More than one-third of cellular proteomes traffic into and across membranes. Bacteria have invented several sophisticated secretion systems that guide various proteins to extracytoplasmic locations and in some cases inject them directly into hosts. Of these, the Sec system is ubiquitous, essential and by far the best understood. Secretory polypeptides are sorted from cytoplasmic ones initially due to characteristic signal peptides. Then they are targeted to the plasma membrane by chaperones/pilots. The translocase, a dynamic nanomachine, lies at the centre of this process and acts as a protein-conducting channel with a unique property; allowing both forward transfer of secretory proteins but also lateral release into the lipid bilayer with high fidelity and efficiency. This process, tightly orchestrated at the expense of energy, ensures fundamental cell processes such as membrane biogenesis, cell division, motility, nutrient uptake and environmental sensing. In the present review, we examine this fascinating process, summarizing current knowledge on the structure, function and mechanics of the Sec pathway.

Key words: channel, chaperone, membrane, protein secretion, signal peptide, translocation.

INTRODUCTION

A bacterial cell produces thousands of different proteins that localize to different sub-cellular compartments [1]. Up to half of the cytoplasmically synthesized proteins either become inserted in a membrane (i.e. plasma membrane) or cross membranes and become released on the other side (i.e. the periplasm or the extracellular milieu) (Figure 1) [2]. Membrane-embedded proteins are frequently transporters, channels involved in energy conversion, sensory transducers etc. Secreted proteins can be hydrolytic enzymes, toxins, small molecule scavengers etc.

The term secretion typically describes complete release of a protein from the cell to the extracellular milieu. In bacteria, secretion studies historically monitored ribosomes to membrane targeting and the translocation event by which secreted proteins cross or integrate into the plasma membrane. Bacterially secreted proteins may become periplasmic or outer membrane residents (Gram-negative bacteria), surface-attached extracellular appendages, cell-wall lipoproteins, might exit the intracellular space or even be injected into eukaryotic host cells.

To tackle the variety of secreted proteins and their destinations, bacterial cells have evolved at least 16 different secretion systems [2]. The need for different secretion systems is dictated by the nature, the folding state and the final destination of the secreted substrates. For example, in the case of a pathogen that injects its toxins directly into the host cytoplasm, it is evident that long bacterial extracellular structures that reach, dock and penetrate the host membranes are needed [3,4]. These structures are rooted in the bacterial plasma membrane and consist of dozens of proteins. In other cases a bigger dynamic channel was necessary to deal with the substrates of the TAT (twin-arginine translocation) secretion system, since they are released folded and usually with a co-factor bound [5,6]. Some preproteins are translocated with bound nucleic acids, so a channel has to be able to accommodate them as well [7]. Bacteria usually have two or more secretion systems.

Of all the bacterial secretion systems, the Sec system, the focus of the present review, mediates the export of most of the secretory and membrane proteins [8] and is by far the best understood. The membrane-embedded Sec channel (SecYEG) is conserved in the three domains of life [9]. The Sec system represents a good general model of the main challenges faced by any protein secretion pathway (Figure 1): how are exported proteins discriminated from cytoplasmic residents (sorting)? How do secretory proteins find the membrane (targeting)? Why is their folding usually prevented in the cytoplasm? How do these large aminoacyl-heteropolymers cross the membrane (translocation) and at what cost (energetics)? Clearly, the mechanical components chosen represent solutions that address these needs.

All secretory, and rarely membrane, proteins that use the Sec pathway are synthesized as ‘preproteins’ with N-terminal signal sequences that are proteolytically removed in a post-translocation step (Figure 2). Signal peptides tell apart secretory proteins from cytoplasmic ones, act as ‘ribosome-to-membrane’ address tags [10] and once at the membrane also act as allosteric keys that activate the Sec channel [11]. A general requirement for Sec preproteins is that they should remain unfolded. Through primary structure features of the preprotein chain and the use of specialized chaperones, almost all secretory proteins avoid folding and are maintained in a non-native state [2,12,13]. Frequently, the Sec core machinery, that includes the SecYEG channel, will handle both incorporation into the plasma membrane as well as secretion across it (Figure 3). In all cases transport is not spontaneous, it requires metabolic energy input. Two main modes of preprotein translocation exist: co- and post-translational. Both
Figure 1 Challenges faced by protein secretion machineries

(1) Exported proteins need to be discriminated from cytoplasmic residents. (2) Secreted proteins must be targeted to translocases. (3) Proteins that cross the lipid bilayer through the Sec system must remain unfolded. (4–5) Protein translocation can only occur at the expense of energy (ATP, PMF).

utilize the SecYEG channel. However, different auxiliary proteins and mechanisms deliver the preprotein cargo to it (Figure 3). In bacteria, the post-translational route is usually followed. Inner membrane proteins are mainly co-translationally targeted. In co-translational translocation, illicit cytoplasmic folding is drastically curtailed, as synthesis of the chain is coupled to its immediate export.

FEATURES OF AN EXITING PREPROTEIN

All Sec-dependent secretory preproteins consist of a signal peptide and a mature domain. A typical such signal peptide (Figure 2) is usually between 20 and 30 aa (amino acids) and has a three-domain structure: an N-terminal domain with 1–8 positive charges, followed by a helical hydrophobic core of 4–16 residues and a slightly polar C-terminal domain containing the SPase (signal peptidase) cleavage site [14,15]. Although Sec signal peptides share common biochemical properties, mature preprotein domains are variable. A mature domain can be as short as 30 aa or longer than 2000 aa with very hydrophilic or very hydrophobic sequences. The inner membrane proteins lack cleavable signal sequences, usually come in a range of sizes, with 1–20 α-helices that contain apolar or hydrophobic aa that will become transmembrane [TMH (transmembrane helix)] [15,16].

THE SEC SYSTEM: AN OVERVIEW OF PARTS AND PATHWAYS

Membrane protein insertion

In the Escherichia coli co-translational system, Ffh protein together with the short 4.5S RNA constitute the SRP (signal recognition particle) (Figure 4A) [17]. SRP docks on the L23 ribosomal subunit, close to the polypeptide exit tunnel [18], binds on the nascent preprotein chain as it emerges (Figures 3A and 5A), and targets the RNC (ribosome nascent chain) complex to its membrane receptor FtsY (Figures 3B and 5B) [17]. FtsY binds on the cytoplasmic loops of SecY and upon GTP hydrolysis promotes the dissociation of the SRP–FtsY complex [19]. Ultimately the protein cargo is delivered to the SecY channel (Figures 3B and 5C). The SRP pathway is essential in bacteria [20].

For a subset of bacterial membrane proteins, translocation involves the essential YidC protein; a membrane protein insertase that acts alone, or in concert with, SecYEG (Figure 3B) [21–23].

Secretory protein translocation

Unlike in eukaryotes, the vast majority of bacterial secretion occurs post-translationally (Figure 3) [24]. In post-translational secretion: (i) the preprotein is targeted to the SecYEG channel after being largely or, in most cases, fully synthesized; (ii) chaperones may be recruited to maintain a non-native state and; (iii) SecA is used, this is an essential ATPase which recognizes preproteins and energizes the translocase [2]. Hereafter, we will focus on the post-translational secretion of preproteins.

Utilizing the Sec machinery to deliver preproteins post-translationally

Post-translational secretion through the Sec system can be dissected in three major stages: (i) targeting of the preprotein to the translocase (SecYEG–SecA) (Figure 3A); (ii) assembly of the SecY–SecA–preprotein complex and preprotein translocation (Figure 3B); and (iii) release of the preprotein followed by either its periplasmic folding or its further translocation through a downstream secretion system (Figure 3C).

Stage 1: from the ribosome to the translocase

Preproteins, either during synthesis or after their release from the ribosome, are recognized by chaperones that prevent their early folding and divert them to the correct membrane location (Figure 3). The most prominent factors for binding the newly synthesized preprotein are the SRP, SecA, TF (trigger factor) and SecB.

TF (Figure 4B), SRP (Figure 4A) and SecA (Figure 4C), all bind to the L23 ribosomal subunit (Figure 5A for SRP and Figure 5D for TF) and therefore can potentially bind the emerging nascent preprotein [25,26]. The SecB chaperone (Figure 4D) is not known to bind directly on the ribosome but binds to ribosome-emerging polypeptides that are larger than ~150 aa [27] and delivers them...
Figure 2  A typical Sec system signal peptide

A summary of features of signal peptides. A total of 425 E. coli secreted proteins were collected from EchoLOCATION and Uniprot [194,195] and analysed. SignalP 3.0 was used to predict the boundaries of the N, H and C regions [196] and the location of the SPase I cleavage site. A subcategory of outer membrane proteins called autotransporters have an extended N region (N-AT) [197]. A typical N-terminal domain usually has positive charges (N) and is followed by a helical hydrophobic (H) core. The C-terminal domain (C) is slightly polar with a preference for cysteine, serine, threonine and alanine residues. This last part includes the SPase I cleavage site; −1 and −3 are its most conserved positions, usually a small aliphatic residue such as alanine and serine (G. Orfanoudaki, M. Papanastasiou and A. Economou, unpublished work). The mature domain of the protein follows the SPase I cleavage site.

Figure 3  Protein secretion in bacteria

Schematic representation of the major modules of the Sec system and the secretion process. (A) For membrane-embedded proteins, a TMH in the nascent polypeptide chain emerging from the ribosome is bound by SRP (co-translational translocation). The SRP–nascent chain–ribosome complex is transferred to SecYEG with the help of FtsY, the SRP receptor. Some proteins may directly use the YidC route (greyed arrow). Secreted proteins that have been largely or fully synthesized will be scanned by TF and through a process that might involve chaperones, such as SecB, will be delivered to soluble or SecYEG-bound SecA (post-translational translocation). SecA may participate in co- as well as post-translational translocation, when membrane proteins with large hydrophilic regions are translocated. (B) Once the SRP–nascent chain–ribosome complex reaches SecYEG, the preprotein, as it is being synthesized, enters the membrane through the SecYEG lateral gate, in some cases with the help of YidC. Following preprotein docking on SecA bound to SecYEG, SecA converts the chemical energy of ATP to mechanical work and transfers the preprotein through SecYEG in 20–30 residue segments [187]. SecDFYaC might help in preprotein release. SPase I (or II for lipoproteins, not shown) cleaves the signal peptide, hence releasing the mature domain to the periplasm. PMF increases the speed of translocation in both co- and post-translational modes. (C) Proteins newly released in the periplasm can interact with chaperones (Skp and SurA shown here as an example) and get transferred to machineries such as the BAM for insertion into the outer membrane (I), fold spontaneously or with the help of periplasmic chaperones that catalyse cysteine oxidation or prolyl isomerization (e.g. Dsb proteins and PPIase (peptidylprolyl isomerase)) (II), get secreted using other specialized systems (III, systems not shown), or become membrane-anchored via small lipid moieties guided by specialized lipoprotein sorting systems (IV).
Figure 4  Structures of Sec system components

Tube models of proteins participating in the secretion of Sec preproteins generated using MacPyMOL. (A) Side view of SRP (Ffh/4.5S RNA) (PDB code 2XXA [39]) indicating its known domains (N, G, M). Ffh is from E. coli; 4.5S RNA from D. radiodurans. (B) Structural model of the E. coli TF (PDB code 1W26 [65]) indicating its known domains. TF exists in solution as a dimer but only a monomer is shown here. (C) Structural model of an E. coli SecA protomer (PDB code 2FSF [99]). SecA domains are: the NBD; IRA2; the PBD and the C-terminal domain (C-domain). The red asterisk denotes the ATP-binding cleft. (D) Structure of the E. coli SecB tetramer (PDB code 1QYN [109]). A shallow groove formed between the two dimers has been proposed to be the preprotein-binding site (red asterisk). (E and F) Cytoplasmic side, top-down views of the protein-conducting channel with closed (E; PDB code 1RH5 [9]) or open (F; PDB code 3MP7 [198]) lateral gate (red arrowhead). SecY contains ten TMHs arranged in a pseudosymmetric clam-shell like structure with five TMHs on each side of the axis (broken line) surrounding the channel (red star). The plug helix (p) blocks the periplasmic face of the channel. SecE resembles a clamp that holds the bundle of SecY TMH together. (G) Structure of the T. thermophilus SecDF (PDB code 3AQP). In this organism, SecDF consists of a single polypeptide chain with 12 transmembrane segments and six periplasmic regions. Domains P1 and P4 and the regions corresponding to separate SecD and SecF proteins are indicated. (H) The structure of the periplasmic domain of the E. coli SPase I (PDB code 1B12 [152]) contains two β-sheets. The catalytic centre is indicated (red asterisk). Two TMHs that anchor SPase I to the membrane were drawn in. Domains I and II face the periplasmic space.

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Complexes potentially formed during preprotein handling by the Sec system components. (A) Model of the ribosome–SRP–signal peptide complex (PDB codes 3J00 and 3J01 [199]); SRP [Ffh (sky blue)/4.5S RNA (grey); PDB code 2J28]; the signal peptide (red cylinder) is from the E. coli FtsQ (PDB code 2J28 [48]). (B) FtsY (PDB code 2XXA [39]) shown bound on the membrane for visualization purposes. The membrane anchor and the ribosome with SRP are missing. (C) A ribosome and translating FtsQ (red tube) that has been translocated into the SecY channel (yellow cylinders; PDB codes 3J00 and 3J01 [199]). Data were derived from a cryo-EM structure. The FtsQ signal peptide is localized to the lateral gate. (D) Model of TF (PDB code 1W26 [65] bound on a ribosome (PDB codes 3J00 and 3J01 [199]). (E) A soluble Bacillus subtilis SecA dimer (PDB code 1M6N [76]). (F) The E. coli SecYEG/SecA holoenzyme modelled after the Thermotoga maritima structure [178]. A model of the LamB signal peptide (red cylinder) is shown bound to SecA. Data were derived from the NMR structure of soluble SecA-signal peptide (based on PDB codes 3DIN [178] and 2VDA [78]). (G) Model of a SecYEG protomer with LamB signal peptide bound based on Hizlan et al. [200]. (The solved cryo-EM structure of a SecYEG dimer with the signal peptide bound on one protomer [200], in this Figure, for simplicity only the protomer with the bound signal peptide is shown.

to SecA [28]. Direct interactions between SecB and SRP or TF have not been reported. The preprotein is finally delivered to the SecYEG channel only through the SecA or SRP.

Previous findings demonstrated that certain preproteins can maintain their non-native translocation-competent state in the absence of any chaperone [11]. This raises the possibility of chaperone-free targeting.

Stage 2: translocation and release

When the ternary complex of SecYEG–SecA–preprotein is formed (Figure 5F), the preprotein is translocated through the SecYEG channel at the expense of ATP by SecA and PMF (protonmotive force) (Figure 3B). SecYEG can associate with another membrane protein complex, SecDFYajC (Figure 3B), although this is not needed for translocation, it has been implicated in several steps of the translocation reaction [29].

Stage 3: periplasmic folding and further trafficking

Upon successful translocation, SPase I (Figures 3B and 4H) cleaves the signal peptide [30]. Release of the mature domain to the periplasm will lead to its folding by periplasmic chaperones (Skp, prolyl isomerases etc.) and cysteine oxidases (Dsb proteins) (Figure 3C, II) [31]. Alternatively, subsequent trafficking to the outer membrane through other secretion machines such as the BAM (β-barrel assembly machinery) system (Figure 3C, I) [32], or the LOL (lipoprotein outer membrane localization) system for lipoproteins (Figure 3C, IV) [33], or even the complete export across the outer membrane (e.g. through the Type II system) might occur (Figure 3C, III) [34].

In the section below, we present the molecular structure of proteins involved in the Sec pathway, their known protein–protein interactions and, finally, we dissect the translocation reaction in distinct mechanistic steps.

THE SEC SYSTEM: THE NUTS AND BOLTS

SRP and its receptor FtsY

Ffh, the proteinaceous moiety of SRP, contains the signal-peptide-binding site, the 4.5S RNA-binding M domain, C-terminally fused to the NG domain (Figure 4A) [35]. The N-terminal domain contains a four-helix bundle [36,37]. Unlike the Ras-like GTPases, the G domain contains an additional characteristic
The complex and is involved in the stimulation of its GTPase activity upon membrane binding [49,50]. The 4.5S RNA structurally supports the interaction with the membrane via two helices and stimulates the GTPase activity of the SRP–FtsY complex upon membrane binding [35,40–48]. An additional FtsY N-terminal domain, the A domain, is a TMH of a nascent membrane protein (Figures 2 and 5A). The C-terminal domain acts as a latch holding the NBD and IRA2 opening of the ATPase motor; (ii) the wing domain; (iii) the conserved helix–loop–helix IRA1; and (iv) a short C-terminal tail [77,82–84]. The PBD and C-terminal domain motions controlled by helicase motor ATP hydrolysis somehow force preproteins to cross the channel [85,86]. The PBD undergoes an almost ∼80° rotational motion of unknown function [87].

Soluble SecA (Figure 5E) has a high affinity for itself (0.76–14 nM) and undergoes monomer to dimer equilibria [88,89]. Given that its total cellular concentration is ∼7 μM [90] (Table 1), cytoplasmic SecA is expected to be mostly dimeric (Figure 5E). Most crystal structures contain SecA dimers [91–99]; however, the orientation of each protomer relative to the other is different [76,93,96,97,100]. Oligomeric organization of SecA during preprotein translocation remains elusive and controversial [87,101,102].

**Trigger factor**

The *E. coli* TF is a 48 kDa dimeric ATP-independent chaperone with an elongated structure like a ‘crouching dragon’ (Figure 4B) [56,57]. TF is not a core component of the Sec system since it binds cytoplasmic proteins as well as appears to be particularly important for the export of outer membrane proteins [58]. Monomeric TF (Figure 4B) interacts via its N-domain (aa 1–149) with the ribosome [56,57]. Its middle region (aa 150–245) is a peptideyl-prolyl cis-trans isomerase [59–61] not essential for chaperone activity in vivo [62–64]. The C domain (aa 246–432) is responsible for chaperone activity [63,65,66]. TF associates with ribosomes (Figure 5D) and interacts with nascent cytoplasmic proteins to assist their folding (folding), prevents folding and aggregation of proteins (holdase) or assists in protein assembly [67,68].

**The SecA ATPase motor**

SecA is a multifunctional nanomotor (Figure 4C). It binds to SecYEG (Figure 5F), preproteins, SecB and phospholipids and mediates the chemo-mechanical energy conversion required for translocation [2].

SecA belongs to the SF2 (superfamily 2) of ‘DExH/D’ (where x is any amino acid) proteins, which include helicases and RNA-modifying enzymes [2,69–74]. The SecA helicase motor consists of an NBD (nucleotide-binding domain) and the IRA2 (intramolecular regulator of ATP hydrolysis) [2,87,70,74]. ATP is sandwiched at the interface of NBD and IRA2, gets hydrolysed and causes helicase motor conformational changes [75–77] (Figure 4C). Two additional domains give SecA its specificity for polypeptides rather than RNA: (i) the PBD (preprotein-binding domain) that is rooted inside the NBD; and (ii) the C-terminal domain that is fused to IRA2. Signal peptides dock on the PBD (Figure 5F) [78–80] thus controlling ATP catalysis [81]. The C-terminal domain acts as a latch holding the NBD and IRA2 together to suppress futile ATP hydrolysis. It is composed of four sub-structures: (i) the scaffold domain, an α-helix that controls the C-terminal domain that is fused to IRA2. Signal peptides dock on the PBD (Figure 5F) [78–80] thus controlling ATP catalysis [81]. The C-terminal domain acts as a latch holding the NBD and IRA2 together to suppress futile ATP hydrolysis. It is composed of four sub-structures: (i) the scaffold domain, an α-helix that controls

<table>
<thead>
<tr>
<th>Component</th>
<th>TF</th>
<th>SRP</th>
<th>SecB</th>
<th>SecA</th>
<th>Ribosomes</th>
<th>SecY</th>
<th>YidC</th>
<th>SecDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular concentration (μM)</td>
<td>40–50 [63]</td>
<td>0.4 [163]</td>
<td>4–20 [111]</td>
<td>5–7 [101]</td>
<td>20 [54]</td>
<td>0.8 [201]</td>
<td>6–8 [202]</td>
<td>0.08 [203]</td>
</tr>
</tbody>
</table>
Table 2 Affinities for the bacterial secretion key components

<table>
<thead>
<tr>
<th>Component</th>
<th>TF</th>
<th>SRP</th>
<th>SecB</th>
<th>SecA</th>
<th>Full-length preprotein</th>
<th>SP preprotein</th>
<th>Mature preprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty ribosome</td>
<td>1 [204]</td>
<td>0.100 [163]</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Ribosomes: nascent preprotein</td>
<td>~0.1 [204]</td>
<td>0.001–0.010 [163]</td>
<td></td>
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<td>SecY</td>
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<tr>
<td>SecY-bound SecA</td>
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<tr>
<td>SecA</td>
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<tr>
<td>Full-length preprotein</td>
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<tr>
<td>SP preprotein</td>
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</tr>
<tr>
<td>Mature preprotein</td>
<td>NM</td>
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Affinity was measured in micromoles. NM, not measured; is used for known interactions to occur with unknown affinities. NM*, there are conflicting reports for the signal peptide (SP)–SecB interaction. Some studies support that SecB binds on the signal peptide [171,207] whereas others support that it does not bind [208]. −, is used for unknown interactions; NA, non-applicable.

albeit inefficiently [11]. In SecY, most of these mutations are located at the central pore and plug [122–125]. Presumably, they loosen the tight assembly of the channel and have also been proposed to affect subunit association of SecYEG, either by stabilizing the open or destabilizing the closed state of the translocase [9,126]. These structural changes may be coincident with the activated state of the translocase known as ‘triggered’ [11].

A small α-helix seals the periplasmic exit of SecY (Figures 4E and 4F) [9]. This ‘plug’ along with the pore constriction keeps SecY closed and prevents ion or water leakage [9,127]. Translocating wild-type preproteins dislocate the plug towards SecE [9]. Cross-linking the plug in the centre of the SecY channel locked the channel in a closed state and abolished preprotein translocation in vitro, suggesting that the plug must move away from the pore during secretion [128]. When the plug was cross-linked to SecE, it was in a presumed open conformation [9], and cells were not viable [129].

Interestingly, SecY with a truncated or deleted plug forms new plug-like structures but these could not stabilize the lateral gate well [130]. The plug was proposed to sense perturbations on SecY caused by the binding of its cytoplasmic partners [131] through a network of hydrogen bonds that interconnect plug residues with the rest of SecY [131]. However, SecA binding did not cause plug relocation [132].

A lateral opening, where the two halves of the clamshell meet (TMHs 1–5 and 6–10), endorses SecY with an additional unique feature. A small loop between TMH5 and TMH6 may act as a hinge allowing lateral gate opening [133] (compare Figure 4E with 4F) [9]. It is thought that this gate is utilized by membrane proteins during their membrane insertion [9]. The cascade of events that lead to lateral gate opening could start with the binding of the preprotein signal peptide [134,135].

The SecDFYajC accessories of the SecYEG channel

The SecD, SecF and YajC heterotrimer can associate with SecYEG (Figure 3B) [29,136]. SecD and SecF are membrane proteins with six transmembrane segments each and extensive important periplasmic domains (Figure 4G) [137]. They are non-essential for life, important in vivo but less so in vitro [138].

SecDF, fused in a single polypeptide in some bacteria (Figure 4G), has been implicated in preprotein release upon completion of translocation [139], in the regulation of SecA membrane cycling [140] and in preventing preprotein backsliding [29,138]. YajC (8 kDa) is non-essential [136,141]. Similar to SecYEG, SecDFYajC also interacts with YidC [142].

Structure and function of SPase

Before preproteins are fully released from the translocase, their signal peptides are cleaved by SPases (Figure 3C) [143]. There are three types of bacterial SPases, I, II and IV. SPase I cleaves signal peptides of newly translocated preproteins [144,145] and is essential for survival [146]. SPase II is involved in the cleavage of lipoprotein signal peptides and SPase IV cleaves signal peptides of prepilins [147,148].

SPase I (35.9 kDa) cleaves signal peptides through a serine–lysine catalytic pair (Figure 4H) [149,150]. Two N-terminal transmembrane segments anchor it to the inner membrane while its catalytic C-terminal region is exposed to the periplasm [151] (Figure 4H). The structure of the soluble part of the E. coli SPase I (Figure 4H) reveals a protein that consists of two large antiparallel β-sheet domains (I and II) [152]. A shallow hydrophobic cleft formed on its surface is probably buried into the membrane, ready to bind and cleave emerging signal peptides [152].

The protein–protein interaction cascade and catalytic events

SRP–FtsY–preprotein–SecYEG

The SRP exhibits a high affinity for ribosomes with nascent polypeptide chains emerging from the tunnel (Figures 3 and 5A) [153] (Table 2). It binds primarily on the L23 ribosomal subunit [26,154], but also associates with L22, L24, L29 and L18 (or L32) subunits and parts of the 23S RNA [48,155,156]. Given their stoichiometries (Table 1), only a small fraction of ribosomes will be occupied by SRP at any given moment.

SRP has high affinity for vacant ribosomes which is increased ~100-fold for translating ribosomes (RNCs) even when the peptide sequence is not yet exposed (Table 2) [157]. When SRP binds an RNC its GTPase activity is stimulated [17] and the RNC is targeted to its membrane receptor FtsY (Figures 5A and 5B) for co-translational secretion [17]. This latter binding further increases the GTPase activity of SRP [16].

So far, FtsY interaction with SRP bound on the ribosome has been visualized only at low resolution with cryo-EM (electron microscopy) (8 Å) [158]. An interesting observation claims that FtsY associates with membrane-bound ribosomes with high affinity even when SRP is absent [159,160].

The ribosome docks on the SecY (Figure 5C) using the same interfaces with SRP [161,162]. This implies that the ribosomal region becomes available for SecY docking only after repositioning of SRP permits it. The presence of the ribosome during co-translational translocation prevents back-slip of the preprotein to the cytoplasm [158].

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SRP binds to RNCs of cytoplasmic proteins but has 10^3-fold less affinity for FtsY [163]. Moreover, RNCs of cytoplasmic proteins induce premature GTP hydrolysis and they become rejected before they reach the SecY channel [163].

TF–preprotein assembly?

Apart from its ribosome interaction (Figure 5D), TF associates and rescues more than 170 proteins from misfolding [68]. One such substrate is the S7 ribosomal protein with which it interacts with a 2:2 stoichiometry [68]. In vitro, TF binds stably cytosolic or secretory proteins and catalyzes their refolding or solubility [164–166]. In the absence of TF and DnaK, many proteins aggregate; once TF is reintroduced they are efficiently rescued [167].

Cytoplasmic proteins seem to bind to an inner cavity in TF; its hydrophobic patches and hydrophilic residues allow TF to be very promiscuous [65,68,168,169]. Monomeric TF, bound on the ribosome, bends in such a way that allows enough space for a newly synthesized protein to create a small folded domain [65,169]. In vivo TF is expected to associate with at least a subset of secretory proteins such as porins but only after ∼110 aa have been synthesized [58].

SecB–preprotein

SecB has a double role in the E. coli Sec pathway: (i) it acts as a holdase preventing preproteins to fold after synthesis [170]; and (ii) it acts as a pilot, delivering the substrate to the membrane-associated SecA [111]. SecB recognizes a consensus motif in substrates; approximately 9 aa long, rich in aromatic and basic residues and poor in acidic residues [171]. Protein binding by SecB is thought to be promiscuous since it recognizes secretory as well as non-secretory proteins [172].

SecA–ribosome

SecA can interact with the L23 ribosomal protein that lies next to the exit tunnel and presumably might grab newly synthesized polypeptide chains as they emerge, although this has not been demonstrated [25]. However, it is clear that SecA can directly bind its substrates post-translationally in solution and deliver them to the translocase channel with or without SecB, in the absence of a translating ribosome (Figure 3A) [25].

SecA–SecB

SecB–preprotein complexes are targeted to soluble or SecYEG-bound SecA (Figure 5F) with high affinity [173,174] (Table 2). SecB was shown to interact with the last 22 aa of the SecA C-terminus [108,175] in an asymmetric manner [28]. Alanine substitutions of 4 aa within this region diminished SecB affinity for SecA even in the presence of zinc [108]. Two SecB regions have been implicated in the SecA–SecB interaction. One is located at the C-terminus of the protein [28,176] and the other, poorly described, involves the SecB β-sheets. Optimal translocation in vitro was proposed to require two SecA homomers to bind per SecB tetramer [28].

SecY–SecA–preprotein assembly

SecA interacts with the cytosolic loops of SecY (Figure 5F) with high affinity (∼40 nM) [177]. A complex of one SecA protomer with one SecYEG was solved (Figure 5F) [86,178]. In this structure, parts of NBD, IRA2 and the C-domain of SecA form a continuous groove with the SecY pore, occupied by the body of PBD. This suggested that this continuous groove might be the path preproteins follow during their secretion. This 1:1 SecA–SecYEG complex could be the minimal functional unit of the translocase [179].

Cycles of ATP hydrolysis drive conformational changes in the SecA motor that are transferred to the other domains [75,76,180,181]. It was proposed that the IRA1 two-helix finger may drive translocation by moving into the cytosolic funnel-like opening [75,76,180,181]. Cross-linking data indicate that SecA captures the preprotein in a clamp, where the preprotein moves forward as an extended polypeptide [75,76,180,181].

SecA alone or SecA with bound preprotein both bind to SecYEG with high affinity (Figure 5F). The oligomeric state of SecA or that of SecYEG and their stoichiometry during preprotein translocation is a topic of debate. Many results support either a monomeric or a dimeric SecA bound to monomeric or dimeric SecYEG, complicating their interpretation [87,101,182].

The structure of a SecA in solution with a signal peptide bound was solved [78]. The relocation of the plug and the lateral gate helices are demonstrated in Figures 4(E) and 4(F) [183].

Chaperone relay during preprotein secretion

TF (Figure 4B) and SecB (Figure 4D) are well characterized E. coli chaperones that interact with secretory proteins [67,170]. In the absence of the TF, SecB interacts with more substrates [167,184] and in this case it has been hypothesized that the outer membrane proteins are diverted to co-translational translocation [185].

Since chaperones might have overlapping functions, it is important to consider the concentration and substrate affinity for each chaperone in the sequence of events.

A potential relay of some key secretion factors can be sketched. SRP, SecA and TF can bind and probably antagonize for the same site of the ribosome (L23 subunit). SRP is at a low cellular concentration (∼0.4 μM), but has a very high affinity for signal peptides and ribosomes and probably binds them first (Table 2). TF is highly abundant and could bind less hydrophobic signal peptides and mature nascent chains that escape from SRP. Current evidence suggests that TF binds commonly after residue 50 (and usually after ∼110 residues) [58] so in vivo signal peptides might generally evade it. Once the length of the nascent polypeptide exceeds 150 aa, SecB can also bind. High intracellular SecB amounts (Table 1) secure its binding to a substrate on the ribosome or after its complete translation. Then SecB could transfer them to soluble or SecY-bound SecA with high affinity [111].

SecA ribosome binding has been hypothesized to secure transfer of the preprotein directly to the membrane [25]. Given that SecA intracellular amounts are low compared with other chaperones (Table 1) and half of it is membrane-associated, there are fewer chances for SecA to occupy the L23 site and to deliver a preprotein to the membrane compared with the other chaperones [101].

Model of secretory protein translocation

The translocation reaction can be described in six distinct and experimentally dissectable steps (Figure 6).

Step 1: SecA docks on SecYEG and the holoenzyme is formed (Figure 6, step I)

Step 2: preproteins are transferred to the translocase with or without chaperoning assistance. The signal peptide and the mature domain bind on their distinct sites on the SecY-bound SecA (Figure 6, step II).
trans and release the preprotein to the multiple repeats of this cycle would complete translocation binding energy of ATP \[124,187\]. The ATP gets hydrolysed and begins in defined segments of 20–30 residues driven by the detaches from the NBD \[82,85,186\], the preprotein translocation ATPase motor breaks, the IRA2 domain becomes disordered and irreversibly trapped within the channel (Figure 6, step IV) \[11\].

the signal peptide. Shortly after this, the mature domain becomes becomes trapped, a step that requires the physical presence of which of the various molecular chaperones actually participate in secretion and how do they interact with the preproteins? What are the exact conformational events that lead direct traffic to the different secretion pathways? How do these conformational events lead to successive movement of the translocating polypeptide chain through the membrane channel? How does the translocase change conformations during translocation? Additional three-dimensional structures of membrane protein complexes and techniques that allow real-time monitoring of highly dynamic procedures will be necessary for the deeper understanding of these fascinating biochemical processes. Better rational understanding of these mechanisms will lead to novel antibacterials, understanding of protein trafficking diseases and improved production of biopharmaceuticals.

Step 3: bound signal peptide lowers the activation energy of the translocase, i.e. it becomes ‘triggered’ (Figure 6, step III) \[11\].

Step 4: following translocase triggering, the mature domain becomes trapped, a step that requires the physical presence of the signal peptide. Shortly after this, the mature domain becomes irreversibly trapped within the channel (Figure 6, step IV) \[11\].

Step 5: a structurally conserved salt bridge at the base of the ATPase motor breaks, the IRA2 domain becomes disordered and detaches from the NBD \[82,85,186\], the preprotein translocation begins in defined segments of 20–30 residues driven by the binding energy of ATP \[124,187\]. The ATP gets hydrolysed and multiple repeats of this cycle would complete translocation and release the preprotein to the trans side of the membrane. When the chain is not engaged by SecA, forward movement is driven by the PMF \[188\], PMF also prevents back-slipping of the translocating chain \[189\] and contributes by correctly orientating the preprotein inside the channel \[190,191\]. In-channel engagement of the chain will displace the SecY plug and the ring will form a gasket-like seal around it. The rate of translocation has been calculated to \(\sim 270\) aa/min \[192\], slightly faster than translation \(760–1260\) aa/min (Figure 6, step V) \[193\].

Step 6: SPase I will cleave the signal peptide and hence allow full release of the preprotein \[144\] to fold in the periplasm or to continue further trafficking (Figure 6, step VI).

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