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Purified SecB protein of *Escherichia coli* retards folding and promotes membrane translocation of the maltose-binding protein *in vitro*

(secretion/soluble translocation factors/posttranslational export/reconstitution/protein folding)

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ABSTRACT **The efficient export of a subset of *Escherichia coli* envelope proteins is dependent upon the product of the *secB* gene. Previous studies indicated that SecB promotes the export of the periplasmic maltose-binding protein (MBP) by preventing premature folding of the precursor MBP in the cytoplasm into an export-incompetent form. In this study, SecB has been purified to homogeneity and shown to be a soluble, cytoplasmic, multimeric protein composed of identical 17-kDa subunits. SecB was required for efficient *in vitro* translocation of MBP into inverted membrane vesicles. The addition of purified SecB to an *in vitro* system prepared from SecB$^-$ cells significantly enhanced MBP translocation. The purified protein also quantitatively retarded folding of precursor MBP into a stable, protease-resistant conformation in the absence of membranes. Finally, the inclusion of excess purified SecB in a SecB$^+$ *in vitro* system significantly prolonged the time in which precursor MBP remained competent for posttranslational import into membrane vesicles.**

A number of components of the protein export machinery of *Escherichia coli* have been identified by genetic approaches [reviewed by Oliver (1)] and by biochemical approaches using *in vitro* protein translocating systems (2–4). The export of a subset of envelope proteins, including the periplasmic maltose-binding protein (MBP), is adversely affected by mutations in *secB*, a nonessential gene that maps near min 81 on the *E. coli* chromosome (5, 6). We have presented (7) evidence that the SecB protein functions as an antifolding factor that specifically interacts with the mature region of the precursor MBP (pre-MBP) to prevent its premature folding in the cytoplasm into a translocation-incompetent form. Kumamoto and Gannon (8) reached a similar conclusion by using a different experimental approach. Thus a specific biochemical function was assigned to the product of an *E. coli sec* gene, which complemented earlier work by Randall and Hardy (9) correlating the folding of pre-MBP into its stable tertiary structure in the cytoplasm with the loss of export competence. The essential role of protein conformation during membrane translocation has been recognized in eukaryotic cells as well (10). For example, unfolding factors that facilitate translocation of proteins into the endoplasmic reticulum and mitochondria have been described (11, 12).

An understanding of protein export in *E. coli* will be greatly aided by the purification and characterization of the individual components that mediate this process and eventual reconstitution of a complete protein translocating system *in vitro*. This study was undertaken to demonstrate the SecB dependence of MBP translocation *in vitro* and to purify biologically active SecB capable of interacting with newly synthesized pre-MBP to retard its folding and promote its import into membrane vesicles.

MATERIALS AND METHODS

***In Vitro* MBP Synthesis and Translocation.** MBP was synthesized *in vitro* by using a coupled *E. coli* transcription–translation system, in the presence or absence of membrane vesicles, and plasmid templates in which the wild-type *malE* or *malEΔ116* alleles were under transcriptional control of the *lacUV5* promoter-operator, as described (7, 13). Cells of strain MC4100 (14) and CK1953 [MC4100 *secB*::Tn*5* (6)] used for the preparation of reaction components (S-30, S-100, membrane-free ribosomes, and inside-out cytoplasmic membrane vesicles) were grown at 30°C to an OD_{600} of 1.0 in TB medium (pH 7.8) containing 0.2% glucose. To assess translocation, half of each sample was treated with proteinase K (55 μg/ml). All of the samples were solubilized by boiling in 1% $NaDodSO_4$, and then the MBP species were immunoprecipitated and analyzed by $NaDodSO_4$/PAGE in 10% polyacrylamide gels and autoradiography (15). The amount of radioactivity present as pre-MBP and mature MBP was quantitated as described (7).

Plasmid Constructions. Plasmid pJW21 was constructed by ligation of the 1.52-kilobase *Pvu* II–*Hin*dIII fragment of plasmid pDC2 [containing the *secB*$^+$ gene (6, 16)] with the large *Pvu* II–*Hin*dIII fragment of vector pZ152 (17), after fragment isolation by low-melting-temperature agarose gel electrophoresis. Plasmid pJW25 (containing the *secB*$^+$ gene under transcriptional control of the T7 RNA polymerase) was constructed by inserting the same 1.52-kilobase fragment of pDC2 into the *Stu* I site of vector pET-7 (18). The direction of *secB* transcription by T7 RNA polymerase was from the *Hin*dIII site toward the *Pvu* II site.

Purification of SecB. Cells of strain BL21(DE3) containing the cloned gene for the T7 RNA polymerase under control of the inducible *lacUV5* promoter (19) and plasmid pJW25 were grown at 30°C to an OD_{600} of 1.5 in LB medium (pH 7.8) and then induced with 1 mM isopropyl thiogalactoside for 2 hr. The cell pellet (4.4 g) was washed once, and then suspended in 20 ml of lysis buffer [20 mM Tris·HCl (pH 7.4) containing 2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and lysozyme at 100 μg/ml]. The cells were incubated at 0°C for 10 min, then $MgSO_4$ (5 mM) and DNase I (10 μg/ml) were added, and, after 10 min of further incubation, the mixture was centrifuged at 30,000 × *g* for 15 min. The pellet was resuspended in lysis buffer and the process was repeated. The supernatant fluids were combined, dialyzed overnight against 20 mM Tris·HCl (pH 7.4) containing 0.1 mM phenyl-

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Abbreviations: MBP, maltose-binding protein; pre-MBP, precursor MBP.

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methylsulfonyl fluoride, and centrifuged at 30,000 × *g* for 20 min. The soluble extract was applied to a 1.5 × 10 cm column of Q-Sepharose (Pharmacia), equilibrated with the same buffer, washed with 30 ml of buffer, and the proteins were eluted with a NaCl gradient of 0–500 mM (total volume, 300 ml). Fractions were assayed by $NaDodSO_4$/PAGE; the SecB-containing fractions (eluted at 0.3 M NaCl) were pooled, precipitated with 80% (wt/vol) ammonium sulfate, and dialyzed overnight against 50 mM potassium phosphate (pH 7.4) containing 150 mM NaCl and 0.1 mM phenylmethylsulfonyl fluoride. The dialyzed extract was concentrated to 2 ml with Centricon concentrators (Amicon; molecular weight cut-off, 10,000) and chromatographed on a 1.5 × 100 cm S-200 HR (Pharmacia) gel filtration column. The peak tubes of SecB-containing fractions were pooled, concentrated with the Centricon concentrators, and rechromatographed on the same S-200 HR column.

Protein concentration was determined by the method of Lowry *et al.* (20) with bovine serum albumin as a standard. Quantitation of purity was determined by use of a LKB laser scanner after staining and destaining $NaDodSO_4$/PAGE gels. Isoelectric focusing was performed as described (21) utilizing Pharmalyte pH 2.5–5.0 ampholytes (Pharmacia).

***In Vivo* Radiolabeling of Cellular Proteins.** Cells were grown in glycerol minimal medium to midlogarithmic phase and radiolabeled with [^{35}S]methionine for 10 min, as described by Rasmussen *et al.* (13). Incorporation of label was terminated by precipitation with trichloroacetic acid. SecB was immunoprecipitated from detergent-solubilized cell extracts and analyzed by $NaDodSO_4$/PAGE and autoradiography. Anti-SecB rabbit IgG was affinity-purified using SecB coupled to Sepharose CL-4B beads (Pharmacia), after DEAE-cellulose column chromatography.

***In Vitro* Assay of MBP Folding.** MBP was synthesized in the absence of membranes, and at each time point an aliquot was removed and quickly diluted into ice-cold 40 mM triethanolamine acetate (pH 7.8) containing 0.5% methionine. Half of each sample was immediately treated with proteinase K (55 μg/ml), and the MBP species were immunoprecipitated and analyzed by $NaDodSO_4$/PAGE as described above. The percent of precursor that was sensitive to proteolysis was calculated as described by Randall and Hardy (9).

RESULTS

SecB Is Required for Maximal MBP Translocation into Membrane Vesicles *in Vitro*. Pre-MBP synthesized *in vitro* in an *E. coli* transcription–translation system in the presence of inside-out cytoplasmic membrane vesicles can be imported into vesicles and converted to mature MBP. The use of a protease-sensitive MBP species designated MBPΔ116 permits import into vesicles to be quantitatively monitored by determining the fraction of total MBP synthesized that is resistant to proteinase K digestion (J.B.W., C. H. MacGregor, D. N. Collier, J. D. Fikes, P.H.R., and P.J.B., Jr., unpublished data). [The *in vivo* export kinetics of MBPΔ116 are identical to those of wild-type MBP. Although MBPΔ116 export is somewhat less SecB-dependent than wild-type MBP, its export is clearly much more efficient in $SecB^+$ cells compared to $SecB^-$ cells (7).] When MBPΔ116 was synthesized by using an S-30 fraction prepared from $SecB^-$ cells, translocation was greatly reduced (9% protected from protease digestion) compared to that obtained with an S-30 fraction from $SecB^+$ cells (48% protected) (Fig. 1). S-100 fractions from either $SecB^+$ or $SecB^-$ cells supplemented with purified wild-type ribosomes promoted normal levels of MBPΔ116 synthesis; virtually all of which remained unprocessed and unprotected from protease digestion in the absence of added membrane vesicles. Vesicles prepared from the $SecB^-$ strain exhibited a somewhat reduced translocation activity when added to a $SecB^+$ S-100 fraction, yet little translocation occurred when the $SecB^-$ membrane vesicles were added to a $SecB^-$ S-100 fraction (18% versus 71% translocated when $SecB^+$ S-100 and membranes were employed). The combination of wild-type membrane vesicles and the $SecB^-$ S-100 fraction also resulted in an intermediate level of MBPΔ116 translocation activity equal to that of $SecB^-$ vesicles used with a $SecB^+$ S-100 fraction (Fig. 1).

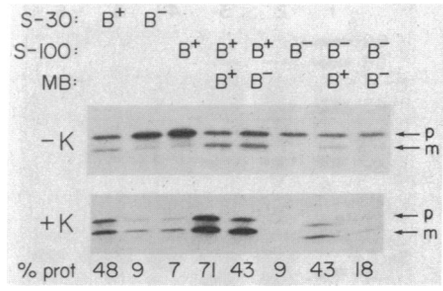

FIG. 1. SecB requirement for *in vitro* MBP translocation. MBPΔ116 was synthesized *in vitro* in the presence of [^{35}S]methionine by using an S-30 fraction, an S-100 fraction plus added wild-type ribosomes, or an S-100 fraction plus added ribosomes and vesicles (MB). Fractions were prepared from wild-type $SecB^+$ cells (strain MC4100; designated B^+) and $SecB^-$ cells [strain CK1953 (*secB*::Tn*5*); designated B^-]. After the reaction mixtures were incubated for 15 min, half of each sample was treated with proteinase K. Subsequently, MBPΔ116 was immunoprecipitated and analyzed by $NaDodSO_4$/PAGE and autoradiography. The lanes containing the protease-treated samples were exposed to film twice as long as untreated samples. The positions of precursor (p) and mature (m) MBPΔ116 are indicated by arrows. The percentage of total MBPΔ116 synthesized that was protected from protease digestion (% prot) (i.e., translocated into vesicles) was calculated after measuring the amount of radioactivity present as pre-MBP and mature MBP before (−K) and after (+K) proteinase K treatment. Note that a small amount of MBPΔ116 was matured and protease-resistant when the $SecB^+$ S-100 was employed without added vesicles, indicating that not all the membranes were removed when this fraction was prepared.

From the above results, it was apparent that SecB was required for efficient *in vitro* translocation of MBP into membrane vesicles. Previous *in vivo* studies have revealed that efficient export of only a subset of *E. coli* envelope proteins is SecB-dependent (5–7). Our *in vitro* analysis was consistent with those observations. In addition to MBP, translocation of the SecB-dependent oligopeptide-binding protein also was found to be deficient when $SecB^-$ fractions were employed, whereas *in vitro* translocation of the SecB-independent ribose-binding protein was unaffected by SecB availability (data not shown).

Purification of SecB. SecB was purified from cells containing the $secB^+$ gene on the multicopy plasmid pJW25 under the transcriptional control of the T7 RNA polymerase. After a 2-hr induction of the T7 RNA polymerase by isopropyl thiogalactoside, a major protein band of ≈17 kDa, representing 30% of the total soluble protein, was easily recognized by $NaDodSO_4$/PAGE. SecB was purified from the crude soluble fraction. An analysis of the purification procedure by $NaDodSO_4$/PAGE is shown in Fig. 2. The total quantity of protein obtained was 42 mg, representing an overall purification of >3-fold with a 50% yield. The purified protein had a pI value of 3.95–4.1, as determined by isoelectric focusing. The molecular mass of native SecB determined by gel filtration chromatography (Sephadex G-200, Sephacryl S-200 HR, and Superose-12) was estimated to be 115 kDa and by Ferguson analysis (22) on non-$NaDodSO_4$ gels was estimated to be 79 kDa (data not shown). Attempts to determine the amino-terminal sequence were unsuccessful, indicating that this terminus was blocked.

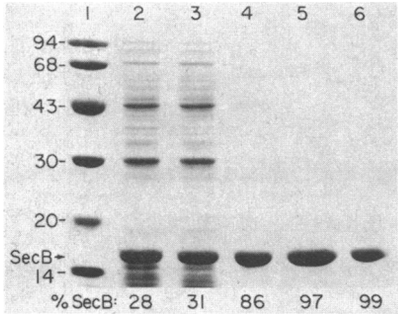

FIG. 2. Purification of SecB. Aliquots from the steps of purification were analyzed on a 12% $NaDodSO_4$/polyacrylamide gel stained with Coomassie blue. The samples applied to each lane contained approximately equivalent amounts of SecB. Lanes: 1, molecular mass standards (in kDa; phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and α-lactalbumin); 2, soluble cell extract; 3, dialyzed cell extract; 4, Q-Sepharose pool; 5, first S-200 HR pool; and 6, second S-200 HR pool. The percentage of SecB (% SecB) in each lane was determined by scanning the gel.

The SecB Protein Is Absent from *secB*::Tn*5* Mutant Cells. To confirm the identity of purified SecB, rabbit antiserum was prepared against the native protein. Affinity-purified IgG antibodies precipitated a single 17-kDa protein from detergent-solubilized [^{35}S]methionine-radiolabeled $SecB^+$ cells (Fig. 3). This polypeptide was not present in extracts prepared from cells of a *secB*::Tn*5* mutant and was precipitated in large amounts from extracts of cells having the $secB^+$ gene on a multicopy plasmid under transcriptional control of either *E. coli* RNA polymerase (pJW21) or T7 RNA polymerase (pJW25).

Purified SecB Promotes *in Vitro* Translocation of MBPΔ116. The addition of purified SecB protein to a $SecB^-$ S-30 fraction significantly elevated the *in vitro* translocation of MBPΔ116 into membrane vesicles (Fig. 4). Although as little as 0.01 μg of SecB protein added to the reaction mixture appeared to stimulate translocation, 0.1 μg clearly resulted in a significant increase in the amount of MBPΔ116 imported into vesicles. Translocation activity reached a maximum 3-fold increase with 1 μg of added SecB. It also appeared that SecB had a somewhat stimulatory effect on the amount of pre-MBP synthesized *in vitro*; however, this increase by itself could not account for the increase in translocation efficiency (J.B.W., unpublished data). The addition of purified SecB to a $SecB^+$ S-30 fraction did not significantly increase translocation efficiency (data not shown).

SecB Availability Affects *in Vitro* Folding of pre-MBP. Folding of newly synthesized wild-type pre-MBP into a stable native tertiary structure can be monitored by the acquisition of resistance to proteinase K digestion (7–9). The folding of pre-MBP in the cytoplasm has been correlated with a loss in the ability of this protein to be translocated across the cytoplasmic membrane (9). We have demonstrated (7) that

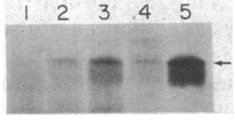

FIG. 3. Radioimmunoprecipitation of SecB. Midlogarithmic-phase cells growing in glycerol minimal medium were radiolabeled with [^{35}S]methionine for 10 min and solubilized, and SecB was immunoprecipitated and analyzed by $NaDodSO_4$/PAGE and autoradiography. Lanes: 1, strain CK1953 (*secB*::Tn*5*); 2, strain MC4100 (haploid $secB^+$); 3, strain MC4100 pJW21; 4, strain BL21(DE3) pJW25, uninduced; and 5, strain BL21(DE3) pJW25 induced for 2 hr with 2 mM isopropyl thiogalactoside.

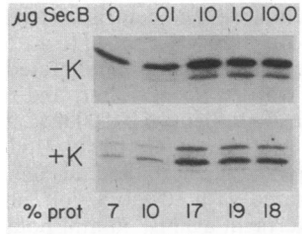

FIG. 4. Stimulation of *in vitro* MBP translocation by purified SecB. MBPΔ116 was synthesized by using a $SecB^-$ S-30 fraction in the presence of various concentrations of purified SecB (10 μg of bovine serum albumin was used as a control in the reaction mixture without added SecB). After 15 min of incubation, half of each sample was treated with proteinase K. Subsequently, MBPΔ116 was immunoprecipitated and analyzed by $NaDodSO_4$/PAGE and autoradiography. The lanes containing the protease-treated samples (+K) were exposed to film for a 5-fold longer period of time than untreated samples (−K). The percentage of total MBPΔ116 synthesized (% prot) that was protected from proteinase K digestion was calculated as described in Fig. 1.

the rate of folding of wild-type pre-MBP synthesized *in vitro* in the absence of membranes was found to be accelerated when a $SecB^-$ S-100 fraction was used and was greatly retarded when the S-100 fraction was prepared from cells harboring the $secB^+$ gene on a multicopy plasmid. The antifolding activity of purified SecB was investigated in this study. At intervals after the initiation of pre-MBP synthesis, aliquots were removed from the reaction and half of each sample was treated with proteinase K. If pre-MBP had completely folded, proteinase K digestion generated mature MBP; whereas if pre-MBP had not completely folded, a sufficient number of residues were accessible to the protease, and no polypeptides were recovered by immunoprecipitation.

When pre-MBP was synthesized using a $SecB^-$ S-100 fraction, with time there was a steady decline in the percent-

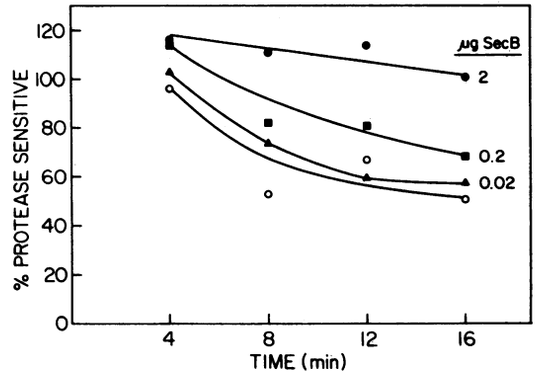

FIG. 5. Folding of pre-MBP synthesized *in vitro* is retarded by purified SecB. The kinetics of folding of pre-MBP was monitored by the loss of sensitivity to proteolytic degradation. Wild-type pre-MBP was synthesized by using a $SecB^-$ S-100 fraction plus added $SecB^-$ ribosomes, in the presence of various concentrations of purified SecB or 2 μg of bovine serum albumin (open circles) as control. Samples were removed from the reaction mixtures at the indicated times, and half of each sample was immediately treated with proteinase K. Subsequently, the MBP was immunoprecipitated and analyzed by $NaDodSO_4$/PAGE and autoradiography. Resistance to proteolysis was indicated by the conversion of pre-MBP to a form that migrates identically to mature MBP. The percentage of the pre-MBP synthesized that was sensitive to proteolytic degradation at each time point was calculated. Note that pre-MBP is continuously synthesized throughout the reaction period. Thus, even in the absence of SecB, 100% of pre-MBP would not be expected to attain a protease-resistant conformation during the time course of this experiment.

age of pre-MBP that retained a protease-sensitive conformation (Fig. 5). When purified SecB was included in the reaction mixture, pre-MBP folding was reduced in a dose-dependent manner. In fact, when 2 μg of SecB was included, folding of the pre-MBP into a proteinase-resistant conformation was virtually undetectable for as long as 16 min after initiating pre-MBP synthesis. Folding of ribose-binding protein also can be monitored by an identical assay (23). We found the addition of SecB to have no measurable effect on the acquisition of proteinase K resistance when this protein was synthesized *in vitro* (data not shown).

Excess SecB Prolongs pre-MBP Competence for Posttranslational Translocation. Posttranslational translocation can be studied independently from cotranslational translocation in an *in vitro* system by the addition of membranes to the reaction mixture only subsequent to the termination of protein synthesis. The effects of the inclusion of excess purified SecB on the capacity of pre-MBP to remain competent for posttranslational translocation during continued incubation at 37°C were investigated. Wild-type pre-MBP was synthesized in the absence of membrane vesicles using a $SecB^+$ S-100 fraction. After 8 min, protein synthesis was terminated by the addition of ribonuclease, and then vesicles were added to individual aliquots of the reaction mixture at intervals to 30 min, and in each case incubation was continued for another 15 min. Since wild-type pre-MBP was synthesized in this experiment, translocation into vesicles was monitored by determining the amount of pre-MBP that was processed to mature MBP.

As shown in Fig. 6, wild-type pre-MBP rapidly became incompetent for posttranslational import into vesicles. Translocation was undetectable if protein synthesis was terminated 20 min prior to the addition of vesicles. In marked contrast, when 2 μg of purified SecB was included in the reaction mixture, the proportion of pre-MBP competent for posttranslational import immediately after the termination of protein synthesis was significantly increased. Furthermore, although translocation activity did diminish somewhat at later time points, the time period that pre-MBP remained translocation-competent was markedly extended, such that import into vesicles was easily detectable when vesicles were added 30 min after protein synthesis termination.

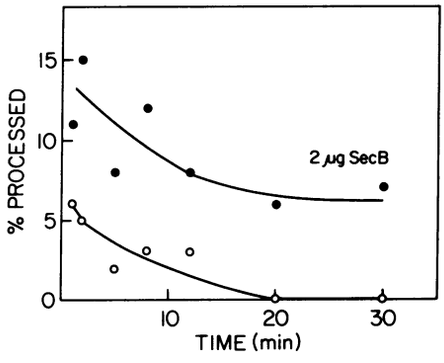

FIG. 6. Effect of purified SecB on posttranslational MBP translocation. Wild-type pre-MBP was synthesized by using a $SecB^+$ S-100 fraction plus added $SecB^+$ ribosomes, and 2 μg of purified SecB (closed circles) or bovine serum albumin (open circles). After 8 min, protein synthesis was terminated and, at the time points indicated, $SecB^+$ membrane vesicles were added to the reaction mixtures. Incubations were continued for another 15 min and then MBP was immunoprecipitated and analyzed by $NaDodSO_4$/PAGE and autoradiography. The percentage of the total MBP synthesized that was processed to mature MBP was calculated after measuring the amount of radioactivity present as pre-MBP and mature MBP. Background values of processing in the absence of added vesicles were subtracted for each time point.

DISCUSSION

A component of the *E. coli* protein-export machinery has been purified and shown to be biologically active by several assays. SecB is an acidic protein with a monomer molecular mass of ≈17 kDa, as determined by its migration on $NaDodSO_4$/PAGE. Analysis of the native protein by gel filtration chromatography and native gel electrophoresis indicated that SecB is probably a multimeric protein of four to six identical subunits. SecB was absent from cells harboring a *secB*::Tn*5* mutation, and fractions prepared from such cells were markedly defective at sponsoring *in vitro* translocation of a subset of *E. coli* periplasmic proteins including MBP, thus mimicking the specific effects of the *secB*::Tn*5* mutation on protein export *in vivo* (5–8). SecB appears to be a soluble cytoplasmic protein for the following reasons: (*i*) active SecB was purified from the soluble fraction of SecB-overproducing cells; (*ii*) a $SecB^+$ S-100 fraction could partially complement membrane vesicles prepared from $SecB^-$ cells for MBP translocation activity; and (*iii*) we had shown (7) that S-100 fractions prepared from $SecB^-$, $SecB^+$, and SecB-overproducing cells exhibited significant differences in the rate of folding of newly synthesized pre-MBP. A $SecB^-$ S-100 fraction also could be complemented by membrane vesicles prepared from wild-type cells. However, since SecB interacts with precursor proteins targeted for export, it was not unexpected that this protein was in both the soluble and crude membrane fractions. Furthermore, since SecB is clearly present in haploid $secB^+$ cells in limiting quantities (7), the soluble and membrane fractions alone probably have insufficient SecB to bring MBP translocation activity in such a complementation assay up to the level obtained when both of these fractions are prepared from $SecB^+$ cells.

The addition of purified SecB to a $SecB^-$ S-30 fraction significantly enhanced translocation of MBP into membrane vesicles, but a translocation efficiency comparable to that exhibited by $SecB^+$ fractions was not attained. This may be an indication that SecB was somehow altered during the purification procedure or was purified away from some other component required for maximal translocation activity. Another possibility is that $SecB^-$ cells suffer alterations in their export machinery such that fractions prepared from these cells exhibit a MBP translocation defect that cannot be totally repaired by the addition of purified SecB. Providing additional SecB to a $SecB^+$ S-30 fraction did not significantly improve MBP translocation efficiency. Thus, a limiting amount of available SecB probably is not responsible for the inability to import MBP into vesicles *in vitro* with 100% efficiency.

Consistent with our previous findings that SecB had an antifolding activity that promoted MBP export (7), we found that purified SecB added to a $SecB^-$ S-100 fraction could significantly retard the folding of newly synthesized pre-MBP into a protease-resistant form. By this assay, folding of pre-MBP was virtually undetectable over the time course of the experiment when 2 μg of SecB was added to the *in vitro* reaction mixture. This effect on pre-MBP folding was identical to that obtained with an S-100 fraction prepared from SecB-overproducing cells (7). Thus, purified SecB must still retain the ability to specifically interact with pre-MBP in such a manner as to significantly affect its folding kinetics. Our earlier studies had strongly suggested that SecB binds to a specific site within the mature moiety of the pre-MBP to effect its antifolding activity, although at this point alternative explanations for this antifolding activity cannot be ruled out (7). The availability of purified biologically active SecB should now allow us to further define the interaction between this protein and unfolded MBP.

Randall and Hardy (9) demonstrated that folding of the pre-MBP in the cytoplasm into a protease-resistant form

correlated with the loss in its ability to be posttranslationally translocated. We subsequently showed that posttranslational MBP export was particularly defective in SecB$^-$ cells and concluded that the antifolding activity of SecB was essential for maintaining the export competence of pre-MBP in the cytoplasm (7). In this study, we have found that the rapid loss in the ability of wild-type pre-MBP synthesized *in vitro* to be posttranslationally imported into vesicles could be significantly slowed by the inclusion of excess purified SecB, well above the haploid SecB level, in the reaction mixture. The interaction between SecB and wild-type pre-MBP *in vivo* is by necessity a transient one since translocation is normally achieved very rapidly. When translocation is prevented, pre-MBP folding in the cytoplasm eventually occurs even in the presence of SecB (7, 8). However, it seems clear from our *in vitro* studies that an elevated level of SecB can markedly affect pre-MBP folding kinetics, possibly by shifting the equilibrium of the pre-MBP–SecB interaction in favor of the association. As an alternative to adding excess SecB, pre-MBP competence for posttranslational import into vesicles also could be significantly prolonged by a mutational alteration in the pre-MBP that adversely affected folding (J.B.W., unpublished data). These findings underscore the importance of an antifolding factor in promoting the posttranslational translocation of MBP.

What about cotranslational MBP export which also is defective in SecB$^-$ cells (7, 8)? Cotranslational translocation of MBP is not initiated until after ≈80% of the nascent chain has been synthesized (25), which includes that portion of the pre-MBP having the SecB interaction site (7). The antifolding activity of SecB might promote cotranslational pre-MBP export by preventing the nascent chain from assuming a conformation that is inhibitory to export or by keeping the nascent chain in a conformation that ensures that the signal peptide is readily accessible to the export machinery. On the other hand, it has been suggested that a possible second function of SecB is to actively facilitate the delivery of nascent pre-MBP to the export machinery in the cytoplasmic membrane, perhaps by directly interacting with a membrane receptor (7, 8). It could be that the loss of this putative second activity is primarily responsible for the defect in cotranslational MBP export in SecB$^-$ cells.

In addition to SecB, other cytoplasmic protein factors from *E. coli* cells that could stimulate *in vitro* protein translocation activity have been identified. The 12S export factor of Müller and Blobel (2) is ≈220 kDa, and the "trigger factor" described by Crooke and Wickner (3) is ≈60 kDa. Weng *et al.* (4) described two factors of ≈60 and ≈120 kDa. None of these appear to be SecB. In addition, the isolation of temperature-sensitive mutants defective in protein export at the nonpermissive temperature led to the identification of SecA, a cytoplasmic 91-kDa protein (24). The role that these various proteins have in promoting co- and/or posttranslational protein export has not yet been determined. Our studies indicate that the inability to attain 100% translocation efficiency of MBP *in vitro* is not associated with the vesicle fraction (J.B.W., unpublished data); rather, some proportion of the newly synthesized pre-MBP may fail to properly interact with one or more of these other soluble factors prior to delivery to the cytoplasmic membrane. Future use of purified SecB and the SecB-specific antiserum should help to define the interactions between MBP, SecB, and the various components of the export machinery.

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