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 Sec-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

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

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Abbreviations: 2HF, Two Helix Finger domain; aa, amino acid; CTL, C-terminal linker; CTT, C-terminal tail; FLD, Flexible Linker Domain; HSD, Helical 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; MBP, Metal Binding Domain; MIC, Minimum Inhibitory Concentration; NBD1, Nucleotide Binding Domain 1; NBD2, Nucleotide Binding Domain 2; OM, Outer Membrane; OMP, Outer Membrane Protein; PDR, 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 (Barks 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 TRANSLLOCATION 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 (Ulbricht 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.](image-url)

(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 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).

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 interconversion 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 disulphide 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 disulphide 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* (Crandall-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 Crandall-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; Findlik et al., 2018; Jamshad et al., 2019; Knüpfert 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.
SecA – a bacterial translocation motor

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

| Domain | Amino acid residues | Function | Binding partners | References |
|--------|---------------------|----------|------------------|------------|
| NBD1   | 1-220 377-416       | ATP binding Autoregulation Substrate binding | ATP, RNA, ribosomes | Crane et al., 2017; Salavati et al., 1997 |
| PBD    | 221-376             | Recognition and binding of substrate protein | Substrate protein, SecYEG | Ernst et al., 2018 |
| IRA2 (NBD2) | 417-621   | Activation of ATP hydrolysis Autoregulation Substrate binding | ATP, RNA, SecB | Papanikolau et al., 2007; Salavati et al., 1997 |
| HSD    | 622-668             | Recognition and binding of substrate protein | Substrate protein, SecYEG, ribosomes | Grady et al., 2012; Ernst et al., 2018 |
| HWD    | 669-755             | Recognition and binding of substrate protein | Substrate protein, SecYEG | Ernst et al., 2018 |
| IRA1 (2HF) | 756-829   | Translocation across the membrane | SecY | Vrontou et al., 2004; 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, Substrate polypeptides, SecB, Phospholipids | Kimsey et al., 1995; Breukink et al., 1995; Fekkes et al., 1997; 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; Kreuzer et al., 2000; Suo et al., 2012). 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; Knüppfert 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
SecA-DEPENDENT PROTEIN TRANSLLOCATION

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 steric 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 anti-microbial 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 (thiouacil-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 thioura 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.

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