturate binding to SecYEG lends strong support for cycling of SecA between the membrane and free cytosolic forms. Finally, the structural data on the SecDF complex suggest that this complex is involved in the stepwise translocation of the preprotein and implies a

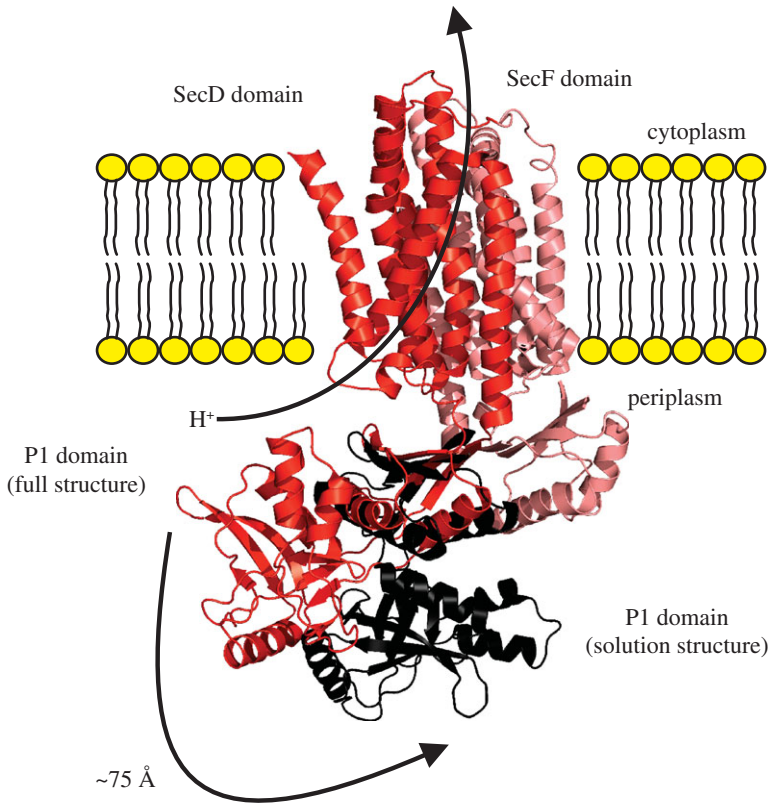

Figure 4. Structure and proposed mechanism of the SecDF complex. *Thermus thermophilus* SecDF (3AQP) is shown with the SecF domain in pink and the SecD domain in red. The structure obtained from the isolated cytoplasmic portion of SecD (3AQO; black) is docked on the transmembrane part of SecD. The proposed turn of the head domain directed by the PMF as indicated by an arrow is shown. The distance indicated is measured from the tip of the head domain in its two conformations.

PMF-dependent pulling on the translocating preprotein. Thus, translocation may be dependent on a 'pushing' force induced by SecA at the cytoplasmic side of the membrane, and a 'pulling' force inflicted by SecDF at the periplasmic side of the membrane. Further detailed analysis of the stepwise translocation mechanisms awaits single-molecule studies as a precise mechanistic resolution of the translocation process by bulk biochemical assays is prevented by their ensemble nature.

6. PERSPECTIVES

Here, we have provided an overview of the recent insights into the structural and biochemical basis of the bacterial protein secretion mechanism. Although the increasing amount of structural information is of significant importance in understanding the underlying mechanisms of protein translocation, these essentially provide only snapshots of protein conformations that need to be verified by accompanying proof of functional relevance. The structural implications of the SecYEG channel have already been extensively tested by various biochemical approaches, resulting in a detailed model of the channel opening mechanism. Nevertheless, the structural basis of the allosteric coupling between channel opening and activation of the SecA ATPase activity is still unknown, nor have the proposed mechanisms for the SecA action been thoroughly tested. Moreover, the recent SecDF structure suggests further mechanistic implications. For instance, the presumed movement of the head domain of SecDF is proposed to pull preprotein out of the translocation channel, but the evidence for such a conformational change is based on a comparison of the structure of the isolated head domain and that of the complete SecDF complex. Further biochemical testing should reveal whether the suggested conformational change is indeed directly coupled to a 'pulling' force on the preprotein. Also, the contact interface between SecDF and SecYEG has not been defined in detail. Information on the spatial organization of the SecYEGDF holotranslocon will yield a further understanding of the cooperation between different players in protein translocation. While the interface of SecYEG with its cytoplasmic partner SecA as shown in the co-crystal is convincing, the dynamics of the SecA dimer and its functional implications are still under debate and the SecA cycling mechanism proposed will require further analysis to reveal the intimate details of the SecA motor action. The increasing sensitivity in fluorescence spectroscopy provides a way to explore such detailed characteristics of the translocation machinery at a single-molecule level.

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