Mutation *prlF1* Relieves the Lethality Associated with Export of β -Galactosidase Hybrid Proteins in *Escherichia coli*

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The 42-1 *lamB-lacZ* gene fusion confers a conditionally lethal, export-dependent phenotype known as maltose sensitivity. A maltose-resistant mutant showing decreased β -galactosidase activity of the hybrid protein, designated *prlF1* (protein localization), was unlinked to the *lamB-lacZ* fusion. This mutation mapped at 70 min on the *Escherichia coli* linkage map and conferred maltose resistance, a 30-fold reduction in β -galactosidase activity, and a 30% decrease in cellular growth rate at 30°C that was independent of the presence of a gene fusion. *prlF1* also decreased the β -galactosidase activity and relieved the maltose sensitivity conferred by fusions of *lacZ* to the gene specifying the periplasmic maltose-binding protein, *malE*. The decrease in β -galactosidase activity, however, was specific for exported hybrid proteins. When export of the hybrid protein was blocked by a signal sequence mutation, *prlF1* decreased the β -galactosidase activity only 2.5-fold. Similarly, *prlF1* did not affect the β -galactosidase activity of fusions of *lacZ* to a gene specifying a nonexported protein, *malK*.

The cytoplasm of *Escherichia coli* is surrounded by two concentric membranes. Between the inner and outer membranes lies the periplasmic space. Synthesis of all periplasmic, inner membrane, and outer membrane proteins occurs within the cytoplasm. A mechanism must therefore exist for the export of these proteins to their specific destinations. Elegant biochemical work has demonstrated that eucaryotic protein localization is a multistep process involving a number of polypeptides and at least one RNA species (19, 20). In procaryotic systems, significant advances toward the understanding of export have been made through genetic analyses (for a review, see reference 17). For example, workers in our laboratory have used fusions between *lamB*, which encodes a major outer membrane protein, and *lacZ*, which encodes β -galactosidase, to define the intragenic information required for export of such hybrids from the cytoplasm (2; S. A. Benson, E. Bremer, and T. J. Silhavy, Proc. Natl. Acad. Sci. U.S.A., in press).

lamB-lacZ fusions can have two phenotypic effects that allow direct selection for mutations affecting export. The first is maltose sensitivity. Strains containing certain *lamB-lacZ* fusions were inhibited by induction of hybrid protein synthesis by maltose, presumably because sequences within the β -galactosidase moiety interfered with the normal export pathway (directed by *lamB* sequences). Upon induction, proteins that normally are exported (including the hybrid protein) accumulate in the cytoplasm as precursors and the cell eventually lyses. If hybrid protein export is prevented by mutation, the cell becomes maltose resistant.

The second trait useful for selecting export-related mutations is the location-dependent alteration in the β -galactosidase activity of the hybrid protein. Strains producing cytoplasmic forms of *LamB-LacZ* hybrid proteins have increased β -galactosidase activities compared with strains producing exported forms. This may be due to a more active conformation taken by the hybrid in the cytoplasm or to its degradation into smaller, more active species (8).

We have obtained intragenic mutations that block hybrid protein export by selecting for maltose resistance and growth on lactose minimal agar. The same selections have been unsuccessful, however, in isolating extragenic mutations with that effect.

Recent work suggested a possible explanation. In both procaryotes and eucaryotes, protein synthesis and export are coupled (9, 19). When certain steps in the export process are blocked, synthesis of exported proteins is decreased markedly (15). We may therefore have been eliminating an important class of mutations because our selections demanded high levels of hybrid-protein β -galactosidase activity. Consequently, we have altered our selections to include maltose-resistant mutants that exhibit low β -galactosidase activities. We describe here one mutation, unlinked to the *lamB-lacZ* fusion, that confers maltose resistance and decreased β -galactosidase activity. This mutation defines a gene, designated *prlF*, whose product may be involved in protein export.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1.

Media and chemicals. Media are described elsewhere (14; T. J. Silhavy, M. L. Berman, and L. Enquist, *Experiments with Gene Fusions*, in press). The sugar concentration was 0.2% in agar minimal media and 0.4% in liquid minimal media, except during the maltose resistance selection, when the liquid minimal media contained 0.8% maltose. We obtained [³⁵S]methionine from Amersham Corp., Arlington Heights, Ill., Formalin-fixed *Staphylococcus aureus* from the Enzyme Center, Boston, Mass., polyacrylamide from Bio-Rad Laboratories, Richmond, Calif., and Kodak XAR film from Eastman Kodak, Rochester, N.Y. *o*-Nitrophenyl- β -D-galactoside came from Sigma Chemical Co., St. Louis, Mo., and 5-bromo-4-chloro-3-indolyl- β -D-galactoside (XG) was from Bachem, Torrance, Calif.

Genetic techniques. Standard techniques were used for P1 transduction, λ induction, and bacterial conjugation (14; Silhavy et al., in press).

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TABLE 1. Bacteria and phages used

Strain	Genotype or relevant genes carried ^a	Origin or reference
Bacterial		
MC4100	F ⁻ <i>araD139</i> Δ (<i>argF-lac</i>)U169 <i>rpsL150 relA1 flbB5301</i> <i>ptsF25 deoC1</i>	3
Pop3186	MC4100 Φ (<i>lamB-lacZ</i>) Hyb42-1 [λ p1(209)]	18
Pop1715	HfrG6 <i>his</i> Δ <i>malB1</i>	10
SFN135	MC4100 <i>argG::Tn10</i>	S. Ferro-Novick
SK2251	F ⁻ <i>gal-6 lacY1 malA1 xyl-7</i> <i>his-1 leuB6 metB1 rpsL104</i> <i>zgi::Tn10 sup-59 T6'</i>	S. R. Kushner
KL720	F ⁻ 102/ <i>leuB6 hisG1 recA1</i> <i>argG6 metB1 lacY1 gal-6</i> <i>malA1 xyl-7 mtl-2 rpsL104</i> <i>tonA2 tsx-1 λ' supE44</i>	K. B. Low
SE1060	MC4100 Φ (<i>lamBS60-lacZ</i>) hyb42-1 [λ p1(209)]	7
BRE1046	MC4100 Φ (<i>malK-lacZ</i>) Hyb1013 (λ <i>placMu1</i>)	E. Bremer
PB72-47	MC4100(λ 72-47)	1
PB4-81	MC4100(λ 4-81)	1
KI2100	MC4100 <i>prlF1</i>	This study
DK2100	Pop3186 <i>prlF1</i>	This study
DK2108	Pop3186 <i>argG::Tn10 recA</i>	This study
DK2105	DK2108 <i>prlF1</i>	This study
DK2172	PB 72-47 <i>prlF1</i>	This study
DK214X	PB 4-81 <i>prlF1</i>	This study
DK2173	DK2105(F'102)	This study
DK2174	DK2108(F'102)	This study
DK2145	BRE1046 <i>argG::Tn10</i>	This study
DK2146	DK2145 <i>prlF1</i>	This study
Phage		
λ p1(209)	<i>trp'BA'-ΔW209-lac' OZYA'</i>	3
λ placMu1	Mu cIts62 cII ⁺ A ⁺ ' <i>ara'</i> Mu 'S' <i>lacZ lacY⁺ lacA'</i>	E. Bremer
λ 72-47	Φ (<i>malE-lacZ</i>) Hyb72-47 <i>lacY⁺ lacA'</i>	1
λ 4-81	Φ (<i>malE-lacZ</i>) Hyb4-81 <i>lacY⁺ lacA'</i>	1

^a Φ , Gene fusion; Hyb, hybrid; prime signs indicate that DNA is deleted on the indicated side(s) of the gene.

Measurement of maltose sensitivity. We used a technique to quantitate maltose sensitivity as a zone of inhibition surrounding filter paper disks saturated with maltose and placed on a lawn of cells (Benson et al., in press).

β -Galactosidase assay. Overnight cultures of cells were grown at 37°C in liquid glycerol minimal medium supplemented with 5% Luria broth. To induce expression of the maltose regulon, the overnight cultures were diluted 1:20 into the same medium containing 0.4% maltose and grown for 3 h at 30°C. We measured β -galactosidase activity of permeabilized cells by using the assay described by Miller (14). Activities were normalized to cell density.

Pulse-labeling and immunoprecipitation. Overnight cultures were grown at 37°C in liquid glycerol minimal medium supplemented with 2% Luria broth. To induce expression of the maltose regulon, we diluted the overnight cultures 1:10 into glycerol minimal medium containing 0.4% maltose and grew them for 3 h at 30°C. Proteins were labeled with 20 μ Ci of [³⁵S]methionine per ml (1,000 Ci/mmol) for 30 s, and the radiolabel was chased by adding a 100,000-fold excess of unlabeled methionine. The hybrid protein was immunoprecipitated with antibody directed against LamB as described by Ito et al. (11). Maltose-binding protein was similarly

immunoprecipitated by using antibody supplied by P. Bassford (University of North Carolina, Chapel Hill). We analyzed immunoprecipitates representing equal numbers of cells on a sodium dodecyl sulfate (SDS)-polyacrylamide gel as described below.

SDS-polyacrylamide gel electrophoresis. Immunoprecipitates and cellular fractions were analyzed on 7.5% polyacrylamide-0.2% bisacrylamide gels, using buffers described by Laemmli (13). The immunoprecipitates were detected by exposing gels treated with sodium salicylate (4) to Kodak XAR film.

Cell fractionation. Overnight cultures were grown at 37°C in liquid glycerol minimal medium supplemented with 5% Luria broth. The overnight cultures were diluted 1:10 into the same medium containing 0.4% maltose, and the cells were grown for 3 h at 30°C. Inner and outer membrane fractions were isolated by isopycnic sucrose density gradient centrifugation (16). Samples representing approximately equal numbers of cells were analyzed on an SDS-polyacrylamide gel as described above.

RESULTS

Selection for mutations unlinked to the *lamB-lacZ* fusion that confer maltose resistance. To avoid obtaining mutants that were maltose resistant and Lac⁻ because of a decreased expression of the hybrid gene, we used the following technique. Growth on lactose requires both the *lacZ* and *lacY* (lactose permease) gene products. *lacY* is located downstream from *lacZ*. Accordingly, any mutation that prevents transcription of the hybrid gene or any polar mutation that prevents synthesis of the hybrid protein will also prevent the expression of *lacY*. Since melibiose is a substrate of lactose permease, and since the additional specific melibiose transport system is not expressed above 37°C (14), *lacY* function can be monitored independently from *lacZ* by scoring for growth on melibiose at 37°C. Thus, by starting with Pop3186, a maltose-sensitive strain carrying the 42-1 *lamB-lacZ* fusion (Mal^s Lac⁺ Mel⁺ at 37°C), we were able to greatly enrich the cell population for the desired mutant by selecting for Mal^r Lac⁻ Mel⁺ cells at 37°C.

Independent colonies of Pop3186 were inoculated into liquid minimal medium containing 0.8% maltose. Growth in this medium enriched for maltose-resistant mutants. These cultures were grown to saturation at 37°C, subcultured into fresh minimal medium containing 0.8% maltose, and grown for 24 h at 37°C. We then spread approximately 10⁷ cells onto minimal agar containing melibiose to select for retention of LacY activity and 5-bromo-4-chloro-3-indolyl- β -D-galactoside (XG) to screen for β -galactosidase activity. These plates were incubated at 37°C for 2 days.

Several hundred colonies with β -galactosidase activities ranging from very low (pale blue) to high (dark blue) appeared on each plate. We were interested in cells with low β -galactosidase activity and therefore picked colonies of this class from each plate for further study. Purification and restreaking on a number of indicator plates showed seven independent mutants to be Lac⁻ Mal^r Mel⁺ at 37°C.

Mapping the mutations. To test for linkage to the *lamB-lacZ* fusion, we isolated a λ transducing phage carrying the *lamB-lacZ* fusion from each of the mutants. Upon relysogenization into MC4100, five of the resulting strains exhibited phenotypes identical to those of the mutants from which the transducing phage originated. Two, however, were Lac⁺ Mal^s, identical to the fusion parent, Pop3186, indicating that the mutations conferring the Mal^r Lac⁻ phenotype mapped in a region of the chromosome unlinked to the fusion.

Mating with HfrG6, which transfers markers in a clockwise direction originating at 66 min on the current *E. coli* linkage map, showed that both mutations mapped between 66 and 1 min. Interestingly, a number of other export-related genes, including *prlA* (6), *prlC* (6), *secB* (12), and *secC* (S. Ferro-Novick, M. Honma, and J. Beckwith, submitted for publication), are located in this region of the chromosome. Our mutations did not map within any of these previously defined genes since they were not cotransduced by P1 at the same frequency with markers linked to each of these genes. Using a Tn10 insertion in *argG* (69 min) that is 94% linked by P1 cotransduction to *secC* (Ferro-Novick et al., submitted for publication), we did find, however, that one mutation, designated *prlF1*, was approximately 9% linked to *argG*.

To precisely map *prlF*, we performed a three-factor cross with a Tn10 insertion within *argG* and between *argG* and *pnp* and found that *prlF* is located at approximately 70 min on the *E. coli* linkage map. *prlC* is approximately 2% linked to *argG* (N. Trun, personal communication), but a Tn10 insertion near *prlC* is not linked to *prlF*. *prlC* must therefore be located at approximately 68 min, on the opposite side of *argG* from *prlF*.

We have been unsuccessful in precisely locating the second mutation conferring Mal^r. It is not linked to any of the above-mentioned markers, and we have not isolated a Tn10 insertion that is cotransducible with this mutation. We suspect the presence of more than one mutation.

Phenotypic effects conferred by *prlF1*. There are three traits conferred by *prlF1*. Two are maltose resistance and decreased β -galactosidase activity of the hybrid protein, and the third, which is independent of the *lamB-lacZ* fusion, is slowed growth at low temperature.

The degree of maltose resistance conferred by *prlF1* is shown in Fig. 1. Lawns of strain Pop3186 without the *prlF1* mutation with 0.5% maltose showed a clear zone of inhibition, and with increasing maltose concentrations, the size of the zone increased. With the *prlF1* mutation, however, even high concentrations of maltose affected cell growth only marginally. Since the *prlF1* mutation confers maltose resistance on strain Pop3186, we expected that precursor accumulation associated with maltose sensitivity would be reduced. This is confirmed in Fig. 2 and 3. In Fig. 2, we pulse-labeled maltose-induced cells for 30 s with [³⁵S]methionine and chased for up to 10 min with excess unlabeled methionine. Maltose-binding protein (MBP), normally a periplasmic protein, was immunoprecipitated from whole-cell extracts and

FIG. 1. Effect of *prlF1* on maltose sensitivity of Pop3186. Filter paper disks saturated with (a) 0.5%, (b) 1.0%, (c) 3%, and (d) 5% maltose were placed on a lawn of Pop3186 with (right) and without (left) the *prlF1* mutation.

FIG. 2. Effect of *prlF1* on maltose-binding protein precursor accumulation in Pop3186 during maltose induction. Immunoprecipitated MBP from cultures of Pop3186 that had been pulsed (30 s) with [³⁵S]methionine and chased for 0, 1, 2, 5, and 10 min with excess unlabeled methionine was run on an SDS-polyacrylamide gel, and an autoradiograph was made. The larger species represents unprocessed precursor MBP; the smaller species represent processed mature MBP.

electrophoresed. In Pop3186, MBP was almost exclusively in the larger-molecular-weight precursor form at all time points, with barely detectable amounts in the mature form, whereas in the same strain with the *prlF1* mutation, it was almost all processed to the mature form after 1 min of chase.

Figure 3 is a Coomassie-blue-stained gel of the whole-cell, soluble, and particulate fractions from maltose-induced cultures of Pop3186 with and without the *prlF1* mutation. Proteins accumulating in association with maltose sensitivity are indicated in the figure by asterisks, and these are reduced or absent in the *prlF1* mutant strain. Whole-cell extracts from uninduced cultures are shown for comparison.

The parent fusion strain, Pop3186, is Lac⁺ on lactose MacConkey and lactose minimal agars at 30°C. The derived strain carrying the *prlF1* mutation is Lac⁻ on both of these media. *prlF1* caused a 30-fold reduction in β -galactosidase activity of the 42-1 fusion strain (Table 2).

Independent of the presence of a fusion, *prlF1* slowed cell growth at low temperatures, as indicated by reduced colony size on agar. We observed this on a variety of media, and it was enhanced at lower temperatures (30°C). At 37°C, slower growth was detectable but was not as striking as at 30°C. The growth defect did not depend on the presence of a *lacZ* fusion (Table 3).

***prlF1* does not affect transcription of the *lamB-lacZ* fusion.** If *prlF1* decreases β -galactosidase activity of the fusion by decreasing its transcription, we would expect it to similarly affect transcription of other genes within the operon, such as *lacY* and *malK* (*malK* is located promoter proximal to *lamB* in the operon and encodes a nonexported protein). Since the mutant retained the ability to grow on melibiose minimal agar at elevated temperatures, we concluded that *lacY* was not affected significantly. To determine the effects on *malK* expression and to quantitate levels of operon transcription, we tested the effect of *prlF1* on the β -galactosidase activity of a *malK-lacZ* fusion. The β -galactosidase activity of a *malK-lacZ* fusion is affected less than twofold by *prlF1* (Table 2, DK2145). We conclude that *prlF1* does not lower the β -galactosidase activity of the 42-1 fusion by decreasing transcription.

***prlF1* specifically lowers the β -galactosidase activity of exported *lacZ* hybrid proteins.** Export of the hybrid protein is blocked in fusion strains containing signal sequence mutations within the *lamB* portion of the hybrid gene (5). We used these strains to determine whether the decreased β -galactosidase activity caused by *prlF1* was associated with hybrid-protein export. The β -galactosidase activity in a fusion strain

FIG. 3. Effect of *prlF1* on the cellular location of the hybrid protein. Strain Pop3186 and its *prlF1* derivative were induced with maltose for 3 h. We prepared supernatant (S) and pellet (P) fractions from whole-cell extracts (CE) by centrifugation at $100,000 \times g$ for 1 h and ran samples on an SDS-polyacrylamide gel. Proteins were stained with Coomassie blue. The hybrid protein (Φ) and a possible stable degradation product are indicated by arrows. Asterisks indicate proteins present in greater amounts in maltose-sensitive strains. Whole-cell extracts from uninduced cultures are in lanes 1 and 5.

carrying the *lamBS60* signal sequence deletion was reduced only approximately two- to threefold by *prlF1* (Table 2). We obtained similar results with another signal sequence point mutation, *lamBS71* (data not shown). Thus, the effect of *prlF1* on the β -galactosidase activity in *lamB-lacZ* fusion strains correlates with hybrid protein export.

The decreased β -galactosidase activity conferred by *prlF1* was not, however, confined to exported *lamB-lacZ* fusions. *malE* encodes periplasmic MBP. Because *malE* is also maltose inducible, strains containing certain *malE-lacZ* fusions are maltose sensitive. Again, the β -galactosidase moiety interfered with the export process, resulting in the accumulation of precursors of other exported proteins. *prlF1* reduced the β -galactosidase activity in the 72-47 *malE-lacZ* fusion strain 300-fold (Table 2). In addition, *prlF1* conferred maltose resistance to this strain. As with the *lamB* signal sequence mutant, the β -galactosidase activity of strains containing a shorter *malE-lacZ* fusion lacking a functional signal sequence (and consequently producing a hybrid protein that remained in the cytoplasm) was affected only twofold (PB4-81). We concluded that the reduction in β -galactosidase activity conferred by *prlF1* was specific for *lacZ* hybrid proteins that were exported.

***prlF1* does not affect the rate of synthesis or degradation of the hybrid protein.** The decreased β -galactosidase activity in

the *prlF1* mutant was not due to a decrease in the amount of hybrid protein (Fig. 3, lanes 2 and 6). These results were confirmed with more sensitive pulse-chase experiments that measured the synthesis and turnover of the hybrid protein. Immunoprecipitation of the hybrid protein from whole-cell extracts with antiserum directed against LamB after a 30-s pulse-labeling with [35 S]methionine, followed by up to 10 min of chase with excess unlabeled methionine, showed no detectable effect of *prlF1* on either the rate of incorporation

TABLE 2. Effect of *prlF1* on the in vitro β -galactosidase activity of various LacZ hybrid proteins^a

Strain	Fusion	Amt of β -galactosidase (U)	
		<i>prlF</i> ⁺	<i>prlF1</i>
Pop3186	$\Phi(lamB-lacZ)$ Hyb42-1	882	29
SE1060	$\Phi(lamBS60-lacZ)$ Hyb42-1	1,085	427
DK2145	$\Phi(malK-lacZ)$ Hyb1013	142	79
PB72-47	$\Phi(malE-lacZ)$ Hyb72-47	62	0.2
PB4-81	$\Phi(malE-lacZ)$ Hyb4-81	1,493	701

^a Cells were grown and induced with maltose as described in the text. β -Galactosidase activities are expressed according to Miller (14).

TABLE 3. Effect of *prlF1* on cell growth^a

Strain	Temp (°C)	Doubling time (min)	
		<i>prlF</i> ⁺	<i>prlF1</i>
Pop3186	30	25	32
Pop3186	37	20	22
MC4100	30	21	35
MC4100	37	19	22

^a Five-milliliter amounts of Luria broth were inoculated with 0.1 ml of an overnight Luria broth culture grown at 37°C and grown for 1 h at 37°C after subculturing in duplicate into Luria broth. Growth rates were determined at 30 and 37°C by measurement of Klett unit turbidity at 20-min intervals.

into, or the disappearance of radiolabel from, the hybrid protein (data not shown). We conclude that *prlF1* does not decrease β -galactosidase activity by decreasing hybrid synthesis or increasing hybrid degradation.

***prlF1* does not affect localization of the hybrid protein to the inner and outer membranes.** Since the β -galactosidase activity of the 42-1 fusion was reduced up to 100-fold by the *prlF1* mutation, whereas the amount of the hybrid protein was not changed, the altered activity may reflect a difference in the localization of the protein. Previous observations support this hypothesis. As mentioned earlier, signal sequence mutations in the *lamB* portion of the gene fusion blocked export of the hybrid protein, and the resulting hybrid protein accumulating in the cytoplasm appeared to be enzymatically more active than membrane-bound forms by both in vitro assay (Table 2, Pop3186 versus SE1060) and lactose indicator plates. Similarly, strains carrying the larger 42-12 *lamB-lacZ* fusion (which contained ca. 60% of the *lamB* gene) were phenotypically Lac⁺. The more efficient export of this hybrid protein to the outer membrane prevents it from assuming an enzymatically active form (8). If *prlF1* caused more efficient localization of the hybrid protein to the outer membrane, we would expect a decrease in β -galactosidase activity. Accordingly, we determined the effect of *prlF1* on the subcellular distribution of the hybrid protein.

There was a small but detectable amount of hybrid protein accumulating in the soluble fraction from Pop3186, and this was accompanied by the appearance of a faster-migrating species slightly larger than authentic β -galactosidase (Fig. 3). This smaller species, probably a degradation product, appeared when β -galactosidase hybrids accumulated in the cytoplasm due to insufficient export information within the hybrid gene, signal sequence mutations, or, as in this case, maltose sensitivity. Examination of the soluble fraction from the *prlF1* fusion strain showed that there was no detectable hybrid protein accumulating in the cytoplasm and consequently no accumulation of the accompanying smaller species (Fig. 3). The decrease in β -galactosidase activity conferred by *prlF1* may have been due to the absence of these cytoplasmic species.

There was little difference between the fusion parent and the *prlF1* mutant in the amount of hybrid protein associated with the particulate (membrane) fraction (Fig. 3). Further fractionation into inner and outer membranes showed approximately equal amounts of the hybrid protein in the inner and the outer membranes from both parental and mutant cells (data not shown). The *prlF1* mutation therefore did not detectably increase the efficiency of hybrid protein export to the outer membrane. Rather, it allowed nearly complete incorporation of the hybrid protein into the membrane

fractions by relieving the lethal effects associated with maltose sensitivity.

***prlF1* is recessive to the wild-type allele.** We studied the effects of a wild-type allele of *prlF1* on the Lac phenotype and growth defect conferred by *prlF1* by transferring F'102 into DK2108 with and without *prlF1*. F'102, which carries the region of the chromosome flanked by *argG* and *argR*, was transferred by mating KL720 with these strains and selecting for Arg⁺. The *prlF1* mutation was recessive to the wild-type gene in maltose sensitivity, in vitro β -galactosidase activity, and growth rate at 30°C (Table 4, DK2173 versus DK2174). However, the growth rate of the merozygote containing two copies of the wild-type allele was markedly slower than that of the corresponding strain containing a single copy (Table 4, DK2108 versus DK2174).

DISCUSSION

We have identified and characterized a mutation that relieves, by a novel mechanism, the maltose sensitivity conferred by the *lamB-lacZ* fusion 42-1. All previously described maltose-resistant derivatives of this fusion strain failed to express the hybrid gene product, or they produced a hybrid protein that remained in the cytoplasm because the intragenic export signals had been either altered or removed by mutation. The *prlF1* mutation, which was not linked to the fusion, did not affect hybrid gene expression and did not block hybrid protein export. These properties suggest that the *prlF1* gene product is involved in cellular protein localization.

Maltose sensitivity is a useful and sensitive indicator of hybrid protein export (5; Benson et al., in press). High-level synthesis of exported LacZ hybrids blocks the localization of other proteins at some undefined step, and this leads ultimately to cell death. Presumably this block occurs because sequences within the normally cytoplasmic protein LacZ are tolerated poorly by the cellular components that mediate the localization process. The *prlF1* mutation relieves the blockade and restores normal protein export but does not prevent export of the hybrid protein itself. It appears that the mutation relieves the detrimental effects of LacZ sequences on protein export.

The *prlF1* mutation dramatically decreased the β -galactosidase activity associated with *lamB-lacZ* or *malE-lacZ* fusions that specified exported hybrid proteins. This effect was not the result of decreased hybrid protein synthesis but rather appeared to be related to the cellular location of the hybrid protein. These hybrid proteins had a higher specific activity in the cytoplasm, perhaps because of the formation of a stable degradation product, than when membrane bound. In the membrane, the hybrid protein may have had difficulty forming the tetrameric structure required for β -

TABLE 4. Complementation analysis of *prlF1*

Strain	<i>prlF</i> alleles	Amt of β -galactosidase ^a (U)	Doubling time ^b (min)
DK2108	<i>prlF</i> ⁺	450	29
DK2105	<i>prlF1</i>	7.4	75
DK2174	F'102 <i>prlF</i> ⁺ / <i>prlF</i> ⁺	197	70
DK2173	F'102 <i>prlF</i> ⁺ / <i>prlF1</i>	238	70

^a β -Galactosidase activities were measured as described for Table 1.

^b Doubling times at 30°C were determined as described for Table 3.

galactosidase activity. The absence of cytoplasmic forms of these hybrid proteins in the *prlF1* mutant probably accounts for the observed decrease in β -galactosidase activity.

The activation of a cytoplasmic protease in the *prlF1* mutant could account for the absence of cytoplasmic hybrid protein and the stable degradation product. This would suggest that the LamB-LacZ hybrid protein accumulating in the cytoplasm because of the presence of a signal sequence mutation (*lamBS60*) would also be degraded rapidly. Since this does not occur, we think this explanation unlikely. We suggest that the absence of cytoplasmic forms of the hybrid protein in *prlF1* strains results from the nearly complete export of the hybrid protein from the cytoplasm. Maltose sensitivity causes the cytoplasmic accumulation of proteins, including the hybrid proteins, that normally are exported. Because *prlF1* confers maltose resistance, this accumulation does not occur.

If, as we propose, the mutation alters a cellular component in a way that makes export of the hybrid protein more complete, then mutations that prevent export should suppress the LacZ⁻ phenotype. This is clearly the case. The *prlF1* mutation has little effect on the β -galactosidase activity of *lamB-lacZ* or *malE-lacZ* fusion strains carrying a mutation that alters or removes the signal sequence. Since signal sequence mutations block an early step in protein localization, it appears that *prlF1* acts after the initiation of the export process.

The largest *lamB-lacZ* fusion, 42-12, contains sufficient information to direct the hybrid protein to the outer membrane with high efficiency. Strains containing this fusion, in contrast to strains containing the 42-1 fusion, exhibit a reduced degree of maltose sensitivity and are LacZ⁻. This finding raised the possibility that *prlF1* causes an increase in the efficiency of hybrid protein export to the outer membrane. Fractionation of the inner and outer membranes, however, revealed no effect of the mutation on the distribution of the 42-1 hybrid protein. Thus, *prlF1* allows nearly complete export of the LamB-LacZ 42-1 hybrid protein from the cytoplasm, but it does not increase the efficiency of hybrid protein export to the outer membrane.

At present we do not know the nature of the *prlF* gene product, nor do we understand its relationship to maltose sensitivity or protein export. The *prlF1* mutation may cause increased production of some component of the export pathway that is rate limiting during high-level expression of exported hybrid proteins. Alternatively, it may alter the interaction between the hybrid protein and components of the export pathway, making export more efficient. Since *prlF1* confers a growth defect that is independent of the presence of a gene fusion, it must affect some normal cellular function and not only hybrid protein export. We have not, however, been able to detect any effect of *prlF1* on normal protein export. Further insights into the function of the *prlF* gene product must await the isolation of defined null mutations.

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LITERATURE CITED

1. Bassford, P. J., Jr., T. J. Silhavy, and J. R. Beckwith. 1979. Use of gene fusion to study secretion of maltose-binding protein into *Escherichia coli* periplasm. *J. Bacteriol.* **139**:19-31.
2. Benson, S. A., and T. J. Silhavy. 1983. Information within the mature LamB protein necessary for localization to the outer membrane of *E. coli* K12. *Cell* **32**:1325-1335.
3. Casadaban, M. J. 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J. Mol. Biol.* **104**:541-555.
4. Chamberlain, J. P. 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. *Anal. Biochem.* **98**:132-135.
5. Emr, S. D., M. N. Hall, and T. J. Silhavy. 1980. A mechanism of protein localization: the signal hypothesis and bacteria. *J. Cell Biol.* **86**:701-711.
6. Emr, S. D., S. Hanley-Way, and T. J. Silhavy. 1981. Suppressor mutations that restore export of a protein with a defective signal sequence. *Cell* **23**:79-88.
7. Emr, S. D., and T. J. Silhavy. 1980. Mutations affecting localization of an *Escherichia coli* outer membrane protein, the bacteriophage λ receptor. *J. Mol. Biol.* **141**:63-90.
8. Emr, S. D., and T. J. Silhavy. 1982. Molecular components of the signal sequence that function in the initiation of protein export. *J. Cell Biol.* **95**:689-696.
9. Hall, M. N., J. Gabay, and M. Schwartz. 1983. Evidence for a coupling of synthesis and export of an outer membrane protein in *Escherichia coli*. *EMBO J.* **2**:15-19.
10. Hofnung, M. 1974. Divergent operons and the genetic structure of the maltose B region in *Escherichia coli* K12. *Genetics* **76**:169-184.
11. Ito, K., P. J. Bassford, Jr., and J. Beckwith. 1981. Protein localization in *E. coli*: is there a common step in the secretion of periplasmic and outer-membrane proteins? *Cell* **24**:707-717.
12. Kumamoto, C. A., and J. Beckwith. 1983. Mutations in a new gene, *secB*, cause defective protein localization in *Escherichia coli*. *J. Bacteriol.* **154**:253-260.
13. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.
14. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
15. Oliver, D. B., and J. Beckwith. 1981. Regulation of a membrane component required for protein secretion in *Escherichia coli*. *Cell* **30**:311-319.
16. Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson. 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*: isolation and characterization of cytoplasmic and outer membrane. *J. Biol. Chem.* **247**:3962-3972.
17. Silhavy, T. J., S. A. Benson, and S. D. Emr. 1983. Mechanisms of protein localization. *Microbiol. Rev.* **47**:313-344.
18. Silhavy, T. J., H. A. Shuman, J. R. Beckwith, and M. Schwartz. 1977. Use of gene fusions to study outer membrane protein localization in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **74**:5411-5415.
19. Walter, P., and G. Blobel. 1981. Translocation of proteins across the endoplasmic reticulum. III. Signal recognition protein (SRP) causes signal sequence-dependent and site-specific arrest of chain elongation that is released by microsomal membranes. *J. Cell Biol.* **91**:557-561.
20. Walter, P., and G. Blobel. 1982. Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature (London)* **299**:691-698.