Biochimica et Biophysica Acta 1843 (2014) 1466–1474

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

SecA-mediated targeting and translocation of secretory proteins ☆

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ARTICLE INFO

Article history:
Received 27 November 2013
Received in revised form 12 February 2014
Accepted 15 February 2014
Available online 25 February 2014

Keywords:
SecA
Protein translocase
SecYEG
Preproteins
ATPase
Motor protein

ABSTRACT

More than 30 years of research have revealed that the dynamic nanomotor SecA is a central player in bacterial protein secretion. SecA associates with the SecYEG channel and transports polypeptides post-translationally to the *trans* side of the cytoplasmic membrane. It comprises a helicase-like ATPase core coupled to two domains that provide specificity for preprotein translocation. Apart from SecYEG, SecA associates with multiple ligands like ribosomes, nucleotides, lipids, chaperones and preproteins. It exerts its essential contribution in two phases. First, SecA, alone or in concert with chaperones, helps mediate the targeting of the secretory proteins from the ribosome to the membrane. Next, at the membrane it converts chemical energy to mechanical work and translocates preproteins through the SecYEG channel. SecA is a highly dynamic enzyme, it exploits disorder–order kinetics, swiveling and dissociation of domains and dimer to monomer transformations that are tightly coupled with its catalytic function. Preprotein signal sequences and mature domains exploit these dynamics to manipulate the nanomotor and thus achieve their export at the expense of metabolic energy. This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

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1. Introduction

In bacteria, protein secretion commonly describes the process by which a polypeptide crosses the semi-permeable cytoplasmic or plasma membrane and is transferred to the *trans* side [1,2]. Additional steps may further guide proteins to the Gram negative outer membrane or, the extra-cellular milieu. Secretion is an essential process that localizes hydrolytic enzymes, motility elements, electron chain transfer components, sensor transducers, transporters etc. in extra-cytoplasmic locations. Proteins that reach their final localization after complete traversal of at least one membrane bilayer are referred to here, collectively, as secretory proteins.

The main conduit for either trans-membrane crossing or membrane insertion of proteins is a single channel, comprising three polypeptides (SecY, SecE and SecG) that assemble into a membrane-embedded trimer. SecYEG is essential, ubiquitous and conserved in all three domains of life and is located in the plasma membrane (Bacteria/Archaea) or, the endoplasmic reticulum (Eukaryotes) [3]. Other specialized systems, have evolved to handle secretion of toxins or components of extracellular organelles, across the outer membrane or across the cell envelope with no periplasmic intermediates [1,4–7].

Sec-dependent polypeptides that cross the membrane are synthesized as pre-proteins: A short N-terminal signal sequence of conserved physicochemical properties is followed by a unique, for each other secretory protein, mature domain of variable length, with no obvious similarities. The latter will form the actual functional, native polypeptide released to the *trans* side of the membrane. This occurs after the cleavage of the signal sequence on the periplasmic face of the membrane [8–10]. Sec-dependent membrane-embedded proteins may, or may not have signal sequences.

During "co-translational" secretion, the Signal Recognition Particle (SRP), composed of the Ffh (Fifty four homologue) protein and the short 4.5S RNA species, recognizes and binds to the ribosomal L23 subunit [4–6] and to the exiting, usually highly hydrophobic, signal sequence [7]. Then RNC (ribosome–nascent chain complex) is delivered to FtsY, its membrane-associated receptor [8,9]. Eventually the SRP–FtsY complex dissociates, at the expense of GTP [10,11]. In *Escherichia coli* this mode of targeting/translocation is mainly taken by the ~1000 inner membrane proteins and by only a handful of secretory proteins [12–14].

The vast majority of the 400–500 secretory proteins of *E. coli*, the best characterized bacterial model, reaches SecYEG post-translationally, after

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http://dx.doi.org/10.1016/j.bbamcr.2014.02.014
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more than two-thirds of the chain has been synthesized at the ribosome [15]. In this case SecA is the SecYEG partner and the energizer of the system [2]. Secretory proteins are targeted to the channel either by binding to cytoplasmically diffusing SecA or on SecY-bound SecA with or without assistance from chaperones like trigger factor (TF) or SecB [16,17]. TF, that also binds to ribosomes, is ubiquitous in Bacteria, while SecB is only found in some proteobacteria [18]. However, neither one is an essential *E. coli* gene [19,20]. Different chaperones, like CsaA, have been proposed to assist targeting in Gram positive bacteria [21]. Alas, we still only poorly understand the underlying networks of complex equilibria that drive such processes. It is anticipated that they are affected by preprotein–chaperone affinity constants, effective cytoplasmic concentrations of the various partners, folding and/or aggregation propensities of preproteins, sub-cellular localization of the secretory RNCs and of the respective mRNAs. In addition, a fundamental property that needs to be satisfied stems from the necessity that any secretory chain must maintain a non-native three-dimensional structure until the narrow pore of the SecYEG channel (0.8–2.5 nm) is negotiated [22–24].

Post-translational protein secretion can be dissected in three distinct and biochemically separable stages: 1) Sorting and targeting: Nascent preproteins are sorted from cytoplasmic resident polypeptides and get from the ribosome to the membrane-embedded translocase either alone or by binding via their signal peptides and/or mature domains to distinct areas on chaperones and/or SecA. 2) Translocase priming and activation: Upon signal peptide binding, SecA reduces the energy requirement for translocase activation and undergoes conformational changes that lead to dimer-to-monomer transition. 3) Multiple rounds of translocation: Monomeric SecA performs multiple rounds of ATP hydrolysis coupled to mechanical translocation of the preprotein mature domain through the lipid bilayer embedded SecYEG pore.

This review will focus on the recent advances of the role of SecA in post-translational protein secretion.

2. SecA protomer structure and domain organization

Crystal structures [25–31] together with biochemical and biophysical studies [2,32,33] reveal the protein's structure and domain organization. SecA belongs to the Superfamily 2 of DExH/D (Asp–Glu–X–His/Asp) proteins, which include various helicases and nucleic acid modifying enzymes [34,35]. Two RecA folds – NBD (Nucleotide Binding Domain) and IRA2 (Intramolecular Regulator of the ATPase2) – comprise the DEAD motor. The two domains sandwich the ATP, which, through binding or hydrolysis, is the metabolic energy source for various translocation steps (Fig. 1A–B).

Members of the Superfamily 2 might share the tertiary structure of the DExH/D motor but their functional specificity is acquired by its extra subunits or domain. Likewise, two domains, absent from any other helicase, confer to SecA specificity for preproteins; the preprotein binding domain (PBD) and the C-domain (Fig. 1A–B) [43,44]. Each one of them is rooted in one of the two motor domains. Moreover, the two appendages physically associate between them. This way SecA recognizes a great variety of preproteins, binds to the channel and dimerizes in a very sophisticated, finely orchestrated manner. Also it has a structural framework through which all domains sense each other.

PBD sprouts out from the NDB through a Stem that includes two anti-parallel beta strands ($stem_{in}$ and $stem_{out}$) and ends up in a bilobate bulb structure. The Stem contacts the NBD, the C-domain and the Bulb.

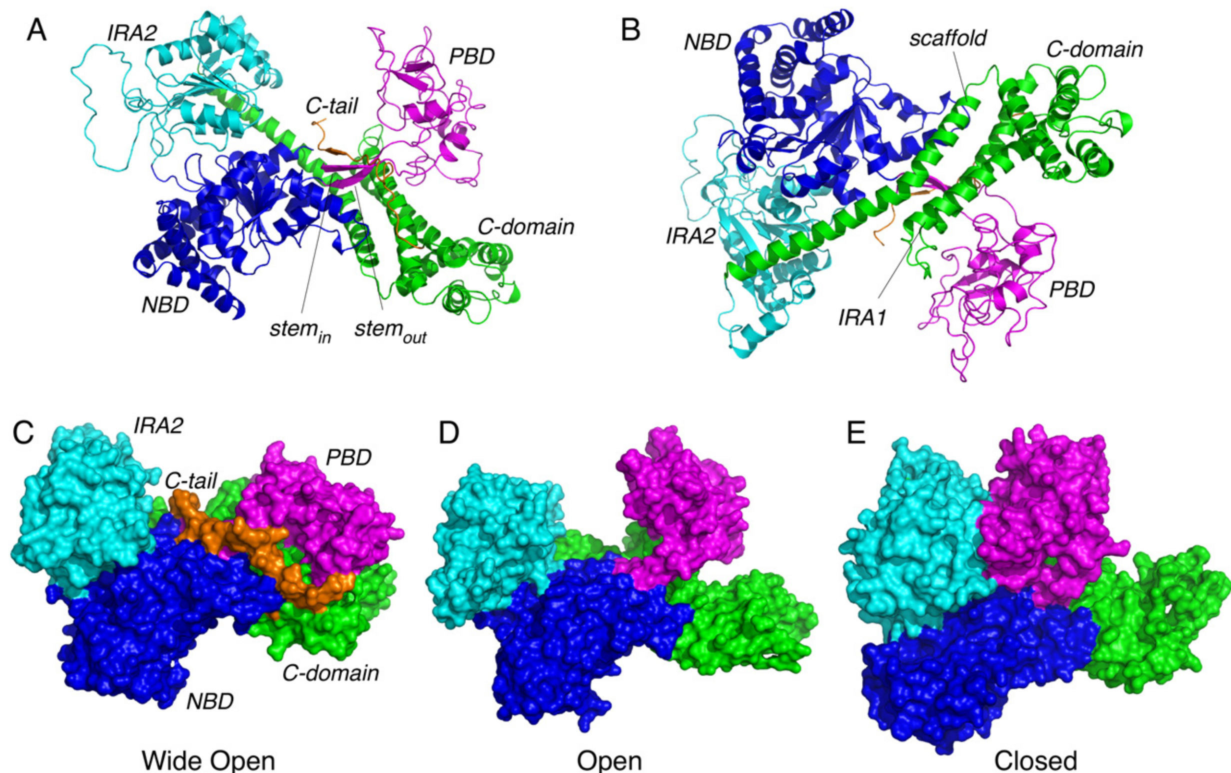

Fig. 1. (A) and (B); Models of the *Escherichia coli* SecA (PDB code: 2FSF) in the Open conformation based on PBD topology [30]. The four domains of SecA are: Nucleotide Binding Domain (NBD; blue), Intramolecular Regulator of ATP-hydrolysis 2 (IRA2; cyan), Preprotein Binding Domain (PBD; purple), C-domain; green. The additional structural elements are noted on each view of the structure. (C), (D), and (E); Space filling models of SecA protomers in three distinctive conformational states: (C) (1M6N; *Bacillus subtilis*) [28], (D) (2FSF; *E. coli*) [30] and (E) (3DIN; *Thermotoga maritima*) [26]. The orange region on the carboxyterminal region of SecA, termed C-tail (visualized only in A), controls preprotein domain docking on the translocase [28,36]. In the translocase complex, SecYEG would be bound at the back of each depicted structure.

The Bulb contributes to the binding of the signal peptide [36] and possibly the mature domain (Fig. 1A–B) [37].

The α-helical C-domain, fused C-terminally to the IRA2 domain of the DEAD motor, contains 4 sub-structures: a) the scaffold domain, a long α-helix that connects the C-domain with both NBD and IRA2, b) the wing domain, c) the conserved and motile helix-loop-helix IRA1 (also known as two helix finger) and d) an extreme C-terminal flexible tail which comprises two regions. The first one interacts with the core of SecA and ends up forming a β-sheet with the two antiparallel β-strands of the Stem [28] (Fig. 1). The second one contains a zinc-finger motif that binds zinc ions, lipids and the SecB chaperone [38–40]. Since the last part of the C-tail is highly motile, it has only been partially resolved crystallographically but it has been analysed by NMR [28,36].

3. The oligomeric state of SecA

Considering its intracellular concentration (5.7–8.2 μM) [41] and inter-protomer affinity (0.001–3 μM) [42–44], SecA is anticipated to function as a dimer (Fig. 2). There are six SecA crystal structures in apparent dimeric assembly [25,27,28,30,31,45] yet, the relative protomer positioning is unique in each one [46]. Several reports suggest that ligand binding [25,27,45], acidic phospholipids or detergents [54] can affect the dimeric state of SecA. Some of these reports are conflicting. Synthetic signal peptides either cause dissociation of the dimer [47] or, promote dimerization of previously monomerized SecA [48] or, have no effect [36]. SecB, in complex with a secretory protein, did not cause dissociation of the SecA dimer. Neither did ATP or ATP-γ-S nor ADP or liposome binding [49,50]. On the contrary, SANS (Small-Angle Neutron Scattering) measurements suggest that ADP–Mg^{2+} or binding of non-hydrolyzable ATP analogue may cause dimer dissociation on lipid-bound SecA [51] although it is not clear whether these relate to on-pathway SecYEG-bound SecA states.

Ensemble FRET studies that were carried out to dissect the oligomeric state of SecA during translocation suggest that it remains dimeric during catalysis [49]. Moreover, a SecA that had its two protomers covalently linked by cross-linkers remained functional, suggesting that complete dissociation of the protomers is not necessary [52–54]. Other studies support that SecA functions as a monomer in the presence of SecYEG [55–57]. In the crystal structure of the SecA–SecYEG complex, a continuous groove created by a single SecA protomer bound to SecY seemed to adequately allow preproteins to travel through it and led the authors to propose it as the minimal functional translocase unit [33,65] (Fig. 3B). However, the functionality of the complex is ambiguous due to the detergents used to achieve membrane solubilization (for a detailed review on the monomer–dimer studies see [46]).

A recent study presented the coupling of the quaternary dynamics of SecA with function [41]. The authors suggest that SecA does not exist as a conformationally unique dimer; various combinations of hydrophobic and/or electrostatic interactions allow sliding as well as rotational motions between the two protomers thus generating multiple possibilities of high affinity dimers with distinct roles (Fig. 2). By studying the functional properties of the otherwise elusive monomer, both in vitro as well as in vivo, the authors propose the following SecA quaternary states: Cytoplasmic SecA mainly exists in two conformationally distinct states: a major "electrostatic dimer" (95%) and a minor salt-resistant dimer (~5%); in the latter, interprotomer interactions, predominantly hydrophobic, stabilize an elongated quaternary state (Figs. 2 and 3A). SecYEG and signal peptide binding, induce a third state, the "triggered dimer". All of these dimers are connected to defined mechanistic steps of translocation. The SecA dimer docks on SecYEG yet, only one of its protomers makes direct contact with the channel. This holoenzyme reduces its activation energy upon signal peptide binding. Subsequent ATP hydrolysis monomerizes SecA. Beyond this point translocation can be completed by the monomer. Once the secretory protein is released the monomer will re-dimerize and a new cycle of events will be repeated (Fig. 4) [41].

4. Flexibility and disorder of the SecA nanomachine

SecA is a highly dynamic enzyme. Its plasticity is synchronized with its catalytic function. Below, we will describe the movements of the DEAD motor, PDB and C-domain.

4.1. The DEAD motor

The motor of SecA is so flexible that attachment of the C-domain provides for a fine mechanism to suppress and regulate its inherent flexibility and ATPase activity [58]. NMR studies on a SecA derivative which lacks the C-domain, revealed that the IRA2 domain remains substantially unstructured even at low temperatures (e.g. 22 °C; [59]). ADP binding stabilizes the DEAD motor, although its dynamic nature is retained and affects allosterically the conformational state of the PBD [59]. A salt bridge, formed between one positively (R566) and one negatively (D212) charged amino acyl residue at the base of the motor, is an internal switch, termed Gate 1. This Gate controls the opening and closure of the nucleotide cleft as well as the signal propagated from the PBD to the motor and that stimulates ATP hydrolysis [58].

4.2. The PBD

In all of the available SecA structures a clamp is formed between PDB and IRA2 (Fig. 1). This clamp becomes largely affected as the motile PBD adopts three different conformations: a) closed [26], b) open [30] and c) wide open [28] (Fig. 1C–E). In the wide open state PBD interacts extensively with the C-domain creating a large cleft [25,28] (Fig. 1E). In the open state PBD undergoes an ~60° rotation and exposes most of its surface to solvent [29,30] (Fig. 1D). Soluble SecA exists mainly in the open conformation (90%) or in the less populated wide open conformation (10%) [36]. On the contrary, the crystallized SecY-bound SecA is in the closed conformation [26] (Fig. 1E). A tripeptide, co-crystallized with *Bacillus subtilis* SecA was bound on the clamp [60]. If the tripeptide

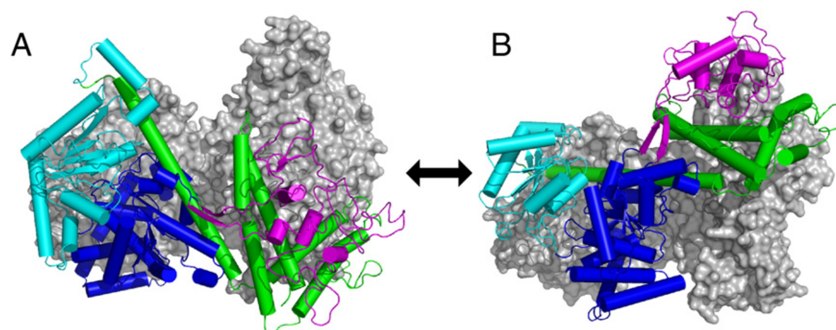

Fig. 2. Models of the interconverting SecA dimers in the cytoplasm. (A) A *ec*SecA dimer modeled after *mt*SecA 1NL3_1 [25] and (B) a *ec*SecA dimer modeled after *bs*SecA 1M6N [25,27,28]. The fixed protomer is coloured grey. The domains of the moving protomer are coloured as in Fig. 1.

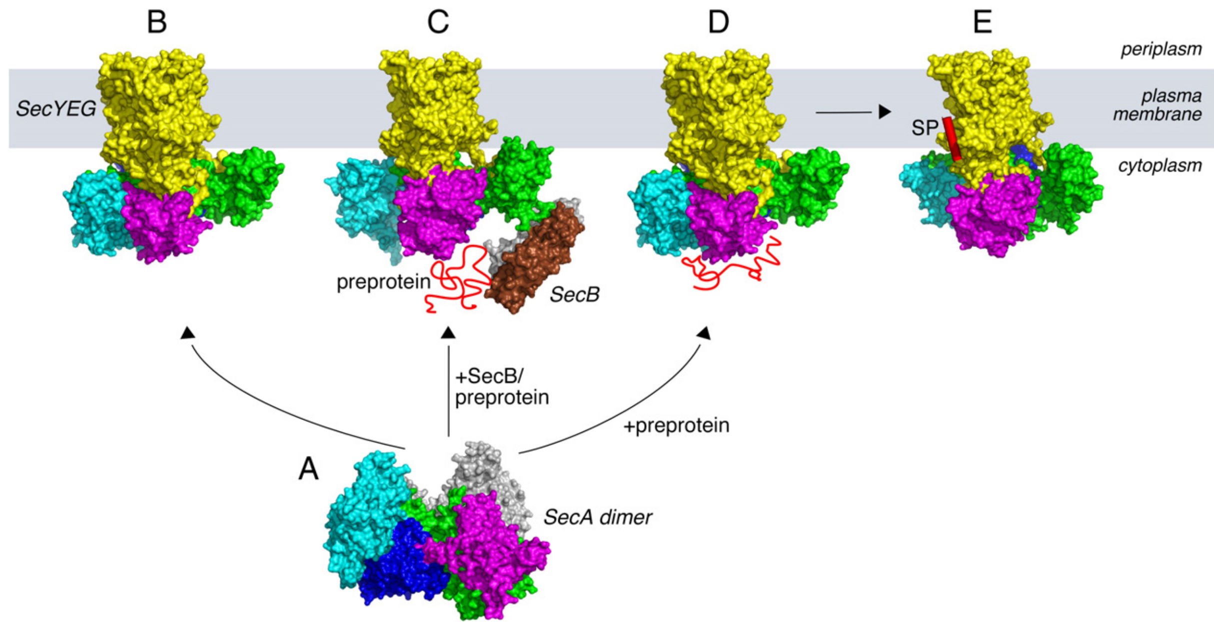

Fig. 3. Space-filling models of protein complexes that form during preprotein translocation. Potential complexes formed during preprotein translocation. (A) Model of the interconverting SecA dimers at the cytoplasm [41]. (B) Model of the translocase complex; SecYEG (yellow); SecA (domains indicated by their respective colour, NBD blue; PBD magenta; IRA2 cyan; C-domain green) (PBD code 3DIN [26]) (C) Hypothetical model of a SecB dimer docked on the translocase based on 3DIN [26] and 1OZB [39]. A preprotein (red line) has been drawn for visualization purposes (D) Cartoon of a preprotein (red line) bound on the translocase. (E) Model of a translocase with bound a LamB signal sequence, based on the cryo-EM structure solved structure [148].

is a true preprotein mimic, PBD motion is anticipated to correlate with preprotein targeting, as it would regulate access of preproteins to the SecA receptor. Moreover, immobilizing PBD, through disulfide cross-links, at different positions interfered with the initiation of the translocation process suggesting that PBD motility might be essential for SecA function [61].

4.3. The C-domain

A part of the signal peptide binding groove is occluded by the C-tail of SecA (Fig. 1E). Binding studies using Isothermal Titration Calorimetry demonstrated that the C-tail must be detached from its position for efficient signal sequence binding to occur [36].

Cross-linking experiments revealed that the loop at the tip of IRA1 (helix two finger) interacts with translocating polypeptides at the entrance of the SecY pore. Specifically, based on the association of the polypeptide chain with the bulky aromatic Tyr794 in *ec*SecA, the authors proposed that IRA1 pushes the peptide through the SecY channel [62]. However, it was recently suggested that the exact positioning of IRA1 might be vital, its motility is not, as its immobilization at the edge of the SecY channel did not affect translocation [63].

A recent study using circular dichroism, tryptophan fluorescence and limited proteolysis demonstrated that SecA undergoes several

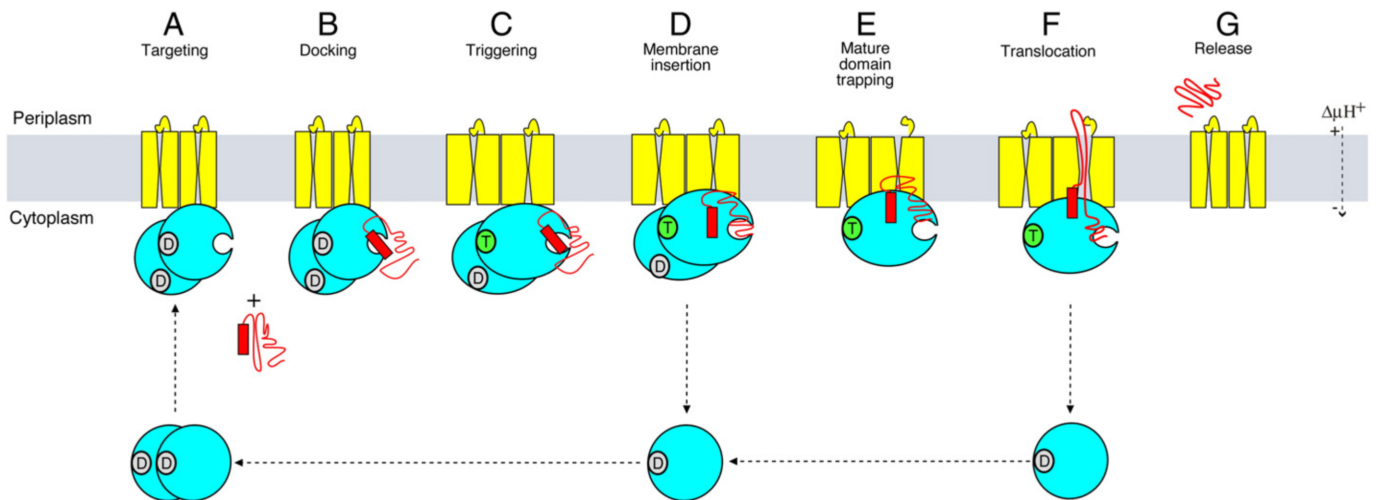

Fig. 4. Schematic representation of preprotein translocation steps. The model represents discrete steps that have been experimentally demonstrated and are described in detail in the text. SecYEG (drawn as dimers; yellow) and SecA (cyan) monomer to dimer transitions (arrows) are shown. Signal sequence (red rectangle), mature domain (red line), ADP (D) and ATP (T) are also indicated. Changes in the orientation of SecA protomers and the shape of SecA and SecYEG indicate conformational rearrangements that lead to the activation of the holoenzyme.

conformational reorganizations like partial unfolding of IRA2, partial dissociation of the scaffold domain from IRA2, and restructuring of the C-terminal region. These changes coincide with the appearance of high SecA ATPase activity and may thus mimic aspects of the translocation competent state [64].

5. SecA and ligands

5.1. SecA and the ribosome

SecA binds to the ribosome with 1:1 stoichiometry and a ~0.9 μM affinity [65]. The scaffold domain is necessary and sufficient to crosslink the protein on the L23 subunit of the ribosomal tunnel [65]. Thus, SecA may recognize the exiting, nascent preproteins and target them to the SecYEG channel. More intriguingly, it was suggested that it might act as a co-translational preprotein aid/selector; by binding on the signal sequences of translating chains it might assist in the transfer of the RNC to the SecYEG channel [66]. However, in such a case SecA would have to compete with binding factors like SRP or TF (see below, Section 6).

5.2. SecA and signal sequence binding

Signal sequences share conserved physicochemical properties. They are usually 15–40 amino acyl residues long and have a tri-partite structure: 1) a positively charged N terminal sequence, 2) a hydrophobic, helical core of 8–12 residues and, 3) a slightly polar, extended C-terminal domain which includes the cleavage site used by the signal peptidase [2,67,68]. Their affinity for SecA varies from medium to low (1–100 μM) [76,77,79,45].

Several studies focus on how signals interact with SecA [47,69,70]. One of them determined the structure of SecA complexed with a signal sequence, using NMR [36]. The signal sequence docks in an elongated groove formed at the interface of PBD and IRA1 and adopts an α-helical structure (Fig. 3E). Its N-terminal part participates in electrostatic interactions with negatively charged residues from the Bulb of the PBD. Its C-terminal region remains unstructured and solvent-exposed [36]. Additional surfaces, from the IRA1 and one from the NBD, were shown to participate in the signal sequence binding by a study that used Förster resonance energy transfer (FRET) experiments [77].

The signal sequence binding groove is relatively long (~28 Å), is mostly hydrophobic and surrounded by charged and polar amino acyl residues. These features and its multipartite structure explain how it can accommodate hundreds of signal sequences of varying charge and length. Taking into account that SecA promiscuously binds a variety of signal sequences using the same groove, one would anticipate a certain degree of degeneracy in the recognition process. At the same time signal sequences must be recognized with high fidelity. Single amino acid substitutions within a signal sequence reduce or even abolish secretion of the preprotein (e.g. proPhoA(L8Q) or proPhoA(L14R)) [16]. Interestingly, the necessity for a preprotein to have a signal sequence can be bypassed if mutant translocases are used. Such mutations, known as *prl* (for *pr*otein *l*ocalization), were isolated either within SecA or in the membrane-embedded translocase subunits [71]. Generally, *prl* are allosteric mutations, which mimic the effect that the signal sequence binding has on the translocase by shifting it to the active, "triggered" conformational state [16]. This allows secretion even of signal-less substrates, albeit very inefficiently [16,41,72], since in downstream strokes of the SecA motor the physical presence of the signal peptide is required.

5.3. SecA and mature domain binding

Preprotein mature domains and signal sequences bind at distinct SecA sites [16] (Fig. 3D,E). A long-standing dogma was that signal sequences are the only features responsible for targeting preproteins. However, it is now clear that mature domains can also be targeted independently of their signal sequences. In some cases they even exhibit better affinity for the translocase when alone than when fused to signal sequences [16]. It appears that for every preprotein separate contributions from the two distinct targeting elements have been fine tuned for optimal binding.

A high-resolution structure for SecA complexed with a mature domain is not currently available. The main technical challenge here is that the mature domain must be solved in a non-native conformation. Do preproteins bind in the clamp that is formed by the IRA2 and PBD domains? (Fig. 1) This was suggested by a study that immobilized, by cross-linking, a translocating preprotein chain on SecY and at various SecA positions [73]. In support of this idea, the structure of SecA with a tripeptide crystallized inside the clamp was solved [60]. However, a highly hydrophobic tripeptide cannot account for the properties of mature domains that span 40–2500 residues and have charged regions interspersed with short hydrophobic patches. On the other hand, it has been suggested that the Bulb of the PBD might be a mature domain binding site, as SecA Bulb mutants fail to load preproteins onto SecYEG [37]. Whether one of these or a different region or even multiple regions, acting in concert, represent the mature domain docking site on SecA it is still under investigation.

5.4. SecA and chaperones

SecB is a tetrameric chaperone organized as a dimer of dimers [74,75], that binds to a SecA dimer in solution with modest affinity (K_D ~1.7 μM) [85], using the zinc-binding site within the last 21C-tail residues (Fig. 3C). Two additional SecA sites, the scaffold domain [76] and the first 11 N-terminal residues [76], were proposed to interact with SecB. Since binding of SecB to SecYEG-bound SecA is much higher (~40 nM), it was proposed to act as a piloting factor for preproteins [77].

TF is a highly abundant bacterial chaperone that is considered important for the secretion of outer membrane proteins [90]. It binds to the ribosomal exit tunnel and to exiting polypeptides [78,79], preferentially of first 100 amino acyl residues [17]. There is no known direct interaction between TF and SecA and it is not clear in any mechanistic detail how TF would contribute to the secretion pathway.

5.5. SecA and SecYEG

The bacterial protein-conducting channel consists of the SecY, SecE and SecG proteins [80,81]. SecY is an essential, conserved, transmembrane protein, present in all domains of life [82,83]. It forms the central export pore and is embraced and stabilized by SecE [84,85]. SecG is not essential for viability or translocation but facilitates the binding of SecA on SecY [86] and its subsequent membrane insertion [87] (see below).

High resolution structures reveal that the central pore of the channel resembles an hourglass, with one opening facing the cytoplasm and the other the periplasmic or extra-cellular space [22,88,89]. The external opening is sealed by an α-helical "plug". The plug, along with the restriction of the pore, inhibits the leakage of small molecules and keeps the channel sealed [90]. During translocation the plug moves aside and the channel may be allowed to expand [91]. An additional feature of SecY is that its ten trans-membrane helices create a clamshell with its opening facing the lipid bilayer. The opening, known as lateral gate, allows the hydrophobic signal sequences and trans-membrane helices to diffuse into the lipid bilayer [92,93].

SecA interacts with the SecYEG channel with high affinity (~20–40 nM) [94] (Fig. 3B). Parts of NBD, IRA2 and the C-domain form a continuous groove with the SecY pore. PBD lies facing the middle of the pore [26]. The stoichiometry of the SecA–SecYEG complex remains controversial [46,95,96] but the 1:1 stoichiometry represented by the crystal structure [26] could represent the minimal translocation unit. A dynamic conversion from a $SecA_2$:$SecY_2$ to a $SecA_1$:$SecY_2$ in the process of translocation was recently proposed [41].

5.6. SecA and nucleotides

The nucleotide binds on NBD and IRA2 residues without causing major rearrangements yet restricting the flexibility of the SecA motor [25,28,30,36]. ATP gets immediately hydrolyzed by soluble SecA and the resulting ADP occupies the nucleotide cleft. Release of ADP becomes a limiting step as it thermally stabilizes the protein [97].

The short lived ATP–SecA state is prolonged at the membrane. The binding energy of ATP allows the membrane-bound SecA to acquire a different conformation. Significant parts of the protein now become protease resistant. ATP hydrolysis (or ADP binding) drives to the previous more protease sensitive SecA state. These steps are known as "membrane insertion" and "de-insertion" states respectively and are both essential for secretion [41,98,99] Repetitive cycles of ATP binding, hydrolysis and ADP release, together with the PMF are required for preprotein translocation [100–102]. The inherent hydrolytic activity of SecA (basal ATPase) increases upon binding to the SecYEG receptor (membrane ATPase) and gets further stimulated in the presence of preproteins (translocation ATPase) [58,103,104].

5.7. SecA and lipids

The inner membrane of *E. coli* is composed by phosphatidylethanolamine (PE) (75–80%), phosphatidylglycerol (PG) (20%) and cardiolipin (CL) (5%). PE stimulates the ATPase activity of SecA and affects protein translocation [105,106]. Ionic phospholipids, like PG and CL, are essential for SecA's ATPase [103], high affinity binding of SecA to SecY [120,121] and membrane insertion [107–109]. Moreover, together with derivatives that have long acyl chains, ionic phospholipids were proposed by in vitro experiment to promote SecA monomerization [48,56], a feature necessary for late steps of the catalytic cycle [41].

5.8. SecA and mRNA

SecA regulates its own translation by binding to its own mRNA. Mg^{2+} and ATP concentrations influence this interaction [110]. An additional mechanism proposed to regulate SecA's intracellular concentration involves the SecM gene (*sec*retion *m*onitor) that is translated prior to SecA on the same transcript. Its 3′ mRNA region causes a transient translational arrest that in turn facilitates the exposure of the Shine–Dalgarno sequence of the succeeding SecA mRNA, thus upregulating its biosynthesis. The arrest is reversed by the active secretion of SecM. Prolonged arrest, due to the lack of SecA molecules, upregulates their synthesis [111,112]. SecM is secreted to the periplasm where it is cleaved by the Prc protease [113], so mainly its role in the cell is to monitor its own secretion and regulate the extent of the translation arrest. SecA also possesses an RNA helicase activity [114] but that is not necessary for protein translocation or regulation of its own translation [115]. It is not clear how wide-spread these regulatory mechanisms are beyond *E. coli*.

6. SecA-mediated preprotein targeting

So far we have discussed the role of SecA during translocation. Can SecA also target secretory preproteins? To address this we need to follow the preproteins as they exit the ribosome. SRP, TF and SecA all bind on the same ribosomal region [65,116,117]. Which one of these factors is going to latch onto the exiting polypeptide? Clearly, the intracellular concentrations of these factors, their stoichiometric excess over the ribosomes, their respective affinities for the ribosome and their involvement in other non-ribosomal processes need to be considered.

SRP is in low cellular amounts (0.4 μM) and is vastly substoichiometric compared to ribosomes (~20 μM). It has a very high affinity for nascent hydrophobic trans-membrane segments and very hydrophobic signal sequences (1–10 nM) [4,118]. SRP probably binds its substrates first, due to its high affinity, and this would sequester those proteins away from the post-translational secretion pathway.

SecA and TF have similar affinities for the ribosome (0.9 and 1 μM respectively; [65,119]) and both have the potential to bind a nascent preprotein. TF is found in the cell at a concentration of 40–50 μM; almost 7 times more abundant than SecA (5.7–8.2 μM) [41,56,120] but is also involved in the general folding of the cytoplasmic proteome. Usually, TF is likely to interact first with the nascent exiting polypeptide. In the absence of available TF, SecA might have a better chance to bind substrates co-translationally and target them to the SecYEG channel [66]. Under the same conditions more SecA associated with the inner membrane [41,56,120].

SecB can bind on preproteins after their first ~150 amino acyl residues are synthesized [121]. SecB is found also in high intracellular concentration (4–20 μM) [122,123] and there are known examples of substrate delivery from SecB to SecA [122]. Baars and colleagues, using a SecB null mutant and comparative proteomics analysis, identified 12 SecB dependent secretory substrates. Six more proteins were already shown to be SecB-dependant [75,124,125]. The rest of the secretome is not affected by the absence of this chaperone [126]. Although it cannot be excluded that SecB binds more preproteins in vivo, this result indicated that more than one chaperone might recognize the same set of secretory proteins. Alternatively, preproteins can be efficiently targeted un-chaperoned. This is supported by findings that preproteins can exist in non-native, translocation-competent states in solution in the absence of any chaperone [16].

7. SecA as a translocation motor

Combining results, that have been accumulated over the past years, from several different labs we will provide an overview of post-translational secretion in bacteria, emphasizing the role of SecA and relying heavily on the *E. coli* paradigm (Fig. 4).

1. ADP–SecA exists as a tight electrostatic dimer in the cytoplasm. It also docks on the SecYEG channel, with a 2:2 stoichiometry, using only one of its two protomers. An approximate 50:50 distribution between the membrane bound and the cytoplasmic SecA is thought to govern this equilibrium in the cell [41] (Fig. 4A).
2. A preprotein diffusing in the cytoplasm either alone or bound to chaperones like SecB, binds stochastically onto SecA [122]. The signal sequence and the mature domain dock independently on their respective SecA docking sites. Unlike the soluble SecA dimer that can accommodate two preprotein molecules, only one is bound on the SecA–SecYEG translocase with high affinity [41] (Fig. 4B).
3. The binding of the signal sequence lowers the activation energy of the holoenzyme (SecA–SecYEG) in a step known as "triggering" [16]. The conformation of the translocase now undergoes an allosteric change; a state mimicked by prl mutations on either SecA or SecY [41] (Fig. 4C).
4. Triggering stabilizes the SecA membrane-inserted state [98] (Fig. 4D). The mature domains of secretory chains are not yet fully engaged in the channel [16].
5. The preprotein becomes "trapped", presumably by forming addition contacts through its mature domain with the SecA triggered dimer [16]. SecA monomerizes at this step and proceeds in catalyzing multiple rounds of ATP hydrolysis (Fig. 4E).
6. ATP hydrolysis by SecA becomes coupled to mechanical work and will eventually mediate complete transfer of the polypeptide chain [48] (Fig. 4E). In every catalytic cycle of ATP hydrolysis 20–30 amino acyl residues of the preprotein are translocated [31].
   To stimulate ATP hydrolysis, binding of the mature domain onto the PBD is allosterically transmitted to the ATPase motor. The base of the motor opens [58], the IRA2 domain becomes disordered and detaches from the NBD. These events loosen up the NBD-IRA2 interface and the previously bound ADP can now diffuse out and is easily

exchanged for ATP [59,102] (Fig. 4E).
PMF affects different stages of the translocation process [100,127,128]. It has been reported that it facilitates the insertion and possibly the orientation of the signal sequences as translocation initiates [129]. Moreover, SecA de-insertion from the membrane is accelerated by PMF either by promoting ADP release from SecA [130,131] or by conformational changes in SecY [132,133] (Fig. 4E).
7. Association of the signal sequence with the lateral gate of SecY causes the periplasmic plug to flip out [91]. Next the signal sequence can diffuse into the bilayer, where the signal peptidase I (SPase II for lipoproteins) whose catalytic domain resides in the *trans* side of the membrane, can cleave it off [134,135]. Signal sequences can later become degraded [136] (Fig. 4F).

The mature domain is released in the periplasm possibly with the aid of SecDF [137]. It either remains there and folds to a native state or, it becomes embedded in the outer membrane or, it gets secreted to the extracellular space [2] (Fig. 4G). Periplasmic proteins fold with the assistance of chaperones like Skp, prolyl isomerases (like PpiA) and cysteine oxidases (like the Dsb protein family) [138,139]. Secretion machines, such as the BAM (β-barrel assembly machinery) and TAM (translocation and assembly module) systems [140–142] or the LOL (lipoprotein outer membrane localization) system for lipoproteins [143], assist the transport to the outer membrane [144]. Specialized machines like the type II and the Curli secretion systems might assist in further extracellular export [145–147].

8. Conclusions and perspectives

Significant progress has been achieved in understanding the role of SecA during translocation. Yet, many questions remain unanswered. Where is the docking site of mature domains on SecA? How does SecA achieve such a high specificity in binding preproteins that have degenerate sequences? How do mature domains promote SecA monomerization during the late stages of translocation? If the mobility of IRA1 is unnecessary, is there some other mechanical event that allows the preprotein to move forward inside the channel or is forward movement explained by a Brownian ratchet mechanism? How does the signal sequence detach from its binding site on SecA to end up in the SecYEG lateral gate?

As far as the role of SecA in targeting is concerned we are missing in vivo data of what is happening in the presence of all chaperones in the complex milieu of the cell. Is SecA a primary means of preprotein delivery to the membrane? Does it already act at the level of the ribosome? What are the signals, unknown so far, that allow non-native preprotein mature domains to be recognized by SecA? High resolution studies in the presence of the membrane and in vivo real time experiments using high resolution optophysics tools will shed light on the role of SecA during targeting and translocation as well as further probe the function of this unique nanomotor.

Acknowledgements

Research in our lab was funded by grants (to AE): Nonaco (Aristeia-Excellence; #1473; Operational Programme for Education and Lifelong Learning co-funded by the European Social Fund and National Resources); KUL-Spa (Onderzoekstoelagen 2013; Bijzonder Onderzoeksfonds; KU Leuven); RiMembR (Vlaanderen Onderzoeksprojecten; #G0C6814N; FWO); StrepSynth (FP7 KBBE.2013.3.6-02: Synthetic Biology towards applications; #613877; EU).

References

[1] A. Economou, P.J. Christie, R.C. Fernandez, T. Palmer, G.V. Plano, A.P. Pugsley, Secretion by numbers: protein traffic in prokaryotes, Mol. Microbiol. 62 (2006) 308–319.
[2] K.E. Chatzi, M.F. Sardis, S. Karamanou, A. Economou, Breaking on through to the other side: protein export through the bacterial Sec system, Biochem. J. 449 (2013) 25–37.
[3] A.R. Osborne, T.A. Rapoport, B. van den Berg, Protein translocation by the Sec61/SecY channel, Annu. Rev. Cell Dev. Biol. 21 (2005) 529–550.
[4] P. Grudnik, G. Bange, I. Sinning, Protein targeting by the signal recognition particle, Biol. Chem. 390 (2009) 775–782.
[5] S.Q. Gu, F. Peske, H.J. Wieden, M.V. Rodnina, W. Wintermeyer, The signal recognition particle binds to protein L23 at the peptide exit of the *Escherichia coli* ribosome, RNA 9 (2003) 566–573.
[6] G.J. Phillips, T.J. Silhavy, The *E. coli* Ffh gene is necessary for viability and efficient protein export, Nature 359 (1992) 744–746.
[7] N. Bradshaw, S.B. Neher, D.S. Booth, P. Walter, Signal sequences activate the catalytic switch of SRP RNA, Science 323 (2009) 127–130.
[8] J. Luirink, C.M. ten Hagen-Jongman, C.C. van der Weijden, B. Oudega, S. High, B. Dobberstein, R. Kusters, An alternative protein targeting pathway in *Escherichia coli*: studies on the role of FtsY, EMBO J. 13 (1994) 2289–2296.
[9] L. Bahari, R. Parlitz, A. Eitan, G. Stjepanovic, E.S. Bochkareva, I. Sinning, E. Bibi, Membrane targeting of ribosomes and their release require distinct and separable functions of FtsY, J. Biol. Chem. 282 (2007) 32168–32175.
[10] P. Peluso, S.O. Shan, S. Nock, D. Herschlag, P. Walter, Role of SRP RNA in the GTPase cycles of Ffh and FtsY, Biochemistry 40 (2001) 15224–15233.
[11] S.B. Neher, N. Bradshaw, S.N. Floor, J.D. Gross, P. Walter, SRP RNA controls a conformational switch regulating the SRP–SRP receptor interaction, Nat. Struct. Mol. Biol. 15 (2008) 916–923.
[12] S.O. Shan, SRP-Mediated Protein Targeting to the Inner Membrane, 2014. (in this issue).
[13] D. Huber, D. Boyd, Y. Xia, M.H. Olma, M. Gerstein, J. Beckwith, Use of thioredoxin as a reporter to identify a subset of *Escherichia coli* signal sequences that promote signal recognition particle-dependent translocation, J. Bacteriol. 187 (2005) 2983–2991.
[14] D. Zhang, M.J. Sweredoski, R.L. Graham, S. Hess, S.O. Shan, Novel proteomic tools reveal essential roles of SRP and importance of proper membrane protein biogenesis, Mol. Cell. Proteomics 11 (2012) (M111 011585).
[15] C. Lee, J. Beckwith, Cotranslational and posttranslational protein translocation in prokaryotic systems, Annu. Rev. Cell Biol. 2 (1986) 315–336.
[16] G. Gouridis, S. Karamanou, I. Gelis, C.G. Kalodimos, A. Economou, Signal peptides are allosteric activators of the protein translocase, Nature 462 (2009) 363–367.
[17] E. Oh, A.H. Becker, A. Sandikci, D. Huber, R. Chaba, F. Gloge, R.J. Nichols, A. Typas, C.A. Gross, G. Kramer, J.S. Weissman, B. Bukau, Selective ribosome profiling reveals the cotranslational chaperone action of trigger factor in vivo, Cell 147 (2011) 1295–1308.
[18] P. Genevaeux, SecB, Trigger Factor and Other Factors Targeting Proteins to the Inner Membrane in *E. coli*, 2014. (in this issue).
[19] C.A. Kumamoto, J. Beckwith, Mutations in a new gene, secB, cause defective protein localization in *Escherichia coli*, J. Bacteriol. 154 (1983) 253–260.
[20] R.S. Ullers, D. Ang, F. Schwager, C. Georgopoulos, P. Genevaux, Trigger factor can antagonize both SecB and DnaK/DnaJ chaperone functions in *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3101–3106.
[21] J.P. Muller, S. Bron, G. Venema, J.M. van Dijl, Chaperone-like activities of the CsaA protein of *Bacillus subtilis*, Microbiology 146 (Pt 1) (2000) 77–88.
[22] B. Van den Berg, W.M. Clemons Jr., I. Collinson, Y. Modis, E. Hartmann, S.C. Harrison, T.A. Rapoport, X-ray structure of a protein-conducting channel, Nature 427 (2004) 36–44.
[23] S. Haider, B.A. Hall, M.S. Sansom, Simulations of a protein translocation pore: SecY, Biochemistry 45 (2006) 13018–13024.
[24] F. Bonardi, E. Halza, M. Walko, F.Du. Plessis, N. Nouwen, B.L. Feringa, A.J. Driessen, Probing the SecYEG translocation pore size with preproteins conjugated with sizable rigid spherical molecules, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 7775–7780.
[25] V. Sharma, A. Arockiasamy, D.R. Ronning, C.G. Savva, A. Holzenburg, M. Braunstein, W.R. Jacobs Jr., J.C. Sacchettini, Crystal structure of Mycobacterium tuberculosis SecA, a preprotein translocating ATPase, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 2243–2248.
[26] J. Zimmer, Y.S. Nam, T.A. Rapoport, Structure of a complex of the ATPase SecA and the protein-translocation channel, Nature 455 (2008) 936–943.
[27] J. Zimmer, W. Li, T.A. Rapoport, A novel dimer interface and conformational changes revealed by an X-ray structure of B. subtilis SecA, J. Mol. Biol. 364 (2006) 259–265.
[28] J.F. Hunt, S. Weinkauf, L. Henry, J.J. Fak, P. McNicholas, D.B. Oliver, J. Deisenhofer, Nucleotide control of interdomain interactions in the conformational reaction cycle of SecA, Science 297 (2002) 2018–2026.
[29] A.R. Osborne, W.M. Clemons Jr., T.A. Rapoport, A large conformational change of the translocation ATPase SecA, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10937–10942.
[30] Y. Papanikolau, M. Papadovasilaki, R.B. Ravelli, A.A. McCarthy, S. Cusack, A. Economou, K. Petratos, Structure of dimeric SecA, the *Escherichia coli* preprotein translocase motor, J. Mol. Biol. 366 (2007) 1545–1557.
[31] D.G. Vassylyev, H. Mori, M.N. Vassylyeva, T. Tsukazaki, Y. Kimura, T.H. Tahirov, K. Ito, Crystal structure of the translocation ATPase SecA from *Thermus thermophilus* reveals a parallel, head-to-head dimer, J. Mol. Biol. 364 (2006) 248–258.
[32] I. Kusters, A.J. Driessen, SecA, a remarkable nanomachine, Cell. Mol. Life Sci. 68 (2011) 2053–2066.
[33] E. Vrontou, A. Economou, Structure and function of SecA, the preprotein translocase nanomotor, Biochim. Biophys. Acta 1694 (2004) 67–80.

[34] E.V. Koonin, A.E. Gorbalenya, Autogenous translation regulation by *Escherichia coli* ATPase SecA may be mediated by an intrinsic RNA helicase activity of this protein, FEBS Lett. 298 (1992) 6–8.
[35] Q. Yang, E. Jankowsky, ATP- and ADP-dependent modulation of RNA unwinding and strand annealing activities by the DEAD-box protein DED1, Biochemistry 44 (2005) 13591–13601.
[36] I. Gelis, A.M. Bonvin, D. Keramisanou, M. Koukaki, G. Gouridis, S. Karamanou, A. Economou, C.G. Kalodimos, Structural basis for signal-sequence recognition by the translocase motor SecA as determined by NMR, Cell 131 (2007) 756–769.
[37] E. Papanikou, S. Karamanou, C. Baud, M. Frank, G. Sianidis, D. Keramisanou, C.G. Kalodimos, A. Kuhn, A. Economou, Identification of the preprotein binding domain of SecA, J. Biol. Chem. 280 (2005) 43209–43217.
[38] E. Breukink, N. Nouwen, A. van Raalte, S. Mizushima, J. Tommassen, B. de Kruijff, The C terminus of SecA is involved in both lipid binding and SecB binding, J. Biol. Chem. 270 (1995) 7902–7907.
[39] J. Zhou, Z. Xu, Structural determinants of SecB recognition by SecA in bacterial protein translocation, Nat. Struct. Biol. 10 (2003) 942–947.
[40] P. Fekkes, J.G. de Wit, A. Boorsma, R.H. Friesen, A.J. Driessen, Zinc stabilizes the SecB binding site of SecA, Biochemistry 38 (1999) 5111–5116.
[41] G. Gouridis, S. Karamanou, M.F. Sardis, M.A. Schärer, G. Capitani, A. Economou, Quaternary Dynamics of the SecA Motor Drive Translocase Catalysis, Molecular Cell, 2013.
[42] R.L. Woodbury, S.J. Hardy, L.L. Randall, Complex behavior in solution of homodimeric SecA, Protein Sci. 11 (2002) 875–882.
[43] I. Kusters, G. van den Bogaart, A. Kedrov, V. Krasnikov, F. Fulyani, B. Poolman, A.J. Driessen, Quaternary structure of SecA in solution and bound to SecYEG probed at the single molecule level, Structure 19 (2011) 430–439.
[44] A.J. Wowor, D. Yu, D.A. Kendall, J.L. Cole, Energetics of SecA dimerization, J Mol Biol 408 (2011) 87–98.
[45] Y. Chen, X. Pan, Y. Tang, S. Quan, P.C. Tai, S.F. Sui, Full-length *Escherichia coli* SecA dimerizes in a closed conformation in solution as determined by cryo-electron microscopy, J. Biol. Chem. 283 (2008) 28783–28787.
[46] M.F. Sardis, A. Economou, SecA: a tale of two protomers, Mol. Microbiol. 76 (2010) 1070–1081.
[47] M. Musial-Siwek, S.L. Rusch, D.A. Kendall, Selective photoaffinity labeling identifies the signal peptide binding domain on SecA, J. Mol. Biol. 365 (2007) 637–648.
[48] J. Benach, Y.T. Chou, J.J. Fak, A. Itkin, D.D. Nicolae, P.C. Smith, G. Wittrock, D.L. Floyd, C.M. Golsaz, L.M. Gierasch, J.F. Hunt, Phospholipid-induced monomerization and signal-peptide-induced oligomerization of SecA, J. Biol. Chem. 278 (2003) 3628–3638.
[49] A.J. Driessen, SecA, the peripheral subunit of the *Escherichia coli* precursor protein translocase, is functional as a dimer, Biochemistry 32 (1993) 13190–13197.
[50] B. Shilton, D.I. Svergun, V.V. Volkov, M.H. Koch, S. Cusack, A. Economou, *Escherichia coli* SecA shape and dimensions, FEBS Lett. 436 (1998) 277–282.
[51] Z. Bu, L. Wang, D.A. Kendall, Nucleotide binding induces changes in the oligomeric state and conformation of Sec A in a lipid environment: a small-angle neutron-scattering study, J. Mol. Biol. 332 (2003) 23–30.
[52] J. de Keyzer, E.O. van der Sluis, R.E. Spelbrink, N. Nijstad, B. de Kruijff, N. Nouwen, C. van der Does, A.J. Driessen, Covalently dimerized SecA is functional in protein translocation, J. Biol. Chem. 280 (2005) 35255–35260.
[53] H. Wang, B. Na, H. Yang, P.C. Tai, Additional in vitro and in vivo evidence for SecA functioning as dimers in the membrane: dissociation into monomers is not essential for protein translocation in *Escherichia coli*, J. Bacteriol. 190 (2008) 1413–1418.
[54] L.B. Jilaveanu, D. Oliver, SecA dimer cross-linked at its subunit interface is functional for protein translocation, J. Bacteriol. 188 (2006) 335–338.
[55] M. Alami, K. Dalal, B. Lelj-Garolla, S.G. Sligar, F. Duong, Nanodiscs unravel the interaction between the SecYEG channel and its cytosolic partner SecA, EMBO J. 26 (2007) 1995–2004.
[56] E. Or, A. Navon, T. Rapoport, Dissociation of the dimeric SecA ATPase during protein translocation across the bacterial membrane, EMBO J. 21 (2002) 4470–4479.
[57] E. Or, D. Boyd, S. Gon, J. Beckwith, T. Rapoport, The bacterial ATPase SecA functions as a monomer in protein translocation, J. Biol. Chem. 280 (2005) 9097–9105.
[58] S. Karamanou, G. Gouridis, E. Papanikou, G. Sianidis, I. Gelis, D. Keramisanou, E. Vrontou, C.G. Kalodimos, A. Economou, Preprotein-controlled catalysis in the helicase motor of SecA, EMBO J. 26 (2007) 2904–2914.
[59] D. Keramisanou, N. Biris, I. Gelis, G. Sianidis, S. Karamanou, A. Economou, C.G. Kalodimos, Disorder–order folding transitions underlie catalysis in the helicase motor of SecA, Nat. Struct. Mol. Biol. 13 (2006) 594–602.
[60] J. Zimmer, T.A. Rapoport, Conformational flexibility and peptide interaction of the translocation ATPase SecA, J. Mol. Biol. 394 (2009) 606–612.
[61] V.A. Gold, S. Whitehouse, A. Robson, I. Collinson, The dynamic action of SecA during the initiation of protein translocation, Biochem. J. 449 (2013) 695–705.
[62] K.J. Erlandson, S.B.M. Miller, Y. Nam, A.R. Osborne, J. Zimmer, T.A. Rapoport, A role for the two-helix finger of the SecA ATPase in protein translocation, Nature 455 (2008) 984–987.
[63] S. Whitehouse, V.A. Gold, A. Robson, W.J. Allen, R.B. Sessions, I. Collinson, Mobility of the SecA 2-helix-finger is not essential for polypeptide translocation via the SecYEG complex, J. Cell Biol. 199 (2012) 919–929.
[64] J.L. Maki, B. Krishnan, L.M. Gierasch, Using a low denaturant model to explore the conformational features of translocation-active SecA, Biochemistry 51 (2012) 1369–1379.
[65] D. Huber, N. Rajagopalan, S. Preissler, M.A. Rocco, F. Merz, G. Kramer, B. Bukau, SecA interacts with ribosomes in order to facilitate posttranslational translocation in bacteria, Mol. Cell 41 (2011) 343–353.
[66] A.L. Karamyshev, A.E. Johnson, 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 (2005) 37930–37940.
[67] R.S. Hegde, H.D. Bernstein, The surprising complexity of signal sequences, Trends Biochem. Sci. 31 (2006) 563–571.
[68] M. Paetzel, Signal Peptide Processing by Signal Peptidases I and II, 2014. (in this issue).
[69] M. Musial-Siwek, S.L. Rusch, D.A. Kendall, Probing the affinity of SecA for signal peptide in different environments, Biochemistry 44 (2005) 13987–13996.
[70] S.M. Auclair, J.P. Moses, M. Musial-Siwek, D.A. Kendall, D.B. Oliver, I. Mukerji, Mapping of the signal peptide-binding domain of *Escherichia coli* SecA using Forster resonance energy transfer, Biochemistry 49 (2010) 782–792.
[71] K.L. Bieker, G.J. Phillips, T.J. Silhavy, The sec and prl genes of *Escherichia coli*, J. Bioenerg. Biomembr. 22 (1990) 291–310.
[72] A.I. Derman, J.W. Puziss, P.J. Bassford Jr., J. Beckwith, A signal sequence is not required for protein export in prlA mutants of *Escherichia coli*, EMBO J. 12 (1993) 879–888.
[73] B.W. Bauer, T.A. Rapoport, Mapping polypeptide interactions of the SecA ATPase during translocation, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 20800–20805.
[74] Z. Xu, J.D. Knafels, K. Yoshino, Crystal structure of the bacterial protein export chaperone secB, Nat. Struct. Biol. 7 (2000) 1172–1177.
[75] C. Dekker, B. de Kruijff, P. Gros, Crystal structure of SecB from *Escherichia coli*, J. Struct. Biol. 144 (2003) 313–319.
[76] L.L. Randall, J.M. Crane, G. Liu, S.J. Hardy, Sites of interaction between SecA and the chaperone SecB, two proteins involved in export, Protein Sci. 13 (2004) 1124–1133.
[77] F.U. Hartl, S. Lecker, E. Schiebel, J.P. Hendrick, W. Wickner, The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane, Cell 63 (1990) 269–279.
[78] V.R. Agashe, S. Guha, H.C. Chang, P. Genevaux, M. Hayer-Hartl, M. Stemp, C. Georgopoulos, F.U. Hartl, J.M. Barral, Function of trigger factor and DnaK in multidomain protein folding: increase in yield at the expense of folding speed, Cell 117 (2004) 199–209.
[79] E. Crooke, B. Guthrie, S. Lecker, R. Lill, W. Wickner, ProOmpA is stabilized for membrane translocation by either purified *E. coli* trigger factor or canine signal recognition particle, Cell 54 (1988) 1003–1011.
[80] L. Brundage, J.P. Hendrick, E. Schiebel, A.J. Driessen, W. Wickner, The purified *E. coli* integral membrane protein SecY/E is sufficient for reconstitution of SecA-dependent precursor protein translocation, Cell 62 (1990) 649–657.
[81] A. Driessen, Structure-Function of the SecYEG Translocase, 2014. (in this issue).
[82] T.A. Rapoport, Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes, Nature 450 (2007) 663–669.
[83] J. Gumbart, K. Schulten, Molecular dynamics studies of the archaeal translocon, Biophys. J. 90 (2006) 2356–2367.
[84] C.R. Harris, T.J. Silhavy, Mapping an interface of SecY (PrlA) and SecE (PrlG) by using synthetic phenotypes and in vivo cross-linking, J. Bacteriol. 181 (1999) 3438–3444.
[85] C.K. Murphy, J. Beckwith, Residues essential for the function of SecE, a membrane component of the *Escherichia coli* secretion apparatus, are located in a conserved cytoplasmic region, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 2557–2561.
[86] K. Nishiyama, T. Suzuki, H. Tokuda, Inversion of the membrane topology of SecG coupled with SecA-dependent preprotein translocation, Cell 85 (1996) 71–81.
[87] H. Suzuki, K. Nishiyama, H. Tokuda, Coupled structure changes of SecA and SecG revealed by the synthetic lethality of the secAcsR11 and delta secG::kan double mutant, Mol. Microbiol. 29 (1998) 331–341.
[88] C. Breyton, W. Haase, T.A. Rapoport, W. Kuhlbrandt, I. Collinson, Three-dimensional structure of the bacterial protein-translocation complex SecYEG, Nature 418 (2002) 662–665.
[89] T. Tsukazaki, H. Mori, S. Fukai, R. Ishitani, T. Mori, N. Dohmae, A. Perederina, Y. Sugita, D.G. Vassylyev, K. Ito, O. Nureki, Conformational transition of Sec machinery inferred from bacterial SecYE structures, Nature 455 (2008) 988–991.
[90] E. Park, T.A. Rapoport, Preserving the membrane barrier for small molecules during bacterial protein translocation, Nature 473 (2011) 239–242.
[91] P.C. Tam, A.P. Maillard, K.K. Chan, F. Duong, Investigating the SecY plug movement at the SecYEG translocation channel, EMBO J. 24 (2005) 3380–3388.
[92] K. Plath, W. Mothes, B.M. Wilkinson, C.J. Stirling, T.A. Rapoport, Signal sequence recognition in posttranslational protein transport across the yeast ER membrane, Cell 94 (1998) 795–807.
[93] S.U. Heinrich, W. Mothes, J. Brunner, T.A. Rapoport, The Sec61p complex mediates the integration of a membrane protein by allowing lipid partitioning of the transmembrane domain, Cell 102 (2000) 233–244.
[94] K. Douville, A. Price, J. Eichler, A. Economou, W. Wickner, SecYEG and SecA are the stoichiometric components of preprotein translocase, J. Biol. Chem. 270 (1995) 20106–20111.
[95] E. Park, T.A. Rapoport, Bacterial protein translocation requires only one copy of the SecY complex in vivo, J. Cell Biol. 198 (2012) 881–893.
[96] K. Deville, V.A.M. Gold, A. Robson, S. Whitehouse, R.B. Sessions, S.A. Baldwin, S.E. Radford, I. Collinson, The oligomeric state and arrangement of the active bacterial translocon, J. Biol. Chem. 286 (2011) 4659–4669.
[97] J.J. Fak, A. Itkin, D.D. Ciobanu, E.C. Lin, X.J. Song, Y.T. Chou, L.M. Gierasch, J.F. Hunt, Nucleotide exchange from the high-affinity ATP-binding site in SecA is the rate-limiting step in the ATPase cycle of the soluble enzyme and occurs through a specialized conformational state, Biochemistry 43 (2004) 7307–7327.
[98] A. Economou, W. Wickner, SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion, Cell 78 (1994) 835–843.

[99] J. Eichler, W. Wickner, Both an N-terminal 65-kDa domain and a C-terminal 30-kDa domain of SecA cycle into the membrane at SecYEG during translocation, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 5574–5581.
[100] E. Schiebel, A.J. Driessen, F.U. Hartl, W. Wickner, Delta mu H+ and ATP function at different steps of the catalytic cycle of preprotein translocase, Cell 64 (1991) 927–939.
[101] S. Karamanou, E. Vrontou, G. Sianidis, C. Baud, T. Roos, A. Kuhn, A.S. Politou, A. Economou, A molecular switch in SecA protein couples ATP hydrolysis to protein translocation, Mol. Microbiol. 34 (1999) 1133–1145.
[102] G. Sianidis, S. Karamanou, E. Vrontou, K. Boulias, K. Repanas, N. Kyrpides, A.S. Politou, A. Economou, Cross-talk between catalytic and regulatory elements in a DEAD motor domain is essential for SecA function, EMBO J. 20 (2001) 961–970.
[103] R. Lill, W. Dowhan, W. Wickner, The ATPase activity of SecA is regulated by acidic phospholipids, SecY, and the leader and mature domains of precursor proteins, Cell 60 (1990) 271–280.
[104] A. Economou, J.A. Pogliano, J. Beckwith, D.B. Oliver, W. Wickner, SecA membrane cycling at SecYEG is driven by distinct ATP binding and hydrolysis events and is regulated by SecD and SecF, Cell 83 (1995) 1171–1181.
[105] A.G. Rietveld, M.C. Koorengevel, B. de Kruijff, Non-bilayer lipids are required for efficient protein transport across the plasma membrane of Escherichia coli, EMBO J. 14 (1995) 5506–5513.
[106] C. van der Does, J. Swaving, W. van Klompenburg, A.J. Driessen, Non-bilayer lipids stimulate the activity of the reconstituted bacterial protein translocase, J. Biol. Chem. 275 (2000) 2472–2478.
[107] E. Breukink, R.A. Demel, G. de Korte-Kool, B. de Kruijff, SecA insertion into phospholipids is stimulated by negatively charged lipids and inhibited by ATP: a monolayer study, Biochemistry 31 (1992) 1119–1124.
[108] N.D. Ulbrandt, E. London, D.B. Oliver, 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 (1992) 15184–15192.
[109] F. van Voorst, C. van der Does, J. Brunner, A.J. Driessen, B. de Kruijff, Translocase-bound SecA is largely shielded from the phospholipid acyl chains, Biochemistry 37 (1998) 12261–12268.
[110] K.M. Dolan, D.B. Oliver, Characterization of *Escherichia coli* SecA protein binding to a site on its mRNA involved in autoregulation, J. Biol. Chem. 266 (1991) 23329–23333.
[111] H. Nakatogawa, A. Murakami, K. Ito, Control of SecA and SecM translation by protein secretion, Curr. Opin. Microbiol. 7 (2004) 145–150.
[112] A. Murakami, H. Nakatogawa, K. Ito, Translation arrest of SecM is essential for the basal and regulated expression of SecA, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 12330–12335.
[113] H. Nakatogawa, K. Ito, Secretion monitor, SecM, undergoes self-translation arrest in the cytosol, Mol. Cell 7 (2001) 185–192.
[114] S.K. Park, D.W. Kim, J. Choe, H. Kim, RNA helicase activity of *Escherichia coli* SecA protein, Biochem. Biophys. Res. Commun. 235 (1997) 593–597.
[115] M.O. Schmidt, R.M. Brosh Jr., D.B. Oliver, *Escherichia coli* SecA helicase activity is not required in vivo for efficient protein translocation or autogenous regulation, J. Biol. Chem. 276 (2001) 37076–37085.
[116] R.S. Ullers, E.N. Houben, A. Raine, C.M. ten Hagen-Jongman, M. Ehrenberg, J. Brunner, B. Oudega, N. Harms, J. Luirink, Interplay of signal recognition particle and trigger factor at L23 near the nascent chain exit site on the *Escherichia coli* ribosome, J. Cell Biol. 161 (2003) 679–684.
[117] R. Singh, C. Kraft, R. Jaiswal, K. Sejwal, V.B. Kasaragod, J. Kuper, J. Berger, T. Mielke, J. Luirink, S. Bhushan, Cryo-electron microscopic structure of SecA bound to the 70S ribosome, J. Biol. Chem. (2014) (in press).
[118] X. Zhang, R. Rashid, K. Wang, S.O. Shan, Sequential checkpoints govern substrate selection during cotranslational protein targeting, Science 328 (2010) 757–760.
[119] A. Rutkowska, M.P. Mayer, A. Hoffmann, F. Merz, B. Zachmann-Brand, C. Schaffitzel, N. Ban, E. Deuerling, B. Bukau, Dynamics of trigger factor interaction with translating ribosomes, J. Biol. Chem. 283 (2008) 4124–4132.
[120] F. Merz, A. Hoffmann, A. Rutkowska, B. Zachmann-Brand, B. Bukau, E. Deuerling, The C-terminal domain of *Escherichia coli* trigger factor represents the central module of its chaperone activity, J. Biol. Chem. 281 (2006) 31963–31971.
[121] L.L. Randall, T.B. Topping, S.J.S. Hardy, M.Y. Pavlov, D.V. Freistroffer, M. Ehrenberg, Binding of SecB to ribosome-bound polypeptides has the same characteristics as binding to full-length, denatured proteins, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 802–807.
[122] L.L. Randall, S.J. Hardy, SecB, one small chaperone in the complex milieu of the cell, Cell. Mol. Life Sci. 59 (2002) 1617–1623.
[123] T.B. Topping, L.L. Randall, Determination of the binding frame within a physiological ligand for the chaperone SecB, Protein Sci. 3 (1994) 730–736.
[124] E.L. Powers, L.L. Randall, Export of periplasmic galactose-binding protein in *Escherichia coli* depends on the chaperone SecB, J. Bacteriol. 177 (1995) 1906–1907.
[125] N.T. Knoblauch, S. Rudiger, H.J. Schonfeld, A.J. Driessen, J. Schneider-Mergener, B. Bukau, Substrate specificity of the SecB chaperone, J. Biol. Chem. 274 (1999) 34219–34225.
[126] L. Baars, A.J. Ytterberg, D. Drew, S. Wagner, C. Thilo, K.J. van Wijk, J.W. de Gier, Defining the role of the *Escherichia coli* chaperone SecB using comparative proteomics, J. Biol. Chem. 281 (2006) 10024–10034.
[127] K. Tani, K. Shiozuka, H. Tokuda, S. Mizushima, In vitro analysis of the process of translocation of OmpA across the *Escherichia coli* cytoplasmic membrane. A translocation intermediate accumulates transiently in the absence of the proton motive force, J. Biol. Chem. 264 (1989) 18582–18588.
[128] A.J. Driessen, Precursor protein translocation by the *Escherichia coli* translocase is directed by the protonmotive force, EMBO J. 11 (1992) 847–853.
[129] A. van Dalen, A. Killian, B. de Kruijff, Delta psi stimulates membrane translocation of the C-terminal part of a signal sequence, J. Biol. Chem. 274 (1999) 19913–19918.
[130] K. Nishiyama, A. Fukuda, K. Morita, H. Tokuda, Membrane deinsertion of SecA underlying proton motive force-dependent stimulation of protein translocation, EMBO J. 18 (1999) 1049–1058.
[131] K. Shiozuka, K. Tani, S. Mizushima, H. Tokuda, The proton motive force lowers the level of ATP required for the in vitro translocation of a secretory protein in *Escherichia coli*, J. Biol. Chem. 265 (1990) 18843–18847.
[132] K. Tani, H. Tokuda, S. Mizushima, Translocation of ProOmpA possessing an intramolecular disulfide bridge into membrane vesicles of *Escherichia coli*. Effect of membrane energization, J. Biol. Chem. 265 (1990) 17341–17347.
[133] N. Nouwen, B. de Kruijff, J. Tommassen, prlA suppressors in *Escherichia coli* relieve the proton electrochemical gradient dependency of translocation of wild-type precursors, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5953–5957.
[134] M. Paetzel, R.E. Dalbey, N.C. Strynadka, Crystal structure of a bacterial signal peptidase apoenzyme: implications for signal peptide binding and the Ser–Lys dyad mechanism, J. Biol. Chem. 277 (2002) 9512–9519.
[135] J.L. Carlos, M. Paetzel, G. Brubaker, A. Karla, C.M. Ashwell, M.O. Lively, G. Cao, P. Bullinger, R.E. Dalbey, The role of the membrane-spanning domain of type I signal peptidases in substrate cleavage site selection, J. Biol. Chem. 275 (2000) 38813–38822.
[136] K. Yamane, S. Matsuyama, S. Mizushima, Efficient in vitro translocation into *Escherichia coli* membrane vesicles of a protein carrying an uncleavable signal peptide. Characterization of the translocation process, J. Biol. Chem. 263 (1988) 5368–5372.
[137] S. Matsuyama, Y. Fujita, S. Mizushima, SecD is involved in the release of translocated secretory proteins from the cytoplasmic membrane of *Escherichia coli*, EMBO J. 12 (1993) 265–270.
[138] W.J. Allen, G. Phan, G. Waksman, Structural biology of periplasmic chaperones, Adv. Protein Chem. Struct. Biol. 78 (2009) 51–97.
[139] J.F. Collet, Periplasmic Oxidative Folding of Secretory Proteins, 2014. (In this issue).
[140] C.L. Hagan, T.J. Silhavy, D. Kahne, β-Barrel membrane protein assembly by the Bam complex, Annu. Rev. Biochem. 80 (2011) 189–210.
[141] T. Lithgow, Integration of Outer Membrane Proteins Through the BAM/TAM Systems, 2014. (In this issue).
[142] J. Selkrig, K. Mosbahi, C.T. Webb, M.J. Belousoff, A.J. Perry, T.J. Wells, F. Morris, D.L. Leyton, M. Totsika, M.D. Phan, N. Celik, M. Kelly, C. Oates, E.L. Hartland, R.M. Robins-Browne, S.H. Ramarathinam, A.W. Purcell, M.A. Schembri, R.A. Strugnell, I.R. Henderson, D. Walker, T. Lithgow, Discovery of an archetypal protein transport system in bacterial outer membranes, Nat. Struct. Mol. Biol. 19 (2012) 506–510 (S501).
[143] W. Zückert, Lipoprotein Sorting, Trafficking and Assembly in the Inner and Outer Membrane, 2014. (In this issue).
[144] S. Okuda, H. Tokuda, Lipoprotein sorting in bacteria, Annu Rev Microbiol 65 (2011) 239–259.
[145] K.V. Korotkov, M. Sandkvist, W.G. Hol, The type II secretion system: biogenesis, molecular architecture and mechanism, Nat. Rev. Microbiol. 10 (2012) 336–351.
[146] O. Francetic, M. Periasamy, Type 2 Secretion, 2014. (In this issue).
[147] M.R. Chapman, M. Evans, Curli Surface Structures: Export, Assembly and the Formation of Biofilms, 2014. (In this issue).
[148] D. Hizlan, A. Robson, S. Whitehouse, V.A. Gold, J. Vonck, D. Mills, W. Kuhlbrandt, I. Collinson, Structure of the SecY complex unlocked by a preprotein mimic, Cell Rep. 1 (2012) 21–28.