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prlA Suppression of Defective Export of Maltose-Binding Protein in *secB* Mutants of *Escherichia coli*

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An *Escherichia coli* strain containing a signal sequence mutation in the periplasmic maltose-binding protein (MBP) (*malE18-1*) and a point mutation in the soluble export factor SecB (*secBL75Q*) is completely defective in export of MBP and unable to grow on maltose (Mal$^-$ phenotype). We isolated 95 spontaneous Mal$^+$ revertants and characterized them genetically. Three types of extragenic suppressors were identified: informational (missense) suppressors, a bypass suppressor conferring the Mal$^+$ phenotype in the absence of MBP, and suppressors affecting the *prlA* gene, which encodes a component of the protein export apparatus. In this study, a novel *prlA* allele, designated *prlA1001* and mapping in the putative second transmembrane domain of the PrlA (SecY) protein, was found. In addition, we isolated a mutation designated *prlA1024* which is identical to *prlA4-2*, the mutation responsible for the signal sequence suppression in the *prlA4* (*prlA4-1 prlA4-2*) double mutant (T. Sako and T. Iino, J. Bacteriol. 170:5389–5391, 1988). Comparison of the *prlA1024* mutant and the *prlA4* double mutant provides a possible explanation for the isolation of these *prlA* alleles.

Identification of components of the *Escherichia coli* secretory apparatus has been facilitated by the isolation of mutants specifically defective in protein localization, using the protein fusion between the maltose-binding protein (MBP) and the cytoplasmic enzyme β-galactosidase (LacZ) (4, 6, 15, 28, 39). In a wild-type cell, export signals of MBP are responsible for localizing the MBP-LacZ hybrid protein to the cytoplasmic membrane. However, the membrane location renders the LacZ portion of the fusion enzymatically inactive (Lac$^-$ phenotype) (4). Selection for Lac$^+$ mutants in this system has yielded a number of signal sequence mutations, which have aided in defining *cis*-acting properties important for protein localization (6, 15). In addition, mutations causing pleiotropic defects in protein export have been isolated, identifying two genes, *secA* (39) and *secB* (28). A number of genetic and biochemical studies (reviewed in references 27 and 40) have helped elucidate the roles of these soluble cytoplasmic factors in protein export.

Isolation of suppressors of signal sequence mutations in *malE* (encoding MBP) or *lamB* (encoding the outer membrane lambda receptor) identified the *prl* (protein localization) loci (3, 17), which participate in the early stages of translocation, prior to the removal of the signal sequence by leader peptidase (47). The most efficient suppressors of signal sequence mutations mapped in the *prlA* gene (17) (also called *secY*), and subsequent isolation of recessive, export-defective alleles (23) in the same gene confirmed its essential role in protein translocation in vivo. Similarly, two independent approaches identified the dominant signal sequence suppressor alleles (48) and recessive, cold-sensitive, export-defective alleles of the gene *secE* (45). Finally, Lac$^+$ selection for mutants in which PhoA-LacZ or LamB-LacZ hybrid proteins are internalized has identified the *secD* and *secF* genes (19, 20). PrlA, SecE, SecD, and SecF are essential integral inner membrane proteins (reviewed in reference 44). There is ample genetic (7, 8) and biochemical (11, 21, 35) evidence suggesting that PrlA and SecE proteins assemble in the membrane and that SecA, a catalytic component of the translocation ATPase (32), associates with this complex.

SecB is a cytoplasmic chaperone required for efficient export of a small subset of *E. coli* proteins (28). Mutations in *secB* cause kinetic export defects, and *secB* null mutants (*secB*::Tn*5*) are nonviable on rich media (29). Binding of SecB to mature domains of precursors such as pre-MBP in the cytoplasm retards their folding (33) and allows rapid and productive interaction with the membrane export apparatus (26). Details of this membrane targeting via SecB are not completely understood. Biochemical evidence suggests that SecB-precursor complex binds to SecA, which in turn delivers the precursor to the export site on the membrane, composed of the PrlA and SecE proteins (21). Genetic experiments on SecA titration agree with such a model, also supporting a role for SecA during precursor translocation across the membrane (8).

In the selection described here, a genetic approach was taken to look for functional interactions between SecB and other components of the export apparatus. Suppressors that restored MBP export in a strain containing both a *malE* signal sequence mutation and a *secB* point mutation were isolated and characterized. We describe here several interesting classes of mutants which emerged from this selection, including a novel allele of *prlA*.

MATERIALS AND METHODS

Bacterial strains and phages. The *E. coli* K-12 strains used in this study are listed in Table 1. Generalized transduction using phage P1 *vir* was described previously (36). To generate pools of random Tn*10* insertions in strain CK2125, phage λNK55 (*b*221 *c*I857ts *O*am *c*III::Tn*10*) was used as described previously (24).

Reagents. L broth and L agar media, maltose tetrazolium agar, and minimal M63 and M9 salts were prepared as described previously (36). Minimal media were supplemented with thiamine (5 μg/ml) and 0.2% sugar or 0.5%

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TABLE 1. Genotypes of *E. coli* strains

Strain	Genotype	Source or reference
MC4100	(F⁻) *Δlac169 araD139 thiA rpsL relA motA*	Laboratory collection
CK2125	MC4100 *secBL75Q malT*(Con) *zjb*::Tn*5 malE18-1*	This study
CK2159	MC4100 *secBL75Q malT*(Con) *zjb*::Tn*5 malE18-1* Sp^r Sm^s	This study
K165	*lacZ*(Am) *phoA*(Am) *supC*(Ts) *trp*(Am) *recA1 rpsL malT*(Am) *rpoH*(Am) *spoT*	B. Bachmann
CK2206	F⁻ *purE lacY malA xyl mtl rpsL dpp*::*kan*	This study
CK2209	MC4100 *malT*(Con) *zjb*::Tn*10 ΔmalE444 secBL75Q*	This study
CKM132	CK2125 *meb*	This study
HS3309	MC4100 Sm^s *malT plp7 zjb*::Tn*10 ΔmalE444*	H. Shuman
JM109	F′ *traD36 lacI^q ΔlacZM15 proAB/recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 Δ(lac-proAB)*	50
CK2103	MC4100 *secB5 malT*(Con) *zjb*::Tn*5 malE18-1* Sp^r Sm^s	This study
OF262	MC4100 *secB*::Tn*5 malT*(Con) *malE18-1* Sp^r Sm^s	This study
CK2158	MC4100 *secBL75Q malT*(Con) *zjb*::Tn*10 ΔmalB101*	This study
CK2162	MC4100 *secBL75Q cysE*::Tn*5 malT*(Con) *malE18-1*	Laboratory collection
OF193	CK2125 *prlA4*	This study
CKM1090	CK2125 *prlA1090*	This study
CKM1001	CK2125 *prlA1001*	This study
CKM1062	CK2125 *prlA1062*	This study
CKM1024	CK2125 *prlA1024*	This study
OF228	MC4100 *pyrE malT*(Con) *malE18-1* Sp^r Sm^s	This study
OF281	MC4100 *malT*(Con) *malE18-1 prlA*$^+$	This study
OF282	MC4100 *malT*(Con) *malE18-1 prlA4*	This study
OF283	MC4100 *malT*(Con) *malE18-1 prlA1024*	This study
OF284	MC4100 *malT*(Con) *malE18-1 prlA1001*	This study
OF262	MC4100 *secB*::Tn*5 malT*(Con) *malE18-1 prlA*$^+$	This study
OF263	MC4100 *secB*::Tn*5 malT*(Con) *malE18-1 prlA4*	This study
OF265	MC4100 *secB*::Tn*5 malT*(Con) *malE18-1 prlA1001*	This study
OF269	MC4100 *secB*::Tn*5 malT*(Con) *malE18-1 prlA1024*	This study
OF140	MC4100 *secB*$^+$ *zjb*::Tn*10 ΔmalB101* Sp^r Sm^s	This study
OF426	MC4100 *secB*$^+$ *malT*(Con) *malE10-1 prlA*$^+$	This study
OF427	MC4100 *secB*$^+$ *malT*(Con) *malE10-1 prlA4*	This study
OF428	MC4100 *secB*$^+$ *malT*(Con) *malE10-1 prlA1024*	This study
OF429	MC4100 *secB*$^+$ *malT*(Con) *malE10-1 prlA1001*	This study
OF430	MC4100 *secB*$^+$ *malT*(Con) *malE16-1 prlA*$^+$	This study
OF431	MC4100 *secB*$^+$ *malT*(Con) *malE16-1 prlA4*	This study
OF432	MC4100 *secB*$^+$ *malT*(Con) *malE16-1 prlA1024*	This study
OF433	MC4100 *secB*$^+$ *malT*(Con) *malE16-1 prlA1001*	This study
OF434	MC4100 *secB*$^+$ *malT*(Con) *malE14-2 prlA*$^+$	This study
OF435	MC4100 *secB*$^+$ *malT*(Con) *malE14-2 prlA4*	This study
OF436	MC4100 *secB*$^+$ *malT*(Con) *malE14-2 prlA1024*	This study
OF437	MC4100 *secB*$^+$ *malT*(Con) *malE14-2 prlA1001*	This study
OF533	MC4100 *secB*$^+$ *malT*(Con) *malE19-1 prlA*$^+$	This study
OF534	MC4100 *secB*$^+$ *malT*(Con) *malE19-1 prlA4*	This study
OF535	MC4100 *secB*$^+$ *malT*(Con) *malE19-1 prlA1024*	This study
OF536	MC4100 *secB*$^+$ *malT*(Con) *malE19-1 prlA1001*	This study
OF418	MC4100 *secB*::Tn*5 prlA*$^+$	This study
OF419	MC4100 *secB*::Tn*5 prlA4*	This study
OF420	MC4100 *secB*::Tn*5 prlA1024*	This study
OF421	MC4100 *secB*::Tn*5 prlA1001*	This study

glycerol, unless otherwise indicated. Amino acids were supplemented as required (36). When appropriate, antibiotics were added to the following concentrations: ampicillin, 100 μg/ml; tetracycline, 20 μg/ml; kanamycin, 25 μg/ml; streptomycin, 150 μg/ml; and spectinomycin, 150 μg/ml. Restriction enzymes, T4 DNA polymerase, and T4 DNA ligase were from New England Biolabs. Tran[^{35}S]-label (1,162 Ci/mmol) was purchased from ICN Biochemicals, and Kodak XAR film was used for autoradiography and fluorography. Rabbit anti-MBP antiserum was described previously (26).

Selection of Mal$^+$ suppressor mutations. One hundred ninety-two single colonies of strain CK2125 [*secBL75Q malT*(Con) *zjb*::Tn*5 malE18-1*] were streaked on maltose minimal plates and grown for 3 days at 37°C, and Mal$^+$ papillae that arose within the streaks were picked. The plates were incubated further for 2 days, and additional Mal$^+$ papillae that arose after a total of 5 days were picked. All papillae were purified on maltose minimal medium at 37°C and then tested for cold sensitivity at room temperature. One mutant, CKM132, exhibited apparent cold sensitivity.

Genetic mapping. For linkage analysis, P1 *vir* phage lysates were prepared from 95 individual Mal$^+$ colonies. The *malE*-linked mutants were identified by transducing the recipient strain CK2158 (*secBL75Q zjb*::Tn*10 ΔmalB101*) with these lysates and selecting for the *zjb*::Tn*5* marker linked to *malE* in the donor. In 62 of the mutants the Mal$^+$ phenotype was cotransducible with Km^r, which allowed us to classify them as *malE* revertants or pseudorevertants. Linkage of Mal$^+$ mutations to *secB* was analyzed for the remaining lysates by using the recipient CK2162 [*cysE*::Tn*5 secBL75Q malT*(Con) *malE18-1*]. After selecting Cys$^+$ transductants on minimal plates, we showed that in two of the mutants the Mal$^+$ phenotype was linked to *cysE*, a marker

mapping next to *secB*, suggesting that the mutations were probably (pseudo)reversions in the *secB* gene. The remaining 31 mutants contained extragenic suppressors of the Mal$^-$ phenotype.

To map the lesion in the cold-sensitive mutant strain CKM132 (*secBL75Q malE18-1 meb*), Tn*10* insertions linked to the *meb* mutation were isolated. The *meb* mutation conferred poor growth at room temperature. Therefore, strain CKM132 was transduced with a P1 lysate grown on a random pool of Tn*10* insertions into the chromosome of the *meb*$^+$ parental strain, CK2125 (*secBL75Q malE18-1*), and Tc^r, large colonies were selected at room temperature. Several Tn*10* insertions were obtained. The approximate map position of one of the Tn*10* insertions was determined by Hfr cross. Three of the *meb*-linked Tn*10* insertions were approximately 10% P1 cotransducible with *rpoH* (min 76), and the fourth Tn*10* insertion was 10% P1 cotransducible with *xyl* (min 80).

Amplification, cloning, and DNA sequence analysis of *prlA* suppressors. DNA sequences containing the *prlA* gene were amplified by polymerase chain reaction (PCR) (38). Amplifications of genomic DNA from boiled bacterial colonies of CK2125 (*prlA*$^+$) and mutant strains CKM1024 and CKM1001 were carried out for 35 cycles (1 min at 95°C, 2 min at 55°C, and 2.5 min at 72°C) in a Perkin-Elmer-Cetus thermal cycler. Primers used for PCR and sequencing of *prlA* were a kind gift of K. Ito. Primer pairs were chosen to amplify partially overlapping *prlA* fragments, each about 0.5 kb in length and each encompassing approximately one-third of the *prlA* coding sequence. Following amplification, DNA was extracted with chloroform, passed through NACS PREPAC minicolumns (GIBCO-BRL), and purified by electrophoretic elution from SeaPlaque agarose gels (FMC, Rockland, Maine). Purified DNA was treated with T4 polymerase and ligated into *Eco*RV-linearized pBR322 (9). Competent cells of strain JM109 were transformed with the ligation mixtures, and the presence of the appropriate inserts in the Ap^r Tc^s transformants was verified by restriction mapping. In this way, the following plasmids were constructed: plasmids pOFY1, pOFY2, and pOFY3, containing the proximal, central, and distal thirds of the *prlA1024* gene, respectively; plasmids pOFY4, pOFY5, and pOFY6, containing the respective fragments from *prlA1001*; and plasmids pOFY7, pOFY8, and pOFY9, containing the respective *prlA*$^+$ DNA fragments. All DNA manipulations, plasmid purification, and bacterial transformation were as described previously (34). Sequencing of the plasmid DNA after alkaline denaturation (51) was done with a Sequenase kit (United States Biochemicals). The sequences of the resulting plasmids were determined by using a set of 10 oligonucleotides (17-mers) complementary to sequences in the *secY* gene.

To determine the sequence of the *prlA4* allele, the gene was PCR amplified from a boiled bacterial colony of strain OF193, using the same primers as above. The amplified DNA was extracted, purified from a SeaPlaque agarose gel, and sequenced directly after alkaline denaturation, using the Sequenase kit.

Labeling, immunoprecipitation, SDS-PAGE, and fluorography. For induction of MBP synthesis, cells were grown in M63 medium supplemented with 0.2% glycerol and 0.4% maltose at 37°C to a density of 2×10^8 to 3×10^8 cells per ml. Cells were pulse-labeled at the same temperature for 15 or 20 s with Tran[^{35}S]-label (10 μCi/ml), and chase was initiated by adding unlabeled methionine (100 μg/ml) and chloramphenicol (0.5 mg/ml). After chase for appropriate times, 1-ml samples were withdrawn and precipitated with trichloroacetic acid (5% final concentration). Immunoprecipitation was done as described previously (30), using IgGSORB (New England Enzyme Center). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 1.5-mm 9% gels was as described previously (31). The gels were treated with sodium salicylate (13) or diphenyl oxazole (10), dried, and exposed for various times at −80°C. Densitometric analysis of the resulting fluorograms was performed with a Molecular Dynamics computing densitometer and ImageQuant software. The obtained values were corrected for the loss of methionine residues in the mature form relative to the precursor form of the protein.

TABLE 2. Summary of Mal$^+$ suppressors isolated (95 total)

Genetic locus	Map position (min)[a]	No. of Mal$^+$ mutants
malE	92	62
secB	81	2
prlA	73	15
metTα	15	1
glyU	62	14
meb	78	1

[a] Map positions are given according to reference 2.

RESULTS

Selection of Mal$^+$ mutants. In wild-type *E. coli* periplasmic MBP is an essential component of the maltose uptake system. Signal sequence mutations in the *malE* gene cause accumulation of the precursor form of MBP in the cytoplasm. In one of the strongest mutations of this type, *malE18-1* (6), arginine (AGG) is substituted for methionine (ATG) at position 18 of the hydrophobic core. Although the amount of periplasmic MBP in this mutant is reduced to less than 5% of the wild-type level (5), this is still sufficient for growth on maltose minimal media.

Mutations in a component of the export apparatus, *secB*, have been shown to affect translocation of MBP (28). For example, a point mutation in the *secB* gene, *secBL75Q* (18), results in a kinetic defect in MBP export. This mutation does not affect growth on maltose, and its effect on MBP processing can be detected only by pulse-labeling experiments. However, in a double mutant, carrying *secBL75Q malE18-1*, export of MBP is completely abolished and cells can no longer grow on maltose. This Mal$^-$ phenotype provided the basis for the selection of spontaneous Mal$^+$ revertants as described in Materials and Methods.

Mapping of Mal$^+$ loci. Spontaneous Mal$^+$ mutants were selected and purified as described in Materials and Methods. A total of 95 Mal$^+$ mutations were mapped as described below, and the summary of the suppressor loci and their map positions is given in Table 2. Linkage analysis using P1 generalized transduction indicated that 62 of the mutants were probably *malE* revertants and that 2 mutants were probably *secB* revertants, as described in Materials and Methods. None of the *malE*- or *secB*-linked revertants were analyzed further in this study. The remaining 31 mutants were clearly unlinked to either of these loci. To test whether these mutations represented lesions in *prlA*, we analyzed the linkage of the Mal$^+$ phenotype to *spc*, which is located within the same operon (46). By transducing the recipient strain CK2159 (Sp^r Sm^s *secBL75Q malE18-1*) to Sm^r, we found that in 15 mutants the Mal$^+$ phenotype was tightly

TABLE 3. Suppression of Mal⁻ phenotype by *prlA* mutants[a]

prlA allele	Phenotype		
	secBL75Q background[b]	*secB*::Tn*5* background[c]	*secB*⁺ background[b]
prlA⁺	Purple	–	Pink
prlA4	Red	+/–	White
Class I mutants			
prlA1001	Pink	+++	White
prlA1003	Pink	+++	White
prlA1008	Pink	+++	White
prlA1041	Pink	+++	White
prlA1055	Pink	+++	White
prlA1059	Pink	+++	White
prlA1090	Pink	+++	White
prlA1091	Pink	+++	White
prlA1092	Pink	+++	White
Class II mutants			
prlA1002	Red	+	White
prlA1012	Red	++	White
prlA1024	Red	++	White
prlA1025	Red	++	White
prlA1062	Red	+	White
prlA1074	Red	+/–	White

[a] Strains used were constructed by transducing *prlA* alleles into strain CK2125 (*malE18-1 secBL75Q*) or OF262 (*malE18-1 secB*::Tn*5*).

[b] Mal phenotype on maltose tetrazolium plates: a gradient of colors is observed, from purple, corresponding to Mal⁻, to red to pink (intermediate Mal⁺) to white (a wild-type Mal⁺ phenotype).

[c] Growth on maltose minimal plates (from – [no growth] to ++++ [wild-type, *malE*⁺ growth]).

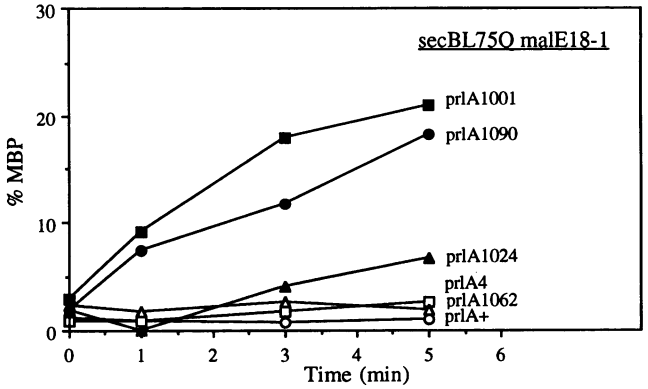

FIG. 1. Suppression of defective MBP export by different *prlA* mutations in strain CK2125 (*secBL75Q malE18-1*). Cells were grown to early log phase in maltose glycerol minimal medium and labeled for 20 s with Tran[^{35}S]-label. Chase was initiated by adding unlabeled methionine and chloramphenicol. After 0, 1, 3, and 5 min, samples were removed, and proteins were precipitated with anti-MBP antiserum and analyzed by SDS-PAGE and fluorography. For each time point, the percentage of radiolabeled MBP immunoprecipitated in the mature form, relative to total immunoprecipitated MBP, was determined (see Materials and Methods).

linked to *spc*. Therefore, these mutants probably contained mutations in *prlA*.

Fifteen mutants contained missense suppressor mutations, one in *metTα* and 14 in the *glyU* gene. Mapping and characterization of these suppressors will be described elsewhere.

The lesion in the mutant CKM132 (*secBL75Q malE18-1 meb*) conferring the cold-sensitive and Mal⁺ phenotypes was shown to permit growth on maltose in the absence of MBP and was designated *meb* (*malE* bypass). An *meb* Δ*malE444* strain grew as well on maltose minimal medium as the original mutant, CKM132. Possible mechanisms of suppression by *meb* could include activation of a cryptic maltose transport system or alteration in the specificity of some other transport system.

Mapping of the *meb* mutation was accomplished as described in Materials and Methods. The site of the lesion was placed in a broad region between *xyl* (min 80) and *rpoH* (min 76), probably around min 78. The lack of suitable markers in this part of the chromosome prevented us from determining the map location more precisely.

Suppression by *prlA* mutations. Fifteen of the Mal⁺ revertants contained extragenic suppressors that were linked to *prlA*. To classify these mutants we tested their phenotypes in the presence of different signal sequence mutations (not shown) and *secB* alleles (Table 3). For comparison, a previously characterized mutation, *prlA4* (16, 17, 43), was included in this analysis. There appeared to be at least two major mutant classes, based on their ability to suppress the *malE18-1* mutation in different *secB* backgrounds. Class I, consisting of nine mutants, contained relatively strong suppressors and allowed good growth on maltose minimal medium. The remaining six mutants (class II) contained weaker suppressors.

For two mutants from each group (CKM1001 [*prlA1001*] and CKM1090 [*prlA1090*] from class I and CKM1024 [*prlA1024*] and CKM1062 [*prlA1062*] from class II), kinetics of MBP processing was analyzed by radiolabeling. For comparison, we also analyzed an isogenic strain, OF193, containing the *prlA4* allele. The data obtained after densitometric scanning of the resulting fluorograms are summarized in Fig. 1. The extremely weak suppressors, *prlA4* and *prlA1062*, were hardly distinguishable from *prlA*⁺ in their ability to promote translocation of the MalE18-1 protein in a *secBL75Q* background. However, the other class II mutant analyzed, the *prlA1024* mutant, showed more efficient translocation in this assay than the *prlA4* or *prlA1062* mutant. The most efficient translocation was observed with the class I *prlA* alleles, consistent with the results of the plate assays. For a more detailed study, we chose one representative from each class of the newly isolated suppressors, *prlA1001* (class I) and *prlA1024* (class II).

Sequence analysis of *prlA* mutations. To demonstrate that these mutants contained lesions in the *prlA* (*secY*) gene and not just *spc*-linked mutations, marker rescue experiments were performed with three overlapping, approximately 0.5-kb-long DNA fragments, which covered the entire *prlA* gene from *prlA*⁺, *prlA1024*, and *prlA1001* alleles, cloned onto pBR322 as described in Materials and Methods. The resulting plasmids (pOFY1 to pOFY9) were transformed into the *prlA*⁺ Mal⁻ strain CK2125 (*secBL75Q malE18-1*) and plated on minimal maltose and glycerol plates containing ampicillin. Only the cells containing plasmids pOFY3 (carrying the distal 548-bp fragment from *prlA1024*) and pOFY4 (a clone of the proximal 550-bp fragment from *prlA1001*) gave rise to Mal⁺ colonies at a frequency of approximately 10^{-5}. In all of the other transformants, Mal⁺ colonies appeared with a frequency of approximately 10^{-7}, which corresponds to the rate of spontaneous mutation. The high frequencies of Mal⁺ colonies in pOFY3 and pOFY4 transformants indicated that they arose by recombination between the cloned fragments and the chromosomal *prlA*⁺ gene.

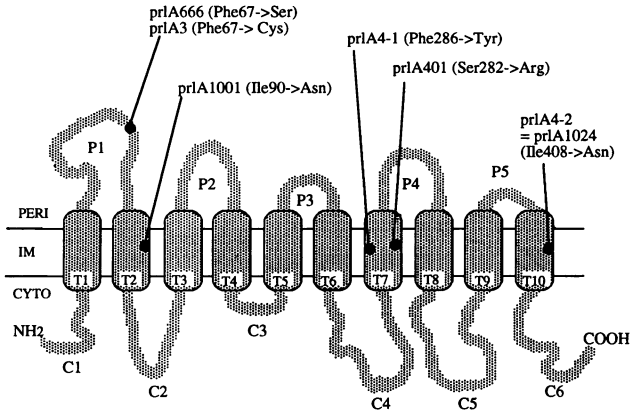

FIG. 2. Positions of *prlA* mutations on the topological map of SecY protein. The model of SecY protein presented here is based on the models proposed previously (1, 22). Putative transmembrane (T1 to T10), periplasmic (P1 to P5), and cytoplasmic (C1 to C6) domains of SecY are indicated. Positions and amino acid substitutions of *prlA* mutations characterized in this study and in previous studies (41, 43) are given.

Sequence analysis of the plasmids used in the marker rescue experiments revealed a single base change in each plasmid. The mutation caused by the class I suppressor *prlA1001* was a T-to-A transversion, causing a change of Ile to Asn at amino acid residue 90 in the second putative transmembrane segment of the PrlA protein (1, 12). With the class II suppressor, *prlA1024*, Asn was substituted for Ile at position 408 in the putative transmembrane domain 10. An identical change had been previously described as *prlA4-2* and was found within the *prlA4* (*prlA4-1 prlA4-2*) double mutant as the lesion responsible for the suppression of *malE* signal sequence mutations (43). We also amplified by PCR the *prlA* gene from the *prlA4* isolate used in these studies and observed that the *prlA4-1* (Tyr-286) and *prlA4-2* (Asn-408) lesions were both present. The positions of these and some of the previously characterized *prlA* mutations on the putative topological map of PrlA are given in Fig. 2.

The mutation responsible for the Mal$^+$ phenotype of *prlA1001* also caused a loss of the recognition sequence for enzyme *Bsa*BI (5′GATN$_4$ATC3′ to 5′GAAN$_4$ATC3′). The remaining eight class I suppressor alleles were tested for the presence of a mutation at the same position. We amplified by PCR the proximal 550-bp region of the *prlA* gene from these mutants and treated the amplified DNA with *Bsa*BI enzyme. We found that the same *Bsa*BI restriction site was missing in all of the class I mutants. This result was further confirmed by treating these DNA fragments with *Sau*3AI enzyme, which has five recognition sites in the first 550 bp of the *prlA*$^+$ sequence and four sites in the same fragment derived from the *prlA1001* allele. In all class I mutants the PCR-amplified proximal fragment of the *prlA* gene had a *Sau*3AI restriction pattern identical to that of *prlA1001*. By marker rescue experiments we confirmed that these fragments contained the lesions responsible for the Mal$^+$ phenotype of class I *prlA* mutants. In conclusion, all of the class I mutants had sustained lesions in or next to residue 90.

To test if the other class II *prlA* mutants contained the same lesion as the *prlA1024* mutant, we also used restriction analysis. In the case of *prlA1024*, the mutation responsible for the Mal$^+$ phenotype creates a recognition site for the enzyme *Mse*I (5′TTAA3′) in the distal part of the *prlA* gene. We amplified by PCR the distal 548 bp of the *prlA* gene from the six class II mutants and treated the amplified DNA with *Mse*I endonuclease. Only the *prlA1025* mutant contained the same change as *prlA1024*, with the *Mse*I site created at the same position, in agreement with the similar phenotype of these mutants (Table 3). The remaining four class II mutants, which were relatively weak suppressors, were not characterized in detail.

***prlA1001* is an unusually strong suppressor of *malE18-1*.** The effects of the newly isolated *prlA* mutations (*prlA1001* and *prlA1024*) on MBP export were compared with those of the previously characterized *prlA4* double mutation (43; see above). First, strain OF228 [MC4100 *pyrE malT*(Con) *malE18-1* Sp^r Sm^s] was used to construct isogenic derivatives containing the relevant *prlA* and *secB* mutations, and the kinetics of MBP processing in these strains was analyzed as shown in Fig. 3. Not surprisingly, *prlA* suppression of the MBP export defect was the highest in the *secB*$^+$ background. Similar to results with the *secBL75Q* background (Fig. 1), the highest rate of MalE18-1 processing was observed with the *prlA1001* mutation, reaching about 92% after 3 min of chase (Fig. 3A). As had been shown previously for *lamB* signal sequence mutations (49), no suppression could be seen in this assay in *secB*::Tn*5* strains (Fig. 3B). However, the relative strengths of the various suppressors could be estimated by growth on maltose minimal plates. In the plate assay, all of the *prlA* mutations could confer some growth to a *secB*::Tn*5* *malE18-1* strain, with the relative level of growth being *prlA1001* > *prlA1024* > *prlA4*. The fact that export could not be observed in the pulse-chase assay for any of these alleles in the absence of SecB, however, indicated that SecB was required for efficient signal sequence suppression.

Surprisingly, the weaker *malE* signal sequence mutations were not suppressed as strongly by *prlA1001* as by the other *prlA* alleles. We analyzed the ability of *prlA* mutations to promote export of MBP in signal sequence mutants carrying *malE10-1*, *malE16-1*, *malE19-1* (6) or *malE14-2* (3). These *malE* mutations map in the hydrophobic core of the signal sequence and result in export defects less severe than that caused by the *malE18-1* mutation. While *malE10-1* and *malE14-2* mutants contain Pro residues substituted for Leu and Ala, at positions 10 and 14, respectively, the *malE16-1* mutant contains Lys at residue 16 in the hydrophobic core (6). The *malE19-1* mutation is an Arg-for-Met substitution at residue 19 and has a phenotype very similar to that of *malE18-1* (6). As shown in Fig. 4, *prlA1001* was less efficient than both *prlA1024* and *prlA4* in suppressing all of these weaker signal sequence mutations. This indicated that there was some allele specificity associated with *prlA1001*.

MBP export in *prlA1024* is faster than in the *prlA4* double mutant. Kinetic analysis of MBP processing in different *malE* signal sequence mutants showed in all cases that *prlA1024* is better than the *prlA4* double mutation in promoting MBP export (Fig. 3 and 4). In addition, when export of MBP containing a wild-type signal sequence was analyzed in the *secB*::Tn*5* strains, different effects of these *prlA* alleles were observed. Export of MBP in the presence of *secB*::Tn*5* in the *prlA*$^+$ background showed a typical severe kinetic defect, with pre-MBP accumulating in an export-incompetent form (Fig. 5) (30). In the strain containing the *prlA4* allele, export of MBP became more defective compared with that in the *prlA*$^+$ strain. At the same time, *prlA1024* improved MBP export in the *secB*::Tn*5* background. Although small and somewhat variable, this effect was observed

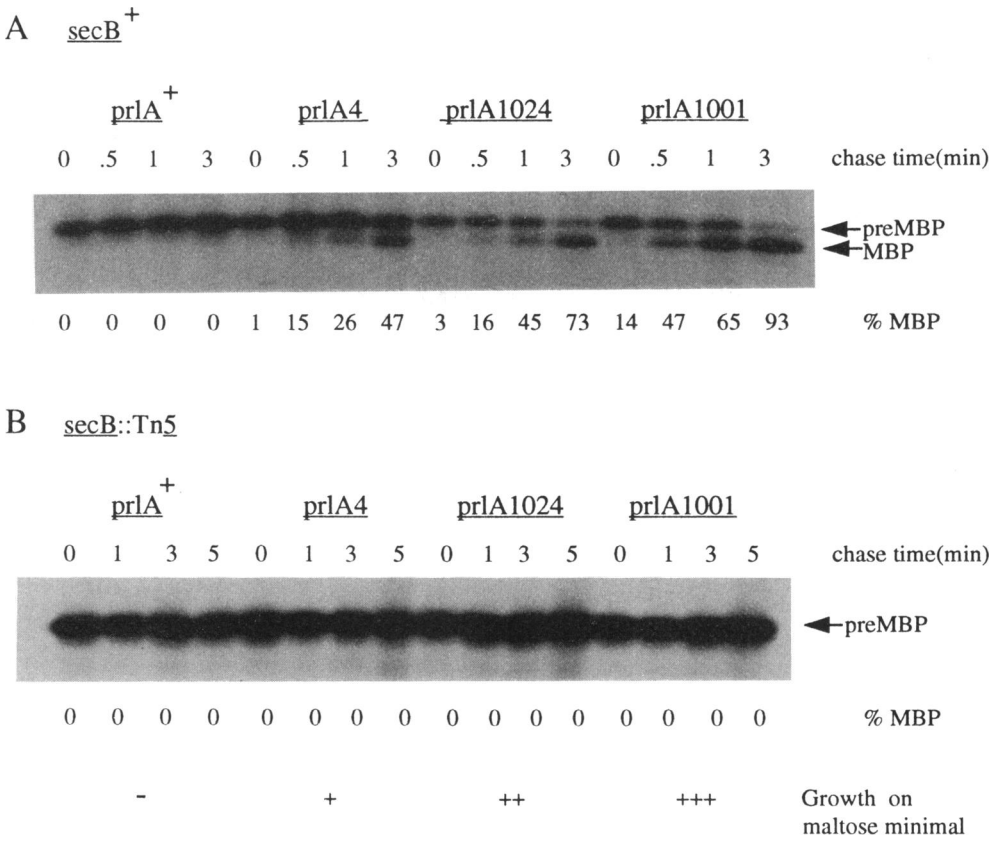

FIG. 3. Suppression of *malE18-1* mutation by *prlA* alleles in the presence of different *secB* mutations. Cells were radiolabeled for 20 s chased for the indicated times, and treated as described in the legend to Fig. 1. The resulting fluorograms are shown, and positions of pre-MBP and MBP are indicated. The percentages of mature relative to total immunoprecipitated MBP were calculated and are given for each lane. (A) Pulse-chase analysis of *prlA*$^+$, *prlA4*, *prlA1024*, and *prlA1001* derivatives of the *secB*$^+$ *malE18-1* strain. (B) Pulse-chase analysis of *prlA*$^+$, *prlA4*, *prlA1024*, and *prlA1001* derivatives of a *secB*::Tn*5* *malE18-1* strain. Growth of *secB*::Tn*5* strains on maltose minimal medium is indicated, ranging from − (no growth) to +++ (nearly wild-type growth).

repeatedly. The *prlA1001* allele did not have a pronounced effect on MBP export in the *secB*::Tn*5* strain.

DISCUSSION

For a subset of *E. coli* proteins, mutations in *secB* exacerbate the leaky export defects caused by signal sequence mutations. In this way more-defective phenotypes are created, which can be utilized in genetic analysis to select for suppressor mutations. Collier and Bassford (14) used this approach in a *malE16-1 secB*::Tn*5* strain and isolated many interesting Mal$^+$ revertants with mutations that mapped in the *malE* gene. However, no second-site suppressors were isolated by using this *secB* null allele. In the selection described here, by using a point mutation in *secB*, a variety of suppressor mutations were isolated. The presence of the *secBL75Q* mutation permitted us to isolate two new suppressor mutations, the *prlA1001* mutation and the *metT*α (*SuAGG*) missense suppressor.

According to the topological model of PrlA (Fig. 2) (1), the *prlA1001* mutation maps at residue 90 in the second putative transmembrane domain (T2) of the protein. Amino acid sequence comparisons of PrlA originating from four prokaryotic species showed that T2 is the most conserved of the 10 transmembrane PrlA domains (25). To our knowledge, *prlA1001* is the first signal sequence suppressor allele mapped in this region. In addition, the existence of a cold-sensitive *secY* allele at residue 84 in the T2 domain has recently been reported (22). Adjacent to the T2 domain, in periplasmic loop 1 (residue 67), two suppressor mutations have been mapped, *prlA3* (17, 43) and *prlA666* (41). Both of these alleles have the ability to suppress mutations which introduce a positive charge in the early mature region of the precursor. Therefore, it has been proposed that this periplasmic domain of PrlA may participate in the interaction with the precursor loop, formed by the distal parts of the signal sequence and the N-terminal region of the mature protein (22). In such a configuration, the T2 domain could come into contact with the hydrophobic core of the signal sequence. The ability of *prlA1001* to strongly suppress a defect caused by a charged residue introduced in the late hydrophobic core would be consistent with this model.

Among the *prlA* mutations analyzed in this study, *prlA1001* was the most efficient in promoting export of MalE18-1 yet was weak relative to *prlA1024* in suppressing other hydrophobic core signal sequence mutations. The eight remaining class I mutants show a phenotype very similar to that of the *prlA1001* mutant and most likely contain lesions in the same position in the T2 domain, on the basis of the restriction analysis described above, further pointing to the importance of this region in recognition of the late hydrophobic portion of the signal sequence. It is interesting

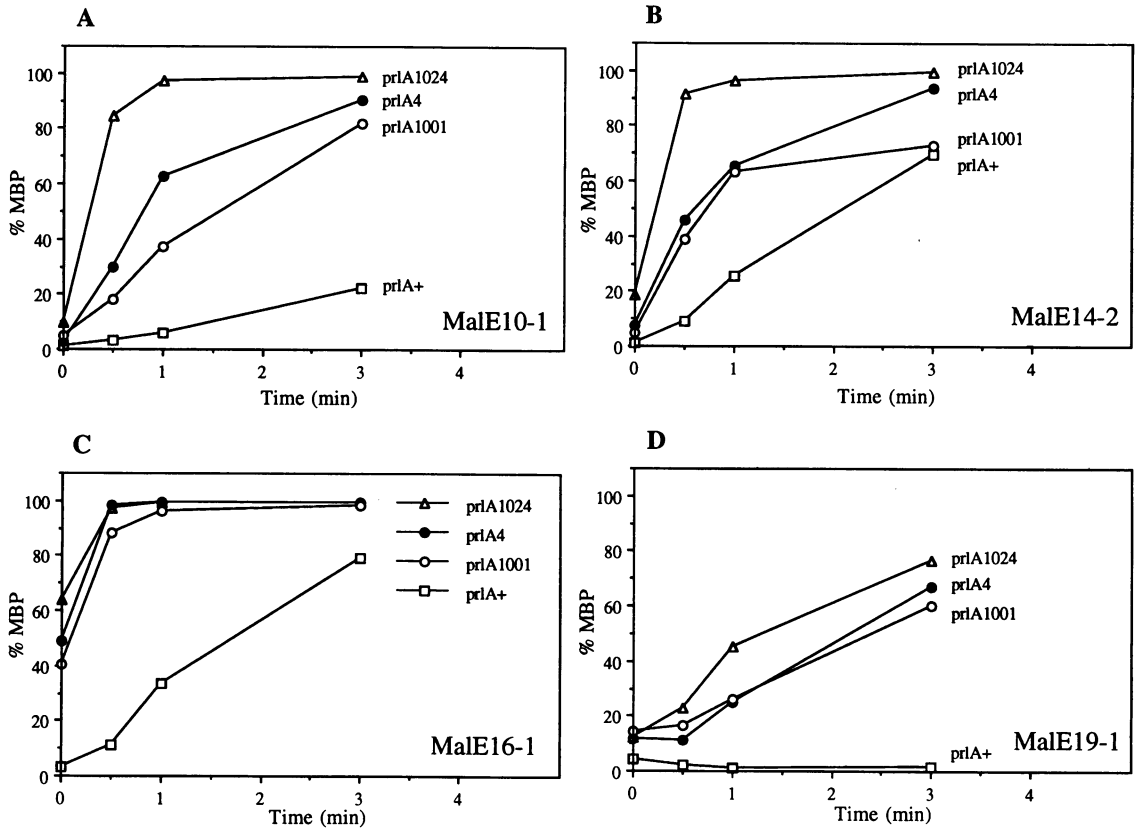

FIG. 4. Suppression of different *malE* signal sequence mutations by *prlA* alleles. Cells grown in maltose glycerol minimal medium were radiolabeled with Tran[^{35}S]-label for 15 s and chased for the indicated times, and immunoprecipitated MBP species were analyzed as described in the legend to Fig. 3. The percentage of mature MBP relative to total immunoprecipitable MBP for each time point was determined by densitometry and plotted as a function of chase time. The effect of *prlA*$^+$, *prlA4*, *prlA1024*, and *prlA1001* alleles on MBP export kinetics was analyzed in *secB*$^+$ *malE10-1* strains (A), *secB*$^+$ *malE14-2* strains (B), *secB*$^+$ *malE16-1* strains (C), and *secB*$^+$ *malE19-1* strains (D).

that allele specificity has been seen previously, as the *prlA3* mutation shows much stronger suppression of the *malE19-1* than of the *malE18-1* mutation (16), although these signal sequence mutations represent identical amino acid changes and lie in adjacent residues (6). The *prlA1001* allele shows the opposite specificity and is more efficient in suppressing the more defective phenotype of the *malE18-1* mutation. It is possible that these two late hydrophobic core mutations define a boundary between two distinct regions of the signal sequence that are differentially recognized by the *prlA1001* and *prlA3* suppressors.

Both *prlA1001* and *prlA1024* mutants have Asn substituted for Ile, in the putative transmembrane helices T2 and T10, respectively. Such a change is likely to increase the beta-turn probability and could induce a kink in the helical structure. Despite the identity of the lesions, there are clear differences between the phenotypes of these suppressors, probably reflecting their positions in PrlA and the roles of the respective segments in the function of the protein.

The lesion found in the class II allele *prlA1024*, mapping in transmembrane domain 10 of PrlA, is identical to the mutation *prlA4-2*, which is responsible for the suppression of signal sequence mutations in the *prlA4* double mutant (*prlA4-1 prlA4-2*) (43). Our results show that the substrate specificities of the single mutant (*prlA1024*) and the double mutant (*prlA4*) are very similar, while the level of suppression by *prlA4* is consistently lower.

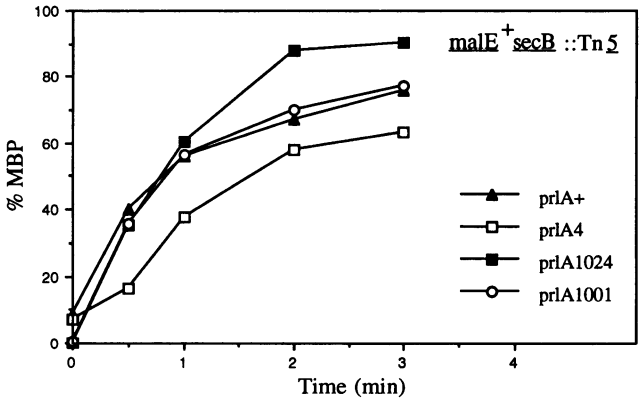

FIG. 5. Effects of different *prlA* alleles on the export of wild-type MBP in a *secB*::Tn*5* null background. Derivatives of a *secB*::Tn*5* *malE*$^+$ strain containing the *prlA*$^+$ (OF418), *prlA4* (OF419), *prlA1024* (OF420), or *prlA1001* (OF421) allele were grown to early log phase in maltose glycerol minimal medium and radiolabeled for 15 s with Tran[^{35}S]-label. Chase was initiated by adding unlabeled methionine and chloramphenicol, and samples were withdrawn at the indicated times and precipitated with trichloroacetic acid. Proteins were immunoprecipitated with anti-MBP antiserum and analyzed by SDS-PAGE and fluorography. The results of densitometric analysis are presented graphically. Values of the percentage of mature relative to total immunoprecipitated MBP were plotted as a function of chase time.

Surprisingly, the *prlA1024* mutation improved to a small extent the export of wild-type MBP in the *secB*::Tn*5* background. The mechanism of this improvement is not clear. In the absence of SecB, the most likely rate-limiting step for MBP export is the targeting of the precursor to the membrane translocation apparatus, and, as a result, a folded, export-incompetent form of MBP accumulates in the cytoplasm (30). One possibility would be that *prlA1024* somehow favors (or stabilizes) the interaction of PrlA with the precursor in the early stages of translocation, i.e., during the entry of the precursor into the translocation site. Suppression of a broad range of signal sequence mutations by *prlA1024* suggests that this effect might involve recognition of some region of the precursor other than the signal sequence.

Our results also indicate that the presence of the *prlA4-1* (Tyr-286) lesion decreases the activity of the *prlA4-2* (Asn-408; i.e., *prlA1024*) suppressor mutation. This effect is readily observable in the cases when MBP export is retarded, either by the presence of a signal sequence mutation or by the absence of SecB. A similar effect of *prlA4-1* and several other mutations located in the same putative domain T7 of PrlA has been previously observed with the non-*E. coli* proteins staphylokinase (42, 43) and streptokinase (37).

We have also observed that the *prlA1024* (Asn-408) mutants grow poorly in the presence of a metabolizable sugar in rich or minimal media. However, the *prlA4* double mutant is less defective in growth. Therefore, under certain conditions, the *prlA4-1* (Tyr-286) mutation may provide a growth advantage to the *prlA4-2* (Asn-408) suppressor, which could explain how the double mutant arose. Several models could explain the growth defect caused by *prlA1024* (Asn-408): (i) a negative effect of this mutation on export of a certain protein(s) that is essential, (ii) attempted export of too many proteins, leading to jamming of the export site, or (iii) an altered interaction(s) of PrlA with another component(s) of the export machinery. Altered interactions are suggested by the synthetic lethality observed in a strain containing the *prlA4* and *prlG1* mutations (7).

prlA suppressor mutations have been mapped so far in three putative transmembrane segments (T2, T7, and T10) and one periplasmic domain (P1). The extensive studies on the T7 region showed that mutations in five residues had an effect on the export of staphylokinase; however, only one of them, *prlA401*, was at the same time capable of suppressing signal sequence defects (42, 43). In this study we obtained nine independent isolates of suppressors in transmembrane domain T2, as well as two mutations identical to the previously described lesion in transmembrane domain T10. The two known suppressors in the P1 domain alter the same amino acid residue (41, 43). It seems very likely, as has been proposed earlier (42), that PrlA makes multiple contacts with the precursor at various stages of translocation. At the same time it seems that a rather limited number of residues of the PrlA protein could participate in those interactions and give rise to suppressors of signal sequence mutations. Clearly, more-extensive studies are required to identify all domains of PrlA involved in these contacts and to elucidate their roles in different stages of translocation.

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