SecB, a molecular chaperone with two faces

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SecB is a molecular chaperone unique to the phylum Proteobacteria, which includes the majority of known Gram-negative bacteria of medical, industrial and agricultural significance. SecB is involved in the translocation of secretory proteins across the cytoplasmic membrane. The crystal structure of the Haemophilus influenzae SecB provides new insights into how SecB simultaneously recognizes its two ligands: unfolded preproteins and SecA, the ATPase subunit of the translocase. SecB uses its entire molecular surface for these two functions, but for preprotein release and its own membrane release, SecB relies on the catalytic activity of SecA. This defines SecB as a translocation-specific molecular chaperone.

In Gram-negative bacteria, periplasmic and outer membrane proteins are synthesized in the cytosol as precursor proteins (preproteins) that contain an amino-terminal signal sequence. Once a major portion of the nascent preprotein has emerged from the ribosome, it is recognized by the soluble chaperone SecB and targeted to SecA, the peripheral ATPase subunit of the translocase (Fig. 1). SecB is a highly acidic homotrimeric protein with a subunit molecular weight of ~17 kDa (Ref. 2). Although SecB is not an essential protein for Escherichia coli, inactivation of secB results in a mild translocation defect of a subset of preproteins and a growth defect on rich medium. The growth defect of the secB-null strain is, however, caused by a polar effect that reduces the expression of a biosynthetic sn-glycerol 3-phosphate dehydrogenase involved in phospholipid synthesis3. SecB is required for the translocation of preproteins into inverted membrane vesicles and their targeting to the SecA protein. SecB maintains the preprotein in a translocation-competent state that will neither fold nor aggregate, an activity shared with general chaperones such as GroEL and DnaK (reviewed in Ref. 1). These general chaperones, however, fail to stimulate translocation as they are unable to target the preprotein to the translocase correctly4. SecB is needed for the translocation of a signal-sequence-less preprotein to compensate for the loss of targeting information normally contained in the signal sequence.

SecB crystal structure

Despite the extensive research on the function of SecB in translocation, many questions have remained unanswered. Why does SecB bind a variety of non-native polypeptides in vitro5 when it is highly selective in vivo2? How does SecB discriminate between its two protein ligands, the preprotein and SecA (Ref. 7)? To understand the chaperone and targeting function, detailed structural information about this molecular chaperone is required. However, SecB has been a difficult protein for crystal structure determination, mainly because of its long-standing resistance to form good diffracting crystals. Xu et al.3 have recently solved this problem by switching from E. coli to Haemophilus influenzae as the source of SecB.

The crystal structure of the tetrameric SecB protein is an exciting development in the field of protein translocation8. The SecB monomer has a simple α + β fold, but the overall rectangular-shaped structure is a tetramer organized as a dimer of dimers (Fig. 2). The quaternary organization agrees well with the observed dynamic dimer–tetramer equilibrium of SecB in solution9.

Selective binding of unfolded preprotein substrates

The SecB-binding frame in preproteins has been assessed by various methods5. An extensive screen of a peptide library for SecB binding10 indicated that a typical peptide substrate (SecB-binding motif) is approximately nine residues long and enriched in aromatic and basic residues; acidic residues are strongly disfavoured. The majority of the binding energy for these peptides results from hydrophobic interactions10.

SecB has been crystallized without a peptide substrate in its binding site, but the structure reveals a long surface-exposed channel on each side of the tetramer that has all the characteristics of a peptide-binding site (Fig. 3a). Xu et al.8 propose that each channel contains two peptide-binding subsites that might recognize distinct features of the preprotein. Subsite 1 is a deep cleft located in a narrow constriction in the middle of the groove (Fig. 3b). It might recognize hydrophobic and aromatic regions of polypeptides, as most of the amino acids that line the surface are aromatic and conserved. The length of this cleft is sufficient to accommodate peptide sizes of the size of the SecB-binding motif11. Subsite 2 is a shallow, open groove with a hydrophobic surface (Fig. 3b). It lacks aromatic residues and might bind extended regions of polypeptides by forming regular main-chain hydrogen bonds. Negatively charged residues are positioned around the groove and this could explain the selectivity for basic residues. The conserved tryptophan residue of SecB has been used to monitor the binding of model substrates5,12 is located at the entry of subsite 1 (Fig. 3b) and has provided evidence for four separate peptide-binding sites on the SecB tetramer.

Specific mutations in E. coli SecB (C76Y, V78F and Q80R) disrupt the interaction between SecB and preproteins11,13. These residues are located on a surface-exposed β-strand, but they point towards the core of the molecule and are not part of the peptide-binding groove8. In these mutants, which show only mild translocation defects13, the tetramer–dimer equilibrium in solution is shifted towards the dimer9. As these residues do not directly contribute to the dimer–dimer interface, it is conceivable that they indirectly disrupt this interface and thereby impair preprotein binding.

The in vitro studies do not explain how SecB differentiates between secretory and cytosolic proteins6,10,12. The signal sequence is not the prime target for SecB binding14. SecB associates with ribosome-bound nascent chains after they have reached a length of ~150 residues5. These chain lengths correspond with the location of SecB-binding sites mapped by proteolysis15. Because SecB recognizes...
peptide sequences that typically occur within core regions of folded proteins\(^{10}\), it binds preferentially to the unfolded conformation of the mature part of preproteins. The long binding regions have been proposed to occupy multiple binding sites on SecB simultaneously, thereby allowing the high binding affinity of non-native preproteins (\(K_d = 5-50 \text{ nM}\))\(^{15}\). To occupy the peptide-binding grooves on both sites, long unstructured polypeptide segments presumably wrap around the chaperone (Fig. 3b). The SecB structure\(^{8}\) provides a first glimpse of how preproteins might bind, but to reveal the molecular details of this interaction, a structure of SecB with bound polypeptide substrate is required.

As to the physiological role of SecB in preventing aggregation of non-native proteins\(^{6,10}\), it is unclear to what extent the cell takes advantage of this general chaperone activity. During protein binding, there is a rapid equilibrium between bound and free states, which will allow partitioning of the polypeptide between folding and rebinding to SecB (Ref. 12). As the signal sequence retards folding of the mature region of proteins\(^{14}\), it will favour re-association with SecB and thus indirectly contribute to the specificity\(^{8}\). However, the dedicated role of SecB in protein translocation is more likely to result from events downstream of the SecB–preprotein interaction, in particular the association of SecA with SecB and the exposed signal sequence.

**High affinity SecA–SecB binding**

SecA bound to the protein-conducting SecYEG channel is primed for the high-affinity interaction with SecB (\(K_d = 10-30 \text{ nM}\))\(^{4,7}\). In solution, SecA and SecB also interact, albeit with much lower affinity (\(K_d = 1.6 \mu\text{M}\))\(^{16}\). The site on SecA that binds SecB comprises the highly conserved carboxy-terminal 22 amino acyl residues of SecA (Refs 7,17). The SecB-binding region of SecA is rich in arginyl and lysyl residues, resulting in a net positive charge. It contains conserved cysteine and histidine residues that coordinate a divalent zinc ion required for the functional interaction between SecB and SecA (Ref. 18).

The well conserved amino acid residues Asp20, Glu24, Leu75 and Glu77 of E. coli SecB are important for the high-affinity SecB–SecA interaction\(^{13,19}\). Mutations L75Q and E77K disrupt the binding of SecB to the membrane-bound SecA (Ref. 19). The corresponding residues in H. influenzae SecB are spatially clustered on a flat solvent-exposed surface on both sides of the SecB tetramer (Fig. 4)\(^{8}\). This surface is negatively charged and thus might interact electrostatically with the positively charged SecB-binding domain on SecA. The 13 amino acid carboxyl terminus of SecB has also been implicated in SecA binding\(^{20}\). Unfortunately, this solvent-exposed acidic region is barely resolved in the SecB structure\(^{8}\), consistent with it being highly mobile\(^{20}\). Interestingly, the (incomplete) \(\alpha\)-helical carboxyl termini protrude out of the core structure as long arms (Fig. 3a), as if they embrace the carboxy-terminal...
The proposed peptide-binding sites on SecB (Ref. 8). The solvent-accessible surface of SecB is coloured based on the polarity of the underlying amino acids: all non-charged polar and charged side-chains, green; all hydrophobic side-chains (except tryptophan), yellow; all backbone atoms, white. The conserved tryptophan residue is shown in blue. The drawing in (a) is the same orientation as in Fig. 2a, and in (b) is rotated by 90° towards the horizontal axis to show the peptide-binding channels on each side of the tetramer. The channels are indicated with a white dot.

**SecB-binding site on SecA.** The low-affinity SecB–SecA interaction in solution requires neither the carboxyl terminus of SecA nor the negatively charged binding cluster on SecB (Ref. 21). At present, the functional significance of this interaction for protein translocation is unclear. This will first require the selection of mutants specifically impaired in low-affinity binding.

The quaternary structure of SecB provides a nice solution to the dilemma how the SecB tetramer binds the homodimeric SecA (Ref. 7). Each SecA subunit could interact with the negatively charged surface formed by one dimer of SecB. The stoichiometry of the SecA–SecB–preprotein ternary complex is 2:4:1 (Ref. 7). The active state of the preprotein-conducting channel has been proposed to consist of a SecA dimer bound to a tetrameric SecYEG complex**2**. In view of the symmetry of the SecA–SecB interaction, it seems plausible that the oligomeric SecYEG channel accepts one preprotein at a time from a SecA–SecB pair.

**Mechanism of preprotein transfer from SecB to SecA**

Preproteins increase the affinity of SecB to SecA and the SecYEG-bound SecA (Ref. 19). The reaction is triggered by the binding of the signal sequence region of the preprotein to SecA. Owing to the tighter SecB–SecA binding, the preprotein is released from SecB and transferred to SecA (Fig. 1)**1**,19. Thus, the SecB–SecA binding reaction somehow modulates the conformation of the preprotein-binding site on SecB. The SecB structure provides no insight into the molecular mechanisms of this transfer reaction. Even with a structure for the ternary SecB–SecA–preprotein complex, this question might be difficult to answer as the transfer reaction requires the high-affinity interaction between SecA and SecB that is observed with the SecYEG-bound SecA. It is of interest to note that some of the mutations that interfere with SecA binding**19 and the tetramer–dimer equilibrium**9 map at different faces of a β-strand**1** that contacts the long α-helices constituting the dimer–dimer interface. Hypothetically, high-affinity binding of SecA to SecB could cause a reduction in the preprotein binding affinity by influencing the SecB dimer–dimer interfacial contact in analogy to the mutations that shift the tetramer–dimer equilibrium. The mature preprotein domain will then move to SecA. As long as SecB remains engaged with the carboxyl terminus of SecA, re-occupancy of the peptide-binding groove of SecB is disfavoured. Concomitantly with the initiation of translocation, SecA uses the binding of ATP to release SecB from the membrane (Fig. 1)**7**.

**Concluding remarks**

On the one hand, the structure suggests that SecB uses its entire molecular surface to interact with its two substrates, the preprotein and SecA (Ref. 8). On the other hand, the biochemical studies have shown that the enzymatic activities of preprotein transfer and SecB release are not contained in the SecB structure**2**,20. A molecular understanding of these events requires a crystal-like structure of SecB with its ligands. This will be a challenge for the future.

**Acknowledgements**

I would like to thank Nico Nouwen for careful reading of the manuscript. Owing to space limitations, only a limited number of references could be cited. The work is supported by the Netherlands Organization for Scientific Research (NWO).

**References**

A marriage of bacteriology with cell biology results in twin arginines

Frank Sargent

The Tat apparatus is a remarkable molecular machine required for the transmembrane translocation of folded proteins. Recent studies have identified jellyfish green fluorescent protein as an excellent reporter protein for the bacterial Tat pathway. Although the genetics of the Tat system are reasonably well-defined in Escherichia coli, the utilization of heterologous proteins as transport substrates promises to facilitate further mechanistic and structural characterization of this system.

The targeting of proteins to their required sites of physiological function is a necessity for all cellular systems. Bacteria are no exception, and subsets of polypeptides are rapidly and efficiently directed to the inner membrane, outer membrane (if present), periplasm (between the two membranes) or the cell exterior. Many proteins destined for translocation across the bacterial inner membrane are synthesized as precursors with amino-terminal signal peptides and transported in an unfolded state by the general secretory (Sec) pathway1 (see pp. 193–196 of this issue for a discussion of the SecB crystal structure). Recently, bacteriologists studying the synthesis of complex enzymes, and cell biologists delineating protein-targeting pathways in chloroplasts, have together unearthed a completely Sec-independent protein-translocation system capable of transporting fully folded proteins across biological membranes.

The Tat protein-transport system A subset of transported proteins is synthesized as precursors with amino-terminal signal peptides bearing the distinctive SRRXFLK 'twin arginine' amino acid sequence motif (where X = any amino acid)2. Concomitantly, the twin arginine signal-peptide-dependent protein translocase (Tat apparatus) is present in all bacteria that synthesize periplasmic proteins with twin arginine signal sequences3, with this system being most clearly defined in Escherichia coli.

In E. coli, four genes (tatA, tatB, tatC and tatE) have been identified that encode components of the Tat translocase4. The TatE protein is not a feature of all bacterial Tat systems; instead, a recent study suggests that E. coli TatE is a poorly expressed duplication of tatA (Ref. 5). The tatB and tatC genes encode essential components of the Tat apparatus. All four E. coli Tat components are integral membrane proteins4 and immunological studies have shown that TatA and TatB interact to form a large membrane-bound complex5, which is also thought to include TatC (Refs 6,7). A completely analogous Tat system is active within the thylakoid membranes of chloroplasts, pointing to the existence of related mechanisms of signal recognition and protein translocation between the two systems8.

Reporter proteins for Tat activity Experimental evidence for the mechanism of protein translocation by the bacterial Tat system has been slow in coming. Progress has been hindered not only by the lack of an in vitro translocation assay but also because of the difficulty in identifying suitable reporter proteins for Tat activity. Reporter enzymes have long been used as model substrates to study protein targeting in bacteria, the classic examples being β-galactosidase (LacZ) and alkaline phosphatase (PhoA).

Unfortunately, these reporter proteins are apparently not suitable for in vivo (or in vitro) studies of Tat activity.

When fused to a bacterial Tat signal sequence, LacZ is not translocated across the inner membrane8. This is probably because the signal peptide–LacZ fusion rapidly forms a very large homotetramer immediately following translation. The resultant chimera is therefore greater in