Overproduction of SecA Suppresses the Export Defect Caused by a Mutation in the Gene Encoding the *Escherichia coli* Export Chaperone SecB

HEATHER A. COOK† AND CAROL A. KUMAMOTO*

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111

Received 10 November 1998/Accepted 1 March 1999

SecB is a cytosolic protein required for rapid and efficient export of particular periplasmic and outer membrane proteins in *Escherichia coli*. SecB promotes export by stabilizing newly synthesized precursor proteins in a nonnative conformation and by targeting the precursors to the inner membrane. Biochemical studies suggest that SecB facilitates precursor targeting by binding to the SecA protein, a component of the membrane-embedded translocation apparatus. To gain more insight into the functional interaction of SecB and SecA, in vivo, mutations in the secA locus that compensate for the export defect caused by the secB missense mutation secBL75Q were isolated. Two suppressors were isolated, both of which led to the overproduction of wild-type SecA protein. In vivo studies demonstrated that the SecBL75Q mutant protein releases precursor proteins at a lower rate than does wild-type SecB. Increasing the level of SecA protein in the cell was found to reverse this slow-release defect, indicating that overproduction of SecA stimulates the turnover of SecBL75Q-precursor complexes. These findings lend additional support to the proposed pathway for precursor targeting in which SecB promotes targeting to the translocation apparatus by binding to the SecA protein.

In the gram-negative bacterium *Escherichia coli*, proteins destined to be localized to the periplasmic space or outer membrane are transported out of the cytoplasm and through the inner membrane via the general export pathway (10, 16). Translocation of proteins across the inner membrane is catalyzed by the preprotein translocase, a multisubunit enzyme complex consisting of the SecA, SecY, SecE, SecG, SecD, SecF, and YajC proteins (17). The core of the translocase consists of an integral domain composed of the SecY, SecE, and SecG proteins and a peripheral domain composed of a dimer of SecA. SecA associates with the membrane through an affinity for acidic phospholipids and for the SecY subunit of the translocase (13, 60). SecA, SecY, and SecE are sufficient for translocation into proteoliposomes reconstituted with purified Sec components (3, 48, 51), although in the absence of the other components of the translocase, translocation is very inefficient (17, 49, 52).

SecA is an ATPase that is found both in the cytoplasm and associated with the inner membrane (4, 38). Cytosolic SecA functions as a repressor of its own translation (12, 57, 58). When SecA is bound to the SecYEG subunits of the translocase, acidic phospholipids and a precursor protein, SecA becomes fully active as an ATPase (39). SecA couples the energy from ATP binding and hydrolysis to protein translocation through repeated cycles of ATP-driven membrane insertion and deinsertion (18).

The initial step in the export process is delivery of the precursor protein to the inner membrane. A number of soluble cytosolic factors, including SecB, GroEL, GroES, DnaK, DnaJ, and the *E. coli* signal recognition particle, are involved in targeting precursors to the membrane (9, 29, 35, 62). Mutations affecting these components result in defective export of subsets of secreted proteins.

The SecB protein is required for efficient export of particular proteins to the periplasmic compartment and outer membrane of *E. coli* (29, 31). In vivo, SecB binds to nascent and fully elongated species of protein precursors (33) and stabilizes them in a nonnative conformation that is essential for translocation across the cytoplasmic membrane (6, 27). In the absence of SecB, export is much slower than in wild-type strains, and a significant amount of precursor protein folds into an export-incompetent conformation. In the case of the SecB ligand pre-maltose binding protein (preMBP), 25% of the protein fails to be exported (34). In addition, in the absence of SecB, export of MBP is completely posttranslational, indicating that SecB is required for cotranslational processing of preMBP (34). These data demonstrate that SecB plays a role in modulating the folding of precursor proteins and, in addition, is required for rapid targeting of precursors to the membrane.

Biochemical analyses suggest that SecB facilitates the targeting of precursor proteins to the translocation apparatus by binding to the SecA protein. Purified soluble SecA interacts with SecB with low affinity in vitro (11, 25). In contrast, SecB binds with high affinity to inner membrane vesicles in a SecA-dependent manner, and the high-affinity binding of SecB is promoted by precursor proteins (25).

Removal of the last 70 amino acids of SecA abolishes the ability of SecA to mediate high-affinity binding of SecB and SecB-PhoE precursor complexes to inner membrane vesicles, suggesting that SecB binds the carboxy terminus of SecA (2). More recently, direct binding of SecB to the C-terminal 22 amino acids of SecA has been demonstrated (20). Expression of a truncated SecA protein missing 66 amino acids of the C terminus reduces the export efficiency of SecB-dependent proteins in vivo (53). Interestingly, export of a SecB-independent protein was not affected by this truncation. Taken together, these results indicate that the C terminus of SecA is required for SecB binding and that efficient targeting of precursor proteins by SecB requires a functional SecB binding site on SecA.
Mutational studies have been used to identify specific residues important for SecB function (22, 28). Amino acid substitutions at Leu-75 or Glu-77 result in a strong defect in the rate of precursor proteins (28). SecBL75Q and SecBE77K are capable of binding unfolded MBP and blocking its refolding in vitro (22). SecBL75Q and SecBE77K are unable to support SecA-dependent membrane binding of the precursor protein proOmpA in vitro due to a defect in SecA binding (19). Thus, these residues may be involved in the formation of a SecA binding site on SecB.

To gain more insight into the SecB-SecA protein interaction, mutations in the secA gene that improve export of MBP in a strain containing the secB missense mutation secBL75Q were isolated. Two suppressor mutations were isolated, and both were found to lead to overproduction of the SecA protein. The effect of overproduction of SecA on the binding and release of precursor proteins from the SecBL75Q mutant protein was analyzed. Precursors were found to be released from SecBL75Q much more slowly than from wild-type SecB. Overproduction of SecA was found to reverse the slow-release defect caused by the secBL75Q mutation. These in vivo results lend additional support to the biochemical data, which indicate that the interaction between SecB and SecA is critical for efficient protein export.

### MATERIALS AND METHODS

**Bacterial strains, phages, and plasmids.** The *E. coli* strains used for these studies are listed in Table 1. Generalized transduction with phage P22 was performed as described previously (46). The plasmids pMF8 and pT7-secA (58) were obtained from Don Oliver, pSR47 (45) and strain SR202 were obtained from Ralph Isberg, pHaeEcEYG (13) was obtained from Bill Wickner, and pBAD22 (24) was obtained from Jon Beckwith.

**Bacterial growth.** L broth, L agar, maltose tetrazolium agar, and M63 and M9 media were prepared as described previously (41). In experiments where the SecYEG complex was overexpressed from plasmid pHAsecEYG, cells were grown in M63 minimal medium containing 0.4% glucose (no methionine or cysteine) as described previously (8). When supplemented with thiamine (5 μg/ml) and 18 amino acids (1/100 of stock) at 37°C, cells were pelleted, washed twice in M63 salts (at 37°C), resuspended in M63 minimal medium containing 0.4% arabinose and ampicillin (at 37°C), and grown for 4 h at 37°C.

**Localized mutagenesis and isolation of Mal** T suppressor mutations. Cells of strain HAC12 (secBL75Q::Tn5 malT10-1 malE10-1) were treated with nitrosoguanidine (62.5 μg/ml) as described previously (59). The mutagenized cells were split into pools. P1vir was prepared on each pool and used to transduce cells of strain OF133 [secBL75Q malT10-1 malE10-1] as described previously (59). Tetracycline-resistant (Tc r) transductants were selected on minimal agar containing tetracycline at 37°C. Tc r transductants were pooled, grown overnight in liquid medium, and plated on minimal maltose agar plates containing tetracycline and sodium citrate. Mal T+ colonies from individual plates were pooled, a P1vir lysate was prepared, and the phage was used to transduce OF133 cells. Tc r transductants were selected and analyzed as described above. Pools containing suppressor mutations showed approximately a 50-fold enrichment of Mal T+ colonies. Individual Mal T+ colonies were purified on minimal medium containing tetracycline, and linkage to the secA locus was analyzed by P1 transduction with the recipient OF133. Approximately 35,000 Tc r transductants were analyzed for growth on maltose by using the enrichment procedure described above.

**Pulse-chase analysis of protein export.** Cells (2 × 10⁹ to 3 × 10⁹ cells/ml) were pulse-labeled for 15 s with Tran35S-label (ICN) (10 μCi/ml at 37°C). The incorporation of label was terminated by the addition of nonradioactive methionine (100 μg/ml) and chloramphenicol (0.5 mg/ml). At various chase times, 1-ml samples were taken and precipitated with trichloroacetic acid (5% final concentration) on ice. MBP and OmpA were immunoprecipitated as previously described (34) with IgSORB (New England Enzyme Center, Inc., Boston, Mass.).

**SDS-PAGE and fluorography.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Laemmli (36). The gels were processed for fluorography with either sodium salicylate (5) or diphenyl oxazole (1).

**Genetic mapping and DNA sequence analysis of suppressors.** DNA manipulations and bacterial transformations were as described previously (41). For mapping the suppressor mutations, DNA fragments encompassing the 5' end of the geneX-secA operon from the end of the envA gene to the SalI restriction site in secA were amplified by PCR (47) from boiled colonies and cloned into pSR47, a suicide plasmid encoding kanamycin resistance (45). To determine whether the amplified fragments contained the mutations, the resulting plasmids were transformed into HAC14 (secBL75Q malE10-1 secA*), and HAC214 (secBL75Q malE10-1 secA1180) or HAC215 (secBL75Q malE10-1 secA4250) where appropriate. Transformants containing plasmid integrations at the secA locus were isolated and tested for growth on minimal maltose medium. The results from these experiments indicated that the lesion conferring Mal T+ in secA4250 mutants was located in the region encompassed by the 5' fragment. To map the mutation more finely, subclones of 5' secA plasmids containing DNA fragments from the end of envA to the EcoRI site in geneX or to the NcoI site in secA were generated. The subclones were tested as described above. Both subclones derived from...
secA1180 conferred Mal⁺ when introduced into strain HAC14 (secA⁺ Mal⁺), indicating that the secA4250 mutation was contained within the 463-bp fragment upstream of the secA gene. This fragment was sequenced, and a single-nucleotide substitution of adenosine for guanine was observed 3 nucleotides upstream of the translational start of geneX.

Attempts to map the secA1180 mutation were unsuccessful. Therefore, the entire geneX-secA region was amplified from the chromosome of HAC214 (secA1180) by PCR and sequenced by the Tufts University sequencing facility. No nucleotide changes were observed, suggesting that the lesion lay outside the region that was analyzed.

Preparation of cell lysates and immunoblotting. For quantitation of total SecA, cell lysates were grown in L broth to an $A_{600}$ of 0.75 to 1.0. The cultures were chased with nonradioactive methionine and chloramphenicol. Samples were taken at the time points indicated, immunoprecipitated with anti-MBP antiserum, and analyzed by SDS-PAGE (10% polyacrylamide) and fluorography as described in Materials and Methods. The positions of preMBP and mature MBP are indicated.

secA4250 conferred Mal⁺ when introduced into strain HAC14 (secA⁺ Mal⁺), indicating that the secA4250 mutation was contained within the 463-bp fragment upstream of the secA gene. This fragment was sequenced, and a single-nucleotide substitution of adenosine for guanine was observed 3 nucleotides upstream of the translational start of geneX.

Results

Isolation of suppressors. MBP is required for the uptake and utilization of maltose as a carbon source. SecB facilitates the export of MBP to the periplasm, and mutations in the secB gene, including the missense mutation secBL75Q, result in a kinetic export defect for MBP. However, posttranslational export of MBP occurs in secB mutants, allowing these strains to grow on maltose. Mutations altering the signal sequence of the gene encoding MBP (malE) also compromise MBP export, but, as seen with secB mutants, malE signal sequence mutants are able to utilize maltose. The presence of both secB and malE signal sequence mutations abolishes MBP export, and the double-mutant cells are unable to grow on maltose (21). In this study, the Mal⁺ phenotype of the secBL75Q malE10-1 double-mutant strain was used in a selection for secA suppressors of the secB missense mutation.

Localized nitrosoguanidine mutagenesis of the secA gene was used to isolate suppressors that enabled strain OF133 (secBL75Q malE10-1) to grow on minimal maltose medium (described in Materials and Methods). Linkage of the suppressor mutation to the secA locus was demonstrated by P1 transduction. Twelve Mal⁺ isolates which carried suppressor mutations linked to the secA gene were obtained.

To determine whether any of the mutations affected the export defect caused by the secBL75Q mutation, MBP export in derivatives of strain CK2163 (secBL75Q malE⁺) was analyzed by pulse-chase labeling experiments. Cells were incubated with Tran³⁵S-label (10 μCi/ml) for 15 s, and the chase was initiated by the addition of nonradioactive methionine and chloramphenicol. Samples were removed at various times and processed for immunoprecipitation with anti-MBP antiserum as described in Materials and Methods. The samples were analyzed by SDS-PAGE and fluorography (Fig. 1A). In wild-
type (secB') cells, export was very rapid, and the majority of MBP was exported during the 15-s pulse. By contrast, only 6% of the MBP was exported in the secBL75Q mutant strain during the pulse. Two strains, HAC216 (secBL75Q secA1180) and HAC217 (secBL75Q secA4250), containing suppressor mutations, showed a reversal of the secBL75Q defect. In both HAC216 (secBL75Q secA1180) and HAC217 (secBL75Q secA4250), the majority of MBP was exported during the pulse (72 and 93%, respectively), and MBP was completely exported by the 30-s chase point. The secA4250 mutation consistently appeared to have a stronger effect than secA1180. Radiolabeling experiments performed with strains carrying the malE10-1 signal sequence mutation and wild-type secB indicated that secA1180 and secA4250 did not suppress the signal sequence mutation (data not shown). Thus, these mutations enabled strain OFl133 (secBL75Q malE10-1) to grow on maltose by suppressing the secBL75Q export defect. The remaining mutants contained suppressors of the malE signal sequence mutation.

Pulse-chase labeling experiments analyzing MBP export in secB::Tn5 strains indicated that both suppressors could improve export in the absence of SecB (data not shown). Although the suppression of the secB::Tn5 defect was very weak, this result indicated that both secA1180 and secA4250 were not allele-specific suppressors of the secBL75Q mutation.

To determine whether HAC214 (secA1180) and HAC215 (secA4250) contained lesions in the secA gene, the region of the secA locus from the end of the envA gene to the end of the secA gene was subjected to genetic mapping experiments and DNA sequence analyses (described in Materials and Methods). The results of these experiments indicated that both suppressors contained lesions outside the secA structural gene (summarized in Materials and Methods).

Overproduction of wild-type SecA protein suppresses secBL75Q. Since the suppressor mutations mapped outside the secA gene, we hypothesized that these mutations might affect secA expression. To determine whether the secA1180 and secA4250 mutations affected the amount of SecA protein synthesized, the total amount of SecA in HAC216 (secA1180) and HAC217 (secA4250) mutant cells was determined. Cells of strains HAC15 (secA'), HAC216 (secA1180), and HAC217 (secA4250) were extracted as described in Materials and Methods. Ten micrograms of total cellular protein was resolved by SDS-PAGE and analyzed by immunoblotting with anti-SecA antiserum. Figure 1B shows that the extracts from the suppressor strains contained more SecA protein than the control extract. Quantitative immunoblotting of dilution series demonstrated that extracts from secA1180 and secA4250 strains contained 2- to 3-fold and 12-fold (n = 2) more SecA, respectively, than extracts from control cells. These data indicated that both mutants were overproducing SecA protein.

To test whether overproduction of wild-type SecA was responsible for the suppressor phenotype, MBP export in the SecA-overproducing strain HAC49 (secBL75Q pMF8 [secA']) and the control strain HAC49 (secBL75Q pBR322 [secA]) was analyzed. pMF8 contains the geneX-secA-mutT operon under control of the operon's natural promoter and results in approximately eightfold overproduction of SecA (data not shown) (58). Figure 1C shows that MBP was exported at a higher rate in the strain containing the SecA-overexpressing plasmid, pMF8, than in the secBL75Q strain with pBR322 (compare lanes a and d). Also, the kinetics of MBP export in strains carrying the plasmid pT7-secA, which contains only the secA gene under control of the φ10 promoter of phage T7, also demonstrated suppression of the export defect caused by secBL75Q (data not shown). Thus, overproduction of SecA alone is sufficient for suppression of secBL75Q.

Overexpression of the SecAEG complex fails to suppress secBL75Q. To determine whether overexpression of other components of the translocation apparatus would suppress the secBL75Q defect, the SecY-SecE-SecG complex (SecYEY) was overexpressed from plasmid pHaseYEY. Overproduction of the SecYEY complex has been shown to increase the amount of functional translocation sites in the membrane and to enhance SecA-dependent translocation in vitro (13, 61). Cells of strains HAC82 (secB' ara') and HAC83 (secBL75Q secA') were transformed with plasmid pHaseYEY or vector pBAD22. pHaseYEY contains the genes for an epitope-tagged SecE, SecY, and SecG under control of the araBAD (arabinose) operon. For overexpression of SecYEY, cells were grown in M63 minimal medium containing 0.4% arabinose and supplemented with ampicillin and 18 amino acids (no cysteine or methionine) at 37°C. After 4 h of induction, samples were taken for analysis of total SecY and SecE by immunoblotting and for analysis of OmpA export (described in Materials and Methods). After 4 h of growth in the presence of arabinose, both SecE and SecY were overproduced at a very high level in pHaseYEY-containing cells (Fig. 2A, lanes b and d). This high level of overexpression did not affect the growth of secB' strains. However, it did dramatically slow the growth of the secBL75Q strain and interfered with the incorporation of label (Fig. 2B, lanes g to i). Nevertheless, as shown in Fig. 2B, overexpression of SecYEY did not interfere with the export of OmpA. OmpA was exported with similar kinetics in strains HAC152 (secBL75Q pBAD22) (Fig. 2B, lanes g to i) and HAC98 (secBL75Q pHaseYEY) (Fig. 2B, lanes j to l). Thus, increasing the number of translocation sites did not suppress the secBL75Q export defect.

Overproduction of SecA improves release of precursor polyepitides from SecBL75Q. Previous studies have shown that substitutions at Leu-75 in SecB, which result in defective MBP export, do not disrupt SecB-preMBP complex formation in vivo (28). Therefore, the secBL75Q mutant is most likely defective in a step subsequent to SecB-precursor complex formation. One possibility is that the SecBL75Q protein may not release precursor proteins normally, resulting in slow turnover of SecB-prefector complexes. To test this hypothesis, polypeptide binding and release from SecB were analyzed. Cells of strains HAC50 (secB'/pBR322) and HAC52 (secBL75Q/pBR322) were incubated with Tran35S-S-label for 15 s, a cytoplasmic extract was prepared, and the extract was subjected to anti-SecB affinity chromatography as described in Materials and Methods. Proteins bound to the column were analyzed by SDS-PAGE and fluorography. After a 15-s pulse-labeling, SecB and proteins bound to SecB were observed in the anti-SecB-bound fraction from extracts of wild-type (secB') cells (Fig. 3, lane a) and mutant (secBL75Q) cells (Fig. 3, lane d). Previous studies demonstrated that the SecB-bound proteins are nascent and fully elongated precursors of LamB, MBP, and the major outer membrane proteins OmpA and OmpF (33). Thus, SecBL75Q formed complexes with SecB-dependent proteins in vivo.

In wild-type cells, when the label was chased with nonradioactive methionine, radiolabeled SecB was observed in the anti-SecB-bound fractions, but SecB-bound precursors, such as pre-
LamB, preMBP, and proOmpA, rapidly disappeared. After 1 min of chase, precursors were barely detectable in the bound fraction (Fig. 3, lane b), and after 2 min, radiolabeled precursors were not observed in the bound fraction (Fig. 3, lane c), indicating that the precursors had been released from SecB. In contrast, when an extract of secBL75Q cells was analyzed, large amounts of radiolabeled precursors were observed in the SecB-bound fraction after 1 min of chase (Fig. 3, lane e). Even after 2 min of chase, a significant amount of preLamB and a small amount of radiolabeled proOmpA and preMBP were bound to SecBL75Q. Thus, the secBL75Q mutation results in slower release of precursors from the mutant SecB protein.

To determine whether overproduction of SecA would reverse the slow-release defect caused by secBL75Q, precursor binding and release were analyzed in strain HAC53 (secBL75Q/pMF8). Analysis of radiolabeled cells demonstrated that after a 15-s pulse-labeling, preLamB, preMBP, and proOmpA were observed in the SecB-bound fraction after 1 min of chase (Fig. 3, lane e). Even after 2 min of chase, a significant amount of preLamB and a small amount of radiolabeled proOmpA and preMBP were bound to SecBL75Q. Thus, the secBL75Q mutation results in slower release of precursors from the mutant SecB protein.

The results of this study demonstrate that overproduction of wild-type SecA protein reverses the defect caused by the secB missense mutation secBL75Q. Overproduction of SecA did not lead to a bypass of the SecBL75Q protein. In cells with normal amounts of SecA, nascent precursor proteins were bound by SecBL75Q but were released at a significantly lower rate than from wild-type SecB. However, when SecA was overproduced eightfold, precursors dissociated from SecBL75Q at close-to-wild-type rates. These findings lend additional support to the biochemical studies which indicate that binding of SecB to SecA is critical for efficient protein export.

SecB promotes rapid export by maintaining precursor proteins in a translocation-competent conformation (6, 54) and by facilitating the delivery of precursors to the translocation machinery via the SecA protein (25). In general, substitutions at Leu-75 in the SecB protein result in a strong export defect, yet changes at this position do not disrupt complex formation between SecB and precursor proteins (28). Furthermore, the SecBL75Q mutant protein exhibits enhanced activity in blocking the folding of unfolded MBP in vitro (22). These results...
mass action. Most likely improves the rate at which precursors are released between SecB and SecA. Therefore, overproduction of SecA cursor complexes (Fig. 3, lanes d to f). Increasing cellular SecA limiting, resulting in the accumulation of cytosolic SecB-pre-

The interaction between SecB and SecA, causing a defect in precursor targeting. The finding that precursors are bound by SecBL75Q but are released at a low rate in vivo indicates that in strains in which the interaction of SecB and SecA is defective, targeting of precursors to the membrane becomes rate-limiting, resulting in the accumulation of cytosolic SecB-pre-cursor complexes (Fig. 3, lanes d to f). Increasing cellular SecA levels would be expected to promote complex formation between SecB and SecA. Therefore, overproduction of SecA most likely improves the rate at which precursors are released by SecBL75Q by improving the SecB-SecA interaction through mass action.

Consistent with the notion that secBL75Q disrupts the SecB-SecA interaction, overexpression of the SecY, SecE, and SecG proteins from a multicopy plasmid did not suppress the export defect of the secBL75Q mutant. Although inner membrane vesicles prepared from cells overexpressing SecYEG show enhanced translocation ATPase and protein translocation activ-

FIG. 3. Defective release of precursors from SecBL75Q is suppressed by overproduction of SecA. Cells were grown in M63 minimal maltose-glycerol medium containing ampicillin. Cells were pulse-labeled with Tran 35S-label for 15 s, and the label was chased with nonradioactive methionine. Samples were taken after the pulse and after 1 and 2 min of chase. Cells were extracted, and the extract was subjected to anti-SecB affinity chromatography as described in Materials and Methods. Proteins bound to the column were eluted and analyzed by SDS-PAGE (12.5% polyacrylamide) and fluorography. Samples were as follows: lane a, HAC50 (secB::pBR322), 15 s pulse; lane b, HAC50, 1-min chase; lane c, HAC50, 2-min chase; lane d, HAC52 (secBL75Q::pBR322), 15 s pulse; lane e, HAC52, 1-min chase; lane f, HAC52, 2-min chase; lane g, HAC53 (secBL75Q::pMF8), 15 s pulse; lane h, HAC53, 1-min chase; lane i, HAC53, 2-min chase. The numbers at the right are molecular weight markers (in thousands). The mobilities of SecB (B), proOmpA (O), preMBP (M), and preLamB (L) are indicated on the left.

suggest that in vivo SecBL75Q is defective at a step in the export pathway that comes after precursor binding.

Biochemical studies suggest that efficient precursor targeting involves binding of SecB to membrane-bound SecA and that the SecBL75Q mutant is defective in binding SecA in vitro (19). Consistent with in vivo studies (28), Fekkes et al. found that purified SecBL75Q could bind the precursor proOmpA but was defective for in vitro translocation and had a lower affinity for membrane-bound SecA than wild-type SecB (19). These data suggest that the secBL75Q mutation disrupts the interaction between SecB and SecA, causing a defect in precursor targeting. The finding that precursors are bound by SecBL75Q but are released at a low rate in vivo indicates that in strains in which the interaction of SecB and SecA is defective, targeting of precursors to the membrane becomes rate-limiting, resulting in the accumulation of cytosolic SecB-pre-cursor complexes (Fig. 3, lanes d to f). Increasing cellular SecA levels would be expected to promote complex formation between SecB and SecA. Therefore, overproduction of SecA most likely improves the rate at which precursors are released by SecBL75Q by improving the SecB-SecA interaction through mass action.

Consistent with the notion that secBL75Q disrupts the SecB-SecA interaction, overexpression of the SecY, SecE, and SecG proteins from a multicopy plasmid did not suppress the export defect of the secBL75Q mutant. Although inner membrane vesicles prepared from cells overexpressing SecYEG show enhanced translocation ATPase and protein translocation activ-

itiess (13), increasing the number of functional translocation sites had no effect on the secBL75Q defect in vivo. Thus, enhancement of steps downstream of the SecB-SecA interac-

Overexpression of SecYEG would be expected to lead to an increase in the amount of SecA bound to SecYEG at the membrane, since there is approximately 10-fold more SecA than SecYEG complexes in cells (14). However, as just discussed, overproduction of SecYEG does not suppress the export defect of secBL75Q mutants. SecB-precursor complexes may be bound by SecA in the cytoplasm prior to targeting, as has been previously suggested (26), and not by SecA bound to SecYEG. Overproduction of SecA has been shown to lead to an increase in cytosolic SecA (reference 4 and unpublished results), and this population may be responsible for suppression.

Overproduction of SecA also improved the rate of export in strains lacking SecB (secB::Tn5). This observation is consistent with the previous findings of Oliver (50). Thus, overproduction of SecA is able to bypass the requirement for SecB altogether. This is in contrast to suppression of the secBL75Q defect, where overproduction of SecA was shown not to bypass SecBL75Q but to reverse the slow-release defect of the mutant protein. In cells with normal SecA levels, in the absence of SecB, export of precursor MBP is much slower than in wild-type cells and is completely posttranslational. Approximately 60% of the intracellular preMBP is exported, indicating that a significant quantity of preMBP is exported in strains lacking SecB. Thus, in secB::Tn5 strains, targeting of precursors to the membrane is most likely the rate-limiting step. It is possible that in the absence of SecB, precursors are bound directly by SecA. If this is the case, then overproduction of SecA could improve export in the absence of SecB by increasing the efficiency with which precursors are bound by SecA.

Since the secA4250 mutation is a G-to-A mutation 3 nucleotides upstream of the translational start site for geneX and strains carrying this mutation express high levels of SecA protein, it seems likely that translation of geneX is affected in secA4250 mutant strains. Initiation regions show a bias in favor of adenosine (A) at most positions, especially downstream of the Shine-Dalgarno sequence (15). Therefore, the secA4250 mutation could improve the efficiency of translation initiation by increasing the adenosine content of the region. This idea is supported by random-mutagenesis studies of the E. coli trp leader region, which demonstrated that A at position −3 from the initiator codon favored translation initiation over guanine (G) at this position.

Improving the efficiency of translation initiation of geneX could lead to overproduction of the SecA protein through a mechanism involving translational coupling. Translational coupling is a common form of regulation in E. coli operons and occurs when the translation of one cistron affects translation initiation of the downstream cistron(s). For some operons, translation of the upstream cistron helps to destabilize mRNA structures which sequester the Shine-Dalgarno sequence and/or the initiator codon (37, 56). Studies of secA regulation demonstrated that secA expression is translationally coupled to that of geneX (44). Translation of the distal region of geneX is thought to open up an RNA secondary structure located in the geneX-secA intergenic region which blocks access to the secA Shine-Dalgarno sequence. Therefore, increasing the amount of geneX translation could increase secA expression by melting the inhibitory RNA structure, enabling ribosomes to bind to the secA Shine-Dalgarno sequence.

In conclusion, the results of this study support the proposed role for SecB in targeting precursor proteins to SecA. Newly
synthesized precursors bound by SecB are guided to the translocation site through the affinity of SecB for SecA. Upon docking at the translocation site, the precursor is transferred to SecA and SecB is released from the membrane, freeing it to bind a newly synthesized precursor.

ACKNOWLEDGMENTS

We thank Jon Beckwith for the generous gifts of plasmid pBAD22 and anti-SecE antisera; Ralph Isberg and Susanna Rankin for providing plasmid pSR47 and strain SR202; Don Oliver for plasmids pMF8 and pT7-secE4; Bill Wickner for plasmid pHasecEYG and anti-SecY antisera; Olivera Franetic, Harvey Kimsey, Lin Randall, Deb Raychaudhuri, and Perry Riggle for helpful discussions; Meckie Pohlschroeder for technical assistance; and Arnold Driessen for valuable discussions and for communicating the method for immunodetection. We are grateful to Linc Sonenshein, Cathy Squires, and Andrew Wright for helpful discussions and for critical reading of the manuscript.

This work was supported by grant GM56415 from the National Institutes of Health (to C.A.K.). Part of the work was performed during the tenure of an American Heart Established Investigator Award (to C.A.K.).

REFERENCES


