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Evidence for Specificity at an Early Step in Protein Export in *Escherichia coli*

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We previously described mutations in a gene, *secB*, which have pleiotropic effects on protein export in *Escherichia coli*. In this paper, we report the isolation of mutants in which the activity of the *secB* gene was eliminated. Null mutations in *secB* affected only a subset of exported proteins. Strains carrying these mutations, although unable to grow on L broth plates, were still viable on minimal media. These *secB* mutations reversed a block in the translation of an exported protein that was caused by the elimination of another component of the secretion machinery, SecA protein. These results suggest that the *secB* product acts at an early step in the export process and is involved in the export of only a subset of cell envelope proteins.

Genetic studies in *Escherichia coli* have revealed the existence of several genes which may code for components of a bacterial machinery for the export of proteins. Mutations in the genes *prlA* (*secY*), *secA*, *secB*, and *secC* have pleiotropic effects on the localization of proteins to the bacterial outer membrane and to the periplasmic space (8, 9, 17, 23, 28). Genes have also been identified for peptidases which cleave the signal sequences from precursors of exported proteins (11, 29, 32). All of these genes map at separate positions on the bacterial chromosome.

The properties of certain conditionally lethal mutations in the *secA* and *secC* genes indicate that, in at least some of its features, the mechanism of protein export in *E. coli* has strong similarities to the mechanism of protein secretion in eucaryotes. The bacterial studies above have shown that there is a feedback mechanism in protein export such that certain mutations which interfere with the export machinery can, in turn, prevent synthesis of exported proteins. Under nonpermissive conditions, a *secA* amber (am) mutation and a *secC* cold-sensitive (cs) mutation inhibit the expression of genes for cell envelope proteins (9, 24). Signal sequence mutations in a gene (*malE*) coding for one of these exported proteins, the periplasmic maltose-binding protein (MBP), reverse the block in synthesis for that protein (9, 18). These in vivo findings are similar to those from in vitro studies with eucaryotic secretory proteins, which have shown that interference with the secretion mechanism causes a pause in continued polypeptide chain elongation of secretory proteins (31). Whether the secretory machinery in procaryotes is entirely comparable to that in eucaryotes is still an open question.

We previously described the selection that yielded mutants defective in the export of proteins (26). This selection is based on an increase in the activity of a protein when its location is altered from the membrane to the cytoplasm. This protein is a hybrid composed of the amino terminus of the periplasmic MBP attached to the normally cytoplasmic β-galactosidase. The signal sequence of MBP initiates the export process and causes the hybrid protein to become associated with the cell envelope. In this location, the hybrid protein is unable to form a normally active β-galactosidase. Selection for Lac^+ derivatives of such a strain yielded mutations in the *secA* and *secB* genes (e.g., *secB1* and *secB2*) which cause some internalization of the hybrid protein. The *secB* mutants isolated in this way exhibit only slight defects in secretion (17), accumulating some precursor of MBP.

Conditionally lethal mutants have been essential to studies of the *sec* and *prlA* genes. However, in our initial studies with *secB*, we were unable to obtain such mutants. In this paper, we describe the isolation and characterization of mutants that have severe effects on the *secB* gene. The results suggest that *secB* is not an essential gene and that its product is required for the normal secretion of only a subset of exported proteins. Furthermore, our findings indicate that the *secB* gene product acts at a very early stage in the process of protein export.

MATERIALS AND METHODS

Bacterial strains and transducing phages. The genotypes of bacterial strains and λ transducing phages are listed in Table 1. Techniques for the growth of cells and phage, bacterial conjugation, and generalized transduction with phage P1 *vir* have been described previously (22).

Isolation of *secB* transducing phage. The phage isolation scheme took advantage of the structure of transposon Tn*5* to allow easy isolation of genes on either side of the gene *cysE*. Phage λ429 (λ *b*221 0.74::IS*50R* c1857 *nin*5 [13]) carries only one IS*50* sequence and can integrate into either of the IS*50* sequences in Tn*5* upon lysogenization. Strain CK104 (MC4100 *cysE*::Tn*5* *thyA*) was lysogenized with λ429, and several lysogens were isolated to ensure that at least some would contain a phage in the IS*50* sequence that was closest to *secB*.

After confirming that the phage had lysogenized within the *cysE* gene, we introduced a *recA1* mutation into four different lysogens by mating with strain KL1699 (Hfr KL16 *recA1*). Phage lysates were prepared from these lysogens by

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TABLE 1. Genotypes of bacterial strains and phages

Strain or phage	Relevant characteristics	Source or reference
Strain		
MC4100	F⁻ Δ*lacU169 araD139 relA rpsL thi*	Lab collection
MM18	MC4100(λ*p*72-47)	2
MM81	MM18 *secB1*	17
MM82	MM18 *secB2*	17
MM52	MC4100 *secA51*	23
BB20	Hfr Cavalli *gpsA20 glpD3 glpR*^{c}*2 phoA8 tonA22* T2(R) *rel-1* (λ)	7
SY822	F⁻ Δ*lacZM445 lacI3* Δ(*recA-srl*)*304 argE his strA mtl xyl*	13
MRi80	MC4100 Δ*rbs-7 pcn*	J. Lopilato (unpublished data)
MM145	MRi80 *secB1*	This study
MM146	MC4100 *srl*::Tn*10 recA1*	This study
MM147	MC4100 *secB1 srl*::Tn*10 recA1*	This study
MM148	MM18 *mtl recA1*/F'ts *mtl*$^+$ *secB2* Tn*10*	This study
MM149	MC4100 *zhe*::Tn*10 malT*^{c}	This study
MM150	MC4100 *zhe*::Tn*10 malT*^{c} *secB7*	This study
MM151	MC4100 *zhe*::Tn*10 malT*^{c} *secB8*	This study
MM152	MC4100 *zhe*::Tn*10 malT*^{c} *secB*::Tn*5*	This study
MM113	MC4100 *secA*(Am) *zci*::Tn*10 trp*(Am) *supF*(Ts) *malT*^{c}	18
MM153	MM113 *secB7*	This study
MM154	MC4100 *secB2 recA1* (λ*p*72-47 *imm*^{21} *P*am3)	This study
MM155	MC4100 *pyrE*	This study
MM156	MC4100 *pyrE*(pCK1)	This study
Phages		
λ421	*b*221 *rex*::Tn*5* c1857 *O*am23 *P*am80	M. Lichten (unpublished data)
λ429	*b*221 (0.74::IS*50R*) c1857 *nin5*	12

heat induction. λ *gpsA*$^+$ transducing phage were isolated by lysogenization of strain BB20 [*gpsA20*(λ)] on minimal medium containing maltose. After induction and purification, λ *gpsA* plaque-forming phage were isolated. λ *gpsA* phage were obtained at a frequency between 10^{-7} and 10^{-8}.

One phage was analyzed in detail. To test whether the phage carried *secB*, it was used to lysogenize strain MM154 [*secB2* (λ72-47 *imm*^{21} *P*am3)] selecting for λ immunity. The lysogens were examined on minimal plates containing lactose and spread with λ *c*I to select for maintenance of the λ *gpsA* phage. The lysogens exhibited a Lac⁻ phenotype, like a *secB*$^+$ strain, suggesting that the phage carried *secB*$^+$.

Construction of pCK1. Procedures for preparation of phage and plasmid DNA, digestion with restriction enzymes, and agarose gel electrophoresis have been described previously (20). DNA derived from λCK68 was digested with *Bgl*II and *Sal*I. pBR322 DNA was digested with *Sal*I and *Bam*HI. The two digests were mixed and ligated with T4 DNA ligase as previously described (20) and transformed into strain BB20. GpsA$^+$ plasmids were identified by replica plating. One was chosen for further study and designated pCK1. When transformed into strain CK105 [MM18 *secB2 recA1*(λ72-47)], the resultant strain exhibited a Lac⁻ (wild-type) phenotype on indicator plates containing lactose.

Isolation of Tn*5* insertions. Transpositions of Tn*5* onto pCK1 or pDC2 were obtained by infecting strains CK106 [MC4100 *recA1*(pCK1)] or CK107 [MC4100 *recA1*(pDC2)] with λ421 (λ *b*221 *rex*::Tn*5* c1857 *O*am *P*am) as previously described (16). Plasmids were isolated from pools of kanamycin-resistant colonies. After transformation into BB20 or MM154 [*secB2 recA1*(λ72-47 *imm*^{21} *P*am3)], insertions into *gpsA* or *secB* were isolated by screening for plasmids that failed to complement the appropriate defect.

Radioactive labeling experiments. Medium M63 was used as the minimal medium for cell growth and was supplemented with sugars and amino acids as noted below. Cultures were radiolabeled with [^{35}S]methionine (approximately 1,200 Ci/mmol) for the following times and temperatures. (i) Cells were grown to early log phase at 30°C in minimal medium containing 0.4% glycerol, 0.4% maltose, and 100 μg of ampicillin per ml. The cells were then labeled with [^{35}S]methionine (20 μCi/ml) for 30 s (see Fig. 2). (ii) Cells were grown at 30°C in minimal medium containing 0.4% glycerol and all amino acids except methionine and were labeled with [^{35}S]methionine (20 μCi/ml) for 30 s or 2 min (see Fig. 3). (iii) Cells were grown in minimal medium containing 0.4% glycerol at 30°C. For strain MM52 minimal medium containing 0.4% glycerol and 0.4% maltose was used, and cells were grown at 30°C and then at 37°C for 2.5 h. Cells were labeled for 2 min with [^{35}S]methionine at 20 μCi/ml at either 30°C (strains MM149, MM150, and MM151) or 37°C (strain MM52) (see Fig. 4). (iv) Cells were grown in medium containing glycerol (0.4%) and all amino acids except methionine at 30°C for 3 h. The cultures were divided, and part was diluted (MM113, 3-fold; MM150, 10-fold; MM153, 2-fold). These diluted cultures were incubated at 41°C for 2.5 h. The undiluted cultures of MM113 [*secB*$^+$ *secA*(Am)] and MM150 [*secB7 secA*$^+$] were labeled at the time of dividing with 20 μCi of [^{35}S]methionine per ml for 1 min at 30°C. The culture of MM153 [*secB7 secA*(Am)] was incubated at 30°C for an additional 2.5 h and labeled with 25 μCi of [^{35}S]methionine per ml for 1 min at 30°C. The cultures grown at 41°C were also labeled with 25 μCi of [^{35}S]methionine per ml for 1 min at 41°C (see Fig. 5). After extraction of labeled cells with sodium dodecyl sulfate at 100°C, immunoprecipitation was performed as previously described (14). Anti-MBP, anti-LamB, anti-OmpF, anti-ribose-binding protein (RBP), and anti-elongation factor G antisera were the gifts of W. Boos, T. Silhavy, R. Taylor, J. Garwin, and P. C. Tai, respectively. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10% polyacrylamide

gels was performed by the method of Laemmli (19), and fluorography was as previously described (5).

For labeling of plasmid-encoded proteins in maxicells, previously described methods were used (12). After being labeled with [^{35}S]methionine (10 μCi/ml of irradiated cells) for 20 min, the cells were washed in 10 mM Tris-hydrochloride (pH 8), suspended in 20% sucrose–0.03 M Tris-hydrochloride (pH 8), and digested with lysozyme at a concentration of 0.1 mg/ml in the presence of 10 mM EDTA for 30 min on ice. Spheroplasts were collected by centrifugation in a Microfuge for 3 min, suspended in 50 mM Tris-hydrochloride (pH 8)–100 mM NaCl–5 mM $MgCl_2$, and extracted by the addition of an equal volume of 1% Triton X-100 in the same buffer. The extract was clarified by centrifugation for 10 min in a Microfuge. After addition of sodium dodecyl sulfate, the extract was analyzed by electrophoresis as described above.

Recombination of the *secB*::Tn*5* mutation onto the bacterial chromosome. Recombination of the *secB*::Tn*5* insertion from pCK11 onto the chromosome was performed essentially as previously described (4). Briefly, pCK11 was transformed into Hfr Cavalli. This strain was mated with strain CK108 [MC4100 *mtl*(pCK1)]. Mtl$^+$ Kan^r recombinants were pooled, and P1 *vir* was grown on the pool. By using these P1 *vir* lysates, strain CK108 was transduced to Mtl$^+$ Kan^r; 3 of 70 transductants were sensitive to tetracycline, indicating that they did not contain the original plasmid, pCK11. These three strains carried the *secB*::Tn*5* mutation on their chromosomes and contained a wild-type copy of *secB* on the plasmid pCK1.

Southern blot analysis was performed by the method of Southern (30).

Selection of *secB* null mutants. Selection of *secB* null mutants was based on the procedures of Austin and Scaife (1). Strain MM148 (MM18 *mtl recA1*/F'ts *mtl*$^+$ *secB2*) is Lac$^-$ because the recessive *secB2* mutation is complemented by the *secB*$^+$ gene on the chromosome. Lac$^+$ derivatives of this strain were selected as previously described (17). Some of these Lac$^+$ mutants were expected to contain mutations that eliminate *secB*$^+$-complementing activity on the chromosome (null mutations in *secB*), revealing the *secB2* mutation on the F′ and allowing the strain to exhibit a Lac$^+$ phenotype. Because a null mutation in *secB* might be lethal to the cell, such mutations might also cause the cell to be dependent on the F′ for growth. Therefore, because the F′ is temperature sensitive for replication, the strain would be temperature sensitive for growth.

The Lac$^+$ mutants were screened for temperature sensitivity on rich medium. By using a *secA*$^+$-transducing phage (λDO20 [25]), temperature-sensitive mutants that contained *secA*(Ts) mutations were identified. The remaining temperature-sensitive mutants not complemented by λDO20 were candidates for strains carrying *secB* null mutations.

Two such mutants were examined in detail. A P1 *vir* lysate was prepared from each strain and used to transduce strain MM155 (MC4100 *pyrE*) to PyrE$^+$. Because *pyrE* is not carried by F'ts *mtl*$^+$ *secB2*, the transductants contained portions of the chromosome from the merodiploid strain.

RESULTS

Cloning and mapping of the *secB* gene. Previous results showed that the *secB* gene is tightly linked to the *cysE* and *gpsA* genes at 81 min on the bacterial chromosome (17). To facilitate the isolation and study of new *secB* mutations, we first mapped the chromosomal location of the gene more precisely by analyzing clones carrying this region. Because the *secB* mutants which had been previously isolated did not cause lethality, it was difficult to select directly for the wild-type copy of the gene. Therefore, the gene was cloned onto a plasmid in two steps: a λ transducing phage carrying *secB* was isolated, and DNA was subcloned from this phage. A phage isolation scheme was devised that would allow isolation of λ phages carrying genes on either side of *cysE* (see above). Phages that carried the *gpsA* gene (the structural gene for glycerol-3-phosphate dehydrogenase [7]) were found to also carry the *secB* gene. When these phages were used to lysogenize *secB2* strain MM154, which produces the hybrid β-galactosidase, the lysogen exhibited a Lac$^-$ phenotype on minimal plates containing lactose, demonstrating that the *secB* mutation was complemented by the transducing phage.

One phage was analyzed in detail. This phage, designated λCK68, contained a chromosomal insert of approximately 7 kilobases. The structure of the phage is shown in Fig. 1. Based on its restriction pattern, the right part of the chromosomal insert was probably derived from the transposon Tn*5*. A 4.5-kilobase *Bgl*II-*Sal*I fragment containing 3 kilobases of chromosomal DNA and 1.5 kilobases of Tn*5* sequences was cloned onto plasmid pBR322 (after digestion of the vector with *Bam*HI and *Sal*I), generating plasmid pCK1 (Fig. 1).

Plasmid pCK1 complemented the *gpsA20*, *secB1*, and *secB2* mutations. Complementation of *secB1* was confirmed by analyzing the secretion of MBP in *secB1* mutant strains (Fig. 2). When pCK1 was present in a strain carrying a *secB* mutation, MBP precursor was not observed, demonstrating that the chromosomal defect was complemented (Fig. 2, lane d). This experiment was performed in a strain carrying a mutation that lowers the copy number of pBR322 plasmid derivatives (J. Lopilato, unpublished data), because pCK1 causes a slight secretion defect when present in high copy number (data not shown).

We also obtained from R. Bell the plasmid pDC2 (6) because it carries the *gpsA* gene. The chromosomal insert on pDC2 was a 5-kilobase *Eco*RI fragment. This plasmid also complemented the *secB2* mutation, as shown by restoration of a Lac$^-$ phenotype to strain MM154 on lactose indicator plates. The restriction map of pDC2 was identical to that of pCK1 in the region carrying *secB* (Fig. 1).

To determine the locations of *secB* and *gpsA* on these plasmids, we inactivated the genes on both plasmids pCK1 and pDC2 by Tn*5* insertion mutagenesis (see above). Plasmids pCK5 and pCK10, derived from pCK1, carry insertions that inactivate *secB* and *gpsA*, respectively. Plasmid pCK11, derived from pDC2, carries an insertion that inactivates *secB*. To confirm that pCK5 does not carry a functional *secB* gene, we examined secretion in a *secB1* mutant strain in the presence of pCK5. The Tn*5* insertion destroyed the ability of the plasmid to complement the chromosomal *secB1* mutation (Fig. 2, lane g). In contrast, the insertion on pCK10 did not destroy complementation (Fig. 2, lane h). Because both pCK5 and pCK11 complement the *gpsA20* mutation, the *secB* and *gpsA* genes must be distinct.

The positions of the Tn*5* insertions in pCK5, pCK10, and pCK11 were mapped by restriction mapping (Fig. 1). *secB* and *gpsA* were close together, and the gene order was *cysE*, *gpsA*, *secB*.

Effects of *secB* null mutants. To determine the role of the *secB* gene in secretion, we next used two approaches to obtain strains that carried chromosomal mutations inactivating the gene. First, we used the Tn*5* insertion on plasmid

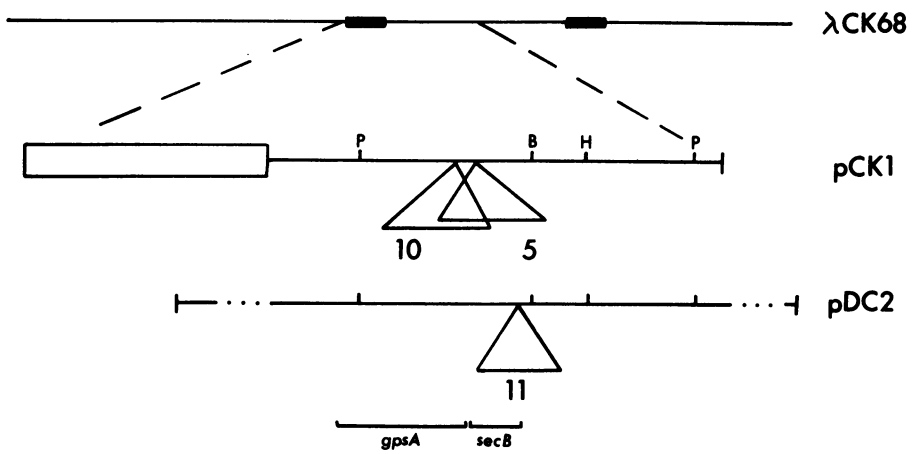

FIG. 1. Structures of *secB* transducing phage and plasmids. The top line shows a diagram of λCK68, the *secB* transducing phage which carries approximately 7 kilobases of chromosomal DNA. IS*50* sequences, derived from the transposon Tn*5*, are represented by blackened boxes. The next line shows the restriction map of the DNA that was cloned into pBR322 to generate pCK1. The open bar represents IS*50* sequences. A 3-kilobase segment of chromosomal DNA is contained on pCK1. Tn*5* insertions on pCK1 are indicated below the map with numbers corresponding to plasmid numbers. The structure of pDC2 is shown for the region including the *secB* gene, and the position of a Tn*5* insertion is indicated by the number below the map. The bottom line shows the approximate locations of the *gpsA* and *secB* genes. Letters refer to restriction sites: P, *Pvu*II; B, *Bam*HI; H, *Hin*dIII.

pCK11 that inactivates the *secB* gene. This mutation was recombined from the plasmid onto the chromosome as described above. Three strains carrying the *secB*::Tn*5* mutation on their chromosomes were obtained. These strains also contained a wild-type copy of the *secB* gene on the plasmid pCK1. To determine whether the *secB*::Tn*5* mutation was a lethal mutation, P1 *vir* was grown on these strains. The P1 lysates were used to transduce two strains, MM156 [MC4100 *pyrE*(pCK1)] and MM155 (MC4100 *pyrE*) to PyrE$^+$. Transductants were screened for kanamycin resistance (encoded on Tn*5*). In the transduction with MM156 [MC4100 *pyrE*(pCK1)], Kan^r transductants were obtained at the expected frequency of 30% (20 of 64). In the transduction with MM155 (MC4100 *pyrE*), Kan^r transductants were obtained at a frequency of 10% (5 of 48). Although this was a lower frequency than in the previous transduction, it was too high to be accounted for by second-site mutations. Therefore, the *secB*::Tn*5* mutation is not lethal in a haploid strain.

Southern blot analysis was used to confirm that the structure of the chromosome of a haploid *secB*::Tn*5* mutant was as predicted based on the structure of pCK11 (data not shown). A Southern blot of *Hin*dIII-digested DNA was probed with labeled pCK1 DNA. With the digests of pCK11 and chromosomal DNA from the *secB*::Tn*5* strain, a 1.5-kilobase fragment was observed. This fragment contained part of *secB* and extended to the right of *secB* (oriented as in Fig. 1). It also contained 1 kilobase of DNA derived from the Tn*5* element. This fragment was not observed in the digest of chromosomal DNA from a *secB*$^+$ strain.

We found that haploid strains with a *secB*::Tn*5* mutation were viable on minimal medium and grew nearly as well as a wild-type strain on minimal medium with glycerol as the sole carbon source. However, the mutant strains were unable to grow on L broth plates. Therefore, the difference in transduction frequencies in the two transductions described above probably reflects the slightly deleterious effects of the *secB*::Tn*5* mutation in a haploid.

secB mutations were also isolated in another way. Because the *secB2* mutation isolated previously was recessive, it was possible to isolate new mutations in *secB* that would cause an inability to complement the *secB2* mutation. Such mutations were isolated in a merodiploid strain, as described above. The phenotype caused by these mutations was identical to the phenotype caused by the *secB*::Tn*5* mutation; therefore, we refer to them as null mutations and to strains carrying these mutations as null mutants. When the null mutations were moved from the merodiploid strain into a haploid strain, 22% (7 of 32) of the PyrE$^+$ transductants received the *secB* mutation and behaved like the *secB*::Tn*5*

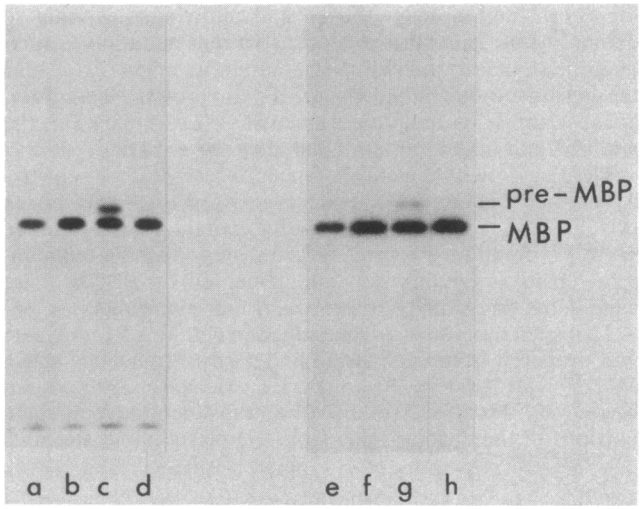

FIG. 2. Complementation of the *secB*1 mutation by plasmids. Cell extracts were immunoprecipitated with anti-MBP antiserum. Strains and their lanes: a, MRi80 (*secB*$^+$)(pBR322); b, MRi80(pCK1); c, MM145 (*secB1*)(pBR322); d, MM145(pCK1); e, MM146 (*secB*$^+$)(pCK5 *secB*::Tn*5*); f, MM146(pCK10 *gpsA*::Tn*5*); g, MM147 (*secB1*)(pCK5); h, MM147(pCK10).

mutant strain. Although they grew well on minimal media, these strains failed to grow on L broth plates.

Secretion was examined in the *secB*::Tn*5* strain. Elimination of functional SecB protein caused a strong defect in secretion of MBP and OmpF protein, a major outer membrane protein (Fig. 3). Secretion of MBP and OmpF protein was similarly defective in two *secB* null mutants (data not shown). Secretion of the outer membrane LamB protein was defective in the two *secB* null mutants that were examined, *secB7* and *secB8* (Fig. 4). However, secretion of RBP was unaffected in the *secB*::Tn*5* strain (Fig. 3) and in the *secB* null mutants (data not shown). In a shorter pulse-labeling experiment, RBP secretion was also identical in the $secB^+$ and *secB*::Tn*5* strains (Fig. 3, lanes a and b). In contrast, the *secA51* mutation causes the precursor form of RBP to accumulate (10). Secretion of the periplasmic enzyme alkaline phosphatase is also unaffected by *secB* mutations (K. Strauch, unpublished data).

The *secB* gene is close to a locus known as *rfa*, which is involved in the biosynthesis of the lipopolysaccharide located in the outer half of the bacterial outer membrane. It seemed possible that *secB* and *rfa* mutations might be affecting the same pathway. *rfa* mutants are resistant to phage U3 (3) which uses lipopolysaccharide as its receptor. However, although the *secB* null mutants plate U3 with a reduced efficiency and the phage plaques are smaller, the strains are still sensitive to the phage, unlike *rfa* mutants.

Order of function of *secB* and *secA*. Previous results suggested that *secB* and *secA* function in the same pathway for secretion (17). The following epistasis experiment provides further support for that conclusion and suggests that *secB* functions first in the pathway.

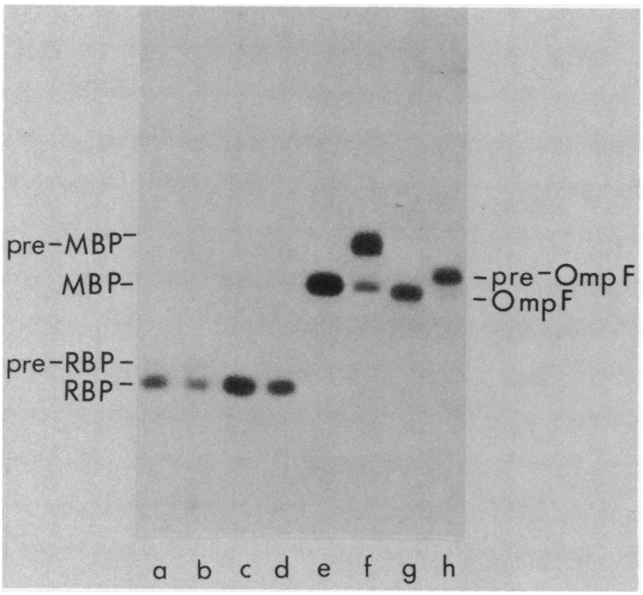

FIG. 3. Effects of the *secB*::Tn*5* mutation on exported proteins. Cells were labeled with [^{35}S]methionine for 30 s (lanes a and b) or 2 min (lanes c to h). Extracts of the cells labeled for 30 s were immunoprecipitated with anti-RBP antiserum (lanes a and b). Extracts of the cells labeled for 2 min were divided and immunoprecipitated with anti-RBP (lanes c and d), anti-MBP (lanes e and f), or anti-OmpF antiserum (lanes g and h). Strains and their lanes: MM149 ($secB^+$) (RBP); b, MM152 (*secB*::Tn*5*) (RBP); c, MM149 (RBP); d, MM152 (RBP); e, MM149 (MPB); f, MM152 (MBP); g, MM149 (OmpF protein); and h, MM152 (OmpF protein).

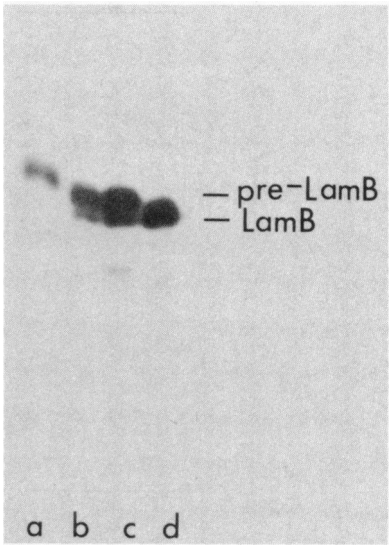

FIG. 4. Effects of *secB7*, *secB8*, and *secA51* mutations on export of LamB protein. Cell extracts were immunoprecipitated with anti-LamB antiserum. Strains and their lanes: a, MM52 (*secA51*); b, MM151 (*secB8*); c, MM150 (*secB7*); and d, MM149 ($secB^+$).

Elimination of SecA protein with an amber mutation in the *secA* gene causes a defect in the translation of secreted proteins (18, 24). If *secB* functions in the same pathway as *secA* but before *secA*, introduction of the *secB7* mutation into a *secA*(Am) strain would result in blockage of the secretion pathway before the effects of the *secA*(Am) mutation are manifested. Because the *secA*(Am) mutation is a lethal mutation, its effects on secretion are examined in the presence of a temperature-sensitive amber suppressor (24). At 30°C, secretion was fairly normal (Fig. 5, lane a). However, after the cells were incubated at 42°C, MBP synthesis was reduced (Fig. 5, lane d) as shown previously. In contrast, the cytoplasmic-protein elongation factor G continued to be synthesized under these conditions. Introduction of the *secB7* mutation into *secA*(Am) strain MM113 prevented the reduction in MBP synthesis (Fig. 5, lane e). In the double mutant, strain MM153, MBP precursor was synthesized in normal amounts.

Identification of SecB protein. By comparison of the products produced from $secB^+$ and *secB*::Tn*5* plasmids in maxicells, we identified the SecB protein. A comparison of products produced from pCK1 and pDC2 ($secB^+$ plasmids) and pCK5 and pCK11 (*secB*::Tn*5* plasmids) is shown in Fig. 6. All of the proteins produced by pCK1 appeared to be produced by pDC2 (except the products from the Tn*5* fragment that is present on pCK1). The same protein was eliminated by both of the Tn*5* insertions that inactivate *secB*. The apparent molecular weight of SecB protein was 12,000, based on its electrophoretic mobility in comparison with standards.

DISCUSSION

These results demonstrate that mutations with severe effects on *secB* cause severe defects in protein secretion. The *secB* mutants obtained in these studies were defective in the secretion of only some proteins. Although MBP, OmpF, and LamB proteins were inhibited in their secretion, RBP and alkaline phosphatase were secreted identically in wild-

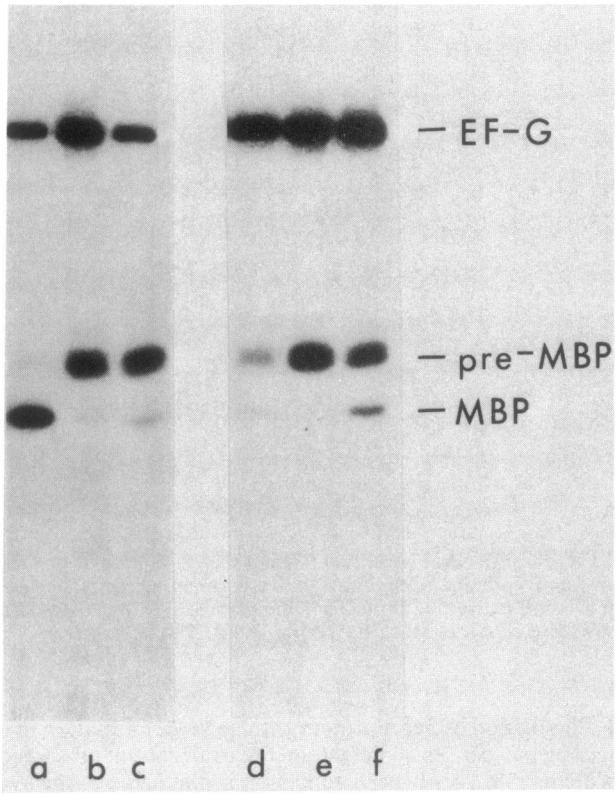

FIG. 5. Synthesis of MBP by *secB7 secA*(Am) double mutants. All extracts were immunoprecipitated with a mixture of anti-elongation factor G and anti-MBP antisera. Strains and their lanes: a, MM113 [*secB*$^+$ *secA*(Am)], 30°C; b, MM153 [*secB7 secA*(Am)], 30°C; c, MM150 (*secB7*), 30°C; d, MM113, 41°C; e, MM153, 41°C; and f, MM150, 41°C.

type and *secB* mutant strains. This suggests that in vivo some of the components of the secretory machinery function to facilitate secretion of subsets of secreted proteins. In contrast, the *secA* and *secC* mutations have much more general effects and affect all of the proteins that were studied here (9, 23). One explanation for these results is that SecB protein functions to initiate the export of specific proteins and to bring those proteins into a common secretion pathway. In fact, it may be the interaction of the SecB protein with ribosomes translating MBP that results in the block in MBP synthesis in the *secA*(Am) and *secC*(Cs) mutants. Proteins such as RBP and alkaline phosphatase may require the function of another SecB-like protein to enter this secretion pathway. This model is consistent with the finding that *secB* appears to function before *secA* and suggests that steps in the export process that are specific for individual proteins occur before steps that are shared by many exported proteins. Furthermore, the nonlethality of the *secB*::Tn*5* strain on minimal media may be because only a subset of exported proteins was affected.

Previous results demonstrated that the synthesis of MBP was blocked in a *secA*(Am) mutant strain under nonpermissive conditions and that mutations in the signal sequence of MBP reverse this effect (18). Therefore, it appears that recognition of the MBP signal sequence by the secretion machinery of the cell is involved in the translation block phenomenon. In the epistasis experiments reported here, the *secB* mutation also reversed the translation block. Because under the nonpermissive conditions SecA protein was not produced, it is unlikely that interaction between SecB and SecA proteins can account for these results. Rather, these results suggest that SecB protein acts before SecA protein in the same pathway. Therefore, SecB protein may be involved in mediating the interaction between the signal sequences of certain proteins and the secretion machinery. The specificity of the *secB* mutations may be a result of selective recognition of signal sequences or other components of exported proteins.

There are other steps in secretion in which the properties of different proteins diverge. For instance, the processing of the signal sequence from the precursor protein may occur predominantly post- or cotranslationally, depending on the protein (15, 27). However, this distinction does not correlate with the sensitivity of proteins to *secB* mutations, because LamB protein and RBP are both processed posttranslation-

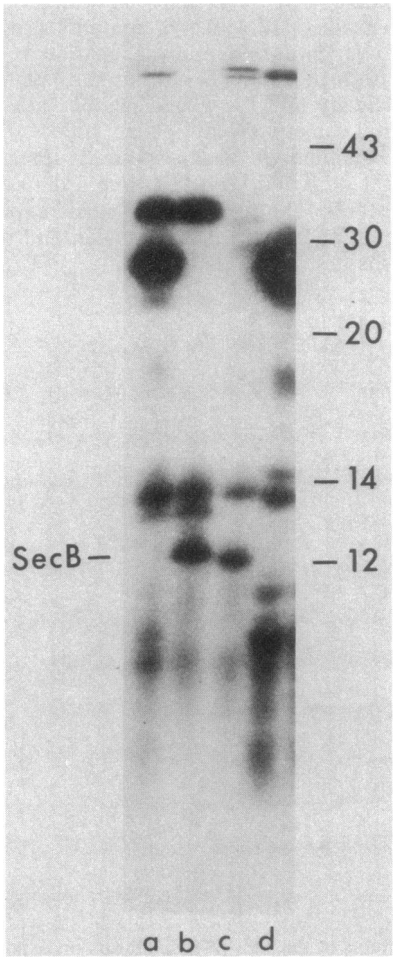

FIG. 6. Identification of SecB protein. All strains were SY822 derivatives carrying plasmids. Labeling and extraction was performed as described in the text, and extracts were analyzed on a 15% polyacrylamide gel. The following proteins were used as molecular weight standards: ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,000), α-lactalbumin (14,000), and cytochrome *c* (12,000). Lanes: a, pCK11 (*secB*::Tn*5*); b, pDC2 (*secB*$^+$); c, pCK1 (*secB*$^+$); and d, pCK5 (*secB*::Tn*5*).

ally and export of the former is dependent on SecB but the latter is not. Similarly, MBP and alkaline phosphatase are processed both post- and cotranslationally, and the former is SecB dependent, but the latter is not.

The *secB* gene mapped close to other genes involved in cell envelope biogenesis, *gpsA* and *rfa*. The properties of Tn*5* insertions in *gpsA* and *secB* showed that these are separate genes. Although mutations in *secB* did affect the sensitivity of the cell to phage U3, a property shared with *rfa* mutants, the phenotype was not as strong. However, we leave open the possibility that there is some connection or common components to the export of proteins and that of lipopolysaccharides.

A pulse-chase labeling experiment with the *secB*::Tn5 mutant strain showed that at least some of the pre-MBP can be chased slowly into mature MBP after 5 to 10 min of chasing with nonradioactive methionine (unpublished data). These results suggest that in the *secB* mutant strain, some export of proteins can occur posttranslationally. Posttranslational secretion has been previously observed in other strains under other conditions (e.g., see references 21, 28, 33).

The Tn*5* insertions that inactivate *secB* are probably located within the *secB* gene. If *secB* was in an operon with another gene, insertions in the other gene might eliminate *secB* function through polar effects on expression. However, this is a less likely explanation because, as shown in Fig. 6, the Tn*5* insertion on pCK11 did not appear to affect production of any protein other than the 12,000-molecular-weight protein. Also, the insertions on pCK5 and pCK11 both eliminated the same small protein.

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