University of Groningen

The Sec system

Driessen, A.J.M.; Fekkes, P.; van der Wolk, J.P.W.

Published in:
Current Opinion in Microbiology

DOI:
[10.1016/S1369-5274\(98\)80014-3](https://doi.org/10.1016/S1369-5274(98)80014-3)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1998

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Driessen, A. J. M., Fekkes, P., & van der Wolk, J. P. W. (1998). The Sec system. *Current Opinion in Microbiology*, 1(2), 216 - 222. [https://doi.org/10.1016/S1369-5274\(98\)80014-3](https://doi.org/10.1016/S1369-5274(98)80014-3)

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

The Sec system

Arnold JM Driessen*, Peter Fekkes and Jeroen PW van der Wolk

Proteins designated to be secreted by *Escherichia coli* are synthesized with an amino-terminal signal peptide and associate as nascent chains with the export-specific chaperone SecB. Translocation occurs at a multisubunit membrane-bound enzyme termed translocase, which consists of a peripheral preprotein-binding site and an ATPase domain termed SecA, a core heterotrimeric integral membrane protein complex with SecY, SecE and SecG as subunits, and an accessory integral membrane protein complex containing SecD and SecF. Major new insights have been gained into the cascade of preprotein targeting events and the enzymatic mechanism of preprotein translocation. It has become clear that preproteins are translocated in a stepwise fashion involving large nucleotide-induced conformational changes of the molecular motor SecA that propels the translocation reaction.

Addresses

Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

*e-mail: a.j.m.driessen@biol.rug.nl

Current Opinion in Microbiology 1998, 1:216-222

<http://biomednet.com/elecref/1369527400100216>

© Current Biology Ltd ISSN 1369-5274

Abbreviations

Δp protonmotive force
NBS nucleotide binding site

Introduction

Protein translocation in *Escherichia coli* is mediated by a cytosolic chaperone, SecB, and a large multisubunit integral membrane protein complex, termed translocase. Translocase consists of a peripheral ATPase, SecA, and at least five integral membrane proteins (i.e. SecY, SecE, SecG, SecD and SecF). This large complex most likely forms the translocation pore along which the preproteins pass the cytoplasmic membrane. The purpose of our review is to summarize the advances in the past few years in our understanding of the mechanism of Sec-mediated preprotein translocation in *Escherichia coli*. Earlier work is reviewed in [1*]. Other protein components involved in targeting such as the *E. coli* SRP and SRP-receptor homologues are not discussed.

Preprotein targeting to the membrane

SecB recognition sites on preproteins and interaction with nascent chains

SecB is a molecular chaperone with a dual function in preprotein translocation. It keeps preproteins in a so-called 'translocation-competent' state and targets them to the SecA subunit of the membrane-bound translocase.

In recent years, research has revealed the sites of interaction between preproteins, SecB and SecA. The global SecB-binding frame in preproteins has been derived from the nature of SecB-protected proteolytic preprotein fragments (see references in [2]). SecB appears to bind to preproteins without specificity for sequence or structural content [3], in contrast to early studies suggesting a preferred interaction with positively charged polypeptide patches. SecB is very selective *in vivo* and binds mainly to a subset of nascent preproteins [4]. With some preproteins, the signal sequence has been shown to retard folding of the mature domain. This has led to the hypothesis that the rate of folding is the basis on which SecB distinguishes between cytosolic and secretory proteins (original kinetic partitioning hypothesis) [3]. This hypothesis is at variance with a number of recent observations. First, SecB interacts with long nascent polypeptide chains [4]. At this stage, no stable protein folding has occurred and one would expect SecB to bind any nascent chain instead of binding to a specific subset. The SecB/nascent chain interaction has also been shown to occur independently of the presence of a signal sequence [5]. Secondly, translocation of some preproteins in which the signal sequences have been removed strictly requires SecB, even though they may not be SecB-dependent as a native preprotein [6,7]. The latter is likely a reflection of the targeting function of SecB, but again argues against the original kinetic partitioning model in which the retardation of folding by the signal sequence is an important element for recognition. Finally, the association between SecB and model proteins is readily reversible, diffusion limited and occurs at rates well beyond that of typical folding [8]. Therefore, SecB will not be able to distinguish between cytosolic and precursor proteins on the basis of the folding behaviour only. Moreover, even when a preprotein escapes the SecB binding at the ribosome, it will be bound in the cytosol before it can fold into a native-like structure. Once SecB has bound an unfolded preprotein, stable folding is prevented in the binding equilibrium by repeated association and dissociation events [9,10]. Taken together, the specificity of the SecB-preprotein interaction *in vivo* remains unclear.

Overlapping preprotein and SecA-binding sites on SecB

Although SecB seems to interact with large preprotein polypeptide areas, mutations in the gene encoding SecB that interfere with preprotein binding are restricted to only a few aminoacyl residues [11]. The hydrophobic nature of the residues are consistent with the fluorescence studies with model peptides that suggest the presence of a hydrophobic binding site [8]. The same region, but at alternating positions compared to the residues involved in preprotein binding, also harbors the mutations that interfere with the recognition of SecB by SecA (P

Fekkes *et al.*, unpublished data). In addition, mutations of negatively charged residues in another region of SecB have been found that strongly reduce translocation without affecting preprotein binding [11]. Because of the prevailing β -structure in SecB and some preprotein substrates, preprotein recognition may be mediated through β - β interaction. The alternating occurrence of mutations that affect preprotein binding versus SecA binding could be explained by an amphiphatic β -sheet with a hydrophobic preprotein binding face and a polar negatively charged SecA binding face. Within the SecB tetramer, four such regions would be present and together they could form the binding sites for the preprotein and SecA. The SecB-binding site on SecA is localized in the carboxyl terminus [12] and corresponds to the last 22 aminoacyl residues of SecA [13••]. This domain contains a high amount of glycyl, prolyl and cysteinyl residues and has a net positive charge, opposite in charge to the site of SecA binding on SecB. The SecA-SecB interaction is, therefore, presumably electrostatic.

Preprotein transfer by SecB to SecA

The importance of the targeting function of SecB is evident when the preprotein signal sequence is either mutated [14] or completely removed [6], thereby reducing the targeting information contained in the signal sequence. Such preproteins are dependent on SecB for translocation. When the SecB-binding domain of SecA is removed, the translocation of preproteins with a defective signal sequence is blocked by SecB instead of stimulated. This is indicative of a need for SecB binding by SecA for preprotein transfer. The interaction between SecB and SecA in the cytosol is of a low affinity, whereas SecB binds with high affinity to the SecYEG-bound SecA [15]. In the presence of a preprotein with a functional signal sequence, the SecB-SecA binding affinity is even enhanced [13••]. This phenomenon is caused by the binding of the preprotein signal sequence to SecA (P Fekkes *et al.*, unpublished data). SecB-mediated targeting of the preprotein to SecA thus also occurs via the signal sequence which is available for SecA interaction as SecB has been shown to bind only the mature part of the preprotein. Targeting presumably proceeds via the following steps: SecB targets the preprotein to the translocase by association with the carboxyl terminus of the SecYEG-bound SecA and the signal sequence associates with SecA to stimulate the interaction between SecA and SecB (Figure 1a). The latter complex is unstable, as it can only be isolated as a ternary complex of SecA, SecB and a preprotein in which the SecB is no longer bound to the preprotein [13••]. The SecB-SecA interaction causes the release of the mature domain of the preprotein from its association with SecB (Figure 1b). The preprotein is thus transferred from SecB to SecA by a synchronous 'hand-shake' mechanism, in which binding of SecB to SecA triggers the transfer event; this probably involves conformational changes of both SecA and SecB. As the SecA-binding site and the preprotein-binding sites

on SecB are overlapping, it seems probable that the tight binding of SecB to the carboxyl terminus of SecA dissociates the preprotein from its SecB-bound state. An important implication of this mechanism is that SecB bound at the translocase will be unable to accept new cargo as long as it remains bound to SecA. Only after the initiation of translocation by the binding of ATP to SecA is SecB released from the membrane to bind a new preprotein in the cytosol (Figure 1c) [13••].

Preprotein translocation across the membrane

The next catalyst in the preprotein translocation cascade is SecA, a large and conformational complex homodimeric protein. SecA plays a central role as it is the site of preprotein entry into the translocase, is the only ATPase essential for preprotein translocation, and interacts with most of the other Sec-proteins (reviewed in [1•]). SecA is present in the cell in large excess of the other Sec-proteins and binds with a high affinity to SecY [16•,17-19].

Nucleotide-induced conformational changes of SecA

SecA contains two essential nucleotide-binding sites (NBSs) [20,21] and undergoes large nucleotide-induced conformational changes during translocation (reviewed in [1•]). The thermal unfolding characteristics of SecA suggest the presence of at least two independent folding domains of near equal size [22•]. The amino and carboxyl domains harbor the high (NBS1) and low (NBS2) affinity nucleotide binding sites, respectively [20]. Binding of ADP at NBS1 enhances the interaction between the amino and carboxyl domain and converts the protein into a more compact state [22•]. In contrast, binding of ATP to NBS1 yields an elongated conformation of SecA with little interaction between the amino and carboxyl domains. Under these conditions, previously unexposed aminoacyl residues become accessible to solvents [23•]. It appears that the energy released during the hydrolysis of ATP at NBS1 is used to drive a thermodynamically unfavourable conformational change, akin to the release of a spring (T den Blaauwen, AJM Driessen, unpublished data). This conformational energy is released upon the exchange of the bound ADP for ATP, and drives the preprotein translocation.

Binding of ADP to NBS2 has an even more dramatic effect on the SecA conformation than ATP binding to NBS1 [22•], resulting in the formation of a highly compact SecA structure. Furthermore, the amount of shared protein surface of the monomers in the SecA dimer is increased and the subunit interaction is tightened. NBS2 is localized either at or near the subunit interface of the SecA protomer [24] in the region that is involved in dimerization [25]. Nucleotide binding at NBS2 may thus modulate the spatial positioning of the two carboxyl domains of the SecA dimer that have been suggested to insert into the membrane during a translocation cycle (see following section).

Figure 1

Schematic representation of the initial stages of preprotein translocation. (a) SecB targets the preprotein to the SecYEG-bound SecA and the signal sequence associates with SecA to stimulate the interaction between SecA and SecB. (b) The SecB-SecA interaction causes the release of the mature preprotein from SecB. The zig-zag symbol represents the region in the SecB that associates with both SecA and preprotein, and changes conformation upon SecA interaction. (c) After SecA binds ATP, resulting in conformational changes in SecA, translocation of the preprotein is initiated and SecB is released from SecA.

Topology and membrane insertion of SecA domains

The idle SecYEG-bound SecA has a complex membrane topology. The extreme carboxy-terminal region of SecA is periplasmically accessible to trypsin [26] whereas parts of both the amino and carboxyl domains are accessible to small chemical reagents [23•], implying either that SecA deeply penetrates the membrane or that the enzyme is accessible from the periplasmic membrane surface via a proteinaceous pore (the SecYEG protein perhaps?). Iodinated SecA has been used to analyse the conformational changes of SecA during preprotein translocation [21,27]. In the presence of preprotein and ATP, or with the nonhydrolysable ATP analogue AMP-PNP alone, SecYEG-bound SecA undergoes a conformational change yielding a stable 30 kDa fragment after proteolysis [21,27,28]. This fragment corresponds to a carboxy-terminal region of SecA [29] and its formation is reversed upon hydrolysis of ATP, whereas it is stabilized by SecD and SecF [21]. Disruption of the membrane destabilizes the 30 kDa fragment and it has been suggested that this SecA domain is deeply membrane-integrated, exposing regions at the periplasmic membrane face [27]. Accordingly, a current working model is that preproteins are translocated across the membrane in a stepwise fashion by repeated nucleotide-induced cycles of SecA membrane insertion and de-insertion.

When Western blotting [22•] or a labelling technique more uniform than iodination is used [30], additional protease-resistant and membrane-protected SecA fragments can be detected. In particular, this concerns the formation of an amino-terminal 65 kDa fragment [31] that together with the carboxy-terminal 30 kDa fragment covers most of the SecA mass. This would imply that almost the entire SecA inserts into or penetrates the membrane, a spectacular event considering that SecA functions as a dimer. As both fragments are shielded from the lipid phase, they may actually insert into the SecYEG pore rather than the membrane [32•]. Although it is evident that conformational changes of SecA are important for preprotein translocation, there are several unambiguities with the current membrane penetration hypothesis. The kinetics of 30 kDa formation are rather slow as compared to preprotein translocation [27], which is surprising as multiple cycles of SecA membrane insertion/de-insertion are required to completely translocate the preprotein. The 30 kDa SecA fragment is accessible from the cytosolic face of the membrane, as a monoclonal antibody directed against this domain reverses the membrane-insertion [33]. Thus, at least part of the membrane-inserted 30 kDa domain is accessible from the cytosolic membrane face. Albeit with lower efficiency, the SecA proteolytic

fragments can also be formed in a nucleotide-dependent manner free in solution [29], demonstrating that these fragments originate from pre-existing SecA domains. Their elevated resistance to proteases during preprotein translocation may just be the result of stabilization of a protein conformation. The AMP-PNP-induced formation of the 30 kDa fragment has also been observed with SecA bound to SecYEG in octylglucoside solution, whereas Triton X-100 destroys the interaction [34•]. Under the same set of conditions that yield the 30 kDa fragment, SecYEG is proteolysed into small remnants. These data critically access the membrane-insertion hypothesis and indicate that the fragments represent tight-folded, protease-resistant SecA domains. This does not rule out that SecA indeed inserts into a translocation channel comprised by SecYEG. It is clear that evidence based on the formation of a protease-resistant conformation only should be taken with precaution. SecA appears to be periplasmically accessible to protease in intact *E. coli* cells overproducing the *secDF* operon [35] but the observation that the amount of accessible SecA by far exceeded the number of high affinity membrane binding sites is as yet unexplained.

Signal sequence proofreading at the initiation of translocation

From the SecA-SecYEG-bound state, preprotein translocation is initiated by the binding of ATP to SecA allowing the membrane-insertion of the signal sequence and part of the mature preprotein region as a hairpin-like structure [36]. The so-called *prl* (for protein localization) class of mutants, which are all isolated as suppressors of signal sequence mutations, have been found in SecA (*prlD*), SecY (*prlA*), SecE (*prlG*) and more recently in SecG (*prlH*) (see references in [7,36–38]). It has been proposed that the *prl* suppressors function not by restoring the recognition of altered signal sequences but rather by preventing the rejection of defective preproteins from the export pathway [39]. According to this hypothesis, SecA, SecY, SecE and SecG would have a proofreading activity. A possible proofreading function has recently been investigated for certain *prlA* mutants (JPW van der Wolk *et al.*, unpublished data). The *prlA4* mutant binds SecA with a much higher affinity than the wild type but has little effect on the SecA translocation ATPase activity. As a result of the tighter interaction, the release of SecA from the SecYEG complex is much slower than normal and one may hypothesize that the prevention of the rejection of the preproteins with defective signal sequences from translocation is caused by a longer retention time of the signal sequence domain in the translocase. In *prlA* strains, but not in *prlD* and *prlG* mutants, translocation is less dependent on the protonmotive force (Δp , the electrochemical gradient of protons across the membrane) [40•]. The lower Δp requirement may well result from the tighter SecA interaction, much akin to the observation that high levels of SecA or ATP can suppress the Δp requirement for translocation.

Sodium azide inhibits preprotein translocation by blocking the SecA translocation ATPase activity, thereby trapping SecA in the ATP-bound state [41••]. Interestingly, the majority of the azide-resistant mutations in SecA are localized in the vicinity of NBS1 and NBS2 [38] suggesting an alteration in the ATPase activity. *Azi* mutants, like the *prlD* mutations, enable the translocation of preproteins with a defective signal sequence [37]. The *prlD* suppressor phenotype of the *azi* mutants may also be caused by a longer retention at the translocation site as found for the *prlA* mutant. In contrast to the *prlA* mutants, this phenomenon would be a result of altered ATPase activity.

Stepwise translocation of the preprotein across the membrane

The SecA-driven translocation of preproteins is a stepwise process [36] manifested by the systematic occurrence of translocation intermediates in intervals of ~5 kDa translocation progress [41••]. A complete catalytic cycle of SecA would occur according to the following scenario: SecA bound to the partially translocated polypeptide chain will translocate ~2.5 kDa of the preprotein upon ATP binding [36,42] (Figure 2a) concomitant with a large conformational change of SecA [21,22•,27,31]. Hydrolysis of ATP subsequently reverses this conformational change and SecA releases the bound preprotein (Figure 2b). Azide blocks preprotein translocation by interfering with this step [41••]. SecA can rebound the exposed portion of the partially translocated preprotein and this step allows the translocation of another 2.5 kDa polypeptide segment (Figure 2c). The Δp can drive the efficient translocation of large polypeptide domains in the absence of SecA by an unknown mechanism [36].

Organization of the integral membrane domain

SecY and SecE are essential subunits of the integral membrane domain of the translocase. SecG [43], SecD and SecF [44] are nonessential proteins, required only at lower temperature. *In vitro*, however, SecG [43] or SecD and SecF [16••] stimulate preprotein translocation in membranes containing SecYE. The *secG* null phenotype can be suppressed by certain enzymes involved in phospholipid biosynthesis (see references in [45••]) but also by overexpression of SecD and SecF, or SecY and SecE [16••]. SecG has been shown to reverse the membrane topology of its two transmembrane segments during the ATP-driven membrane-insertion of SecA [45••]. This remarkable phenomenon also occurs when SecA binds the nonhydrolysable AMP-PNP and the tantalizing suggestion has been made that SecG 'lubricates' the translocation pore for the insertion of a SecA domain [45••].

SecD and SecF can be immunoprecipitated together with YajC as an heterotrimeric subcomplex [16••]. YajC is encoded by a gene in the *secDF* operon that so far has no known function in preprotein translocation. In mild detergent, the SecDFYajC complex can be co-immunoprecipitated with the SecYEG complex. These

Figure 2

Current Opinion in Microbiology

Schematic representation of the catalytic steps during the intermediate stages of preprotein translocation. **(a)** After the SecYEG-bound SecA binds ATP, the partially translocated polypeptide chain is translocated ~2.5 kDa and a large conformational change occurs in SecA. **(b)** ATP hydrolysis reverses this conformational change and SecA releases the bound polypeptide chain. This step is blocked by azide. **(c)** SecA can rebind the exposed portion of the partially translocated polypeptide chain allowing the translocation of another 2.5 kDa polypeptide segment.

data indicate that, although the core of the translocase is formed by the SecYEG complex, the translocase may also exist as a large complex. In the absence of SecD and SecE, cells are unable to maintain a Δp [46]. Recent evidence indicates that SecD and SecE prevent backward sliding of the preprotein in the translocation channel [47*].

Conclusions

Many molecular details of the preprotein translocation process have been revealed during the past year. In particular, the mechanism by which the SecA protein drives the preprotein across the membrane at the expense of ATP is a significant finding. Knowledge on the structure of the translocase is urgently needed to reveal the intimate details of the mechanism. High-resolution electron-microscopical analysis of the endoplasmic reticulum Sec61p complex shows ring-like structures, suggesting that the membrane domain of the translocase consists of three to four Sec61p trimers with a central pore [48**]. Such ring-structures are induced by binding of the ribosome or Sec62/63/71/72p to the Sec61p trimer. Does the SecYEG complex also assemble into a large oligomer in order to form a functional translocase, for instance, when it binds SecA? How are integral membrane proteins integrated into the *E. coli* membrane and does this involve the lateral opening of the translocase to release the membrane segments into the lipid phase? Other issues to be resolved concern the molecular mechanism of proofreading, how preproteins are released at the trans-side of the translocase, the role of the low-affinity lipid bound SecA in

translocation, and the mechanism by which non-bilayer lipids stimulate translocation [49]. Finally, an unresolved aspect concerns the catalytic mechanism by which the Δp drives translocation. Efficient and complete Δp -driven preprotein translocation has been observed only under artificial conditions (i.e. when SecA is removed from the translocation sites) [36]. It remains to be established, however, whether ATP-driven preprotein translocation in the presence of a Δp is indeed a continuous process or if it involves the same set of intermediate steps as ATP-driven translocation.

Acknowledgements

Research related to this review was supported by a PIONIER grant of the Netherlands Organization for Scientific Research (NWO), the Life Sciences Foundation (SLW), the Netherlands Foundation for Chemical Research (SON), and the European Community (BIO2-CT-930254 and BIO4-CT-960097).

References and recommended reading

Papers of particular interest, published within the annual period of review have been highlighted as:

- of special interest
 - of outstanding interest
1. Driessen AJM: **Translocation of proteins across the bacterial cytoplasmic membrane.** In *Handbook of Biophysics*, vol 2. *Transport Processes in Membranes*. Edited by Konings WN, Kaback HR, Lolkema JS. Amsterdam: Elsevier; 1996:759-790. A recent and comprehensive review on bacterial protein translocation.
 2. Smith VF, Hardy SJS, Randall LL: **Determination of the binding frame of the chaperone SecB within the physiological ligand oligopeptide-binding protein.** *Prot Sci* 1997, 6:1746-1755.

3. Randall LL, Hardy SJS: High selectivity with low specificity: how SecB has solved the paradox of chaperone binding. *Trends Biochem Sci* 1995, 20:65-69.
 4. Kumamoto CA, Francetić O: Highly selective binding of nascent polypeptides by an *Escherichia coli* chaperone *in vivo*. *J Bacteriol* 1993, 175:2184-2188.
 5. Randall LL, Topping TB, Hardy SJS, Pavlov MY, Freistoffer DV, Ehrenberg M: Binding of SecB to ribosome-bound polypeptides has the same characteristics as binding to full-length, denatured proteins. *Proc Natl Acad Sci USA* 1997, 94:802-807.
 6. Flower AM, Doebele RC, Silhavy TJ: PrfA and PrfG suppressors reduce the requirement for signal sequence recognition. *J Bacteriol* 1994, 176:5607-5615.
 7. Prinz WA, Spiess C, Ehrmann M, Schierle C, Beckwith J: Targeting of signal sequence less proteins for export in *Escherichia coli* with altered protein translocase. *EMBO J* 1996, 15:5209-5217.
 8. Fekkes P, Den Blaauwen T, Driessen AJM: Diffusion-limited interaction between unfolded polypeptides and the *Escherichia coli* chaperone SecB. *Biochemistry* 1995, 34:10078-10085.
 9. Zahn R, Perrett S, Sternberg G, Fersht AR: Catalysis of amide proton exchange by the molecular chaperones GroEL and SecB. *Science* 1996, 272:642-645.
 10. Topping TB, Randall LL: Chaperone SecB from *Escherichia coli* mediates kinetic partitioning via a dynamic equilibrium with its ligands. *J Biol Chem* 1997, 272:19314-19318.
 11. Kimsey HH, Dagarag MD, Kumamoto CA: Diverse effects of mutations on the activity of the *Escherichia coli* export chaperone SecB. *J Biol Chem* 1995, 270:22831-22835.
 12. Breukink E, Nouwen N, van Raalte A, Mizushima S, Tommassen J, De Kruijff B: The C terminus of SecA is involved in both lipid binding and SecB binding. *J Biol Chem* 1995, 270:7902-7907.
 13. Fekkes P, Van der Does C, Driessen AJM: The molecular chaperone SecB is released from the carboxy-terminus of SecA during initiation of precursor protein translocation. *EMBO J* 1997, 16:6105-6113.
- This paper, together with that of Breukink *et al.*, 1995 [12], demonstrates that SecB binds to the carboxyl terminus of SecA. The authors provide evidence that SecB is released from the SecYEG-bound SecA at an early stage of the translocation reaction and that, under those conditions, the carboxyl terminus of SecA is no longer available for SecB binding. As the carboxyl terminus of the tightly-membrane associated form of SecA is accessible to trypsin and chemical reagents added from the periplasmic face [23*,26], the region may have altered its location at the initiation of translocation.
14. Francetić O, Kumamoto CA: *Escherichia coli* SecB stimulates export without maintaining export competence of ribosome-binding protein signal sequence mutants. *J Bacteriol* 1996, 178:5954-5959.
 15. Hartl F-U, Lecker S, Schiebel E, Hendrick JP, Wickner W: The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane. *Cell* 1990, 63:269-279.
 16. Duong F, Wickner W: Distinct catalytic roles of the SecYE, SecG and SecDFyajC subunits of preprotein. *EMBO J* 1997, 16:2756-2768.
- First evidence that the integral membrane domain of the translocase consists of two subcomplexes that perhaps assemble and disassemble on demand.
17. Manting EH, van der Does C, Driessen AJM: SecA and SecY are associated subunits of the *Escherichia coli* precursor protein translocase. *J Bacteriol* 1997, 179:5699-5704.
 18. Matsumoto G, Yoshihisa T, Ito K: SecY and SecA interact to allow SecA insertion and protein translocation across the *Escherichia coli* plasma membrane. *EMBO J* 1997, 16:6384-6393.
 19. Snyders S, Ramamurthy V, Oliver D: Identification of a region of interaction between *Escherichia coli* SecA and SecY proteins. *J Biol Chem* 1997, 272:11302-11306.
 20. Mitchell C, Oliver D: Two distinct ATP-binding domains are needed to promote protein export by *Escherichia coli* SecA ATPase. *Mol Microbiol* 1993, 10:483-497.
 21. Economou A, Pogliano JA, Beckwith J, Oliver DB, Wickner W: SecA membrane cycling at SecYEG is driven by distinct ATP binding and hydrolysis events and is regulated by SecD and SecE. *Cell* 1995, 83:1171-1181.
 22. Den Blaauwen T, Fekkes P, De Wit JG, Kuiper W, Driessen AJM: Domain interactions of the peripheral preprotein translocase subunit SecA. *Biochemistry* 1996, 35:11994-12004.
- The authors report on the SecA domain organization and show that SecA consists of at least two domains that interact in a nucleotide-dependent manner. The data indicate that SecA functions as a molecular motor.
23. Ramamurthy V, Oliver D: Topology of integral membrane form of *Escherichia coli* SecA protein reveals multiple periplasmically exposed regions and modulation by ATP binding. *J Biol Chem* 1997, 272:23239-23246.
- A clear demonstration, by the use of membrane-impermeable cysteine-reactive agents and single cysteine mutants of SecA, that various regions of the membrane-integrated form of SecA are accessible from the periplasmic face of the membrane, thus indicating a complex topology.
24. Van der Wolk JPW, Boersma A, Knoche M, Schäfer H-J, Driessen AJM: The low affinity ATP-binding site of the *Escherichia coli* SecA dimer is localized at the subunit interface. *Biochemistry* 1997, 36:14924-14929.
 25. Hirano M, Matsuyama S, Tokuda H: The carboxyl-terminal region is essential for SecA dimerization. *Biochem Biophys Res Commun* 1996, 229:90-95.
 26. Van der Does C, Den Blaauwen T, De Wit JG, Manting EH, Groot NA, Fekkes P, Driessen AJM: SecA is an intrinsic subunit of the *Escherichia coli* preprotein translocase and exposes its carboxy-terminus to the periplasm. *Mol Microbiol* 1996, 22:619-629.
 27. Economou A, Wickner W: SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. *Cell* 1994, 78:835-843.
 28. Douville K, Price A, Eichler J, Economou A, Wickner W: SecYEG and SecA are the stoichiometric components of preprotein translocase. *J Biol Chem* 1995, 270:20106-20111.
 29. Price A, Economou A, Duong F, Wickner W: Separable ATPase and membrane insertion domains of the SecA subunit of preprotein translocase. *J Biol Chem* 1996, 271:31580-31584.
 30. Chen X, Xu H, Tai PC: A significant fraction of functional SecA is permanently embedded in the membrane. SecA cycling on and off the membrane is not essential during protein translocation. *J Biol Chem* 1996, 271:29698-29706.
 31. Eichler J, Wickner W: Both an N-terminal 65 kDa domain and a C-terminal 30 kDa domain of SecA cycle into the membrane at SecYEG during translocation. *Proc Natl Acad Sci USA* 1997, 94:5574-5581.
 32. Eichler J, Brunner J, Wickner W: The protease-protected 30 kDa domain of SecA is largely inaccessible to the membrane lipid phase. *EMBO J* 1997, 16:2188-2196.
- Tests the hypothesis that the 30 kDa domain of SecA is membrane-integrated by probing its localization by means of a photoaffinity crosslinking lipid analog. The 30 kDa domain is shielded from the lipid phase and presumably present in an aqueous or proteaceous environment.
33. Den Blaauwen T, De Wit JG, Gosker H, Van der Does C, Breukink E, De Leij L, Driessen AJM: Inhibition of preprotein translocation and reversion of the membrane inserted state of SecA by a carboxyl terminus binding mAb. *Biochemistry* 1997, 36:9159-9168.
 34. Van der Does C, Manting EH, Kaufmann A, Lutz M, Driessen AJM: Interaction between SecA and SecYEG in micellar solution and formation of the membrane-inserted state. *Biochemistry* 1998, 37:201-210.
- By using a detergent-solubilized SecA-SecYEG complex, the authors confirm the findings of Eichler *et al.*, 1997 [32*] and further show that the 30 kDa SecA fragment is also not protected by the SecYEG protein. It is concluded that this SecA fragment represents a stable conformation of a pre-existing domain.
35. Kim YJ, Rajapandi T, Oliver DB: SecA protein is exposed to the periplasmic surface of the *E. coli* inner membrane in an active state. *Cell* 1994, 78:845-853.
 36. Schiebel E, Driessen AJM, Hartl F-U, Wickner W: $\Delta\mu$ H⁺ and ATP function at different steps of the catalytic cycle of preprotein translocase. *Cell* 1991, 64:927-939.
 37. Bost S, Belin D: prl mutations in the *Escherichia coli* secG gene. *J Biol Chem* 1997, 272:4087-4421.
 38. Huie JL, Silhavy TJ: Suppression of signal sequence defects and azide resistance in *Escherichia coli* commonly results from the same mutations in secA. *J Bacteriol* 1995, 177:3518-3526.

39. Osborne RS, Silhavy TJ: **PrfA suppressor mutations cluster in regions corresponding to three distinct topological domains.** *EMBO J* 1993, 12:3391-3398.
40. Nouwen N, de Kruijff B, Tommassen J: **PrfA suppressors in *Escherichia coli* relieve the proton electrochemical gradient dependency of translocation of wild-type precursors.** *Proc Natl Acad Sci USA* 1996, 93:5953-5957.
Shows that, in a *prfA* mutant strain, preprotein translocation exhibits a reduced protonmotive force requirement.
41. Van der Wolk JPW, De Wit JG, Driessen AJM: **The catalytic cycle of the *Escherichia coli* SecA ATPase comprises two distinct preprotein translocation events.** *EMBO J* 1997, 16:7297-7304.
This paper demonstrates that a catalytic preprotein translocation cycle of SecA consists of two distinct translocation steps. Each of these steps allows the translocation of ~20-25 amino acid residues of the SecA-bound preprotein. One step is driven by the binding of SecA to the translocating polypeptide chain and the other step is driven by the binding of ATP to SecA.
42. Uchida K, Mori H, Mizushima M: **Stepwise movement of preproteins in the process of translocation across the cytoplasmic membrane of *Escherichia coli*.** *J Biol Chem* 1995, 270:30862-30868.
43. Nishiyama K, Hanada M, Tokuda H: **Disruption of the gene encoding p12 (SecE) reveals the direct involvement and important function of SecE in the protein translocation of *Escherichia coli* at low temperature.** *EMBO J* 1994, 13:3272-3277.
44. Pogliano JA, Beckwith J: **SecD and SecF facilitate protein export in *Escherichia coli*.** *EMBO J* 1994, 13:554-561.
45. Nishiyama KI, Suzuki T, Tokuda H: **Inversion of the membrane topology of SecE coupled with SecA-dependent preprotein translocation.** *Cell* 1996, 85:71-81.
This is an excellent paper showing that SecE undergoes a remarkable membrane topology inversion when SecA binds ATP.
46. Arkowitz RA, Wickner W: **SecD and SecF are required for the proton electrochemical gradient stimulation of preprotein translocation.** *EMBO J* 1994, 13:954-963.
47. Duong F, Wickner W: **The SecDFyajC domain of preprotein translocase controls preprotein movement by regulating SecA membrane cycling.** *EMBO J* 1997, 16:4871-4879.
This paper demonstrates that SecD and SecF prevent the backward sliding of a translocating polypeptide chain and, together with Economou *et al.*, 1995 [21], shows that SecD and SecF stabilize the 30 kDa SecA domain.
48. Hanein D, Matlack KE, Jungnickel B, Plath K, Kalies KU, Miller KR, Rapoport TA, Akey CW: **Oligomeric rings of the Sec61p complex induced by ligands required for protein translocation.** *Cell* 1996, 87:535-544.
This is the first low-resolution structural information on a preprotein translocation pore, showing ring-like structures with a central pore-like opening. As the SecYEG complex has a similar function and subunit organization to the endoplasmic reticulum Sec61p, it is probable that the bacterial translocase has a similar overall structure.
49. Rietveld AG, Koorengel, MC, de Kruijff B: **Non-bilayer lipids are required for efficient protein transport across the plasma membrane of *Escherichia coli*.** *EMBO J* 1995, 14:5506-5513.