Sec Protein-Conducting Channel and SecA

Sluis, E.O. van der; Nouwen, N.; Driessen, A.J.M.

Published in:
Molecular Machines Involved in Protein Transport across Cellular Membranes

Publication date:
2007

2

Sec Protein-Conducting Channel and SecA

ELI O. VAV DER SLUIS • NICO NOUWEN • ARNOLD J.M. DRIESSEN

Department of Molecular Microbiology
Groningen Biomolecular Sciences and Biotechnology Institute
University of Groningen
9751 NN Haren, The Netherlands

I. Abstract

The Sec machinery facilitates protein translocation membrane insertion and into biological membranes of organisms from all three domains of life. The mechanism of the cotranslational mode of translocation is conserved across the domains, whereas the components involved in posttranslational translocation differ. In addition, significant differences are observed in the composition of the Sec machinery within the bacterial domain. Here, we will review these differences in an evolutionary context, and discuss the latest insights into the structure and dynamics of the translocon and the bacterial motor protein SecA, with emphasis on their oligomeric state(s) during protein translocation.

II. Introduction

Every cell contains at least one membrane that separates the cytoplasm from the extracellular environment and its intracellular organelles. Embedded within these membranes is a variety of different transport systems that selectively allow passage of molecules, thereby enabling the cell to carefully

ISSN NO: 1874-6047
DOI: 10.1016/S1874-6047(07)25002-4

control the (bio)chemical composition on both sides of the membrane. Proteins are the largest and most complex molecules that are transported across membranes, and several different transport systems exist that can handle this class of substrates. The Sec machinery is the only protein transport system that is conserved across all three domains of life. It enables protein translocation across the cytoplasmic membrane of bacteria and archaea, the endoplasmic reticulum (ER) membrane of eukarya, and the thylakoid membrane of photosynthetic eukarya [1].

A property that distinguishes the Sec machinery from other transport systems is its ability to transport substrates toward two different cellular compartments: the aqueous environment on the trans side of the membrane or the hydrophobic environment of the membrane itself. In line with that property, the spectrum of substrates that is transported by the Sec machinery ranges from highly hydrophobic to highly hydrophilic proteins. The only feature that all substrates have in common is a hydrophobic N-terminal signal sequence or a transmembrane segment (membrane anchor signal) that ensures substrate recognition and initiation of the translocation process. Most signal sequences are cleaved off by a signal peptidase to convert the preprotein into the mature form, whereas N-terminal transmembrane segments remain attached to the substrate.

The most conserved part of the Sec machinery is the ''translocon,'' a membrane integrated channel that allows the passage of the (pre)proteins across the hydrophobic lipid bilayer [2]. All translocons consist of three evolutionarily related subunits, but nevertheless archaeal and eukaryotic translocons can be distinguished from bacterial and thylakoid translocons on the basis of their amino acid sequences [3]. The translocon can associate with different partners to mediate two conceptually different modes of protein translocation: cotranslational and posttranslational translocation. The first is mainly employed for the insertion of integral membrane proteins (IMPs), and the latter mainly for translocation of secretory proteins [4]. Cotranslational translocation requires the translocon to associate with the ribosome, allowing a direct coupling between synthesis and translocation of the (pre)protein [5]. This process is conserved in all domains of life [6] and driven by ongoing protein synthesis at the ribosome. To prevent synthesis of membrane proteins in the cytoplasm, ribosome nascent chain complexes (RNCs) are targeted to the translocon via the signal recognition particle (SRP) in conjunction with its membrane-bound receptor (SR) [7]. In eukaryotes, protein synthesis is slowed down or arrested until the nascent chain has been transferred from SRP to the translocon [8]. For more details on the mechanism of SRP-dependent targeting, the reader is referred to one of the reviews that have appeared [9, 10].

Posttranslational protein translocation occurs by definition after protein synthesis has been completed and requires the translocon to associate with

a motor protein to provide the driving force for the translocation reaction. In this mode of translocation, the Sec machineries in the various domains of life differ substantially from each other. Posttranslational translocation in bacteria and chloroplasts is driven by the *cis*-acting ATPase SecA [11], whereas in ER membranes it is driven by a *trans*-acting Hsp70-like ATPase termed BiP or Kar2 [12]. Given this topological difference, the molecular mechanism underlying posttranslational translocation is expected to differ largely between the ER and the bacterial cytoplasmic membrane. Post-translational protein translocation has also been suggested to occur in archaea, but these organisms lack a SecA homologue and no apparent energy source is available for a trans-acting motor protein.

III. Outline

The overall mechanisms of the two modes of protein translocation have been unraveled by groundbreaking studies in the early nineties, employing reconstituted systems from *Escherichia coli* and *Saccharomyces cerevisiae*. The last 5 years have led to a tremendous increase in our insights into the structural basis of protein translocation through the elucidation of high-resolution crystal structures from individual components [13–17] and low- to medium-resolution electron microscopy (EM) structures of a variety of functional complexes [18–21]. These structural and biochemical data have yielded detailed insights into the molecular mechanism underlying protein translocation. The purpose of this review is to present an overview of our current understanding of the structural dynamics of the bacterial Sec machinery during protein translocation. We will focus on conformational changes that occur within the translocon, how they might be induced by (pre)proteins, the ribosome or SecA, and we will highlight major unresolved questions. Some of these issues have received considerable attention in reviews [2, 22–24], and therefore additional emphasis will be on two issues that have not been addressed extensively, that is variations that are observed between Sec machineries of different bacteria and the controversy concerning the oligomeric state(s) of the translocon and SecA during protein translocation.

IV. Variation and Evolution of the Sec Machinery

A. The Canonical Bacterial Sec Machinery

In addition to the motor protein SecA and the three translocon proteins (SecY, SecE, and SecG), the Sec machinery of the vast majority of bacteria consists of YidC, SecD, SecF, and YajC. YidC is involved in the insertion of

IMPs into the lipid bilayer by contacting the transmembrane segments of nascent IMPs shortly after they leave the SecYEG translocon [25]. In addition, YidC functions independently of SecYEG in the integration of small IMPs such as the F_oC subunit of ATP synthase and the bacteriophage coat protein M13 [26]. The mitochondrial YidC homologue Oxa1p from *S. cerevisiae* has been shown to directly interact with the ribosome [27, 28] but thus far ribosome binding has not been demonstrated for YidC, while the cytoplasmic domain of Oxa1p implied in ribosome binding is absent in YidC.

With the exception of some lactic acid bacteria, all completely sequenced bacterial genomes encode for the proteins SecD, SecF, and YajC. SecDFYajC forms a trimeric complex that is involved in protein translocation and associates with SecYEG [29, 30]. Two studies have indicated that SecDF might be both functionally and physically coupled to SecG [31, 32], but the exact function of SecDFYajC has remained elusive [33]. It has been proposed that SecDFYajC is involved in release of preproteins from the translocon, regulation of SecA cycling, and maintenance of the proton motive force. The latter proposal has been shown to be based on a polar effect of the growth conditions used with a SecDF depletion strain, rather than on the functional defects of the depletion of SecDF itself [34]. Further experiments are required to (dis)prove the other proposed functions of SecDF. In contrast to SecD and SecF, YajC is not required for cell viability. YajC alone has been shown to exist as a homooligomeric complex in the inner membrane of *E. coli* [35], but the functional importance of this complex is unknown.

B. Evolutionary History of the *E. coli* Sec Machinery

Although the most intensively studied bacterial Sec machinery is that from *E. coli*, some characteristics of this system are not representative for the vast majority of bacteria. There are at least three components that distinguish the *E. coli* Sec machinery from that of other bacteria: SecB, SecM, and SecE. The tetrameric cytoplasmic protein SecB is a secretion specific chaperone that prevents intracellular aggregation of (pre)proteins [36]. SecB slows down the folding of preproteins by binding to their mature region [37], and it targets them to the extreme C-terminus of SecYEG-bound SecA [38]. Once translocation of the preprotein has been initiated, SecB is released from the translocon and able to start a new targeting cycle [11]. SecB is not essential for cell viability [39], but it is thought to be required for translocation of a subset of (pre)proteins [40]. Thus far, no clear amino acid motifs have been identified that render (pre)proteins SecB dependent [41], but it has been shown that SecB-binding sites are enriched in aromatic and basic residues [42].

The second component that distinguishes *E. coli* from most other bacteria is SecM, a small regulatory protein (formerly known as gene X) that is encoded directly upstream of SecA [43]. Under secretion-deficient conditions, SecM induces a pause in translation of the *secM–secA* messenger RNA by means of an arrest sequence in its C-terminus [44] that is sensed by the interior of the ribosome [45]. This results in prolonged exposure of the SecA ribosome-binding site and consequently an upregulation of the amount of cellular SecA. In addition, SecM is involved in localizing the expression of SecA to the vicinity of SecYEG [46]. SecM contains a signal sequence at its N-terminus, and thus the ribosome carrying a *secM–secA* messenger and the arrested nascent chain is targeted to the translocon. The SecA molecules that are subsequently synthesized in the vicinity of SecYEG are more active in protein translocation than SecA molecules that are synthesized without a functional *secM* gene in *cis* [46]. This SecA population possibly corresponds to the ''membrane integral'' form of SecA [47, 48]. SecM is not required for cell viability provided that sufficient SecA is supplied *in trans* [43].

SecE is the third component that distinguishes *E. coli* from many other bacteria; *E. coli* SecE consists of three transmembrane segments (TMSs), whereas most of its homologues are single spanning membrane proteins [49]. The additional two TMSs might specifically facilitate protein translocation at low temperatures, since *E. coli* cells containing a variant of SecE lacking these two TMSs are cold sensitive for growth [50].

An extensive genome analysis has revealed that SecB, SecM, and SecE with three TMSs are not unique to *E. coli* as they are present in several other proteobacteria, but not in any other bacterial divisions [51]. It is tempting to speculate that an optimized Sec machinery could be particularly beneficial to the frequently pathogenic proteobacteria, but it should be noted that the microbial genome-sequencing projects are strongly biased toward pathogenic organisms in general. Interestingly, the genomic distribution of SecB, SecM, and SecE with three TMSs reveals a part of the evolutionary history of the *E. coli* Sec machinery. By combining the genomic distribution with the phylogenetic relationships between the proteobacterial subdivisions in which each component is present (Figure 2.1), it was revealed that the Sec machinery has most likely evolved in the following successive steps: within the proteobacteria, the canonical Sec machinery (containing only SecYEG, SecA, SecDFYajC, and YidC) was first supplemented with SecB, then SecE was extended with two TMSs, and finally SecM was introduced. Hence, the *E. coli* Sec machinery represents the end product of a stepwise evolutionary process. Intermediate compositions with only SecB or SecB in combination with a three TMS-containing SecE are also observed, but neither the extended SecE nor SecM is ever observed without SecB, and SecM is

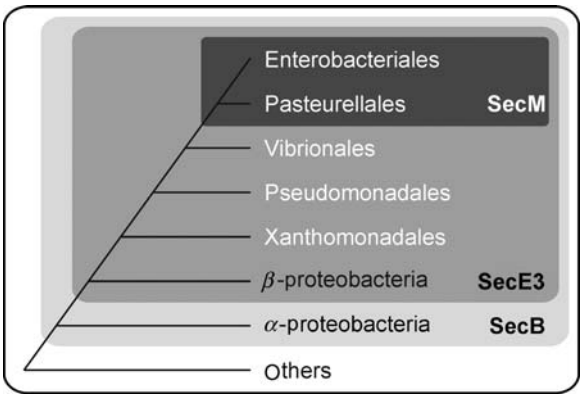

FIG. 2.1. Genomic distribution of accessory features of the Sec machinery in proteobacteria in combination with bacterial phylogeny. The distribution suggests that the Sec machinery has evolved in a stepwise fashion by sequentially acquiring SecB, the SecE extension, and SecM [51].

never observed without extended SecE. It has been proposed that both SecE with three TMSs and SecM could specifically improve SecB-dependent protein translocation by maximizing the amount of SecYEG-bound SecA that forms the receptor for preprotein–SecB complexes. This can be accomplished in two ways: (1) by increasing the affinity of SecA for SecYEG (via SecE) or (2) by carefully regulating and localizing the expression of SecA (via SecM) [51]. Further biochemical studies are required to investigate the possible synergistic contribution of SecB, SecM, and extended SecE to protein translocation. What should be kept in mind is that the Sec machinery of the model organism *E. coli* is of much greater complexity than that of most other bacteria.

C. SEC PARALOGUES

Noncanonical compositions of the Sec machinery-containing paralogues of one or more components are also observed in many bacteria. Several genomes of organisms belonging to the divisions Actinobacteria (e.g., *Mycobacterium tuberculosis*) [52] and Firmicutes (e.g., *Listeria monocytogenes* and *Streptococcus gordonii*) [53, 54] encode for paralogues of SecA, and few of those bacteria encode for paralogues of SecY, SecE, and/or SecG as well. The genomes of the proteobacteria *Gluconobacter oxydans* and *Francisella tularensis* encode for SecB paralogues [51]. The genomic distribution of these paralogues has not yet been investigated in an

evolutionary context, and SecA2 is the only paralogue that has been studied genetically. It has been shown in both *M. tuberculosis* [55] and in *L. monocytogenes* [56] that SecA2 is important for pathogenicity but not for viability. These observations have led to the speculation that the accessory Sec machinery components of these Gram-positive bacteria might be functional equivalents of the pathogenicity related Type II–IV secretion systems found in many Gram-negative bacteria [56]. The thus far identified SecA2-dependent substrates do not have any functional characteristics in common. However, several substrates contain an atypical signal sequence or become glycosylated before translocation [56–62]. Interestingly, some SecA2-dependent substrates do not contain a signal sequence at all [55, 56]. It will be of great interest to investigate these and other features that distinguish SecA2 and the other paralogues from the canonical Sec machinery.

V. SecA Structure, Function, and Dynamics

A. The Involvement of SecA in Cotranslational Protein Translocation

The motor protein SecA is one of the largest and most complex bacterial proteins. It consists of multiple domains and it interacts with nearly all the other components involved in protein translocation: (pre)proteins, SecYEG, SecB, nucleotides, the cytoplasmic membrane, and possibly the ribosome. Although co- and posttranslational translocation reactions are mostly studied as individual pathways in *S. cerevisiae* and *E. coli*, respectively, there are several indications that the two pathways overlap. Most IMPs are translocated cotranslationally, but several IMPs contain large extracytoplasmic domains that are translocated in a SecA-dependent manner [25, 63–66]. This implies that SecA and the ribosome can either bind to the translocon simultaneously or that they can bind alternating to the translocon. Although simultaneous binding of SecA and the ribosome to SecYEG is structurally difficult to envisage (see Sections 15.4 and 17), it has been shown that ribosomes and SecA do not compete for binding to SecYEG [67]. In addition, it has been demonstrated that SecA has a low but intrinsic ribosome-binding capacity, either alone [68, 69] or in conjunction with SecYEG [67]. Interestingly, ATP hydrolysis by SecA appears to induce the release of the ribosome from the translocon [67]. In this context, it should be stressed that during translocation of a large extracytoplasmic domain of an IMP by SecA, the ribosome would remain tethered to the translocon via the nascent chain rather than being truly released. The latter

would favor rebinding of the ribosome to the translocon for cotranslational continuation of the translocation process. Taken together, the co- and posttranslational protein translocation pathways are likely to be intertwined. Therefore, *in vitro* membrane protein insertion studies with SecA-dependent membrane proteins of varying topologies are eagerly awaited to further unravel this intricate process. In particular, special attention should be paid to the role of YidC and SecDFyajC during membrane insertion of SecA-dependent IMPs.

B. The Overall Mechanism of Posttranslational Protein Translocation

In contrast to its possible role in cotranslational protein translocation, the role of SecA in posttranslational translocation is understood in much more detail due to extensive biochemical studies with purified components. This has resulted in the following widely accepted working model (Figure 2.2): In SecB-containing organisms, the cycle of posttranslational translocation starts with binding of a (pre)protein–SecB complex to SecYEG-bound SecA [11], on which the preprotein is transferred to SecA [70]. In organisms lacking SecB, the preproteins either bind directly to SecYEG-bound SecA, or are targeted to the translocon via binding to cytoplasmic or lipid-bound SecA. The subsequent binding of ATP to SecYEG-bound SecA induces a conformational change that results in insertion of the signal sequence into the translocon, and release of SecB (if present). At the same time, SecA is thought to insert partially into the translocon [71], and around 2.5 kDa of the mature domain of the preprotein is translocated [72, 73]. ATP hydrolysis results in release of the (pre)protein

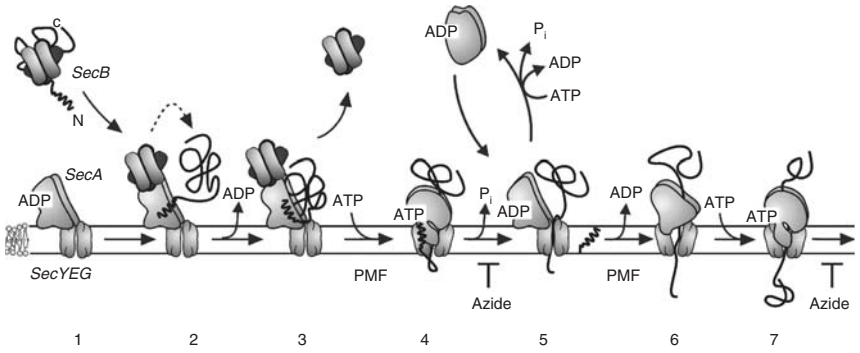

Fig. 2.2. Schematic representation of posttranslational protein translocation in *E. coli*. See text for details.

from SecA and deinsertion of SecA from the translocon, in a step that can be inhibited by the commonly used antibacterial compound azide [74]. Next, rebinding of SecA to the partially translocated polypeptide chain can drive the translocation of another 2.5 kDa of the mature preprotein domain [72, 73]. Depending on the length of the (pre)protein, multiple cycles of ATP binding and hydrolysis and SecA binding and release are required to completely translocate the substrate across the membrane.

C. Structure of the SecA Protomer

The working model described above is still rather abstract, but our insight into the molecular details of the mechanism has become increasingly clear due to the availability of crystal structures from SecA [13, 17, 75], SecB [14, 15, 76], and an archaeal SecYEG homologue [16]. Three different crystal structures of SecA are available, two from *B. subtilis* and one from *M. tuberculosis*. The actual motor function of SecA, that is conversion of chemical energy into movement, is initiated by a ''DEAD motor'' core that is also present in DNA/RNA helicases [77]. The DEAD motor consists of two similarly folded domains that are referred to as nucleotide-binding folds (NBF1 and NBF2), each resembling the recombination protein RecA. At the interface of these two domains a single ATP molecule can be bound and hydrolyzed, which induces the conformational changes in SecA that ultimately results in the translocation of preproteins. SecA interacts with preproteins via the preprotein-binding domain (PBD, also referred to as ''preprotein cross-linking domain'' (PPXD) [78]) that is inserted into the amino acid sequence of NBF1 (Figure 2.3A), but forms a separate domain in the SecA structure [79, 13] (Figure 2.3B). The remainder of the SecA structure can be subdivided into four regions: the helical scaffold domain (HSD), the helical wing domain (HWD), the C-terminal linker (CTL), and the SecB-binding domain ''SecAc.'' The HSD forms a long scaffold to which NBF1, NBF2, the PBD, and the HWD are connected, the HWD is a loosely attached domain with unknown function, and the CTL forms the connection with SecAc at the extreme C-terminus [13] (Figure 2.3A and B).

1. Oligomeric State of SecA

In order to understand the working mechanism of any protein on a molecular level, it is not only essential to know its structure and the exact location of the interaction sites for all its ligands but also to elucidate the functional oligomeric state of the protein itself. The oligomeric state of both SecA and SecYEG during protein translocation has become a controversial

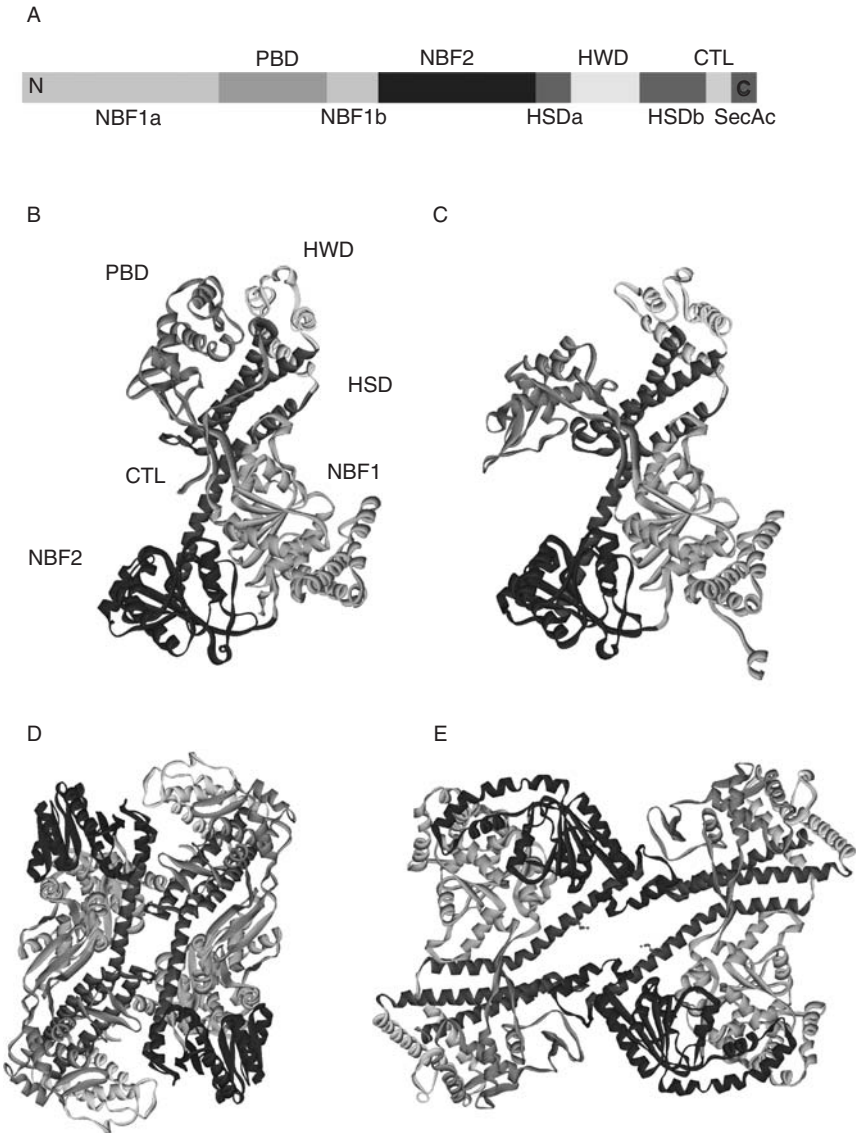

FIG. 2.3. Structure of SecA. (A) Schematic overview of the domain structure of SecA. NBF: nucleotide-binding fold; PBD: preprotein-binding domain; HSD: helical scaffold domain; HWD: helical wing domain; CTL: C-terminal linker; SecAc: SecB-binding motif. (B) Crystal structure of SecA protomer from *B. subtilis* with individual domains colored as in (A) [13]. (C) Crystal structure of SecA from *B. subtilis* in an open conformation, possibly representing the (pre)protein-bound state [75]. The conformational changes with respect to the structure

topic, and the complexity of the matter is schematically depicted in Figure 2.4. In an attempt to enlighten both discussions, we will address the topics individually, starting with SecA. For clarity, we have grouped the experimental data according to the following three subquestions:

1. What is the oligomeric state of soluble SecA?
2. What is the oligomeric state of SecYEG-bound SecA?
3. What is the oligomeric state of translocation-engaged SecA?

2. *The Oligomeric State of Soluble SecA*

It has been shown with various techniques that purified SecA exists in a dynamic equilibrium between a monomeric and a dimeric form, and the dissociation constant (K_D) has been estimated to be around 0.1 μM under physiological conditions [80]. The cellular concentration of SecA is $\sim$8 μM [81], and thus SecA is expected to be largely dimeric *in vivo*. Higher order SecA oligomers have also been reported, but only under nonphysiological conditions or with truncated SecA mutants [17, 82]. Three reports have shown that translocation ligands can induce monomerization of SecA dimers, which raises the question whether the cellular predominant SecA dimer is also the functional state. Fluorescence- and cross-linking studies with purified SecA have shown that the monomer–dimer equilibrium can be shifted toward the monomer by the addition of certain lipids or detergents [83, 84], or signal peptides [83, 85], although a different view has been

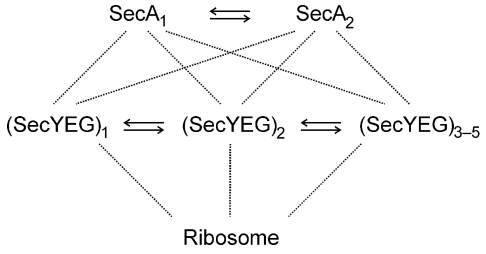

FIG. 2.4. Schematic overview depicting the complexity of the debate concerning the oligomeric states of SecA and SecYEG. The experimentally demonstrated equilibria between the different oligomeric states are indicated by arrows, and all the possible interactions are indicated by dashed lines. See text for details.

depicted in (B) are indicated by arrows. (D) Crystal structure of dimeric SecA from *B. subtilis* that most likely represents the physiologically active dimer [13]. The two intradimeric HSD–HSD contacts that are maintained during protein translocation are depicted in red [95]. (E) Crystal structure of *M. tuberculosis* SecA. (See color plate.)

published, suggesting that signal peptides induce oligomerization of SecA [84]. Lipid-bound SecA has been shown to exist mainly in a dimeric form that can be dissociated on binding of nucleotides [86]. Although all these studies underscore the dynamic and sensitive nature of the SecA monomer–dimer equilibrium, it remains questionable whether any of these observed changes in oligomeric state are functionally relevant since no SecYEG nor (pre)proteins were present in these studies.

3. The Oligomeric State of SecYEG-Bound SecA

The oligomeric state of SecA while bound to SecYEG detergent solution has been addressed by native gel electrophoresis and gel filtration [87–89]. It was shown that both monomeric and dimeric SecA can bind to SecYEG, provided that SecYEG is stabilized in a dimeric form either by covalent linkage [88] or by an antibody [89]. Unstabilized SecYEG in detergent only retains monomeric SecA after a preprotein has been trapped inside the channel before solubilization of the membrane [88]. These results should be interpreted carefully, however, since the monomer–dimer equilibrium of SecA has been shown to be highly sensitive to detergents [83].

The oligomeric state of SecA bound to membrane-embedded SecYEG has been addressed by chemical cross-linking [90] and surface plasmon resonance (SPR) [91]. Dimeric SecA can be detected after binding to urea-treated inverted membrane vesicles (IMVs) [90], but could not be detected with SecYEG-containing proteoliposomes [83]. The concentration of SecA added in the latter experiment however was far below physiological (5 nM vs 8 μM), and thus the results obtained with the IMVs appear to be more reliable. Chemical cross-linking of the population of SecA that copurifies with IMVs revealed mainly SecA monomers [90], while the fraction of SDS-resistant dimers dramatically increases on overexpression of SecYEG [91]. SPR measurements also suggest that SecA is dimeric while it is bound to membrane-embedded SecYEG, since wild-type SecA binds to SecYEG-overexpressing IMVs similarly to a covalently cross-linked SecA dimer [91]. Taken together, these data indicate that both monomeric and dimeric SecA can bind to SecYEG.

4. The Oligomeric State of Translocation-Engaged SecA

Activity assays are obviously the most relevant experiments to assess the oligomeric state of SecA during protein translocation. In order to investigate the functional requirement of dimeric SecA, several studies have characterized SecA mutants with disturbed dimerization properties. Removal of the N-terminal eight amino acids of SecA does not influence its oligomeric state [92], but SecA has been reported to be predominantly monomeric when

the first 11 amino acids are removed [90, 93, 94]. Alternatively, monomeric SecA can be obtained by mutating 6 residues in the C-terminal region of a SecA truncate that lacks 70 residues from its extreme C-terminus [83]. It should be noted that these monomeric SecA mutants are not incapable of dimerization per se, as the mutations have shifted the monomer–dimer equilibrium in solution substantially toward the monomeric state [93]. In the two assays that measure SecA activity, for example the *in vitro* preprotein translocation assay and the precursor-stimulated SecA ATPase assay, all monomeric SecA mutants show either a very low activity or no activity at all. Although the low residual *in vitro* activity has been interpreted as being significant in some reports [83, 94], it seems more likely that the residual activity is caused by a small fraction of SecA dimers that can still be formed or by traces of copurified wild-type SecA from the expression host.

Activity assays with covalently dimerized SecA have yielded varying results. SecA dimers cross-linked via endogenous cysteines located in the SecB-binding domain (SecAc) [91] or via a pair of engineered cysteines in the HSD (Arg^{637} and Gln^{801}) [95] were shown to be nearly fully active in protein translocation and preprotein-stimulated SecA ATPase activity. Although these observations alone do not directly imply that SecA functions as a dimer, it does show that monomerization is not required for functionality as proposed earlier [83].

Perhaps the most convincing experiment that assesses the functional oligomeric state of SecA-involved heterodimers of active and inactive SecA monomers [96]. If SecA would function as a monomer, these heterodimers are expected to have half the activity of wild-type SecA. However, it was observed that these heterodimers are completely inactive, strongly suggesting that SecA is functional as a dimer.

5. Summary of Oligomeric States SecA

Taken together from our point of view, the experimental data showing that SecA dimers dissociate on binding of translocation ligands are not necessarily related to protein translocation, since they might simply reflect the sensitive nature of the monomer–dimer equilibrium. The data supporting the proposal that SecA functions as a monomer are in our opinion either; obtained under conditions too distant from physiological; explainable by a conformational change of SecA, or misinterpreted. On the other hand, the experimental data supporting the SecA dimer as a functional unit are more convincing and more abundant. Furthermore, there are no experimental data disproving a functional SecA dimer, whereas *in vivo* and *in vitro* experiments in different laboratories demonstrate that monomeric

SecA variants are inactive. Finally, it has been shown that SecB targets preproteins to dimeric SecA, and that this targeting greatly stimulates the efficiency of protein translocation [38]. Combined with the notion that cellular SecA is predominantly dimeric, we assume that SecA functions as a dimer in posttranslational protein translocation at SecYEG.

We speculate that the physiological relevance of the binding of monomeric SecA to SecYEG and the sensitive nature of the monomer–dimer equilibrium could be related to (pre)protein targeting to SecYEG. As mentioned above, in organisms lacking SecB, (pre)proteins might first bind to cytoplasmic or lipid-bound SecA, and subsequently transferred to the translocon. If one SecA protomer would remain permanently bound to SecYEG, the dimerization of SecA could play a role in the initiation of translocation via this SecB-independent targeting process.

D. Structure of the Functional SecA Dimer

With our current insight that SecA functions as a dimer, the next question is at which side of a SecA protomer the intradimeric interactions take place. Two interactions observed in various crystal structures have been proposed to represent a physiological dimer interface [13, 17]. The overall arrangement of both of these SecA dimers is very similar; the two elongated SecA monomers are arranged side-by-side in an antiparallel fashion (Figure 2.3D and E). This antiparallel arrangement is supported by fluorescence resonance energy transfer (FRET) [97] and cross-linking studies [94, 95]. The difference between both dimers lies in the SecA surface that contacts the neighboring protomer. The proposed *B. subtilis* dimer is relatively compact and the dimer interface comprises a large surface (5442 $Å^2$) [13], whereas the *M. tuberculosis* dimer is relatively flat, comprises a smaller surface (2822 $Å^2$), and contains a cavity at the dimer interface [17]. One dimer arrangement can be converted into the other by rotating each protomer $\sim 75^\circ$ around its long axis. Although it is conceivable that such rotations could play a role in the cycle of SecA-driven protein translocation, the observation that a SecA dimer that is fixed in the *B. subtilis* arrangement (Figure 2.3D) still supports efficient protein translocation [95] suggests that at least the *B. subtilis* dimer is part of the conformational cycle of SecA. Thus, it can be concluded that the HSDs of two SecA protomers can be considered as a single scaffold domain in the SecA dimer, and that none of the conformational changes that SecA undergoes during protein translocation is severely hampered by the intradimeric HSD–HSD cross-links. Whether the *M. tuberculosis* dimer arrangement (Figure 2.3E) also represents a functional intermediate remains to be established.

E. Conformational Changes Within SecA

Several regions in SecA have been shown to be dynamic [13, 98–105], but detailed structural information is only available on two conformational changes: one that can be inferred from SecA's similarity to helicases and another that has been observed directly with X-ray crystallography [75]. As mentioned previously, the DEAD-motor core of SecA (NBF1 and NBF2) is homologous to that of SFI and SFII helicases, and therefore the nucleotide-induced conformational changes are assumed to be similar in all three protein families. SecA has been crystallized with bound ADP and in the nucleotide free state, but these structures differ only slightly in the orientation of side chains that are involved in nucleotide binding. Unfortunately, attempts to crystallize SecA in the functionally important ATP-bound state have failed thus far. In addition to conformations that are very similar to those of nucleotide free and ADP-bound SecA, the helicase DEAD motors have been crystallized in two substantially different conformations. First, the SFII helicase MJ0669 has been crystallized without nucleotides in an open conformation in which the two NBFs are separated from each other by a large cleft [106]. Second, the SFI helicase PcrA has been crystallized in the ATP-bound state in which the two NBFs have undergone an $\sim 10^{\circ}$ rotation relative to each other compared to the ADP-bound state [107]. All three distinct conformations as observed in different DEAD motors (open, closed, and closed-rotated) are assumed to underlie the ATPase cycle of SecA as well. Given the observation that a SecA dimer in which the two HSDs are cross-linked is still active, the relative reorientations of NBF1 and NBF2 that are required for ATP binding and hydrolysis are apparently not influenced by these disulfide-bonded cross-links. When the mobility of NBF1 is restricted by a disulfide cross-link to the HWD of the neighboring protomer however, the SecA dimer is inactive [95].

The conformational change of SecA that has been visualized by X-ray crystallography does not involve the DEAD-motor or nucleotide, and it takes place in the opposite end of a SecA protomer [75]. *B. subtilis* SecA has been crystallized in two different conformations, and a comparison of both conformations reveals the following movements in a protomer: the HSD and HWD undergo a small rotation, and the PBD undergoes a large ($\sim 60^{\circ}$) rotation combined with a rigid body translation away from the HSD and HWD (Figure 2.3B and C). This results in opening of a groove at the PBD–HSD/HWD interface (Figure 2.3C) that has been proposed to form the actual preprotein-binding site since its physicochemical characteristics are similar to that of peptide-binding sites from other proteins with broad substrate specificities. Assuming that this conformation of SecA represents a (pre)protein-bound state and knowing that *B. subtilis* does not contain a SecB protein

it could represent either a SecYEG-bound form, a lipid-bound form, or a soluble form. In the latter two cases, it might represent the earlier proposed (monomeric) form of SecA that was suggested to be involved in SecB-independent targeting of (pre)proteins to a SecYEG-bound protomer. As at present it is unclear whether the observed conformational changes can take place in the *B. subtilis* dimer arrangement, the conformation in the crystal structure could also represent (one of) the SecYEG-bound SecA protomer(s) after receiving a (pre)protein. The location of the CTL that connects the SecB-binding domain SecAc to the HWD suggests how binding of a SecB-(pre)protein complex could be mechanistically coupled to the conformational change in SecA (see Section 12).

F. SecA–SecB Interaction

The interaction between SecA and SecB has been investigated in great detail. Since an excellent review on the SecA–SecB interaction has appeared [108], we will only discuss the most important findings and a possible relation to conformational changes in SecA. It has been shown that the extreme C-terminus of SecA (SecAc) contains a dedicated SecB-binding site that is formed by a small cysteine-rich domain that chelates a zinc ion [109]. This highly conserved domain is also found in organisms lacking SecB, which might be related to the fact that the C-terminus is also involved in lipid binding [110]. The SecAc domain is not resolved in any of the available SecA crystal structures, but its structure has been determined in isolation by NMR [111, 112] and in complex with *Haemophilus influenzae* SecB by X-ray crystallography [15]. The latter structure revealed that two SecAc domains are bound to opposite sides of one SecB tetramer, on a surface that was previously shown to be crucial for SecB-binding to SecA [70, 113]. The SecAc domain is stabilized by the zinc ion that is coordinated by three cysteines and one histidine, explaining why SecA mutants in which these residues are either mutated [114] or cross-linked [91] are unable to support SecB-dependent protein translocation.

The approximate position of the SecB tetramer bound to SecA in the *B. subtilis* dimer arrangement has been estimated by docking of the SecB–SecAc complex onto the SecA structure [108]. It seems likely however that on binding of a preprotein–SecB complex to SecA, the transfer of the (pre)protein requires (or induces) a substantial conformational change in SecA [70]. This conformational change possibly corresponds to the one that is observed by X-ray crystallography [75]. Binding of SecB to the highly mobile SecAc domain could displace the CTL that connects SecAc to the HWD. Since the CTL is part of the PBD-hinge region in the closed conformation of SecA and it meanders partially underneath the PBD

(Figure 2.3B), this displacement could directly induce the observed rigid body movement of the PBD that results in opening of the proposed (pre) protein-binding groove (Figure 2.3C). Furthermore, CTL displacement could be directly responsible for the small rotation of the HWD/HSD that coincides with opening of the groove. Although *B. subtilis* does not contain SecB, it has been shown that *E. coli* SecA undergoes a similar conformational change [75]. In organisms lacking SecB, displacement of the CTL is expected to be induced by an alternative mechanism. This could involve the interaction of SecAc with lipids [110] or binding of SecA to SecYEG [115].

G. SecA–Membrane Interaction

A detailed understanding of SecA binding to the membrane is fundamental for understanding the molecular mechanism of SecA-driven protein translocation. However, whereas binding of SecA to *E. coli* membranes has been studied extensively, surprisingly little is known about the region(s) of SecA that interact(s) with the membrane. The lipid-binding region of SecA has been localized to its C-terminal 70 amino acids [110], but the SecYEG-binding region of SecA has not been identified in detail. Far western experiments using SecA fragments mapped the SecYEG-binding region to the N-terminal part of the SecA protomer, comprising both NBFs and the PBD [116]. Moreover, binding experiments with SecA fragments have demonstrated that the same N-terminal region of SecA comprises the high-affinity SecYEG-binding site, whereas the remaining C-terminal one-third of SecA does not bind to SecYEG [116]. However, the exact SecYEG interaction sites within the N-terminal region have not been determined yet. The relatively new technique of cysteine-directed cross-linking in combination with mass spectrometry appears to be the most suitable biochemical approach to identify the exact regions in SecA that interact with SecYEG. In addition, medium- and high-resolution structural studies on SecYEG–SecA complexes will contribute to answering this critical question.

VI. SecYEG Structure, Function, and Dynamics

A. Structure of the SecYEG Protomer

The structure–function relationship of the translocon has been extensively studied in *E. coli* and *S. cerevisiae*. The recently solved high-resolution translocon structure from the archaeon *Methanococcus jannaschii* [16] was a major breakthrough in the field. Despite the fact that archaeal translocon subunits are more similar to eukaryotic than to bacterial ones [3], they are

commonly named after the bacterial subunits. Since no significant sequence similarity can be detected between SecG and its archaeal counterpart Sec(61)β [117], the eukaryotic nomenclature is applied to the latter, resulting in the hybrid term SecYEβ. In agreement with its universal conservation, the overall structure of *M. jannaschii* SecYEβ is nearly identical to that of *E. coli* SecYEG [118]. The two complexes differ only slightly in conformation [119], and the *E. coli* translocon contains three additional TMSs compared to that from *M. jannaschii*: two from SecE (Section IV) and one from SecG. The center of the complex is formed by SecY, whereas SecE and SecG are located at the periphery (Figure 2.5A and B). The structure of

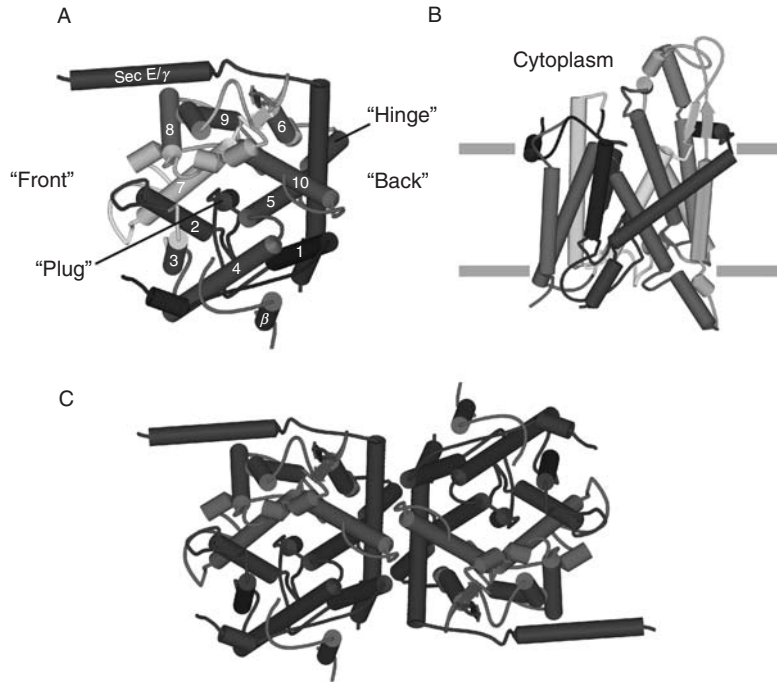

FIG. 2.5. Structure of SecYEβ from *M. jannaschii* [16]. (A) Cytoplasmic view showing the arrangement of transmembrane segments in different colors. SecE is depicted in purple, Secβ in pink. Sides referred to as ''front'' and ''back'' are indicated. (B) View from within the plane of the membrane showing the two cytoplasmic loops that extend into the cytoplasm and have been shown to interact with the ribosome and SecA: C4 and C5, connecting TMS6 with TMS7 and TMS8 with TMS9, respectively. (C) Back-to-back dimer arrangement of SecYEβ protomers as observed for *E. coli* SecYEG in two-dimensional crystals [118]. The N-terminal halves of SecY are depicted in blue, the C-terminal halves in red, and SecE and Secβ in purple and pink, respectively. (See color plate.)

M. jannaschii SecYEβ consists of two distinct domains that are similarly folded. Each domain is composed of a bundle of five TMSs, formed by the N- or C-terminal half of the SecY sequence, respectively. The two halves of SecY are held together by SecE: the conserved TMS of SecE crosses the membrane diagonally [120], and contacts both SecY halves at the same side where they are connected by the extracytoplasmic loop between TMS5 and TMS6. This side of the SecYEG protomer is referred to as the ''back.'' The amphipathic cytoplasmic helix of SecE [121] runs parallel to the membrane surface along the C-terminal half of SecY (Figure 2.5A). Two of the cytoplasmic loops of SecY protrude far into the cytoplasm: the C4 loop connecting TMS6 with TMS7, and the C5 loop connecting TMS8 with TMS9 (Figure 2.5B). The extracytoplasmic loops on average are considerably shorter, and two of those fold back into the membrane region: the E4 loop connecting TMS7 with TMS8, and the E1 loop connecting TMS1 with TMS2. The latter is highly conserved, folds back between the two SecY halves, and is referred to as the ''plug'' domain [16].

At first sight, there is no obvious region in the channel that is large enough to allow passage of unfolded proteins. For this reason, it has been concluded that the structure represents the closed conformation of SecYEβ. However, on the basis of two domain structure of the channel and the observation that signal sequences of (pre)proteins can be cross-linked to TMS2 and TMS7 [122–124] at the domain interface, it was proposed that insertion of the signal sequence between TM2 and TM7 results in separation of the two halves of SecY and displacement of the ''plug'' that blocks the proposed pore from the extracellular side and that the substrates pass through the center of the channel [16]. Molecular dynamics simulations have revealed that the opening that is created by this mechanism is indeed large enough to allow passage of unfolded and even α-helical proteins [125]. In the opened state, nascent IMPs (and signal sequences) could leave the translocon laterally toward the lipid bilayer via the TMS2–TMS7 interface. The possible mechanisms by which SecA or the ribosome could induce channel opening will be discussed below, but first we will address the oligomeric state(s) of the translocon.

1. Oligomeric States of SecYEG

As outlined above for SecA, knowledge of the functional oligomeric state of a protein is of fundamental importance for understanding its mechanism of action. Also the oligomeric state of SecYEG is heavily debated (Figure 2.4). In an attempt to enlighten this discussion, we will give an overview of the relevant experimental data. For clarity, we have

subdivided the assessment of the oligomeric state of SecYEG into three subquestions:

1. What is the oligomeric state of SecYEG in the absence of ligands?
2. What is the oligomeric state of SecYEG with bound SecA?
3. What is the oligomeric state of SecYEG with a bound ribosome?

2. Oligomeric State of SecYEG in Absence of Ligands

The oligomeric state of SecYEG in the absence of ligands has been addressed with several cross-linking studies and fluorescence resonance energy transfer (FRET). All these studies indicate that at least two copies of SecY [126–128], SecE [129, 130], and SecG [131] are present in a single complex. However, whether such an oligomeric complex contains two or more copies of each subunit can not be distinguished. More accurate information on the oligomeric state of purified SecYEG has been obtained in detergent solution by density centrifugation [132], analytical ultracentrifugation [133], gel filtration [89], native gel electrophoresis [87], and negative stain EM [132, 134, 135]. Several of these studies indicate that SecYEG exists in a dynamic equilibrium between monomers, dimers, and larger oligomers. The latter group includes presumed trimers, tetramers, and pentamers. Similar results were obtained with SecYEG reconstituted into lipid bilayers [130].

The observation of trimeric/tetrameric purified SecYEG complexes, per se, does not necessarily imply that these oligomeric states are also functionally relevant. Concerning this aspect three critical comments should be given. First, most of the experimental conditions that addressed the oligomeric state of SecYEG involve high concentrations of (overexpressed) SecYEG, and these might lead to nonphysiological distributions of the oligomeric states [136]. Second, the removal of SecYEG from a potential ''supercomplex'' with SecDFYajC and/or YidC in the membrane [29, 33, 137] might expose surfaces on SecYEG that in absence of these subunits could form an interaction site for self-association. Third and most importantly, the oligomeric state of SecYEG during protein translocation, that is with bound ligands, might differ from that in a ''resting'' state.

3. Oligomeric State of SecYEG with Bound SecA

SecA has been shown to bind to both dimeric [88, 89] and tetrameric SecYEG [130, 134], but not to SecYEG monomers [88, 89]. Binding of SecA induces a shift in the SecYEG equilibrium, both in detergent solution [89] and in lipid bilayers [130]. In addition (membrane insertion of) SecA has been shown to increase the amount of SecYEG dimers and proposed

tetramers at the expense of SecYEG monomers [130, 134]. Constitutive SecYEG dimers that were created by covalent linkage [88] (N. Nouwen, unpublished data) or via disulfide cross-linking [127] were shown to be active in posttranslational protein translocation. Taken together, all these data indicate that in contrast to an earlier proposal [138] SecYEG functions in posttranslational translocation as an oligomeric complex. The exact oligomeric state however is difficult to assess, as pro- and contraarguments can be given for both dimers and higher order oligomers.

4. Oligomeric State of SecYEG with a Bound Ribosome

The oligomeric state of the translocon is not necessarily the same during the post- and cotranslational translocation modes. The oligomeric state of the translocon during cotranslational translocation has been studied in both bacteria and eukarya, mainly by EM. Early EM studies of rough ER membranes revealed the existence of large ringlike particles that were estimated to contain three to four translocons [135]. Importantly, the formation of these particles from purified and membrane-reconstituted translocons was induced by the addition of ribosomes. Several subsequent cryo-EM studies on eukaryotic ribosome-bound translocons revealed that irrespective of the presence of an arrested nascent chain, similarly sized particles bind to ribosomes [18, 20, 21, 139, 140, 141]. Recently, however, a cryo-EM reconstruction of an *E. coli* ribosome-bound translocon was presented that was estimated to consist of only two SecYEG protomers, despite the fact that the overall size of this translocon is similar to the other reconstructions [19]. Given the universal conservation of cotranslational protein translocation and the observation that the ribosome–translocon interaction is conserved across the three domains of life [142], it seems unlikely that this difference reflects a property that distinguishes the bacterial translocon from its eukaryotic counterparts. A conclusive assessment of the oligomeric state of the ribosome-bound translocon is limited by the medium resolution of the currently available cryo-EM structures.

5. Summary Oligomeric States SecYEG

Taken together, the oligomeric state of SecYEG during both co- and posttranslational protein translocation is at least dimeric, but the exact number of protomers constituting an active translocon remains controversial. Biochemical data assessing the oligomeric state of SecYEG during cotranslational translocation in particular and higher resolution three-dimensional structures of ribosome-bound translocons are eagerly awaited to resolve this critical issue.

C. Arrangement of SecYEG Protomers Within an Oligomeric Assembly

Since the oligomeric state of SecYEG during both co- and posttranslational translocation is at least dimeric, it is relevant to assess the arrangement of SecYEG protomers within a dimeric assembly. By fitting the high-resolution structure of *M. jannaschii* SecYEβ into a previously solved three-dimensional reconstruction of *E. coli* SecYEG based on two-dimensional crystals [118], it was revealed that the conserved TMS of SecE is located at the dimer interface. Several cross-linking studies showed a similar localization of SecE in SecYEG complexes within *E. coli* inner membrane vesicles [129]. Importantly, several covalent linkages of constitutive SecY dimers that do not interfere with activity [88, 127] span the same dimer interface, suggesting that this so-called back-to-back arrangement could represent a physiological SecYEG dimer. Tetrameric assemblies of SecYEG have been proposed to consist of two back-to-back dimers arranged side-by-side (a dimer of dimers) [140], such that SecG and the amphipathic helix of SecE are located at the interface of the two dimers. However, this specific tetrameric arrangement is not supported by structural data, while SecG-dependent tetramerization is only supported by scarce biochemical evidence [87].

On the basis of cryo-EM reconstruction of ribosome-bound *E. coli* SecYEG, a radically different dimer arrangement of SecYEG protomers was proposed [19]. For generation of stable RNCs, the SecM translation arrest sequence was used and the complex that was isolated consists of the 70S ribosome (50S and 30S subunit) carrying a nascent single-spanning membrane protein, mRNA, three tRNAs, and two translocons. One of the translocons is bound to the arrested nascent chain at the polypeptide exit tunnel as observed in previous studies, but the other is bound to the mRNA via an interaction that is most likely nonphysiological. On the basis of normal mode flexible fitting (NMFF) of SecYEG into the observed electron densities, it was proposed that the two translocons represent SecYEG dimers in a front-to-front arrangement in an open and a closed conformation, respectively (Figure 2.6B and A). Importantly, these analyses suggested that the conformational change underlying opening of the channel indeed involves separation of the two SecY halves. Prominent electron density that most likely corresponds to the arrested nascent chain was observed at the TMS2–TMS7 interface of the two neighboring SecY molecules (black cross in Figure 2.6B), rather than at the TMS2–TMS7 interface of a single SecY. This led the authors to propose that after being inserted into a single SecYEG protomer at the interface of the two SecY halves, nascent membrane proteins leave the translocon laterally via the

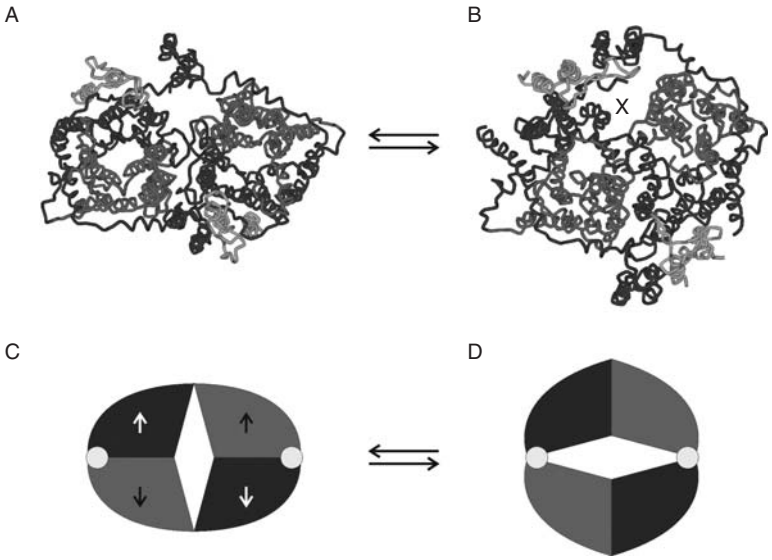

FIG. 2.6. Front-to-front dimer arrangements of *E. coli* SecYEG [19]. (A) Closed conformation of the front-to-front dimer, nonphysiologically bound to mRNA in the cryo-EM structure. (B) Open conformation of the front-to-front dimer bound to the arrested nascent chain at the ribosomal exit tunnel. The black cross indicates the position of the electron density that possibly corresponds to the arrested nascent chain. In (A) and (B), the N-terminal halves of SecY are depicted in blue, the C-terminal halves in red, SecE in pink, and SecG in green. (C) and (D) Schematic representation of the proposed ribosome/SecA-induced opening mechanism. A simultaneous interaction of the ribosome or SecA with the N-terminal (blue) and C-terminal (red) domain of one or two SecY molecules could induce opening of the translocon via outward directed forces. The proposed hinge region (loop E3 connecting TMS5 and TMS6) is represented by yellow circles, the proposed outward directed forces are indicated by arrows. The large clefts within both states of the translocon are merely for illustrative purposes. (See color plate.)

interface of two SecY molecules. Furthermore, the front-to-front arrangement will allow the formation of a large consolidated pore that could be required for hairpin insertion of (pre)proteins and/or translocation of substrates containing bulky side chains or internal disulfide bonds [143, 144]. Although other cryo-EM studies consistently indicated translocon oligomeric states of a higher order than dimers and a front-to-front arrangement of protomers was unanticipated, the proposed model provides many explanations for previously obtained biochemical results. Future biochemical and structural studies are required to experimentally validate the proposed front-to-front model.

D. Induction of Conformational Changes in SecYEG

Assuming that the proposed open conformation of dimeric SecYEG represents a physiologically active translocon, the question is how the ribosome or SecA can induce opening of the channel. Interestingly, the ribosome and SecA interact with similar regions of the translocon, suggesting that they might share a common opening mechanism. The ribosome interacts with the translocon via three distinct connections. In agreement with biochemical studies [145, 146], two connections are similarly formed by the pairs of long cytoplasmic loops of SecY (C4 and C5, connecting TMS6 with TMS7 and TMS8 with TMS9, respectively, black arrows in Figure 2.6C). The third connection is mediated by the cytoplasmic loop of SecG and the N-terminus two transmembrane segments of SecE (one of the white arrows in Figure 2.6C). SecA has been shown to interact with the C5 loop of SecY as well (EvdS *et al*. submitted for publication), with SecG [147], and with the interface between TMS4 and C3 of SecY (EvdS *et al*. submitted for publication) that is in direct contact with SecG [127]. Importantly, the two regions of interaction are located in different domains of a single SecYEG protomer, and thus separation of the two SecY domains could be induced by a simultaneous interaction with both of them (Figure 2.6C and D). In the front-to-front arrangement, separation of the two SecY domains mainly takes place at the dimer interface, and thus opening of a single protomer will be directly transmitted to the neighboring protomer.

It should be noted, however, that the features that mediate the third ribosome–translocon connection (SecG/Secβ and the SecE extension) are not essential for viability or protein translocation [148, 149, 50]. Thus, ribosome-induced opening of the translocon might be primarily mediated by the two C4/C5 connections, while the third connection plays an auxiliary role. This would explain the mere stimulatory role of Secβ on posttranslational protein translocation [150]. The stimulatory role of SecG can be explained similarly, but the SecA-induced opening mechanism differs in at least one aspect from the ribosome-induced opening mechanism, that is the SecA ''membrane insertion'' cycle. The SecA interaction site in the N-terminal half of SecY (the TMS4–C3 interface) appears to be part of the region where SecA inserts at least partially into the translocon. SecG is in proximity of this region and might thus facilitate membrane cycling of SecA [151, 152]. It seems unlikely however that SecG completely inverts its membrane topology during protein translocation via SecYEG as proposed previously [153], as topologically fixed SecG has been shown to be equally active as wild-type SecG [154]. The different conformations of SecG that are observed *in vitro* most likely represent conformational changes within this highly dynamic region of the translocon.

E. The Role of the Plug

In addition to separation of the two SecY domains, the opening mechanism of the translocon is thought to involve displacement of the ''plug'' domain formed by the E1 loop [16]. This proposal is based on the location of the plug domain at the extracellular end of the pore region in the closed conformation of the channel, and the observation that it has the potential to be cross-linked to the C-terminal region of SecE, located ~20 Å away [155]. The mobile nature of the plug domain has been confirmed by molecular dynamics simulations [125] (Gumbart and Schulten, Biophysical Journal, in press), homology modeling [119], and a cross-linking approach [156]. In the latter study, it was shown that the plug domain is displaced during protein translocation, providing the first experimental evidence for its proposed function. An interesting observation that provides a novel hypothesis on the mechanism of SecA-induced opening of the translocon was recently made with a peptide scanning approach (EvdS *et al.*, in preparation). It was shown that SecA directly interacts with peptides derived from the plug domain, suggesting that displacement of the plug domain in bacteria might be directly induced by SecA.

VII. Concluding Remarks

To summarize, our understanding of the molecular mechanism of protein translocation in bacteria has increased dramatically during the past few years, and long held schematic models are slowly beginning to take shape on a detailed structural level. However, a ''molecular movie'' of protein translocation is not expected in the near future because of the tremendous complexity of the process. New insights have to be provided by a combination of structural, biophysical, and biochemical studies. Considering the large amount of unresolved questions, research on the Sec machinery is expected to remain an exciting area in biology throughout the next decade.

References

1. Pohlschroder, M., Prinz, W.A., Hartmann, E., and Beckwith, J. (1997). Protein translocation in the three domains of life: Variations on a theme. *Cell* 91:563–566.
2. Osborne, A.R., Rapoport, T.A., and Van den Berg, B. (2005). Protein translocation by the Sec61/SecY channel. *Annu Rev Cell Dev Biol* 21:529–550.
3. Cao, T.B., and Saier, M.H. (2003). The general protein secretory pathway: Phylogenetic analyses leading to evolutionary conclusions. *Biochim Biophys Acta* 1609:115–125.

4. Ulbrandt, N.D., Newitt, J.A., and Bernstein, H.D. (1997). The *E. coli* signal recognition particle is required for the insertion of a subset of inner membrane proteins. *Cell* 88:187–196.
5. Kalies, K.U., Gorlich, D., and Rapoport, T.A. (1994). Binding of ribosomes to the rough endoplasmic reticulum mediated by the Sec61p-complex. *J Cell Biol* 126:925–934.
6. Pohlschroder, M., Hartmann, E., Hand, N.J., Dilks, K., and Haddad, A. (2005). Diversity and evolution of protein translocation. *Annu Rev Microbiol* 59:91–111.
7. Rapiejko, P.J., and Gilmore, R. (1997). Empty site forms of the SRP54 and SR alpha GTPases mediate targeting of ribosome-nascent chain complexes to the endoplasmic reticulum. *Cell* 89:703–713.
8. Mason, N., Ciufo, L.F., and Brown, J.D. (2000). Elongation arrest is a physiologically important function of signal recognition particle. *EMBO J* 19:4164–4174.
9. Luirink, J., and Sinning, I. (2004). SRP-mediated protein targeting: Structure and function revisited. *Biochim Biophys Acta* 1694:17–35.
10. Wild, K., Halic, M., Sinning, I., and Beckmann, R. (2004). SRP meets the ribosome. *Nat Struct Mol Biol* 11:1049–1053.
11. Hartl, F.U., Lecker, S., Schiebel, E., Hendrick, J.P., and Wickner, W. (1990). The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane. *Cell* 63:269–279.
12. Brodsky, J.L., Goeckeler, J., and Schekman, R. (1995). BiP and Sec63p are required for both co- and posttranslational protein translocation into the yeast endoplasmic reticulum. *Proc Natl Acad Sci USA* 92:9643–9646.
13. Hunt, J.F., Weinkauf, S., Henry, L., Fak, J.J., McNicholas, P., Oliver, D.B., and Deisenhofer, J. (2002). Nucleotide control of interdomain interactions in the conformational reaction cycle of SecA. *Science* 297:2018–2026.
14. Xu, Z., Knafels, J.D., and Yoshino, K. (2000). Crystal structure of the bacterial protein export chaperone secB. *Nat Struct Biol* 7:1172–1177.
15. Zhou, J., and Xu, Z. (2003). Structural determinants of SecB recognition by SecA in bacterial protein translocation. *Nat Struct Biol* 10:942–947.
16. Van den Berg, B., Clemons, W.M., Jr., Collinson, I., Modis, Y., Hartmann, E., Harrison, S.C., and Rapoport, T.A. (2004). X-ray structure of a protein-conducting channel. *Nature* 427:36–44.
17. Sharma, V., Arockiasamy, A., Ronning, D.R., Savva, C.G., Holzenburg, A., Braunstein, M., Jacobs, W.R., Jr., and Sacchettini, J.C. (2003). Crystal structure of *Mycobacterium tuberculosis* SecA, a preprotein translocating ATPase. *Proc Natl Acad Sci USA* 100:2243–2248.
18. Beckmann, R., Spahn, C.M., Eswar, N., Helmers, J., Penczek, P.A., Sali, A., Frank, J., and Blobel, G. (2001). Architecture of the protein-conducting channel associated with the translating 80S ribosome. *Cell* 107:361–372.
19. Mitra, K., Schaffitzel, C., Shaikh, T., Tama, F., Jenni, S., Brooks, C.L., III, Ban, N., and Frank, J. (2005). Structure of the *E. coli* protein-conducting channel bound to a translating ribosome. *Nature* 438:318–324.
20. Menetret, J.F., Neuhof, A., Morgan, D.G., Plath, K., Radermacher, M., Rapoport, T.A., and Akey, C.W. (2000). The structure of ribosome-channel complexes engaged in protein translocation. *Mol Cell* 6:1219–1232.
21. Morgan, D.G., Menetret, J.F., Radermacher, M., Neuhof, A., Akey, I.V., Rapoport, T.A., and Akey, C.W. (2000). A comparison of the yeast and rabbit 80 S ribosome reveals the topology of the nascent chain exit tunnel, inter-subunit bridges and mammalian rRNA expansion segments. *J Mol Biol* 301:301–321.
22. de Keyzer, J., Van der Does, C., and Driessen, A.J.M. (2003). The bacterial translocase: A dynamic protein channel complex. *Cell Mol Life Sci* 60:2034–2052.

23. Veenendaal, A.K., Van der Does, C., and Driessen, A.J.M. (2004). The protein-conducting channel SecYEG. *Biochim Biophys Acta* 1694:81–95.
24. Vrontou, E., and Economou, A. (2004). Structure and function of SecA, the preprotein translocase nanomotor. *Biochim Biophys Acta* 1694:67–80.
25. Urbanus, M.L., Scotti, P.A., Froderberg, L., Saaf, A., de Gier, J.W., Brunner, J., Samuelson, J.C., Dalbey, R.E., Oudega, B., and Luirink, J. (2001). Sec-dependent membrane protein insertion: Sequential interaction of nascent FtsQ with SecY and YidC. *EMBO Rep* 2:524–529.
26. Samuelson, J.C., Jiang, F., Yi, L., Chen, M., de Gier, J.W., Kuhn, A., and Dalbey, R.E. (2001). Function of YidC for the insertion of M13 procoat protein in *Escherichia coli*: Translocation of mutants that show differences in their membrane potential dependence and Sec requirement. *J Biol Chem* 276:34847–34852.
27. Jia, L., Dienhart, M., Schramp, M., McCauley, M., Hell, K., and Stuart, R.A. (2003). Yeast Oxa1 interacts with mitochondrial ribosomes: The importance of the C-terminal region of Oxa1. *EMBO J* 22:6438–6447.
28. Szyrach, G., Ott, M., Bonnefoy, N., Neupert, W., and Herrmann, J.M. (2003). Ribosome binding to the Oxa1 complex facilitates co-translational protein insertion in mitochondria. *EMBO J* 22:6448–6457.
29. Duong, F., and Wickner, W. (1997). Distinct catalytic roles of the SecYE, SecG and SecDFyajC subunits of preprotein translocase holoenzyme. *EMBO J* 16:2756–2768.
30. Duong, F., and Wickner, W. (1997). The SecDFyajC domain of preprotein translocase controls preprotein movement by regulating SecA membrane cycling. *EMBO J* 16:4871–4879.
31. Kato, Y., Nishiyama, K., and Tokuda, H. (2003). Depletion of SecDF-YajC causes a decrease in the level of SecG: Implication for their functional interaction. *FEBS Lett* 550:114–118.
32. Rain, J.C., Selig, L., De Reuse, H., Battaglia, V., Reverdy, C., Simon, S., Lenzen, G., Petel, F., Wojcik, J., Schachter, V., Chemama, Y., Labigne, A., *et al.* (2001). The protein-protein interaction map of *Helicobacter pylori. Nature* 409:211–215.
33. Nouwen, N., and Driessen, A.J.M. (2002). SecDFyajC forms a heterotetrameric complex with YidC. *Mol Microbiol* 44:1397–1405.
34. Nouwen, N., Van der Laan, M., and Driessen, A.J.M. (2001). SecDFyajC is not required for the maintenance of the proton motive force. *FEBS Lett* 508:103–106.
35. Stenberg, F., Chovanec, P., Maslen, S.L., Robinson, C.V., Ilag, L.L., von Heijne, G., and Daley, D.O. (2005). Protein complexes of the *Escherichia coli* cell envelope. *J Biol Chem* 280:34409–34419.
36. Lecker, S.H., Driessen, A.J.M., and Wickner, W. (1990). ProOmpA contains secondary and tertiary structure prior to translocation and is shielded from aggregation by association with SecB protein. *EMBO J* 9:2309–2314.
37. Gannon, P.M., Li, P., and Kumamoto, C.A. (1989). The mature portion of *Escherichia coli* maltose-binding protein (MBP) determines the dependence of MBP on SecB for export. *J Bacteriol* 171:813–818.
38. Fekkes, P., Van der Does, C., and Driessen, A.J.M. (1997). The molecular chaperone SecB is released from the carboxy-terminus of SecA during initiation of precursor protein translocation. *EMBO J* 16:6105–6113.
39. Shimizu, H., Nishiyama, K., and Tokuda, H. (1997). Expression of *gpsA* encoding biosynthetic sn-glycerol 3-phosphate dehydrogenase suppresses both the LB-phenotype of a *secB* null mutant and the cold-sensitive phenotype of a *secG* null mutant. *Mol Microbiol* 26:1013–1021.

40. Delepelaire, P., and Wandersman, C. (1998). The SecB chaperone is involved in the secretion of the Serratia marcescens HasA protein through an ABC transporter. *EMBO J* 17:936–944.
41. Baars, L., Ytterberg, A.J., Drew, D., Wagner, S., Thilo, C., Van Wijk, K.J., and de Gier, J.W. (2005). Defining the role of the *E. coli* chaperone SecB using comparative proteomics. *J Biol Chem* 281:10024–10034.
42. Knoblauch, N.T., Rudiger, S., Schonfeld, H.J., Driessen, A.J.M., Schneider-Mergener, J., and Bukau, B. (1999). Substrate Specificity of the SecB Chaperone. *J Biol Chem* 274:34219–34225.
43. Rajapandi, T., Dolan, K.M., and Oliver, D.B. (1991). The first gene in the *Escherichia coli secA* operon, gene X, encodes a nonessential secretory protein. *J Bacteriol* 173:7092–7097.
44. Nakatogawa, H., and Ito, K. (2001). Secretion monitor, SecM, undergoes self-translation arrest in the cytosol. *Mol Cell* 7:185–192.
45. Nakatogawa, H., and Ito, K. (2002). The ribosomal exit tunnel functions as a discriminating gate. *Cell* 108:629–636.
46. Nakatogawa, H., Murakami, A., Mori, H., and Ito, K. (2005). SecM facilitates translocase function of SecA by localizing its biosynthesis. *Genes Dev* 19:436–444.
47. Chen, X., Xu, H., and Tai, P.C. (1996). A significant fraction of functional SecA is permanently embedded in the membrane. SecA cycling on and off the membrane is not essential during protein translocation. *J Biol Chem* 271:29698–29706.
48. Eichler, J., Rinard, K., and Wickner, W. (1998). Endogenous SecA catalyzes preprotein translocation at SecYEG. *J Biol Chem* 273:21675–21681.
49. Schatz, P.J., Riggs, P.D., Jacq, A., Fath, M.J., and Beckwith, J. (1989). The *secE* gene encodes an integral membrane protein required for protein export in *Escherichia coli*. *Genes Dev* 3:1035–1044.
50. Schatz, P.J., Bieker, K.L., Ottemann, K.M., Silhavy, T.J., and Beckwith, J. (1991). One of three transmembrane stretches is sufficient for the functioning of the SecE protein, a membrane component of the *E. coli* secretion machinery. *EMBO J* 10:1749–1757.
51. Van der Sluis, E.O., and Driessen, A.J.M. (2006). Stepwise evolution of the Sec machinery in Proteobacteria. *Trends Microbiol* 14:105–108.
52. Braunstein, M., Brown, A.M., Kurtz, S., and Jacobs, W.R., Jr. (2001). Two nonredundant SecA homologues function in mycobacteria. *J Bacteriol* 183:6979–6990.
53. Lenz, L.L., and Portnoy, D.A. (2002). Identification of a second Listeria secA gene associated with protein secretion and the rough phenotype. *Mol Microbiol* 45: 1043–1056.
54. Bensing, B.A., and Sullam, P.M. (2002). An accessory *sec* locus of *Streptococcus gordonii* is required for export of the surface protein GspB and for normal levels of binding to human platelets. *Mol Microbiol* 44:1081–1094.
55. Braunstein, M., Espinosa, B.J., Chan, J., Belisle, J.T., and Jacobs, R. (2003). SecA2 functions in the secretion of superoxide dismutase A and in the virulence of *Mycobacterium tuberculosis*. *Mol Microbiol* 48:453–464.
56. Lenz, L.L., Mohammadi, S., Geissler, A., and Portnoy, D.A. (2003). SecA2-dependent secretion of autolytic enzymes promotes *Listeria monocytogenes* pathogenesis. *Proc Natl Acad Sci USA* 100:12432–12437.
57. Bensing, B.A., Gibson, B.W., and Sullam, P.M. (2004). The *Streptococcus gordonii* platelet binding protein GspB undergoes glycosylation independently of export. *J Bacteriol* 186:638–645.
58. Bensing, B.A., Takamatsu, D., and Sullam, P.M. (2005). Determinants of the streptococcal surface glycoprotein GspB that facilitate export by the accessory Sec system. *Mol Microbiol* 58:1468–1481.

59. Takamatsu, D., Bensing, B.A., and Sullam, P.M. (2004). Four proteins encoded in the gspB-secY2A2 operon of *Streptococcus gordonii* mediate the intracellular glycosylation of the platelet-binding protein GspB. *J Bacteriol* 186:7100–7111.
60. Takamatsu, D., Bensing, B.A., and Sullam, P.M. (2004). Genes in the accessory sec locus of *Streptococcus gordonii* have three functionally distinct effects on the expression of the platelet-binding protein GspB. *Mol Microbiol* 52:189–203.
61. Chen, Q., Wu, H., and Fives-Taylor, P.M. (2004). Investigating the role of secA2 in secretion and glycosylation of a fimbrial adhesin in *Streptococcus parasanguis* FW213. *Mol Microbiol* 53:843–856.
62. Machata, S., Hain, T., Rohde, M., and Chakraborty, T. (2005). Simultaneous deficiency of both MurA and p60 proteins generates a rough phenotype in *Listeria monocytogenes*. *J Bacteriol* 187:8385–8394.
63. Tian, H., Boyd, D., and Beckwith, J. (2000). A mutant hunt for defects in membrane protein assembly yields mutations affecting the bacterial signal recognition particle and Sec machinery. *Proc Natl Acad Sci USA* 97:4730–4735.
64. Traxler, B., and Murphy, C. (1996). Insertion of the polytopic membrane protein MalF is dependent on the bacterial secretion machinery. *J Biol Chem* 271:12394–12400.
65. Van der Laan, M., Nouwen, N., and Driessen, A.J.M. (2004). SecYEG proteoliposomes catalyze the $\Delta\psi$-dependent membrane insertion of FtsQ. *J Biol Chem* 279:1659–1664.
66. Neumann-Haefelin, C., Schafer, U., Muller, M., and Koch, H.G. (2000). SRP-dependent co-translational targeting and SecA-dependent translocation analyzed as individual steps in the export of a bacterial protein. *EMBO J* 19:6419–6426.
67. Zito, C.R., and Oliver, D. (2003). Two-stage binding of SecA to the bacterial translocon regulates ribosome-translocon interaction. *J Biol Chem* 278:40640–40646.
68. Karamyshev, A.L., and Johnson, A.E. (2005). Selective SecA association with signal sequences in ribosome-bound nascent chains: A potential role for SecA in ribosome targeting to the bacterial membrane. *J Biol Chem* 280:37930–37940.
69. Liebke, H.H. (1987). Multiple SecA protein isoforms in *Escherichia coli*. *J Bacteriol* 169:1174–1181.
70. Fekkes, P., de Wit, J.G., Van der Wolk, J.P., Kimsey, H.H., Kumamoto, C.A., and Driessen, A.J.M. (1998). Preprotein transfer to the *Escherichia coli* translocase requires the co-operative binding of SecB and the signal sequence to SecA. *Mol Microbiol* 29:1179–1190.
71. Economou, A., and Wickner, W. (1994). SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. *Cell* 78:835–843.
72. Schiebel, E., Driessen, A.J.M., Hartl, F.U., and Wickner, W. (1991). $\Delta\mu$ H+ and ATP function at different steps of the catalytic cycle of preprotein translocase. *Cell* 64:927–939.
73. Van der Wolk, J.P., de Wit, J.G., and Driessen, A.J.M. (1997). The catalytic cycle of the *Escherichia coli* SecA ATPase comprises two distinct preprotein translocation events. *EMBO J* 16:7297–7304.
74. Economou, A., Pogliano, J.A., Beckwith, J., Oliver, D.B., and Wickner, W. (1995). SecA membrane cycling at SecYEG is driven by distinct ATP binding and hydrolysis events and is regulated by SecD and SecF. *Cell* 83:1171–1181.
75. Osborne, A.R., Clemons, W.M., Jr., and Rapoport, T.A. (2004). A large conformational change of the translocation ATPase SecA. *Proc Natl Acad Sci USA* 101:10937–10942.
76. Dekker, C., de Kruijff, B., and Gros, P. (2003). Crystal structure of SecB from *Escherichia coli*. *J Struct Biol* 144:313–319.
77. Koonin, E.V., and Gorbalenya, A.E. (1992). Autogenous translation regulation by *Escherichia coli* ATPase SecA may be mediated by an intrinsic RNA helicase activity of this protein. *FEBS Lett* 298:6–8.

78. Papanikou, E., Karamanou, S., Baud, C., Frank, M., Sianidis, G., Keramisanou, D., Kalodimos, C.G., Kuhn, A., and Economou, A. (2005). Identification of the preprotein binding domain of SecA. *J Biol Chem* 280:43209–43217.
79. Kimura, E., Akita, M., Matsuyama, S., and Mizushima, S. (1991). Determination of a region in SecA that interacts with presecretory proteins in *Escherichia coli*. *J Biol Chem* 266:6600–6606.
80. Woodbury, R.L., Hardy, S.J., and Randall, L.L. (2002). Complex behavior in solution of homodimeric SecA. *Protein Sci* 11:875–882.
81. Akita, M., Shinaki, A., Matsuyama, S., and Mizushima, S. (1991). SecA, an essential component of the secretory machinery of *Escherichia coli*, exists as homodimer. *Biochem Biophys Res Commun* 174:211–216.
82. Dempsey, B.R., Economou, A., Dunn, S.D., and Shilton, B.H. (2002). The ATPase domain of SecA can form a tetramer in solution. *J Mol Biol* 315:831–843.
83. Or, E., Navon, A., and Rapoport, T. (2002). Dissociation of the dimeric SecA ATPase during protein translocation across the bacterial membrane. *EMBO J* 21:4470–4479.
84. Benach, J., Chou, Y.T., Fak, J.J., Itkin, A., Nicolae, D.D., Smith, P.C., Wittrock, G., Floyd, D.L., Golsaz, C.M., Gierasch, L.M., and Hunt, J.F. (2003). Phospholipid-induced monomerization and signal-peptide-induced oligomerization of SecA. *J Biol Chem* 278:3628–3638.
85. Musial-Siwek, M., Rusch, S.L., and Kendall, D.A. (2005). Probing the affinity of SecA for signal peptide in different environments. *Biochemistry* 44:13987–13996.
86. Bu, Z., Wang, L., and Kendall, D.A. (2003). Nucleotide binding induces changes in the oligomeric state and conformation of SecA in a lipid environment: A small-angle neutron-scattering study. *J Mol Biol* 332:23–30.
87. Bessonneau, P., Besson, V., Collinson, I., and Duong, F. (2002). The SecYEG preprotein translocation channel is a conformationally dynamic and dimeric structure. *EMBO J* 21:995–1003.
88. Duong, F. (2003). Binding, activation and dissociation of the dimeric SecA ATPase at the dimeric SecYEG translocase. *EMBO J* 22:4375–4384.
89. Tziatzios, C., Schubert, D., Lotz, M., Gundogan, D., Betz, H., Schagger, H., Haase, W., Duong, F., and Collinson, I. (2004). The bacterial protein-translocation complex: SecYEG dimers associate with one or two SecA molecules. *J Mol Biol* 340:513–524.
90. Jilaveanu, L.B., Zito, C.R., and Oliver, D. (2005). Dimeric SecA is essential for protein translocation. *Proc Natl Acad Sci USA* 102:7511–7516.
91. de Keyzer, J., Van der Sluis, E.O., Spelbrink, R.E., Nijstad, N., de Kruijff, B., Nouwen, N., Van der Does, C., and Driessen, A.J.M. (2005). Covalently dimerized SecA is functional in protein translocation. *J Biol Chem* 280:35255–35260.
92. Karamanou, S., Sianidis, G., Gouridis, G., Pozidis, C., Papanikolau, Y., Papanikou, E., and Economou, A. (2005). *Escherichia coli* SecA truncated at its termini is functional and dimeric. *FEBS Lett* 579:1267–1271.
93. Randall, L.L., Crane, J.M., Lilly, A.A., Liu, G., Mao, C., Patel, C.N., and Hardy, S.J. (2005). Asymmetric binding between SecA and SecB two symmetric proteins: Implications for function in export. *J Mol Biol* 348:479–489.
94. Or, E., Boyd, D., Gon, S., Beckwith, J., and Rapoport, T. (2005). The bacterial ATPase SecA functions as a monomer in protein translocation. *J Biol Chem* 280:9097–9105.
95. Jilaveanu, L.B., and Oliver, D. (2006). SecA dimer cross-linked at its subunit interface is functional for protein translocation. *J Bacteriol* 188:335–338.
96. Driessen, A.J.M. (1993). SecA, the peripheral subunit of the *Escherichia coli* precursor protein translocase, is functional as a dimer. *Biochemistry* 32:13190–13197.

97. Ding, H., Hunt, J.F., Mukerji, I., and Oliver, D. (2003). *Bacillus subtilis* SecA ATPase exists as an antiparallel dimer in solution. *Biochemistry* 42:8729–8738.
98. Ding, H., Mukerji, I., and Oliver, D. (2003). Nucleotide and phospholipid-dependent control of PPXD and C-domain association for SecA ATPase. *Biochemistry* 42:13468–13475.
99. Ding, H., Mukerji, I., and Oliver, D. (2001). Lipid and signal peptide-induced conformational changes within the C-domain of *Escherichia coli* SecA protein. *Biochemistry* 40:1835–1843.
100. Chou, Y.T., Swain, J.F., and Gierasch, L.M. (2002). Functionally significant mobile regions of *Escherichia coli* SecA ATPase identified by NMR. *J Biol Chem* 277:50985–50990.
101. Ulbrandt, N.D., London, E., and Oliver, D.B. (1992). Deep penetration of a portion of *Escherichia coli* SecA protein into model membranes is promoted by anionic phospholipids and by partial unfolding. *J Biol Chem* 267:15184–15192.
102. Den Blaauwen, T., Fekkes, P., de Wit, J.G., Kuiper, W., and Driessen, A.J.M. (1996). Domain interactions of the peripheral preprotein translocase subunit SecA. *Biochemistry* 35:11994–12004.
103. Schmidt, M., Ding, H., Ramamurthy, V., Mukerji, I., and Oliver, D. (2000). Nucleotide binding activity of SecA homodimer is conformationally regulated by temperature and altered by *prlD* and *azi* mutations. *J Biol Chem* 275:15440–15448.
104. Baud, C., Karamanou, S., Sianidis, G., Vrontou, E., Politou, A.S., and Economou, A. (2002). Allosteric communication between signal peptides and the SecA protein DEAD motor ATPase domain. *J Biol Chem* 277:13724–13731.
105. Vrontou, E., Karamanou, S., Baud, C., Sianidis, G., and Economou, A. (2004). Global co-ordination of protein translocation by the SecA IRA1 switch. *J Biol Chem* 279(21)**:** 22490–22497.
106. Story, R.M., Li, H., and Abelson, J.N. (2001). Crystal structure of a DEAD box protein from the hyperthermophile *Methanococcus jannaschii*. *Proc Natl Acad Sci USA* 98:1465–1470.
107. Velankar, S.S., Soultanas, P., Dillingham, M.S., Subramanya, H.S., and Wigley, D.B. (1999). Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. *Cell* 97:75–84.
108. Zhou, J., and Xu, Z. (2005). The structural view of bacterial translocation-specific chaperone SecB: Implications for function. *Mol Microbiol* 58:349–357.
109. Fekkes, P., de Wit, J.G., Boorsma, A., Friesen, R.H., and Driessen, A.J.M. (1999). Zinc stabilizes the SecB binding site of SecA. *Biochemistry* 38:5111–5116.
110. Breukink, E., Nouwen, N., Van Raalte, A., Mizushima, S., Tommassen, J., and de Kruijff, B. (1995). The C terminus of SecA is involved in both lipid binding and SecB binding. *J Biol Chem* 270:7902–7907.
111. Dempsey, B.R., Wrona, M., Moulin, J.M., Gloor, G.B., Jalilehvand, F., Lajoie, G., Shaw, G.S., and Shilton, B.H. (2004). Solution NMR structure and X-ray absorption analysis of the C-terminal zinc-binding domain of the SecA ATPase. *Biochemistry* 43:9361–9371.
112. Matousek, W.M., and Alexandrescu, A.T. (2004). NMR structure of the C-terminal domain of SecA in the free state. *Biochim Biophys Acta* 1702:163–171.
113. Kimsey, H.H., Dagarag, M.D., and Kumamoto, C.A. (1995). Diverse effects of mutation on the activity of the *Escherichia coli* export chaperone SecB. *J Biol Chem* 270:22831–22835.
114. Rajapandi, T., and Oliver, D. (1994). Carboxy-terminal region of *Escherichia coli* SecA ATPase is important to promote its protein translocation activity *in vivo*. *Biochem Biophys Res Commun* 200:1477–1483.

115. Van Voorst, F., Van der Does, C., Brunner, J., Driessen, A.J.M., and de Kruijff, B. (1998). Translocase-bound SecA is largely shielded from the phospholipid acyl chains. *Biochemistry* 37:12261–12268.
116. Dapic, V., and Oliver, D. (2000). Distinct membrane binding properties of N- and C-terminal domains of *Escherichia coli* SecA ATPase. *J Biol Chem* 275:25000–25007.
117. Kinch, L.N., Saier, M.H., Jr., and Grishin, N.V. (2002). Sec61beta–a component of the archaeal protein secretory system. *Trends Biochem Sci* 27:170–171.
118. Breyton, C., Haase, W., Rapoport, T.A., Kuhlbrandt, W., and Collinson, I. (2002). Three-dimensional structure of the bacterial protein-translocation complex SecYEG. *Nature* 418:662–665.
119. Bostina, M., Mohsin, B., Kuhlbrandt, W., and Collinson, I. (2005). Atomic model of the *E. coli* membrane-bound protein translocation complex SecYEG. *J Mol Biol* 352:1035–1043.
120. Veenendaal, A.K.J., Van der Does, C., and Driessen, A.J.M. (2002). The core of the bacterial translocase harbors a tilted transmembrane segment 3 of SecE. *J Biol Chem* 277:36640–36645.
121. Beswick, V., Baleux, F., Huynh-Dinh, T., Kepes, F., Neumann, J.M., and Sanson, A. (1996). NMR conformational study of the cytoplasmic domain of the canine Sec61 gamma protein from the protein translocation pore of the endoplasmic reticulum membrane. *Biochemistry* 35:14717–14724.
122. Plath, K., Mothes, W., Wilkinson, B.M., Stirling, C.J., and Rapoport, T.A. (1998). Signal sequence recognition in posttranslational protein transport across the yeast ER membrane. *Cell* 94:795–807.
123. Wang, L., Miller, A., Rusch, S.L., and Kendall, D.A. (2004). Demonstration of a specific *Escherichia coli* SecY-signal peptide interaction. *Biochemistry* 43:13185–13192.
124. Plath, K., Wilkinson, B.M., Stirling, C.J., and Rapoport, T.A. (2004). Interactions between Sec complex and prepro-alpha-factor during posttranslational protein transport into the endoplasmic reticulum. *Mol Biol Cell* 15:1–10.
125. Tian, P., and Andricioaei, I. (2006). Size, motion and function of the SecY translocon revealed by molecular dynamics simulations with virtual probes. *Biophys J* 90:2718–2730.
126. Mori, H., Tsukazaki, T., Masui, R., Kuramitsu, S., Yokoyama, S., Johnson, A.E., Kimura, Y., Akiyama, Y., and Ito, K. (2003). Fluorescence resonance energy transfer analysis of protein translocase. SecYE from *Thermus thermophilus* HB8 forms a constitutive oligomer in membranes. *J Biol Chem* 278:14257–14264.
127. Van der Sluis, E., Nouwen, N., and Driessen, A.J.M. (2002). SecY-SecY and SecY-SecG contacts revealed by site-specific crosslinking. *FEBS Lett* 527:159–165.
128. Veenendaal, A.K.J., Van der Does, C., and Driessen, A.J.M. (2001). Mapping the sites of interaction between SecY and SecE by cysteine scanning mutagenesis. *J Biol Chem* 276:32559–32566.
129. Kaufmann, A., Manting, E.H., Veenendaal, A.K.J., Driessen, A.J.M., and Van der Does, C. (1999). Cysteine-directed cross-linking demonstrates that helix 3 of SecE is close to helix 2 of SecY and helix 3 of a neighboring SecE. *Biochemistry* 38:9115–9125.
130. Scheuring, J., Braun, N., Nothdurft, L., Stumpf, M., Veenendaal, A.K., Kol, S., Van der Does, C., Driessen, A.J.M., and Weinkauf, S. (2005). The oligomeric distribution of SecYEG is altered by SecA and translocation ligands. *J Mol Biol* 354:258–271.
131. Nagamori, S., Nishiyama, K., and Tokuda, H. (2000). Two SecG molecules present in a single protein translocation machinery are functional even after crosslinking. *J Biochem* 128:129–137.

132. Meyer, T.H., Ménétret, J.F., Breitling, R., Miller, K.R., Akey, C.W., and Rapoport, T.A. (1999). The bacterial SecY/E translocation complex forms channel-like structures similar to those of the eukaryotic Sec61p complex. *J Mol Biol* 285:1789–1800.
133. Collinson, I., Breyton, C., Duong, F., Tziatzios, C., Schubert, D., Or, E., Rapoport, T., and Kuhlbrandt, W. (2001). Projection structure and oligomeric properties of a bacterial core protein translocase. *EMBO J* 20:2462–2471.
134. Manting, E.H., Van der Does, C., Remigy, H., Engel, A., and Driessen, A.J.M. (2000). SecYEG assembles into a tetramer to form the active protein translocation channel. *EMBO J* 19:852–861.
135. Hanein, D., Matlack, K.E., Jungnickel, B., Plath, K., Kalies, K.U., Miller, K.R., Rapoport, T.A., and Akey, C.W. (1996). Oligomeric rings of the Sec61p complex induced by ligands required for protein translocation. *Cell* 87:721–732.
136. Woolf, P.J., and Linderman, J.J. (2003). Self organization of membrane proteins via dimerization. *Biophys Chem* 104:217–227.
137. Scotti, P.A., Urbanus, M.L., Brunner, J., de Gier, J.W., von Heijne, G., Van der Does, C., Driessen, A.J.M., Oudega, B., and Luirink, J. (2000). YidC, the *Escherichia coli* homologue of mitochondrial Oxa1p, is a component of the Sec translocase. *EMBO J* 19:542–549.
138. Yahr, T.L., and Wickner, W.T. (2000). Evaluating the oligomeric state of SecYEG in preprotein translocase. *EMBO J* 19:4393–4401.
139. Beckmann, R., Bubeck, D., Grassucci, R., Penczek, P., Verschoor, A., Blobel, G., and Frank, J. (1997). Alignment of conduits for the nascent polypeptide chain in the ribosome-Sec61 complex. *Science* 278:2123–2126.
140. Menetret, J.F., Hegde, R.S., Heinrich, S.U., Chandramouli, P., Ludtke, S.J., Rapoport, T.A., and Akey, C.W. (2005). Architecture of the ribosome-channel complex derived from native membranes. *J Mol Biol* 348:445–457.
141. Morgan, D.G., Menetret, J.F., Neuhof, A., Rapoport, T.A., and Akey, C.W. (2002). Structure of the mammalian ribosome-channel complex at 17A resolution. *J Mol Biol* 324:871–886.
142. Prinz, A., Behrens, C., Rapoport, T.A., Hartmann, E., and Kalies, K.U. (2000). Evolutionarily conserved binding of ribosomes to the translocation channel via the large ribosomal RNA. *EMBO J* 19:1900–1906.
143. Tani, K., Tokuda, H., and Mizushima, S. (1990). Translocation of ProOmpA possessing an intramolecular disulfide bridge into membrane vesicles of *Escherichia coli*. Effect of membrane energization. *J Biol Chem* 265:17341–17347.
144. De Keyzer, J., Van der Does, C., and Driessen, A.J.M. (2002). Kinetic analysis of the translocation of fluorescent precursor proteins into *Escherichia coli* membrane vesicles. *J Biol Chem* 277:46059–46065.
145. Raden, D., Song, W., and Gilmore, R. (2000). Role of the cytoplasmic segments of Sec61alpha in the ribosome-binding and translocation-promoting activities of the Sec61 complex. *J Cell Biol* 150:53–64.
146. Cheng, Z., Jiang, Y., Mandon, E.C., and Gilmore, R. (2005). Identification of cytoplasmic residues of Sec61p involved in ribosome binding and cotranslational translocation. *J Cell Biol* 168:67–77.
147. Nagamori, S., Nishiyama, K., and Tokuda, H. (2002). Membrane topology inversion of SecG detected by labeling with a membrane-impermeable sulfhydryl reagent that causes a close association of SecG with SecA. *J Biochem (Tokyo)* 132:629–634.
148. Nishiyama, K., Hanada, M., and Tokuda, H. (1994). Disruption of the gene encoding p12 (SecG) reveals the direct involvement and important function of SecG in the protein translocation of *Escherichia coli* at low temperature. *EMBO J* 13:3272–3277.

149. Finke, K., Plath, K., Panzner, S., Prehn, S., Rapoport, T.A., Hartmann, E., and Sommer, T. (1996). A second trimeric complex containing homologs of the Sec61p complex functions in protein transport across the ER membrane of *S. cerevisiae*. *EMBO J* 15:1482–1494.
150. Kalies, K.U., Rapoport, T.A., and Hartmann, E. (1998). The beta subunit of the Sec61 complex facilitates cotranslational protein transport and interacts with the signal peptidase during translocation. *J Cell Biol* 141:887–894.
151. Suzuki, H., Nishiyama, K., and Tokuda, H. (1998). Coupled structure changes of SecA and SecG revealed by the synthetic lethality of the secAcsR11 and delta secG::kan double mutant. *Mol Microbiol* 29:331–341.
152. Matsumoto, G., Mori, H., and Ito, K. (1998). Roles of SecG in ATP- and SecA-dependent protein translocation. *Proc Natl Acad Sci USA* 95:13567–13572.
153. Nishiyama, K., Suzuki, T., and Tokuda, H. (1996). Inversion of the membrane topology of SecG coupled with SecA-dependent preprotein translocation. *Cell* 85:71–81.
154. Van der Sluis, E.O., Van der Vries, E., Berrelkamp, G., Nouwen, N., and Driessen, A.J.M. (2006). Topologically fixed SecG is fully functional. *J Bacteriol* 188:1188–1190.
155. Harris, C.R., and Silhavy, T.J. (1999). Mapping an interface of SecY (PrlA) and SecE (PrlG) by using synthetic phenotypes and *in vivo* cross-linking. *J Bacteriol* 181:3438–3444.
156. Tam, P.C., Maillard, A.P., Chan, K.K., and Duong, F. (2005). Investigating the SecY plug movement at the SecYEG translocation channel. *EMBO J* 24:3380–3388.