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The Folding Properties of the *Escherichia coli* Maltose-Binding Protein Influence Its Interaction with SecB In Vitro

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It has been proposed that the cytoplasmic SecB protein functions as a component of the *Escherichia coli* protein export machinery by serving as an antifolding factor that retards folding of the precursor maltose-binding protein (preMBP) into a translocation-incompetent form. In this study, it was found that SecB directly interacts with wild-type preMBP and various mutationally altered MBP species synthesized in vitro to form a SecB-MBP complex that can be precipitated with anti-SecB serum. The association of SecB with wild-type preMBP was relatively unstable; such a complex was formed only when SecB was present cotranslationally or after denaturation of previously synthesized preMBP and was detected with only low efficiency. In marked contrast, MBP species that were defective in the ability to assume the stable conformation of wild-type preMBP or that exhibited significantly slower folding kinetics formed much more stable complexes with SecB. In one case, we demonstrated that SecB did not need to be present cotranslationally for complex formation to occur. Formation of a complex between SecB and MBP was clearly not dependent on the MBP signal peptide. However, we were unable to detect complex formation between SecB and MBP lacking virtually the entire signal peptide but having a completely intact mature moiety. This MBP species folded at a rate considerably faster than that of wild-type preMBP. The propensity of this mutant protein to assume the native conformation of mature MBP apparently precludes a stable association with SecB, whereas an MBP species lacking a signal peptide but exhibiting altered folding properties did form a complex with SecB that could be precipitated with anti-SecB serum.

Translocation of proteins across bacterial, endoplasmic reticulum, and mitochondrial membranes requires that the newly synthesized polypeptide exhibit a translocation-competent conformation that is thought to represent a largely unfolded or loosely folded state (for recent reviews, see references 1, 26, and 29). For proteins whose translocation is not tightly coupled to translation, it has been proposed that cytosolic activities capable of unfolding or retarding the folding of proteins render the polypeptides competent for translocation (1, 4–6, 8–11). In a previous study of protein export in the bacterium *Escherichia coli*, Randall and Hardy (28) correlated the folding of precursor maltose-binding protein (preMBP) in the cytoplasm into a stable, tertiary structure with the loss of export competence. Subsequently, we presented biochemical and genetic evidence that the cytoplasmic SecB protein functions as a component of the *E. coli* export machinery by acting as an antifolding factor that interacts with certain precursor proteins to retard their folding prior to export from the cytoplasm (5, 6, 35). SecB is thought to be one of a class of cytoplasmic proteins, called chaperones (13), that can serve to maintain the export competence of precursor polypeptides in *E. coli*. Other proteins postulated to have a similar function include the trigger factor characterized by Crooke et al. (8–10) and GroEL (2).

SecB is a nonessential, tetrameric protein composed of identical 16.4-kilodalton subunits that is required for the efficient export of a subset of *E. coli* envelope proteins, including the MBP (5, 19, 20, 21, 35). In cells lacking SecB, MBP export appears to represent a race between delivery of the newly synthesized, export-competent polypeptide to the export machinery in the cytoplasmic membrane and folding of preMBP in the cytoplasm into an export-incompetent conformation (5, 22, 28, 35). Purified SecB protein retards folding of preMBP and can prolong the time in which preMBP remains competent for posttranslational import into membrane vesicles in vitro (35). The nature of the SecB-MBP interaction in maintaining preMBP export competence is not known. We had originally proposed that SecB bound directly to the mature moiety of the newly synthesized preMBP (5). Very recently, three different studies have demonstrated that SecB directly associates in a 1:1 molar ratio with several different precursor proteins, including preMBP, preLamB, proOmpA, and prePhoE, to form a soluble complex that presumably represents a transient intermediate in the export process (18, 23, 32). In marked contrast to our previous proposal, Watanabe and Blobel (33) recently presented evidence indicating that SecB binds specifically to the emerging signal peptide of newly synthesized preMBP. It was suggested that SecB functions as the procaryotic equivalent of the signal recognition particle previously identified in studies of protein targeting to the rough endoplasmic reticulum of eucaryotic cells (for a review, see reference 30).

We have further investigated the interaction of SecB with preMBP. Our studies indicate that the association of these two proteins to form a complex is not dependent on the signal peptide but is strongly influenced by the folding properties of the mature MBP moiety.

MATERIALS AND METHODS

In vitro MBP synthesis. MBP was synthesized in vitro by using a coupled *E. coli* transcription-translation system and plasmid templates in which the *malE* alleles were under

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TABLE 1. MBP species tested for the ability to interact with SecB

MBP species	Encoded by plasmid:	Mutational alteration	Export competent?	Reference
MBP$^+$	pJF2	Wild type: 26-residue signal peptide and 370-residue mature moiety	Yes	15
MBPΔ323	pJF32	Deleted from residue 7 of signal peptide through residue 89 of mature moiety	No	5
MBP2261	pJW13	Asp substituted for Tyr at residue 283 of mature protein	Yes	34
MBPΔ57-145	pDNC155	Deleted for residues 57 through 145 of mature moiety	Yes	5
MBPΔ2-26	pUZ226	Deleted for residues 2 through 26 of signal peptide	No	34
MBPΔ2-26/2261	pJW41	MBPΔ2-26 signal peptide and MBP2261 mature moiety	No	This study

transcriptional control of the *lacUV5* promoter-operator, as described previously (5, 34, 35). Plasmids pDNC155 (MBPΔ57–145), pJF2 (wild-type MBP), pJF32 (MBPΔ323), pJW13 (MBP2261), and pUZ226 (MBPΔ2–26) were described previously (5, 15, 34). Plasmid pJW41 (MBPΔ2–26/2261) was constructed by ligating the small *Eco*RI-*Bgl*II fragment of pUZ226 (containing the 5′ end of the *malE* gene with the signal peptide coding region deleted) with the large *Eco*RI-*Bgl*II fragment of pJW13 (containing the *malE2261* mutation). The MBP species encoded by these various plasmids are described in Table 1. Plasmid pAI12, a derivative of pACYC encoding wild-type ribose-binding protein (17), was provided by Linda Randall. Reaction components were prepared from haploid *secB*$^+$ cells (strain MC4100 [3]), SecB-overproducing cells (strain MC4100 pDC2 [20]), and SecB$^-$ cells (strain CK1953 [20]). The preparation of purified SecB from SecB-overproducing cells has been described previously (35).

Radioimmunoprecipitation reactions. The radiolabeled translation reaction products were immediately diluted 50-fold into buffer containing 50 mM Tris hydrochloride (pH 8.0), 150 mM NaCl, 0.1 mM EDTA, 2% Triton X-100, and either preimmune rabbit serum, polyclonal rabbit anti-SecB serum (35), or polyclonal rabbit anti-MBP serum (14). The mixtures were incubated for 16 h at 4°C with mixing. The immune complexes were precipitated with IgGsorb (Enzyme Center, Boston, Mass.) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography using 10% polyacrylamide gels, as described previously (14). The amount of MBP immunoprecipitated with anti-SecB serum was quantitated by counting the radioactivity eluted from gel slices (5) and then calculated relative to the amount of MBP immunoprecipitated with anti-MBP serum. It should be noted that for unknown reasons, radioimmunoprecipitation reactions using extracts prepared from in vitro translation reactions tend to give significantly higher background levels than do similar reactions using extracts prepared from whole cells. In quantitating the immunoprecipitation of proteins by immune serum, the amount of the same protein precipitated by preimmune serum was first subtracted. Hence, because of the higher background levels, this calculation probably underestimated to some degree the amount of protein that was specifically precipitated.

RESULTS

MBP synthesized in vitro can be precipitated with anti-SecB serum. Wild-type preMBP (preMBP$^+$) and various mutant MBP species were synthesized in an *E. coli* coupled transcription-translation system, using an S-100 fraction prepared from SecB-overproducing cells. Membranes were not present in the reaction mixture. Extracts containing the in vitro reaction products were prepared and used in an immunoprecipitation reaction with preimmune rabbit serum or rabbit anti-SecB or anti-MBP serum (see Materials and Methods). The precipitates obtained were then analyzed by SDS-PAGE and autoradiography (Fig. 1A) A small but still significant amount (7%) of preMBP$^+$ was specifically precipitated by the anti-SecB serum as compared with the amount precipitated by anti-MBP serum. In marked contrast, an internally truncated MBP species designated MBPΔ323 (Table 1) was much more efficiently immunoprecipitated by anti-SecB serum (42%). When SecB was omitted from the in vitro reaction by using an S-100 fraction prepared from SecB$^-$ cells, specific immunoprecipitation of MBPΔ323 with anti-SecB serum was not observed (Fig. 1B). In addition, when the precipitation of MBPΔ323 was performed in the presence of an excess of purified SecB, the amount of radiolabeled MBP precipitated was greatly reduced (Fig. 1B). These latter two experiments established that the precipitation of MBP by anti-SecB serum was dependent on the SecB protein.

We considered it likely that the more efficient precipitation of MBPΔ323 by anti-SecB serum compared with preMBP$^+$ was related to the folding properties of these two MBP species (see Discussion). The role of folding in the ability of MBP to form a stable complex with SecB was further investigated by use of additional MBP species with altered folding properties. MBP2261 has a single amino acid substitution at residue 283 of the mature protein. Liu et al. (24) have demonstrated that purified mMBP2261 refolds after denaturation at a rate significantly slower than that of wild-type mature MBP. A substantial proportion (32%) of preMBP2261 synthesized in vitro was specifically immunoprecipitated with anti-SecB serum (Fig. 1A). MBPΔ57–145, missing residues 57 to 145 from the mature MBP, is unable to assume the stable, tertiary conformation of MBP$^+$ and exhibits other properties indicating that its folding is significantly altered compared with that of MBP$^+$ (5, 7, 24; see Discussion). Precursor MBPΔ57-145 also was more efficiently precipitated by anti-SecB serum that was preMBP$^+$ (Fig. 1A).

Since MBPΔ323 is missing virtually the entire signal peptide, the efficient formation of a stable complex between this MBP species and SecB suggested that SecB specifically interacts with the mature moiety of MBP, as suggested in our earlier study (5). Thus, we further assessed the role of the signal peptide in formation of the stable SecB-MBP complex through use of two MBP species that lack the entire 26-residue signal peptide but contain either the intact mature moiety (MBPΔ2–26) or the mature moiety containing the same single amino acid substitution as does MBP2261 (MBPΔ2–26/2261). MBPΔ2–26 synthesized in vitro was unable to form a stable complex with SecB under the experimental conditions used, as demonstrated by the failure to specifically immunoprecipitate this protein (less than 1%) with anti-SecB serum (Fig. 1A). In marked contrast, large

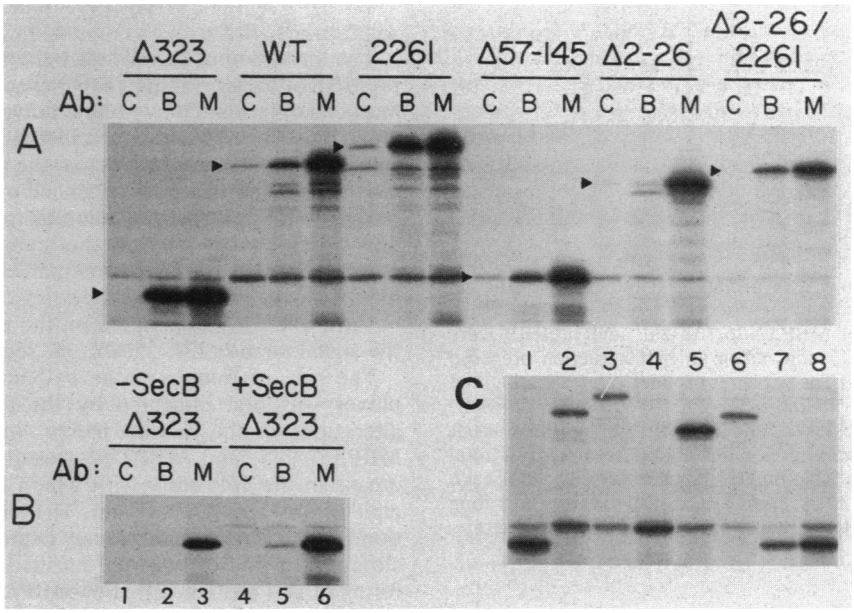

FIG. 1. SecB-MBP complex formation obtained by using an S-100 fraction prepared from SecB-overproducing cells. (A) MBPΔ323 (Δ323), wild-type preMBP (WT), MBP2261 (2261), MBPΔ57–145 (Δ57–145), MBPΔ2–26 (Δ2–26), and MBPΔ2–26/2261 (Δ2–26/261) were synthesized in vitro for 15 min, using an S-100 fraction prepared from SecB-overproducing cells. The reaction products were precipitated with preimmune rabbit serum (C), anti-SecB serum (B) or rabbit anti-MBP serum (M). The immune complexes were subsequently analyzed by SDS-PAGE and autoradiography. Positions of the full-length MBP species are indicated by arrowheads. For all MBP species, note the presence of truncated forms migrating below the full-length protein that represent translational intermediates. (B) For lanes 1 to 3, MBPΔ323 was synthesized in the absence of SecB, using an S-100 fraction prepared from SecB$^-$ cells; for lanes 3 to 6, MBPΔ323 was synthesized by using an S-100 fraction prepared from SecB-overproducing cells. After synthesis, the reaction products were precipitated in the presence of 10 μg of purified SecB (35). The autoradiograph in panel C shows the in vitro reaction products used for each set of immunoprecipitation experiments (presented in the same order, i.e., lane 1 shows the products encoded by plasmid pJF32 [MBPΔ323]; lane 2, shows those encoded by plasmid pJF2 [wild-type preMBP], etc.). Note that MBPΔ57–145 migrates on SDS-PAGE identically to precursor β-lactamase that is encoded by each of the plasmids listed in Table 1.

amounts of MBPΔ2–26/2261 were immunoprecipitated with anti-SecB serum (20%). Also, a faster-migrating, truncated form of MBPΔ2-26 synthesized in the same reaction with full-length MBPΔ2–26 was efficiently precipitated by anti-SecB serum. Similar results were observed for truncated forms (probably representing nascent chains) of other MBP species synthesized in vitro.

In a separate experiment, we compared the ability of anti-SecB serum to precipitate either preMBP$^+$ or wild-type precursor ribose-binding protein (preRBP$^+$) synthesized in vitro by using an S-100 fraction prepared from SecB-overproducing cells. RBP export in vivo is known to be SecB independent (5, 19). Approximately 8% of preMBP$^+$ was precipitated by anti-SecB serum (Fig. 2), whereas immunoprecipitation of preRBP$^+$ was not detectable.

We also synthesized preMBP$^+$, MBPΔ323, MBPΔ2–26, and preRBP$^+$ in vitro in the presence of a significantly lower concentration of SecB by using an S-100 fraction prepared from a haploid *secB*$^+$ strain (35). The amounts of preMBP$^+$ and MBPΔ323 precipitated by anti-SecB serum were both lower than the amount obtained with an S-100 fraction from SecB-overproducing cells (21 and 2% specifically precipitated, respectively) (Fig. 3). However, in this instance it was also apparent that MBPΔ323 was much more efficiently precipitated than was preMBP$^+$. Once again, specific precipitation of MBPΔ2–26 and preRBP$^+$ by anti-SecB serum was not detected. In addition, specific precipitation of truncated forms of both wild-type preMBP and MBPΔ2–26 by anti-SecB serum was evident.

SecB does not complex with preMBP$^+$ when added post-translationally. After in vitro synthesis in the absence of membranes, preMBP$^+$ will fold into a stable, tertiary structure, as monitored by the acquisition of proteinase K resistance. The rate of folding of wild-type preMBP is accelerated when SecB is absent and greatly retarded when excess SecB is present (5). Thus, the ability of MBP synthesized in vitro to form a complex with SecB that was presented posttrans-

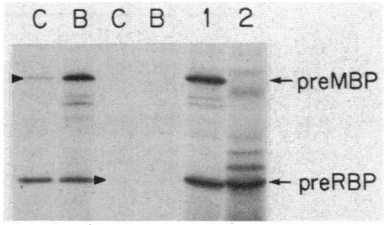

FIG. 2. Demonstration that precursor RBP is not precipitated with anti-SecB serum. Wild-type preMBP (first two lanes) and wild-type preRBP (second two lanes) were synthesized in vitro for 15 min, using an S-100 fraction prepared from SecB-overproducing cells. The reaction products were precipitated with preimmune rabbit serum (lanes C) or rabbit anti-SecB serum (lanes B) and then analyzed by SDS-PAGE and autoradiography. Positions of the full-length preMBP and preRBP species are indicated by arrows at the right and arrowheads at the left. Lanes 1 and 2 represent the in vitro reaction products for plasmid pJF2 (wild-type preMBP) and pAI12 (wild-type preRBP). It is important to note that plasmid pAI12 does not encode β-lactamase, which migrates almost at the same position as preRBP and can be easily seen in the first, second, and fifth lanes.

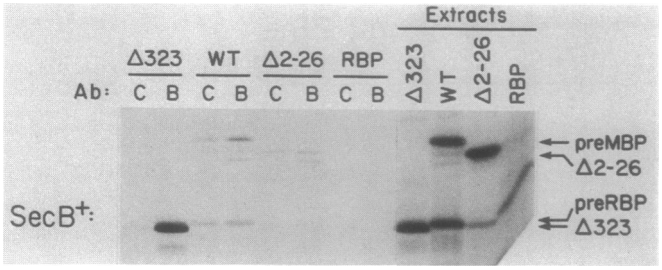

FIG. 3. SecB-MBP complex formation obtained by using an S-100 fraction prepared from haploid secB$^+$ cells. MBPΔ323 (Δ323), wild-type preMBP (WT), MBPΔ2–26 (Δ2–26), and preRBP were synthesized in vitro for 15 min, using an S-100 fraction prepared from haploid *secB*$^+$ cells. The reaction products were precipitated with preimmune rabbit serum (lanes C) or anti-SecB serum (lanes B) and then analyzed by SDS-PAGE and autoradiography. Positions of the full-length MBP species are indicated by arrows. The total products for each in vitro reaction are shown at the right. For each of the MBP species, note the presence of truncated forms migrating below the full-length proteins that represent translational intermediates.

lationally was assessed. MBPΔ323, preMBP$^+$, and MBPΔ2–26 were synthesized in the absence of SecB, protein synthesis was terminated, and then purified SecB protein (35) was added to the reaction mixture. A substantial portion (19%) of the MBPΔ323 formed a complex with SecB, as indicated by immunoprecipitation with anti-SecB serum (Fig. 4). In marked contrast, specific immunoprecipitation of preMBP$^+$ or MBPΔ2–26 with anti-SecB serum was not detected when SecB was added posttranslationally. When preMBP$^+$ was first denatured in 5 M urea and subsequently diluted out of urea in the presence of SecB, we were once again able to detect a SecB-preMBP complex by immunoprecipitation with anti-SecB serum (10%) (Fig. 4). Urea denaturation had no effect on the ability of MBPΔ323 to form a stable complex with SecB. MBPΔ2–26 did not form an immunoprecipitable complex with SecB under these conditions of denaturation and renaturation.

DISCUSSION

The formation of complexes between SecB and wild-type preMBP or various mutant MBP species synthesized in vitro has been investigated by precipitating SecB-MBP complexes with anti-SecB serum. The binding of SecB to MBP was found not to be dependent on the signal peptide per se. However, as particularly evidenced by marked differences in our ability to demonstrate complex formation between SecB and various MBP species, the stability of the SecB-MBP complex under our experimental conditions was clearly influenced by the folding properties of the MBP. The latter, in turn, is clearly dependent on the presence or absence of the signal peptide (24, 25, 27, 34; see below).

The role of folding in the stability of SecB-MBP complexes was first indicated by the finding that mutational alterations in the mature moiety of preMBP (MBPΔ323, MBPΔ57–145, and MBP2261) resulted in a significant increase in the efficiently with which MBP was immunoprecipitated by anti-SecB serum. The single amino acid alteration in MBP2261 has previously been shown to significantly slow the folding of both the purified mature and precursor forms of this protein (24). For MBPΔ323 and MBPΔ57–145, the deletion of a large number of residues would certainly be expected to significantly alter the folding properties of these proteins. In fact, these two MBP species are unable to form the tertiary structure characteristic of wild-type MBP, as indicated by their sensitivity to proteinase K (12) and their marked in vivo instability (5). It seems highly likely that the lack of wild-type folding properties facilitated the more stable interaction of these three mutant MBP species with SecB. MBPΔ57–145 and MBP2261, both of which are synthesized with an intact MBP signal peptide, are exported to the periplasm of cells with wild-type kinetics, and MBP2261 also can facilitate maltose uptake (5, 7). Therefore, these altered MBPs would be expected to interact normally with the protein export machinery. It is interesting that their export in vivo is significantly less SecB dependent than is that of wild-type MBP (5). In vitro studies have demonstrated that preMBP$^+$ rapidly becomes incompetent for posttranslational translocation into inverted cytoplasmic

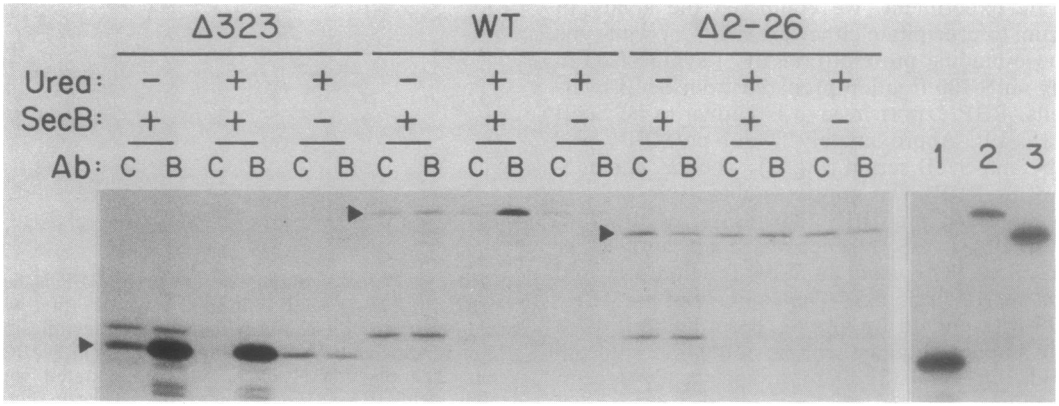

FIG. 4. Demonstration that SecB does not form a complex with wild-type preMBP when added posttranslationally. Wild-type preMBP (WT), MBPΔ323 (Δ323), and MBPΔ2–26 (Δ2–26) were synthesized in vitro, using an S-100 fraction prepared from SecB$^-$ cells. After 15 min, protein synthesis was terminated. The incubations were continued for 5 min at 37°C, and then either 8 μg of purified SecB was added to the 25-μl reaction or the sample was denatured with urea. Urea was added to a final concentration of 5 M, the sample was incubated at room temperature for 30 min, and then the sample was diluted fivefold into buffer containing either purified SecB or bovine serum albumin. After 10 min of incubation at 4°C with the SecB, the reaction products were immunoprecipitated with preimmune serum (lanes C), anti-SecB serum (lanes B), or anti-MBP serum (lanes 1 to 3) and analyzed by SDS-PAGE and autoradiography. Positions of preMBP, MBPΔ323, and MBPΔ2–26 are indicated by arrowheads.

membrane vesicles, whereas both MBPΔ57–145 and MBP2261 remain competent for significantly longer periods (34). Each of these properties is thought to be due to the altered folding character of the mutant proteins, compared with that of preMBP$^+$, as is the finding that these two proteins form a more stable complex with SecB than does preMBP$^+$.

Although only a small fraction of preMBP$^+$ synthesized in vitro could be demonstrated to complex with SecB by immunoprecipitation with anti-SecB serum, our previous studies monitoring the effect of SecB on preMBP$^+$ folding in vitro indicated that this interaction is more likely to include virtually 100% of the preMBP molecules (5, 35). However, preMBP$^+$ folding was monitored in the presence of a great excess of SecB. The equilibrium of the SecB-preMBP$^+$ association must be determined in part by the molar ratio of SecB to MBP and in part by the propensity of MBP species to assume a conformation that cannot maintain this interaction. We found that when haploid levels of SecB were present, less complex was detected with MBP than in the presence of excess SecB. Furthermore, Liu et al. (25) recently reported that the molar ratio of purified SecB to purified mature MBP2261 must be equal to or greater than 4 in order for SecB to effectively retard the folding of this MBP species. Once excess SecB is complexed with antibody, preMBP$^+$ folding probably results in dissociation of the great majority of SecB-preMBP complexes. Because of the altered folding properties of MBPΔ323, MBPΔ57–145, and MBP2261, they do not as readily assume a conformation that disrupts or prevents the interaction with SecB. Thus, these MBP species were precipitated with much greater efficiency by anti-SecB serum. The failure to recover closer to 100% of the latter proteins as complexes with SecB may indicate that these MBP species still have some capacity to assume a structure that is unable to stably interact with SecB. This view is consistent with an earlier finding that both MBPΔ57–145 and MBP2261 eventually lose competence for posttranslational import into vesicles in vitro, although at a markedly slower rate than does preMBP$^+$ (35).

The correlation between the stability of the SecB-MBP complex and the folding properties of the MBP was further demonstrated by our finding that an interaction between SecB and preMBP$^+$ was detectable only when SecB was present during MBP synthesis or when preMBP$^+$ than had been synthesized in the absence of SecB was denatured and subsequently diluted out of denaturant in the presence of SecB. Results similar to the latter have been described for the interaction of trigger factor and the export-competent form of purified proOmpA in vitro (8, 10). It would seem that prior folding of preMBP$^+$ prevents a stable interaction with SecB, whereas an MBP species such as MBPΔ323 with its altered folding properties can be shown to interact nearly as well with SecB added 5 min after translation termination. Our earlier studies indicated that SecB has as antifolding activity but is unable to unfold preMBP$^+$ that had previously assumed a protease-resistant conformation (5, 35).

MBPΔ2–26 is also a mutant MBP species that exhibits folding kinetics that are different from those of the wild-type preMBP. When this protein lacking the signal peptide is synthesized in vitro, it folds into a stable, protease-resistant conformation at a rate far exceeding that of preMBP$^+$ (34), a finding consistent with the results of Park et al. (27), who demonstrated that the presence of the signal peptide significantly retards the refolding of denatured, purified MBP. We were unable to detect an interaction between SecB and MBPΔ2–26, even after the latter had been denatured and diluted in the presence of SecB. Although these results could be interpreted to indicate that the signal peptide was required for an interaction with SecB, such an interaction was clearly demonstrated with MBPΔ2–26/2261 harboring an additional alteration that slows folding of the mature MBP or, alternatively, a truncated form of MBPΔ2–26 that was a by-product of the in vitro synthesis reaction. Liu et al. (25) found that purified SecB is able to slow the folding of purified, denatured mature MBP$^+$ (= MBPΔ2–26), but only if SecB was present in great molar excess and the temperature of the renaturation reaction was reduced from 25 to 5°C. Thus, it appears that it is the particularly strong propensity of MBPΔ2–26 to assume its final tertiary conformation that prevents the formation of a stable, immunoprecipitable complex with SecB.

The failure to detect a complex between SecB and MBPΔ2–26 in vitro also supports data obtained from in vivo studies. We had previously demonstrated that the synthesis of export-defective MBP species such as MBPΔ323 markedly interferes with the export of SecB-dependent proteins in *E. coli* cells. This interference is relieved in cells overexpressing SecB, leading to the conclusion that MBPΔ323 obstructs normal protein export in haploid *secB*$^+$ cells by binding for an extended period of time the small amount of available SecB (5). In marked contrast, synthesis of MBPΔ2–26 does not interfere with normal protein export in vivo (25; J. W. Puziss and P. J. Bassford, Jr., unpublished observations). This is despite the fact that this protein species retains the site between residues 151 and 186 of the mature moiety that was shown to be sufficient for mediating this interference phenomenon (5). Presumably, stable binding of SecB to this region of MBPΔ2–26 is prevented by the rapid folding of the protein.

The finding that the interaction between SecB and preMBP$^+$ is relatively unstable is not inconsistent with the antifolding role proposed for SecB in promoting MBP export. By necessity, the interaction of SecB with preMBP must be a transient one, since under normal circumstances newly synthesized preMBP is rapidly secreted into the periplasm. We have previously suggested that SecB functions to retard, rather than prevent, the folding of preMBP (5, 35), and in vivo studies have shown that preMBP$^+$ whose export is blocked does fold into a stable conformation in the cytoplasm of SecB$^+$ cells (28). A very stable interaction between SecB and preMBP$^+$ would probably be strongly inhibitory to the export process. In fact, the export kinetics of wild-type MBP in SecB-overproducing cells are somewhat slower than those exhibited in haploid *secB*$^+$ cells (5, 20).

In a recent series of articles, Watanabe and Blobel (31–33) reported that SecB is a component of a previously characterized cytosolic export factor for preprotein import into inverted *E. coli* membrane vesicles in vitro. Most recently, they concluded that SecB binds to the signal peptide of a truncated MBP species and functions as a procaryotic equivalent of signal recognition particle (33). They were unable to demonstrate an interaction of SecB with the mature MBP or intact preMBP$^+$. We previously had concluded that SecB specifically interacted with the mature moiety of preMBP on the basis of the interference phenomenon described above that depleted cells of SecB, as well as the fact that alterations in the mature MBP moiety reduced the SecB dependence of the MBP for export (5). Gannon et al. (16) also concluded that it was the mature moiety of preMBP$^+$ that rendered this protein SecB dependent, and as mentioned above, Liu et al. (25) demonstrated that purified SecB could interact with

either purified mMBP$^+$ or mMBP2261 to retard folding. The experiments in this study demonstrated that SecB can specifically interact with MBP species from which the MBP signal peptide has been largely (MBPΔ323) or completely (MBPΔ2–26/2261) deleted. Furthermore, the interaction of SecB with MBP is not noticeably improved for those MBP species that retain the intact signal peptide (preMBP$^+$, preMBP2261, and preMBPΔ57–145). In fact, the association of SecB with preMBP$^+$ having an intact signal peptide was significantly less than its association with MBPΔ2–26/2261 lacking a signal peptide. It has been suggested that hydrophobicity may be one important element in binding of SecB to precursor proteins (23), so some interaction of SecB with the MBP signal peptide as assayed by Watanabe and Blobel (33) might not be unexpected. This question clearly requires additional investigation.

Finally, since various proteins are exported in a SecB-independent manner (e.g., RBP), does this mean that SecB does not interact with these proteins? We found that preRBP$^+$ was not specifically precipitated by anti-SecB serum. Furthermore, export-defective preRBP does not interfere with normal protein export in vivo (D. N. Collier, S. M. Strobel, and P. J. Bassford, Jr., unpublished data). These results suggest that there is some specificity to the interaction of SecB with precursor proteins. This view is also consistent with the data of Collier et al. (5) that identified a specific region of the mature MBP that interacted with SecB. On the other hand, Kumamoto (18) reported that some RBP synthesized in vivo could be bound to an anti-SecB affinity column. The interaction of SecB with preRBP species exhibiting altered folding properties, now under way in this laboratory, may provide additional insight into this question.

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