Sec, drugs and rock'n'roll: antibiotic targeting of bacterial protein translocation

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A large number of bacterial proteins are active in extracytoplasmic locations. Targeting and membrane translocation of the vast majority of these secretory and membrane polypeptides is mediated by the Sec pathway. Protein secretion requires the co-ordinated and sequential action of targeting factors on the *cis*-side of the membrane, a complex membrane-embedded protein translocase and maturation enzymes on the *trans*-side. Recently, significant advances in the molecular genetics and biochemistry of the Sec pathway have revealed that several of the Sec pathway components are essential for bacterial viability and/or pathogenicity. Moreover, several biochemical assays and structural insights have become available. Importantly, some of the Sec components are unique to bacteria. These developments raise the possibility that the bacterial protein translocase and other Sec pathway components could become formidable targets for antibacterial drug discovery.

Keywords: *ATPase, DEAD helicase, lipoproteins, membrane proteins, protein translocase, SecA, SecB, SecDF, SecYEG, signal peptidase, signal recognition particle, YidC*

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Review

1. Introduction

Genomic analyses revealed that more than one third of the cellular proteome comprises membrane and secretory proteins [1,2,3]. Extracytoplasmic proteins (either integral membrane or secretory proteins or lipoproteins) build the surrounding membranous structures and cell walls, allow the cell to communicate with other cells, participate in the acquisition of nutrients from the surrounding milieu or are involved in pathogenesis and/or in antipathogen response. The vast number and functional importance of these interactions makes protein secretion a cellular process essential for viability and/or pathogenicity.

Complex cellular processes acquire fidelity, accuracy, unidirectionality and speed through use of multisubunit molecular machines and highly specific protein-protein interactions. Bacteria have evolved numerous distinct protein trafficking machineries and pathways that allow them to export an astonishing array of substrates: hydrolytic enzymes (e.g., amylases, proteases, lipases), toxins (e.g., pertussis, haemolysins, colicins), appendages for attachment to surfaces (e.g., pili, adhesins), protein-wrapped DNA (e.g., Agrobacterium T-DNA), scavenger proteins (e.g., haeme binders), hormone-like signals (e.g., *Enterococcus* pheromone) and even regulatory

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proteins (e.g., the flagellar FlgM anti-σ factor). Proteins can be exported in a folded native state or, more frequently, in a partially unfolded form.

The vast majority of proteins and lipoproteins traffic across and into the bacterial membrane by making use of the Sec (for secretion) pathway [4], the only protein trafficking system that is essential for cell viability. Most Sec pathway proteins are conserved in the bacterial domain and some are also found in eukaryotes and in the archaea [5,6,201]. The process involves three biochemically distinct and temporally successive steps, each catalysed by specialised enzymes [4,7-10]:

- membrane targeting, involving chaperone-like proteins
- transmembrane crossing catalysed by the protein translocase (also known as 'translocon')
- maturation of the translocated protein catalysed by peptidases followed by periplasmic folding and release (i.e., in the case of secretory proteins) or lateral escape to the lipid phase and membrane integration (i.e., membrane proteins) mediated by folding facilitators

Commercially available antibiotics target no more than 15 bacterial enzymes and macromolecular complexes [11]. Practically all of the newly developed antibiotics of the past 20 years, with few exceptions (e.g., the protein synthesis inhibitor linezolid [12]), are only ameliorated derivatives of the original functional classes. However, the disquieting resurgence of antibiotic resistance (e.g., to vancomycin [13]) has prompted renewed efforts to discover not only new chemical classes of antibiotics but also to develop new approaches that take advantage of recent technological breakthroughs in genomics, enzymology and chemical synthesis [14-21]. These developments may lead to much needed targeting of other essential bacterial functions. Here, one such essential bacterial enzyme, the bacterial protein translocase and its accessory components, is discussed in the context of its potential as a chemotherapeutic target.

2. Why target the protein translocase?

Bacterial Sec translocase as an antibiotic target has several exceptionally attractive features:

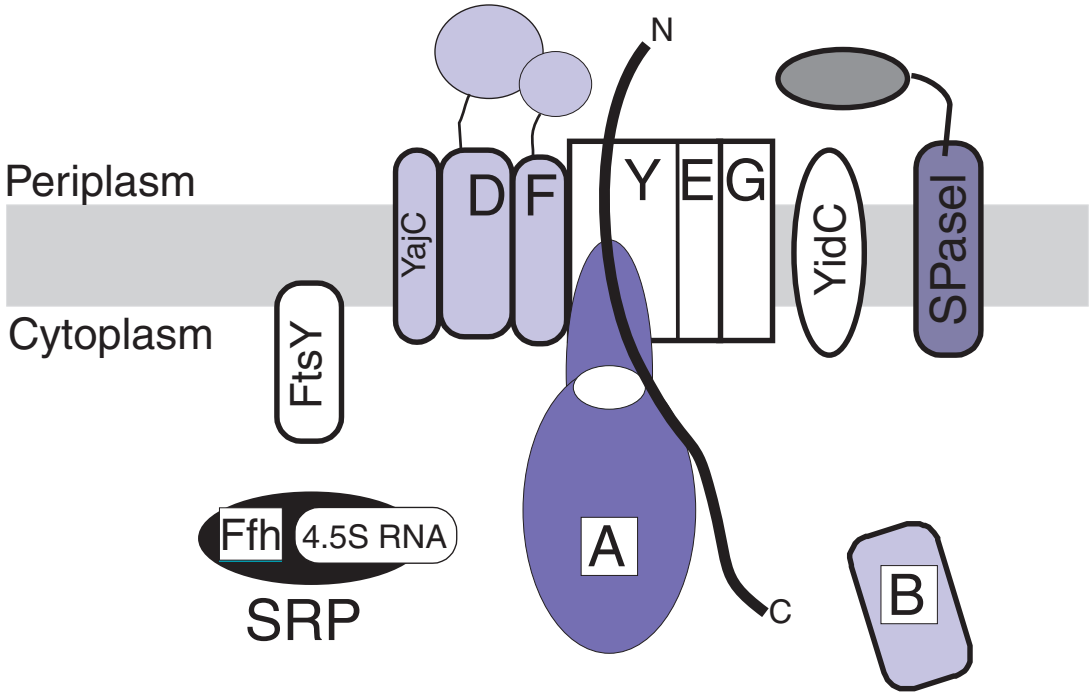
- translocase is essential for cell viability
- translocase catalyses the export of toxins, adhesins, pili and other virulence factors [22] and inhibition of the export of such factors could combat infection [23]
- translocase contains some subunits essential for function that are completely absent from eukaryotes
- due to the translocase subcellular topology it may be accessible from the periplasm without the need for the drug to be first taken up by the cytoplasm
- several translocase inhibitors (although non-marketable as antibiotics) have been identified: sodium azide [24], phenylethyl alcohol [25], the fatty acid biosynthesis inhibitor cerulelin [26] and others [27]
- *in vitro* and *in vivo* colorimetric assays have been developed (although further optimisation may be necessary)

A number of efforts have already been carried out by the pharmaceutical industry to develop Sec translocase antibacterials with modest success [27,28]. Some of these efforts are still ongoing. One of the obvious difficulties of this endeavour stems from the involvement of hydrophobic membranes in the Sec system and this makes *in vitro* high-throughput screening (HTS) assay development cumbersome. Moreover, it raises the chances of isolating generic membrane structure perturbants rather than specific translocase inhibitors. Recent molecular knowledge of the translocase has provided us with novel tools for renewed HTS efforts and has laid the foundations for others that need further development. Drawing on the recent molecular understanding of the Sec enzymes and also on the knowledge available from other enzymes with similar activities, I will discuss here types of inhibitors I anticipate to answer future HTS efforts. Finally, I will argue that much of this recent knowledge has not yet been taken advantage of and that therefore the potential for translocase as a drug target remains largely untapped.

2.1 Machines and enzymes of the Sec pathway

Secretory proteins possess reasonably hydrophobic N-terminal transit peptides (termed signal or leader peptides) while integral membrane proteins contain one or more hydrophobic patches of 20-30 amino-acyl residues. These features allow the cell to sort exported substrates from cytoplasmic proteins. Secretory proteins are recognised and delivered to the

Figure 1: Schematic representation of the bacterial translocase and accessory proteins of a Gram-negative bacterium. Sec proteins are only denoted with a capital letter. SRP (Signal Recognition Particle) is composed of two subunits: a 4.5S RNA species and the GTPase Ffh protein. The SecB secretion-specific chaperone is not present in Gram-positive bacteria. Signal peptidase I (SPase I; also known as Lep, leader peptidase) is responsible for proteolytic processing of translocated signal peptides at the *trans* side of the membrane. Another peptidase (Signal Peptidase II; not shown) processes only lipoprotein signal peptides. Gram-positive bacteria have more than 4 different signal peptidase polypeptides [3]. YidC has been isolated in complex with the translocase core [104] but may also operate as an independent exporter [37].



translocase at the membrane with the assistance of two cytoplasmic secretion-specific chaperone-like factors (**Figure 1**), SRP (Signal Recognition Particle [29]) and SecB [30] and/or by house keeping chaperones. SRP (a complex of a 4.5S RNA species with the Ffh protein [29]) binds the signal peptide of some secretory proteins and reaches the membrane by forming complexes with its cognate receptor, FtsY GTPase, a protein shown to partition between cytoplasm and membrane. SecB binds to the mature domain of pre-proteins and delivers them to the membrane by direct binding to the translocase [9,10].

Translocase is comprised of four subunits (SecA, SecY, SecE and SecG) that assemble in a functional and essential core (**Figure 1**) [9,10]. SecA is a peripheral membrane protein while SecYEG are polytopic integral membrane proteins. SecA possesses ATP binding and hydrolytic activities [31,32]. SecYEG may oligomerise into dimers or tetramers [33] and appear in low resolution electron microscopy images as doughnut-shaped with a putative indentation or trough in the middle [33,34]. Accessory subunits SecD, SecF, YajC and YidC (all polytopic membrane proteins) assemble with the SecYEGA core [35,36]. Such assemblies may form specialised export devices for different substrates. The exact quantitation of these translocase subspecies in the membrane, their dynamics and their physiological importance remain elusive. YidC is also thought to be capable of functioning on its own to translocate inner membrane proteins [37].

Following partial translocation to the periplasmic phase of the membrane, signal peptides are cleaved off by the action of Signal peptidase II (lipoproteins) or I (all other secretory proteins). Amazingly, Gram-positive bacteria possess at least three more signal peptidases with apparent specificity for particular substrates. It can not be excluded that signal peptidases form dynamic complexes with the translocase. Finally, enzymes, which could be considered to be components of the secretion pathway proper but which will not be discussed here, including periplasmic folding facilitators and disulfide bond formation enzymes [4], complete the export process.

2.2 Mechanistic model of bacterial translocation

We now have a complete inventory of the Sec pathway proteins and the reaction has been reconstituted *in vitro* [9,10]. These pioneering enzymology and genetic efforts have resulted in a coarse multi-step mechanistic model for bacterial Sec-mediated protein translocation. (**Figure 2** [32,38,39]). A central mechanistic aspect of this model is the SecA membrane cycling hypothesis (for detailed descriptions see [7,10,32,38-40]). Briefly, cytoplasmic SecA is targeted to the membrane by virtue of its affinity for lipids and the SecY protein (**Figure 2**, step 1). Initiation of protein translocation stems from the formation of cognate complexes between translocase, substrate and chaperones at the inner phase of the plasma membrane. Pre-protein substrates are delivered to the membrane by SecB or the SRP. The SecB-pre-protein complex binds directly to SecA. The SRP-pre-protein complex probably binds to the membrane at FtsY (not shown) and substrates are subsequently delivered to the translocase (step 2). Some inner membrane protein substrates may not make use of SecA at all. ATP binds to SecA and its binding energy drives a SecA conformational change. SecA appears to penetrate deeper into the membrane plane at SecYEG in a reaction termed 'membrane insertion'. Concomitantly, short segments of the substrate (approximately 20-30 amino-acyl residues) are transferred into the membrane plane. The inserted state of both SecA and the substrate is stabilised by SecDF (step 3). ATP is hydrolysed and the translocated polymeric substrate is partially released from SecA (step 4). When substrates are not bound to SecA further translocation may be driven by the proton motive force (step 5). ATP hydrolysis also causes SecA 'de-insertion' and the enzyme is recycled (step 6). SecA can either return to the cytoplasmic pool (step 7) or it can bind to a succeeding segment of the polypeptide and reinitiate the insertion/de-insertion cycle (step 8). Multiple repeated cycles of this piston-like motion are expected to allow processive movement of the protein translocase on the polymeric substrate [7] thereby leading to catalysis of complete translocation of an average 200-300 amino-acyl residue substrate. The SecA membrane cycling model is generally accepted today although some aspects of this mechanism have been challenged in one study [41].

Despite significant progress in understanding the Sec-pathway at the level of basic enzymology and cell biology, several central questions remain before we can claim molecular understanding of the system. Clearly, quantitative examination of the proposed enzymatic features, detailed topologies and conformational dynamics as well as the coupling of energy to mechanical movement remain elusive and will require novel biophysical and structural insight.

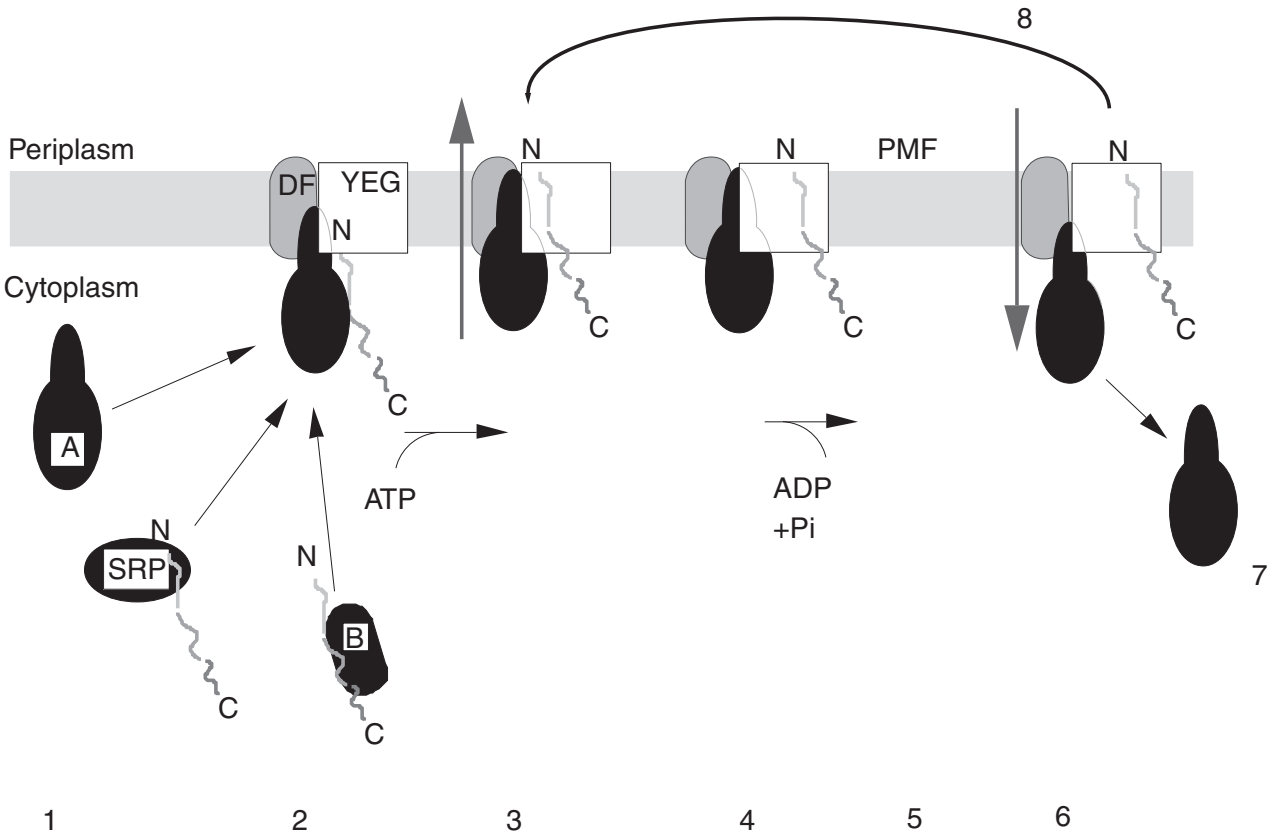
3. HTS assays

Chemical and natural product libraries, collectively representing several million compounds, are currently available to the pharmaceutical industry. It is possible that molecules with the potential to serve as lead compounds for several targets are present in these libraries waiting to be unearthed. In HTS for novel drug discovery, as is also true for genetic selection or enzyme purification, an appropriate assay system is one of the most crucial rate limiting steps. Numerous assays are currently available for assaying protein translocase for both *in vitro* and *in vivo* screens. Only a limited number of these assays have been developed for HTS and this may be partly responsible for the limited results.

3.1 *In vivo* assays

In vivo screening for inhibitors is a very powerful approach that ensures that the putative compounds to be isolated are bioactive and therefore problems with bacterial uptake sometimes seen with *in vitro*-developed drugs are circumvented. One of the original *in vivo* Sec pathway assays utilised for HTS was simple and took advantage of the upregulation of the SecA protein under conditions of compromised translocation. A β-galactosidase reporter fused to SecA permits facile detection of elevated SecA biosynthesis. This indirect assay has only yielded compounds that perturb membrane integrity [27]. Efforts to develop more direct indicators of *in vivo* secretion have also begun. Enzymes like alkaline phosphatase become active only upon translocation to the periplasm and can be easily assayed with chromogenic substrates [42]. Green fluorescent protein (GFP) is a powerful tool to study bacterial macromolecular trafficking [43] and provides another alternative as an easily detectable secretion substrate. GFP has been shown to be efficiently secreted across bacterial membranes once fused to the appropriate targeting signals [44,45], including Sec signal peptides [46]. In *Escherichia coli* bacteria, GFP was fused to the C-terminus of maltose binding protein signal peptide. The fusion protein was secreted to the periplasm but did not fluoresce suggesting improper folding [46].

Figure 2: Model of bacterial protein translocation through SecA membrane cycling. (see text for details). Signal peptidase, YidC, YajC have been omitted for simplicity.



Optimisation of such a system, e.g., by using a different fusion partner or strains overexpressing periplasmic folding facilitators could yield a useful tool for HTS.

Another interesting approach has made use of the easily detectable avidin-biotin chemistry. Avidin has been targeted and secreted into the lumen of eukaryotic organelles [47]. In *E. coli* a genetic method that allows biotinylation of polypeptides upon secretion [48] could also be exploited.

3.2 *In vitro* assays

While *in vivo* screens have the potential of yielding hits against all possible targets of a pathway in an unbiased way, *in vitro* approaches can also be deployed once sufficient molecular knowledge has been amassed. Such approaches can be very focused (e.g., on one target enzyme only). Bacterial Sec translocase and the complete protein secretion reaction has been completely reconstituted *in vitro* [32,49] and several enzymatic detection assays have become available. These include an *in vitro* translocation reaction that is unsuitable for HTS scale-up due to the use of low amounts of radioactive precursor, several handling steps and detection involving protein electrophoresis. A more popular approach has been the membrane and substrate-stimulated ATPase activity of SecA (translocation ATPase) that can be monitored spectrophotometrically using a simple colorimetric method detecting released phosphate [31]. Another widely used *in vitro* HTS detection method, fluoresence, has received little attention in the study of protein translocase (for some examples see [50,51]) and needs to be developed further. Finally, in the last decade equipment taking advantage of surface plasmon resonance (SPR) optical biosensors has become widely available and elegant methodologies have been developed for the study of membrane proteins immobilised on a biosensor surface [52]. In view of the several protein-protein interactions necessary for translocase function, SPR may prove to be very rewarding. SecA binding to lipid [53] and SecYEG [54] biosensors has been reported and several SecA interdomain interactions have been examined [55].

4. Potential Sec pathway targets

4.1 Potential targets: membrane targeting factors

SecB is an important low molecular weight chaperone that is organised in tetrameric assemblies [30] and is unique to most Gram-negative bacteria. SecB would therefore not be a broad range target. Apparently, this protein evolved to optimise secretion in the complex Gram-negative cell envelope. It was originally thought that SecB was essential for bacteria to survive in rich media when the demands or protein export are elevated but this effect was later shown to be indirect [56]. Recently, a high resolution structure of SecB was presented [30] and this could facilitate rational drug design. Small compounds and peptides that inhibit other chaperones have been identified [57,58].

The other important targeting factor, Signal Recognition Particle and its FtsY membrane receptor, have been to a significant extent conserved in evolution [29]. Nevertheless the proteins and RNA involved are sufficiently diverged to expect that specific drugs may be developed. Targeting SRP with *in vitro* tools is currently difficult since reconstitution of the reaction *in vitro* is poor. Furthermore the substrates that utilise SRP are mainly inner membrane proteins whose insertion can not be easily monitored in HTS assays and because their insertion is largely co-translational, they require the presence of synthesising ribosomes. This entails the danger of giving rise to false-positive protein synthesis inhibitors. An alternative rational design strategy could take advantage of the high resolution structural information that has become available for all of the subunits of the particle (including the mammalian one) and for a segment of its FtsY receptor [59]. Powerful software recently developed by Ribotargets (Cambridge, UK [202]) allows molecular docking *in silico* of large libraries of chemical compounds to RNA and proteins.

4.2 Potential targets: translocase subunits

Assuming that appropriate assay tools were available what translocase targets would one want to aim for and/or expect to get inhibitors against? When it comes to considering potential targets for antibacterial chemotherapy one obviously seeks to identify compounds that are uniquely effective against bacteria but would not have any harmful effect on humans. One logical pursuit is to identify essential proteins (ideally enzymes) present in bacteria but absent from humans. This effort has been significantly aided by the recent explosion in bacterial genomics and proteomics data. In addition, several successful targets represent enzymes that are ubiquitous in the three domains of life but that have diverged sufficiently to allow selective targeting. Bacterial secretion offers several possibilities since it is catalysed by some enzymes not present in humans as well as others that have evolved substantial sequence variation. Focused *in vitro* and *in silico* efforts together with more unbiased *in vivo* screens could turn up potential inhibitors for a large number of the participating proteins.

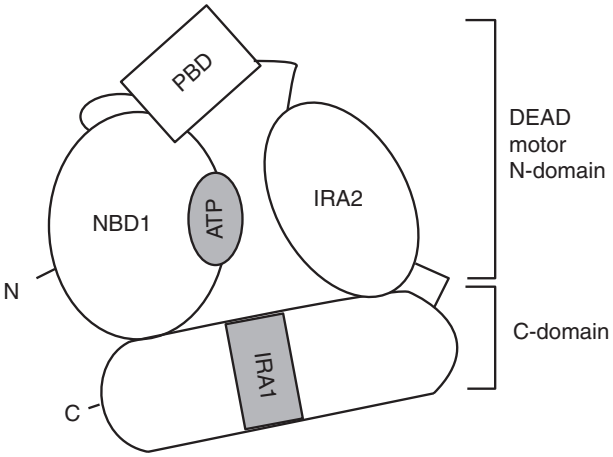
4.2.1 SecA

The ATPase motor of the translocase is an essential protein unique to bacteria SecA. It is an elongated [60], multi-domain protein containing an ATPase N-terminal domain of 68kDa and a C-terminal domain of 34kDa (**Figure 3**). Detailed mapping of interdomain interactions has revealed the basic structural plan of the protein allowing a combination of rigidity and flexibility [53,55]. The modular architecture of SecA permits physical interactions with almost all of the other components of the reaction and as a result the enzyme possesses several measurable enzymatic activities (**Table 1**). These observations render SecA a highly attractive candidate for chemotherapeutic targeting and raise the possibility that several different types of inhibitors affecting distinct SecA sub-reactions can be potentially identified. Furthermore, a high resolution structure of SecA has presented at meetings should become available shortly.

4.2.1.1 SecA ATPase activity

ATP hydrolysis by SecA is essential for translocase to work [31,32]. The ability to hydrolyse ATP resides in the N-terminal domain shown to have homology to the motor domain of DEAD proteins ([55], see below). Several inhibitors of ATP and GTP hydrolysing enzymes are known. One example is NSC630668-R/1 that inhibits the Hsp70-family ATPase motor of the yeast endoplasmic reticulum and prevents protein translocation [55]. In another example aurovertins inhibit the F_1-ATPase [61,62]. Sodium azide, a compound toxic to humans, is also a potent inhibitor of both the F_1-ATPase [62] and SecA [24]. *In vitro* experiments have suggested that high levels of sodium azide reduce the ATPase activity of SecA [24]. In addition, high-throughput screening efforts by a

Figure 3: Model of domain organisation of the SecA protomer (after [53, 55]). Two essential IRA (Intramolecular Regulator of ATP hydrolysis) elements are present in the SecA protein: IRA1, a molecular switch, suppresses ATP hydrolysis in cytoplasmic SecA and primes the enzyme for membrane-induced catalysis upon SecA interaction with SecY. IRA2 is a positive activator of catalysis at the nucleotide binding domain NBD1 and contains two conserved DEAD RNA helicase motifs. PBD denotes the Pre-protein Binding Domain proposed by chemical cross-linking studies [79].



number of companies employing the *in vitro* translocation ATPase assay have identified inhibitors of the SecA ATPase activity. However, these small-size inhibitors were of limited value since they were shown to be general inhibitors of many other ATPases. This problem has been encountered several times before with enzymes that interact with ATP (see, e.g., [21,63]) since they carry conserved nucleotide-binding domains [64]. Such problems may be subsequently overcome by chemical modification. Interestingly, it was recently shown that apart from the conserved nucleotide binding domain, SecA possesses additional regulatory elements that are essential for function and contain two signature motifs of the DEAD protein family [55]. Some of these sequences are unique to the SecA family. This raises the possibility that one might be able to identify allosteric rather than catalytic drugs that disrupt the ATPase activity of SecA by targeting regions other than those present in human ATPases. An additional possibility is to attempt to identify molecules, such as RNA aptamers [21] which would be small enough to cross the bacterial cell walls but be large enough to generate multiple contacts with the SecA ATPase domain and therefore be more specific catalytic antagonists.

4.2.1.2 SecA RNA helicase activity

One of the many enzymatic activities that render SecA a central enzyme for cellular metabolism in Gram-negative bacteria is its ability to regulate the translation of its own mRNA by binding to a site upstream of its Shine-Dalgarno sequence [65]. SecA biosynthesis also appears to be regulated in Gram-positive bacteria although the mechanism may be distinct [66]. It was proposed that SecA may possess RNA helicase activity, based on sequence similarity to DEAD helicases [67], and this exciting hypothesis was confirmed experimentally [68]. Although the physiological importance of the RNA helicase activity of SecA remains speculative if it is indeed involved in regulating SecA biosynthesis, it would be expected to be important for bacterial viability. Interestingly, the N-terminal DEAD motor domain of SecA is essential for its polypeptide translocation function [53,55]. The same DEAD motor would be anticipated to catalyse the RNA-related functions of SecA. Other DEAD helicases have been targets of drug development efforts since they are important virulence determinants. The hepatitis C virus NS3 protein is a DEAD-family RNA helicase whose structure has been determined [69] and is thought to aid replication of the viral genome. Vertex pharmaceuticals [203], Schering-Plough [69,70,204], Roche [71,205] and others [72] are actively involved in the development of drugs that target the NS3 helicase activity. One interesting class of compounds that could inhibit the RNA helicase activity of SecA is the peptide-nucleic acids (PNAs) [73], either acting by direct targeting of the enzymatic activity or by binding to the SecA mRNA recognition sequences and preventing SecA binding. PNAs that inhibit a DNA helicase [74] and others that target bacterial mRNA [75] have been identified. PNAs do not efficiently reach the cytoplasm of Gram-negative bacteria *in vivo* [76] and may therefore be better suited for Gram-positive targets. Finally, another class of inhibitors, thiopeptide or streptogramin derivatives, bind specifically to RNA [77] and may affect SecA RNA helicase activity.

4.2.1.3 SecA substrate binding

Bacterial protein secretion of soluble enzymes and integration of at least some membrane proteins initiates upon interaction binding of the exported substrate to SecA at the membrane [9,10]. One defined

Table 1: Biochemical properties and enzymatic activities of the SecA protein.

Property	Reference
Dimerisation	[53,81]
RNA binding	[99]
ATP-dependent RNA unwinding	[68]
secA mRNA translational control	[99]
ATP binding and hydrolysis activities (basal, membrane, translocation, lipid)	[31,99]
SecB binding	[38]
Signal peptide and mature domain binding	[31,78,79]
SecY binding	[38]
Phospholipid binding (nucleotide regulated)	[101]
Lipid bilayer penetration	[50]
Membrane insertion/de-insertion (cycling) at SecYEG	[39]
GroEL binding	[102]
CsaA binding	[103]

interaction is that between SecA and the signal peptide. Synthetic signal peptides bind to SecA and induce its ATPase activity [78]. Conceivably, such an activity could be used to develop fluorogenic or optical biosensor-based HTS assays to identify inhibitors of this interaction. Signal peptides are not extensively conserved in terms of their primary sequence although they have conserved biophysical properties across organisms. It is unlikely that signal peptide mimics can be used as drugs since they may perturb human cell membranes and are probably toxic. To overcome this caveat the signal peptide binding site on SecA can be specifically targeted by steric inhibitors. Binding sites on SecA for the mature, hydrophilic part of the secretory substrates [31,79] may be an even better candidate, although the nature of such sites remains totally unknown at this point. Such sites on SecA could be inhibited by peptide antibiotics similar to the ones targeted to the chaperone DnaK [57].

4.2.1.4 SecA dimerisation and interdomain interactions

The SecA protein acts as a dimer [80]. The ability of the protein to dimerise has been mapped to its 34kDa C-terminal domain [53,81]. Although the mechanistic significance of this higher order organisation is not known, it is conceivable that it is essential for SecA function and this is the form of the actively translocating enzyme [80]. Therefore, inhibitors of SecA oligomerisation would be expected to also inhibit SecA function. A number of dimeric enzymes are inhibited by dimerisation poisons [82-86]. Recent progress has allowed the development of several elegant tools that aim at identifying specific disruptors of protein-protein interactions [86-90]. SecA is a multidomain protein with several interdomain interactions dictating its function [53,55]. Although few protein-protein interaction inhibitors are used commercially to date [86], knowledge of the SecA structure may aid future discovery of such interdomain interaction drugs. Given the fact that the enzyme has several ligands, allosteric changes must be important as a means of co-ordinating the order of SecA-related biochemical events [53,55]. Another possibility would therefore be the identification of allosteric inhibitors [19] that would prevent interconversions between the different functional states.

4.2.2 SecYEG and YidC

SecY, SecE and YidC are not obvious antibiotic targets since these are polytopic membrane proteins and are largely conserved throughout the three karyotypic domains. Furthermore, there are no specific biochemical assays to detect distinct SecYE activities that could be used in an HTS assay. SecG on the other hand is a small membrane protein that is not conserved (although similar-sized subunits are part of the eukaryotic translocase [91]). Deletion of SecG is lethal in only few *E. coli* strains tested [92], although SecG is important for translocase function under compromising conditions [36]. SecG was proposed to undergo a substantial topological inversion during catalysis [93]. This very interesting change in topology may be important for facilitating SecA membrane cycling. Inhibitors of this flipping reaction could have a drastic effect in translocase catalysis by slowing down or preventing SecA cycling. A possible type of inhibitor with obvious advantages could bind to SecG from the periplasmic side thus preventing its topology reversion.

4.2.3 SecDF

The SecD and SecF proteins are not found in eukaryotes. They are present in the cell in complexes also containing YajC, a small, non-essential protein of

unknown function [35,94]. SecD and SecF are not catalytically essential for the process of secretion [36,40] but their deletion results in severely compromised protein translocation and severely reduced growth [94]. These two proteins are thought to optimise the reaction cycle of SecA by stabilising the inserted state of SecA and of the secretory substrate intermediate [35,36,40], but the exact mechanism of function remains elusive. However, these proteins have a characteristic feature: they both harbour very large soluble domains that sprout out of the integral membrane body of the proteins and into the periplasm (see **Figure 1**). Interestingly, anti-SecD antibodies added exogenously from the periplasmic face were shown to cause stalling of secretory intermediates *in statu translucanti* and as a result inhibit *in vitro* protein translocation [95]. These observations suggest that it may be possible to successfully target the periplasmic domains of SecDF. The obvious advantage of this approach is ease of access to exogenously added drugs without any need for uptake. Inefficient uptake sometimes prevents powerful *in vitro* inhibitors from being used *in vivo* as antibiotics. Moreover, the polar nature of these SecDF domains makes it possible to isolate them relatively easily allowing their subsequent structural characterisation in the absence of the integral membrane moiety.

4.3 Potential targets: signal peptidases

Maturation of translocated polypeptides after proteolytic removal of the signal peptide is essential for membrane biogenesis. The availability of simple *in vitro* assays and the fact that the catalytic domain of the processing enzymes SPase I and II are exposed to the periplasmic space prompted early HTS efforts. Several inhibitors have been identified for both bacterial peptidases [96-98]. Recently an atomic resolution structure of the periplasmically exposed catalytic domain of SPase I in complex with a β-lactam inhibitor has become available [28,98] and this represents a major step towards the optimisation and development of available lead compounds.

4.4 Potential targets: the importance of kinetics

Protein secretion requires the co-ordinated and sequential interaction of several proteinaceous factors. This requirement makes the cell extremely vulnerable to even slight reduction of rates within the Sec pathway. It is for this reason that an elaborate system making use of translational SecA regulation has evolved. Whenever secretion is compromised by mutation, lowering of ATP or proton motive force levels, exogenous addition of antitranslocase antibodies it leads to severely compromised or lethal phenotypes. This is thought to be the result of a 'log-jam' effect [94], i.e., pre-protein intermediates are stalled *in statu translocanti* and occupy the exit sites preventing other molecules from being exported. This observation is particularly important since it raises the hope that distinct 'kinetic' drugs affecting several different steps of the Sec pathway may be deleterious to bacterial secretion. Furthermore, this characteristic also opens up the possibility of the development of synergistic drugs for combination therapies. We may need to get accustomed to this idea as 'classic' cellular targets become saturated and a significant portion of them are still recalcitrant to drug inhibition.

5. Expert opinion

Past efforts to target the bacterial Sec pathway have been met with limited success. However, recent advances in the understanding of the basic biology of the Sec pathway and of the translocase in particular allow significant optimism for the development of novel anti-Sec pathway and anti-translocase drugs. High resolution structures for some of the components of the pathway have been obtained while others are being prepared. Enzymological findings suggest that several proteins and protein-protein interactions in the Sec pathway could be specifically targeted and a bevy of *in vitro* biochemical subreactions have been described. Even modest enzymatic inhibition may have dramatic kinetic effects on secretion. Several *in vitro* and *in vivo* assays are now available and more can be easily developed from existing ones and adapted to HTS automation. These exciting developments bring to the fore the bacterial Sec pathway as an attractive target for antibiotic development. However, in practice, the only good target is a "drugable" one. To achieve this, focused and extensive efforts in anti-translocase drug discovery will be required and newly developed assays and knowledge must be embraced.

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- •• of considerable interest

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