ACTA ABP BIOCHIMICA POLONICA

Vol. 68, No 3/2021
427–436
https://doi.org/10.18388/abp.2020_5761

Review

# SecA – a multidomain and multitask bacterial export protein*

Patrycja Ambroziak, Iga Rzepka and Joanna Skorko-Glonek✉

Department of General and Medical Biochemistry, Faculty of Biology, University of Gdańsk, Gdańsk, Poland

**Most bacterial secretory proteins destined to the extracytoplasmic space are secreted posttranslationally by the Sec translocase. SecA, a key component of the Sec system, is the ATPase motor protein, directly responsible for transferring the preprotein across the cytoplasmic membrane. SecA is a large protein, composed of several domains, capable of binding client preproteins and a variety of partners, including the SecYEG inner membrane channel complex, membrane phospholipids and ribosomes. SecA-mediated translocation can be divided into two major steps: (1) targeting of the preproteins to the membrane translocation apparatus and (2) transport across the membrane through the SecYEG channel. In this review we present current knowledge regarding SecA structure and function of this protein in both translocation steps. The most recent model of the SecA-dependent preprotein mechanical translocation across the bacterial cytoplasmic membrane is described. A possibility of targeting SecA with inhibitory compounds as a strategy to combat pathogenic bacteria will be discussed as well.**

**Keywords**: protein export, SecA ATPase, protein structure, SecA regulation

**Received**: 30 June, 2021; revised: 27 July, 2021; accepted: 28 July, 2021; available on-line: 31 August, 2021

✉e-mail: joanna.skorko-glonek@ug.edu.pl

***This paper is dedicated to Professor Wacław Tadeusz Szybalski on the 100th anniversary of his birth**

**Acknowledgements of Financial Support**: This work was supported by the University of Gdansk grant no. D0001.6000.2.2020.

**Abbreviations**: 2HF, Two Helix Finger domain; aa, amino acid; CTL, C-terminal linker; CTT, C-terminal tail; FLD, Flexible Linker Domain; HSD, Helix Scaffold Domain; HWD, Helical Wing Domain; IM, Inner Membrane; IRA1, Intramolecular Regulator of ATPase 1; IRA2, Intramolecular Regulator of ATPase 2; LH, Linker Helix; LUV, Unilamellar Vesicles; MBD, Metal Binding Domain; MIC, Minimum Inhibitory Concentration; NBD1, Nucleotide Binding Domain 1; NBD2, Nucleotide Binding Domain 2; OM, Outer Membrane; OMP, Outer Membrane Protein; PDB, Protein Binding Domain; Pi, inorganic phosphate; SD, Shine-Dalgarno sequence; SRP, Signal Recognition Particle; TAT, Twin-Arginine Translocation; VAR, Variable Domain

## INTRODUCTION

In bacteria, all proteins are synthesized in the cytoplasm. However, it is estimated that approximately 20% of proteins is located outside the cytoplasmic compartment (Li *et al.,* 2014; Cranford-Smith & Huber, 2018). Proteins destined for the periplasm or cellular membranes, as well as proteins secreted to the extracellular milieu, have to cross the inner (cytoplasmic) membrane (IM) via dedicated transport systems. There are two general protein export pathways, termed Sec and twin-arginine translocation (TAT), and specialized secretion systems typical for pathogenic bacteria (Christie, 2019).

The TAT system primarily secretes folded proteins that contain posttranslational modifications (Berks *et al.,* 2005). The Sec translocon exports a majority (approximately 95%) of the envelope proteins (Orfanoudaki & Economou, 2014), and it transports proteins before they acquire a stable tertiary structure (Chatzi *et al.,* 2014). In a model Gram negative bacterium *Escherichia coli*, this system consists of two principal components, the SecA motor and the channel complex, composed of the SecY, SecE and SecG proteins (SecYEG) (Crane & Randall, 2017; Cranford-Smith & Huber, 2018).

SecA is a protein typical for bacteria. In eukaryotes, SecA homologs are found in chloroplasts but are absent in mitochondria (Pohlschröder *et al.,* 1997). SecA is a crucial component of the Sec translocon and its function is required for translocation of most secretory proteins in *E. coli* (Oliver & Beckwith, 1981; Oliver & Beckwith, 1982a; Oliver & Beckwith, 1982b). This protein is localized both in the cytoplasm and IM. Early studies involving cell lysis and fractionation revealed that 50% of the total SecA cellular pool resides in the cytosol, while the other half is associated with the IM (Cabelli *et al.,* 1991). However, latest research based on the super-resolution microscopy indicates that the cytosolic pool of SecA is significantly lower and more than 90% is associated with the IM (Seinen *et al.,* 2021).

SecA performs a dual role in protein translocation: (1) it participates in recruitment and delivery of suitable substrates to the Sec channel and (2) it acts as an ATP-dependent nanomotor to move a polypeptide across the IM (Cranford-Smith & Huber, 2018). While performing its functions, SecA contacts many components of the bacterial cell. These include substrate preproteins, the SecYEG channel, ribosomes, membrane lipids, SecB chaperone, and RNA (Crane & Randall, 2017; Jamshad *et al.,* 2019). This is possible due to the presence of several domains with distinct binding capabilities and functions in this protein. Moreover, the SecA structure is highly dynamic and it undergoes substantial changes at each step of the translocation process, possibly including a change in the oligomerization level (reviewed in Kusters & Driessen, 2011). This makes the SecA protein a fascinating subject for biochemical studies.

The *secA* gene was originally identified 40 years ago in *E. coli* (Oliver & Beckwith, 1981; Oliver & Beckwith, 1982a). Despite four decades of studies in the field of protein export in bacteria, several issues are still subject to debate. In particular, the exact mechanism of how SecA performs its functions is not clear yet. In this review we will present current data on the action of this protein in the process of preprotein targeting and translocation across the cytoplasmic membrane. It should be noted that the vast majority of data regarding functioning of SecA, as well as the whole Sec translocon, comes from studies performed on the *E. coli* model. For this

reason, in this work we will present an overview of data obtained mainly for this model bacterium. Since SecA function is essential for bacterial viability and virulence, this protein is regarded as an attractive candidate for antibacterial drug design (Segers & Anné, 2011).

## A BRIEF OVERVIEW OF THE Sec-DEPENDENT TRANSLOCATION PROCESS

Secretory and membrane proteins are targeted for the Sec-dependent translocation by the internally encoded signal sequences of conserved physicochemical properties. In the case of the integral IM proteins, the signal is encoded within one of its transmembrane domains (Luirink *et al.,* 2005; Schibich *et al.,* 2016) while OMPs, soluble periplasmic proteins, and lipoproteins possess a cleavable N-terminal signal sequence (Hegde & Bernstein, 2006). Nascent polypeptides can be exported from the cytoplasm either during their synthesis (cotranslationally) or after the protein synthesis is completed (posttranslationally) (Fig. 1). In *E. coli*, the integral IM protein insertion generally occurs in a cotranslational manner (Ulbrandt *et al.,* 1997; Beck *et al.,* 2001), while transport of most outer membrane proteins (OMPs), lipoproteins and soluble periplasmic proteins tends to be posttranslational (Danese & Silhavy, 1998; Sikdar *et al.,* 2017).

The cotranslational protein export usually is coupled with the translation process. In this pathway, the N-terminal signal sequence of the synthesized protein is recognized by the signal recognition particle (SRP) at the ribosome exit tunnel (Poritz *et al.,* 1990; Walter *et al.,* 1981; Walter *et al.,* 1981a; Gilmore *et al.,* 1982) and is subsequently directed to FtsY, a membrane-bound SRP-receptor (Luirink *et al.,* 1994; Bahari *et al.,* 2007). The whole complex is then moved to the Sec machinery where both processes, translation and translocation, occur simultaneously and the GTP hydrolysis based processive power of protein synthesis drives the translocation (Fig. 1A).

The posttranslational translocation is independent of the translation process and is mediated by the SecA ATPase. In this pathway, the preprotein released from the ribosome must be delivered to the Sec complex. The SecB chaperone, as well as SecA, play an important role at this stage (Hartl *et al.,* 1990; Cranford-Smith & Huber, 2018) (Fig. 1Ba).

A third variant of the preprotein targeting, termed the uncoupled cotranslational mode, was also proposed. It assumes that the protein synthesis and translocation are not mechanistically linked, but the preprotein is delivered to the Sec channel during its synthesis on the ribosome. In this case, SecA binds the nascent substrates at the ribosome, before they interact with SecB. SecB appears to function downstream from recognition by SecA and is possibly required to release SecA from its complex with the ribosome (Huber *et al.,* 2016) (Fig. 1Bb).

It is also important to note that the Sec translocon exports proteins in the unfolded form (Arkowitz *et al.,* 1993). In the coupled cotranslational process, the polypeptide emerging from the ribosome is routed directly to the translocation channel. Therefore, there are no issues with maintaining the preprotein structure appropriate for

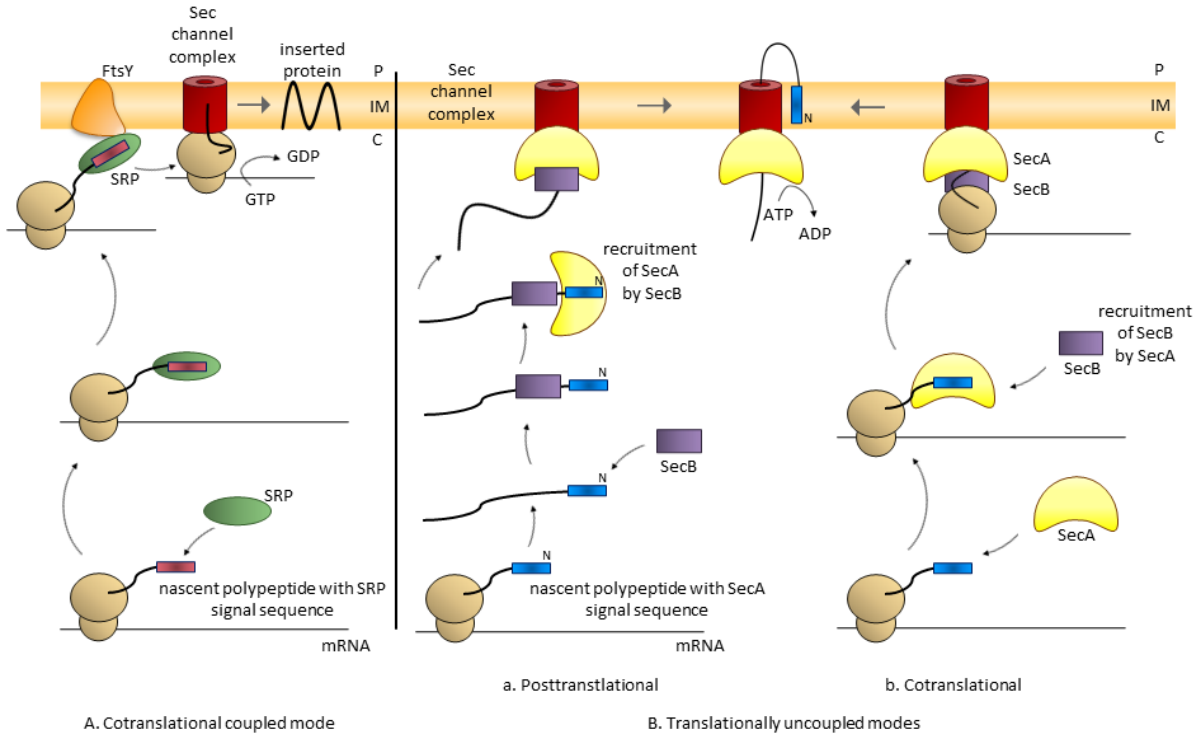


**Figure 1. Schematic overview of the Sec-dependent protein export modes.**
(**A**) While the protein is synthesized at the ribosome, the SRP signal peptide is recognized and bound by SRP. SRP interacts with its receptor in the IM (FtsY protein) and delivers its cargo to the Sec channel complex. Then, the protein is translocated into the IM in a translationally coupled manner. (**B**) In the translationally uncoupled modes, the preprotein recognition and delivery to the Sec channel complex can occur either when the protein synthesis is completed (**a**) or when it is still synthesized (b). In the first case, the SecB chaperone binds a preprotein and then it recruits SecA. In the co-translational variant (**b**), SecA recognizes and binds a nascent polypeptide at the ribosome and then it recruits SecB. After delivery to the Sec channel complex, the SecA nanomotor drives translocation of preproteins across the IM, at the expense of ATP hydrolysis.

the translocation process. However, proteins exported posttranslationally require assistance to maintain their structure competent for the Sec-dependent secretion. This function is performed by cytoplasmic chaperones, in particular the ribosome associated trigger factor and SecB, as well as the SecA protein itself (Fig. 1) (Cranford-Smith & Huber, 2018).

## SECA DOMAIN ORGANIZATION

At present, much of our understanding concerning mechanism of SecA action comes from structural studies of E. coli SecA. This protein has been studied in detail and its crystal structure is available (Papanikolau *et al.,* 2007). E. coli SecA is a large protein composed of 901 amino acids (102 kDa) which are organized into several domains (Fig. 2). The amino acid residues 12-830 of SecA are considered as a catalytic core of this protein, which is essential for bacterial viability and maintaining the translocation process (Or *et al.,* 2005; Na *et al.,* 2015).

The N-terminal part of SecA (aa residues: 1-621) consists of three domains: the Nucleotide Binding Domain (NBD1), the Precursor Binding Domain (PDB) and the Intramolecular Regulator of ATPase 2 (IRA2, also known as NBD2) (Papanikolau *et al.,* 2007).

NBD1 and IRA2 together form a so called DEAD motor domain. Presence of this domain places the SecA protein in the Superfamily 2 of DexH/D (Asp-Glu-X-His/Asp, where X stands for any amino acid) proteins, known to include helicases and enzymes that modify nucleic acids (Koonin & Gorbalenya, 1992; Papanikolau *et al.,* 2007). The spatial organisation of NBD1 and IRA2 domains forms a clamp, and ATP hydrolysis occurs at the interface between these two domains (Sato *et al.,* 1996; Hunt *et al.,* 2002). The NBD1 domain contains two high-affinity ATP binding sites, the highly conserved Walker A (aa residues 83-139) and Walker B (aa residues: 205-227) motifs (Matsuyama *et al.,* 1990; Mitchell & Oliver, 1993; Economou *et al.,* 1995). IRA2 contains two sub-structures: VAR (variable region), partially responsible for the SecA ATPase activity (Das *et al.,* 2012), and the Linker Helix (LH) that connects IRA2 with the C-domain (Papanikolau *et al.,* 2007). This domain plays a regulatory role by activating ATP hydrolysis and nucleotide turnover in NBD1 (Sianidis *et al.,* 2001).The third mentioned domain, the Precursor Binding Domain (PBD), is important for substrate protein recognition and binding, as well as ATPase activation. It is rooted within NBD1 and emerges from between β-strands 5 and 6 of NBD1 (Kimura *et al.,* 1991; Kourtz & Oliver, 2000; Papanikolau *et al.*, 2007; Chada *et al.,* 2018). PBD is composed of a stem-like and bulb-like structures. The first one forms contacts with the NBD and C-domains, while the latter structure mainly contributes to substrate protein binding (Papanikou *et al.,* 2005; Cooper *et al.,* 2008). PBD and IRA2 form another clamp in the N-terminal part of the protein, and the properties of this clamp are strongly influenced by the structural state of NBD1 (discussed later in the text).

The C-terminal domain of SecA (aa residues 622-901) consists of 4 sub-domains: the Helix Scaffold Domain (HSD), the Helical Wing Domain (HWD), the Intramolecular Regulator of ATPase 1 (IRA1) and the C-terminal tail (CTT) (Papanikolau *et al.,* 2007). HSD is a long α-helix which is connected to IRA2 via LH and spreads throughout the DEAD motor (binding both NBD and IRA2); in consequence, it interconnects all SecA domains (Papanikolau *et al.,* 2007). IRA1, also termed 2HF (two-helix-finger) domain, interacts with the SecY central pore (Karamanou *et al.,* 1999; Zimmer & Rapoport, 2006; Erlandson *et al.,* 2008). CTT, also known as a C-terminal linker (CTL), is relatively long and contains two sub-structures: the Flexible Linker Domain (FLD) and the Metal Binding Domain (MBD) (Hunt *et al.,* 2002; Jamshad *et al.,* 2019). The FLD domain seems to play an autoinhibitory role by interacting with the catalytic core of SecA (Jamshad *et al.,* 2019). MBD is responsible for interactions with the SecB chaperone, phospholipids and ribosomes, which result in an increased affinity for substrate polypeptides (Breukink *et al.,* 1995; Kimsey *et al.,* 1995; Fekkes *et al.,* 1997; Jamshad *et al.,* 2019). Moreover, the MBD domain contains a conserved zinc and iron binding cysteine motif (Matousek & Alexandrescu, 2004; Cranford-Smith *et al.,* 2020). Presence of metal ions in this domain stabilize the SecA structure and enable efficient binding to the SecB chaperone (Fekkes *et al.,* 1999; Zhou & Xu 2003).

However, it should be noted that despite an overall evolutionary preservation of the SecA protein and its domain organization in bacteria, the SecA polypeptide

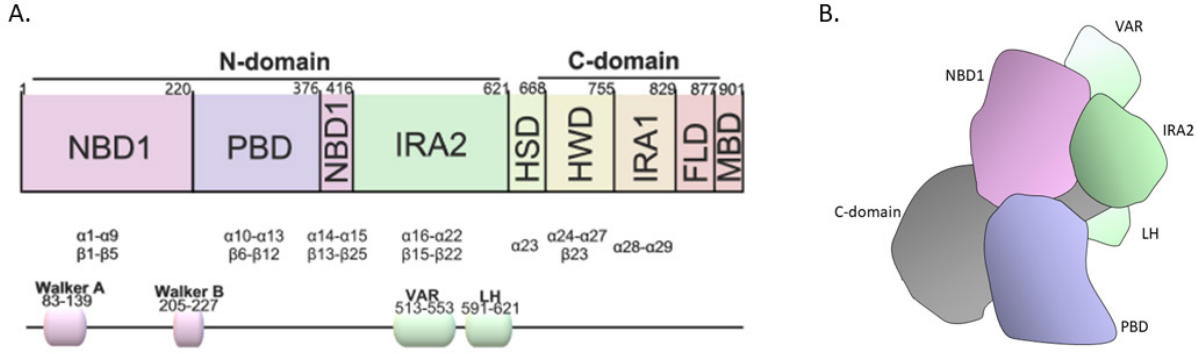


**Figure 2. Domain organization of the SecA protein.**
(**A**) The N-terminal part (aa residues 1-619) consists of three domains: NBD1 (aa residues 1-220) with Walker A (aa residues 83-139) and Walker B (aa residues 205-227) motifs; PDB (aa residues 221-376) and IRA2, also known as NBD2 (aa residues 417-621) with two sub-structures: VAR (aa residues 513-553) and LH (aa residues 591-621). The C-terminal part (aa residues 622-901) consists of four domains: HSD (aa residues 622-668), HWD (aa residues 669-755), IRA1, also known as 2HF (aa residues 756-823), and CTT (aa residues 832-901) with two sub-domains: FLD (aa residues 832-880) and MBD (aa residues 881-901). The domain secondary structure components (α-helices and β-sheets) are denoted as α/β and accompanied by the corresponding structure number. (**B**) Schematic representation of SecA tertiary structure. Color coding: NBD1, pink; PDB, purple; IRA2 (with VAR and LH), green; the C-domain (with HSD, HWD, IRA1 and CTT), grey.

may differ in length and net charge of amino acids. Recent bioinformatics analysis of the 425 SecA homologs derived from bacterial species representing all bacterial phyla revealed that majority of them tend to carry a negative charge, but the length of the amino acid sequence vary due to the presence of deletions or insertions. It was proposed that specific features in the amino acid sequences can reflect the specificity of function, and/or reaction mechanism of SecA in a given organism (Del Val & Bondar, 2020).

## OLIGOMERIC STATE OF SecA

Despite the abundance of reports on the SecA quaternary structure, the oligomeric state of this protein during its functioning still remains unclear. Generally, SecA is mainly purified from cells in a dimeric form and many studies indicate that this protein functions as a dimer (Akita *et al*., 1991; Driessen, 1993; de Keyzer *et al.,* 2005; Jilaveanu, *et al.,* 2005; Wang *et al.,* 2008; Kusters *et al.,* 2011; Gouridis *et al.,* 2013). However, there are also reports suggesting that in the course of ligand binding and protein substrate translocation, SecA changes its oligomeric state and temporarily monomerizes (Or *et al.,* 2002; Or *et al.,* 2005; Gouridis *et al.,* 2013; Roussel & White, 2020). Furthermore, the *in vitro* data indicates that the SecA oligomeric state can be shifted towards monomers by binding ligands, such as lipids, detergents, signal peptides or nucleotides (Or *et al.,* 2002; Benach *et al.,* 2003; Bu *et al.,* 2003; Musial-Siwek *et al*., 2005).

According to the crystal structure of a SecA dimer (Papanikolau *et al.,* 2007), its subunits are associated in an anti-parallel way, with the dimerization interface located almost exclusively in the DEAD motor domains (NBD1, IRA2). Additionally, the K475, W519, and P529 residues of protomer α and E141, M161, A525, L526 residues of protomer β form stabilizing contacts. Also, the IRA2 domain of one protomer interacts with the ATP groove in the DEAD motor of another SecA protomer. The interdomain contacts are mainly hydrophobic, further stabilized by hydrogen bonds (Papanikolau *et al.,* 2007).

Even though SecA mainly operates as a dimer, it is expected that various forms of dimers are assembled. Moreover, high dynamics of the quaternary structure may be coupled with different functions played by SecA at each stage of protein export. It was proposed that the soluble cytoplasmic SecA adopts one of the two conformationally distinct forms: an "electrostatic dimer" (majority of SecA molecules, 95%) and a salt-resistant dimer (5%). Binding of substrate protein and attachment to SecYEG induces interconvertion to the third state, named the "triggered dimer" (Gouridis *et al.,* 2013). This mechanism seems to be supported by experiments in which the SecA protomers were "immobilized" in the dimer by disulfide cross-linking, thus preventing the oligomer rearrangements. Thus modified SecA was deficient in lipid binding and showed weaker ATPase activity. The effect was fully reverted by reduction of disulphides (Or & Rapoport, 2007). Whether the SecA molecule disassembles to monomers or remains as a dimer in the SecA-SecYEG complex is a matter of dispute, as data supporting both possibilities can be found in the literature (Sardis & Economou, 2010).

Additional controversy is related to the SecA oligomeric state during binding to the lipid bilayer. In the recently published data, it was shown that the disulfide crosslinked dimers bind only weakly to large lipid vesicles (LUV), while SecA binds to LUV only as a monomer (Roussel & White, 2020). In contrast, a report based on single-molecule visualization inside living cells indicates that SecA associates with the IM as a homodimer (Seinen *et al.,* 2021).

## STRUCTURAL DYNAMICS OF SecA

SecA is a highly dynamic protein and it undergoes numerous but highly coordinated structural changes in the course of preprotein delivery and translocation across the IM.

How is ligand binding communicated to the DEAD ATPase motor? As could be seen in a linear SecA domain scheme (Fig. 2A), both domains of the DEAD motor are physically linked to the two domains implicated in substrate binding (PBD and C-terminal). The NBD1 domain is interrupted by insertion of the PDB domain, while IRA2 is connected with the C-terminal domain by LH. Moreover, HSD provides a contact interface between the DEAD motor and the C-domain (Papanikolau *et al.,* 2007) (Fig. 2B). Such structural organization has its consequences. First, a structural framework for highly coordinated processes is formed: from substrate binding to its translocation across the IM. Second, multiple contacts between domains and their high flexibility provide basis for precise regulation of SecA. A remarkable protein plasticity is observed at the secondary, tertiary and quaternary SecA structure levels. First of all, the catalytic (DEAD) and preprotein binding domains are highly flexible. The motor domain requires stabilization; otherwise, the isolated SecA DEAD domain is largely unstructured. Such stabilization is achieved due to attachment to the C-terminal domain. Also, ADP binding stabilizes the DEAD domain structure, at the same time affecting structure of PBD. The PBD domain is mobile and it can adopt three different conformations: (1) closed, (2) open and (3) wide open, as judged from crystal structure of *E. coli* SecA and its homologs from *Bacillus subtilis* and *Thermotoga maritima* (Cranford-Smith & Huber, 2018). Motility of the PBD domain seems to be essential for SecA functioning. This feature is most probably linked with regulation of access for preprotein substrates by opening and closing a clamp formed between PBD and IRA2 (Gold *et al.,* 2013). Several other biochemical and biophysical studies reveal presence of other flexible SecA regions. These include the HSD, HWD and IRA1 domains, as well as the extreme C-terminal part which is particularly flexible and was not traced by crystallography (reviewed in Chatzi *et al.,* 2014).

Finally, SecA undergoes cyclic conformational changes during ATP binding and hydrolysis, a process which is strictly connected with the preprotein threading across the Sec channel (described in detail in Cranford-Smith & Huber, 2018).

## SecA BINDING PARTNERS

Structural organization of SecA into domains with several subdomains facilitates interactions with several ligands, such as ribosomes, phospholipids, ATP, substrate polypeptides, the SecYEG channel, SecB protein and RNA (Table 1) (Crane & Randall, 2017; Findik *et al.,* 2018; Jamshad *et al.,* 2019; Knüpffer *et al.,* 2019). First of all, SecA binds its substrates, preproteins with appropriate signal sequences, and ATP as an energy source for translocation. SecA recognizes precursor polypeptides both, by interacting with a signal sequence at the substrate N-terminal end and by binding certain sequences

**Table 1. The *E. coli* SecA domains, their functions and binding partners.**

| Domain | | Amino acid residues | Function | Binding partners | References |
|---|---|---|---|---|---|
| NBD1 | | 1-220<br>377-416 | ATP binding<br>Autoregulation<br>Substrate binding | ATP,<br>RNA,<br>ribosomes | Crane *et al.,* 2017;<br>Salavati *et al.,* 1997 |
| PBD | | 221-376 | Recognition and binding of substrate protein | Substrate protein,<br>SecYEG | Ernst *et al.,* 2018, |
| IRA2 (NBD2) | | 417-621 | Activation of ATP hydrolysis<br>Autoregulation<br>Substrate binding | ATP,<br>RNA,<br>SecB | Crane *et al.,* 2017;<br>Papanikolau *et al.,* 2007;<br>Salavati *et al.,* 1997 |
| HSD | | 622-668 | Recognition and binding of substrate protein | Substrate protein,<br>SecYEG,<br>ribosomes | Grady *et al.,* 2012;<br>Ernst *et al.,* 2018 |
| HWD | | 669-755 | Recognition and binding of substrate protein | Substrate protein,<br>SecYEG | Grady *et al.,* 2012;<br>Ernst *et al.,* 2018 |
| IRA1 (2HF) | | 756-829 | Translocation across the membrane | SecY | Vrontou *et al.,* 2004;<br>Ernst *et al.,* 2018 |
| C-terminal tail | FLD | 833-877 | Autoinhibitory role | SecA | Jamshad *et al.,* 2019 |
| | MBD | 878-901 | Increasing affinity for substrate polypeptide | Ribosomes,<br>Substrate polypeptides,<br>SecB,<br>Phospholipids | Kimsey *et al.,* 1995;<br>Breukink *et al.,* 1995;<br>Fekkes *et al.,* 1997;<br>Jamshad *et al.,* 2019 |

within the mature part of a substrate protein (Gelis *et al.,* 2007; Grady *et al.,* 2012; Chatzi *et al.,* 2017). SecA recognizes many signal peptides of various primary sequences, but sharing a common organization scheme: the N-terminal positively charged residues followed by a hydrophobic core, and the C-terminal hydrophilic region containing a signal peptidase cleavage site (von Heijne, 1985; Gierasch, 1989). The N-terminal signal sequence forms an α-helix and binds to the groove formed by PBD and HSD of SecA (Gelis *et al.,* 2007; Grady *et al.,* 2012; Zhang *et al.,* 2016). As SecA transports preproteins in an unfolded state, it is necessary to stabilize their structure prior to translocation through the Sec channel. This function is played by cytoplasmic chaperones, including SecB. SecA binds to this chaperone using several spatially distant regions: the MBD domain of the C-terminal tail, the N-terminal 2-11 amino acid residues and linker helix of the IRA2 domain (aa 600-610) (Breukink *et al.,* 1995; Kimsey *et al.,* 1995; Fekkes *et al.,* 1997; 1999; Randall *et al.,* 2005; Suo *et al.,* 2011). Formation of the SecA-SecB complex causes a release of the preprotein from interactions with SecB, and allows for its binding to SecA (Crane *et al.,* 2006). Cotranslational SecA-dependent translocation is possible by direct binding to ribosomal uL23, which together with uL24 and uL29 form the ribosomal exit tunnel. In these interactions, at least two parts of SecA are involved: the N-terminal helix formed by the 1-38 aa residues and two lysine residues (Lys625 and Lys633) of the HSD domain (Suo *et al.,* 2011; Singh *et al.,* 2014; Knüpffer *et al.,* 2019). Recently published data indicates that the MBD domain of SecA also interacts with ribosomes (Jamshad *et al.,* 2019).

SecA binds to SecYEG by its amphipathic, positively charged N-terminus, via a phospholipid-bound intermediate. It has been suggested that the SecA interaction with SecYEG is preceded by SecA binding to acidic phospholipids in the lipid bilayer (Floyd *et al.,* 2014; Koch *et al.,* 2016). This interaction with phospholipids leads to conformational changes in SecA which result in an increased affinity to the SecYEG channel (Koch *et al.,* 2016). During protein translocation through the Sec channel, the IRA1 domain of SecA is inserted into SecY, but the exact role of this interaction is not fully understood yet (described in Komarudin & Driessen, 2019; Ma *et al.,* 2019).

## SecA REGULATION

Owing to great importance of the SecA function in the bacterial cell, both its level and activity should be tightly regulated. Estimations of the SecA cellular content vastly differ in numbers. Depending on experimental approach, the predicted number of SecA copies per cell ranges from approximately 50 to up to more than 10,000 (Oliver & Beckwith, 1982b; Akita *et al.,* 1991; Or *et al.,* 2002; Taniguchi *et al.,* 2010; Li *et al.,* 2014; Schmidt *et al.,* 2016). A recent work nicely demonstrates that SecA is not a very abundant protein and its estimated copy number ranges between 37 and 336 (average of 126) per cell, in the exponentially growing *E. coli* culture under optimal conditions (Seinen *et al.,* 2021). The cellular level of SecA increases in the stationary growth phase (Yang *et al.,* 2013), and in response to a secretion defect (Oliver & Beckwith 1982b). The SecA content is mainly regulated at the level of translation by a mechanism involving both, the *secM* mRNA and the SecM protein (reviewed in Nakatogawa *et al.,* 2004). The *secA* and *secM* genes belong to the same transcriptional unit, with *secM* located upstream of *secA*. The *secM-secA* transcript forms a stem loop secondary structure which contains the Shine-Dalgarno (SD) ribosome binding site of *secA*. In the stem loop, the *secA* SD sequence is not available for interaction with ribosomes. A key feature of the *secM* translation process is an elongation arrest occurring at the Pro166 codon (just before the stem loop forming sequences). According to the current model of SecA regulation, at the state of *secM* translation arrest, the stem-loop unfolds and the *secA* SD sequence becomes well exposed for translation initiation. Under physiological conditions, the SecM-elongation arrest is transient: the N-terminus of the nascent SecM polypeptide is recognized by SRP and guided to the Sec channel for export. When SecM undergoes translocation, the

elongation arrest is cancelled. The stem-loop becomes reformed when translation of both, SecM and SecA, is terminated. Under unfavorable conditions and impaired protein secretion, ribosome stalling at the *secM* mRNA is prolonged, which results in an increased SecA synthesis (Nakatogawa *et al.,* 2004).

Additionaly, the SecA level in the bacterial cell is also controlled by autoregulation. SecA contains an RNA binding site (between NBD and IRA2) and can bind its own mRNA. This interaction blocks the SD ribosome binding site and it may furthermore lead to dissociation of an existing translation initiation complex formed by 30 S tRNAfMet-*secM-secA* RNA (Rajapandi *et al.,* 1991; Salavati & Oliver 1995; Schmidt *et al.,* 2001; Nakatogawa *et al.,* 2004).

Recent studies suggest the existence of an additional regulation mechanism which involves the CTT domain (Jamshad *et al.,* 2019). Based on research on the SecA variants deprived of one or both CTT sub-domains (FLD and MBD), a new model of SecA self-regulation was proposed. In this model, the FLD domain interacts with the catalytic core of SecA in the PBD region and affects its conformation. As a result, protein substrate binding is disabled. When MBD and the catalytic core bind to a ribosome, the FLD and PBD interaction becomes disrupted. This allows SecA to recognize and bind a polypeptide with a suitable signal sequence while it emerges from the ribosome. SecA-substrate protein interaction displaces FLD from the groove formed by PBD and leads to conformational changes which release SecA from the ribosome. It has been suggested that this kind of autoregulation prevents binding of SecA to non-substrate proteins (Jamshad *et al.,* 2019).

## SecA-DEPENDENT PROTEIN TRANSLOCATION

SecA is an ATPase and during protein export it undergoes repeated cycles of ATP hydrolysis and nucleotide exchange. ATP binding and hydrolysis induce pronounced conformational changes in the SecA molecule. These cycles of the nucleotide binding and hydrolysis driven conformational changes are strictly linked with the function of SecA, regarded as a molecular nanomotor for preprotein transport across the Sec channel (Schiebel *et al.,* 1991; Economou & Wickner, 1994; van der Wolk *et al.,* 1997). However, it must be noted that despite tremendous wealth of data describing SecA structure and activity, the exact mechanism of the SecA-mediated translocation is not fully understood. As many as four models of the SecA action have been proposed: (1) the "Brownian-Ratchet", (2) the "Push and Slide", (3) the "Reciprocating Piston" and (4) the "Power-Stroke", as reviewed in (Komarudin & Driessen, 2019). All models are based on the SecA ATP/ADP exchange induced conformational rearrangements which strongly affect affinity of SecA to the SecY protein and substrate polypeptides. During translocation, interaction of the IRA1 domain with SecY appears to play a key role. The major differences between the models are in the exact use of energy released during ATP hydrolysis, active or passive role of SecA during polypeptide movement across the Sec channel, and finally the SecA oligomeric status.

In the "Brownian Ratchet" model, the IRA1 domain of SecA controls opening and closing of the Sec channel, while the preprotein crosses the membrane by diffusion. In the ATP-bound state, SecA keeps the protein-conducting channel open, while ATP hydrolysis leads to contraction of the channel. Conversion of the channel from the closed to open state is coordinated with dimensions of the transported polypeptide region. Presence of large aromatic side chains and short α-helices prevents the preprotein from passing through the narrow channel due to sterical constraints. Contact of these regions with IRA1 induces ADP to ATP exchange and opening of the channel. Subsequent closing of the channel prevents the backward movement of the substrate (Allen *et al.,* 2016; Catipovic, 2020). Another model, called the "Push and Slide", assumes that conformational changes of IRA1 occurring in the process of ATP binding and hydrolysis enable pushing the substrate polypeptide into the transducing channel and its subsequent sliding across the membrane. In particular, in the ATP bound state, IRA1 enters the Sec channel, interacts with the substrate and pushes it. Following ATP hydrolysis, IRA1 releases the substrate and retracts from SecY, enabling the preprotein to move forward and backward in the channel (Erlandson *et al.,* 2008; Zimmer *et al.,* 2008; Bauer *et al.,* 2014). The third proposed model, the "Reciprocating Piston", assumes a change in the SecA oligomerization state during translocation. SecA binds to the SecYEG channel as a dimer, however, ATP hydrolysis induces its monomerization. The SecA protomer remains attached to the SecYEG channel and prevents backsliding of preprotein. Then, another SecA monomer binds to the SecYEG-SecA monomer-preprotein complex and a subsequent binding of ATP promotes preprotein translocation (Zimmer *et al.,* 2008; Kusters & Driessen, 2011).

Recently published data support the fourth model, named the "Power-Stroke" (Catipovic *et al.,* 2019; Catipovic, 2020; Gupta *et al.,* 2020). According to this model, during translocation process IRA1 undergoes serious conformational changes caused by ATP binding and hydrolysis. These conformational changes move IRA1 deeper into the Sec channel and push the polypeptide through it. After hydrolysis, in an ADP-bound state, IRA1 retracts from SecY and is prepared for another ATP binding and hydrolysis cycle. Such retraction of IRA1 might result in retraction of the polypeptide from the Sec channel. However, there is a mechanism which prevents the backward movement of the polypeptide during translocation. During ATP hydrolysis, rotation of SecA PBD towards NBD2 results in a "clamp" formation. This clamp tightens around the substrate polypeptide while IRA1 is in a "resetting" state, and therefore the polypeptide cannot move forward. After ATP hydrolysis and release of inorganic phosphate (Pi), the clamp relaxes and the polypeptide passively slides through the SecY channel (Catipovic *et al.,* 2019; Catipovic, 2020; Gupta *et al.,* 2020).

A characteristic feature of the SecA mechanism of action during protein translocation is processivity, reflected in successive cycles of SecA binding and dissociation from SecYEG. The importance of this on/off cycling seems to depend on the length of the translocating substrate, and SecA processivity does not appear to be crucial in the case of translocations of short proteins (Young & Duong, 2019).

There are also reports demonstrating a possibility that SecA can also function independently of the SecYEG translocon. It was shown that SecA can penetrate *E. coli* anionic phospholipid bilayers *in vitro*. This observation led to a hypothesis that SecA can form a ring-like pore structures in the IM, which are able to translocate preprotein substrates by itself (without engagement of SecYEG). These ring-like pore structures seem to be formed by the dimeric form of SecA (Wang *et al.,* 2003). In this SecA-only-channel, one protomer functions as

a conducting channel, while the second one acts as an ATPase (Hsieh *et al.,* 2013). The HSD domain seems to be crucial for formation of these ring-like pore structures (Hsieh *et al.,* 2017). Although the SecA-only conducting channels possess lower translocating efficiency than the SecA-SecYEG channels, it is possible that they are able to translocate IM proteins which do not contain signal peptides (Hsieh *et al.,* 2011; Hsieh *et al.,* 2013; You *et al.,* 2013).

## SecA AS A THERAPEUTIC TARGET

The *secA* gene is regarded to be essential for cell viability in all bacterial species studied thus far. In pathogenic species, most virulence factors and toxins are secreted via the Sec translocon. Furthermore, SecA is conserved among both, the Gram negative and positive bacteria, while no close structural homologs were identified in humans (Segers & Anné, 2011; Rao *et al.,* 2014). This features make the SecA protein an attractive target for antibacterial drug design. Several reports indicate that SecA is druggable and structurally distinct classes of SecA inhibitors were identified (reviewed in Chaudhary *et al.,* 2015; Jin *et al.,* 2018). Moreover, the results of numerous studies performed on a variety of bacterial pathogens indicate that inhibition of SecA leads to antimicrobial effects, including growth inhibition, and more importantly also to attenuated secretion of virulence factors (for example Sugie *et al.,* 2002; Huang *et al.,* 2012; Cui *et al.,* 2013; Jin *et al.,* 2015; Jin *et al.,* 2016; Walsh *et al.,* 2019). The non-competitive inhibitors of the SecA ATPase activity seem to be very promising. In particular, SCA-15 (thiouracil-pyrimidine analog) and two triazole-pyrimidine compounds SCA-107 and SCA-112 were shown to exhibit a certain selectivity for SecA, as they did not affect activity of other tested ATPases (Jin *et al.,* 2015; 2016). Subsequent research led to the development of other thiouracil-based SecA inhibitors. These compounds contained acyl thiourea or triazolo-thiadiazole moieties and showed high inhibitory activity against the SecA protein (Cui *et al.,* 2017; Cui *et al.,* 2017a).

The major issue is membrane permeability for the drug molecule which can severely affect accessibility of SecA for the inhibitory compounds. It is a particular problem in case of the Gram-negative pathogens, whose cell is protected by two membranes. As a result, the minimal inhibitory concentration (MIC) values of the tested compounds were much higher for the Gram negative than Gram positive bacteria. Therefore, the best inhibitory results in the Gram negative pathogens were obtained in the case of mutants with increased membrane permeability or in the presence of membrane permeabilizers (Jin *et al.,* 2015; Jin *et al.,* 2016).

The studies performed thus far indicate that SecA can be regarded as a valid target for the development of new antibacterial drugs. However, further optimization of compounds in terms of potency and selectivity towards SecA will be required to minimize the toxicity issues. Moreover, further refinement of the inhibitor penetration into bacterial cells is needed.

## CONCLUSIONS

In the last 40 years since the SecA protein was identified, a huge amount of research has been carried out on the structure and function of this protein. The universal conservation of SecA among bacterial species underlines importance of the SecA-mediated pathway and indicates evolutionary preservation of the translocation mechanism in bacteria (for example: Cao & Milton, 2003; Segers & Anné, 2011).

The great plasticity of SecA is the basis of its functioning. Structural changes allow for the coordination of the processes of recognition and binding of a substrate molecule, its delivery to the translocation channel, and then its active movement to the other side of the membrane in an ATP hydrolysis dependent process (for example: Chatzi *et al.,* 2014).

The indispensability of the SecA function for cell viability, as well as the importance of the Sec secretion-dependent virulence factors for pathogenicity of numerous bacterial pathogens, make this protein a good candidate for a therapeutic target (for example: Or *et al.,* 2005; Na *et al.,* 2015).

However, it must be pointed out that several aspects of the SecA structure and mechanism of substrate recognition, delivery and translocation still need to be clarified. This mainly concerns the final establishment of the SecA-dependent translocation mechanism, SecA oligomer rearrangements, and structure and function of the C-terminal part of the protein.

## REFERENCES


Akita M, Shinkai A, Matsuyama S, Mizushima S (1991) SecA, an essential component of the secretory machinery of *Escherichia coli*, exists as homodimer. *Biochem Biophys Res Commun* **174**: 211–216. https://doi.org/10.1016/0006-291X(91)90507-4

Allen WJ, Corey RA, Oatley P, Sessions RB, Baldwin SA, Radford SE, Tuma R, Collinson I (2016) Two-way communication between SecY and SecA suggests a Brownian ratchet mechanism for protein translocation. *eLife* **5**: e15598. https://doi.org/10.7554/eLife.15598

Arkowitz RA, Joly JC, Wickner W (1993) Translocation can drive the unfolding of a preprotein domain. *EMBO J* **12**: 243–253.

Bahari L, Parlitz R, Eitan A, Stjepanovic G, Bochkareva ES, Sinning I, Bibi E (2007) Membrane targeting of ribosomes and their release require distinct and separable functions of FtsY*. *J Biol Chem* **282**: 32168–32175. https://doi.org/10.1074/jbc.M705429200

Bauer BW, Shemesh T, Chen Y, Rapoport TA (2014) A "Push and Slide" Mechanism Allows Sequence-Insensitive Translocation of Secretory Proteins by the SecA ATPase. *Cell* **157**: 1416–1429. https://doi.org/10.1016/j.cell.2014.03.063

Beck K, Eisner G, Trescher D, Dalbey RE, Brunner J, Müller M (2001) YidC, an assembly site for polytopic *Escherichia coli* membrane proteins located in immediate proximity to the SecYE translocon and lipids. *EMBO Rep* **2**: 709–714. https://doi.org/10.1093/embo-reports/kve154

Benach J, Chou Y-T, Fak JJ, Itkin A, Nicolae DD, Smith PC, Wittrock G, Floyd DL, Golsaz CM, Gierasch LM, Hunt JF (2003) Phospholipid-induced monomerization and signal-peptide-induced oligomerization of SecA. *J Biol Chem* **278**: 3628–3638. https://doi.org/10.1074/jbc.M205992200

Berks BC, Palmer T, Sargent F (2005) Protein targeting by the bacterial twin-arginine translocation (Tat) pathway. *Curr Opin Microbiol* **8**: 174–181. https://doi.org/10.1016/j.mib.2005.02.010

Breukink E, Nouwen N, van Raalte A, Mizushima S, Tommassen J, de Kruijff B (1995) The C terminus of SecA is involved in both lipid binding and SecB binding. *J Biol Chem* **270**: 7902–7907. https://doi.org/10.1074/jbc.270.14.7902

Bu Z, Wang L, Kendall DA (2003) 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**: 23–30. https://doi.org/10.1016/s0022-2836(03)00840-4

Cabelli RJ, Dolan KM, Qian LP, Oliver DB (1991) Characterization of membrane-associated and soluble states of SecA protein from wild-type and SecA51(TS) mutant strains of *Escherichia coli*. *J Biol Chem* **266**: 24420–24427

Cao TB, Milton HS (2003) The general protein secretory pathway: phylogenetic analyses leading to evolutionary conclusions. *Biochim Biophys Acta* **1609**: 115–125. https://doi.org/10.1016/S0005-2736(02)00662-4.

Catipovic MA (2020) Mechanism of Translocation by the Bacterial ATPase SecA. Doctoral dissertation, Harvard University, Graduate School of Arts & Sciences

Catipovic MA, Bauer BW, Loparo JJ, Rapoport TA (2019) Protein translocation by the SecA ATPase occurs by a power-stroke

mechanism. *EMBO J* **38**: e101140. https://doi.org/10.15252/embj.2018101140
Chada N, Chattrakun K, Marsh BP, Mao C, Bariya P, King GM (2018) Single-molecule observation of nucleotide induced conformational changes in basal SecA-ATP hydrolysis. *Sci Adv* **4**: eaat8797. https://doi.org/10.1126/sciadv.aat8797
Chatzi KE, Sardis MF, Economou A, Karamanou S (2014) SecA-mediated targeting and translocation of secretory proteins. *Biochim Biophys Acta* **1843**: 1466–1474. https://doi.org/10.1016/j.bbamcr.2014.02.014
Chatzi KE, Sardis MF, Tsirigotaki A, Koukaki M, Šoštarić N, Konijnenberg A, Sobott F, Kalodimos CG, Karamanou S, Economou A (2017) Preprotein mature domains contain translocase targeting signals that are essential for secretion. *J Cell Biol* **216**: 1357–1369. https://doi.org/10.1083/jcb.201609022
Chaudhary AS, Chen W, Jin J, Tai PC, Wang B (2015) SecA: a potential antimicrobial target. *Future Med Chem* **7**: 989–1007. https://doi.org/10.4155/fmc.15.42
Christie PJ (2019) The Rich Tapestry of Bacterial Protein Translocation Systems. *Protein J* **38**: 389–408. https://doi.org/10.1007/s10930-019-09862-3
Cooper DB, Smith VF, Crane JM, Roth HC, Lilly AA, Randall LL (2008) SecA, the motor of the secretion machine, binds diverse partners on one interactive surface. *J Mol Biol* **382**: 74–87. https://doi.org/10.1016/j.jmb.2008.06.049
Crane JM, Randall LL (2017) The Sec system: protein export in *Escherichia coli*. *EcoSal Plus* **7**. https://doi.org/10.1128/ecosalplus.ESP-0002-2017
Crane JM, Suo Y, Lilly AA, Mao C, Hubbell WL, Randall LL (2006) Sites of interaction of a precursor polypeptide on the export chaperone SecB mapped by site-directed spin labeling. *J Mol Biol* **363**: 63–74. https://doi.org/10.1016/j.jmb.2006.07.021
Cranford-Smith T, Huber D (2018) The way is the goal: how SecA transports proteins across the cytoplasmic membrane in bacteria. *FEMS Microbiol Lett* **365**. https://doi.org/10.1093/femsle/fny093
Cranford-Smith T, Jamshad M, Jeeves M, Chandler RA, Yule J, Robinson A, Alam F, Dunne KA, Aponte Angarita EH, Alanazi M, Carter C, Henderson IR, Lovett JE, Winn P, Knowles T, Huber D (2020) Iron is a ligand of SecA-like metal-binding domains *in vivo*. *J Biol Chem* **295**: 7516–7528. https://doi.org/10.1074/jbc.RA120.012611
Cui J, Jin J, Hsieh Y-H, Yang H, Ke B, Damera K, Tai PC, Wang B (2013) Design, synthesis and biological evaluation of rose bengal analogues as SecA inhibitors. *Chem Med Chem* **8**: 1384–1393. https://doi.org/10.1002/cmdc.201300216
Cui P, Li X, Zhu M, Wang B, Liu J, Chen H. (2017) Design, synthesis and antibacterial activities of thiouracil derivatives containing acyl thiourea as SecA inhibitors. *Bioorg Med Chem Lett* **27**: 2234–2237. https://doi.org/10.1016/j.bmcl.2016.11.060
Cui P, Li X, Zhu M, Wang B, Liu J, Chen H (2017a) Design, synthesis and antimicrobial activities of thiouracil derivatives containing triazolo-thiadiazole as SecA inhibitors. *Eur J Med Chem* **127**: 159–165. https://doi.org/10.1016/j.ejmech.2016.12.053
Danese PN, Silhavy TJ (1998) Targeting and assembly of periplasmic and outer-membrane proteins in *Escherichia coli*. *Annu Rev Genet* **32**: 59–94. https://doi.org/10.1146/annurev.genet.32.1.59
Das S, Grady LM, Michtavy J, Zhou Y, Cohan FM, Hingorani MM, Oliver DB (2012) The variable subdomain of *Escherichia coli* SecA functions to regulate SecA ATPase activity and ADP release. *J Bacteriol* **194**: 2205–2213. https://doi.org/10.1128/JB.00039-12
de Keyzer J, van der Sluis EO, Spelbrink REJ, Nijstad N, de Kruijff B, Nouwen N, van der Does C, Driessen AJM (2005) Covalently dimerized SecA is functional in protein translocation. *J Biol Chem* **280**: 35255–35260. https://doi.org/10.1074/jbc.M506157200
Del Val C, Bondar AN, (2020) Diversity and sequence motifs of the bacterial SecA protein motor. *Biochim Biophys Acta Biomembr*.1862(10): 183319. https://doi.org/10.1016/j.bbamem.2020.183319.
Driessen AJ (1993) SecA, the peripheral subunit of the *Escherichia coli* precursor protein translocase, is functional as a dimer. *Biochemistry* **32**: 13190–13197. https://doi.org/10.1021/bi00211a030
Economou A, Pogliano JA, Beckwith J, Oliver DB, 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. https://doi.org/10.1016/0092-8674(95)90143-4
Economou A, Wickner W (1994) SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. *Cell* **78**: 835–843. https://doi.org/10.1016/S0092-8674(94)90582-7
Erlandson KJ, Miller SBM, Nam Y, Osborne AR, Zimmer J, Rapoport TA (2008) A role for the two-helix finger of the SecA ATPase in protein translocation. *Nature* **455**: 984–987. https://doi.org/10.1038/nature07439
Fekkes P, de Wit JG, Boorsma A, Friesen RHE, Driessen AJM (1999) Zinc Stabilizes the SecB Binding Site of SecA. *Biochemistry* **38**: 5111–5116. https://doi.org/10.1021/bi982818r
Fekkes P, van der Does C, Driessen AJ (1997) The molecular chaperone SecB is released from the carboxy-terminus of SecA during initiation of precursor protein translocation. *EMBO J* **16**: 6105–6113. https://doi.org/10.1093/emboj/16.20.6105
Findik BT, Smith VF, Randall LL (2018) Penetration into membrane of amino-terminal region of SecA when associated with SecYEG in active complexes: Penetration of SecA N Terminus into a Membrane Bilayer. *Protein Sci* **27**: 681–691. https://doi.org/10.1002/pro.3362
Floyd JH, You Z, Hsieh Y-H, Ma Y, Yang H, Tai PC (2014) The dispensability and requirement of SecA N-terminal aminoacyl residues for complementation, membrane binding, lipid-specific domains and channel activities. *Biochem Biophys Res Commun* **453**: 138–142. https://doi.org/10.1016/j.bbrc.2014.09.080
Gelis I, Bonvin AMJJ, Keramisanou D, Koukaki M, Gouridis G, Karamanou S, Economou A, Kalodimos CG (2007) Structural basis for signal-sequence recognition by the translocase motor SecA as determined by NMR. *Cell* **131**: 756–769. https://doi.org/10.1016/j.cell.2007.09.039
Gierasch LM (1989) Signal sequences. *Biochemistry* **28**: 923–930. https://doi.org/10.1021/bi00429a001
Gilmore R, Walter P, Blobel G (1982) Protein translocation across the endoplasmic reticulum. II. Isolation and characterization of the signal recognition particle receptor. *J Cell Biol* **95**: 470–477. https://doi.org/10.1083/jcb.95.2.470
Gold VAM, Whitehouse S, Robson A, Collinson I (2013) The dynamic action of SecA during the initiation of protein translocation. *Biochem J* **449**: 695–705. https://doi.org/10.1042/BJ20121314
Gouridis G, Karamanou S, Sardis MF, Schärer MA, Capitani G, Economou A (2013) Quaternary dynamics of the SecA motor drive translocase catalysis. *Mol Cell* **52**: 655–666. https://doi.org/10.1016/j.molcel.2013.10.036
Grady LM, Michtavy J, Oliver DB (2012) Characterization of the *Escherichia coli* SecA signal peptide-binding site. *J Bacteriol* **194**: 307–316. https://doi.org/10.1128/JB.06150-11
Gupta R, Toptygin D, Kaiser CM (2020) The SecA motor generates mechanical force during protein translocation. *Nat Commun* **11**: 3802. https://doi.org/10.1038/s41467-020-17561-2
Hartl FU, Lecker S, Schiebel E, Hendrick JP, 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. https://doi.org/10.1016/0092-8674(90)90160-g
Hegde RS, Bernstein HD (2006) The surprising complexity of signal sequences. *Trends Biochem Sci* **31**: 563–571. https://doi.org/10.1016/j.tibs.2006.08.004
Hsieh Y, Zhang H, Lin B, Cui N, Na B, Yang H, Jiang C, Sui S, Tai PC (2011) SecA alone can promote protein translocation and ion channel activity: SecYEG increases efficiency and signal peptide specificity. *J Biol Chem* **286**: 44702–44709. https://doi.org/10.1074/jbc.M111.300111
Hsieh Y, Zhang H, Wang H, Yang H, Jiang C, Sui S, Tai PC (2013) Reconstitution of functionally efficient SecA-dependent protein-conducting channels: transformation of low-affinity SecA-liposome channels to high-affinity SecA-SecYEG-SecDF·YajC channels. *Biochem Biophys Res Commun* **431**: 388–392. https://doi.org/10.1016/j.bbrc.2013.01.042
Hsieh Y-H, Huang Y-J, Zhang H, Liu Q, Lu Y, Yang H, Houghton J, Jiang C, Sui S-F, Tai PC (2017) Dissecting structures and functions of SecA-only protein-conducting channels: ATPase, pore structure, ion channel activity, protein translocation, and interaction with SecYEG/SecDF•YajC. *PloS One* **12**: e0178307. https://doi.org/10.1371/journal.pone.0178307
Huang Y-J, Wang H, Gao F-B, Li M, Yang H, Wang B, Tai PC (2012) Fluorescein analogues inhibit SecA ATPase: the first sub-micromolar inhibitor of bacterial protein translocation. *Chem Med Chem* **7**: 571–577. https://doi.org/10.1002/cmdc.201100594
Huber D, Jamshad M, Hanmer R, Schibich D, Döring K, Marcomini I, Kramer G, Bukau B (2016) SecA cotranslationally interacts with nascent substrate proteins *in vivo*. *J Bacteriol* **199**: e00622-16. https://doi.org/10.1128/JB.00622-16
Hunt JF, Weinkauf S, Henry L, Fak JJ, McNicholas P, Oliver DB, Deisenhofer J (2002) Nucleotide control of interdomain interactions in the conformational reaction cycle of SecA. *Science* **297**: 2018–2026. https://doi.org/10.1126/science.1074424
Jamshad M, Knowles TJ, White SA, Ward DG, Mohammed F, Rahman KF, Wynne M, Hughes GW, Kramer G, Bukau B, Huber D (2019) The C-terminal tail of the bacterial translocation ATPase SecA modulates its activity. *eLife* **8**. https://doi.org/10.7554/eLife.48385
Jilaveanu LB, Zito CR, Oliver D (2005) Dimeric SecA is essential for protein translocation. *Proc Natl Acad Sci U S A* **102**: 7511–7516. https://doi.org/10.1073/pnas.0502774102
Jin J, Cui J, Chaudhary AS, Hsieh Y-H, Damera K, Zhang H, Yang H, Wang B, Tai PC (2015) Evaluation of small molecule SecA inhibitors against methicillin-resistant *Staphylococcus aureus*. *Bioorg Med Chem* **23**: 7061–7068. https://doi.org/10.1016/j.bmc.2015.09.027

Jin J, Hsieh Y-H, Chaudhary AS, Cui J, Houghton JE, Sui S-F, Wang B, Tai PC (2018) SecA inhibitors as potential antimicrobial agents: differential actions on SecA-only and SecA-SecYEG protein-conducting channels. *FEMS Microbiol Lett* **365**. https://doi.org/10.1093/femsle/fny145

Jin J, Hsieh Y-H, Cui J, Damera K, Dai C, Chaudhary AS, Zhang H, Yang H, Cao N, Jiang C, Vaara M, Wang B, Tai PC (2016) Using chemical probes to assess the feasibility of targeting seca for developing antimicrobial agents against gram-negative bacteria. *ChemMedChem* **11**: 2511–2521. https://doi.org/10.1002/cmdc.201600421

Karamanou S, Vrontou E, Sianidis G, Baud C, Roos T, Kuhn A, Politou AS, Economou A (1999) A molecular switch in SecA protein couples ATP hydrolysis to protein translocation. *Mol Microbiol* **34**: 1133–1145. https://doi.org/10.1046/j.1365-2958.1999.01686.x

Kimsey HH, Dagarag MD, Kumamoto CA (1995) Diverse effects of mutation on the activity of the *Escherichia coli* export chaperone SecB. *J Biol Chem* **270**: 22831–22835. https://doi.org/10.1074/jbc.270.39.22831

Kimura E, Akita M, Matsuyama S, Mizushima S. (1991) Determination of a region in SecA that interacts with presecretory proteins in *Escherichia coli*. *J Biol Chem* **266**: 6600–6606

Knüpffer L, Fehrenbach C, Denks K, Erichsen V, Petriman N-A, Koch H-G (2019) Molecular mimicry of SecA and signal recognition particle binding to the bacterial ribosome. *mBio* **10**. https://doi.org/10.1128/mBio.01317-19

Koch S, de Wit JG, Vos I, Birkner JP, Gordiichuk P, Herrmann A, van Oijen AM, Driessen AJM (2016) Lipids activate SecA for high affinity binding to the SecYEG complex. *J Biol Chem* **291**: 22534–22543. https://doi.org/10.1074/jbc.M116.743831

Komarudin AG, Driessen AJM (2019) SecA-mediated protein translocation through the SecYEG channel. *Microbiol Spectr* **7**. https://doi.org/10.1128/microbiolspec.PSIB-0028-2019

Koonin EV, Gorbalenya AE (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. https://doi.org/10.1016/0014-5793(92)80009-6

Kourtz L, Oliver D (2000) Tyr-326 plays a critical role in controlling SecA-preprotein interaction. *Mol Microbiol* **37**: 1342–1356. https://doi.org/10.1046/j.1365-2958.2000.02078.x

Kusters I, Driessen AJM (2011) SecA, a remarkable nanomachine. *Cell Mol Life Sci CMLS* **68**: 2053–2066. https://doi.org/10.1007/s00018-011-0681-y

Kusters I, van den Bogaart G, Kedrov A, Krasnikov V, Fulyani F, Poolman B, Driessen AJM (2011) Quaternary structure of SecA in solution and bound to SecYEG probed at the single molecule level. *Structure* **19**: 430–439. https://doi.org/10.1016/j.str.2010.12.016

Li G-W, Burkhardt D, Gross C, Weissman JS (2014) Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. *Cell* **157**: 624–635. https://doi.org/10.1016/j.cell.2014.02.033

Luirink J, ten Hagen-Jongman CM, van der Weijden CC, Oudega B, High S, Dobberstein B, Kusters R (1994) An alternative protein targeting pathway in *Escherichia coli*: studies on the role of FtsY. *EMBO J* **13**: 2289–2296

Luirink J, von Heijne G, Houben E, de Gier J-W (2005) Biogenesis of inner membrane proteins in *Escherichia coli*. *Annu Rev Microbiol* **59**: 329–355. https://doi.org/10.1146/annurev.micro.59.030804.121246

Ma C, Wu X, Sun D, Park E, Catipovic MA, Rapoport TA, Gao N, Li L (2019) Structure of the substrate-engaged SecA-SecY protein translocation machine. *Nat Commun* **10**: 2872. https://doi.org/10.1038/s41467-019-10918-2

Matousek WM, Alexandrescu AT (2004) NMR structure of the C-terminal domain of SecA in the free state. *Biochim Biophys Acta BBA - Proteins Proteomics* **1702**: 163–171. https://doi.org/10.1016/j.bbapap.2004.08.012

Matsuyama S, Kimura E, Mizushima S (1990) Complementation of two overlapping fragments of SecA, a protein translocation ATPase of *Escherichia coli*, allows ATP binding to its amino-terminal region. *J Biol Chem* **265**: 8760–8765. https://doi.org/10.1016/S0021-9258(19)38953-7

Mitchell C, Oliver D (1993) Two distinct ATP-binding domains are needed to promote protein export by *Escherichia coli* SecA ATPase. *Mol Microbiol* **10**: 483–497. https://doi.org/10.1111/j.1365-2958.1993.tb00921.x

Musial-Siwek M, Rusch SL, Kendall DA (2005) Probing the affinity of SecA for signal peptide in different environments. *Biochemistry* **44**: 13987–13996. https://doi.org/10.1021/bi050882k

Na B, You Z, Yang H, Tai PC (2015) Characterization of the minimal length of functional SecA in *Escherichia coli*. *Biochem Biophys Res Commun* **456**: 213–218. https://doi.org/10.1016/j.bbrc.2014.11.061

Nakatogawa H, Murakami A, Ito K (2004) Control of SecA and SecM translation by protein secretion. *Curr Opin Microbiol* **7**: 145–150. https://doi.org/10.1016/j.mib.2004.01.001

Oliver DB, Beckwith J (1982a) Regulation of a membrane component required for protein secretion in *Escherichia coli*. *Cell* **30**: 311–319. https://doi.org/10.1016/0092-8674(82)90037-x

Oliver DB, Beckwith J (1982b) Identification of a new gene (*secA*) and gene product involved in the secretion of envelope proteins in *Escherichia coli*. *J Bacteriol* **150**: 686–691. https://doi.org/10.1128/jb.150.2.686-691.1982

Oliver DB, Beckwith J (1981) *E. coli* mutant pleiotropically defective in the export of secreted proteins. *Cell* **25**: 765–772. https://doi.org/10.1016/0092-8674(81)90184-7

Or E, Boyd D, Gon S, Beckwith J, Rapoport T (2005) The bacterial ATPase SecA functions as a monomer in protein translocation. *J Biol Chem* **280**: 9097–9105. https://doi.org/10.1074/jbc.M413947200

Or E, Navon A, Rapoport T (2002) Dissociation of the dimeric SecA ATPase during protein translocation across the bacterial membrane. *EMBO J* **21**: 4470–4479. https://doi.org/10.1093/emboj/cdf471

Or E, Rapoport T (2007) Cross-linked SecA dimers are not functional in protein translocation. *FEBS Lett* **581**: 2616–2620. https://doi.org/10.1016/j.febslet.2007.04.083

Orfanoudaki G, Economou A (2014) Proteome-wide subcellular topologies of *E. coli* polypeptides database (STEPdb). *Mol Cell Proteomics MCP* **13**: 3674–3687. https://doi.org/10.1074/mcp.O114.041137

Papanikolau Y, Papadovasilaki M, Ravelli RBG, McCarthy AA, Cusack S, Economou A, Petratos K (2007) Structure of dimeric SecA, the *Escherichia coli* preprotein translocase motor. *J Mol Biol* **366**: 1545–1557. https://doi.org/10.1016/j.jmb.2006.12.049

Papanikou E, Karamanou S, Baud C, Frank M, Sianidis G, Keramisanou D, Kalodimos CG, Kuhn A, Economou A (2005) Identification of the preprotein binding domain of SecA. *J Biol Chem* **280**: 43209–43217. https://doi.org/10.1074/jbc.M509990200

Pohlschröder M, Prinz WA, Hartmann E, Beckwith J (1997) Protein translocation in the three domains of life: variations on a theme. *Cell* **91**: 563–566. https://doi.org/10.1016/s0092-8674(00)80443-2

Poritz MA, Bernstein HD, Strub K, Zopf D, Wilhelm H, Walter P (1990) An *E. coli* ribonucleoprotein containing 4.5S RNA resembles mammalian signal recognition particle. *Science* **250**: 1111–1117. https://doi.org/10.1126/science.1701272

Rajapandi T, Dolan KM, Oliver DB (1991) The first gene in the *Escherichia coli secA* operon, gene X, encodes a nonessential secretory protein. *J Bacteriol* **173**: 7092–7097. https://doi.org/10.1128/jb.173.22.7092-7097.1991

Randall LL, Crane JM, Lilly AA, Liu G, Mao C, Patel CN, Hardy SJS (2005) Asymmetric binding between SecA and SecB two symmetric proteins: implications for function in export. *J Mol Biol* **348**: 479–489. https://doi.org/10.1016/j.jmb.2005.02.036

Rao C V S, De Waelheyns E, Economou A, Anné J (2014) Antibiotic targeting of the bacterial secretory pathway. *Biochim Biophys Acta* **1843**: 1762–1783. https://doi.org/10.1016/j.bbamcr.2014.02.004

Roussel G, White SH (2020) Binding of SecA ATPase monomers and dimers to lipid vesicles. *Biochim Biophys Acta BBA – Biomembr* **1862**: 183112. https://doi.org/10.1016/j.bbamem.2019.183112

Salavati R, Oliver D (1995) Competition between ribosome and SecA binding promotes *Escherichia coli* secA translational regulation. *RNA N Y N* **1**: 745–753.

Sardis MF, Economou A (2010) SecA: a tale of two protomers. *Mol Microbiol* **76**: 1070–1081. https://doi.org/10.1111/j.1365-2958.2010.07176.x

Sato K, Mori H, Yoshida M, Mizushima S (1996) Characterization of a potential catalytic residue, Asp-133, in the high affinity ATP-binding site of *Escherichia coli* SecA, translocation ATPase. *J Biol Chem* **271**: 17439–17444. https://doi.org/10.1074/jbc.271.29.17439

Schibich D, Gloge F, Pöhner I, Björkholm P, Wade RC, von Heijne G, Bukau B, Kramer G (2016) Global profiling of SRP interaction with nascent polypeptides. *Nature* **536**: 219–223. https://doi.org/10.1038/nature19070

Schiebel E, Driessen AJM, Hartl F-U, Wickner W (1991) ΔμH+ and ATP function at different steps of the catalytic cycle of preprotein translocase. *Cell* **64**: 927–939. https://doi.org/10.1016/0092-8674(91)90317-R

Schmidt A, Kochanowski K, Vedelaar S, Ahrné E, Volkmer B, Callipo L, Knoops K, Bauer M, Aebersold R, Heinemann M (2016) The quantitative and condition-dependent *Escherichia coli* proteome. *Nat Biotechnol* **34**: 104–110. https://doi.org/10.1038/nbt.3418

Schmidt MO, Brosh RM, Oliver DB (2001) *Escherichia coli* SecA helicase activity is not required *in vivo* for efficient protein translocation or autogenous regulation. *J Biol Chem* **276**: 37076–37085. https://doi.org/10.1074/jbc.M104584200

Segers K, Anné J (2011) Traffic jam at the bacterial sec translocase: targeting the SecA nanomotor by small-molecule inhibitors. *Chem Biol* **18**: 685–698. https://doi.org/10.1016/j.chembiol.2011.04.007

Seinen A-B, Spakman D, van Oijen AM, Driessen AJM (2021) Cellular dynamics of the SecA ATPase at the single molecule level. *Sci Rep* **11**: 1433. https://doi.org/10.1038/s41598-021-81081-2

Sianidis G, Karamanou S, Vrontou E, Boulias K, Repanas K, Kyrpides N, Politou AS, Economou A (2001) Cross-talk between catalytic and regulatory elements in a DEAD motor domain is essential for SecA function. *EMBO J* **20**: 961–970. https://doi.org/10.1093/emboj/20.5.961

Sikdar R, Peterson JH, Anderson DE, Bernstein HD (2017) Folding of a bacterial integral outer membrane protein is initiated in the periplasm. *Nat Commun* **8**: 1309. https://doi.org/10.1038/s41467-017-01246-4

Singh R, Kraft C, Jaiswal R, Sejwal K, Kasaragod VB, Kuper J, Bürger J, Mielke T, Luirink J, Bhushan S (2014) Cryo-electron microscopic structure of SecA protein bound to the 70S ribosome. *J Biol Chem* **289**: 7190–7199. https://doi.org/10.1074/jbc.M113.506634

Sugie Y, Inagaki S, Kato Y, Nishida H, Pang C-H, Saito T, Sakemi S, Dib-Hajj F, Mueller JP, Sutcliffe J, Kojima Y (2002) CJ-21, 058, a new SecA inhibitor isolated from a fungus. *J Antibiot (Tokyo)* **55**: 25–29. https://doi.org/10.7164/antibiotics.55.25

Suo Y, Hardy SJS, Randall LL (2011) Orientation of SecA and SecB in complex, derived from disulfide cross-linking. *J Bacteriol* **193**: 190–196. https://doi.org/10.1128/JB.00975-10

Taniguchi Y, Choi PJ, Li G-W, Chen H, Babu M, Hearn J, Emili A, Xie XS (2010) Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science* **329**: 533–538. https://doi.org/10.1126/science.1188308

Ulbrandt ND, Newitt JA, Bernstein HD (1997) The *E. coli* signal recognition particle is required for the insertion of a subset of inner membrane proteins. *Cell* **88**: 187–196. https://doi.org/10.1016/s0092-8674(00)81839-5

van der Wolk JPW, de Wit JG, Driessen AJM (1997) The catalytic cycle of the *Escherichia coli* SecA ATPase comprises two distinct preprotein translocation events. *EMBO J* **16**: 7297–7304. https://doi.org/10.1093/emboj/16.24.7297

von Heijne G (1985) Signal sequences. The limits of variation. *J Mol Biol* **184**: 99–105. https://doi.org/10.1016/0022-2836(85)90046-4

Walsh SI, Peters DS, Smith PA, Craney A, Dix MM, Cravatt BF, Romesberg FE (2019) Inhibition of protein secretion in *Escherichia coli* and Sub-MIC effects of arylomycin antibiotics. *Antimicrob Agents Chemother* **63**: e01253-18. https://doi.org/10.1128/AAC.01253-18

Walter P, Blobel G (1981) Translocation of proteins across the endoplasmic reticulum III. Signal recognition protein (SRP) causes signal sequence-dependent and site-specific arrest of chain elongation that is released by microsomal membranes. *J Cell Biol* **91**: 557–561. https://doi.org/10.1083/jcb.91.2.557

Walter P, Ibrahimi I, Blobel G (1981a) Translocation of proteins across the endoplasmic reticulum. I. Signal recognition protein (SRP) binds to in-vitro-assembled polysomes synthesizing secretory protein. *J Cell Biol* **91**: 545–550. https://doi.org/10.1083/jcb.91.2.545

Wang H, Na B, Yang H, Tai PC (2008) 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**: 1413–1418. https://doi.org/10.1128/JB.01633-07

Wang H-W, Chen Y, Yang H, Chen X, Duan M-X, Tai PC, Sui S-F (2003) Ring-like pore structures of SecA: Implication for bacterial protein-conducting channels. *Proc Natl Acad Sci U S A* **100**: 4221–4226. https://doi.org/10.1073/pnas.0737415100

Yang C-K, Lu C-D, Tai PC (2013) Differential expression of secretion machinery during bacterial growth: SecY and SecF decrease while SecA increases during transition from exponential phase to stationary phase. *Curr Microbiol* **67**: 682–687. https://doi.org/10.1007/s00284-013-0421-7

You Z, Liao M, Zhang H, Yang H, Pan X, Houghton JE, Sui S-F, Tai PC (2013) Phospholipids induce conformational changes of SecA to form membrane-specific domains: AFM structures and implication on protein-conducting channels. *PloS One* **8**: e72560. https://doi.org/10.1371/journal.pone.0072560

Young J, Duong F (2019) Investigating the stability of the SecA-SecYEG complex during protein translocation across the bacterial membrane. *J Biol Chem* **294**, 3577–3587. https://doi.org/10.1074/jbc.RA118.006447

Zhang Q, Li Y, Olson R, Mukerji I, Oliver D (2016) Conserved SecA signal peptide-binding site revealed by engineered protein chimeras and förster resonance energy transfer. *Biochemistry* **55**: 1291–1300. https://doi.org/10.1021/acs.biochem.5b01115

Zhou J, Xu Z (2003) Structural determinants of SecB recognition by SecA in bacterial protein translocation. *Nat Struct Mol Biol* **10**: 942–947. https://doi.org/10.1038/nsb980

Zimmer J, Li W, Rapoport TA (2006) A novel dimer interface and conformational changes revealed by an X-ray structure of *B. subtilis* SecA. *J Mol Biol* **364**: 259–265. https://doi.org/10.1016/j.jmb.2006.08.044

Zimmer J, Nam Y, Rapoport TA (2008) Structure of a complex of the ATPase SecA and the protein-translocation channel. *Nature* **455**: 936–943. https://doi.org/10.1038/nature07335