New suppressors of signal-sequence mutations, *prlG*, are linked tightly to the *secE* gene of *Escherichia coli*

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Analysis of more than 100 extragenic suppressors of the *lamB14D* signal-sequence mutation (changes Val in the hydrophobic core region at position 14 to Asp) has revealed alterations that appear to lie at *prlA* (*secY*) and *secA* (*prlD*), two loci known to be mutable to suppressor alleles, and a new suppressor termed *prlG*. One allele of the new suppressor class, *prlG1*, has been characterized in some detail. This suppressor counteracts, to some degree, the export defect conferred by a variety of signal-sequence mutations in two different genes, *lamB* and *malE*. Genetic analysis shows that the dominant suppressor mutations are linked tightly to, and probably allelic with, the gene *secE*. This result, coupled with data obtained with conditional-lethal alleles of *secE* (see accompanying paper by Schatz et al.), argues strongly that *SecE* is an important component of the cellular protein export machinery in *Escherichia coli*.

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Escherichia coli strains carrying signal-sequence mutations have been used in genetic selections with the objective of identifying genes encoding components of the cellular export machinery. The rationale of such selections is that if the signal sequence interacts with one or more components of the export machinery, then it should be possible to obtain suppressor mutations in genes encoding these components that will restore some level of function to the altered signal sequence. This approach has been applied successfully with signal-sequence mutations in two genes of the maltose regulon, *lamB* (Emr et al. 1981; Trun and Silhavy 1987) and *malE* (Bankaitis and Bassford 1985; Ryan and Bassford 1985).

The *lamB* gene specifies an outer-membrane protein that acts both as a maltoporin to facilitate transport of maltose (Luckey and Nikaido 1980) and dextrans, and as the receptor for the bacteriophage λ (Randall-Hazelbauer and Schwartz 1973). Accordingly, *LamB*⁻ strains exhibit a Dex⁻ (inability to use dextrans as a carbon source) λ ^r phenotype. Such strains can still use maltose because this sugar can cross the outer membrane via other porin proteins. The *malE* gene specifies the periplasmic maltose-binding protein that is required absolutely for both maltose and dextrin transport (Shuman 1982).

Starting with a strain that carries a signal-sequence mutation in *lamB*, suppressor mutations have been identified by selecting Dex⁺. These suppressors lie in

two genes, *prlA* and *prlC* (Emr et al. 1981). Similar selections with *malE* signal-sequence mutations have revealed suppressors also at *prlA* and, in addition, *prlD* (Bankaitis and Bassford 1985; Ryan and Bassford 1985). Subsequent studies have shown that *prlA* is allelic with *secY* (Shiba et al. 1984) and that at least some of the *prlD* alleles lie in the *secA* gene (Fikes and Bassford 1989). Conditional-lethal mutations at *secY* and *secA* cause a pleiotropic defect in protein export (Oliver and Beckwith 1981; Ito et al. 1983) and this combination of genetic data, coupled with recent biochemical evidence (Bacallo et al. 1986; Fandl and Tai 1987; Cabelli et al. 1988), establishes that these proteins are components of the cellular export machinery. The *prlC* suppressors exert their effects in a manner that is different from *prlA* and *prlD*, and the function of the wild-type *PrlC* protein is not yet understood (Trun and Silhavy 1989).

Evidence suggests that the signal-sequence may perform multiple functions during the export process (Stader et al. 1986; Thom and Randall 1988). Accordingly, different signal-sequence mutations may cause export defects for different mechanistic reasons. If this is true, then different signal-sequence mutations may yield different types of suppressors. A test of this hypothesis requires that selection for suppressors be performed with a spectrum of signal-sequence mutations. In the past this has been problematic because most signal-sequence mutations do not cause a severe block in export and, thus, do not confer distinctive phenotypes; indeed, some are indistinguishable from the wild type phenotypically. In the case of *lamB*, for example, previous selections

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were done with a deletion mutation that removes 12 amino acids from the signal sequence. It seemed possible that new suppressors might be revealed if less drastic mutations were employed.

Using highly purified dextrans, we have been able to devise more stringent conditions for selecting suppressors of *lamB* signal-sequence mutations (Trun and Silhavy 1987). Analysis of a large number of suppressors of the mutation *lamB14D* again has yielded mutations that appear to reside in *prlA* and *secA* but, in addition, a new locus (termed *prlG*) has been uncovered. Genetic analysis suggests that these mutations are allelic to *secE*, a gene described in the accompanying paper (Schatz et al. 1989).

Results

Mutagenesis and selection of Dex⁺ revertants of STA14D

STA14D carries a point mutation in *lamB* that changes the valine residue at position 14 of the signal sequence to an aspartic acid residue. This substitution causes a pronounced defect in the export of *lamB* (Stader et al. 1986; Stader and Silhavy 1988). Dex⁺ revertants of STA14D were obtained either spontaneously or following UV irradiation as described in Materials and methods. In a typical experiment, 10⁸ cells were spread on dextrin minimal agar, and after 2 days several hundred revertants could be identified. It is not possible from these data to calculate an accurate reversion frequency because the *lamB14D* mutation is leaky. Accordingly, there is substantial background growth. We observed, however, that UV irradiation increased the number of revertants by 20-fold.

Following selection, 6–8 Dex⁺ colonies were picked from each plate and their phenotypes on dextrin MacConkey agar were compared. If isolates from the same plate had identical phenotypes they were assumed to be siblings, and only one was examined further. Isolates that exhibited a very weak Dex⁺ phenotype on MacConkey agar were discarded. Of 123 Dex⁺ revertants characterized, 46 occurred spontaneously and 77 were obtained following UV irradiation.

Initial characterization of revertants

Besides the desired suppressor mutation, at least three other mutational events could confer a Dex⁺ phenotype to STA14D: (1) Mutations that alter the structural gene for another outer membrane porin and thus bypass the requirement of LamB for dextrin entry (Benson and DeCloux 1985), (2) true revertants of the *lamB14D* mutation, or (3) intragenic suppressor mutations that restore function to the altered LamB signal sequence. To distinguish these possibilities, the following tests were employed.

Bypass mutations are identified simply. Since they do not cause LamB export to the outer membrane, the revertant cells remain resistant to λ . Mutations of this type are quite rare. Indeed, strains containing a deletion

within *lamB* revert at frequencies 100-fold lower than that observed with STA14D under the conditions we employ.

True revertants or strains that carry intragenic suppressor mutations can be recognized because the Dex⁺ mutation lies in *lamB*. To identify these, we used a Tn10 insertion that is linked to *lamB* by 45% in a transduction cross. A P1 lysate grown on a strain carrying this insertion linked to *lamB14D* was used to transduce each of the revertants to Tet^r. If the Dex⁺ mutation lies at *lamB*, then we expect that ~45% of the Tet^r transductants would become Dex⁺, owing to loss of the suppressor. In this manner, we determined that 21 (17%) of the 123 revertants contain mutations linked tightly to *lamB*. These were not studied further. These experiments demonstrate also that the remaining 102 must contain an extragenic suppressor mutation.

Mapping the suppressor mutations

Extragenic suppressor mutations that restore function to an altered signal sequence are likely to reside in genes that specify a component of the cellular export machinery such as *prlA* (Emr et al. 1981), *secA* (Oliver and Beckwith 1981), *SecB* (Kumamoto and Beckwith 1983), or *SecD* (Gardel et al. 1987). Indeed, suppressors have been identified at *prlA* (Emr et al. 1981) and *secA* (Fikes and Bassford 1989) already. In addition, suppressors have been found at *prlC* (Emr et al. 1981; Trun and Silhavy 1987). To determine whether the extragenic suppressors mapped to any of these loci, we employed strains, which contain a Tn10 linked to each in a transduction cross analogous to that described above, to identify intragenic suppressor mutations at *lamB*. In this manner we found 83 (81%) suppressors linked to *prlA* and 12 (12%) linked to *secA*. Although these suppressors have not been characterized further, we suspect that they represent new alleles of *prlA* and *secA*, respectively.

It was somewhat surprising that no additional *prlC* suppressors were identified, as some alleles of this gene are known to suppress *lamB14D* (Trun and Silhavy 1987, 1989). However, *prlC* suppression of *lamB14D* is weak, and it is possible that these suppressors were discarded in our initial screening. A negative result from *secB* was not unexpected because it has been reported that SecB interacts with precursors at sites other than the signal sequence (Collier et al. 1988; Trun et al. 1988). None of the suppressors mapped to *secD*.

Mapping the new locus, prlG

To facilitate mapping of the seven remaining suppressor mutations, a Tn10 insertion that was linked genetically to the suppressor locus was identified as described in Materials and methods. Initially, this was done with two of the suppressors that exhibited the highest level of suppression in phenotypic tests with *lamB14D*. Subsequent transductions with a particular Tn10 insertion that was 90% linked to one suppressor revealed that it was also 90% linked to the other. Indeed, it was found

that all of the seven remaining suppressors exhibited 90% linkage to the same Tn10 insertion. Given these results, it seems likely that all seven are alleles of the same gene, and tentatively we have termed this gene *prlG*.

Because Tn10 insertions confer a phenotype that can be selected simply, we decided to localize *prlG* on the chromosome indirectly by first mapping the linked Tn10. Initial experiments involved patch matings (see Materials and methods) with three different Hfr strains, HfrG6, AL205, and AL210, carrying the particular Tn10 insertion. We found that HfrG6 (origin 66; clockwise transfer) could transfer the Tn10 efficiently, whereas AL205 (origin 61; counterclockwise transfer) and AL210 (origin 97; clockwise transfer) could not. This suggests that the Tn10 lies between 66 and 97 min. To map the location of the Tn10 insertion more precisely, matings between HfrG6 and P210 (*argE thr-1*) were performed selecting either for Arg⁺ or Thr⁺. Analysis of the exconjugants revealed linkage of the Tn10 to *argE*. This was confirmed by P1 transduction, which shows linkage of 46%.

The prlG1 suppressor is dominant

Known suppressors of signal-sequence mutations are dominant in diploid analysis. This reflects the fact that these mutations are missense mutations that alter, rather than abolish, function. To test the behavior of *prlG1* in diploid analysis, we use the F' factor KLF10. This episome carries chromosomal DNA from *metB* to *malK* and therefore should carry the wild-type *prlG*⁺ gene. LG9.1 is a *prlG1 lamB14D* strain into which *recA :: kan argE :: Tn10* were introduced by transduction. The F' factor was introduced into this strain by mating with E7127 and selection for ArgE⁺. Exconjugants were purified and their Dex phenotype and sensitivity to phage λ were determined. We found these phenotypes to be identical to the parent strain LG9.1, therefore we conclude that *prlG1* is dominant to the wild type.

prlG1 is linked tightly to, and probably allelic with, secE

During the course of this work, Riggs et al. (1988) identified a new *sec* gene *secE*, by isolating mutants in which expression of a *secA-lacZ* gene fusion is increased. In addition to causing *secA* derepression, the *secE* mutation confers a cold-sensitive growth defect that appears to result from a pleiotropic defect in protein export. Of special interest was the fact that *secEcs501* exhibited 50% linkage with *argE* in a transduction cross, raising the possibility that this mutation and *prlG1* might affect the same gene. Consistent with this hypothesis is the fact that *prlG1* and *secEcs501* are greater than 90% linked as measured by P1 transduction.

To map *prlG1* more precisely with respect to *secE*, we took advantage of a specialized λ transducing phage isolated by S. Sullivan and M. Gottesman (pers. comm.). This phage, λ U101, contains a 2.2-kb chromosomal in-

sert with two genes, *secE* and *U*, the latter identified originally as a suppressor of *nusA1*. [In the accompanying paper by Schatz et al. (1989) λ U101 is described in more detail.] We wished to determine whether λ U101 carried *prlG*⁺ also.

Because the *prlG1* suppressor mutation is dominant, complementation tests with λ U101 cannot be used directly to determine whether the phage carries *prlG*⁺. Instead, we asked whether or not the chromosomal DNA carried by the transducing phage is homologous to *prlG1* by determining whether this phage can be used to exchange alleles of *prlG* by recombination. λ U101 is *cl857* and *int*⁻. Accordingly, lysogens occur primarily by recombination at the region of homology resulting in a λ prophage that is flanked by chromosomal DNA in direct repeat. When the prophage excises, chromosomal DNA either to the right or to the left will be lost depending on the position at which the recombination event occurs. If the phage carries *prlG*⁺, then it should be possible to exchange the chromosomal *prlG1* with the wild-type allele carried by the prophage. To test this possibility, λ U101 was lysogenized into LG9 (*lamB14D prlG1*) by selecting immunity at 30°C. As expected, the lysogen JS5 formed red (Dex⁺) colonies on dextrin MacConkey agar. As a control, we also constructed a lysogen in a wild-type background, JS4, which also formed red colonies on dextrin MacConkey agar. Cultures of these strains were grown to midlogarithmic phase at 30°C and then were shifted to 39°C for 90 min to induce prophage excision. Dilutions of the cultures were plated on dextrin MacConkey agar and incubated overnight at 39°C. All colonies arising from JS4 were red (Dex⁺) and non-immune. In contrast, about one-third to one-half of the colonies arising from JS5 were white (Dex⁻); the rest were red, but all were nonimmune. We conclude from this result that λ U101 carries chromosomal DNA that is homologous to *prlG1*. Thus, *prlG1* must be linked tightly to *secE* and given the export-related phenotype of *prlG1*, we think it likely that the suppressor is an allele of *secE*.

Suppression of other signal-sequence mutations by prlG1

To assess the spectrum of signal-sequence mutations suppressed by *prlG1*, strains were constructed containing the suppressor mutation in combination with 11 *lamB* and 4 *malE* signal-sequence mutations. The relevant phenotypes of these strains then were compared with the phenotypes of the various mutants without the suppressor. Results obtained with the *lamB* signal-sequence mutations are shown in Table 2. We find that *prlG1* has an ameliorating effect in most of the *lamB* signal-sequence mutants, however, in no case is pronounced suppression observed. Data obtained with the *malE* signal-sequence mutants were similar. Some suppression was observed with *malE14-1*, Δ 12-18, and 18-1; none was observed with *malE10-1* (data not shown). Again, the phenotypic effects were slight.

To provide biochemical evidence for the suppression

Table 1. *Bacterial strains*

Strain	Description	Source	Strain	Description	Source
<i>E. coli:</i>			<i>E. coli:</i>		
AL205	HfrKL16 origin 61' counterclockwise	N. Allgood	STA104	STA1000 <i>malF</i> :: Tn10	this study
AL210	HfrH $\Delta(lac)$ <i>opp</i> origin 97' clockwise	N. Allgood	STA105	STA102 <i>malF</i> :: Tn10	this study
BW6175	HfrPK3 <i>argE</i> :: Tn10 <i>thr-1 leuB6 thi-1 lacY1 aziI5 tonA21 supE44</i>	B. Wanner	STA106	STA104 <i>prlG1</i>	this study
ECB475	MC4100 <i>proC</i> :: Tn10 <i>tsx</i> :: Tn5	Gardel et al. (1987)	STA107-131 (odd #)	STA1000 <i>lamB</i> signal sequence mutations	this study
HfrG6his	HfrG6 <i>his</i> origin 66' clockwise	laboratory stock	STA108-132 (even #)	STA1000 <i>prlG1 lamB</i> signal sequence mutations	this study
JS3	STA14D <i>malF</i> :: Tn10	this study	STA133	STA1000 <i>malE10-1</i>	this study
LG1	STA1000 Tn10 90% linked to <i>prlG1</i>	this study	STA134	STA133 <i>prlG1</i>	this study
LG3	STA14D Tn10 90% linked to <i>prlG1</i>	this study	STA139	STA1000 <i>malE14-1</i>	this study
LG9	STA14D <i>prlG1</i> (original isolate)	this study	STA140	STA139 <i>prlG1</i>	this study
LG10	LG9 Tn10 90% linked to <i>prlG</i> ⁺	this study	STA1000	<i>F⁻ araD139 Δ(argF-lac)U169 rpsL150 relA1 f1bB-5301 ptsF25 deoC1 thiA1</i>	Stader and Silhavy (1988)
MC4100	<i>F⁻ araD139 Δ(argF-lac)U169 rpsL150 relA1 f1bB-5301 ptsF25 deoC1 thiA1</i>	Casadaban (1976)			
MCR106	MC4100 <i>lamB</i> s106	Emr and Silhavy (1980)			
MM152	MC4100 <i>zhe</i> :: Tn10 <i>malT secB</i> :: Tn10	Kumamoto and Beckwith (1985)			
NT106	MC4100 <i>lamB</i> Δ 60 <i>zhc</i> :: Tn10	N. Trun			
NT192	STA1000 <i>lamB</i> Δ 78 <i>secB</i> :: Tn5	N. Trun			
NT350	MC4100 <i>lamB</i> Δ 60 <i>zha</i> :: Tn10	Trun and Silhavy (1987)			
P210	AB1133 <i>ompF</i> <i>F⁻ thr-1 leuB6 proA2 lacY1 supE44 galK2 hisG4 rpsL31 mnt11 argE3 thi-1 xyl-5 ara14</i>	R. Misra			
PB1101	MC4100 <i>malE18-1</i>	Bedouelle et al. (1980)			
RL361	MC4100 <i>malE</i> Δ 12-18	Bankaitis et al. (1984)			
STA14D	STA1000 <i>lamB</i> 14D	Stader and Silhavy (1988)			
STA100	STA14D <i>argE</i> :: Tn10	this study			
STA101	STA14D <i>prlG</i> ⁺	this study			
STA102	STA14D <i>prlG1</i>	this study			
STA103	STA14D <i>malF</i> :: Tn10	this study			

of the export defect caused by the signal-sequence mutations, we performed pulse-chase assays to monitor the rate of signal-sequence processing. In most cases, little, if any, effect was observed, this is not terribly surprising in light of the phenotypes. Phenotypic effects provide a much more sensitive method for detecting small changes in export efficiency than does the biochemical pulse-chase assay. With two *lamB* signal-sequence mutations, 17D and 17R, increased rates of processing were observed (Fig. 1). These two mutations are notable because they confer moderate export defects; in other words, they are quite leaky. Accordingly, it is possible that suppression is observed more easily with these mutations under the experimental conditions employed. In any event, results with these mutations demonstrate that the suppressor works by increasing export of the mutant LamB proteins.

There is a slight, but measurable, negative effect of the *prlG1* mutation on the export of wild-type LamB. The effect can be seen both in Dex and phage sensitivity phenotypes (Table 2) as well as in the pulse-chase assay (Fig. 1). This negative effect is not observed with wild-type maltose-binding protein. Although the significance of this effect is not yet clear, it provides further evidence that the *prlG1* mutation affects an export-related process.

Discussion

The *lamB14D* signal-sequence mutation confers a Dex⁻ λ^+ phenotype because the mutant *lamB* precursor is exported poorly (Emr and Silhavy 1980; Stader et al. 1986; Stader and Silhavy 1988). The *prlG* suppressors relieve this defect by restoring export of the mutant precursor, thus allowing a greater percentage of LamB molecules to reach the outer membrane. This increase in functional

Figure 1. Kinetics of LamB processing in *prlG* strains. Cells were pulse-labeled as described previously (Stader et al. 1986). Chase times are as indicated. LamB was immune-precipitated and subjected to SDS-polyacrylamide gel electrophoresis, followed by autoradiography. B⁺ is wild-type *lamB*; B17D and B17R are *lamB* signal-sequence mutants; G⁺ is wild-type *prlG*; G1 is the *prlG1* suppressor allele.

LamB is evidenced by enhanced use of dextrans and a greater sensitivity to phage λ . In addition, with certain *lamB* signal-sequence mutations, an increased rate of signal-sequence processing is observed. This provides biochemical evidence for an increase in export because the enzyme responsible for this cleavage event is located at the outer surface of the inner membrane (Zwizinski and Wickner 1980).

It seems likely that suppressors of signal-sequence mutations, such as *prlG*, work by broadening the specificity of the cellular protein export machinery so that recognition of the mutant precursor is restored. The fact that the suppressor mutations are dominant in diploid analysis is consistent with such an altered activity and it argues against an effect caused by functional impairment. Broadened specificity could reflect a direct physical interaction between the cellular component affected by the suppressor mutation and the signal sequence, or it could occur by a more indirect means. For example, the export machinery may be a complex of different proteins and alterations in one could affect function of another. Genetic evidence to support a direct interaction would come from the demonstration of allele specificity, i.e., a certain suppressor would be specific for a particular signal-sequence mutation. However, all known suppressors exhibit rather broad specificity for signal-sequence mutations and *prlG* is no exception. The *prlG1* suppressor affects a variety of signal-sequence mutations in both *lamB* and *malE*. This fact, however, should not be construed as indicating the lack of direct interaction. Given the great sequence diversity of signal sequences, inability to demonstrate allele specificity need not be surprising. The specificity of the wild-type export machinery must be quite broad as well. Thus, the potential for direct interaction between the components defined by suppressor mutations and the signal sequence remains an attractive possibility.

We have demonstrated that the *prlG1* mutation lies within a 2.2-kb segment of chromosomal DNA. This

DNA segment is known to encode two proteins, SecE and 'U' (Schatz et al. 1989). Although we have no direct

Table 2. Effects of the *prlG1* suppressor in various *lamB* signal-sequence mutants

<i>lamB</i> allele	<i>prlG</i> allele	Dex MacConkey	λ Sensitivity		
			LB	glucose	maltose
+	+	1	1	1	1
+	1	2	1	1	2
6S	+	1	1	3	1
6S	1	3	1	3	1
$\Delta 60$	+	5	5	5	5
$\Delta 60$	1	4	5	4	3
$\Delta 78$	+	4	4	4	3
$\Delta 78$	1	2	2	2	1
12D	+	3	3	4	1
12D	1	2	1	1	1
13D	+	2	3	4	1
13D	1	1	1	2	1
14D	+	5	5	5	5
14D	1	4	3	3	1
15E	+	4	4	4	2
15E	1	3	2	3	1
16E	+	4	4	5	2
16E	1	3	4	5	2
17D	+	1	2	2	1
17D	1	2	1	1	1
17R	+	1	2	2	1
17R	1	1	2	1	1
19K	+	4	3	3	1
19K	1	2	2	1	1
19R	+	4	4	5	3
19R	1	3	5	5	4

Phenotypes were scored on a scale of 1 to 5, with 1 corresponding to wild type and 5 corresponding to a total defect (i.e., the phenotype of a *lamB* deletion strain). Phenotypes scored as 2, 3, or 4 are intermediate, with 2 more closely resembling the wild type, and with 4 more closely resembling the deletion strain. See Materials and methods for details.

evidence, we think it likely that the *prlG* suppressors are alleles of *secE*. Conditional-lethal mutations in *secE* show defects in protein export (Riggs et al. 1988; Schatz et al. 1989), and this relationship is compelling. Moreover, we note that *prlG1* causes a slight export defect for wild-type LamB, a phenotype that clearly is analogous to that seen with known *secE* alleles. In contrast, mutations in *U* have no known effects on protein export. The fact that two completely different genetic approaches appear to have identified the same gene is striking, and it seems that this protein must play an important role in protein export.

Results presented here validate the use of suppressors of signal-sequence mutations for the identification of genes that specify components of the export machinery. More than 100 extragenic suppressors of *lamB14D* were characterized, and all were found to be linked tightly to genes whose products function in protein export; 81% at *prlA/secY*, 12% at *secA/prlD*, and 7% at *prlG/secE*. Clearly, there is a high probability of success. Given the extent to which this approach has been applied in *E. coli*, it could be argued that most, if not all, of the target genes (i.e., genes that can be mutated to give a suppressor phenotype) have been identified. In other organisms, however, this approach could prove quite useful.

Materials and methods

Bacterial strains and phages

All strains are derivatives of *E. coli* K12 and are described in Table 1. The bacterial strains used in this study, the STA100 series, are all derived from STA14D (Stader and Silhavy 1988) and were constructed by P1 transduction.

To introduce *prlG1* into the STA14D background, the strain was first transduced to Tet^r with a P1 lysate grown on BW6175 (*argE* :: Tn10). The resulting strain STA100 subsequently was transduced to Arg⁺ with a P1 lysate grown on LG9 (*prlG1*). The resulting strain, STA102 (*prlG1 lamB14D*) is phenotypically λ^s.

The various *lamB* signal-sequence mutations were introduced into the STA14D background in a two-step procedure. First, strain STA102 was transduced to Tet^r with a P1 lysate grown on TST6 (*malF* :: Tn10). This yielded two strains, STA105 (*lamB14D*) and STA106 (*lamB*⁺). The P1 lysates grown on the various *lamB* mutants then were used to transduce *Mal*⁺. Acquisition of the tightly linked *lamB* allele was verified by marker rescue in all cases.

Strains carrying *lamB* signal-sequence mutations fall into two phenotypic classes; those that confer a λ^r phenotype (*lamB* alleles 12D, 13E, 14D, 15E, 16E, 19K, 19R, Δ78, and Δ60) and those that are λ^s (*lamB* alleles 6S, 17D, and 17R). To simplify identification of the desired transductant in the *Mal*⁺ transduction described in the preceding paragraph, STA105 was used as the recipient for alleles that confer a λ^s phenotype and STA106 was used for alleles that confer a λ^r phenotype. In this manner, acquisition of the desired *lamB* allele could be scored by a change in phage sensitivity in the marker-rescue experiments.

Media and chemicals

Liquid and solid media were prepared as described previously (Silhavy et al. 1984). Dextrins were obtained from Pfansteil Lab-

oratories, Inc. (Waukegan, Illinois) as a malto-oligosaccharide mixture and used in the preparation of media as described by Trun and Silhavy (1987). Tetracycline was purchased from U.S. Biochemical Corp. (Cleveland, Ohio) and used at a concentration of 25 μg/ml.

Genetic techniques

P1 transduction, conjugation, and other standard techniques were carried out as described previously (Miller 1972; Silhavy et al. 1984). 'Patch' mating was done by growing a patch of donor (Tet^r) cells overnight at 37°C on an L-agar plate and replica-plating the patches onto a plate that had been spread with a lawn of the recipient (Str^r) cells. The plate was incubated at 37°C for 2 hr, then replica-plated onto an L-plate containing tetracycline to select for the recipients and streptomycin to counterselect the donor. The exconjugates were incubated overnight, purified by streaking, and screened for the appropriate markers.

Isolation of Dex⁺ spontaneous revertants

The spontaneous mutants were isolated either by streaking isolated colonies of STA14D to dextrin minimal agar or by spreading 10⁸ cells that had been grown overnight in L-broth. The agar plates were incubated at 37°C for 2 days at which time revertants could be recognized as papillae that grew out of the dense background. The papillae were picked and were purified several times on dextrin minimal agar.

Isolation of UV-induced Dex⁺ revertants

UV irradiation was employed to yield 99.9% killing as described by Miller (1972). Briefly, overnight cultures were harvested and resuspended in 0.1 M MgSO₄. The cells were then exposed to UV irradiation of 1500 ergs/mm² followed by the addition of L-broth. The irradiated cells then were grown overnight in the dark at 37°C. Cells were then spread onto dextrin minimal agar and revertants were purified as described above.

Phenotypic characterization of pseudorevertants

LamB phenotypes of strains carrying a suppressor and a particular signal-sequence mutation were scored in several ways. To assess an increase in maltoporin function, the strains were streaked on dextrin MacConkey agar. On this media, increased LamB function correlates with an increased red color. An increase in phage receptors at the cell surface was detected by cross-streaking the mutant strains against λvir on various media; L-agar, maltose, or glucose minimal agar, and scoring sensitivity. On such media LamB synthesis is uninduced, induced, or repressed, respectively. Wild-type strains score as sensitive on all media; strains that express very little LamB at the cell surface are sensitive only when LamB synthesis is induced fully. *malE* phenotypes were scored on maltose MacConkey agar.

Isolation of strains with Tn10 linked to the *prlG* suppressor

A P1 lysate prepared on a population of cells containing random Tn10 insertions was used to transduce strains containing the *prlG* suppressors to Tet^r. Transductants that were Tet^r, Dex⁺, Mal⁺, and λ^r contain a Tn10 linked to the wild-type allele of the suppressor. P1 was grown on these strains and used to transduce the *prlG* mutants to obtain a Tn10 linked to the sup-

pressor locus. The suppressor then was moved to a clean genetic background by transducing STA14D to Tet^r and scoring for Dex⁺.

Pulse labeling and immune precipitation

Cell cultures were grown and pulse-labeled at 37°C as described previously (Stader et al. 1986).

SDS–polyacrylamide gel electrophoresis and autoradiography

Gel electrophoresis and autoradiography were carried out as described previously (Stader et al. 1986).

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