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SecB, one small chaperone in the complex milieu of the cell

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Abstract. SecB is only one of a plethora of cytosolic chaperones in *E. coli* whose common property is that they bind nonnative proteins. It plays a crucial role during protein export via the general secretory pathway by modulating the partitioning of precursors between folding or aggregation and delivery to the membrane-bound translocation apparatus. In this latter role SecB demonstrates specific binding to a unique partner, SecA. SecB has the potential to participate in functions outside of export acting as a general nonspecific chaperone to provide buffering capacity of the nonnative state of proteins in the cytosolic pool. We discuss the interactions of SecB with its many binding partners in light of its recently determined structure, emphasizing both kinetic and thermodynamic parameters.

Key words. Chaperone; SecB; export; secretion.

Introduction

In the Gram-negative bacterium *Escherichia coli*, two systems have been identified that transfer proteins into the cytoplasmic membrane itself, or through that membrane into the periplasmic space between the cytoplasmic and outer membranes. A striking difference that distinguishes the two systems from each other is that one, the Tat system, handles proteins that must completely fold in the cytosol to acquire necessary cofactors (for review see [1]) whereas the general secretory, or Sec, system (for review see [2]) cannot handle folded proteins at all, but must transfer polypeptides before they acquire a stably folded structure [3]. Thus, as newly synthesized proteins partition among the various pathways in the cell, those that travel through the Sec system will be subject to kinetic considerations, whereas those that follow the Tat pathway will be governed solely by thermodynamic parameters. This difference is reflected not only in the nature of the crucial parameters but also in the architecture of the systems. The Tat system comprises four integral membrane proteins that provide transport across the membrane barrier. Proper delivery to this system is assured since the precursors carry signal sequences that specifically bind to that translocation apparatus; no soluble factors are required. In contrast the Sec system can only transfer unstructured polypeptides. Thus, in addition to a pathway through the membrane, it must either have a means to unfold proteins or to capture them before they acquire stable structures. Although both the energy of ATP hydrolysis and protonmotive force are consumed during transfer, there is no evidence for an unfolding activity. On the contrary, it has been demonstrated in vivo that precursors are captured before they fold [3]. The Sec system achieves this early capture by making use of a diverse group of soluble chaperones. SecB, the subject of this review, is one of those chaperones.

Interplay among chaperones

The emphasis on kinetic partitioning as it applies to the Sec pathway should not be taken to mean that affinities between the components of the Sec system are not important. Dissociation constants are thermodynamic parameters that dictate the probability that at equilibrium given proteins are in complex, but there are overriding kinetic aspects at play during export. Once a precursor has folded, no matter how strong the binding is to the translocon, the

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precursor will not be translocated. The final partitioning between the cytosol and transfer through the membrane is determined kinetically, by the rate of passage through the Sec pathway relative to the rate of the competing, essentially irreversible reactions, folding and aggregation.

Figure 1 is a simplified illustration of some of the many pathways open to newly synthesized polypeptides, with an emphasis on the Sec system. SecB is only one of numerous chaperones in the cell, and thus it cannot be considered in isolation. The balance among the many possible interactions a newly synthesized polypeptide might make is influenced by the interplay of a multitude of factors. As a family, chaperones are united by their remarkable ability to selectively and rapidly bind polypeptides in nonnative states. Although the interactions between a ligand and different chaperones will each be characterized by different molecular contacts and therefore different affinities, to some degree chaperones are in competition for the same pool of ligands. The binding to some chaperones such as GroE is not readily reversible, ATP must be hydrolyzed for release and so this binding might be considered a committed step in the pathway of folding. However, GroE has also been shown to be involved in the export of β-lactamase via the Sec system [4]. Similarly, the DnaK-DnaJ-GrpE chaperone machine plays a role in export of some proteins but also is crucial in regulation of the heat shock response [1, 5]. Perhaps the difficulty in assigning different chaperones to different functions is that the cell does not have neatly compartmentalized roles for them. There seems to be a great deal of overlap and interplay. For example, in SecB null cells, the heat shock chaperones DnaK and DnaJ can substitute for SecB in the export of several proteins which normally depend on SecB [6, 7]. In addition, at 30 °C SecB is needed for the export of alkaline phosphatase, whereas at higher temperatures DnaK and DnaJ are used instead. This might simply be because at 30 °C the cellular levels of DnaK and DnaJ are too low to fulfill the need for a chaperone, whereas they are more highly expressed at elevated temperatures. The secretion of alkaline phosphatase is also affected by depletion of Ffh, a component of the bacterial SRP [8], which like SecA can deliver precursors to the Sec translocon (for review see [9]). Ribose-binding protein also displays a conditional need for SecB if the efficiency of export is compromised [10]. This can be explained in several ways. When export occurs rapidly the spontaneous folding rate of ribose-binding protein may be slow enough to allow partitioning into the export pathway. Alternatively, a different chaperone might be present in quantities that are sufficient to handle the normal level of precursor; but if export is slow, SecB may become important to cope with the greater amount of precursor ribose-binding protein in the pool waiting to exit. In other words, the unfolded polypeptides will bind to what is available. If the chaperone with the highest affinity for a specific ligand is not present in sufficiently high concentration, a chaperone with lower affinity that is abundantly present may serve the function. As pointed out previously by Jagger and Richards [11], 'you can't always get what you want, but if you try sometimes, you just might find, you get what you need.'

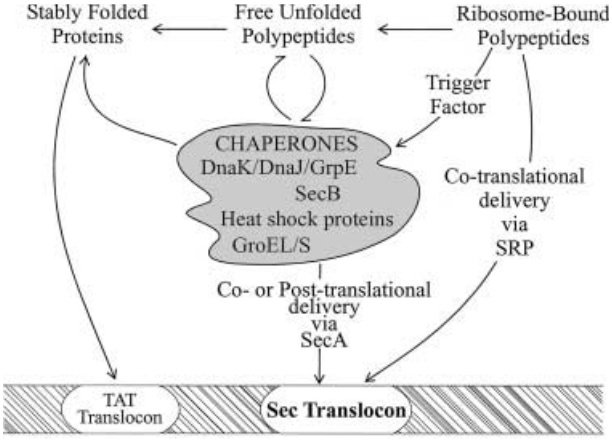


Figure 1. Multiple pathways open to newly synthesized polypeptides. Nonnative polypeptides can be bound by chaperones and directed to the membrane-associated Sec translocon either cotranslationally or posttranslationally. Binding of unfolded polypeptides by chaperones is fully reversible. The essentially irreversible steps are release from the ribosome and protein folding. The Tat translocon transfers native, folded protein.

Potential for roles outside of export

A protein that needs to be held in an unstructured state to be competent for export might interact transiently with several chaperones before it reaches an irreversible, committed step. The irreversible step in the Sec system occurs when a precursor is transferred to SecA and ATP is bound to initiate translocation [12]. When ligand-bound SecB interacts with SecA, the irreversible transfer occurs only if the ligand carries a functional leader sequence that itself interacts with SecA [12]. Ligands that are in complex with SecB, whether nascent chains or fully elongated polypeptides, are in rapid equilibrium with the free pool [13, 14]. Although affinities for nonnative polypeptides are in the nanomolar range, the rate constants of association are near collision limited, and therefore the off rates are also very high [15–17]. This means that SecB could be transiently occupied by cytosolic proteins that would rapidly partition to the folded state without blocking the export pathway. Indeed, it is possible that if conditions were to arise in the cell such that SecB became so complexed with nonsecretory polypeptides that the efficiency of export were compromised, the production of SecB would increase. Müller [18] has shown that SecB production is regulated in response to overproduction of the pre-

cursors, which are dependent on SecB for export. Therefore, in addition to acting as a chaperone to pass polypeptides into the Sec system via specific interaction with SecA, SecB might also act as a general buffer of the unfolded state of polypeptides in the pool. This idea finds support in the observation that the loss of heat shock chaperones GroEL, GroES, DnaK or DnaJ resulted in increased production of SecB [19]. SecB, like the heat shock chaperones, might engage in functions completely unrelated to export such as dissociation of aggregates of misfolded polypeptides and redirection of such species to correct folding. Such potential has been documented. Through a thermodynamic coupling, SecB used the energy of binding to monomers to pull the equilibrium and disrupt aggregates of insulin B chains [20]. In vivo upon dissociation from SecB, the monomer might then either fold properly or be shunted down a pathway to degradation. SecB might even actively facilitate folding in vivo, since in vitro interaction between SecB and an intermediate along the folding pathway of barnase resulted in transient disruption and reformation of secondary structure [21]. Such an activity could correct improperly formed structures.

The cellular milieu is too complex to allow prediction of an order of binding among the chaperones. This would depend on whether the binding reflects the thermodynamically most stable interaction or a kinetic advantage. In addition, the effects of macromolecular crowding in the cell have profound effects that make it difficult to relate parameters determined in dilute, ideal conditions to interactions in the cytosol. For a thorough treatment of macromolecular crowding and macromolecular confinement in physiological media, the reader is referred to two excellent recent reviews [22, 23]. While effects of excluded volume will retard diffusional motion and slow the encounter of reacting partners, crowding favors association because of the resulting increase in accessible volume for the other molecules. Macromolecular crowding will influence the equilibrium and kinetics of any reaction which results in a change in the volume occupied by the reactants. Such reactions include not only the association of proteins but also the folding of polypeptides. Another factor to consider is local concentration. This is of particular importance to interactions of nascent polypeptides with trigger factor, which is a chaperone associated with ribosomes [24]. In free solution SecB has a higher affinity for tested ligands than does trigger factor, but during elongation the chain will encounter trigger factor because it is bound to the ribosome, whereas reaction with SecB would require collision between the ribosome and SecB. SecB does bind nascent chains [25], but not until they have elongated to approximately 150 amino acids [13]. Therefore, shorter nascent chains would preferentially bind trigger factor, whereas later in elongation or after release from the ribosome, SecB has the higher affinity [26].

Structure of SecB

SecB is a tetrameric chaperone comprising four identical subunits each of molar mass 17,200 Da [27]. The X-ray crystal structure of SecB from *Hemophilus influenzae* [28], which has 55% sequence identity with SecB from *E. coli,* has confirmed biochemical studies that indicated the molecule is organized as a dimer of dimers [29, 30]. Each monomer has a simple $\alpha+\beta$ fold consisting of two α helices and four β strands (fig. 2A). The four β strands are organized into an antiparallel β sheet that lies on the surface of the molecule, with the α helices below the sheet. Two monomers are paired in a dimer via strand β1 and helix α1 of each monomer. As a result of this pairing there is an eight-stranded antiparallel β sheet on the surface of the dimer (fig. 2B). The tetramer is formed by association of the dimers via the helices (fig. 2C). The extreme C-terminal helix of each subunit displays a different number of turns and is not packed against the tetramer after the third turn, consistent with proton nuclear magnetic resonance studies indicating that the C-terminal 13 residues in *E. coli* SecB are highly mobile [31].

The tetrameric structure of the wild-type chaperone is very stable, with an equilibrium constant at pH 7.6 well below 20 nM [29]. Three variant forms of SecB each having a single aminoacyl substitution at Cys76, Val78 or Gln80 manifest equilibria shifted toward dimer under physiological conditions [29]. The corresponding residues in the crystal structure of *H. influenzae* SecB are located on a surface β strand with the side chains pointing toward the interior of the molecule. The side chains are not directly involved in contacts at the dimer interface; therefore, the effect on the equilibrium is likely to arise from a conformational change that is propagated to the interface. Data for thermal unfolding of tetrameric SecB could be fit to a two-state model in which the folded tetramer is directly converted to unfolded monomer [32], in agreement with an independent study using mass spectrometry that showed that thermal denaturation resulted in dissociation directly into monomers without a dimeric

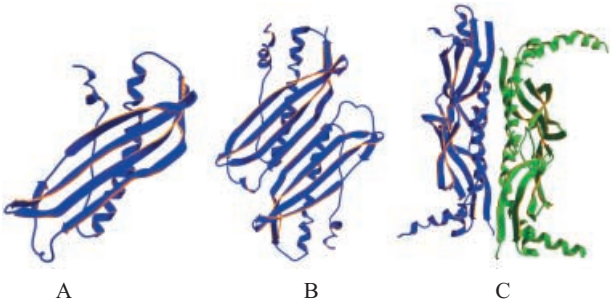


Figure 2. Structure of *Hemophilus influenzae* SecB. (*A*) Ribbon representation of a monomer. (*B*) Ribbon representation of dimer. (*C*) Ribbon representation of SecB tetramer; the view is orthogonal to the view of dimer

intermediate [27]. In contrast, at alkaline pH, a dimeric state was populated [27]. Taken together with the crystal structure that definitively shows SecB is a dimer of dimers, these data suggest that the contacts that stabilize the dimer at the interface must also stabilize the intramolecular contacts within the monomers of the dimers. This would explain why attempts to purify the forms of SecB that have the substitutions which destabilize the dimer interface resulted in aggregation of the variant proteins and recovery in inclusion bodies [29].

Two types of interaction

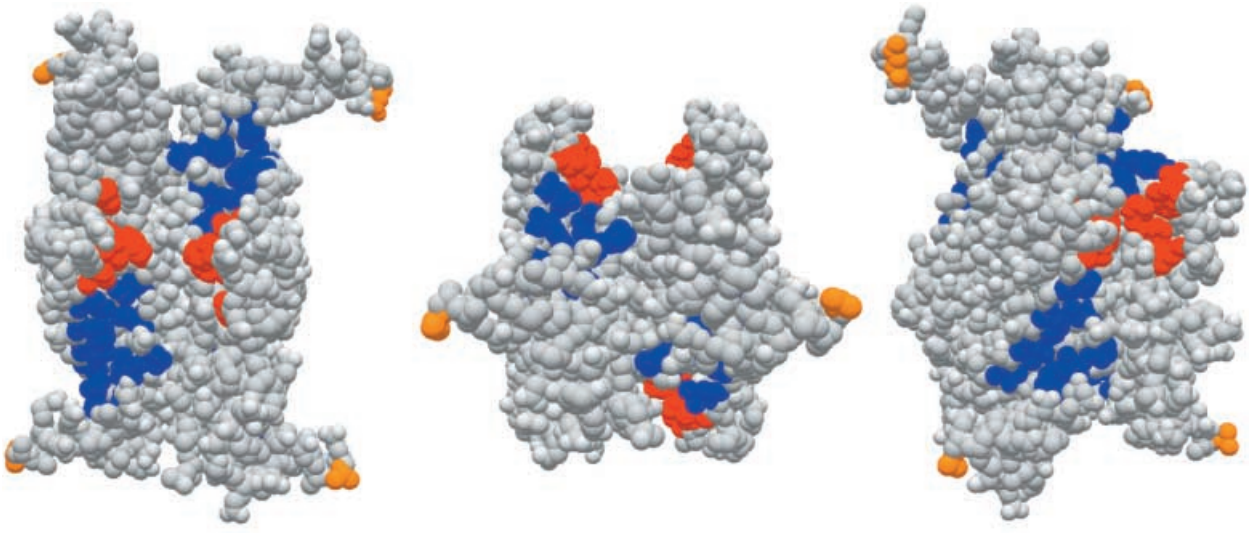
SecB participates in interactions of two fundamentally different types. Its function as a chaperone requires interaction with the diverse group of polypeptides united by the fact that they are unstructured, whereas its role in delivery of precursors to the membrane translocase requires interaction with a unique partner, SecA. The ability to bind many different nonnative polypeptides demands a site that can tolerate diverse sequences of amino acids. In contrast, formation of a complex with SecA is dictated by mutual recognition of specific conformations of aminoacyl side chains.

Promiscuous ligand binding

As a chaperone, SecB binds unfolded polypeptides with high affinity but interacts with proteins in their folded native state either not at all or with very low affinity [15, 21, 33]. The exported proteins that SecB binds are synthesized as precursors containing leader sequences at their amino termini. The leader is crucial to export since it binds directly to SecA and triggers the translocation step [12, 34, 35]. The leader does play a role in the binding of precursors to SecB, but it is indirect. It has been shown that the leader of precursor maltose-binding protein is not directly recognized, but rather retards folding so that SecB can interact with the unfolded mature portion of the protein [36]. The leader is not necessary for binding at all if the polypeptide folds sufficiently slowly, thereby accounting for the ability of SecB to bind proteins not directed to the Sec system. Stability of complexes between ligands and SecB in vivo is not simply predicated by affinity to the unstructured polypeptides. The system is not at equilibrium, and thus interaction with SecB is in part dictated by a kinetic partitioning between binding and folding (for further discussion of kinetic partitioning see [37]). A calculation of relative stabilities will illustrate what is meant when we say that in vivo the binding between SecB and its natural ligands is most likely kinetically determined. The free energy of stabilization of maltose-binding protein is 12.9 kcal mol^{-1} [38]. Since at 37 °C each order of magnitude of concentration above the dissociation constant will provide 1.4 kcal mol^{-1} of energy of stabilization of a complex, the cellular concentration of SecB would need to be nine orders of magnitude higher than the dissociation constant (K_d ~ 30 nM [39]) for the energy of stabilization of the complex to exceed that of the folded protein. Even taking into account the effects of excluded volume on thermodynamic activity, it is unlikely that free SecB (estimates of the concentration of total SecB range from 4 μM to 20 μM [40, 41]) could reach molar activities. In accord with this idea, when export was blocked in vivo so that equilibrium between binding and folding would be achieved, the complexes dissociated. In addition, it was shown that a complex between SecB and a slow-folding variant of maltose-binding protein was longer lived than that between wild-type maltose-binding protein and SecB. Incomplete nascent chains, which cannot fold, remained complexed with SecB [42]. If SecB were to encounter a polypeptide that either could not fold or folded with an energy of stabilization so low that it would be less than the energy available from binding SecB, then a complex could form that would be thermodynamically stable. All complexes are characterized by binding energy, a thermodynamic parameter described by the K_d. Only under equilibrium conditions can one determine the true K_d as opposed to an apparent K_d. To examine binding in the absence of kinetic partitioning, polypeptide ligands can be modified so they cannot fold, for example by carboxamidomethylation of cysteines in ribonuclease A or bovine pancreatic trypsin inhibition.

Molecular details of chaperone binding

Let us now consider what is known about the molecular detail of ligand binding. SecB was crystallized without a ligand in the binding site, but examination of the structure reveals two surface grooves that lie across the dimer interface, one on each side of the dimer (fig. 3A). These grooves have all the characteristics necessary to rationalize the extensive body of data gathered on binding of ligands to SecB. Surveys of peptides as potential ligands for SecB led to the conclusion that SecB has a preference for unstructured peptides that contain basic and aromatic residues and have a minimum length of nine amino acids [43, 44]. SecB can bind several ligands simultaneously if they are small. For very short polypeptides, such as bovine pancreatic trypsin inhibitor, which is 58 aminoacyl residues in length, the tetramer binds four molecules [43, 45]. In all cases studied, the physiological ligands of SecB, which are all polypeptides of molar mass 30,000 Da or greater, were bound with a stoichiometry of one polypeptide ligand per SecB tetramer. This was demonstrated by titration calorimetry for maltose-binding protein and galactose-binding protein [39], by both analytical centrifu-



A B C

Figure 3. The peptide binding channel of SecB. (*A*) The two subsites for peptide binding as proposed by Xu et al. [28] with the residues lining subsite 1 colored red and those lining subsite 2 colored blue. The C-terminal residue on each subunit is colored yellow. (*B*) A view of the structure shown in (*A*), rotated 90° toward the viewer around the horizontal axis to illustrate the groove and the possible path for the ligand to cross over between the C-terminal helices. (*C*) A view of the structure in (*A*). rotated ~135° clockwise around the vertical axis to reveal the possible path between the subsites around the side.

gation and mass spectrometry for oligopeptide-binding protein [46, 47] and by sedimentation velocity in sucrose gradients for OmpA and PhoE [26]. Ligands of length intermediate between the short peptides and the natural ligands display different stoichiometries. Studies of barnase (110 amino acids) [17], ribonuclease A (124 amino acids) and lactalbumin (123 amino acids) [45] indicate a binding of one ligand per monomer. However, a form of staphylococcus nuclease which has been modified so it will not fold and is very near the same size (131 amino acids) as the ribonucleases studied binds with a stoichiometry of three molecules per tetramer when assessed in solution by analytical centrifugation. When analyzed by mass spectrometry, only two molecules are observed bound to a tetramer of SecB [48]. Since mass spectrometry is carried out in vacuum, it is likely that the third molecule was associated predominantly through hydrophobic interactions that would be unstable in the absence of water.

Xu et al. [28] have proposed that the putative binding grooves can each be divided into subsites (fig. 3A). The midsection of each channel is deep and lined with aromatic amino acids. At each end the channels are shallow and more open, with hydrophobic floors. The short polypeptide ligands studied might independently occupy the grooves, with each groove accommodating up to two molecules. In order to fill the grooves on both sides of the tetramer with one ligand, it would need to wrap around the chaperone. Three natural ligands that bind with a stoichiometry of one do make contact with SecB over sufficient length to simultaneously fill both grooves. The binding frame for each of these ligands, maltose-binding protein [49], galactose-binding protein [50] and oligopeptide-binding protein [46], was shown to lie in the middle of the primary sequence and to span a stretch of 150 aminoacyl residues or more. The idea that the channel across the dimer interface is the site of binding of ligands is consistent with the fact that the variant forms of SecB that have their equilibria shifted toward dimer were first described as having defective interactions in vivo with precursor proteins [51].

If the ligand does wrap around the tetramer to occupy both grooves simultaneously, it could take one of two possible pathways. It might pass between the C-terminal α helices across the top of the molecule as it is oriented in figure 3B or it might pass under the C-terminal helices and across the side as illustrated in figure 3C. By either route the ligand would approach the C-terminal helices, consistent with observations that binding of peptides protects SecB from proteolysis at a site in this helix [43] and that removal of the helix (residues 143 to 155 in *E. coli*) results in a small, twofold decrease in affinity for ligand [52]. As suggested recently [53] the C-terminal helix may fold back over the binding site to form a cap in a manner similar to that seen in DnaK, which is another chaperone that must accommodate diverse peptides in its binding channel [54].

Specific binding to SecA

SecB can directly pass precursors to SecA at the membrane translocase because it binds to SecA specifically. In

solution, the interaction is characterized by a K_d of approximately micromolar [55], whereas if the SecA is associated with the translocase at the membrane, the affinity is higher (K_d ~ 30 nM). The binding is even tighter if SecB is carrying a precursor (K_d ~ 10 nM [56]), presumably in part because the leader itself interacts with SecA. If as proposed the C-terminal helices of SecB did fold back over the bound ligand, the cap formed would not only stabilize the complex but would also allow SecB to present the same surface to SecA no matter what ligand was bound. This surface cap might provide one of the two separate sites on SecB that have been shown to be involved in binding SecA [41], since in vivo studies have implicated the extreme C-terminus in interactions with SecA [31]. The other site of interaction has been shown both genetically and biochemically to be between the extreme C-termini of the homodimeric SecA and negatively charged patches on the eight-stranded β sheets created by the dimerization of SecB monomers [51, 56, 57]. The two identical sites on opposite sides of the tetramer are well positioned to interact with dimeric SecA and facilitate transfer of the ligand from SecB.

We have discussed the role of SecB as a chaperone in maintaining polypeptides in an unfolded state and the role in delivery of precursors directly to the translocase at the membrane via its affinity for SecA. Recent evidence indicates SecB is also capable of activating SecA so that export occurs more efficiently [41, 58].

Summary

There has been much discussion over the precise role of SecB. It is time to step back and reset the question: as stated, it is not answerable. The body of knowledge that has accumulated over the years since the discovery of SecB indicates that SecB has multiple roles. Better questions than what is the primary role of SecB, would be, how many roles does SecB play, and are all its functions related to export? and Are all of its functions related to export? As a chaperone it binds promiscuously to nonnative proteins. As a factor in export it specifically recognizes SecA. Depending on the ligand and the specific circumstances, the binding can be kinetically or thermodynamically determined. Perhaps we should avoid forcing SecB into a specific category and appreciate that it is one among many chaperones that interact in the complex cellular mileu, which is intricately balanced to optimize function.

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1 Berks B. C., Sargent F. and Palmer T. (2000) The Tat protein export pathway. Mol. Microbiol. **35:** 260–274
2 Mori, H., and Ito, K. (2001) The Sec protein-translocation pathway. Trends Microbiol. **9:** 494–500
3 Randall L. L. and Hardy S. J. (1986) 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. **46:** 921–928
4 Kusukawa N., Yura T., Ueguchi C., Akiyama Y. and Ito K. (1989) Effects of mutations in heat-shock genes groES and groEL on protein export in *Escherichia coli.* EMBO J. **8:** 3517– 3521
5 Wild J., Rossmeissl P., Walter W. A. and Gross C. A. (1996) Involvement of the DnaK-DnaJ-GrpE chaperone team in protein secretion in *Escherichia coli*. J. Bacteriol. **178:** 3608–3613
6 Altman E., Kumamoto C. A. and Emr S. D. (1991) Heat-shock proteins can substitute for SecB function during protein export in *Escherichia coli*. EMBO J. **10:** 239–245
7 Wild J., Altman E., Yura, T. and Gross C. A. (1992) DnaK and DnaJ heat shock proteins participate in protein export in *Escherichia coli*. Genes Dev. **6:** 1165–1172
8 Phillips G. J. and Silhavy T. J. (1992) The *E. coli* ffh gene is necessary for viability and efficient protein export. Nature **359:** 744–746
9 Herskovits A. A., Bochkareva E. S. and Bibi E. (2000) New prospects in studying the bacterial signal recognition particle pathway. Mol. Microbiol. **38:** 927–939
10 Kim J., Lee Y., Kim C. and Park C. (1992) Involvement of SecB, a chaperone, in the export of ribose-binding protein. J. Bacteriol. **174:** 5219–5227
11 Jagger M. and Richards K. (1969) You can't always get what you want. Gideon Music, London
12 Fekkes P., de Wit J. G., van der Wolk J. P., Kimsey H. H., Kumamoto C. A. and Driessen A. J. (1998) Preprotein transfer to the *Escherichia coli* translocase requires the co-operative binding of SecB and the signal sequence to SecA. Mol. Microbiol. **29:** 1179–1190
13 Randall L. L., Topping T. B., Hardy S. J., Pavlov M. Y., Freistroffer D. V. and Ehrenberg M. (1997) Binding of SecB to ribosome-bound polypeptides has the same characteristics as binding to full-length, denatured proteins. Proc. Natl. Acad. Sci. USA **94:** 802–807
14 Topping T. B. and Randall L. L. (1997) Chaperone SecB from *Escherichia coli* mediates kinetic partitioning via a dynamic equilibrium with its ligands. J. Biol. Chem. **272:** 19314–19318
15 Hardy S. J. and Randall L. L. (1991) A kinetic partitioning model of selective binding of nonnative proteins by the bacterial chaperone SecB. Science **251:** 439–443
16 Fekkes P., den Blaauwen, T. and Driessen A. J. (1995) Diffusion-limited interaction between unfolded polypeptides and the *Escherichia coli* chaperone SecB. Biochemistry **34:** 10078– 10085
17 Stenberg G. and Fersht A. R. (1997) Folding of barnase in the presence of the molecular chaperone SecB. J. Mol. Biol. **274:** 268–275
18 Müller J. P. (1999) Effects of pre-protein overexpression on SecB synthesis in *Escherichia coli*. FEMS Microbiol. Lett. **176:** 219–227
19 Müller J. P. (1996) Influence of impaired chaperone or secretion function on SecB production in *Escherichia coli*. J. Bacteriol. **178:** 6097–6104
20 Panse V. G., Vogel P., Trommer W. E. and Varadarajan R. (2000) A thermodynamic coupling mechanism for the disaggregation of a model peptide substrate by chaperone SecB. J. Biol. Chem. **275:** 18698–18703
21 Zahn R., Perrett S. and Fersht A. R. (1996) Conformational states bound by the molecular chaperones GroEL and SecB: a hidden unfolding (annealing) activity. J. Mol. Biol. **261:** 43–61
22 Ellis R. J. (2001) Macromolecular crowding: obvious but underappreciated. Trends. Biochem. Sci. **26:** 597–604

23 Minton A. P. (2001) The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. J. Biol. Chem. **276:** 10577–10580
24 Lill R., Crooke E., Guthrie B. and Wickner W. (1988) The 'trigger factor cycle' includes ribosomes, presecretory proteins and the plasma membrane. Cell **54:** 1013–1018
25 Kumamoto C. A. and Francetic O. (1993) Highly selective binding of nascent polypeptides by an *Escherichia coli* chaperone protein in vivo. J. Bacteriol. **175:** 2184–2188
26 Lecker S., Lill R., Ziegelhoffer T., Georgopoulos C., Bassford P. J. Jr, Kumamoto C. A. et al. (1989) Three pure chaperone proteins of *Escherichia coli* – SecB, trigger factor and GroEL – form soluble complexes with precursor proteins in vitro. EMBO J. **8:** 2703–2709
27 Smith V. F., Schwartz B. L., Randall L. L. and Smith R. D. (1996) Electrospray mass spectrometric investigation of the chaperone SecB. Protein Sci. **5:** 488–494
28 Xu Z., Knafels J. D. and Yoshino K. (2000) Crystal structure of the bacterial protein export chaperone SecB. Nat. Struct. Biol. **7:** 1172–1177
29 Múren E. M., Suciu D., Topping T. B., Kumamoto C. A. and Randall L. L. (1999) Mutational alterations in the homotetrameric chaperone SecB that implicate the structure as dimer of dimers. J. Biol. Chem. **274:** 19397–19402
30 Topping T. B., Woodbury R. L., Diamond D. L., Hardy S. J., and Randall L. L. (2001) Direct demonstration that homotetrameric chaperone SecB undergoes a dynamic dimer-tetramer equilibrium. J. Biol. Chem. **276:** 7437–7441
31 Volkert T. L., Baleja J. D. and Kumamoto C. A. (1999) A highly mobile C-terminal tail of the *Escherichia coli* protein export chaperone SecB. Biochem. Biophys. Res. Commun. **264:** 949–954
32 Panse V. G., Swaminathan C. P., Aloor J. J., Surolia A. and Varadarajan R. (2000) Unfolding thermodynamics of the tetrameric chaperone, SecB. Biochemistry **39:** 2362–2369
33 Randall L. L., Topping T. B. and Hardy S. J. (1990) No specific recognition of leader peptide by SecB, a chaperone involved in protein export. Science **248:** 860–863
34 Thom J. R. and Randall L. L. (1988) Role of the leader peptide of maltose-binding protein in two steps of the export process. J. Bacteriol. **170:** 5654–5661
35 Lill R., Dowhan W. and Wickner W. (1990) The ATPase activity of SecA is regulated by acidic phospholipids, SecY, and the leader and mature domains of precursor proteins. Cell **60:** 271–280
36 Liu G., Topping T. B. and Randall L. L. (1989) Physiological role during export for the retardation of folding by the leader peptide of maltose-binding protein. Proc. Natl. Acad. Sci. USA **86:** 9213–9217
37 Randall L. L. and Hardy S. J. (1995) High selectivity with low specificity: how SecB has solved the paradox of chaperone binding. Trends Biochem. Sci. **20:** 65–69
38 Fox J. D., Kapust R. B. and Waugh D. S. (2001) Single amino acid substitutions on the surface of *Escherichia coli* maltose-binding protein can have a profound impact on the solubility of fusion proteins. Protein Sci. **10:** 622–630
39 Randall L. L., Topping T. B., Suciu D. and Hardy S. J. (1998) Calorimetric analyses of the interaction between SecB and its ligands. Protein Sci. **7:** 1195–1200
40 Watanabe M. and Blobel G. (1989) Cytosolic factor purified from *Escherichia coli* is necessary and sufficient for the export of a preprotein and is a homotetramer of SecB. Proc. Natl. Acad. Sci. USA **86:** 2728–2732
41 Woodbury R. L., Topping T. B., Diamond D. L., Suciu D., Kumamoto C. A., Hardy S. J. et al. (2000) Complexes between protein export chaperone SecB and SecA. Evidence for separate sites on SecA providing binding energy and regulatory interactions. J. Biol. Chem. **275:** 24191–24198
42 Khisty V. J. and Randall L. L. (1995) Demonstration in vivo that interaction of maltose-binding protein with SecB is determined by a kinetic partitioning. J. Bacteriol. **177:** 3277–3282
43 Randall L. L. (1992) Peptide binding by chaperone SecB: implications for recognition of nonnative structure. Science **257:** 241–245
44 Knoblauch N. T., Rudiger S., Schonfeld H. J., Driessen A. J., Schneider-Mergener J. and Bukau B. (1999) Substrate specificity of the SecB chaperone. J. Biol. Chem. **274:** 34219– 34225
45 Panse V. G., Swaminathan C. P., Surolia A. and Varadarajan R. (2000) Thermodynamics of substrate binding to the chaperone SecB. Biochemistry **39:** 2420–2427
46 Smith V. F., Hardy S. J. and Randall L. L. (1997) Determination of the binding frame of the chaperone SecB within the physiological ligand oligopeptide-binding protein. Protein Sci. **6:** 1746–1755
47 Bruce J. E., Smith V. F., Liu C., Randall L. L. and Smith R. D. (1998) The observation of chaperone-ligand noncovalent complexes with electrospray ionization mass spectrometry. Protein Sci. **7:** 1180–1185
48 Randall L. L., Hardy S. J., Topping T. B., Smith V. F., Bruce J. E. and Smith R. D. (1998) The interaction between the chaperone SecB and its ligands: evidence for multiple subsites for binding. Protein Sci. **7:** 2384–2390
49 Topping T. B. and Randall L. L. (1994) Determination of the binding frame within a physiological ligand for the chaperone SecB. Protein Sci. **3:** 730–736
50 Khisty V. J., Munske G. R. and Randall L. L. (1995) Mapping of the binding frame for the chaperone SecB within a natural ligand, galactose-binding protein. J. Biol. Chem. **270:** 25920–25927
51 Kimsey H. H., Dagarag M. D. and Kumamoto C. A. (1995) Diverse effects of mutation on the activity of the Escherichia coli export chaperone SecB. J. Biol. Chem. **270:** 22831–22835
52 Diamond D. L. and Randall L. L. (1997) Kinetic partitioning. Poising SecB to favor association with a rapidly folding ligand. J. Biol. Chem. **272:** 28994–28998
53 Randall L. L. and Hardy S. J. (2000) The promiscuous and specific sides of SecB. Nat. Struct. Biol. **7:** 1077–1079
54 Zhu X., Zhao X., Burkholder W. F., Gragerov A., Ogata C. M., Gottesman M. E. et al. (1996) Structural analysis of substrate binding by the molecular chaperone DnaK. Science **272:** 1606–1614
55 den Blaauwen T., Terpetschnig E., Lakowicz J. R. and Driessen A. J. (1997) Interaction of SecB with soluble SecA. FEBS Lett. **416:** 35–38
56 Fekkes P., van der Does C. and Driessen A. J. (1997) The molecular chaperone SecB is released from the carboxy-terminus of SecA during initiation of precursor protein translocation. EMBO J. **16:** 6105–6113
57 Breukink E., Nouwen N., van Raalte A., Mizushima S., Tommassen J. and de Kruijff B. (1995) The C terminus of SecA is involved in both lipid binding and SecB binding. J. Biol. Chem. **270:** 7902–7907
58 Kim J., Miller A., Wang L., Muller J. P. and Kendall D. A. (2001) Evidence that SecB enhances the activity of SecA. Biochemistry **40:** 3674–3680