MINI-REVIEW

SecB Protein: A Cytosolic Export Factor That Associates with Nascent Exported Proteins

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Abstract

Soluble factors participate in protein translocation across a variety of biological membranes. The *Escherichia coli* soluble protein SecB (the product of the *secB* gene) is involved in the export of periplasmic and outer membrane proteins. The isolation of *secB* mutations permitted the demonstration that SecB is required for rapid and efficient export of certain proteins. Consistent with the results of these genetic studies, purified SecB has been shown to stimulate protein translocation across *E. coli* inner membrane vesicles *in vitro*. This article presents a review of these past studies of SecB, speculation on the role of SecB in protein translocation, and a comparison of SecB and other factors, trigger factor and GroEL.

Key Words: Protein translocation; *secB* mutants; chaperone proteins; translocation competence; trigger factor; GroEL.

Introduction

The study of protein translocation across different biological membranes has revealed that the mechanisms that ensure proper protein targeting and that permit a protein to traverse a membrane are highly related. For example, the sorting sequences, termed signal or leader sequences, that are found on the amino termini of bacterial exported proteins and mammalian secretory proteins are remarkably similar in properties (von Heijne, 1985), although they lack primary sequence conservation (Kaiser *et al.*, 1987). The similarities in mechanism permit a human secretory protein, expressed in the bacterium

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E. coli, to be recognized as a secretory protein and successfully exported across the bacterial inner membrane (Talmadge *et al.*, 1980).

Soluble factors participate in the translocation of proteins across bacterial inner membranes, mammalian and yeast endoplasmic reticulum, and yeast mitochondrial membranes. A mammalian protein/RNA complex, termed Signal Recognition Particle (SRP), is required for the translocation of nascent proteins across microsomal membranes (Walter *et al.*, 1984). In yeast, the hsp70 heat-shock proteins are involved in movement of proteins across endoplasmic reticulum membranes and mitochondrial membranes (Deshaies *et al.*, 1988; Chirico *et al.*, 1988). Soluble factors also participate in protein export across the *E. coli* inner membrane. Two soluble proteins, SecA (Oliver and Beckwith, 1981) and SecB (Kumamoto and Beckwith, 1983), have been identified as export factors by genetic analysis. Reconstituted systems containing vesicles derived from the *E. coli* inner membrane have been used to describe other cytosolic factors that stimulate protein translocation. These factors include 12S export complex (Müller and Blobel, 1984), which was recently shown to contain SecB (Watanabe and Blobel, 1989a), trigger factor (Crooke and Wickner, 1987), CTF (cytoplasmic translocation factor; Weng *et al.*, 1988), and GroEL (Bochkareva *et al.*, 1988).

Soluble factors probably play a role in targeting precursor proteins to the translocation apparatus that resides within the membrane. Mammalian SRP has been shown to bind to an endoplasmic reticulum receptor termed SRP receptor (Walter *et al.*, 1984) or docking protein (Meyer *et al.*, 1982). The interactions of SRP and its receptor bring the nascent precursor molecule into the proximity of the translocation site. Receptors for other soluble factors have not yet been identified.

Soluble factors also influence the conformation of the precursor molecule. Randall and Hardy (1986) and Eilers and Schatz (1986) showed that the tightly folded conformation of a mature protein is incompatible with its translocation across a membrane. Therefore, to be competent for translocation, the precursor protein must possess an alternative, non-native, possibly completely unfolded conformation. By binding to a nascent precursor, a soluble factor could prevent the molecule from folding into its most stable mature conformation. The *E. coli* proteins SecB, trigger factor, and GroEL are thought to bind to precursor molecules and influence their folding; these proteins have been termed chaperone proteins (Lecker *et al.*, 1989).

This article will focus primarily on studies of SecB. The involvement of SecB in protein export was first observed by genetic analysis (Kumamoto and Beckwith, 1983). Independently, SecB was purified from extracts of wild type cells on the basis of its activity in stimulating protein translocation *in vitro* (Watanabe and Blobel, 1989a), strengthening the conclusion that SecB is an

export factor. Recent results demonstrate that SecB associates with nascent
forms of exported proteins (Kumamoto, 1989; Lecker *et al.*, 1989; Watanabe
and Blobel, 1989b). This association probably plays a role in targeting the
nascent precursors to the membrane and in preventing the folding of the
exported protein into its mature conformation.

Genetic Analysis of *secB*

The isolation of the first mutants in *secB* (and also in *secA*) was made
possible by a simple yet extraordinarily powerful mutant selection procedure
designed by Beckwith (Oliver *et al.*, 1982). This selection relied on the
unusual properties of a hybrid protein that contained most of the sequence
of maltose binding protein (MBP; a periplasmic protein) fused to enzymati-
cally active β-galactosidase (normally a cytoplasmic protein). The hybrid
protein appeared to initiate the export process and became associated, in a
poorly defined way, with the inner membrane. In this unusual location, the
specific activity of the β-galactosidase portion of the hybrid protein was
extremely low and cells carrying the hybrid gene exhibited a Lac⁻ phenotype
on lactose indicator media. If the signal sequence of the MBP portion of the
hybrid protein was inactivated by mutation, the hybrid protein did not
appear to initiate export and remained cytoplasmic. In this location, the
specific activity of the β-galactosidase portion was much greater, permitting
the cells to exhibit a Lac⁺ phenotype. It would be expected that a mutation
of the cellular apparatus could similarly cause some of the hybrid protein to
accumulate in the cytoplasm, where it would be more active and cause a Lac⁺
phenotype. Significantly, it was not necessary for the cellular apparatus to be
completely defective in order for the mutation to cause a sufficient accumu-
lation of cytoplasmic hybrid protein. Thus, it was possible to isolate mutants
with subtle phenotypes.

By characterizing a large collection of Lac⁺ mutants, derived from a
strain carrying the hybrid protein, we demonstrated that all of the mutants
obtained had defects in protein export (Kumamoto and Beckwith, 1983).
Many of the mutations were linked to the gene encoding the hybrid protein;
DNA sequencing of some of them showed that they were signal sequence
mutations (Kumamoto, Froshauer, and Beckwith, unpublished). Some of the
mutations affected the *secA* gene and the remainder affected the *secB* gene.
These *secB* mutations were the first to be found in the *secB* gene. Consistent
with their isolation by the Lac⁺ selection, the *secB* mutants exhibited weak
defects in protein export (Kumamoto and Beckwith, 1983).

In order to determine whether *secB* played an important role in export,
stronger *secB* mutations were sought. The strategy for isolation of *secB* null

mutations, in which the *secB* gene was completely inactivated, was based on the idea that *secB* might be an essential gene. Therefore, the mutant hunt was conducted using a merodiploid strain. After isolating an F′ that carried *secB* and recombining one of the Lac⁺ mutations, *secB2*, onto the F′, the F′*secB2* was transferred into a *secB⁺* strain that carried the gene encoding the MBP-β-galactosidase hybrid protein (Kumamoto and Beckwith, 1985). The *secB2* mutation was recessive (Kumamoto and Beckwith, 1983) and the phenotype of the merodiploid was Lac⁻ (i.e., wild type). By selecting for Lac⁺ derivatives, mutations that completely inactivated *secB* were obtained (Kumamoto and Beckwith, 1985). For example, one of these mutations, *secB8*, was found to be a nonsense mutation (Kumamoto and Nault, 1989).

The *secB* null mutants were found to be viable in a haploid strain. Haploid *secB⁻* strains grew well on minimal medium, but were unable to grow on rich medium. The basis for the selective growth defect is unknown, but it has been consistently observed for many different *secB* null mutants (Reuveny and Kumamoto, unpublished). To rule out the possibility that a truncated, partially functional SecB was produced by these mutants, an insertion mutation was constructed (Kumamoto and Beckwith, 1985). Insertion of the transposon, Tn*5*, into the 4th codon of SecB (Kumamoto and Nault, 1989), did not cause a lethal mutation, and the *secB*::Tn*5* strain exhibited the same growth defect on rich media. As expected, the *secB*::Tn*5* mutant contains no detectable SecB protein (Kumamoto *et al.*, 1989).

All of the *secB* null mutants exhibited a much stronger defect in export of pre-MBP and pre-OmpF (an outer membrane protein) than the original Lac⁺ mutants (Kumamoto and Beckwith, 1985). The results were consistent with the hypothesis that, *in vivo*, SecB was important for export of at least some proteins.

As a prelude to the characterization of the *secB* gene product, it was necessary to complete the characterization of the *secB* gene and to demonstrate that *secB* encoded a protein product. A specialized transducing phage that carried the *secB⁺* gene was isolated (Kumamoto and Beckwith, 1985) by taking advantage of a small lambda phage that carried a portion of Tn*5* (Isberg *et al.*, 1982). Using a Tn*5* insertion that was near *secB* as a "portable region of homology" (Kleckner *et al.*, 1977), the small lambda phage was integrated into the chromosome near *secB*. A *recA* mutation was introduced to incapacitate the homologous recombination system, and phage were induced from the lysogen. Illegitimate recombination resulted in transducing phage that carried portions of the bacterial chromosome, including *secB* and the neighboring gene *gpsA* (Clark *et al.*, 1980) encoding glycerol-3-phosphate dehydrogenase (Kumamoto and Beckwith, 1985). A 4.5-kb² DNA fragment

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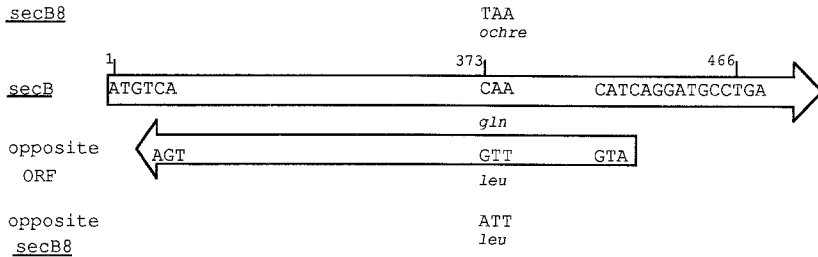²Abbreviations: kb, kilobase; bp, base pair; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

secB8

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                                        TAA
                                        ochre
              1                    373            466
secB    ATGTCA                      CAA    CATCAGGATGCCTGA

                                    gln
opposite      AGT                   GTT    GTA
  ORF                               leu

opposite                            ATT
  secB8                             leu
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Fig. 1. Diagram of the *secB* open reading frame and the opposite open reading frame. The *secB* gene is illustrated schematically. Arrows indicate open reading frames. Nucleotide sequences at the beginnings and ends of each open reading frame are shown. Numbers indicate nucleotide positions in the sequence. The nucleotide and amino acid changes caused by the *secB8* mutation are shown above and below the wild type sequences.

encoding *secB*, *gpsA*, and other unknown genes was subcloned onto a plasmid, generating pCK1, and smaller fragments of the pCK1 insert were subcloned. The smallest piece of DNA that was capable of complementing the *secB2* mutation, when provided with a promoter, was a 630-bp fragment (Kumamoto and Nault, 1989).

DNA sequence analysis of the 630-bp fragment revealed that the DNA encoded two open reading frames which were virtually superimposable (Kumamoto and Nault, 1989). The *secB* open reading frame is longer than the opposite open reading frame by 3 bp at one end and 6 bp at the other end (Fig. 1).

To determine definitely which open reading frame corresponded to *secB*, *secB* null mutations were characterized. The mutations were recombined from the chromosome onto a plasmid by Hfr mobilization. A plasmid that carried DNA from the *secB* region of the chromosome, but which had sustained a deletion of approximately 1 kb removing the *secB* gene and inactivating the *gpsA* gene (Gannon and Kumamoto, unpublished), was transformed into an Hfr strain that carried a *secB* mutation. Plasmid recombinants were selected in which the deletion had been replaced by the chromosomal region, yielding a plasmid that was phenotypically GpsA$^+$ SecB$^-$. The *secB* lesion was mapped by restriction fragment exchange, and the relevant restriction fragment was sequenced. We found that the null mutation *secB8* was caused by a nucleotide substitution that changed a Gln codon to an ochre codon (Fig. 1; Kumamoto and Nault, 1989). On the opposite strand, the base change did not change the coding of the open reading frame because it affected a third base position (Fig. 1). Therefore, the *secB* open reading frame was identified as the one that had sustained the ochre mutation.

This analysis permitted the demonstration that *secB* encodes a 16-kDa protein which is highly negatively charged and probably soluble. The protein

was not strikingly similar to other proteins in the NBRF database. The phenotype of the *secB* null mutants indicated that the 16-kDa SecB protein was involved in the protein export process.

Characterization of SecB, the Protein Product of *secB*

An independent way to demonstrate the involvement of SecB in protein export would be to demonstrate that purified SecB protein stimulates protein translocation *in vitro*. To this end, SecB was purified from extracts of SecB overproducing strains by Weiss *et al.* (1988), and by Kumamoto *et al.* (1989). As mentioned above, SecB was also purified from extracts of wild-type cells on the basis of activity in an *in vitro* translocation assay (Watanabe and Blobel, 1989a).

Purified SecB exhibited both co-translational and post-translational translocation activity. In our studies, SecB was incubated with a partially purified preparation of pro-OmpA (the precursor of an outer membrane protein), inner membrane vesicles, ATP, and other cofactors. SecB stimulated the post-translational translocation of some of the pro-OmpA into the vesicles where it was protected from protease treatment (Kumamoto *et al.*, 1989). (This reconstituted system is reviewed in detail elsewhere in this volume.) In this experiment, purified SecB was the only soluble protein added and the purified SecB was not as active as a crude cytosolic extract of *E. coli*, suggesting that additional soluble factors might be needed for optimal translocation activity. The inner membrane vesicles probably contained a small amount of the other soluble factors, such as SecA or CTF, already bound to translocation sites. If these amounts were suboptimal, the activity of purified SecB might appear to be lower than the activity of a crude cytosolic extract.

In their studies of pre-MBP$_\Delta$116 (an MBP derivative containing an internal deletion), Weiss *et al.* (1988) showed that extracts prepared from strain CK1953, the *secB*::Tn5 mutant (Kumamoto and Beckwith, 1985), were inactive in supporting co-translational translocation of preMBP$_\Delta$116. When purified SecB was added to the CK1953 extract, a small amount of translocation activity was recovered (Weiss *et al.*, 1988), suggesting that SecB participated in co-translational translocation of pre-MBP$_\Delta$116.

SecB that was purified from wild-type cells was active in stimulating post-translational translocation of pre-MBP* (a prematurely terminated derivative of pre-MBP) and pre-LamB (the precursor of an outer membrane protein) (Watanabe and Blobel, 1989a). For these studies, pre-MBP* was translated *in vitro* using a wheat germ cell-free system. Post-translational translocation of pre-MBP* was strictly dependent upon a soluble extract

of *E. coli* cells. The active component was purified to a single 16-kDa polypeptide, and amino terminal sequencing revealed that this protein was SecB. In the pre-MBP* post-translational translocation assay, purified SecB was as active as the crude cytosol. However, for post-translational translocation of pre-LamB, SecB was less active than the crude cytoplasmic extract. These latter results suggested that an additional soluble factor(s) might be needed for maximal activity, as was observed in the studies of pro-OmpA translocation.

Consistent with the activity exhibited by purified SecB, depletion of SecB from cell extracts using anti-SecB antibody inactivated the extract (Watanabe and Blobel, 1989b). Addition of purified SecB restored the ability to stimulate translocation of pre-MBP*.

Despite a monomer molecular weight of 16 kDa, native wild-type SecB exhibited a much larger apparent molecular weight on gel filtration columns (Watanabe and Blobel, 1989a; Kumamoto *et al.*, 1989). These results suggested that SecB might be either oligomeric or unusually elongated. During purification of SecB from wild-type cell extracts, the apparent molecular weight decreased from approximately 150 to 64 kDa based on gel filtration (Watanabe and Blobel, 1989a). However, no evidence for a SecB-associated polypeptide was found, so it was suggested that SecB existed in alternative oligomeric forms. These results suggested that SecB was not found in a complex with other polypeptides, similar to SRP. In addition, SecB was almost exclusively cytosolic (Kumamoto *et al.*, 1989; Watanabe and Blobel, 1989b).

In summary, SecB could be purified from extracts on the basis of its activity (Watanabe and Blobel, 1989a), and SecB stimulated translocation of several different protein precursors *in vitro* (Weiss *et al.*, 1988; Watanabe and Blobel, 1989a; Kumamoto *et al.*, 1989). These results strongly support the conclusions based on studies of *secB* mutants and demonstrate that SecB participates directly in protein export.

Functions of SecB

To determine the role of SecB in protein export, the phenotype of the *secB*::Tn5 mutant strain was characterized (Kumamoto and Beckwith, 1985). The mutation caused precursor forms of certain proteins such as MBP, OmpA, and LamB to accumulate. However, other proteins such as ribose binding protein were found to be unaffected by the *secB* mutation.

In the *secB*::Tn5 mutant, co-translational processing of pre-MBP nascent chains was not observed (Kumamoto and Gannon, 1988), unlike in wild type cells (Josefsson and Randall, 1981). However, fully elongated

pre-MBP molecules were capable of translocating across the cell membrane, and processed, mature MBP was formed at a very slow rate (Kumamoto and Gannon, 1988). The slow, post-translational processing of pre-MBP indicated that these molecules were competent for export, but were delayed in moving to or across the membrane by the absence of SecB.

Randall and Hardy (1986) demonstrated the importance of precursor conformation in determining whether a precursor molecule would be capable of translocating across the *E. coli* inner membrane. Folding of an intracellular pre-MBP molecule into a tightly folded, protease-resistant conformation was correlated with the loss of its ability to be translocated. In contrast, pre-MBP molecules that were capable of being translocated were sensitive to protease treatment. These molecules appeared to possess an alternative, less tightly folded conformation, possibly a completely unfolded conformation that was devoid of tertiary structure.

The *secB*::Tn5 mutation caused an increase in the amount of tightly folded intracellular pre-MBP *in vivo* (Kumamoto and Gannon, 1988) and *in vitro* (Collier *et al.*, 1988), indicating that SecB influences the conformation of pre-MBP. In both of these studies, pre-MBP conformation was monitored by protease sensitivity. *In vivo*, approximately 25% of the pre-MBP molecules attained the protease-resistant conformation immediately after synthesis (Kumamoto and Gannon, 1988). The remaining 75% of the molecules were protease sensitive and, as mentioned above, underwent post-translational translocation and processing (Kumamoto and Gannon, 1988). When *in vitro* translocation of pre-MBP was conducted with extracts prepared from CK1953 (*secB*::Tn5), tightly folded (protease resistant) pre-MBP molecules appeared rapidly (Collier *et al.*, 1988) and the addition of purified SecB protein to the CK1953 extract prevented the effect (Weiss *et al.*, 1988).

Recently, purified SecB has been shown to block the folding of denatured pre-MBP in a reaction involving only the two purified proteins (Liu *et al.*, 1989). In these studies, pre-MBP folding was monitored directly, using increased fluorescence as an indicator of the folded state (Park *et al.*, 1988). Addition of purified SecB completely blocked the refolding of denatured pre-MBP. These results directly demonstrate the postulated effect of SecB on folding of pre-MBP.

The effects of the *secB*::Tn5 mutation on pre-MBP conformation could be explained if, under normal conditions, SecB bound to intracellular pre-MBP and prevented folding. If SecB binding occurred while the pre-MBP molecules were nascent, the association could assist in targeting the nascent molecules to the translocation machinery in the membrane and could stimulate co-translational translocation and processing. Consistent with these hypotheses, association of SecB with precursors of exported proteins has

been demonstrated *in vivo* (Kumamoto, 1989) and *in vitro* (Lecker *et al.*, 1989; Watanabe and Blobel, 1989b).

When labeled cell extracts were fractionated with an anti-SecB column (anti-SecB antibody bound to protein A-Sepharose beads), SecB and a variety of additional labeled proteins were observed in the fraction that bound to the column (Kumamoto, 1989). Immunoprecipitation of the bound fraction demonstrated that pre-MBP, pre-LamB, and pre-OmpA species had bound, but β-galactosidase (a cytoplasmic protein) species had not bound. Partial protease digests of the bound pre-MBP material revealed that nascent forms of pre-MBP were present in the anti-SecB bound fraction. These results were consistent with the interpretation that both nascent and fully elongated forms of exported protein precursors associate with SecB intracellularly. If the cells were pulse labeled and then chased, the additional proteins were quickly lost from the anti-SecB bound fraction. However, if CCCP was included during the chase period, to block protein export, turnover of the SecB complexes was significantly slower (Kumamoto, unpublished). These results are consistent with the interpretation that the proteins associated with SecB were predominantly, if not exclusively, exported protein precursors. While some of the intracellular pre-MBP could be sedimented by centrifugation of the extract at $438,000\,g$, most of the SecB-associated pre-MBP remained soluble after this treatment, indicating that most of the SecB complexes were soluble. Thus, SecB association with nascent pre-MBP probably precedes membrane association.

When purified SecB was incubated with either denatured pro-OmpA or denatured pre-phoE (another outer membrane protein), complexes formed *in vitro* (Lecker *et al.*, 1989). Purified SecB also formed a complex with pre-MBP*, the prematurely terminated form of pre-MBP (Watanabe and Blobel, 1989b). These results demonstrate that SecB is a precursor binding protein.

Under certain conditions, SecB has been shown to form complexes with mature proteins that lack their signal sequences. Upon dilution from urea, denatured OmpA, the mature protein, formed a complex with SecB (Lecker *et al.*, 1989). In contrast, the mature form of pre-MBP* did not bind SecB (Watanabe and Blobel, 1989b). Denaturation of MBP* prior to SecB addition was not reported; however, it was noted that MBP* was not protease resistant.

These apparently contradictory results may be explained by the results of Randall and coworkers (Liu *et al.*, 1989) who showed that the rate of MBP folding affected the ability of SecB to bind and block folding. If denatured, mature MBP was incubated with SecB at room temperature, SecB did not prevent its folding, indicating that a stable complex had not formed. However, if the mature MBP contained a mutation that slowed its folding

(Liu *et al.*, 1988), or if the incubation was conducted at lower temperature, so that MBP folding was slower, SecB readily bound to MBP and blocked folding. Thus, conditions that slowed the folding of MBP stabilized its interactions with SecB. Because OmpA is a membrane protein, it might not fold rapidly into its most stable conformation in an aqueous environment and it might remain capable of binding SecB. These data show that the presence of a signal sequence is not obligatory for SecB binding. However, the signal sequence probably participates, perhaps by modulating the rate of folding (Randall and Hardy, 1989; Laminet and Pluckthun, 1989). It seems likely that under normal conditions, both the signal sequence and the mature sequence contribute to the binding of SecB.

   Recent studies have demonstrated that precursor forms of the SecB-independent proteins, ribose-binding protein and alkaline phosphatase, can be found in association with SecB in labeled cell extracts (Kumamoto, unpublished). Thus, SecB-independent proteins are capable of associating with SecB. To determine what features distinguish SecB-independent and SecB-dependent proteins, we analyzed the behaviour of hybrid proteins that contained portions of the SecB independent protein alkaline phosphatase and the SecB-dependent protein MBP (Gannon *et al.*, 1989). Exchanging the signal sequence had no effect on SecB-dependence, but inclusion of regions of MBP mature sequence caused alkaline phosphatase to exhibit SecB-dependence. For example, a hybrid protein containing the signal sequence of pre-MBP, 230 amino acids of mature MBP, followed by the entire sequence of mature alkaline phosphatase, was dependent upon SecB for rapid and efficient export. A hybrid protein containing the pre-MBP signal sequence and 34 amino acids of mature was minimally SecB-dependent, while a hybrid protein containing the pre-MBP signal sequence and 74 amino acids of mature MBP sequence exhibited an intermediate phenotype. The results indicated that the properties of mature sequence govern SecB-dependence and the inclusion of more mature MBP sequence gradually gave rise to a SecB-dependent molecule.

   To explain these results and the fact that SecB-independent proteins may utilize SecB during export in wild-type cells, we propose the model illus-trated in Fig. 2, incorporating results and hypotheses presented previously. In order for a protein to translocate across the membrane rapidly (i.e., co-translationally or immediately after translation), we suggest that the conformation of the nascent protein must be one that causes the signal sequence to be presented to the membrane in an appropriate conformation. Alternative conformations may exist in which the signal sequence is less accessible to the membrane. For example, the signal sequence may interact with the mature sequence in order to retard folding. The equilibrium between the two forms (step 2 in Fig. 2) depends upon the properties of the mature
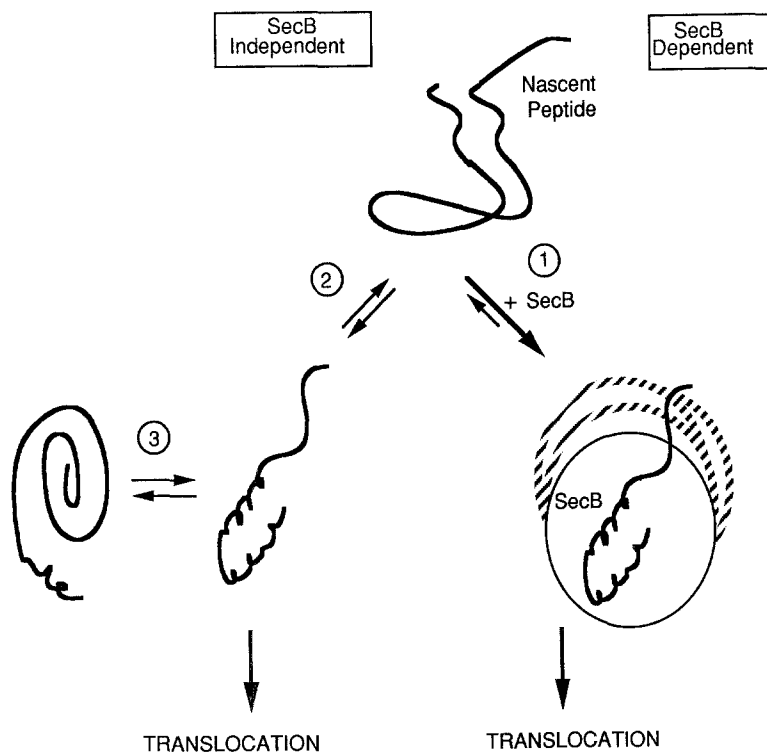
Fig. 2. Proposed effects of SecB binding on the conformations of a nascent exported protein. Proposed conformations of the nascent peptide are diagrammed. From left to right: Export-incompetent conformation in which the mature sequence has formed a tightly folded structure. Translocationally active conformation in which the signal sequence is highly accessible for interaction with the membrane-bound translocation apparatus. Alternative conformation in which the signal sequence is unaccessible and is interacting with the mature sequence. Translocationally active conformation in a complex with SecB; transitions to other conformations are prevented by association with SecB.

sequence. However, binding of SecB shifts the equilibrium to favor the translocationally active conformation (step 1 in Fig. 2). Therefore, SecB binding favors signal sequence accessibility and favors association of the precursor with the translocation machinery in the membrane, consistent with the previous demonstration that SecB is required for signal sequence recognition by a membrane component, PrlA (Trun *et al.*, 1988). Binding of SecB also prevents the competing reaction, folding into a tightly folded, export-incompetent conformation (step 3 in Fig. 2). In the absence of SecB, the concentration of molecules possessing the translocationally active conformation depends upon the two equilibria, 2 and 3. The SecB-independent proteins may be those that have a high probability of spontaneously forming

the translocationally active conformation and therefore are not dependent upon SecB for export.

A mutation of the mature sequence of MBP has been observed to improve export of pre-MBP in strain CK1953 (*secB*::Tn*5*) (Collier *et al.*, 1988). This mutation slows the folding of MBP (Liu *et al.*, 1988). Although the mutation caused more *efficient* export of mutant pre-MBP in CK1953 (perhaps by affecting step 3 in Fig. 2), the improved export was not significantly faster than the export of wild type pre-MBP in CK1953 (both may be limited by step 2 in Fig. 3). This phenotype is considerably different from the rapid export of truly SecB-independent proteins in CK1953. Thus, we suggest that signal sequence accessibility determines the rate of association of pre-MBP with the translocation site and that SecB acts to ensure accessibility; we consider this activity to represent a targeting function.

SecB and Other Chaperones

SecB is probably not the only chaperone protein that participates in protein export. Two other factors, trigger factor and GroEL, are thought to be involved in translocation of pro-OmpA and β-lactamase (a periplasmic protein).

Trigger factor was originally identified by its ability to stabilize pro-OmpA for translocation *in vitro* (Crooke and Wickner, 1987). Purified pro-OmpA, when rapidly diluted out of urea, is capable of translocating across inner membrane vesicles without the addition of soluble *E. coli* factors (Crooke *et al.*, 1988). This contrasts with the pre-MBP* preparation that was used in the assay for SecB purification (Watanabe and Blobel, 1989a). Incubation of diluted pro-OmpA in nondenaturing buffer, for 2 hr at room temperature, resulted in the loss of its ability to translocate (Crooke *et al.*, 1988). Presumably, folding of pro-OmpA into a translocation-incompetent form had occurred. When urea was removed from denatured pro-OmpA by dialysis, the pro-OmpA was also inactive for translocation. Addition of a soluble extract of *E. coli* (S100) directly to inactive pro-OmpA did not restore translocation of pro-OmpA. However, if the S100 extract was present during the dialysis, pro-OmpA was stabilized in a form that was active for translocation; the active component of this S100 extract was termed trigger factor (Crooke and Wickner, 1987). These results indicated that fully elongated, denatured pro-OmpA could spontaneously form the translocationally active conformation. During incubation under nondenaturing conditions, either during dialysis or upon dilution, the pro-OmpA conformation changed and pro-OmpA became inactive for translocation. A soluble factor(s) was able to stabilize the pro-OmpA translocationally active conformation.

Fractionation of the S100 extract by velocity sedimentation showed that the activity that stabilized pro-OmpA during dialysis sedimented with an apparent molecular weight of approximately 60 kDa (Crooke and Wickner, 1987). This activity, trigger factor, was purified from extracts using the dialysis assay to monitor purification. The purified polypeptide exhibited a molecular weight of 73 kDa (Lill *et al.*, 1988). The close correspondence between the monomer molecular weight and the size of the active, native component determined by velocity sedimentation, suggests that trigger factor is active as a monomer. *E. coli* ribosomes could also be used as a source for preparation of active 73-kDa trigger factor, showing that trigger factor is peripherally associated with ribosomes (Lill *et al.*, 1988).

If urea was removed from pro-OmpA by rapid dilution, purified SecB and purified GroEL also stabilized the translocationally active form of pro-OmpA (Lecker *et al.*, 1989). Unlike trigger factor, SecB and GroEL are thought to be active as oligomers. Thus, these factors might not be stable under the harsh conditions used in the original assays and purification of trigger factor, so that their activities may not have been detected in those studies.

Purified trigger factor and purified pro-OmpA can form complexes (Lecker *et al.*, 1989). Incubation of trigger factor with denatured pro-OmpA, conditions under which trigger factor exhibits its activity, resulted in formation of complexes that could be detected by velocity sedimentation. A second protein, pre-PhoE, failed to bind to trigger factor under identical conditions. While only two proteins have been compared, these results suggest that trigger factor may be a very specific chaperone. Trigger factor-deficient mutants have not yet been reported, so the *in vivo* significance of trigger factor for pro-OmpA export has not been determined.

GroEL is a general stimulator of oligomeric protein assembly and is involved in a variety of assembly reactions (Friedman *et al.*, 1984; Hemmingsen *et al.*, 1988). Synthesis of GroEL is stimulated by heat shock, although GroEL is essential for growth at all temperatures (Fayet *et al.*, 1989). *groE* temperature-sensitive mutations caused defects in the export of β-lactamase but not OmpA or MBP, at the nonpermissive temperature (Kusukawa *et al.*, 1989). The phenotypes of the *groE* mutants resembled that of the *secB*::Tn5 mutant except for the specificity of effects. β-lactamase is SecB-independent and GroE-dependent, whereas MBP and OmpA are SecB-dependent and GroE-independent. *In vitro*, GroEL can be crosslinked to both newly synthesized pre-β-lactamase and newly synthesized chloramphenicol acetyl-transferase, a cytoplasmic protein (Bochkareva *et al.*, 1988). Incubation of purified GroEL with denatured pro-OmpA, pre-PhoE, and OmpA resulted in formation of complexes that dissociated after ATP hydrolysis (Lecker *et al.*, 1989). These results indicate a role for GroEL in translocation of certain protein precursors.

In comparison with trigger factor and GroEL, SecB bound to precursors with higher affinity; pro-OmpA that was complexed with either trigger factor or GroEL readily exchanged into a SecB complex (Lecker *et al.*, 1989). These findings suggest that trigger factor and GroEL might act as backup systems and associate with excess precursor proteins, when SecB is saturated. The precursors could subsequently be exchanged into SecB complexes for delivery to the membrane. In this model, we suggest that SecB may be the chaperone that is specialized for targeting exported protein precursors to the membrane. Under certain conditions, trigger factor, which is associated with ribosomes, might act before SecB and bind to nascent pro-OmpA immediately after its translation has begun. GroEL appears to participate in a wide variety of protein assembly reactions, but may be available to maintain translocation competence if SecB is saturated or if SecB fails to recognize a particular precursor. Future research will provide a more complete picture of the function of the various chaperones by clarifying the specificity of chaperone binding to precursors, and the interrelationships between the chaperones and other export factors, both soluble and membrane bound.

Acknowledgments

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