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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

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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.

* Corresponding author. Mailing address: Department of Molecular Biology and Microbiology, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111. Phone: (617) 636-0404. Fax: (617) 636-0337. E-mail: CKUMAMOT@opal.tufts.edu.

† Present address: Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester, MA 01605.

TABLE 1. Genotypes of *E. coli* strains

Strain	Genotype	Source
MC4100	F$^-$ Δ*lac-169 araD139 thiA rpsL relA motA*	Lab collection
CK2163	MC4100 *secBL75Q malE*$^+$	Lab collection
OF133	MC4100 *secBL75Q malE10-1 malT*(Con)	Lab collection
HAC10	MC4100 *zab-1*::Tn*10 secA*$^+$	This study
HAC12	MC4100 *secBL75Q zjb*::Tn*5* Δ*malB101 zab-1*::Tn*10 secA*$^+$ malT(Con)	This study
HAC13	MC4100 *secB*$^+$ *malE10-1 malT*(Con) *zab-1*::Tn*10 secA*$^+$	This study
HAC14	MC4100 *secBL75Q malE10-1 malT*(Con) *zab-1*::Tn*10 secA*$^+$	This study
HAC15	MC4100 *secBL75Q zab-1*::Tn*10 secA*$^+$	This study
HAC49	MC4100 *secBL75Q recA1 srl*::Tn*10*	This study
HAC50	MC4100 *secB*$^+$ *recA1 srl*::Tn*10*/pBR322	This study
HAC52	MC4100 *secBL75Q recA1 srl*::Tn*10*/pBR322	This study
HAC53	MC4100 *secBL75Q recA1 srl*::Tn*10*/pMF8	This study
HAC82	MC4100 *secB*$^+$ *ara*$^+$ *recA1 srl*::Tn*10*	This study
HAC83	MC4100 *secBL75Q ara*$^+$ *recA1 srl*::Tn*10*	This study
HAC97	MC4100 *secB*$^+$ *ara*$^+$ *recA1 srl*::Tn*10*/pHA*secEYG*	This study
HAC98	MC4100 *secBL75Q ara*$^+$ *recA1 srl*::Tn*10*/pHA*secEYG*	This study
HAC151	MC4100 *secB*$^+$ *ara*$^+$ *recA1 srl*::Tn*10*/pBAD22	This study
HAC152	MC4100 *secBL75Q ara*$^+$ *recA1 srl*::Tn*10*/pBAD22	This study
HAC214	MC4100 *secBL75Q malE10-1 malT*(Con) *zab-1*::Tn*10 secA1180*	This study
HAC215	MC4100 *secBL75Q malE10-1 malT*(Con) *zab-1*::Tn*10 secA4250*	This study
HAC216	MC4100 *secBL75Q malE*$^+$ *zab-1*::Tn*10 secA1180*	This study
HAC217	MC4100 *secBL75Q malE*$^+$ *zab-1*::Tn*10 secA4250*	This study

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 export in vivo but do not compromise complex formation between SecB and 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 P1*vir* 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, pHA*secEYG* (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 salts were prepared as previously described (46). Minimal media were supplemented with thiamine (5 μg/ml) and with 0.5% glycerol or with the combination of 0.2% glycerol and 0.4% maltose. Some cultures were supplemented with 18 amino acids (no methionine or cysteine) as described previously (8). When appropriate, antibiotics were added to the following concentrations: ampicillin, 100 μg/ml; chloramphenicol, 25 μg/ml; kanamycin, 10 μg/ml; and tetracycline, 20 μg/ml.

In experiments where the SecYEG complex was overexpressed from plasmid pHA*secEYG*, cells were grown in M63 minimal medium containing 0.4% glucose, ampicillin (100 μg/ml), and 18 amino acids (1/100 of stock) at 37°C. When pHA*secEYG*-containing strains reached a cell density of 2×10^8 to 3×10^8 cells/ml, the 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$^+$ suppressor mutations. Cells of strain HAC12 (*secBL75Q zjb*::Tn*5* Δ*malB101 zab-1*::Tn*10 secA*) were treated with nitrosoguanidine (62.5 μg/ml) as described previously (59). The mutagenized cells were split into pools. P1*vir* was prepared on each pool and used to transduce cells of strain OF133 [*secB75Q malT*(Con) *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$^+$ colonies from individual plates were pooled, a P1*vir* 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$^+$ colonies. Individual Mal$^+$ 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 (2×10^8 to 3×10^8 cells/ml) were pulse-labeled for 15 s with Tran^{35}S-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 *Sal*I restriction site in *secA* (5′*secA*) and from the *Sal*I site to the end of the *secA* gene (3′*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$^+$ 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 *Eco*RI site in *geneX* or to the *Nco*I site in *secA* were generated. The subclones were tested as described above. Both subclones derived from

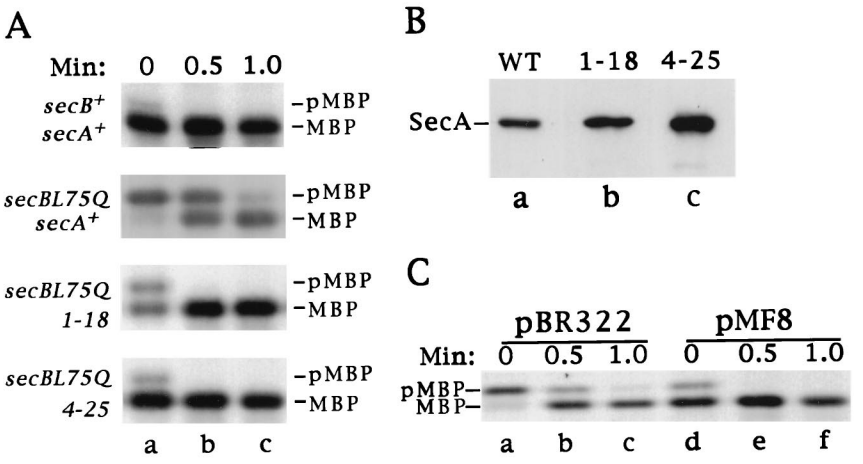

FIG. 1. (A) Suppression of *secBL75Q* by *secA1180*, *secA4250*, and SecA overproduction. Cells were grown in maltose-glycerol minimal medium and labeled for 15 s with Tran^{35}S-label. Chase was initiated with nonradioactive methionine and chloramphenicol. Samples were taken at the time points indicated, immunoprecipitated with anti-MBP antiserum, and analyzed by SDS-PAGE (10% polyacrylamide) and fluorography as described in Materials and Methods. The positions of preMBP (pMBP) and mature MBP are indicated. (B) Total SecA levels in *secA1180* and *secA4250* mutant cells. Strains HAC15 (*secA*$^+$), HAC216 (*secA1180*), and HAC217 (*secA4250*) were grown in L broth, and clarified cell lysates were made as described in Materials and Methods. Samples containing 10 μg of total protein were separated by SDS-PAGE, transferred to an Immobilon PVDF membrane, and probed with anti-SecA antiserum. WT, wild type. (C) Overproduction of wild-type SecA from plasmid pMF8 suppresses *secBL75Q*. Cells were grown in maltose minimal medium and subjected to pulse-chase analysis as described for panel A. 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*.

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.

Antisera. Anti-MBP, anti-OmpA, and anti-SecB antisera have been described previously (32). For preparation of anti-SecA antiserum, SecA protein was purified as described previously (7). Rabbits were immunized with denatured SecA as described previously (32), except that Hunter's TiterMax (CytRx Corporation, Norcross, Ga.) was used as an adjuvant. Anti-SecE and anti-SecY antisera were the kind gifts of Jon Beckwith and Bill Wickner, respectively.

Preparation of cell lysates and immunoblotting. For quantitation of total SecA, cells were grown in L broth to an A_{600} of 0.75 to 1.0. The cultures were poured over crushed ice, cells were harvested by centrifugation at 7,000 rpm for 10 min at 4°C in a Beckman JA-14 rotor, and the pellet was resuspended in 1/100th volume of lysis buffer (10 mM Tris-acetate [pH 7.6], 50 mM KCl, 10 mM Mg acetate, 1 mM dithiothreitol) plus 17.4 μg of phenylmethylsulfonyl fluoride per ml and 2 μg of DNase I per ml. Cells were lysed in a prechilled French pressure cell (two passes at 14,000 lb/in^2), and the extracts were clarified by centrifugation at 10,000 rpm (12,000 × *g*) for 10 min. Protein concentrations were determined by the method of Lowry et al. (40) in the presence of SDS. Clarified extracts were subjected to SDS-PAGE, transferred to Immobilon polyvinylidene difluoride (PVDF) membranes (Millipore), and probed with polyclonal anti-SecA antiserum. The blot was developed with the Western-Light chemiluminescent detection system (Tropix, Bedford, Mass.) as recommended by the manufacturer.

Detection of SecB complexes. Cells were grown in M63 minimal medium supplemented with 0.2% glycerol, 0.4% maltose, and 100 μg of ampicillin per ml at 37°C. Cultures were labeled with Tran^{35}S-label (ICN), and the label was chased with nonradioactive methionine (2.2×10^{-5} M) as described previously (54). Cells were harvested over ice, converted to spheroplasts, and extracted as described previously (54). Material which was bound nonspecifically to the protein A-Sepharose matrix was removed, and cell membranes were pelleted as follows: 0.1 ml of protein A-Sepharose (1:1 slurry in phosphate-buffered saline [PBS]) was added to the extract, and the extract was centrifuged for 15 min at 83,000 rpm with a Beckman TLA 100.2 rotor at 4°C.

Chromatography was performed at 4°C. Anti-SecB antibodies were bound to a column of protein A-Sepharose as previously described (30). Precleared extracts were passed slowly through the anti-SecB column, and the column was washed once with 0.5 ml of PBS–0.1% Tween 20 and twice with 0.5 ml of PBS–0.5% Tween 20. Proteins bound to the column were eluted with 200 μl of boiling SDS buffer (250 mM Tris-HCl [pH 6.8], 4.0% SDS, 30% glycerol, 10% β-mercaptoethanol), and the column was washed twice with 100 μl of 10 mM Tris-HCl (pH 8)–0.5% Tween 20. The washes were pooled with the eluent.

Densitometry. Densitometric analysis was performed with a Molecular Dynamics computing densitometer and ImageQuant 3.3 software. When the amount of exported MBP or exported OmpA was calculated, the obtained values were corrected for the loss of methionine residues in the mature form relative to the precursor form of the protein.

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 Tran^{35}S-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 OF133 (*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*::Tn*5* strains indicated that both suppressors could improve export in the absence of SecB (data not shown). Although the suppression of the *secB*::Tn*5* 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 were comparable to those seen in HAC216 (*secA1180*) and HAC217 (*secA4250*) (Fig. 1A). Export of the SecB-dependent proteins preLamB, proOmpA, and precursor galactose binding protein was also improved in strains containing pMF8 (data not shown).

Pulse-chase experiments analyzing export in strains containing 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 SecYEG complex fails to suppress *secBL75Q*. To determine whether overproduction of other components of the translocation apparatus would suppress the *secBL75Q* defect, the SecY-SecE-SecG complex (SecYEG) was overexpressed from plasmid pHA*secEYG*. Overproduction of the SecYEG 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 ara*$^+$) were transformed with plasmid pHA*secEYG* or vector pBAD22. pHA*secEYG* contains the genes for an epitope-tagged SecE, SecY, and SecG under control of the P_{BAD} promoter of the *araBAD* (arabinose) operon. For overexpression of SecYEG, 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 pHA*secEYG*-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 SecYEG 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*/pHA*secEYG*) (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 polypeptides 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-precursor 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 Tran^{35}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-

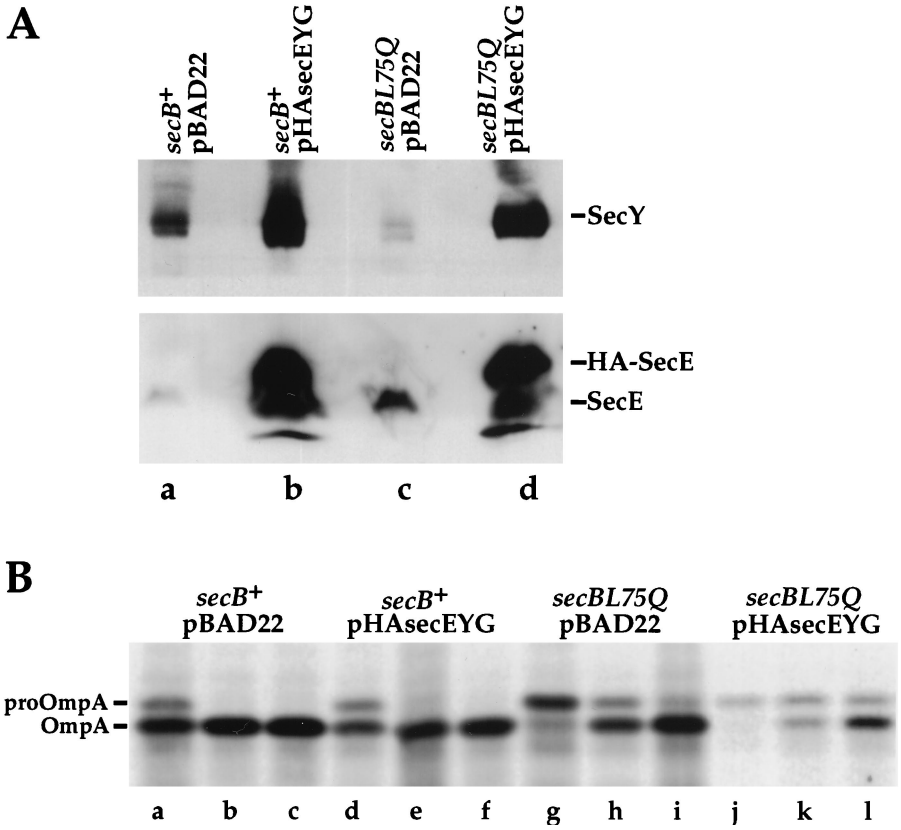

FIG. 2. Overexpression of the SecYEG complex does not improve export in *secBL75Q* mutant strains. Cells were grown in minimal arabinose medium supplemented with ampicillin and a mix of 18 amino acids. (A) Immunoblots of total SecY and SecE protein. Samples (1 ml) were precipitated with trichloroacetic acid and analyzed by high-Tris SDS-PAGE (3). Proteins were transferred to a PVDF membrane and probed with anti-SecY and anti-SecE antisera. (B) Pulse-chase analysis of OmpA export. Cells were labeled with Tran^{35}S-label for 15 s, and the label was chased with nonradioactive methionine and chloramphenicol. Samples were removed at the times indicated and treated as for Fig. 1, except that the samples were immunoprecipitated with anti-OmpA antiserum. Samples are as follows: lane a, HAC151 (*secB*$^+$/pBAD22), 15-s pulse; lane b, HAC151, 0.5-min chase; lane c, HAC151, 1.0-min chase; lane d, HAC97 (*secB*$^+$/pHA*secEYG*), 15-s pulse; lane e, HAC97, 0.5-min chase; lane f, HAC97, 1.0-min chase; lane g, HAC152 (*secBL75Q*/pBAD22), 15-s pulse; lane h, HAC152, 0.5-min chase; lane i, HAC152, 1.0-min chase; lane j, HAC98 (*secBL75Q*/pHA*secEYG*), 15-s pulse; lane k, HAC98, 0.5-min chase; lane l, HAC98, 1.0-min chase.

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 preLamB, preMBP, and proOmpA 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 bound to SecBL75Q in extracts from cells overproducing SecA (Fig. 3, lane g). This result indicated that precursor proteins were not bypassing SecBL75Q in cells with high levels of SecA protein. After 1 min of chase, small amounts of preLamB, preMBP, and proOmpA were observed in the bound fraction (Fig. 3, lane h), and after 2 min of chase, these precursors could barely be detected (Fig. 3, lane i). These data indicated that increasing SecA levels in the cell reversed the defective release of precursors from SecBL75Q.

DISCUSSION

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

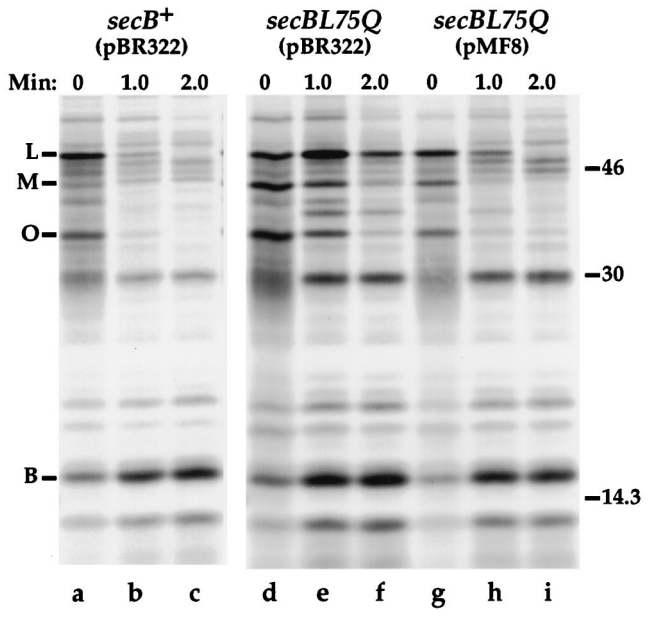

FIG. 3. Defective release of precursors from SecBL75Q is suppressed by overproduction of SecA. Cells were grown in M63 minimal maltose-glycerol medium containing ampicillin. Cells were pulse-labeled with Tran^{35}S-label for 15 s, and the label was chased with nonradioactive methionine. Samples were 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-precursor 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 activities (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 interaction does not lead to suppression of *secBL75Q*.

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 cytosol 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*::Tn*5*). 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*::Tn*5* 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.

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REFERENCES

1. **Bonner, W. M., and R. A. Lasky.** 1974. Detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46:**83–88.
2. **Breukink, E., N. Nouwen, A. van Raalte, S. Mizushima, J. Tommassen, and B. de Kruijff.** 1995. The C terminus of SecA is involved in both lipid binding and SecB binding. J. Biol. Chem. **270:**7902–7907.
3. **Brundage, L., J. P. Hendrick, E. Scheibel, A. J. M. Driessen, and W. Wickner.** 1990. The purified *E. coli* integral membrane protein SecY/E is sufficient for reconstitution of SecA-dependent precursor protein translocation. Cell **62:**649–657.
4. **Cabelli, R. J., K. M. Dolan, L. Qian, and D. B. Oliver.** 1991. Characterization of membrane-associated and soluble states of SecA protein from wild-type and secA52(Ts) mutant strains of *Escherichia coli*. J. Biol. Chem. **266:**24420–24427.
5. **Chamberlain, J. B.** 1979. Fluorographic detection of radioactivity in polyacrylamide gels with water soluble fluor, sodium salicylate. Anal. Biochem. **98:**132–135.
6. **Collier, D. N., V. A. Bankaitis, J. B. Weiss, and P. J. Bassford, Jr.** 1988. The antifolding activity of SecB promotes the export of the *E. coli* maltose-binding protein. Cell **53:**273–283.
7. **Cunningham, K., R. Lill, E. Crooke, M. Rice, K. Moore, W. Wickner, and D. Oliver.** 1989. SecA protein, a peripheral protein of the *Escherichia coli* plasma membrane, is essential for the functional binding and translocation of proOmpA. EMBO J. **8:**955–959.
8. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **De Gier, J.-W. L., Q. A. Valent, G. Von Heijne, and J. Luirink.** 1997. The *E. coli SRP*: preferences of a targeting factor. FEBS Lett. **408:**1–4.
10. **Den Blaauwen, T., and A. J. M. Driessen.** 1996. Sec-dependent preprotein translocation in bacteria. Arch. Microbiol. **165:**1–8.
11. **Den Blaauwen, T., E. Terpetschnig, J. R. Lakowicz, and A. J. M. Driessen.** 1997. Interaction of SecB with soluble SecA. FEBS Lett. **416:**35–38.
12. **Dolan, K. M., and D. B. Oliver.** 1991. Characterization of *Escherichia coli* SecA protein binding to a site on its mRNA involved in autoregulation. J. Biol. Chem. **266:**23329–23333.
13. **Douville, K., A. Price, J. Eichler, A. Economou, and W. Wickner.** 1995. SecYEG and SecA are the stoichiometric components of the preprotein translocase. J. Biol. Chem. **270:**20106–20111.
14. **Driessen, A., J. G. de Wit, W. Kuiper, J. P. W. van der Wolk, P. Fekkes, C. van der Does, K. van Wely, E. Manting, and T. den Blaauwen.** 1995. SecA, a novel ATPase that converts chemical energy into a mechanical force to drive precursor protein translocation. Biochem. Soc. Trans. **23:**981–985.
15. **Dreyfus, M.** 1988. What constitutes the signal for the initiation of protein synthesis on *Escherichia coli* mRNAs? J. Mol. Biol. **204:**79–94.
16. **Duong, F., J. Eichler, A. Price, M. R. Leonard, and W. Wickner.** 1997. Biogenesis of the gram-negative bacterial envelope. Cell **91:**567–573.
17. **Duong, F., and W. Wickner.** 1997. Distinct catalytic roles of the SecYE, SecG and SecDFyajC subunits of preprotein translocase holoenzyme. EMBO J. **16:**2756–2768.
18. **Economou, A., and W. Wickner.** 1994. SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. Cell **78:**835–843.
19. **Fekkes, P., J. G. de Wit, J. P. W. van der Wolk, H. H. Kimsey, C. A. Kumamoto, and A. J. M. Driessen.** 1998. Preprotein transfer to the Escherichia coli translocase requires the cooperative binding of SecB and the signal sequence to SecA. Mol. Microbiol. **29:**1179–1190.
20. **Fekkes, P., C. van der Does, and A. K. M. Driessen.** 1997. The molecular chaperone SecB is released from the carboxy-terminus of SecA during initiation of precursor protein translocation. EMBO J. **16:**6105–6113.
21. **Francetic, O., M. P. Hanson, and C. A. Kumamoto.** 1993. *prlA* suppression of defective export of maltose-binding protein mutants of *Escherichia coli*. J. Bacteriol. **175:**4036–4044.
22. **Gannon, P. M., and C. A. Kumamoto.** 1993. Mutations of the molecular chaperone protein SecB which alter the interaction between SecB and Maltose-binding Protein. J. Biol. Chem. **268:**1590–1595.
23. **Gold, L., D. Pribnow, T. Schneider, S. Shinedling, B. Swebilius Singer, and G. Stormo.** 1981. Translation initiation in prokaryotes. Annu. Rev. Microbiol. **35:**365–403.
24. **Guzman, L.-M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J. Bacteriol. **177:**4121–4130.
25. **Hartl, F.-U., S. Lecker, E. Schiebel, J. P. Hendrick, and W. Wickner.** 1990. The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane. Cell **63:**269–279.
26. **Hoffschulte, H. K., B. Drees, and M. Müller.** 1994. Identification of a soluble SecA/SecB complex by means of a subfractionation cell-free export system. J. Biol. Chem. **269:**12833–12839.
27. **Khisty, V. J., and L. L. Randall.** 1995. Demonstration in vivo that interaction of maltose-binding protein with SecB is determined by a kinetic partitioning model. J. Bacteriol. **177:**3277–3282.
28. **Kimsey, H. H., M. D. Dagarag, and C. A. Kumamoto.** 1995. Diverse effects of mutation on the activity of the *Escherichia coli* export chaperone SecB. J. Biol. Chem. **270:**22831–22835.
29. **Kumamoto, C., and J. Beckwith.** 1985. Evidence for specificity at an early step in protein export in *Escherichia coli*. J. Bacteriol. **154:**267–274.
30. **Kumamoto, C. A.** 1989. *Escherichia coli* SecB protein associates with exported proteins *in vivo*. Proc. Natl. Acad. Sci. USA **86:**5320–5324.
31. **Kumamoto, C. A.** 1990. SecB protein: a cytosolic export factor that associates with nascent exported proteins. J. Bioenerg. Biomembr. **22:**337–351.
32. **Kumamoto, C. A., L. Chen, J. Fandl, and P. C. Tai.** 1989. Purification of the Escherichia coli secB gene product and demonstration of its activity in an *in vitro* protein translocation system. J. Biol. Chem. **264:**2242–2249.
33. **Kumamoto, C. A., and O. Francetic.** 1993. Highly selective binding of nascent polypeptides by an *Escherichia coli* chaperone protein in vivo. J. Bacteriol. **175:**2184–2188.
34. **Kumamoto, C. A., and P. M. Gannon.** 1988. Effects of *Escherichia coli secB* mutations on pre-maltose binding protein conformation and export kinetics. J. Biol. Chem. **263:**11554–11558.
35. **Kusukawa, N., T. Yura, C. Ueguchi, Y. Akiyama, and K. Ito.** 1989. Effects of mutations in heat-shock genes *groES* and *groEL* on protein export in *Escherichia coli*. EMBO J. **8:**3517–3521.
36. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
37. **Lesage, P., C. Chiaruttini, M. Graffe, J. Doddon, M. Milet, and M. Springer.** 1992. Messenger RNA secondary structure and translational coupling in the *Escherichia coli* operon encoding translation initiation factor IF3 and the ribosomal proteins, L35 and L20. J. Mol. Biol. **228:**366–386.
38. **Lill, R., R. Cunningham, L. Brundage, K. Ito, D. Oliver, and W. Wickner.** 1989. The SecA protein hydrolyzes ATP and is an essential component of the protein translocation ATPase of *E. coli*. EMBO J. **8:**961–966.
39. **Lill, R., W. Dowhan, and W. Wickner.** 1990. The ATPase activity of SecA is regulated by acidic phospholipids, SecY, and the leader and mature domains of precursor proteins. Cell **60:**271–280.
40. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
41. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Matteucii, M. D., and H. L. Heyneker.** 1983. Targeted random mutagenesis: the use of ambiguously synthesized oligonucleotides to mutagenize sequences immediately 5′ of an ATG initiation codon. Nucleic Acids Res. **11:**3113–3121.
43. **McCabe, P. C.** 1990. Production of single-stranded DNA by asymmetric PCR, p. 76–83. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, Inc., San Diego, Calif.
44. **McNicholas, P., R. Salavati, and D. Oliver.** 1997. Dual regulation of *Escherichia coli secA* translation by distinct upstream elements. J. Mol. Biol. **265:**128–141.
45. **Merriam, J. J., R. Mathur, R. Maxfield-Boumil, and R. R. Isberg.** 1997. Analysis of the *Legionella pneumophila fliI* gene: intracellular growth of a defined mutant defective for flagellum biosynthesis. Infect. Immun. **65:**2497–2501.
46. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
47. **Mullis, K., F. Faloona, S. Scharf, R. Daiki, G. Horn, and H. Erlich.** 1986.

Specific enzyme amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biol. **51:**263–273.
48. **Murphy, C. K., E. J. Stewart, and J. Beckwith.** 1995. A double counterselection for the study of essential genes in *Escherichia coli*. Gene **155:**1–7.
49. **Nishiyama, K., M. Hanada, and H. Tokuda.** 1994. Disruption of the gene encoding p12 (SecG) reveals the direct involvement and important function of SecG in the protein translocation of *Escherichia coli* at low temperature. EMBO J. **13:**3272–3277.
50. **Oliver, D.** 1998. Personal communication.
51. **Oliver, D. B., and J. Beckwith.** 1982. Regulation of a membrane component required for protein secretion in *Escherichia coli*. Cell **30:**311–319.
52. **Pogliano, J. A., and J. Beckwith.** 1994. SecD and SecF facilitate protein export in *Escherichia coli*. EMBO J. **13:**554–561.
53. **Rajapandi, T., and D. B. Oliver.** 1994. Carboxy-terminal region of Escherichia coli SecA ATPase is important to promote its protein translocation activity *in vivo*. Biochem. Biophys. Res. Commun. **200:**1477–1483.
54. **Randall, L. L., and S. J. S. Hardy.** 1986. Correlation of competence for export with lack of tertiary structure of the mature species: a study *in vivo* of maltose-binding protein in *E. coli*. Cell **46:**921–928.
55. **Rose, R. E.** 1988. The nucleotide sequence of pACYC184. Nucleic Acids Res. **16:**355.
56. **Saito, K., and M. Nomura.** 1994. Post-transcriptional regulation of the *str* operon in *Escherichia coli*: structural and mutational analysis of the target site for translational repressor S7. J. Mol. Biol. **235:**125–139.
57. **Schmidt, M. G., K. M. Dolan, and D. B. Oliver.** 1991. Regulation of *Escherichia coli* SecA mRNA translation by a secretion-responsive element. J. Bacteriol. **173:**6605–6611.
58. **Schmidt, M. G., and D. B. Oliver.** 1989. SecA protein autogenously represses its own translation during normal protein secretion in *Escherichia coli*. J. Bacteriol. **171:**643–649.
59. **Silhavy, T. J., M. L. Berman, and L. E. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
60. **Snyders, S., V. Ramamurthy, and D. Oliver.** 1997. Identification of a region of interaction between Escherichia coli SecA and SecY proteins. J. Biol. Chem. **272:**11302–11306.
61. **Van der Does, C., T. den Blaaudwen, J. G. de Wit, E. H. Mantig, N. A. Groot, P. Fekkes, and A. J. M. Driessen.** 1996. SecA is an intrinsic subunit of the *Escherichia coli* preprotein translocase and exposes its carboxyl terminus to the periplasm. Mol. Microbiol. **22:**619–629.
62. **Wild, J., E. Altman, T. Yura, and T. Gross.** 1992. DnaK and DnaJ heat shock proteins participate in protein export in *Escherichia coli*. Genes Dev. **6:**1165–1172.