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Mutations in a New Gene, *secB*, Cause Defective Protein Localization in *Escherichia coli*

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We isolated a new class of *Escherichia coli* mutants with pleiotropic defects in protein secretion. Using a previously described selection procedure (Oliver et al., Ann. Microbiol. [Paris] **133A**:105–110, 1982), we obtained a large collection of strains containing mutations that affect protein localization. In many cases, the lesions causing the secretion defects were mapped in or near the previously identified gene, *secA* (Oliver and Beckwith, Cell **25**:765–772, 1981). However, the selection also yielded mutants with mutations in a new locus, which was designated *secB*. These *secB* mutants were defective in the localization of maltose-binding protein and, in at least one case, OmpF protein. Double mutants with lesions in both *secA* and *secB* had strong defects in the secretion of maltose-binding protein and OmpF protein. The *secB* locus mapped near *cysE* at min 80.5 on the *E. coli* genetic map. The properties of *secB* mutants suggest that the *secB* product could be a component of the *E. coli* secretory apparatus.

In recent years the mechanism of protein secretion has been studied in great detail in both eucaryotic (23) and procaryotic (17) systems. There appear to be striking similarities among organisms in the initial steps of the process of protein transfer across membranes. Although many of the fundamental advances in understanding protein secretion have been made in eucaryotic systems, the gram-negative bacteria, *Escherichia coli* and *Salmonella typhimurium* offer the significant advantage of highly developed genetic systems. Already the characterization of bacterial mutants defective in secretion has provided direct evidence that signal sequences are required for secretion (2, 9). Furthermore, the analysis of chain termination and mutants with deletion mutations in certain genes has shown that very little, if any, of the amino acid sequence of proteins other than the signal sequence is necessary to promote traversal of the cytoplasmic membrane (13).

Genetic studies on secretion in *E. coli* were also aimed at understanding the nature of the cellular machinery required for secretion and involved the isolation and characterization of mutations having pleiotropic effects on protein export. Mutations in one locus, *prlA*, are suppressors of signal sequence mutations (8); that is, they restore the secretion of several different proteins with defective signal sequences (7). Ito (11) found a temperature-sensitive lethal mutation in or near the *prlA* gene that causes the cell to accumulate precursors of normally secreted proteins. Another class of mutations in a gene now termed *envZ* decrease the amounts of a number of periplasmic and outer membrane proteins (24, 25). The *expA* mutation has similar properties (6). However, neither *envZ* nor *expA* mutants accumulate precursors of secreted proteins, indicating that the effects of these mutations on secretion may not be direct.

We previously described a selection procedure that yields mutants with pleiotropic defects in secretion (22). The selection is based on the fact that a hybrid protein arising from a hybrid gene, with the amino terminus of the maltose-binding protein (MBP) gene fused to the gene coding for β-galactosidase, is localized to the cytoplasmic membrane, where it has abnormally low basal β-galactosidase activity, causing a Lac^- phenotype. Selection for Lac^+ derivatives yields mutants in which some of the hybrid protein is localized to the cytoplasm, where it has higher enzymatic activity. This procedure provides a direct selection for mutants altered in secretion and also allows the detection of mutants with only a 5 to 10% defect in the process. Since mutations that cause strong defects in secretion would probably be lethal to the cell, this sensitive selection procedure permits the isolation of mutants that might otherwise be difficult to obtain.

Using this selection, Oliver and Beckwith (20) obtained one class of mutants that have slight defects in secretion at 30°C but are strongly affected at 42°C. These mutants which are termed *seeA* in analogy to the *see* mutants of *Saccharomyces cerevisiae* (19), are conditional

TABLE 1. Bacterial strains

Strain	Relevant characteristics	Source or reference
MC4100	F⁻ *lacU169 araD139 thiA rpsL relA*	Lab collection
MM18	MC4100 (λp 72-47)	1
MM52	MC4100 *secA51*	20
MM81	MM18 *secB1*	This study
MM82	MM18 *secB2*	This study
MM83	MM18 *secB3*	This study
MM84	MC4100 *secB1*	This study
MM85	MC4100 *secB2*	This study
MM86	MC4100 *secB3*	This study
MM87	MM82 *zia*::Tn*10*	This study
MM88	MC4100 *leu*::Tn*10 secA51*	This study
MM89	MM84 *leu*::Tn*10 secA51*	This study
MM90	MM85 *leu*::Tn*10 secA51*	This study
MM91	MM18 *cysE*::Tn*5*	This study
MM92	MM18 *zia*::Tn*10 pyrE*	This study
MM93	MM18 *mtl-2*	This study
MM94	MM82 *mtl-2 zia*::Tn*10*	This study
MM95	MM82 *cysE*::Tn*5 recA56*	This study
MM96	MM18 *recA56*	This study
MM97	MM82 *recA56*	This study
MM60	MC4100 *leu*::Tn*10*	Lab collection
MM56	MC4100 *leu*::Tn*10 secA51 ompF*::Tn*5*	Lab collection
XAc	F⁻ *ara* Δ(*lac-pro*)*XIII argE*(Am) *nalA* Rif^r *thi*	Lab collection
XAc-10	XAc *malT cysE*::Tn*5 mtl-2 recA1*	This study
IO7011.1	F⁻ *trpR trpA his metE ilvD bgl*⁺ Str^r	Lab collection
MS272	F⁻ *purE lacY malA xyl-7 mtl-2* Str^r	R. Isberg
CK101	F′ ts *lac* Tn*10*/MC4100 *thyA zia*::Tn*10*	This study
CK102	Hfr MC4100 *thyA zia*::Tn*10* (F′ts *lac* Tn*10*, integrated)	This study
CK103	F′ts *mtl secB*/XAc-10	This study

lethals. The *secA* gene maps at min 2.5 on the *E. coli* chromosome (20). Studies on the *secA* protein suggest that it is a component of the secretory apparatus of *E. coli* (21).

In this paper, we describe a more detailed analysis of the mutants obtained in the Lac⁺ selection with the hybrid protein. These studies demonstrate the existence of a new class of mutants with pleiotropic defects in secretion. The mutants define a new gene, *secB*, whose product may be involved in the secretory apparatus of *E. coli*.

MATERIALS AND METHODS

Genetic techniques. Bacterial strains are listed in Table 1. The media and techniques used, such as for P1 transduction and bacterial conjugation, have been described previously (18). Lac⁺ derivatives of strain MM18 were isolated by previously described methods (20). To bias the selection against signal sequence mutants, we picked only those papillae that arose after 3 days of incubation. These were purified on minimal lactose plates. Previously described methods were used to cure these mutants of the λ transducing phage carrying the *malE-lacZ* fusion (20).

Isolation of Tn*10* insertion linked to *secB*. Approximately 5,000 derivatives of strain MM85 (*secB2*) containing random Tn*10* insertions were pooled (15). A P1 lysate was prepared from the mixture. Strain MM18 was transduced with this lysate, selecting for tetracycline resistance on minimal lactose plates. Larger colonies were picked and purified. P1 transduction was used to confirm that the Tn*10* insertions were linked to the original *secB2* mutation.

Isolation of Hfr strains. We used the strain carrying the Tn*10* linked to *secB* to isolate an Hfr strain with its origin of transfer near *secB* by the methods of Chumley et al. (4). Briefly, strain CK101 was grown at 42°C in M63 medium containing lactose and thymine. Stable Lac⁺ colonies were isolated from this culture on MacConkey plates with lactose at 42°C. These were Hfr strains with the F′ integrated in the chromosome via the Tn*10* genetic homology.

Isolation of F′ episome. Episomes carrying *secB* were isolated from the Hfr strain described above by selecting for episomes carrying the closely linked marker *cysE*. Strain CK102 was mated with strain XAc-10 (*cysE recA malT*) at 30°C in L broth. After being pelleted and resuspended in M63 medium, undiluted mating mixture was plated on minimal plates supplemented with glycerol (0.2%), arginine (80 μg/ml), and glycylproline (30 μg/ml) and spread with λ *vir*. λ *vir* was included to bias the selection for smaller episomes that did not carry *malT*⁺. Out of 50 episomes analyzed, 2 were Tet^r and carried *mtl*, *cysE*, and *secB* but not *xyl* or *lac*. One of these episomes was designated F′ts *mtl secB*.

The *secB2* mutation was crossed onto the F′ episome. Strain CK103 (F′ts *mtl secB*/XAc-10) was crossed with strain MM82 (*secB2 recA*⁺). A saturated culture of this diploid strain was diluted and plated on minimal lactose plates containing tetracycline. Larger colonies were picked and purified; these were homogenotes. To show that these F′ episomes carried the *secB2* mutation, we crossed them into strain MM18 (*secB*⁺ *recA*⁺). Recombination between chromosome and episome gave rise to *secB*⁻ derivatives.

Labeling, immunoprecipitation, and polyacrylamide gel electrophoresis. Cells were grown in M63 medium containing 0.4% glycerol and, for the analysis of MBP, 0.4% maltose. Cultures were incubated with [^{35}S]methionine (approximately 1,200 Ci/mmol) at 20 μCi/ml for various times. Unless otherwise noted, immunoprecipitation was performed as previously described (12). Immunoprecipitates were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (16), followed by fluorography with sodium salicylate (3). Antisera to MBP, OmpF protein, and ribose-binding protein (RBP) were the gifts of W. Boos, R. Taylor, and J. Garwin, respectively.

Fractionation of cells. Cells were treated with lysozyme-EDTA to release periplasmic contents (14). Spheroplasts were lysed by three cycles of freezing and thawing followed by centrifugation. This treatment gave a soluble fraction (cytoplasm) and a fraction that sedimented (membrane). SDS was added to all

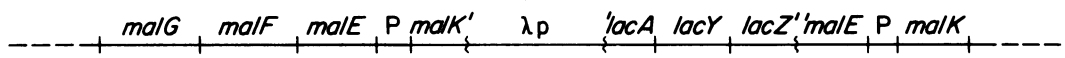


FIG. 1. Structure of strain MM18 lysogen. We showed by restriction mapping that λp72-47 carries only a portion of *malK* (C. Kumamoto and S. Froshauer, unpublished data).

fractions to a final concentration of 1%. After being heated at 100°C for 2 min, samples were immunoprecipitated.

β-Galactosidase assay. The procedure for assay of β-galactosidase activity has been described (18).

RESULTS

Selection for mutants with higher basal β-galactosidase activity yielded only three classes. Strain MM18 carries a hybrid gene in which a substantial portion of the gene (*malE*) for the periplasmic MBP is attached to the gene (*lacZ*) for the cytoplasmic β-galactosidase. This hybrid gene is carried on a λ transducing phage, which is integrated into the chromosome by genetic homology with the *mal* genes (Fig. 1). Because this strain has a Lac^- phenotype, mutant derivatives with higher basal β-galactosidase activity could be selected. Among these we expected to find mutants defective in localization of the *malE-lacZ* fusion protein, as described above. Lac^+ selections were carried out on cultures of 58 independent clones of strain MM18, and 187 Lac^+ mutants were genetically characterized (Table 2). As a first step, we determined whether the mutations were genetically linked to the fusion. In some cases, the fusion strains were cured of the λ fusion phage by exposure to UV light. The λ phage will excise by homologous recombination between repeated segments of the *mal* genes. If the mutation causing the Lac^+ phenotype is in the hybrid gene, in many cases the chromosome will no longer contain a mutation when the phage excises. These cured strains, when relysogenized with the original λ fusion phage, will be Lac^-. Alternatively a λ fusion phage lysate was made from the mutant strain and used to transduce strain MC4100, the parent of strain MM18, to Lac^+. If the mutation were linked to the fusion, some of the phage from the lysate should, after integration, give Lac^+ derivatives. In this way it was shown that 71 of the mutations causing the Lac^+ phenotype were linked to the *malE-lacZ* fusion. We presume that many, if not all, of these are signal sequence mutations.

TABLE 2. Distribution of Lac^+ mutants[a]

Mutant class	No. (% of total)
Linked to fusion	71 (38)
Linked to *secA* (nonlethal)	84 (45)
Linked to *secA* (temperature sensitive)	19 (10)
Linked to *secB* (nonlethal)	13 (7)

[a] Mutants were isolated from strain MM18 by selecting for higher β-galactosidase activity. They were grouped into classes based on the genetic location of the mutation conferring this phenotype. The mutations were mostly independent.

Of the mutations, 103 were in or near the *secA* gene. This was shown by their P1 cotransduction with a Tn*10* transposon inserted near the *secA* gene. This class included both 19 conditional lethal (temperature-sensitive) and 84 nonlethal mutations.

The remaining 13 mutations mapped in a third region of the chromosome. A Tn*10* insertion was isolated in which Tn*10* was 90% P1 cotransducible with one of the mutations (see above). P1 transduction showed that all the other mutations in this class were similarly linked to the Tn*10*. The locus defined by these mutations was designated *secB*.

***secB* mutants defective in protein localization.** Since *secA* mutants obtained by this selection have pleiotropic effects on secretion (20), we examined *secB* mutants for similar properties. It should be pointed out that the studies with *secA* were facilitated by the conditional lethal nature of some of the mutations, which made it possible to look for effects on secretion when the defect was strong (at 42°C). In contrast, nonlethal mutations, such as the ones found so far in *secB*, would probably not cause such pronounced defects.

We first examined the secretion of MBP in the *secB* mutants. The strains were first cured of the transducing phage carrying the *malE-lacZ* fusion. The cured mutants were labeled with [^{35}S]methionine, extracted, and immunoprecipitated with anti-MBP antiserum. Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis. Although strain MC4100, the wild-type parent, contains only mature MBP (Fig. 2, lane a), three independently isolated *secB* mutants accumulated a small amount of precursor to MBP (lanes b through d). All *secB* mutants analyzed showed accumulation of the precursor (not shown). Since the differences in the amounts of precursor (Fig. 2) were reproducible, the *secB* mutants seemed to differ from each other.

A fractionation experiment with strain MM84 (*secB1*) showed that the accumulation of MBP precursor was due to a defect in secretion. After being labeled, cells were fractionated into periplasmic, membrane, and cytoplasmic fractions.

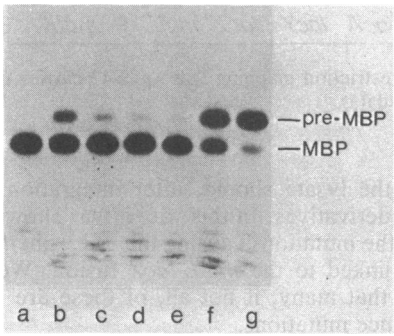


FIG. 2. Analysis of MBP in mutant strains at 30°C: (a) MC4100; (b) MM84 (*secB1*); (c) MM85 (*secB2*); (d) MM86 (*secB3*); (e) MM52 (*secA51*); (f) MM89 (*secA51 secB1*); (g) MM90 (*secA51 secB2*).

The fractions were immunoprecipitated and analyzed as described above. Again, strain MC4100 contained only mature MBP, and it was found almost entirely in the periplasmic fraction (Fig. 3). The small amount seen in the cytoplasmic fraction was probably contamination due to incomplete release of periplasmic contents. When strain MM84 was fractionated, pre-MBP was found in the cytoplasmic and membrane fractions and mature MBP was found predominantly in the periplasm (Fig. 3). Pre-MBP was greatly enriched relative to MBP in the cytoplasmic fraction.

In addition, the *secB1* mutation caused mutants to accumulate the precursor form of OmpF protein, one of the major proteins of the outer membrane. After being labeled, cell extracts were immunoprecipitated with anti-OmpF antiserum. With strain MC4100, only one band,

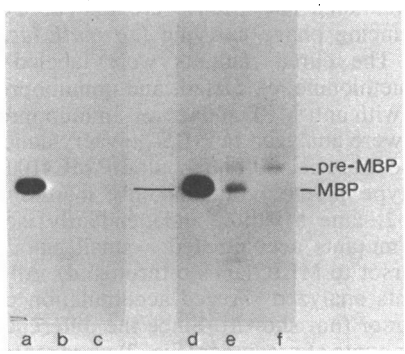


FIG. 3. Location of MBP and pre-MBP. Fractions from cells labeled for 30 s were immunoprecipitated with anti-MBP antiserum and electrophoresed on a 9% polyacrylamide gel. (a, b, and c) Strain MC4100, periplasmic (a), cytoplasmic (b), and membrane (c) fractions; (d, e, and f) strain MM84, periplasmic (d), cytoplasmic (e), and membrane (f) fractions.

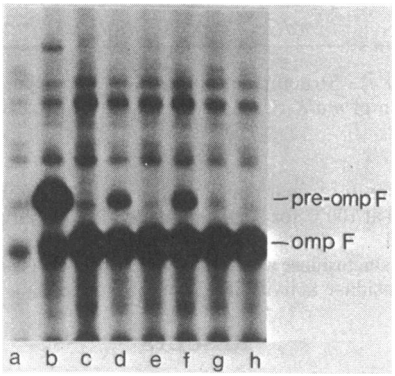


FIG. 4. Analysis of OmpF protein in mutants. Cells were labeled for 2 min at 37°C (strain MM52) or 30°C (all other strains). Extracts were immunoprecipitated with anti-OmpF antiserum with phenylmethylsulfonyl fluoride (150 μg/ml). Immunoprecipitates were analyzed on a 9% polyacrylamide gel. Strains: (a) MM56 (*ompF*::Tn*5*); (b) MM52 (*secA51*); (c and e) MC4100; (d and f) MM84 (*secB1*); (g) MM85 (*secB2*); (h) MM86 (*secB3*).

mature OmpF protein, was specifically precipitated (Fig. 4). Immunoprecipitation of a strain carrying an *ompF*::Tn*5* mutation showed that some proteins were precipitated nonspecifically; however, when strain MM84 extracts were analyzed, a second, slower-migrating protein species was detected (Fig. 4). This band comigrated electrophoretically with the putative OmpF precursor that accumulated in strain MM52 (*secA51*) cells at the nonpermissive temperature (Fig. 4). Therefore, the *secB1* mutation caused cells to accumulate precursor to OmpF protein. *secB2* and *secB3* mutants did not contain detectable amounts of pre-OmpF protein (Fig. 4). Secretion of RBP was also examined. As compared with the wild type, none of the *secB* mutants analyzed accumulated detectable amounts of pre-RBP (Fig. 5). In contrast, *secA51* mutants accumulated almost entirely pre-RBP at the nonpermissive temperature.

Since overproduction of the SecA protein has been seen when secretion is blocked (21), it is conceivable that the *secB* mutations might act by causing an overproduction of SecA protein, which disrupts the secretion process. However, analysis of the rate of SecA protein synthesis by labeling cells and immunoprecipitating them with anti-SecA protein antiserum showed that there was no detectable change in the rate of synthesis (data not shown). Therefore, the *secB* mutations do not act by regulating the synthesis of SecA protein.

***secA51* mutation enhances the defect of *secB* mutants.** To further examine the relationship of *secB* and *secA*, we introduced the *secA51* condi-

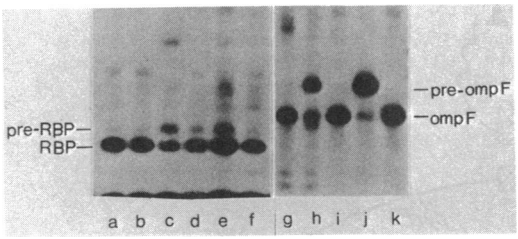


FIG. 5. Analysis of OmpF protein and RBP. Cells were labeled for 2 min at 30°C. Extracts were immunoprecipitated with 150 μg of phenylmethylsulfonyl fluoride per ml, with anti-RBP antiserum (a through f) or anti-OmpF antiserum (g through k). Immunoprecipitates were analyzed by electrophoresis in SDS on a 10% polyacrylamide gel. Strains: (a) MC4100; (b and k) MM85 (*secB2*); (c and j) MM90 (*secA51 secB2*); (d and i) MM52 (*secA51*); (e and h) MM89 (*secA51 secB1*); (f and g) MM84 (*secB1*).

tional lethal mutation into two *secB* mutant strains by P1 transduction, using strain MM88 as the donor. The double mutants grew less well at the high and intermediate temperatures than did the *secA51* parent strain. The double mutants were pulse-labeled with [^{35}S]methionine at 30°C, the permissive temperature, for 30 s, immunoprecipitated with anti-MBP antiserum, and electrophoresed in SDS–9% polyacrylamide gels. Both double mutants accumulated significantly more pre-MBP than either *secB* or *secA* mutants alone (Fig. 2). The amount of precursor seen appeared to be more than could be accounted for by the additive effects of the two mutations.

This synergism of effects was seen even more strongly when OmpF protein was examined. After being labeled at 30°C, extracts were immunoprecipitated with anti-OmpF antiserum and analyzed as before. *secA51 secB* double mutants accumulated almost entirely pre-OmpF protein (Fig. 5). In contrast, the *secB2* mutant did not accumulate detectable amounts of precursor (Fig. 5), and *secA51* and *secB1* mutants accumulated only a small amount, which could be seen after longer exposure (data not shown). When the same extracts were precipitated with anti-RBP antiserum, the *secA51 secB2* double mutant accumulated significantly more pre-RBP than did either of the mutants carrying only one of these mutations. The amount of pre-RBP seen with the *secA51 secB1* double mutant was probably not significantly different from that seen with the *secA51* mutant (Fig. 5).

secB mutation located near min 80.5 on the _E. coli_ genetic map. Since *secB* mutants did not have a readily selectable phenotype, they were mapped indirectly by first isolating a Tn*10* insertion near *secB* and mapping the location of the Tn*10*. This approach also permitted the isolation of an Hfr with its origin close to *secB* and an F′ episome carrying the *secB* gene. A pool of random Tn*10* insertions in a *secB* mutant was used to prepare a P1 lysate. Strain MM18 was transduced with this lysate to tetracycline resistance on minimal lactose plates. *secB* mutants formed slightly larger colonies on these plates than did the wild-type parent strain, MM18. By screening many of the larger colonies, we isolated several strains containing Tn*10* insertions linked to *secB*, and one of these was used for mapping. The Tn*10* insertion used was 90% P1 cotransducible with *secB*.

After introduction of the Tn*10* into Hfr Cavalli by P1 transduction, time-of-entry experiments were used to show that the Tn*10* was transferred after *argE* (min 89). The location of the Tn*10* insertion was more precisely mapped by using an F′ts *lac* Tn*10* to construct an Hfr strain from the strain bearing it. When the episome integrated via Tn*10* homology, an Hfr strain was generated with an origin of transfer very close to *secB* (Fig. 6). This Hfr strain (CK102) was found to transfer *argE* (min 89), *ilv* (min 84), and *pyrE* (min 81), but not *cysE* (min 80.5), *mtl* (min 80), or *xyl* (min 79), suggesting that the Tn*10* insertion was located between *cysE* and *pyrE*. P1 transduction analysis showed linkage between the Tn*10* and *cysE*, *pyrE*, *mtl*, and *xyl*, confirming the information from the Hfr strain.

secB was found to be 92% P1 cotransducible with *cysE* and was not transferred by CK102. Reciprocal three-factor crosses, with *mtl* used as the selected outside marker, confirmed the order *mtl*, *secB*, and Tn*10* (Fig. 7). The approximate map position of *secB* is shown in Fig. 6A. We recently isolated a λ transducing phage carrying both *secB* and *gpsA*, the origin of which confirmed the gene order shown (Fig. 6) (unpublished data).

Merodiploid studies showed that the *secB2* mutation was recessive. To perform these experiments, we isolated an F′ episome carrying *mtl*, *cysE*, and *secB* but not *xyl* or *lac* from strain CK102. When the episome was transferred into strain MM95, the resultant diploid had the wild-type phenotype when analyzed on lactose indicator plates. This result was confirmed by β-galactosidase assays. The merodiploid strain was found to have 1.8 U of activity, compared with 1.4 U for strain MM96 (*secB*$^+$) and 5.9 U for strain MM97 (*secB2*). All strains used in this experiment were *recA*$^-$ to prevent recombination between the chromosome and episome.

Using this episome, we also obtained evidence suggesting that the different *secB* mutations were allelic. First, the *secB2* mutation was crossed onto the episome. When this episome was mated into strain MM81 (*secB1*) or MM83 (*secB3*), the resultant diploids had mutant phenotypes. In contrast, when an episome carrying

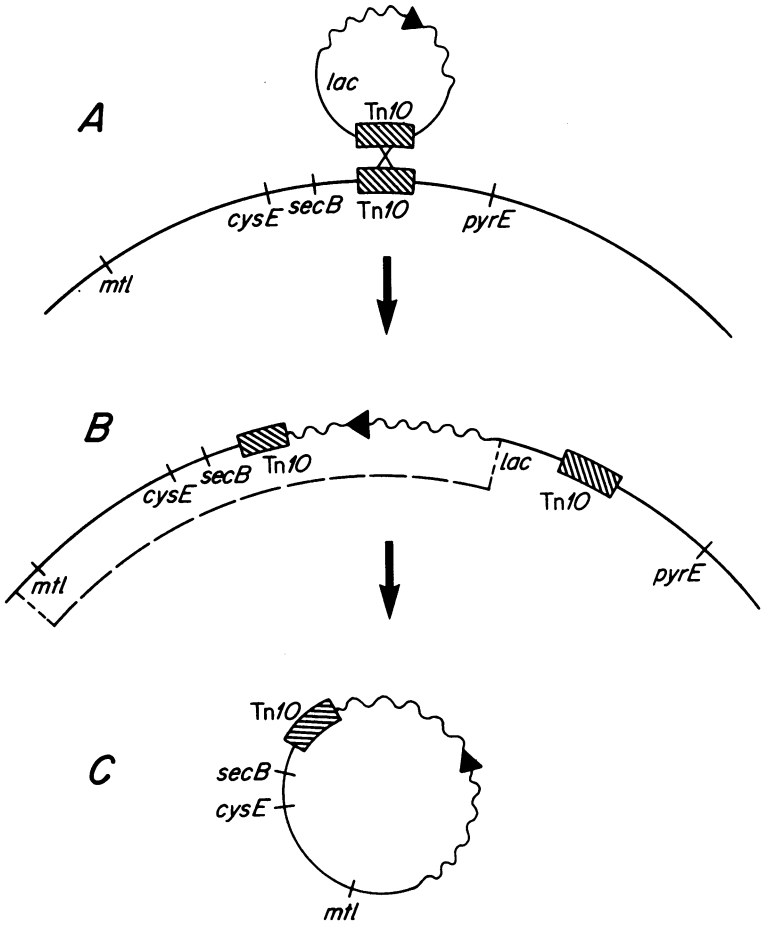


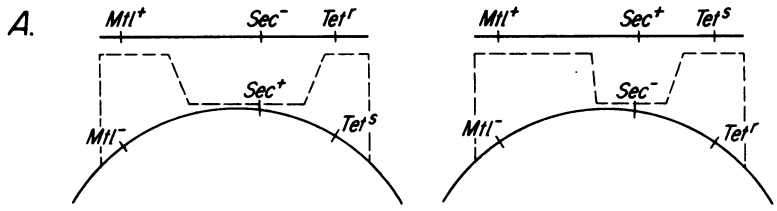
FIG. 6. Construction of strains CK102 and CK103. (A) Strain CK101. Selection of stable Lac$^+$ derivatives of strain CK101 at 42°C yielded the Hfr strain CK102 (B). Mating strains CK102 and XAc-10 yielded an F′ (C). The presumed excision event generating the F′ is shown as a dotted line in panel B. Tn*10* elements are shown as cross-hatched boxes and F-factor sequences as wavy lines. Since *secB* and *cysE* have not been ordered, the approximate map position of *secB* is shown.

secB$^+$ was crossed into these strains, the resultant diploids had wild-type phenotypes.

DISCUSSION

We characterized a new genetic locus, *secB*, which is defined by mutants having pleiotropic defects in secretion. Two general classes of explanations for the properties of *secB* mutants can be proposed. First, the *secB* gene could code for a component of the secretory machinery of the cell, and the mutations would directly block export. Second, the *secB* mutations could in some indirect fashion interfere with protein export; for example, they might eliminate some step in membrane biogenesis (e.g., lipid biosynthesis), which could in turn interfere with export of proteins. Another possibility is that the altered *secB* gene might produce an altered envelope protein that blocks secretion in a fashion similar to that of hybrid proteins that have this effect (12). The finding that *secB* mutations are recessive to wild-type *secB*$^+$ makes this explanation unlikely.

Several additional lines of argument suggest that *secB* mutations directly interfere with protein secretion. The identification of only two genetic loci, *secA* and *secB*, after extensive screening of Lac$^+$ derivatives of strain MM18 suggests that mutations that affect membrane biogenesis in general are not being picked up by this procedure. Furthermore, *secB* mutations do not seem to affect secretion nonspecifically by poisoning cellular metabolism. When different *secB* mutants were compared, the only one with a slight growth defect when cultured on plates was strain MM86. Since strain MM84 accumulated the most pre-MBP and pre-OmpF protein



B. DISTRIBUTION OF RECOMBINANTS

Sec^-Tet^s	17	0
Sec^-Tet^r	38	116
Sec^+Tet^s	107	36
Sec^+Tet^r	2	13

FIG. 7. Three-factor crosses. Strains MM93 and MM94 were transduced to Mtl^+ by using P1 lysates made from strain MM87 or MC4100, respectively. (A) Diagram of the crosses. The donors are drawn above and the recipients below; the dotted lines show crossovers that generated the minority classes. (B) Distribution of recombinants.

(Fig. 2 and 4), there was no correlation between the secretion defect and growth of the cells. Rather, strain MM86 may be more defective in the secretion of proteins essential for growth.

The results obtained with double mutants also supported the hypothesis that *secB* may code for a component of the secretory apparatus, as Oliver and Beckwith (21) presented evidence that the *secA* protein is directly involved in secretion. If the *secB* product acted totally independently of the *secA* product, it might be expected that double mutants would show the additive effects of the two mutations. In fact neither double mutant strain behaved this way; strains MM89 and MM90 both showed greater than additive effects on the secretion of MBP and OmpF protein. Also, strain MM90 (*secA51 secB2*) was more defective in secretion than was strain MM89 (*secA51 secB1*) for all three proteins examined. In contrast, strain MM85 ($secA^+$ *secB2*) was less defective in secretion than strain MM84 ($secA^+$ *secB1*) for MBP and OmpF protein. *secA* and *secB* gene products could be envisioned as participating in the same or parallel pathways for secretion. However, we feel that the simplest explanation for both the synergistic effects and the allele specificity is that the two products are involved in the same pathway. In addition, the allele specificity studies suggest that the *secA* and *secB* gene products might interact.

secB mutations probably affect secretion rather than proteolytic processing of precursors. Pulse-chase experiments showed that a pool of stable pre-MBP was present even after 9 min of being chased (unpublished data). Therefore, it does not appear that the *secB* mutants simply process precursors at a slower rate. However, if processing and secretion are tightly coupled, a processing defect could cause cytoplasmic accumulation of the precursor. In this case the cytoplasmic precursor might be stable.

Unlike the *secA51* mutation, *secB* mutations caused only a slight defect in secretion of MBP and had demonstrable effects on a restricted spectrum of proteins. Even at the permissive temperature, *secA51* had a more general effect on secretion. The apparent difference in the properties of *secA* and *secB* mutants may merely reflect the fact that *secA51* is a conditional lethal mutation and *secB* is not. Perhaps nonlethal *secA* mutations would have properties similar to those of the *secB* mutations. Alternatively, *secA* and *secB* gene products may have fundamentally different roles in secretion. For example, *secB* may code for an auxiliary factor required for the secretion of only a subset of secreted proteins. In this case, *secB* might not be essential for growth. We are currently seeking mutations that eliminate *secB* activity to test this hypothesis.

The *secB* gene maps near *gpsA*, which codes for the glycerol-3-phosphate dehydrogenase required for phospholipid biosynthesis. Since a defect in *gpsA* might cause an altered membrane, leading to a secretion defect, we tested the *gpsA20* mutation (5) to determine whether it was allelic to the *secB* mutations. When a *gpsA20* mutant lysogenized with λ72-47 was crossed with F'ts *mtl* carrying the *secB2* mutation, the resultant diploid was no longer auxotrophic for glycerol 3-phosphate and had the $secB^+$ phenotype (unpublished data). Although intragenic complementation is possible, this result suggests that *gpsA* and *secB* are separate genes.

Using the selection for internalization of a hybrid protein, we identified two loci, *secA* and *secB*, that seem to be involved in the secretion process. However, it is possible that other genes

are required for secretion but do not yield mutants with a Lac^+ phenotype. Mutations in genes coding for proteins required in later steps of secretion might not cause internalization of the hybrid protein and therefore would not have been detected in our selection. In addition, the evidence suggests that translation and secretion may be coupled (10). Mutations in genes coding for proteins required in the very early steps of secretion might block translation of the hybrid protein and would also not be isolated as Lac^+ mutants. Alternative selection or screening procedures must be developed to identify additional genes.

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