Involvement of SecB, a Chaperone, in the Export of Ribose-Binding Protein

JEONGHO KIM, YOUNGHEE LEE, CHANGHOON KIM, AND CHANKYU PARK*

Department of Life Science, Korea Advanced Institute of Science and Technology, Yuseong-Ku, Daejeon, Korea

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Ribose-binding protein (RBP) is an exported protein of Escherichia coli that functions in the periplasm. The export of RBP involves the secretion machinery of the cell, consisting of a cytoplasmic protein, SecA, and the integral membrane translocation complex, including SecE and SecY. SecB protein, a chaperone known to mediate the export of some periplasmic and outer membrane proteins, was previously reported not to be involved in RBP translocation even though small amounts of in vitro complexes between SecB and RBP have been detected. In our investigation, it was shown that a dependence on SecB could be demonstrated under conditions in which export was compromised. Species of RBP which carry two mutations, one in the leader that blocks export and a second in the mature protein which partially suppresses the export defect, were shown to be affected by SecB for efficient translocation. Five different changes which suppress the effect of the signal sequence mutation −17LP are all located in the N domain of the tertiary structure of RBP. All species of RBP show similar interaction with SecB. Furthermore, a leaky mutation, −14AE, generated by site-specific mutagenesis causes reduced export in the absence of SecB. These results indicate that SecB can interact with RBP during secretion, although it is not absolutely required under normal circumstances.

The ribose-binding protein (RBP) of Escherichia coli functions in the periplasm as a component of a high-affinity transport system for ribose (38) and as a primary receptor for bacterial chemotaxis toward ribose (1). RBP is exported by components involved in the translocation of other secreted proteins, such as maltose-binding protein (MBP), bacteriophage lambda receptor (LamB), and other periplasmic and outer membrane proteins. However, it has been shown that the translocation of RBP synthesized in the cytoplasm is independent of translocation of the export component SecB (5, 13, 15), although some interaction between SecB and RBP has been demonstrated in vitro (8, 12, 14).

SecB function was originally identified by a genetic defect in protein secretion. The protein exists as an oligomer with a monomeric subunit of 16.6 kDa (36), which appears to be required for the export of a subset of proteins that are secreted via a SecA and SecY (PrlA)-dependent pathway (9). The periplasmic protein MBP and outer membrane proteins LamB, OmpA, and OmpF are SecB dependent, whereas β-lactamase, lipoprotein, phage M13 coat protein, and RBP are SecB independent (13). SecB has been proposed to function as a molecular chaperone that maintains protein folding intermediates, perhaps structurally similar to the molten globule (21), by preventing the protein from attaining its final conformation (4). Other chaperones, for example, GroEL and Hsp70, are also known to be involved in the export of β-lactamase (3) and in the import of protein into the mitochondria (7), respectively. However, the cross-specificity of these proteins for their target substrates with respect to protein translocation appears to be limited (13, 17).

It is not yet clear how an interaction between a chaperone and an exported protein occurs. The best-studied example is the interaction between SecB and the precursor MBP. Several studies indicate that SecB interacts with the mature moiety of precursor MBP (8, 19, 29, 37). In contrast, Watanabe and Blobel (35) demonstrated that SecB binds to the signal sequence of precursor MBP but not to the mature portion. Other studies showed that both the signal and mature regions are required for the interaction with SecB (2). Export of proteins to the periplasm of E. coli requires that the polypeptides be maintained in a structure that is competent for translocation (4, 28). SecB, as a chaperone, is involved in the translocation of some exported proteins. However, previous observations indicated that export of wild-type RBP was not significantly affected when the secB gene was disrupted by a transposon insertion (15), even though some interaction between SecB and RBP could be demonstrated in vitro (8, 11).

To resolve these seemingly contradictory observations, we investigated the effect of SecB on RBP translocation in vivo. It was found that, under conditions in which export of RBP was less than optimal, a requirement for SecB could be demonstrated. Mutant RBPs carrying both a signal sequence mutation and a suppressor mutation in the mature region which partially restores export were significantly affected by SecB. It was also observed that when the translocation of wild-type RBP was suppressed by the presence of an uncoupler, carbonyl cyanide m-chlorophenylhydrazone (CCCP), it could be improved by the presence of SecB, suggesting that there is a secretory interaction between the RBP and SecB in vivo.

MATERIALS AND METHODS

Bacterial strains and genetic techniques. All bacterial strains used in this study are derivatives of E. coli K-12 (Table 1). The strain harboring secB::Tn5 was constructed by P1 transduction as described by Miller (22). P1 lysate was prepared from strain MM152 (secB::Tn5) and used to transduce strain CP626 to secB::Tn5. Kanamycin-resistant transductants were selected on H1 minimal medium (25) containing 0.4% glucose and 50 μg of kanamycin per ml and tested

* Corresponding author.
TABLE 1. Bacterial strains and plasmids

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<th>Strain or plasmid</th>
<th>Relevant characteristics*</th>
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**Plasmids**

pACYC184 cat tet (4.2 kb) pAI12 pACYC184 rbsB+ (8.1 kb) pAI24 pBR322 rbsB+ (4.7 kb) S. Park (10) pAI27 pAI12 rbsB103 (−17LP) 32 pHE100 pAI24 (−14AE) This study pHO7 pAI27 rbsB201 (50VE) 32 pHO19 pAI27 rbsB209 (75VF) This study pHO38 pAI27 rbsB202 (248AT) 32 pTS15 pAI27 rbsB202 (27AT) 32 pTS38 pAI27 rbsB212 (36VE) 32

* For rbsB alleles, the resulting change in RsbB is shown in parentheses (e.g., −17LP indicates a change from leucine to proline at position −17 in the protein).

Colonies that tested positive for ribose taxis due to the reversions were checked to see whether the suppressor mutation lay in the coding region for the signal sequence or the mature region of the rbsB gene. Plasmid DNAs, derivatives of pAI27 (8.1 kb, Table 1), were isolated from the suppressor strains, and the locations of suppressor mutations were determined by standard recombinant DNA techniques (32). Plasmids that were able to suppress the rbsB103 mutation were sequenced to determine their mutational changes.

DNA sequencing. Plasmid DNAs were purified by the polyethylene glycol method (20), and the nucleotide sequences were determined with the T7 polymerase kit from U.S. Biochemical Co. Three DNA primers, designed to cover the entire rbsB gene in approximately 300-bp intervals, were synthesized with a Pharmacia oligonucleotide synthesizer.

Osmotic shock treatment. A modification of the method of Neu and Heppel was used to obtain periplasmic RBP (24). Cells were grown to mid-log phase (approximately \(3 \times 10^8\) cells per ml) in M9 minimal salts medium containing chloramphenicol (30 µg/ml). Then 1.5 \(\times 10^8\) cells were harvested and washed twice with 30 mM NaCl. The pellet was suspended in 0.2 ml of 30 mM Tris-Cl (pH 7.3), and the suspension was mixed with 40% sucrose and 4 µl of 0.01 M EDTA and shaken occasionally for 10 min. Cells were again collected and rapidly resuspended in 0.5 ml of ice-cold 0.5 mM MgCl₂ solution with a micropipette. The suspension was stored on ice for 5 min. The shocked cells were removed by centrifugation, and the supernatant was stored for analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE and Western immunoblot analysis. SDS-PAGE was performed by the method of Laemmli (18) with a slight modification. Proteins separated on an SDS-12% polyacrylamide gel were transferred to a nitrocellulose filter (Schleicher & Schuell) (20). Rabbit anti-RBP was diluted 1:500 and used in conjunction with goat anti-rabbit immunoglobulin G-horseradish peroxidase conjugate (Bio-Rad) as a secondary antibody after dilution to 1:1,000 to 1:2,000. 4-Chloro-1-naphthol (Sigma Chemical Co.) was used as a substrate for the peroxidase.

Pulse labeling and immunoprecipitation. The pulse-labeling method of Ryan and Bassford (30) was used, as follows. Cells were grown to a density of \(10^8\) cells per ml in M9 minimal salts medium containing 0.4% glycerol, 0.01% thiamine, and 30 µg of chloramphenicol per ml at 35°C with vigorous shaking. The cells were radiolabeled for 1 min by the addition of 30 µCi of \([35S]\)methionine per ml. An equal volume of nonradioactive 0.75% methionine was subsequently added, and at the indicated times, 0.8 ml of culture was pipetted into 0.7 ml of 10% trichloroacetic acid.

For the experiments with CCCP, we followed the conditions used by Altman et al. (2). Cells were grown to a density of \(10^8\) cells per ml, and then CCCP was added at a final concentration of 50 µM and incubated for 1 min prior to pulse labeling. Samples were then pulse-labeled for 2 min with 30 µCi of \([35S]\)methionine per ml and chased with unlabeled methionine, added to a final concentration of 0.375% (wt/vol). After 1 min, β-mercaptoethanol was added to a final concentration of 0.05% (wt/vol), and at the indicated times, 0.8-ml samples were removed and processed by trichloroacetic acid precipitation as described above.

The samples were then harvested and washed with 0.5 ml of acetone. The pellets were suspended in 50 µl of 10 ml Tris-Cl (pH 8.0)–1 mM EDTA–1% SDS and solubilized by
boiling for 4 min. After centrifugation, 40 μl of supernatant was taken, mixed with 1 ml of 50 mM Tris-Cl (pH 8.0)–0.15 M NaCl–0.1 mM EDTA–2% Triton X-100, and stored overnight at 4°C. Pellets were obtained by centrifugation and resuspended in 150 μl of 0.5 M NaCl–0.5% Triton X-100–10 mM Tris-Cl (pH 8.0)–5 mM EDTA–1% SDS. Pellets were again obtained from the resuspension by centrifugation and washed with 150 μl of 10 mM Tris (pH 8.0). They were then analyzed by SDS-PAGE and autoradiography. Bands were sliced from the gels, and the relative band intensities were measured with an LKB 2202 Ultrascan laser densitometer with an LKB 2220 recording integrator, Berthold LB 2842 C Automatic TLC linear analyzer, or Beckmann LS 3801 scintillation counter.

Site-directed mutagenesis with oligonucleotides. Site-directed mutagenesis was performed by the method of Kunkel (16). Mutagenic primers were synthesized with a Pharmacia LKB Gene Assembler Plus DNA synthesizer, purified by 7 M urea–15% polyacrylamide gel electrophoresis, and separated on a Sep-Pak C18 reversed-phase column (20). An M13mp19 phage carrying the wild-type gene was constructed by inserting the 2.3-kb HindIII-Bel fragment of pA112 into the HindIII and BamHI sites in the multiple cloning region of M13mp19. A uracil-containing template was obtained by propagating phage in strain RZ1032 (ung dut). Mutational changes in all plasmids constructed from the mutant oligonucleotide primers were confirmed by DNA sequencing.

RESULTS

SecB and RBP export. Wild-type RBP is exported in the secB::Tn5 strain as efficiently as in the secB+ strain (Fig. 1A), as shown previously by Kumamoto and Beckwith (15).

FIG. 1. SecB-dependent translocation of RBP. Cells were pulse-labeled with [35S]methionine for 1 min and chased with unlabeled methionine. At the indicated times, samples were removed, immunoprecipitated, and analyzed by 15% polyacrylamide–SDS gel electrophoresis and autoradiography. The amount of exported protein was calculated as the percentage of total RBP (precursor plus mature) that was mature RBP, as described in detail in Materials and Methods. (A) Strain HO205 (secB+ rbsB*) was used as the secB+ host, and HO206 (secB::Tn5 rbsB*) was used as the secB mutant host. Precursor and mature forms of RBP are indicated as pre-RBP and mRBP, respectively. (B) Strain HO224 (secB+, –17LP/50VE) was used as the secB+ host, and HO213 (secB::Tn5, –17LP/50VE) was used as the secB mutant host. Precursor RBP carrying 50VE as well as –17LP is indicated as pre-RBP, and mature RBP carrying 50VE is indicated as mRBP.

Even early in the chase, virtually all RBP was processed regardless of whether SecB was present. Our conditions for examining RBP are basically the same as those used by Kumamoto and Beckwith (15) except that the cells were labeled for a longer period, 60 s. In the secB mutant, normal levels of RBP were exported to the periplasm (Fig. 2, lane 1). Likewise, the chemotactic responses to ribose that are mediated by RBP were normal (data not shown).

In contrast, export of the mutant RBP with two changes, –17LP (leucine [L] changed to proline [P] at residue –17 of the signal sequence) and 50VE, in both the signal and mature sequences was dependent on the presence of SecB (Fig. 1B). The 50VE mutation in the mature region was isolated by its ability to suppress the signal sequence defect –17LP (rbsB103 [10]) and was shown to reduce the rate of protein folding (32). Since no processing of precursor RBP was observed with the signal sequence mutation alone in either the presence or absence of SecB, the suppressor change in the mature protein makes it possible for SecB to improve export. The change which slows folding might make a binding site in the mature region accessible to SecB; alternatively, it is possible that the change in the mature protein creates a binding site for SecB. However, the latter possibility does not seem likely, since an extragenic suppressor can also render export of RBP SecB dependent. The extragenic suppressor, which was mapped in a location where there are no export-related genes (10a), of the export defect of the rbsB103 signal sequence mutation was tested for its effects on the translocation of RbsB103 protein in both a secB+ and secB mutant background. No export of the mutant RBP to the periplasm, as assessed by the ability of the strain to demonstrate a chemotactic response on a ribose swarm plate, was detected in the secB mutant strain (data not shown), whereas a chemotactic response was clearly present in a secB+ background. However, the presence of the prlA402 suppressor, a mutation in SecY, in a secB mutant host allowed the RbsB103 mutant to exhibit a ribose-tactic response. The reason for the absence of SecB dependence in the prlA402 suppressor mutant is probably that the suppressor efficiency of prlA402 is slightly better than that of the other extragenic suppressors, as judged by the amount of RBP found in the periplasm (10a). The level of periplasmic
RBP in the strains carrying extragenic suppressors was substantially lower than that in strains carrying the intragenic suppressors described here. These observations clearly established that *secB* affects RBP export in vivo, which led us to study this interaction further.

**Effect of SecB on RBP mutations isolated as suppressors of the signal sequence mutation.** The system for the isolation of intragenic suppressors employed here was previously described in detail by Teschke et al. (32). *rbsB103*, a mutation which changes leucine to proline at residue −17 of the signal sequence (−17LP), completely blocks the export of RBP into the periplasm. From the strain carrying the *rbsB103* mutation, we were able to isolate, by positive selection, a suppressor mutation which allows precursor RBP to be translocated and which functions as a transport component. From a strain carrying the *rbsB103* mutation on a plasmid, revertants were isolated on minimal medium containing 0.05% ribose. Most of the revertants were located in the region coding for the signal sequence, while some of them were found in the region encoding the mature portion of RBP. Five of the intragenic suppressor mutations described here in detail cause changes in the mature protein (Table 1).

Although their effects on the translocation of RbsB103 protein are variable, the suppressor alleles all result in accumulation of 5 to 20% of the wild-type level of RBP in the periplasm (Fig. 2). Within this range of periplasmic RBP levels, no difference in ribose-tactic response could be observed. Precursors were often observed in osmotic shock fluid (Fig. 2), probably owing to lysis of a small fraction of cells during the shock procedure. It is noted that both precursor and mature RBP with the suppressor mutations 50VE and 75VG were present in lower amounts than the RBPs coded by other suppressor species. This decreased amount likely reflects the instability of the proteins, which was observed in pulse-chase experiments. The label gradually disappeared during a long-term chase. In previous studies (32), it was shown that the suppressor mutants 50VE and 27AT have folding rates that are slower by 13- and 19-fold, respectively, than the folding rate of wild-type RBP. Thus, it seems likely that other intragenic suppressor mutants also have slower folding rates, although their folding properties have not yet been examined. Although the exact mechanism of suppressor exerted by a suppressor mutation in the mature region of RBP on the signal sequence defect is not well defined, it has been proposed (32) that retardation of folding in mutant RBP could increase the probability that the precursor is targeted to the translocation site in the cytoplasmic membrane before it folds into a site that is incompatible with translocation. Most of the suppressor changes, including the two already described, are located in the N-terminal region of mature RBP; 27AT in the first α-helix (helix I [23]), 36VE in the second β-sheet, 50VE in helix II, 75VG in helix III, and 248AT in helix IX, which is the only one located towards the carboxyl end of the sequence. All of the residues where the suppressor changes were found are in the N domain of the RBP three-dimensional structure (Fig. 3). Considering that the suppressor mutations were isolated spontaneously in random locations, their presence in only the N domain suggests that the folding of the N domain is more crucial for accessibility of the amino-terminal signal sequence to the translocation complex than that of the C domain.

In order to look at the SecB dependence of the suppressor proteins, we measured their export kinetics by pulse-chase experiments. In a secB<sup>+</sup> background, most suppressor proteins are processed to 47 to 53% of the level of the mature protein after a 10-min chase (Fig. 4). However, only about 23 to 38% of the precursors are processed in a *secB* mutant cell (Fig. 4). Regardless of the locations of the changes in the primary structure, there is not much difference in the efficiency of processing among the suppressor proteins, suggesting that the ability of the mutated proteins to interact with SecB does not depend on the particular residues which are altered. Replacement of the mutant signal sequence for the suppressor proteins −17LP/27AT and −17LP/50VE with the signal sequence of the wild type eliminated the requirement for SecB for efficient translocation (data not shown). It may be that SecB effects in these situations are masked due to an exceedingly high rate of translocation of precursor protein containing a wild-type signal sequence in both the secB<sup>+</sup> and secB background. This is in contrast to the observation made for MBP, that in secB mutant cells the export of mutant MBP carrying a suppressor mutation (Y283D) with a wild-type signal sequence was significantly more efficient than that of wild-type MBP.

**SecB effect on leaky signal sequence mutation.** Our next question was whether a mutation in the mature sequence is necessary for the export of a precursor to be affected by SecB. If the role of the suppressor is simply to provide a critical situation in which secB function can be manifested, a change in signal sequence which slows export might have a similar consequence. During our study, a new signal sequence mutation in RBP, −14AE, was created by site-directed mutagenesis. This signal sequence mutation causes
a leaky defect in protein translocation, so that the processing of precursor occurs at about 30% of the wild-type level after a 10-min chase. This property allowed us to examine its translocation process by a pulse-chase experiment that could not be done for the rbsB103 signal sequence mutation, since translocation of the protein was blocked almost completely. As shown in Fig. 5, a considerable defect in precursor processing was observed for the -14AE mutant in secB cells, indicating that the presence of SecB can facilitate RBP translocation even without a mutational change in the mature sequence. In secB cells, 10% of the total RBP carrying the -14AE mutation is processed, whereas 30% is translocated if wild-type SecB is present. An interesting feature here is that the export kinetics of the mutation reaches a plateau very early in the chase. Such a rapid initial rate was not observed for the suppressor RBP described previously. The difference might be due to the folding characteristics of the suppressor proteins in that alterations of the mature protein slow the folding to facilitate the translocation of RBP in an early chase period.

The role played by the signal sequence mutation -14AE in creating a situation in which SecB dependence is observed appears to be different from that of the suppressor mutation located in the mature protein. In previous reports (33), it has been suggested that the leader peptide is involved in more than one step of export. Two mutations in MBP, 14-1 (-13AE) and 18-1 (-9MR), allow the precursor to be associated with the cytoplasmic membrane even though it remains on the cytoplasm side (33). However, a mutant precursor, MalEΔ2-26, which lacks the signal sequence, abolished this association, which seems to be a step prior to translocation. Supposing that the role of the leader during entry into the membrane is to modulate the folding process of the precursor to assume a translocation-competent state that is recognized by the export machinery, an alteration in the leader that affects the folding property of precursor may also eliminate its membrane association. The MalEΔ2-26 deletion can be regarded as one such example, although the mutant protein is basically identical to the mature protein because of a deletion removing most of its signal sequence. In our previous work (32), we showed that the mutational change in precursor RbsB103, which causes a defect in export, does not alter the ability of the signal sequence to retard folding. If the rate of folding of the precursor protein is altered by the -14AE signal sequence mutation, we would predict that the altered precursor would fold faster than the wild type, which is indeed the reverse of what we have observed for intragenic suppressor proteins. This implies that the retardation of folding resulting from the intragenic suppressor mutation is not a requirement for interaction with SecB. We have also found that translocation of the -14AE mutant precursor is enhanced by the presence of the 36VE suppressor mutation (data not shown), and the export of this precursor carrying both mutations is dependent on SecB.

Translocation of wild-type RBP is affected by SecB in the presence of CCCP. Our results suggest that a change in neither the mature protein nor the signal sequence itself is directly responsible for creating an interaction with SecB. Rather, it seems that any constraints that reduce the efficiency of membrane translocation would allow us to observe...
SecB-dependent export of RBP. In other words, under circumstances in which the translocation process occurs at a very low efficiency, i.e., extremely rate limiting, we would probably see the effect of SecB. If this is the case, the SecB effect could be detected for the wild-type RBP when we apply a condition that inhibits the translocation process. Since dissipation of proton motive force is known to inhibit export in vivo, we examined the export of RBP after treatment with 50 μM CCCP used to block translocation and with β-mercaptoethanol used to resume translocation at slower rate. In the pulse-chase experiment with wild-type RBP, we observed a reduced rate of processing in the secB mutant host compared with the wild type host (Fig. 6). Although it is obvious that the export of wild-type RBP is improved by SecB, the magnitude of the difference in processing caused by SecB is smaller than that observed for a protein carrying a signal sequence mutation alone or with a suppressor change, as shown in previous experiments. We have also made an attempt to decrease the export rate by lowering the temperature to 25 and 17°C during the pulse-chase experiment. In both cases, processing still occurs fairly rapidly, to give more than 97% mature protein after a 30-s chase. No SecB effect was seen under those circumstances.

**DISCUSSION**

The fact that SecB affects the export rate of RBP has been demonstrated by several approaches. By using a mutant...
RBP defective in export, it was shown that RBP translocation was less efficient when SecB was not present. This might be due to the existence of not only the signal sequence mutation, which slows down the export rate of RBP and thus causes accumulation of more RBP in the translocation-incompetent conformation, but also the suppressor mutation in the mature region that restores translocation competency by retarding protein folding. At first, we assumed that the SecB effect might result from the mutational change in the mature portion of RBP, since previous reports (14, 15) failed to provide evidence for SecB interaction with wild-type RBP. However, the fact that proteins carrying suppressor changes, which occurred in five different locations in the mature region (Fig. 4), exhibited only marginal variations in SecB effect led us to suspect that these changes may not be primarily responsible for creating affinity-binding sites for SecB. Furthermore, it was shown that a leaky mutation in the signal sequence alone rendered the export of RBP dependent on SecB, and when the export rate of wild-type RBP was greatly reduced by disrupting the proton motive force (39), export also became SecB dependent.

These results indicate that neither a change in the signal sequence nor a change in the mature region is absolutely necessary for interaction with SecB. It appears that any type of constraint imposed upon the translocation process could provide a situation in which a SecB effect can be found. In this regard, CCCP, a signal sequence mutation, and even a secA(Ts) mutation seem to play similar roles in blocking the translocation process. They all result in an accumulation of precursor protein in the cytosol, increasing a chance for the precursor to interact with SecB. In fact, it was observed that turnover of the SecB-pre-MBP complexes was significantly slower when CCCP was present (12). Kumamoto and Beckwith (14) noticed that there was more accumulation of pre-RBP in the secA(Ts) secB double mutant than in the secA(Ts) mutant, which could also be interpreted as a manifestation of the SecB effect. A similar interpretation might be given to the −14AE signal sequence mutation, for which we observed a SecB effect. However, in the case of the rbsB103 mutation, which blocks more than 98% of the wild-type level of export, the secondary mutation residing in the mature protein was required for the precursor RBP to be processed. An increase in translocation efficiency caused by the suppressor mutation could be explained on the basis of a kinetic partitioning (8) between folding of the pre-RBP in the cytoplasm and productive translocation. As reported previously (32), the two suppressor mutant proteins which were examined both showed slower folding than wild-type RBP. According to the model, it appears that the suppressor mutations increase the translocation competency of the precursor by slowing the spontaneous folding of the precursor inside the cytosol. Therefore, the suppressor mutation is thought to indirectly lessen the degree of translocation blockage caused by a tight signal sequence mutation like rbsB103. We suspected that the suppressor mutation might also alter the interaction with the chaperone, since it changes the folding characteristics of RBP. However, the data indicate that such alteration is not likely to occur to a significant extent, since a mutant RBP with the leaky signal sequence mutation but without a mutation in the mature protein exhibited a degree of SecB dependency similar to that found among the suppressor proteins. Yet we are not able to exclude the possibility of an increase in SecB interaction with the suppressor proteins in a nonspecific manner due to the presence of mutational changes. Indeed, the differences in the export rates of suppressor mutants (Fig. 4) in secB+ and secB strains are slightly greater than that seen in the wild type treated with CCCP (Fig. 6), although the −14AE mutant gave an increase similar (Fig. 5) to that with the suppressor proteins.

The intragenic suppressors described here all appear to behave as folding attenuators in correcting the export defect. Like the previously reported suppressor mutations 27AT and 50VE (32), three more changes, 36VE, 75VG, and 248AT, identified in this work, occur at sites buried inside the radius of the signal sequence, since they are adjacent to a hydrophobic interior or interact with nearby hydrophobic residues, mostly isoleucines. It seems likely that they play a crucial role in the formation of a hydrophobic core in the folding pathway of RBP. Even though the five suppressors are evenly distributed in the secondary structural units involved in the packing of the N domain (Fig. 3), their effects likely involve an alteration in the rate-limiting step of the folding pathway leading to the formation of the N domain.

As a theoretical approach toward understanding the role of SecB in the translocation process, we have made an attempt to formulate a quantitative model which could explain our pulse-chase results (Fig. 4). The processing of precursor RBP as it is translocated across the cytoplasmic membrane has been treated as a simple dilution of precursor molecules which disappear from the cytoplasm at a certain constant rate. To describe the behavior of precursor RBP, two kinetic aspects were taken into consideration by adopting the coefficients $k_e$ for export and $k_f$ for folding of the precursor RBP. A precursor molecule synthesized by the ribosome in the cytosol is postulated to have two different fates: to be processed to produce the mature form for release into the periplasm after translocation or to remain in the cytoplasm as stably folded proteins. Involved in the former process are proteins such as SecA, SecY, and sometimes SecB, known to constitute a secretory pathway. This process, although it occurs through multiple steps, proceeds at a certain rate, characterized here by the rate constant $k_e$. During or before entering this process, a newly synthesized precursor tends to fold in the cytosol and fails to be translocated, which is represented collectively as $k_f$, a coefficient of folding. Such an abortive folding of the precursor may generate a mature protein inside the cell by nonspecific degradation of signal peptide. However, since degradation occurs very slowly in our experimental conditions, we ignored it for the sake of simplicity. An essential feature of the model is incorporated into two differential equations: the formation of mature protein over time is formulated as $d[P]/dt = k_e[P]$, and the disappearance of precursor is formulated as $-d[P]/dt = (k_e + k_f) \times [P]$, where $m$ and $P$ refer to mature and precursor proteins, respectively. Solving these equations yielded the following relationship, $m = [k_e/(k_e + k_f)] \times [1 - e^{-k_f \times t}] \times P_o$, where $P_o$ is the amount of precursor synthesized, relating the formation of mature protein to time after the chase. In Fig. 7, the data points acquired for one of the suppressor proteins are displayed with a theoretical curve that was fitted by adjusting the values for the coefficients $k_e$ and $k_f$ according to the above equation. The result indicates that the theoretical equations based upon our assumptions explain the experimental data nicely. From the calculation of coefficients, it is noted that the presence of SecB considerably increases the rate constant of export, two- to threefold, compared with a small increase in the folding coefficient. This suggests the role of SecB as an active component of the secretory process leading to proficient export of secretory protein. However, SecB does not seem to significantly reduce the probability of...
Supporting evidence was obtained by an experiment in which anti-SecB antiserum was used to pull down the complex between SecB and MalE2261 proteins (37). Whether RBP suppressor mutants are better able to bind SecB still remains to be answered. In fact, there are some discrepancies in the properties regarding SecB interaction between MBP and RBP. MBP translocation is known to be SecB dependent in that its processing is substantially reduced in the absence of SecB protein, yet some portion of pre-MBP can be processed without SecB. For RBP, the vast majority of precursors seem to be exported without the aid of SecB, and thus it has been regarded as SecB independent; yet RBP can interact with SecB, which is apparent in the critical situations described here. Therefore, the concept of SecB dependency must be applied in relative terms. In other words, interaction with SecB is not an absolute requirement but increases the efficiency of export. Thus, the critical role of SecB is manifested in particular physiological conditions which influence the effectiveness of membrane traffic. An interesting conjecture is that cells could change their repertoire of secreted protein by modulating the relative amount of each chaperone, such as SecB, GroEL, etc. This might explain why the specificity of chaperones for secreted proteins is not rigid (7, 13, 17). An extreme case is that when the DnaK chaperone is overproduced, even a fusion protein between LamB and LacZ which is normally confined to the cytoplasm is exported to the periplasm (26). It appears that the export of RBP is not affected by DnaK (7a).

The study of RBP-MBP hybrid proteins, aiming at comparison of export characteristics between RBP and MBP (5), revealed that a determinant for SecB interaction resides in the mature portion of MBP but not in the same part of RBP even with an MBP signal. That experiment repeatedly confirmed that there is very little, if any, SecB interaction in RBP export in vivo, since it was not detectable. They also demonstrated that the export defect of the −17LP mutant, but not that of the −14AR or −11LK mutant, could be suppressed by the prlA402 mutation in SecY, and the efficiency of the suppressor mutation was not diminished in SecB− cells. According to Trun et al. (34), prlA-mediated suppression requires SecB function, which argues against an observation made by Collier et al. (5). Our finding on the existence of an RBP-SecB interaction appears to resolve this puzzle.

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