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MicroReview

The structural view of bacterial translocation-specific chaperone SecB: implications for function

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Summary

SecB is a molecular chaperone that functions in bacterial post-translational protein translocation pathway. It maintains newly synthesized precursor polypeptide chains in a translocation-competent state and guides them to the translocon via its high-affinity binding to the ligand as well as to the membrane-embedded ATPase SecA. Recent advances in elucidating the structures of SecB have enabled the examination of protein function in the structural context. Structures of SecB from both *Haemophilus influenzae* and *Escherichia coli* support the early two-subsite polypeptide-binding model. In addition, the detailed molecular interaction between SecB and SecA was revealed by a structure of SecB in complex with the C-terminal zinc-containing domain of SecA. These observations explain the dual role of SecB plays in the translocation pathway, as a molecular chaperone and a specific targeting factor. A model of SecB–SecA complex suggests that the binding of SecA to SecB changes the conformation of the polypeptide binding sites in the chaperone, enabling transfer of precursor polypeptides from SecB to SecA. Recent studies also show the presence of a second zinc-independent SecB binding site in SecA and the new interaction might contribute to the function of SecB.

Introduction

The role of molecular chaperones in protein folding has been well established over the last decade. In general, molecular chaperones have a particular affinity for non-native conformations that exist in protein folding intermediates. Interaction between folding intermediates and chaperone stabilizes folding intermediates and prevents them from aggregation. Molecular chaperones also play important roles in protein translocation because the majority of proteins are translocated across the membrane as unfolded polypeptides. Therefore, it is important for them to be kept in an unfolded state to remain competent for translocation.

SecB is a molecular chaperone specialized in the post-translational protein translocation pathway of some proteobacteria. It binds to newly synthesized precursor polypeptides (preproteins) and stabilizes them in an unfolded and non-aggregated state after they exit from the ribosome translation tunnel (Lecker *et al*., 1989; 1990; Liu *et al*., 1989; Breukink *et al*., 1992). It also delivers preproteins to the membrane-embedded translocon via its specific interaction with SecA, an ATPase that provides part of the actual energy for translocation (Hartl *et al*., 1990; de Cock and Tommassen, 1992; Hoffschulte *et al*., 1994; Fekkes *et al*., 1998). The role of SecB in preprotein export was first revealed by Kumamoto and Beckwith (1983; 1985) when they found that *secB* mutations resulted in translocation defects for a subset of secretory proteins in *Escherichia coli*. Extensive work has been carried out on SecB as its first identification (Weiss *et al*., 1988; Kumamoto, 1989). Several excellent reviews on the function of SecB have appeared in the past few years (Randall and Hardy, 1995; 2002; Fekkes and Driessen, 1999; Kim and Kendall, 2000; Driessen, 2001; Driessen *et al*., 2001). In this review, we will focus on advances in our understanding of the molecular mechanism of SecB function based on recent structural analyses (Xu *et al*., 2000; Dekker *et al*., 2003; Zhou and Xu, 2003).

SecB structure

In solution, SecB exists as a tetramer with a molecular mass of 69 KDa. The X-ray crystal structures of SecB from both *E. coli* and *Haemophilus influenzae* (Xu *et al*., 2000; Dekker *et al*., 2003) have shown that the molecule is organized as a dimer of dimers, consistent with data from biochemical studies (Fig. 1) (Muren *et al*., 1999; Topping *et al*., 2001). The monomer unit contains a four-stranded antiparallel β-sheet followed by a pair of antiparallel α-

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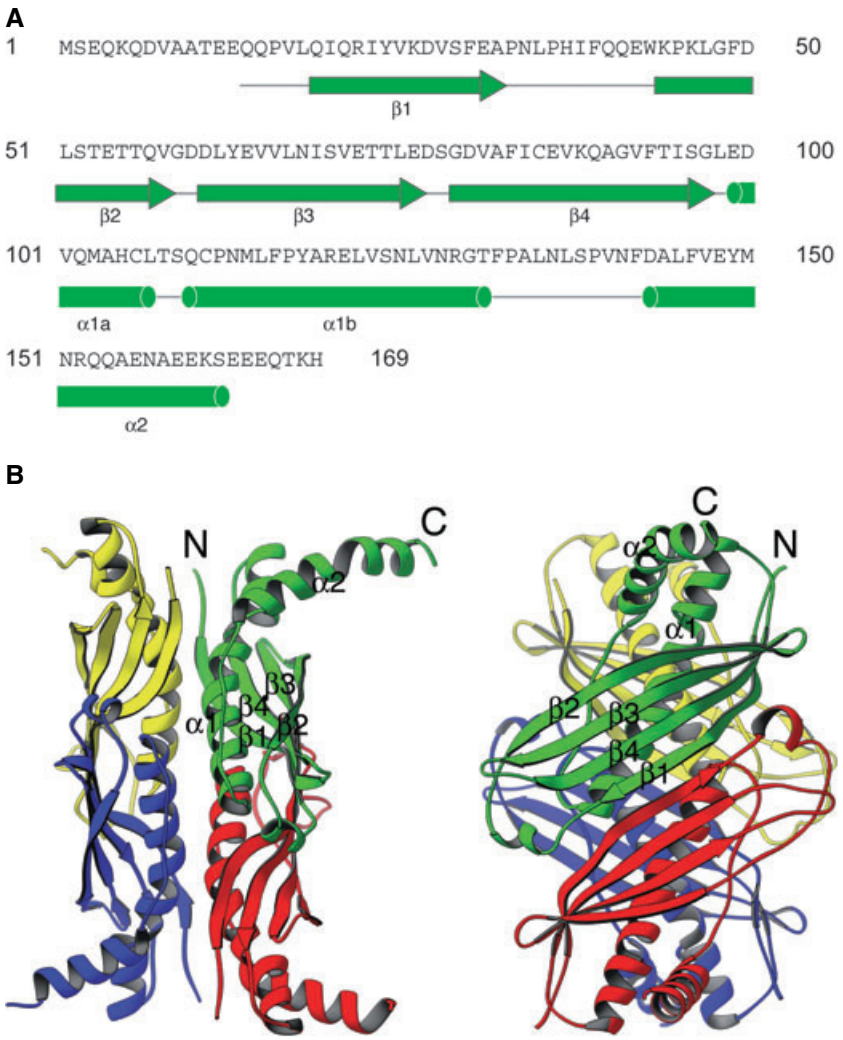

Fig. 1. The crystal structure of the SecB molecule.
A. Protein sequence and secondary structure assignment of *H. influenzae* SecB. Secondary structure elements are indicated underneath the sequence: α-helices are drawn as cylinders; β-strands as arrows; and other elements as grey lines.
B. Ribbon drawings of the SecB tetramer are shown in two orthogonal views. Each subunit in the tetramer is coloured differently. The secondary structural elements for the green subunit are labelled as in (A): α-helices as coils, β-strands as arrows and other elements as thick lines. One dimer consists of the green and red subunits while the other consists of the blue and yellow subunits.

helices. The dimer is a flat molecule formed by a 180° rotation of one monomer with respect to the other and pairing them together via strand β1 and helix α1. As a result of the pairing, there is a surface-exposed antiparallel β-sheet on one face of the SecB dimer. The dimer is mainly stabilized by main chain hydrogen bonds between the two antiparallel β1 strands from the two monomers. Two SecB dimers associate to form a tetramer by sandwiching four long α1 helices between the eight-stranded antiparallel β-sheets. The tetramer is very stable, with an estimated tetramer–dimer equilibrium constant at pH 7.6 well below 20 nM (Muren *et al*., 1999). The dimer–dimer interface is stabilized by polar interactions involving sidechains from the four α1 helices.

The amino acid sequence identity between *E. coli* SecB and *H. influenzae* SecB is 59% and, as expected, the two structures are very similar. Differences between them are found mainly in the loop regions. There is a two-residue difference in the connecting loop between strands β3 and β4: residues 76–79 in *H. influenzae* SecB and residues 69–70 in *E. coli* SecB (Xu *et al*., 2000; Dekker *et al*., 2003). This flexible β-hairpin region is close to the negatively charged SecA-binding surface of SecB (see below) and may affect interaction with SecA.

The extreme C-terminus of SecB subunits is not visible in the electron density maps of either of the crystal structures. Proton nuclear magnetic resonance (NMR) spectroscopy demonstrated that this region is highly mobile (Volkert *et al*., 1999). Deletion of the C-terminal tail produces a stable truncated SecB protein that retains its ability to bind to non-native polypeptide *in vitro* but causes a defect in protein export when overproduced *in vivo*. The defect can be alleviated by overproduction of SecA, suggesting that the C-terminal tail of SecB may interact with SecA. Deletion of the C-terminal tail also leads to a twofold decrease in affinity for non-native polypeptide (Diamond and Randall, 1997). As peptide binding to SecB protects this tail region from proteolysis (Randall, 1992),

it is likely that the tail contributes to peptide binding. A structure of SecB–peptide complex will provide the necessary molecular details about how the C-terminal tail of SecB packs against the rest of the structure and regulates the preprotein translocation process.

Polypeptide binding

In the Sec-dependent pathway, protein translocation cannot occur if newly synthesized polypeptide chains are either folded or aggregated (Randall and Hardy, 1986; Weiss *et al*., 1988; de Cock *et al*., 1992). To keep newly synthesized proteins in the translocation-competent state, SecB recognizes the non-native conformation within the mature regions of preproteins and binds with high affinity (the dissociation constant is around 5 nM to 50 nM) (Randall and Hardy, 1995; Randall *et al*., 1997; 1998; Topping and Randall, 1997). Although SecB does not directly bind to the leader/signal sequence of preproteins (Randall *et al*., 1990), the presence of this sequence is nevertheless crucial for export. First, the leader sequence can significantly slow down the rate of spontaneous folding of preproteins and, thereby, increase the probability of binding by SecB (Hardy and Randall, 1991). Second, leader sequences bind to a specific domain of SecA and are thought to be important for the transfer of preproteins from SecB to SecA (Lill *et al*., 1990; Fekkes *et al*., 1998; Baud *et al*., 2002).

Randall and colleagues carried out extensive studies to define the SecB-binding frame within the preprotein. By analysing proteolytic digestion fragments of complexes between SecB and its natural ligands maltose-binding protein, galactose-binding protein or oligopeptide-binding protein, the SecB-binding frame was found to be located in the mature region of the three preproteins and to span a stretch of approximately 150–170 residues (Topping and Randall, 1994; Khisty *et al*., 1995; Randall and Hardy, 1995; Smith *et al*., 1997). Based on their early studies of a SecB-binding peptide library (Randall, 1992), a model was proposed for the interaction of SecB with its ligand (Randall and Hardy, 1995). The model suggests that there are two types of peptide-binding motifs or structures that can be bound by SecB: flexible stretches of polypeptide of approximately 15 residues in length and exposed hydrophobic regions within the non-native polypeptide. The initial interaction occurs at the extended and flexible binding site. Saturation of these binding sites induces a conformational change in SecB that leads to exposure of hydrophobic sites for ligand binding.

Consistent with Randall's hypothesis (Randall and Hardy, 1995), it was proposed that there are two peptide binding subsites in SecB based on the crystal structure of *H. influenzae* protein (Fig. 2) (Xu *et al*., 2000). A similar situation was observed more recently in the crystal structure of *E. coli* SecB (Dekker *et al*., 2003). Subsite 1 corresponds to the deep section of the peptide-binding channel of SecB and may recognize the hydrophobic and aromatic region within the non-native polypeptide because most of the residues lining subsite 1 are aromatic and conserved. Conformational variation among the different subunits of SecB suggests that this region is structurally flexible and provides a possible explanation for the necessary plasticity for peptide binding. Subsite 1 is large enough to accommodate a hypothesized nine-residue 'SecB-binding motif' (Knoblauch *et al*., 1999). Subsite 2 is much shallower and more open than subsite 1. It is there-

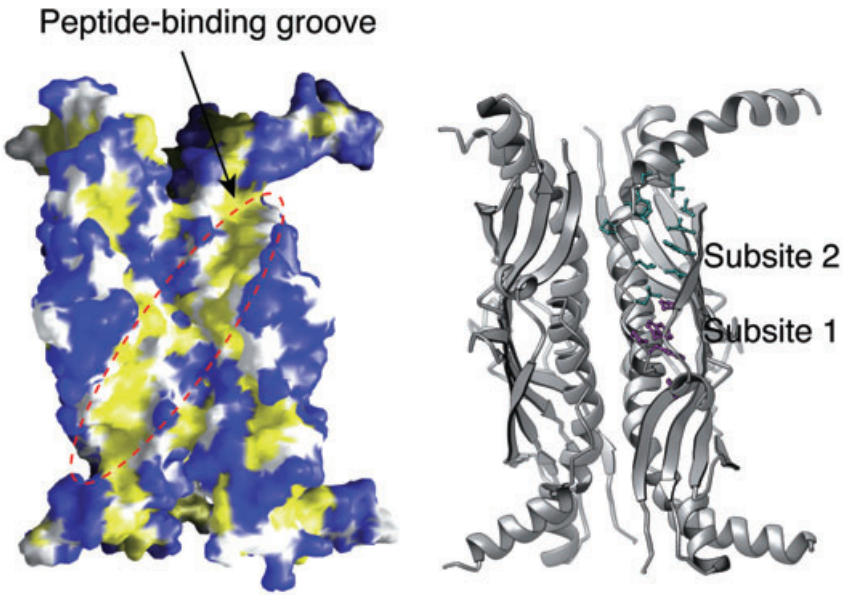

Fig. 2. The peptide-binding groove within the SecB molecule. The left panel shows the molecular surface of SecB, coloured according to the underlying atoms: all backbone atoms, white; all non-charged polar and charged side-chain atoms, blue; all hydrophobic side-chain atoms, yellow. The right panel shows the ribbon drawing of SecB in the same orientation as in the left panel. Residues lining the peptide-binding groove in one of the subunits are coloured: subsite 1, magenta; subsite 2, cyan. The residues lining the two sites are all hydrophobic.

fore able to accommodate a more extended region within the non-native polypeptide. In the crystal structure of *H. influenzae* SecB, the N-terminal region of a neighbouring molecule in the crystal lattice inserts itself into subsite 2 (J. Zhou and Z. Xu, unpubl. results). It interacts with subsite 2 by adopting an extended conformation and forming regular main chain hydrogen bonds with strand β2 of the β-sheet. The pattern of interaction likely holds true for the non-native polypeptide ligand.

The molecular symmetry within SecB gives rise to four identical binding sites. While affinity for individual sites might be low, simultaneous occupancy of multiple binding sites will ensure high-affinity binding of polypeptide to SecB. As mentioned above, the SecB-binding frame of its natural ligands spans a stretch of approximately 150–170 residues (Topping and Randall, 1994; Khisty *et al*., 1995; Smith *et al*., 1997). While the same SecB molecule does not necessarily occupy the entire stretch of residues, one can speculate that the long unstructured polypeptide segments might wrap around the chaperone to occupy the binding sites on both sides of SecB. It is sufficiently long to loop out from one side through either the path across the top of chaperone or the path across the side of the chaperone. In both cases, the ligand makes close contact with the extreme C-terminal part of SecB. This could explain why polypeptide binding protects these protease-sensitive C-terminal tails (Randall, 1992) and why deletion of the tail part of SecB decreases its affinity for polypeptide ligands (Diamond and Randall, 1997).

SecA recognition

SecB directs the bound preprotein into the translocation pathway via its specific interaction with membrane-bound SecA (Fekkes *et al*., 1998). SecB binds to SecA with low affinity in solution (dissociation constant is about 1.6 μM) (den Blaauwen *et al*., 1997). The binding affinity increases significantly when SecA is bound to the membrane-embedded translocon SecYEG complex (the dissociation constant is 10–30 nM) (Fekkes *et al*., 1997). The binding is even tighter (dissociation constant around 10 nM) if SecB is loaded with a polypeptide ligand (Hartl *et al*., 1990; Fekkes *et al*., 1997). The SecB binding site on SecA is localized primarily at the extreme C-terminus of SecA, although additional sites have also been suggested (Woodbury *et al*., 2000) (see below for more discussion). Removal of the C-terminal 22 residues of SecA causes a deficiency in SecB-mediated preprotein translocation (Fekkes *et al*., 1997).

The crystal structure of *H. influenzae* SecB in complex with the last 27 C-terminal residues of *H. influenzae* SecA (SecAc) provides details about the molecular interaction between the two proteins (Fig. 3) (Zhou and Xu, 2003). SecB uses the solvent-exposed surface of the eight-stranded β-sheet formed by two of the four subunits to interact with one SecAc peptide. In the crystal structure, the SecAc peptide is mainly stabilized by a bound zinc atom. The zinc atom is co-ordinated by three highly conserved cysteines and a histidine. Substitution of these residues in SecA by serine abolishes the ability of SecB to promote preprotein translocation (Rajapandi and Oliver, 1994). The well-structured SecA C-terminal region is necessary for SecB interaction, as the interaction is disrupted by treatment of SecA with a zinc chelator and restored by the addition of $ZnCl_2$ (Fekkes *et al*., 1999). Two recent solution NMR structures of *E. coli* SecAc peptide suggest that structural changes in SecAc upon binding to SecB are minimal (Dempsey *et al*., 2004; Matousek and Alexandrescu, 2004). The crystal structure also shows four residues in *H. influenzae* SecAc, Arg878, Asn879, Lys889 and Lys879, contribute significantly to the binding of SecB. Replacement of any of these residues by alanine in SecA abolishes its binding to SecB (Zhou and Xu, 2003).

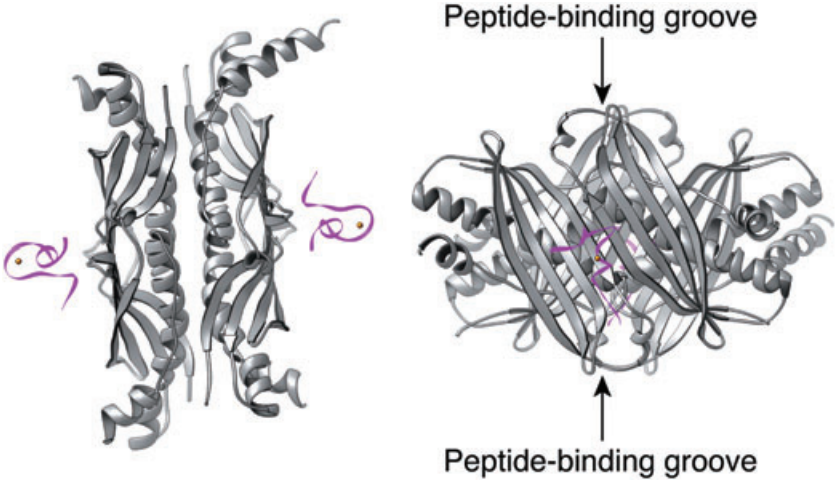

Fig. 3. The crystal structure of the SecB–SecAc complex. Ribbon drawings of the complex are shown in two different views. One SecB tetramer (grey) is in complex with two SecA C-terminal peptides (residues 876–899, magenta ribbons). Each SecAc molecule contains one bound zinc ion (golden sphere).

To visualize how SecB might interact with SecA, the crystal structure of *Bacillus subtilis* SecA (Hunt *et al*., 2002) was manually docked onto the structure of *H. influenzae* SecB–SecAc complex (Fig. 4) (Zhou and Xu, 2003). Although *B. subtilis* does not possess SecB, high sequence identity (46%) between SecA molecules from *B. subtilis* and *H. influenzae* suggests that the two proteins are likely to share a very similar overall structure. In addition, a hybrid '*B. subtilis* SecA' with its C-terminal 27 residues substituted with the corresponding sequence from *H. influenzae* SecA binds to *H. influenzae* SecB (J. Zhou and Z. Xu, unpubl. results). Three major constraints were used in the docking procedure. First, the C-terminal tails of SecA (not seen in any of the three published structures: Hunt *et al*., 2002; Sharma *et al*., 2003; Osborne *et al*., 2004) project into a space beneath the core of the SecA dimer based on the positions of the observed C-terminal ends in the SecA structure. Second, a symmetric complex is assumed to form between SecB and SecA. This requires that the twofold axis of SecA dimer be aligned with the twofold axis relating the two SecA-binding surfaces within a SecB tetramer. Third, the distance between the observed C-terminal ends in the SecA structure and the start of the SecAc segments needs to fit about 15 amino acids missing between these two points of connection. If these 15 amino acids all adopt extended backbone conformations, they could extend over a span of about 50 Å. In this model, the distance between the two points of connection (dashed line in Fig. 4) is 37 Å. Although the docking procedure is only approximate, the resulting model allows us to speculate about the interaction between SecB and SecA on a structural term.

The crystal structure of SecA (Fig. 4) (Hunt *et al*., 2002; Sharma *et al*., 2003; Osborne *et al*., 2004) shows that it contains two nucleotide-binding motifs (NBF I and NBF II) with a preprotein cross-linking domain (PPXD) (Kimura *et al*., 1991) inserted in between at the N-terminal two-thirds of the sequence. The C-terminal one-third of the sequence consists of the α-helical scaffold (HSD) and the helical wings domains (HWD) as well as the extreme C-terminal zinc-containing domain. As shown in Figs 4 and 5, SecB–preprotein complex binds to SecA from the bottom direction via the zinc-binding domains of SecA (step 2 in Fig. 5). One of the SecB polypeptide-binding grooves located on the top of the molecule is sandwiched between SecB and SecA. This positions SecB-bound preprotein directly beneath the SecA molecule and near its preprotein cross-linking domain, which makes direct preprotein transfer feasible. One could imagine that the simultaneous binding of the C-terminal tails of the SecA dimer to SecB changes the relative orientation of the two eight-stranded β-sheets in SecB, and therefore the conformation of the polypeptide-binding groove. This could lead to a decrease in the affinity of SecB for the bound preprotein, resulting in the release of preprotein from SecB and subsequent transfer to SecA (step 3 in Fig. 5).

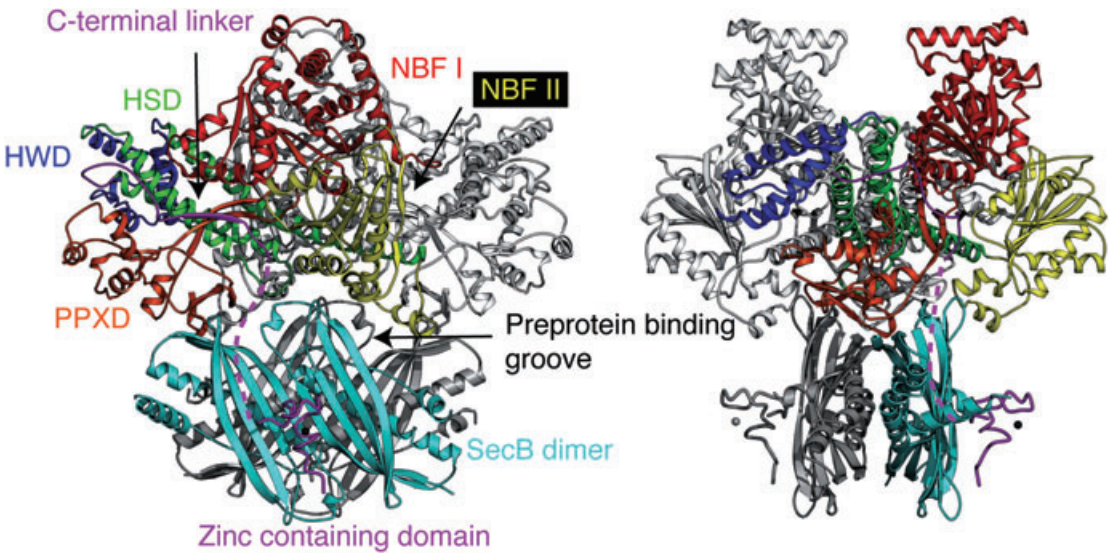

Fig. 4. A hypothetical model of SecA–SecB molecular complex. The hypothetical SecA–SecB complex is shown in two orthogonal views. It was generated by manually docking the C-terminal linker of *B. subtilis* SecA structure onto the N-terminus of *H. influenzae* SecAc of the SecB–SecAc complex structure (see text for details). In both views, SecA dimer is on the top and SecB tetramer is on the bottom. One SecA monomer is coloured white while the other is coloured based on its previously defined domains (Hunt *et al*., 2002): NBF I, red; PPXD, orange; NBF II, yellow; HSD, green; HWD, blue; and C-terminal linker, magenta. One SecB dimer is coloured grey while the other is coloured cyan and its associated SecAc magenta. Roughly, a total of 15 residues are missing between the visible end of the SecA structure and the beginning of the SecAc structure (dashed line). One of the preprotein-binding grooves of SecB is sandwiched between SecB and SecA.

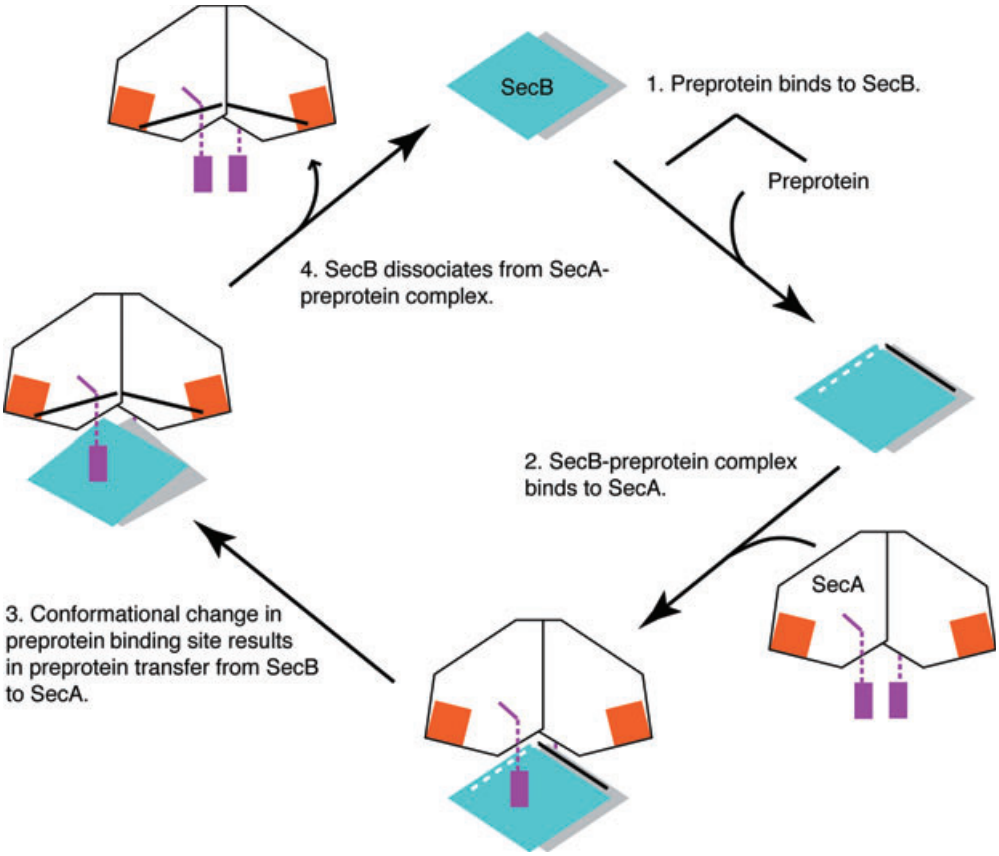

Fig. 5. Model of the SecB-mediated preprotein targeting in the bacterial protein translocation pathway. SecB tetramer is shown as two diamonds coloured cyan and grey. SecA dimer is coloured white except the preprotein cross-linking domains (orange) and the C-terminal zinc-containing domains (magenta). Majority of SecA molecules that interact with SecB–preprotein complex are membrane-associated. It is likely that SecA exists in monomer–dimer equilibrium.

The proposed SecA–SecB interaction model assumes that SecA symmetrically interacts with SecB as a dimer and the interaction is mediated via the C-terminal zinc-containing domain. This is supported by the SecAc–SecB complex structure as well as binding studies using various SecA and SecB mutants (Fekkes *et al*., 1997; 1999; Zhou and Xu, 2003). In particular, both C-terminal tails of SecA dimer appear to be necessary to form a tight SecA–SecB complex as heterodimers of wild-type SecA and a C-terminal truncated form of SecA are defective in SecB binding (Fekkes *et al*., 1997). Recent studies using chimeric SecA proteins also suggest that the C-terminal tail of SecA is necessary and sufficient to mediate the specific interaction between SecB and SecA. *B. subtilis* lacks a SecB proteins and its SecA does not bind to either *E. coli* or *H. influenzae* SecB. By genetically switching the C-terminal tail of *B. subtilis* SecA to the corresponding part from either *E. coli* SecA or *H. influenzae* SecA, the new hybrid '*B. subtilis* SecA' can now bind to the cognate SecB, depending on which tail the chimeric protein contains (J. Zhou and Z. Xu, unpubl. data).

In addition to the above-mentioned interaction, Randall and colleagues discovered a second site of SecA–SecB interaction. By analysing complexes formed between *E. coli* SecA and variants of *E. coli* SecB, they found that the translocation defective variants of SecB still form a complex with SecA, even though they cannot interact with the C-terminal tail of SecA (Woodbury *et al*., 2000). This hypothesis was further supported by their titration calorimetry and sedimentation velocity centrifugation data (Randall *et al*., 2004). The second binding site was now mapped to the C-terminal α-helix of SecB and the dimer interfacial region of SecA (Randall *et al*., 2005). They also showed that this additional interaction between SecA and SecB might serve to destabilize the SecA dimer, leading to the asymmetric interaction between the two proteins. Most of the asymmetric binding observed by Randall and colleagues requires the disruption of the interaction involving the zinc-binding domain. It is unclear to what extent this secondary binding contributes to the overall interaction between two wild-type proteins. However, at a certain stage of the SecB function cycle, SecA–SecB interaction

must be weakened to ensure the release of SecB from the membrane. This would facilitate the C-terminal helix of SecB to interact with the dimer interface of SecA. It is possible that the interaction then weakens the SecA dimer and leads to conformational changes in SecA, which might also contribute to the transfer of preprotein from SecB to SecA (Jilaveanu *et al*., 2005; Or *et al*., 2005; Randall *et al*., 2005). To fully address the functional role of the C-terminal independent SecA–SecB interaction will require the determination of its structure.

Other functions of SecB

Besides its function in the Sec-dependent protein translocation pathway, SecB is also the chaperone for the secretion of the HasA haemophore through the type I protein translocation pathway in *Serratia marcescens* (Delepelaire and Wandersman, 1998; Sapriel *et al*., 2003). HasA is a protein secreted by the Gram-negative bacteria under iron starvation conditions to assist the utilization of external haem as an iron source. Efficient secretion of HasA requires the protein in an unfolded state, which is maintained by the binding of SecB. *In vitro* studies have shown that SecB specifically binds to unfolded HasA and slows down its folding rate significantly (Wolff *et al*., 2003). Therefore, it is likely that SecB uses the hydrophobic polypeptide-binding groove to recognize unfolded HasA. Elimination of SecB in *S. marcescens* affects the secretion of both HasA and proteins translocated through the Sec-dependent pathway (Sapriel *et al*., 2003). Although the chaperoning mechanism of SecB in the two pathways appears to be similar, the downstream interaction partners are different. The SecB residues implicated in SecA binding are not important for HasA secretion. Further biochemical and structural characterization of the type I pathway-specific protein–protein interactions in SecB will help elucidate the mechanism.

SecB may also act as a general chaperone, as it can bind to denatured luciferase and facilitates the subsequent refolding of the protein by the DnaK/DnaJ system (Knoblauch *et al*., 1999). Recent studies showed that overproduction of SecB can suppress the temperature-sensitive and the aggregation-prone phenotypes caused by elimination of both DnaK and trigger factor in *E. coli* (Ullers *et al*. 2004). It should be noted that this reflects a rather aberrant condition in which the cognate folding machinery is highly compromised, while a rescue is observed only upon overproduction of SecB. Whether this reflects a genuine *in vivo* function of SecB remains to be seen. The structural basis for this activity of SecB is unknown but likely involves the polypeptide binding site used in protein translocation pathway.

SecB has also been shown to interact directly with bacterial ribosome-bound chaperone trigger factor (Ha *et al*., 2004). The dissociation constant between *E. coli* SecB and *E. coli* trigger factor is ~6 μM, as determined by surface plasmon resonance. However, complex formation between the two proteins was not observed in a gel filtration chromatography experiment using purified materials. Isothermal titration calorimetry suggested that the dissociation constant is much lower (in the mM range) (J. Zhou and Z. Xu, unpubl. results). If they do interact, which part of SecB structure is involved in interaction? Little is known for the physiological role this interaction might play *in vivo*. Could it be possible that SecB receives the newly synthesized preprotein from trigger factor rather than through random collision in the cytosol? Clearly, further studies are necessary to clarify these issues.

Conclusions

The recent crystal structures of SecB and its complex with the C-terminal tail of SecA have shed new light on the molecular mechanism by which the small bacterial chaperone SecB functions in protein translocation. SecB employs hydrophobic, solvent-exposed surfaces to stabilize the non-native conformation that exists in preproteins. The predominant interactions between SecB and preproteins involve non-specific main chain hydrogen bonds and hydrophobic interactions. As SecB does not seem to recognize any particular sequence motifs within preproteins, this structural feature of SecB ensures that different preprotein ligands can be recognized. In contrast, the interaction between SecB and SecA is highly specific. Not surprisingly, the protein–protein interface within the SecB–SecA complex comprises highly conserved residues. Interactions between the two proteins are mediated by specific side-chain hydrogen bonds. Replacement of these residues by alanines has a drastic effect on the stability of the molecular complex.

As neither crystal structure contains polypeptide ligands, the exact mode of SecB–polypeptide interaction is not known. In particular, the structural basis for the function of SecB C-terminus in protein translocation is not clear. However, it is not difficult to imagine that a large stretch of polypeptide binds to the hydrophobic SecB peptide-binding groove in an extended conformation. The structure of SecB–SecAc complex suggests that SecA likely modulates the conformation of the polypeptide-binding groove upon its interaction with SecB, thereby promoting the release of preproteins from SecB. While the C-terminal tail of SecA plays a critical role in determining the specific interaction with SecB, other structural elements have also been shown to promote complex formation independent of the C-terminal tail. Elucidating the structure of SecB in complex with full-length SecA is the next logical step to further our understanding of this important event in bacterial protein translocation.

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