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SecB—A chaperone dedicated to protein translocation†

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SecB is a molecular chaperone in Gram-negative bacteria dedicated to the post-translational translocation of proteins across the cytoplasmic membrane. The entire surface of this chaperone is used for both of its native functions in protein targeting and unfolding. Single molecule studies revealed how SecB affects the folding pathway of proteins and how it prevents the tertiary structure formation and aggregation to support protein translocation.

I. Introduction

In the late 1970s, the definition *chaperone* was given for the first time to a nucleoplasmin that assisted the assembly of nucleosomes from folded histones and DNA.[1] In modern molecular biology, chaperones are defined as proteins that support the non-covalent (un)folding or (dis)assembly of proteins and protein complexes. Chaperones are not part of the final protein structures when they perform their normal biological functions. Today, over 30 heterogeneous chaperone families have been described and many are heat shock proteins. The latter reflects an increased need for chaperone function when proteins are subjected to denaturation by environmental stresses. One major function of molecular chaperones is to prevent proteins from aggregating into non-functional structures. Another essential function of chaperones is their involvement in the translocation of proteins across biological membranes (Fig. 1). In this review we will discuss SecB, a chaperone that is specifically involved in protein translocation across the cytoplasmic membrane by the Sec-system in bacteria.

II. History of SecB findings

In the late 1980s most components of the *Escherichia coli* protein translocation machinery (Sec-system) (Fig. 1) were identified by genetic approaches. Since the Sec-system fulfils an important function, most of the genes that constitute this system are essential for cell viability. Consequently, most Sec mutants were identified by screening for conditional lethal mutations that cause a pleiotropic defect in protein export. In 1983, Kumamoto and Beckwith found mutations in a gene that eliminated the export of a subset of periplasmic proteins only.[2] *E. coli* cells with a disruption of this gene, termed *SecB*, were found to be unable to grow on rich Luria broth media, while growth is unaffected on minimal media. Thus in contrast to many other Sec proteins, SecB is not essential for cell viability. Pulse chase experiments showed that mutations in the *SecB* gene lead to a retardation or a block of the processing of the signal sequence of maltose binding protein, preMBP.[3] In these mutants, the preMBP was found to accumulate in the cytosol and to fold into a protease resistant state. These observations led to the suggestion that SecB is responsible for stabilizing MBP in a translocation competent state. In 1988, Collier *et al.* demonstrated that SecB slows down the folding rate of *in vitro* synthesized preMBP.[4] This was the first indication for the *anti*-folding activity of SecB. SecB was for the first time purified in Phil Bassford's laboratory, and in *in vitro* translocation assays using the purified protein, SecB was shown to stimulate the translocation of preMBP into inside-out membrane vesicles of *E. coli*.[5,6] Based on further *in vitro* studies using purified SecB, preMBP and mature MBP, Hardy and Randall in the early nineties suggested that SecB discriminates between secretory and cytosolic proteins by the rate of folding and proposed the so called "kinetic partitioning model" for SecB functioning.[7] In this model, the slow folding of secretory proteins, partially because of the presence of the signal sequence, gives a greater time window for secretory proteins to interact with SecB. In the same period, Topping and Randall defined the SecB binding frame in the mature region of preproteins, and excluded the signal sequence as binding partner.[8] SecB already at an early stage during protein synthesis associates with nascent secretory proteins that emerge from the ribosome.[9,10]

Another important finding was the observation that SecB is not only equipped with anti-folding activity but that it also acts as a preprotein targeting factor that binds directly to the membrane associated SecA, the motor domain of the Sec-translocase.[11] It was shown that the C-terminus of SecA is required for SecB binding,[12] and that this domain contains a zinc ion that is critical for the binding reaction.[13] Catalytic details of the SecB-targeting cycle and the mechanism of preprotein transfer to SecA were resolved.[11,14] A major break-through was the elucidation of the three-dimensional structure of SecB by Xu and co-workers in 2000,[15] followed by

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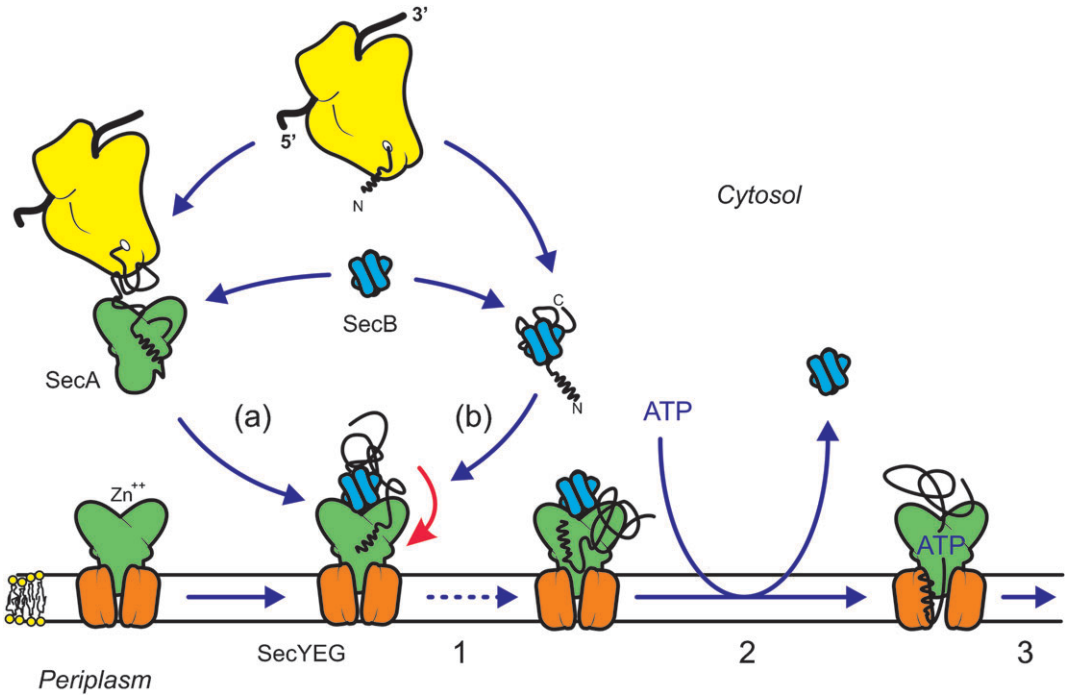

Fig. 1 Bacterial protein translocation and SecB targeting cycle. There are two possible ways for a nascent secretory polypeptide chain to enter the SecB targeting cycle. Cytosolic SecA may at an early phase bind the signal sequence of the preprotein (a) and either in the presence or absence of a SecA interaction, SecB binds to unfolded mature domain (b) thereby keeping the preprotein in an unfolded state. Next, the tertiary or binary complex is targeted to the translocon (SecYEG). Targeting involves the high affinity binding of SecB to the carboxyl terminus of the SecYEG-bound SecA. Binding of the signal sequence domain of the preprotein to SecA tightens the SecB–SecA interaction and releases the preprotein from SecB (1). Upon binding of ATP to SecA, preprotein translocation is initiated and SecB is released from complex into the cytosol where it can associate with newly synthesized preproteins in the cytosol (2). Translocation of the preprotein through the SecYEG pore is powered by cycles of ATP binding and hydrolysis by SecA and by the proton motive force (3).

the structure of SecB in complex with a peptide that corresponds to the SecB binding domain of SecA.[16] In recent years, comparative proteomic studies have expanded the list of SecB dependent preprotein substrates,[17] while site-directed spin labelling has been used to precisely map the sites of interaction of preproteins onto SecB.[18] Finally, single molecule measurements on the impact of SecB on the secretory protein folding pathway provided direct evidence for the anti-folding and anti-aggregation activity of SecB.[19]

III. Structural and functional studies of SecB

A. SecB structure

The SecB chaperone was crystallized from two bacteria, *Haemophilus influenza*[15] and *E. coli*.[20] (Fig. 2). These proteins exhibit 55% sequence identity and have a similar structure. SecB is a tetramer, organized as a dimer of dimers.[21,22] Each monomer has a molecular mass of 17 200 dalton and is composed of a simple α/β fold with four β-strands and two α-helices.[23] The four subunits interact *via* their α-helices and expose together an eight stranded β-sheet surface on each side of the stable and tightly packed tetramer. At pH 7.6, the equilibrium constant of the dissociation of the tetramer into dimers is about 20 nM indicating that the tetramer is quite stable.[21] A number of mutations in SecB (Cys76, Val78 and Gln80) lead to a destabilization of the tetramer yielding dimers under physiological conditions.[21] All tetramer destabilizing mutant residues are positioned in the β-sheets and face the inside of the molecule. Most likely these mutations cause a conformational change that lead to a distortion of the dimer–dimer interface. An alkaline pH and high salt concentrations also disturb the tetrameric structure and lead to an equilibrium shift to the dimeric state.[23] In this dimeric state, SecB is unable to bind preproteins.

SecB is a highly acidic protein. In the polarity surface profile of the SecB tetramer, a long groove is noticeable (Fig. 3, dashed yellow circle). This surface exposed groove is proposed to be the peptide-binding channel. The groove is composed of two subsites. Subsite one is formed by conserved aromatic residues that are positioned in a deep cleft close to the outside of the channel. It has been suggested that this subsite recognises the hydrophobic regions of peptide substrates. The second subsite is located in the middle of the groove and is relatively shallow. It also has a hydrophobic character but lacks aromatic residues. This subsite might be involved in peptide binding through the formation of hydrogen bonds with extended regions of the bound substrate. Around the anticipated peptide binding sites the surface is mostly negatively charged. This feature could explain the selectivity of SecB for peptides with basic residues.[24]

B. Binding of preproteins to SecB

Polypeptide chains that emerge from the ribosome tunnel exit are exposed to a pool of molecular chaperones that promote

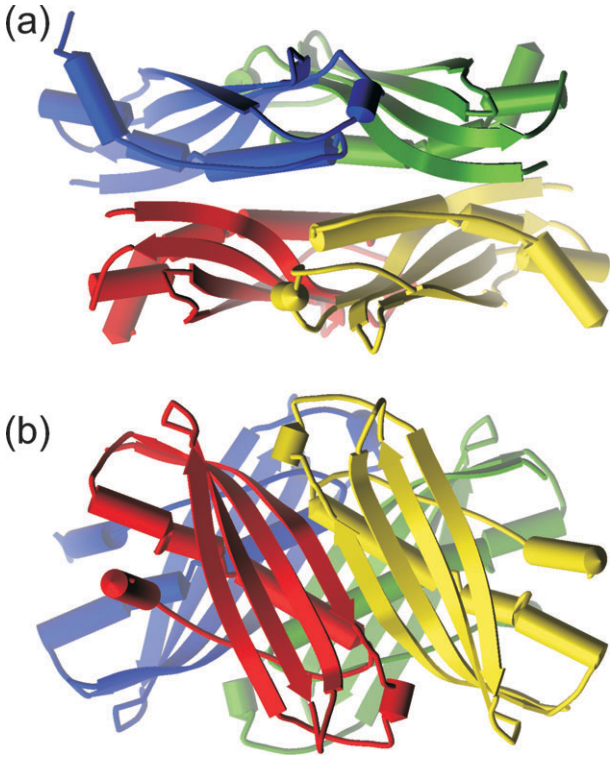

Fig. 2 The crystal structure of the SecB tetramer. Ribbon drawing of the *E. coli* SecB tetramer based on the coordinates deposited in the Protein Data Base as 1QYN in two orthogonal views. (a) Front view, showing the four stranded-sheets of each monomer and the packing of the dimer. (b) Side view, showing the dimer–dimer interface formed by the α-helices. Each subunit in the tetramer is depicted in a different colour.

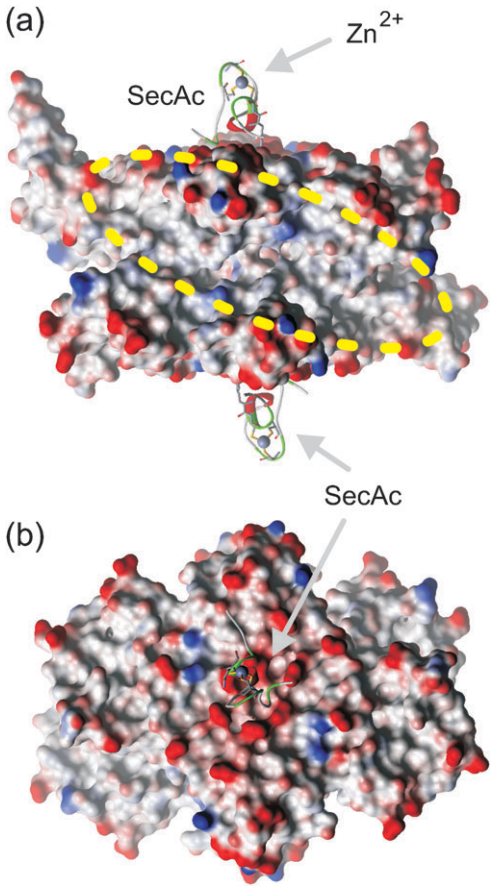

Fig. 3 The solvent accessible surface of the tetrameric SecB, shown as side (a) and front (b) view. Indicated are the carboxyl-terminal SecB-binding domains of SecA (residues 876–899) (Protein Data Base: 1OZB) that associates on both sides of SecB to the flat anionic surface formed by β-sheets. Both ribbon drawn peptides contain a zinc ion. The solvent accessible surface of the SecB tetramer is coloured according to the surface potential; red: negative electrostatic potential; blue: positive electrostatic potential. The dash yellow circle indicates the long groove that has been proposed to harbour the polypeptide binding sites.

folding and/or targeting for integration into or translocation across the cytoplasmic membrane. About 25–30% of all proteins in *E. coli* fulfil their function outside of the cell and thus these have to be transported across the cytoplasmic membrane to reach their final destination such as the periplasm, the outer membrane or the external medium. There are two main pathways for protein transport across the cytoplasmic membrane in bacteria: *i.e.*, the twin-arginine translocation (TAT) pathway[25] and the general secretion (Sec) system.[26] In most bacteria, only a subset of proteins is transported *via* the TAT system, and these proteins first acquire their final fold in the cytosol prior to translocation.[25] In contrast, proteins that are exported *via* the Sec-system need to be unfolded.[27] Many of these are targeted to the Sec-system in a post-translational manner which means that they are first synthesised to their full length before they are passed through the translocation channel (see Fig. 1: SecB preprotein targeting cycle). In the absence of chaperones, unfolded proteins tend to fold or aggregate in the cytosol. Indeed, in a SecB deletion strain, many secretory proteins are found as full length precursors in protein aggregates and inclusion bodies. Besides SecB, other more general chaperones can associate with unfolded secretory proteins such as Trigger Factor (TF) or DnaK.[28,29] However, only SecB seems to fulfil a specific role in protein export as it has both *anti*-folding activity and the ability to target secretory proteins to SecA.

SecB is found only in a subgroup of α-, β- and γ-proteobacteria which include most of the medical and industrial relevant Gram-negative bacteria such as *Pseudomonas*, *Neisseria* and *Haemophilus*.[30] SecB is absent in Gram-positive bacteria.[31] A SecB homolog was found in the archaeon *Methanococcus jannaschii*, but this appears exceptional, as so far it is not found in other archaea.[32] *In vivo*, it has been shown that SecB binds to a subset of nascent secretory proteins only. Direct evidence for SecB dependence was shown for eighteen proteins in *E. coli* using a combination of pulse labelling protein secretion studies and comparative proteomics. These SecB-dependent proteins are: MBP, GBP, PhoE, LamB, OmpF, OmpA, DegP, FhuA, FkpA, OmpT, OmpX, OppA, TolB, TolC, YbgF, YgiW and YncE.[17] All these proteins are synthesized with an N-terminal signal sequence that directs them to the Sec-system. However, SecB does not recognize or bind to the signal sequence of preproteins, but associates with internal polypeptide stretches in the critical core regions that drive protein folding.[33] A weak consensus binding motif has

been defined by means of a peptide scan of preprotein substrates. The SecB consensus motif has a length of about nine amino acid residues that are enriched in aromatic and basic residues whereas acidic residues are strongly disfavoured. In the tested preproteins the SecB-binding peptides correspond to regions that are buried in the native folded protein structure.[33] The identified consensus binding motif, however, is not specific for preproteins per se and thus does not explain why *in vivo* only a subset of preproteins associate with SecB. Typically, peptides that correspond to the consensus motif are present at 20–30 amino acid residue intervals in all cellular proteins.

Although SecB plays a role in the post-translational translocation of preproteins, a stable association with SecB has been found for nascent polypeptide chains of preMBP with a length of about 150 residues.[10] It should be noted that this length of the nascent chain corresponds with results of an earlier study that mapped the SecB binding sites in preMBP in the extreme N-terminal half of the preprotein.[8,23] *In vitro*, SecB appears rather unselective and binds many different proteins provided that they are unfolded. For instance, SecB binds to stabilized molten globular-like proteins, like barstar,[34] bovine pancreas trypsin inhibitor (BPTI), α-lactalbumin or ribonuclease A (RNaseA).[35] These small polypeptides bind in a four to one ratio to the tetrameric SecB, which implies that one polypeptide chain is bound per SecB monomer. Calorimetric measurements suggested that 7 up to 29 amino acyl residues of the polypeptide substrate are bound by SecB[35,36] and that this binding occurs with a dissociation constant that is in the micromolar range.[37] However, natural unfolded preproteins attach to the SecB tetramer in a one to one ratio and with high affinity showing a dissociation constant in the nanomolar range.[36,38]

The exact molecular mechanism of preprotein recognition by SecB has remained obscure. The selectivity for the substrate binding was suggested to occur by kinetic partitioning of substrates between protein folding and SecB association.[7] The kinetic partitioning model is based on the observation that SecB blocks the refolding of the precursor form of MBP and of slow folding MBP mutants, but not of the faster folding wild type MBP or fast folding cytosolic proteins.[7] In this respect, the signal sequence slows down the folding of the mature preprotein domain and thus indirectly will promote the association of the preprotein with SecB. However, the molecular basis of kinetic partitioning is not clear and likely relates to the combined effect of folding rates and binding affinity.[39,40] Strikingly, the SecB dependence for translocation is not affected by mutations that interfere with folding of MBP or alternatively mutations that cause the formation of a more stably folded structure of MBP.

Another important issue relates to the folding state of the preprotein when bound to SecB. Earlier biochemical studies suggested that SecB-bound preproteins are devoid of stable tertiary structure but that they contain native like-secondary structure.[7] Indeed, a recent single molecule study shows that SecB binds the unfolded state of MBP that lacks any stable tertiary structure.[19] In this study, MBP was mechanically unfolded with optical tweezers by coupling the protein between two polystyrene beads. The (un)folding pathway of MBP was studied in the absence and presence of SecB. The data indicate a folding pathway with a large variety of transitions and modes of folding (summarised in Fig. 4), which are each affected differently by SecB. The extended MBP peptide is compacted to a molten globule state either in the presence or absence of SecB. In the absence of SecB MBP folding proceeds from the molten globule to a core intermediate, but SecB prevents the formation of stable tertiary interactions thereby maintaining the molten globule-like state. This effect is more general and applies also to other chaperones such as GroEL.[41,42] Once the core intermediate of MBP has formed SecB cannot bind and it therefore has no effect on the folding of the external α-helices onto the surface of the core structure. SecB also prevents the stable aggregation interactions that occur at high local MBP concentrations. Although there is no structural information on the SecB:preprotein complex, in the bound state, the core folding region of the preprotein may wrap around the SecB tetramer binding into the long polypeptide binding grooves on both sides of the SecB tetramer. A mutant of SecB in which a leucine centrally positioned in the polypeptide binding pocket was replaced by arginine showed a reduced holdase activity. The mutation lowered the polypeptide binding affinity, and likely because of an increased dissociation rate it allows for partial folding of the SecB bound polypeptide chain (Bechtluft *et al.*, unpublished data). This further underscores the delicate balance between tight polypeptide binding and the chaperone function of SecB.

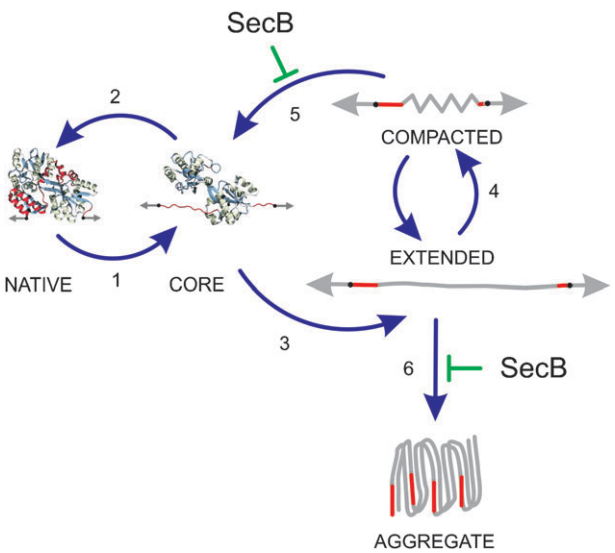

Fig. 4 Effect of SecB on the folding pathway of MBP observed by optical tweezers experiments. When mechanical force is applied to the termini of the native protein, several C-terminal α-helices detach from the tightly folded core (1). This transition is reversible (2). When the force is further increased the whole structure collapses and the polypeptide chain is fully extended (3). When the tension on the protein is lowered it relaxes and forms a compacted but molten globule like state that lacks stable tertiary structure (4). In the presents of the SecB chaperone the refolding of MBP to the core and native state in prevented (5). With a quadruple MBP often a tight and irreversible form of folded and aggregated protein is observed after relaxation (6). SecB prevents this aggregation of the four MBP repeats. Adapted from ref. 18.

C. Targeting of preproteins to the SecA subunit of the translocase

After binding to the preprotein, SecB targets the preprotein substrate to the motor domain of the translocase, SecA. SecA is the 102 kDa ATPase motor that is organized as a homodimer. SecA exists in a membrane-bound state, a soluble cytosolic state, and is also found to be associated with the ribosome.[26] Soluble SecA exhibits a low binding affinity for SecB with a K_d in the micromolar range.[43] Membrane binding of SecA to the protein conducting pore (SecYEG), greatly enhances the binding affinity for SecB (K_d of 30 nM) while the SecYEG-bound SecA recognized the binary SecB-preprotein complex with an even higher affinity (K_d of 10 nM).[11,14] Under the latter conditions, the signal sequence of the preprotein has a major impact on this affinity transition, as the signal sequence alone binds strongly to SecA and thus contributes to the formation of the ternary SecA–SecB-preprotein complex. The location of the SecB binding site on SecA was determined by truncation analysis. The high affinity binding site consists of only the C-terminal 22 amino acids of SecA.[12] This region of SecA is highly conserved in bacteria, even in organisms that lack a SecB homolog. The SecB binding region of SecA has a net positive charge due to the high abundance of lysyl and arginyl residues. Furthermore, the C-terminus of SecA includes three cysteines and a histidine that chelate a zinc atom that is essential for high affinity SecB binding.[13] The structure of this peptide was solved by NMR confirming a ligation of the zinc atom by the cysteines and histidine.[44] Also, the crystal structure of this C-terminal peptide of SecA bound to SecB has been solved.[16] The structure suggests that two SecA peptides bind to one tetramer of SecB at the acidic eight β-strand SecB surface. The interactions at the surface are most likely electrostatic interactions and involve hydrogen-bonding and intermolecular salt bridges. The residues that are involved in this SecA-SecB interactions were investigated by site directed mutagenesis in SecA of *E. coli*. Four conserved residues were found to be essential for high affinity interactions: one neutral polar asparagine 882 and three positively charged residues (Arg881, Lys891 and Lys 893). Mutagenesis of these residues to alanine disrupts the SecB binding, even without losing the ability to bind zinc.[16]

Even though the structures of SecB and SecA display a two-fold symmetry, NMR measurements of the SecA–SecB complex suggest that the contacts needed for stabilizing the complex are asymmetric.[45] In total there are three areas of contact in the complex of a SecB tetramer with two protomers of SecA. The first stabilizing interactions are between the zinc containing extreme C-terminus of SecA and the negatively charged flat β-sheet surface of SecB.[13] A second interaction involves the C-terminal region of the chaperone and the interfacial region of the SecA dimer.[45,46] The third area of contact is less defined but involves residues lying on the β-sheets of SecB as well as along the interface of the dimer of dimers. Interestingly, in solution the low-affinity SecB–SecA binding does not depend on the C-terminal end of SecA nor does it involve the anionic binding cluster on SecB.[47] However, for functional binding of SecB to the SecYEG-bound SecA protein, these regions of interaction are pivotal.[14]

The anionic binding cluster in SecB that interacts with the C-terminus of SecA harbours a number of conserved residues that are essential for SecA binding. In the *E. coli* SecB these residues[3,48] (Asp20, Glu24, Leu75 and Glu77) are positioned in a cluster on the outer β-stranded surface of the chaperone. Mutations of these residues result in a reduced affinity of SecB for SecA, whereas preprotein binding by SecB remains unaffected.[14] The mutations cause a preprotein translocation defect which is likely due to a defect in preprotein transfer from SecB to SecA. The structural analysis of the corresponding *H. influenzae* SecB–SecA interaction site demonstrated that the SecB surface is negatively charged and that it electrostatically interacts with the C-terminal end of SecA. This interaction between SecA and SecB is specific, taken into account that all the involved residues are highly conserved among the bacteria. Also the C-terminus of SecB, which is barely resolved in the structure due to its high mobility, plays an important role in SecA binding.[49] These α-helical C-termini protrude from the core structure as long arms and fit to interact with their counterpart, the C-terminal SecB-binding site on SecA. The SecB–SecA peptide crystals showed two SecB tetramers in the asymmetric unit, one without and one with bound SecA peptide.[16] Free in solution there is an equilibrium between monomers and dimers of SecA with a K_d of 0.1 to 1 nM depending on the ionic strength and temperature of the solution.[47] To be active *in vitro*, two SecA protomers must be bound to the tetrameric SecB for the complex to be active in preprotein translocation.[45] A recent study showed that a dimeric SecA is crucial to obtain a maximal coupling efficiency between ATP hydrolysis and translocation.[50]

The subunit stoichiometry of the functional SecA–SecB–preprotein complex is 2 : 4 : 1. Once the binary SecB–preprotein complex associates with the SecYEG-bound SecA, SecB needs to release the preprotein to SecA. In the presence of a preprotein, SecB exhibits a higher binding affinity for the SecYEG-bound SecA, and this phenomenon relates to the ability of SecA to bind the signal sequence of the exposed preprotein since high affinity SecB binding can be mimicked with synthetic signal sequences only.[51] This binding event likely fulfils an important role in the transfer mechanism, and in the stable ternary complex of SecB–preprotein–SecA, the preprotein is likely no longer bound to SecB but transferred to SecA. The exact mechanism of transfer is, however, unclear. It is of interest to note that binding of SecB to SecA results in a partial activation of the SecA ATPase activity.

The release of SecB from SecA is directly coupled to translocation. As soon as ATP binds to SecA, preprotein translocation is initiated and SecB is released from SecA and returns to the cytosol where it is able to associate with a new preprotein.[51] It has been suggested that the release of SecB is linked to a dissociation of the SecA dimer into a monomer at the SecYEG complex. The dissociation of SecA would result in a loss of the high affinity binding of SecB that requires two of the C-termini of the SecA dimer.

In the past, suppressor mutants of core components of the translocation machinery as SecY (PrlA) and SecA (PrlD) have been selected that restore the translocation of precursor

proteins even with a defective or missing signal sequence.[52] The translocation of these signal sequence-less preproteins is completely dependent on SecB, even for preproteins that under normal conditions are translocated independent of SecB.[53–56] These observations can be linked to the targeting function of SecB to SecA. When the intrinsic targeting information of the signal sequence for SecA is lost the supportive targeting function of SecB to SecA is more prominently required.[14]

D. Other functions of SecB

SecB has been proposed to play a more general role as a chaperone in the cytosol where it may act as a holdase of unfolded polypeptides. Depletion of the heat shock proteins DnaKJ and GroEL/GroES results in an increased SecB production.[57] *In vitro*, SecB has the capacity to assist DnaK in the folding of luciferase.[33] Overexpression of SecB can complement the growth defect of a DnaK/Trigger factor double knockout strain and the SecB present in a cellular lysate of this mutant strain can be chemically crosslinked to nascent chains of both preproteins and cytoplasmic proteins.[29] Although the aforementioned studies suggest a more general chaperone role of SecB in the cell, evidence sofar is solely based on cells that are severely compromised in the functionality of the regular cellular protein folding machinery. It therefore remains to be demonstrated that SecB fulfils a general chaperone function under normal conditions.

Besides the chaperone function for preproteins transported *via* the Sec-secretion system SecB also plays a role in the secretion of the hemophore, HasA, that is part of the heme acquisition system of *Serratia marcecens*. HasA is not translocated *via* the Sec-pathway, but is secreted by a specific ABC transporter. Nevertheless, HasA translocation is found to be strictly dependent on SecB.[58] This relates to the ability of SecB to maintain HasA in a translocation competent state. HasA is a protein that rapidly folds and binding of SecB results in an almost complete inhibition of folding.[59] Slow folding mutants of HasA are secreted independently of SecB.[60] Point mutations in SecB that affect the interaction with SecA have no effect on HasA secretion, but SecB mutations that affecting its oligometric structure and thus prevents preproteins to bind to SecB, block HasA secretion.[61] SecB thus functions as a specific chaperone for HasA export and maintains this protein in a translocation competent state prior to export.

Another function of chaperones is their ability to dissolve aggregates of misfolded proteins as observed for several heat shock chaperones. One study reports such function for SecB showing that SecB dissolves aggregates of insulin B chains.[62] However, in single molecule measurements, SecB was found to be unable to dissolve an aggregated form of MBP.[19]

IV. Concluding remarks

In twenty-six years of SecB research, many of the intimate features of this chaperone have been elucidated. Importantly, the dual function of SecB in protein translocation explains to a large extent its role in this process, *i.e.*, maintaining preproteins in an unfolded, molten globule like state, and targeting of preproteins to SecA. The entire molecular surface of the SecB chaperone is used for interaction with its partners, *i.e.*, preprotein substrates and SecA. For the SecB–SecA complex now three different sites of interaction have been described that play a crucial role in the functional interaction and efficient transfer of the preprotein to the translocase. Also, for the preprotein–SecB complex, sites of interaction have been mapped but the molecular basis for the high selectivity of SecB for a subset of preproteins in the cell remains a challenge for the future.

Abbreviations

BPTI	bovine pancreas trypsin inhibitor
preMBP	precursor of Maltose Binding Protein
RNaseA	ribonuclease A
TAT	Twin arginine translocase

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References

1 R. A. Laskey, B. M. Honda, A. D. Mills and J. T. Finch, Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA, *Nature*, 1978, **275**, 416–20.
2 C. A. Kumamoto and J. Beckwith, Mutations in a new gene, SecB, cause defective protein localization in *Escherichia coli*, *J. Bacteriol.*, 1983, **154**, 253–60.
3 P. M. Gannon and C. A. Kumamoto, Mutations of the molecular chaperone protein SecB which alter the interaction between SecB and maltose-binding protein, *J. Biol. Chem.*, 1993, **268**, 1590–5.
4 D. N. Collier, V. A. Bankaitis, J. B. Weiss and P. J. Bassford, Jr, The antifolding activity of SecB promotes the export of the *E. coli* maltose-binding protein, *Cell*, 1988, **53**, 273–83.
5 J. B. Weiss, P. H. Ray and P. J. Bassford, Jr, Purified SecB protein of *Escherichia coli* retards folding and promotes membrane translocation of the maltose-binding protein *in vitro*, *Proc. Natl. Acad. Sci. U. S. A.*, 1988, **85**, 8978–82.
6 J. B. Weiss and P. J. Bassford, Jr, The folding properties of the *Escherichia coli* maltose-binding protein influence its interaction with SecB *in vitro*, *J. Bacteriol.*, 1990, **172**, 3023–9.
7 S. J. Hardy and L. L. Randall, A kinetic partitioning model of selective binding of nonnative proteins by the bacterial chaperone SecB, *Science*, 1991, **251**, 439–43.
8 T. B. Topping and L. L. Randall, Determination of the binding frame within a physiological ligand for the chaperone SecB, *Protein Sci.*, 1994, **3**, 730–6.
9 F. U. Hartl and M. Hayer-Hartl, Molecular chaperones in the cytosol: from nascent chain to folded protein, *Science*, 2002, **295**, 1852–8.
10 L. L. Randall, T. B. Topping, S. J. Hardy, M. Y. Pavlov, D. V. Freistroffer and M. Ehrenberg, Binding of SecB to ribosome-bound polypeptides has the same characteristics as binding to full-length, denatured proteins, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 802–7.
11 F. U. Hartl, S. Lecker, E. Schiebel, J. P. Hendrick and W. Wickner, The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane, *Cell*, 1990, **63**, 269–79.

12 E. Breukink, N. Nouwen, A. van Raalte, S. Mizushima, J. Tommassen and B. de Kruijff, The C terminus of SecA is involved in both lipid binding and SecB binding, *J. Biol. Chem.*, 1995, **270**, 7902–7.
13 P. Fekkes, J. G. de Wit, A. Boorsma, R. H. Friesen and A. J. Driessen, Zinc stabilizes the SecB binding site of SecA, *Biochemistry*, 1999, **38**, 5111–6.
14 P. Fekkes, J. G. de Wit, J. P. van der Wolk, H. H. Kimsey, C. A. Kumamoto and A. J. Driessen, Preprotein transfer to the *Escherichia coli* translocase requires the co-operative binding of SecB and the signal sequence to SecA, *Mol. Microbiol.*, 1998, **29**, 1179–90.
15 Z. Xu, J. D. Knafels and K. Yoshino, Crystal structure of the bacterial protein export chaperone SecB, *Nat. Struct. Biol.*, 2000, **7**, 1172–7.
16 J. Zhou and Z. Xu, Structural determinants of SecB recognition by SecA in bacterial protein translocation, *Nat. Struct. Biol.*, 2003, **10**, 942–7.
17 L. Baars, A. J. Ytterberg, D. Drew, S. Wagner, C. Thilo, K. J. van Wijk and J. W. de Gier, Defining the role of the *Escherichia coli* chaperone SecB using comparative proteomics, *J. Biol. Chem.*, 2006, **281**, 10024–34.
18 J. M. Crane, Y. Suo, A. A. Lilly, C. Mao, W. L. Hubbell and L. L. Randall, Sites of interaction of a precursor polypeptide on the export chaperone SecB mapped by site-directed spin labeling, *J. Mol. Biol.*, 2006, **363**, 63–74.
19 P. Bechtluft, R. G. van Leeuwen, M. Tyreman, D. Tomkiewicz, N. Nouwen, H. L. Tepper, A. J. Driessen and S. J. Tans, Direct observation of chaperone-induced changes in a protein folding pathway, *Science*, 2007, **318**, 1458–61.
20 C. Dekker, B. de Kruijff and P. Gros, Crystal structure of SecB from *Escherichia coli*, *J. Struct. Biol.*, 2003, **144**, 313–9.
21 E. M. Muren, D. Suciu, T. B. Topping, C. A. Kumamoto and L. L. Randall, Mutational alterations in the homotetrameric chaperone SecB that implicate the structure as dimer of dimers, *J. Biol. Chem.*, 1999, **274**, 19397–402.
22 T. B. Topping, R. L. Woodbury, D. L. Diamond, S. J. Hardy and L. L. Randall, Direct demonstration that homotetrameric chaperone SecB undergoes a dynamic dimer-tetramer equilibrium, *J. Biol. Chem.*, 2001, **276**, 7437–41.
23 V. F. Smith, B. L. Schwartz, L. L. Randall and R. D. Smith, Electrospray mass spectrometric investigation of the chaperone SecB, *Protein Sci.*, 1996, **5**, 488–94.
24 J. Kim and D. A. Kendall, Identification of a sequence motif that confers SecB dependence on a SecB-independent secretory protein *in vivo*, *J. Bacteriol.*, 1998, **180**, 1396–401.
25 F. Sargent, The twin-arginine transport system: moving folded proteins across membranes, *Biochem. Soc. Trans.*, 2007, **35**, 835–47.
26 A. J. Driessen and N. Nouwen, Protein translocation across the bacterial cytoplasmic membrane, *Annu. Rev. Biochem.*, 2008, **77**, 643–67.
27 L. L. Randall and S. J. Hardy, Correlation of competence for export with lack of tertiary structure of the mature species: a study *in vivo* of maltose-binding protein in *E. coli*, *Cell*, 1986, **46**, 921–8.
28 E. Schaffitzel, S. Rudiger, B. Bukau and E. Deuerling, Functional dissection of trigger factor and DnaK: interactions with nascent polypeptides and thermally denatured proteins, *Biol. Chem.*, 2001, **382**, 1235–43.
29 R. S. Ullers, J. Luirink, N. Harms, F. Schwager, C. Georgopoulos and P. Genevaux, SecB is a bona fide generalized chaperone in *Escherichia coli*, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 7583–8.
30 P. Fekkes and A. J. Driessen, Protein targeting to the bacterial cytoplasmic membrane, *Microbiol. Mol. Biol. Rev.*, 1999, **63**, 161–73.
31 K. H. van Wely, J. Swaving, R. Freudl and A. J. Driessen, Translocation of proteins across the cell envelope of Gram-positive bacteria, *FEMS Microbiol. Rev.*, 2001, **25**, 437–54.
32 S. C. Ha, T. H. Lee, S. S. Cha and K. K. Kim, Functional identification of the SecB homologue in Methanococcus jannaschii and direct interaction of SecB with trigger factor, *Biochem. Biophys. Res. Commun.*, 2004, **315**, 1039–44.
33 N. T. Knoblauch, S. Rudiger, H. J. Schonfeld, A. J. Driessen, J. Schneider-Mergener and B. Bukau, Substrate specificity of the SecB chaperone, *J. Biol. Chem.*, 1999, **274**, 34219–25.
34 V. G. Panse, J. B. Udgaonkar and R. Varadarajan, SecB binds only to a late native-like intermediate in the folding pathway of barstar and not to the unfolded state, *Biochemistry*, 1998, **37**, 14477–83.
35 V. G. Panse, C. P. Swaminathan, A. Surolia and R. Varadarajan, Thermodynamics of substrate binding to the chaperone SecB, *Biochemistry*, 2000, **39**, 2420–7.
36 L. L. Randall, T. B. Topping, D. Suciu and S. J. Hardy, Calorimetric analyses of the interaction between SecB and its ligands, *Protein Sci.*, 1998, **7**, 1195–200.
37 L. L. Randall, Peptide binding by chaperone SecB: implications for recognition of nonnative structure, *Science*, 1992, **257**, 241–5.
38 P. Fekkes, T. den Blaauwen and A. J. Driessen, Diffusion-limited interaction between unfolded polypeptides and the *Escherichia coli* chaperoneSecB, *Biochemistry*, 1995, **34**, 10078–85.
39 D. Tomkiewicz, N. Nouwen and A. J. Driessen, Kinetics and energetics of the translocation of maltose binding protein folding mutants, *J. Mol. Biol.*, 2008, **377**, 83–90.
40 B. Krishnan, S. R. Kulothungan, A. K. Patra, J. B. Udgaonkar and R. Varadarajan, SecB-mediated protein export need not occur *via* kinetic partitioning, *J. Mol. Biol.*, 2009, **385**, 1243–56.
41 R. Horst, E. B. Bertelsen, J. Fiaux, G. Wider, A. L. Horwich and K. Wuthrich, Direct NMR observation of a substrate protein bound to the chaperonin GroEL, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 12748–53.
42 G. Stan, G. H. Lorimer, D. Thirumalai and B. R. Brooks, Coupling between allosteric transitions in GroEL and assisted folding of a substrate protein, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 8803–8.
43 T. den Blaauwen, E. Terpetschnig, J. R. Lakowicz and A. J. Driessen, Interaction of SecB with soluble SecA, *FEBS Lett.*, 1997, **416**, 35–8.
44 B. R. Dempsey, M. Wrona, J. M. Moulin, G. B. Gloor, F. Jalilehvand, G. Lajoie, G. S. Shaw and B. H. Shilton, Solution NMR structure and X-ray absorption analysis of the C-terminal zinc-binding domain of the SecA ATPase, *Biochemistry*, 2004, **43**, 9361–71.
45 L. L. Randall, J. M. Crane, A. A. Lilly, G. Liu, C. Mao, C. N. Patel and S. J. Hardy, Asymmetric binding between SecA and SecB two symmetric proteins: implications for function in export, *J. Mol. Biol.*, 2005, **348**, 479–89.
46 L. L. Randall, J. M. Crane, G. Liu and S. J. Hardy, Sites of interaction between SecA and the chaperone SecB, two proteins involved in export, *Protein Sci.*, 2004, **13**, 1124–33.
47 R. L. Woodbury, T. B. Topping, D. L. Diamond, D. Suciu, C. A. Kumamoto, S. J. Hardy and L. L. Randall, Complexes between protein export chaperone SecB and SecA. Evidence for separate sites on SecA providing binding energy and regulatory interactions, *J. Biol. Chem.*, 2000, **275**, 24191–8.
48 H. H. Kimsey, M. D. Dagarag and C. A. Kumamoto, Diverse effects of mutation on the activity of the *Escherichia coli* export chaperone SecB, *J. Biol. Chem.*, 1995, **270**, 22831–5.
49 T. L. Volkert, J. D. Baleja and C. A. Kumamoto, A highly mobile C-terminal tail of the *Escherichia coli* protein export chaperone SecB, *Biochem. Biophys. Res. Commun.*, 1999, **264**, 949–54.
50 C. Mao, S. J. Hardy and L. L. Randall, Maximal efficiency of coupling between ATP hydrolysis and translocation of polypeptides mediated by SecB requires two protomers of SecA, *J. Bacteriol.*, 2009, **191**, 978–84.
51 P. Fekkes, C. van der Does and A. J. Driessen, The molecular chaperone SecB is released from the carboxy-terminus of SecA during initiation of precursor protein translocation, *EMBO J.*, 1997, **16**, 6105–13.
52 P. N. Danese and T. J. Silhavy, Targeting and assembly of periplasmic and outer-membrane proteins in *Escherichia coli*, *Annu. Rev. Genet.*, 1998, **32**, 59–94.
53 O. Francetic, M. P. Hanson and C. A. Kumamoto, prlA suppression of defective export of maltose-binding protein in SecB mutants of *Escherichia coli*, *J. Bacteriol.*, 1993, **175**, 4036–44.
54 N. J. Trun, J. Stader, A. Lupas, C. Kumamoto and T. J. Silhavy, Two cellular components, PrlA and SecB, that recognize different sequence determinants are required for efficient protein export, *J. Bacteriol.*, 1988, **170**, 5928–30.
55 A. I. Derman, J. W. Puziss, P. J. Bassford Jr and J. Beckwith, A signal sequence is not required for protein export in prlA: mutants of *Escherichia coli*, *EMBO J.*, 1993, **12**, 879–88.

56 O. Francetic and C. A. Kumamoto, *Escherichia coli* SecB stimulates export without maintaining export competence of ribose-binding protein signal sequence mutants, *J. Bacteriol.*, 1996, **178**, 5954–9.
57 J. P. Muller, Influence of impaired chaperone or secretion function on SecB production in *Escherichia coli*, *J. Bacteriol.*, 1996, **178**, 6097–104.
58 P. Delepelaire and C. Wandersman, The SecB chaperone is involved in the secretion of the Serratia marcescens HasA protein through an ABC transporter, *EMBO J.*, 1998, **17**, 936–44.
59 L. Debarbieux and C. Wandersman, Folded HasA inhibits its own secretion through its ABC exporter, *EMBO J.*, 2001, **20**, 4657–63.
60 N. Wolff, G. Sapriel, C. Bodenreider, A. Chaffotte and P. Delepelaire, Antifolding activity of the SecB chaperone is essential for secretion of HasA, a quickly folding ABC pathway substrate, *J. Biol. Chem.*, 2003, **278**, 38247–53.
61 G. Sapriel, C. Wandersman and P. Delepelaire, The N terminus of the HasA protein and the SecB chaperone cooperate in the efficient targeting and secretion of HasA *via* the ATP-binding cassette transporter, *J. Biol. Chem.*, 2002, **277**, 6726–32.
62 V. G. Panse, P. Vogel, W. E. Trommer and R. Varadarajan, A thermodynamic coupling mechanism for the disaggregation of a model peptide substrate by chaperone SecB, *J. Biol. Chem.*, 2000, **275**, 18698–703.