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Characterization and *in Vivo* Cloning of *prlC*, a Suppressor of Signal Sequence Mutations in *Escherichia coli* K12

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ABSTRACT

The *prlC* gene of *E. coli* was originally identified as an allele, *prlC1*, which suppresses certain signal sequence mutations in the genes for several exported proteins. We have isolated six new alleles of *prlC* that also confer this phenotype. These mutations can be placed into three classes based on the degree to which they suppress the *lamB* signal sequence deletion, *lamBs78*. Genetic mapping reveals that the physical location of the mutations in *prlC* correlates with the strength of the suppression, suggesting that different regions of the gene can be altered to yield a suppressor phenotype. We also describe an *in vivo* cloning procedure using λp*lac*Mu9H. The procedure relies on transposition and illegitimate recombination to generate a specialized transducing phage that carries *prlC1*. This method should be applicable to any gene for which there is a mutant phenotype.

LAMB is localized to the outer membrane where it functions in maltodextrin transport and as the receptor for bacteriophage λ (SZMELCMAN *et al.* 1976; RANDALL-HAZELBAUER and SCHWARTZ 1973). It is synthesized initially as a precursor with a typical amino-terminal signal sequence. If a small deletion internal to the signal sequence is present in the *lamB* gene, export is blocked and the mutant protein is found in the cytoplasm. Cells carrying this deletion in *lamB* (*lamBs60*) are phenotypically resistant to λ and unable to grow on maltodextrins as a sole carbon source (Dex^-) (EMR and SILHAVY 1980). EMR, HANLEY-WAY and SILHAVY (1981) used these phenotypes to isolate second site revertants which are now capable of exporting the mutant LamB protein. The rationale for this selection is that the pseudorevertants should carry suppressor mutations that affect the protein export machinery of *Escherichia coli*. These suppressor mutations were mapped to two distinct genetic loci, *prlA* and *prlC*. We describe here the further characterization and cloning of *prlC*.

The *prlC* gene maps to 71 min on the *E. coli* chromosome, linked to *argR*. The original *prlC* mutation, *prlC1*, was shown to suppress signal sequence mutations in both LamB and maltose-binding protein (*malE*) (EMR and BASSFORD 1982). A second allele, *prlC2*, was also identified by repeating the original selection (J. SCHULTZ, personal communication). To provide further evidence that *prlC* specifies a component of the export machinery and to give insight into the nature of the gene product, we have used a technique that we call localized spontaneous mutagenesis to isolate six additional *prlC* mutations. To facilitate identification and characterization of the *prlC* gene product, we have devised a general *in vivo* cloning method that takes advantage of the λ-Mu hybrid phage, λp*lac*Mu9H. This method relies on transposition and illegitimate recombination to generate a λ specialized transducing phage that carries *prlC*.

MATERIALS AND METHODS

Media, reagents and enzymes: Liquid and solid media were as described previously (SILHAVY, BERMAN and ENQUIST 1984). Dextrins were obtained from Pfanstiel Laboratories, Inc. (Waukegan, Illinois) as a malto-oligosaccharide mixture. This mixture contains approximately 35% maltotriose, which must be removed prior to use. A 100% (w/v) solution of the malto-oligosaccharide mix was prepared in distilled water. The solution was dialized (Spectrapore membrane tubing (Los Angeles); molecular weight diffusion limit of 1000) against 40 liters of distilled water for six hours with two changes of water. The resulting dextrin solution was filter sterilized using a 0.45 μm Nalgene filter (Nalge Co., Rochester, New York). The exact composition of this solution is unknown and was titered empirically for both indicator and minimal media. A concentration of the dextrin solution was chosen so that MC4100 (*lamB*$^+$) and NT1051 (*lamBs60 prlC1*) give a positive reaction and MCR106 (Δ*lamB*) and NT1060 (*lamBs60*) give a negative reaction.

Ampicillin, tetracycline and kanamycin were used at 25, 25 and 50 μg/ml, respectively. When tetracycline or kanamycin was added to MacConkey or tetrazolium indicator plates, one third of these concentrations was used. Antibiotics were purchased from U.S. Biochemical Corp. (Cleveland, Ohio). All enzymes were purchased from New England Biolabs (Beverly, Massachusetts) and used according to the manufacturer's specifications.

Strains: Bacteria, phage and plasmids are described in Table 1. All bacteria are derivatives of *E. coli* K12. pTA108 is a derivative of pRA101 (ARMSTRONG *et al.* 1984). and pUC8 (VIEIRA and MESSING 1982). pRA101 was cleaved at

TABLE 1

Bacterial strains, bacteriophage and plasmids

Strain	Description	Source
(a) Strains		
MC4100	F- *araD139* Δ(*argF-lac*)*U139 rpsL150 relA1 flbB-5301 ptsF25 deoC1*	CASADABAN (1976)
MBM7014	F- *araCam araD* Δ(*argF-lac*)*U139 trpam rpsL relA malBam thi supF*	BERMAN and BECKWITH (1979)
NT1106	MC4100 Δ(*lamB*)106	EMR and SILHAVY (1980)[a]
JS1013	MC4100 *lamBs78*	EMR and SILHAVY (1980)[a,b]
NT113	NT1060 *recA56 sr1C300*::Tn*10*	CSONKA and CLARK (1980)[a]
NT116	NT1060 Φ(*isx-lacZ*)hyb	T. PALVA[a]
NT350	NT1060 *zga*::Tn10 (50% linked)[c]	This study
NT1060	MC4100 *lamBs60*	EMR and SILHAVY (1980)[a]
NT1051	NT1060 *prlC1*	This study
NT105	NT1060 *prlC2 zga*::Tn*10*[c]	This study
NT205	NT1060 *prlC5 zga*::Tn*10*[c]	This study
NT207	NT1060 *prlC7 zga*::Tn*10*[c]	This study
NT208	NT1060 *prlC8 zga*::Tn*10*[c]	This study
NT211	NT1060 *prlC11 zga*::Tn*10*[c]	This study
NT212	NT1060 *prlC12 zga*::Tn*10*[c]	This study
NT218	NT1060 *prlC18 zga*::Tn*10*[c]	This study
PC110	NT1051 *zga*::λplacMu9H-110 (70% linked to *prlC1*)[c]	This study
PC111	NT1051 *zga*::λp*lac*Mu9H-111 (85% linked to *prlC1*)[c]	This study
PC113	NT1051 *zga*::λp*lac*Mu9H-113 (89% linked to *prlC1*)[c]	This study
PC115	NT1051 *zga*::λp*lac*Mu9H-115 (80% linked to *prlC1*)[c]	This study
(b) Bacteriophage		
λp*lac*Mu9H	λp*lac*Mu9 *h82*[d]	BREMER, SILHAVY and WEINSTOCK (1985)
λpMu507H	*cIts857 Sam7 h82*[d] Mu A^+ B^+	BREMER *et al.* (1984)
λpTC101	*prlC1*	This study
λvir		Laboratory stock
P1		Laboratory stock
(c) Plasmids		
pRA101	pSC101 derivative	ARMSTRONG *et al.* (1984)
pUC8		VIEIRA and MESSING (1982)
pTA108	See Figure 3	This study
pTC100	*prlC*$^+$ (constructed by recombination)	This study
pTC101	*prlC1* (constructed *in vitro*)	This study
pTC102	*prlC2* (constructed by recombination)	This study
pTC105	*prlC5* (constructed by recombination)	This study
pTC107	*prlC7* (constructed by recombination)	This study
pTC108	*prlC8* (constructed by recombination)	This study
pTC111	*prlC11* (constructed by recombination)	This study
pTC112	*prlC12* (constructed by recombination)	This study
pTC118	*prlC18* (constructed by recombination)	This study

[a] References are for mutations which distinguish strains from MC4100. All of these mutations were moved into MC4100 and its derivatives by P1 transduction. This was done to insure that all strains are isogenic.

[b] The *lamBs78* mutation was transduced into MC4100 by J. STADER (personal communication).

[c] The precise location of these insertions with respect to outside markers is unknown. However, they are co-transduced with *prlC* by the indicated linkages. The *zga*::Tn10 insertion is the same in all cases and shows 50% linkage to *prlC*.

[d] The *h82* mutation was crossed on to both phage for this study. This was done so that the phage and its derivatives could be used to infect LamB$^-$ strains.

the unique *Bam*HI site. The *Bam*HI site was then filled out with T4 DNA polymerase. pUC8 was cleaved with *Hae*II and the 424-bp fragment containing the *lac* DNA was gel purified. The *Hae*II ends were made flush with T4 DNA polymerase and the fragment inserted into the filled-out *Bam*HI site of pRA101. The resulting plasmid, pTA108, contains the *lac* DNA and multiple cloning sites from pUC8 (*Eco*RI — *Sma*I — *Bam*HI — *Sal*I — *Pst*I — *Hind*III) inserted into the low copy number plasmid pRA101, as shown in Figure 3. pTC101 is a derivative of pTA108 and is described in Results. All other plasmids are derivatives of pTC101.

Phage manipulations: λ resistance or sensitivity was detected by cross-streaking cells against λvir on maltose minimal agar. Stocks of λp*lac*Mu9H were prepared on MC4100 and of λpMu507H on MBM7014. The use of the helper phage λpMu507H was as described previously (BREMER *et al.* 1984). P1 transductions were also carried out as previ-

ously described (SILHAVY, BERMAN and ENQUIST 1984).

Localized spontaneous mutagenesis: NT350 was streaked on an LB plate and incubated at 37° overnight. The next day, 20 tubes containing 5 ml of LB were inoculated from 20 individual colonies and grown overnight at 30°. The cultures were spun down and washed three times with 5 ml of M63 minimal media. Each 5 ml culture [4×10^{10} colony forming units (CFU)] was plated on one dextrin M63 minimal agar plate. The plates were incubated in a wet box (SILHAVY, BERMAN and ENQUIST 1984) for 5 days at 30°. All of the colonies from a single plate (500–1000) were pooled and washed three times with M63 minimal media. Each of the 20 pools were inoculated at a 1:20 dilution into dextrin M63 minimal media and allowed to grow for 6 hr (*ca.* five doublings). P1 was prepared on the pools and used to transduce NT1060 (*lamBs60*) to Tet^r on dextrin MacConkey tetracycline plates at 30°. Tet^r Dex$^+$ transductants were purified for further characterization.

Isolation of a λp*lac*Mu9H pool: A fresh overnight culture of NT1051 in LB was spun down and resuspended in half the volume in 10 mM $MgSO_4$. Half a milliliter of cells were infected with λp*lac*Mu9H and λpMu507H at a multiplicity of infection (MOI) of 5. After incubation at 37° for 30 min, the cultures were washed four times in 5 ml of LB + 50 mM sodium citrate. The cells were resuspended in 5 ml of LB and incubated at 37° with aeration for 2 hr. Ten aliquots each of a 5-fold and 50-fold dilution of the culture were plated on LB + kanamycin and incubated overnight at 37°. The 5-fold dilutions contained *ca.* 5000 CFU/plate and the 50-fold dilutions *ca.* 500 CFU/plate. The colonies from the 20 plates were pooled and washed five times in LB + 50 mM sodium citrate. P1 was grown on the pool and used to transduce NT1060 (*lamBs60*) to Kan^r and the dextrin phenotype was scored on dextrin MacConkey indicator agar. Nine thousand transductants were screened and, of these, 0.5% were Kan^r Dex$^+$. The linkage between λp*lac*Mu9H and *prlC1* was determined for twenty insertions by P1 transduction.

Excision of λp*lac*Mu9H: UV induction of λ lysogens was performed as described (SILHAVY, BERMAN and ENQUIST 1984). The resulting low titer lysate (10^5–10^6 PFU/ml) was used to prepare a secondary lysate by infecting NT1051 at an MOI of <0.1.

Characterization of the excised pool: The test for Mu immunity was performed as previously described (BREMER *et al.* 1984). Detection of LacZ activity was accomplished by adding 0.1 ml of a 20 mg/ml solution of 5-bromo-4-chloro-3-indoyl-β-D-galactoside (XG) to 3 ml of top agar. The formation of λ lysogens and release of phage from these lysogens has also been described (SILHAVY, BERMAN and ENQUIST 1984).

Transductional allele exchange: Plasmids were introduced into strains by standard transformation procedures (SILHAVY, BERMAN and ENQUIST 1984). After plasmids carrying the *prlC* suppressor mutations were identified by transduction, plasmid DNA was isolated and used to transform NT113 to Amp^r Dex$^+$. This was done to ensure homogeneity of the plasmids because all of the *prlC* mutations are dominant. In pairwise crosses of the mutants, one *prlC* allele resides on the chromosome and the other on a derivative of the plasmid, pTC100.

Steady state labeling, immune precipitations and SDS-PAGE: Growth of cells and induction of LamB were as previously described (STADER, BENSON and SILHAVY 1986). Cells were labeled with 10 μCi [^{35}S]methionine (New England Nuclear) per milliliter of culture for 2 min at 30° and combined with 0.5 ml of kill solution [0.4 mg/ml chloramphenicol (Sigma), 0.4 M sodium azide (Sigma), 0.02 M dinitrophenol (Sigma)] at 0°. Immunoprecipitation of LamB was carried out as described (STADER, BENSON and SILHAVY 1986). Samples were electrophoresed by the method of LAEMMLI (1970) on a 10% polyacrylamide gel. The gel was fluorographed with 1 M sodium salicylate as described by CHAMBERLAIN (1979). Autoradiograms were scanned on a Hoeffer GS300 Transmittance/Reflectance Scanning Densitometer (Hoeffer Scientific Instruments, Inc., San Francisco) and peaks were integrated using the Hoeffer Scientific GS350 program interfaced with an IBM PC XT.

RESULTS

Isolation of new alleles of *prlC* by localized spontaneous mutagenesis: In the original isolation procedure for *prl* mutations, a strain carrying *lamBs60* was plated on dextrin minimal agar and spontaneous mutants isolated. This led to a ratio of approximately 50 *prlA* mutations for every *prlC* mutation identified (EMR, HANLEY-WAY and SILHAVY 1981). We wished to be able to enrich for *prlC* mutations against this background. To accomplish this, we started with the strain, NT350, that contains a Tn*10* 50% linked to the *prlC*$^+$ locus. Twenty independent cultures of NT350 were plated on dextrin minimal agar and Dex$^+$ colonies selected. Each plate yielded 500–1000 Dex$^+$ pseudorevertants. Because *lamBs60* is a deletion, true reversion is not possible. P1 was grown on all 20 pools and used to transduce NT1060 (*lamBs60*) to Tet^r on dextrin MacConkey agar containing tetracycline. Six of the 20 pools yielded Tet^r Dex$^+$ colonies. To ensure that each mutant arose independently, one Tet^r Dex$^+$ colony from each pool was purified and further tested as a possible *prlC* mutant.

The linkage between the Dex$^+$ mutation and the Tn*10* was determined by P1 transduction. In all six new mutants, this linkage is the expected value of 50%. In addition, the mutants are sensitive to λvir, indicating that the Dex$^+$ mutation is a result of LamB being exported to the outer membrane. The frequency of Dex$^+$ pseudorevertants is approximately 5×10^{-9} per cell per generation. Thus, very few genes can be mutated to give this phenotype. Because of these data, and results presented below, we believe that all six mutants do indeed contain mutations in the *prlC* gene. However, because all of the suppressor mutations are dominant, it is not possible to verify this prediction by complementation analysis. Accordingly, we cannot rigorously exclude the possibility of more than a single closely-linked gene that can be altered by mutation to yield a suppressor phenotype.

Strategy of *in vivo* cloning with λp*lac*Mu9H: λp*lac*Mu9H is a hybrid λ-Mu phage previously described by BREMER *et al.* (1984) that can be used for the isolation of *lacZ* fusions. For *in vivo* cloning, its salient features are: (1) it can be propagated as a λ

phage, (2) it contains enough Mu information so that it can transpose randomly into the bacterial chromosome, (3) it is marked with a Kan^r determinant, and (4) it contains a complete deletion of *attP*. By taking advantage of its ability to transpose, it is possible to isolate chromosomal insertions of λp*lac*Mu9H that are near any gene of interest. Because the phage has been inserted by a transposition event, it is not possible for it to excise from the chromosome by homologous recombination. When excision occurs, it must be the result of an illegitimate event. This aberrant excision can be such that the phage will inherit chromosomal DNA from either or both sides of the original insertion. We reasoned that if the original insertion of λp*lac*Mu9H is close enough to the gene of interest, a plaque forming phage that now carries this gene should be present within a pool of excised phage. This phage could then be identified by standard genetic means.

Isolation of λp*lac*Mu9H insertions near *prlC1*: A random pool of 50,000 independent λp*lac*Mu9H insertions was generated on NT1051 which carries the *prlC1* allele. We chose to clone *prlC1* because it is dominant to wild type and it confers a selectable phenotype, *i.e.*, Dex$^+$ in a strain carrying a *lamB* signal sequence mutation. P1 was grown on the pool and used to transduce NT1060 (*lamBs60*), selecting for the Kan^r determinant on the phage and scoring for the presence of *prlC1* (Dex$^+$). In this manner, insertions of λp*lac*Mu9H near *prlC* were identified. Nine thousand transducts were screened and the frequency of insertion near *prlC1* was 0.5%. Twenty of these were subsequently checked for the linkage between λp*lac*Mu9H and *prlC1*. Because the λp*lac*Mu9H (*ca.* 40 kb of DNA) was moved by P1 transduction (P1 packages *ca.* 90 kb of DNA), the majority of insertions near *prlC1* (14/20) exhibit greater than 70% linkage between the phage and gene. Based on size estimates of λp*lac*Mu9H, it should be possible for the phage to carry approximately 10–11 kb of chromosomal DNA and still be capable of forming plaques. Therefore, those insertions of λp*lac*Mu9H which show the greatest linkage to *prlC* were chosen for further study. Although accurate predictions are difficult, we think it unlikely that plaque-forming transducing phage can be obtained if genetic linkage is <70%.

Excision of λp*lac*Mu9H: Following induction with UV light, five different types of excision events are expected (Figure 1). Figure 1(a) shows those phage which have retained both ends of the Mu DNA intact and have acquired chromosomal DNA from both sides. This is not the desired phage for two reasons. First, because both ends of Mu are still present, the phage can still transpose. Second, the chromosomal DNA flanking the phage is now inverted with respect

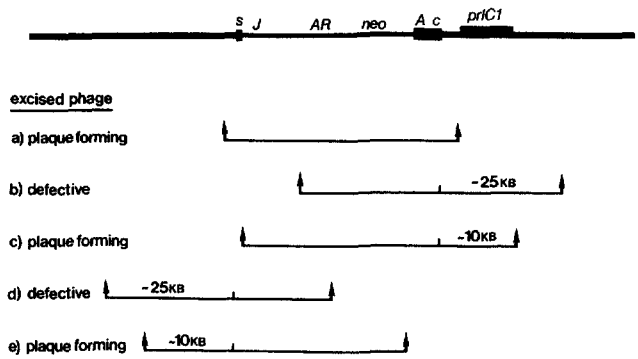

FIGURE 1.—Excision of λp*lac*Mu9H. The structure of λp*lac*Mu9H when it has transposed into the chromosome is shown at the top. Thin lines indicate the λ DNA and heavy lines the flanking chromosomal DNA. The orientation of λp*lac*Mu9H with regard to *prlC1* is shown in only one of the two possible orientations. The relevant features of the five types of excised phage are discussed in the text.

to its original orientation and contains a novel joint between the two inverted DNA segments. Both of these properties can greatly complicate further genetic analysis. The second and fourth excision events are those which create defective phages. A defective phage can accommodate approximately 25 kb of chromosomal DNA either from the left (d) or the right (b) of the original insertion. Because of the difficulty of manipulating a defective phage, plaque forming phage from the third and fifth excision events are preferred. Again, plaque forming phage can carry chromosomal DNA from either the left (e) or the right (c) of the original insertion. These phage can carry only about 10–11 kb of chromosomal DNA.

The lysogenic strains PC110, PC111, PC113 and PC115 were induced by treatment with UV. These strains carry λp*lac*Mu9H linked to *prlC1* by 70, 85, 89 and 80%, respectively. In all cases, the resulting phage lysate contains 10^5 to 10^6 plaque-forming units (pfu)/ml. The excision event needed to generate a defective phage is less stringent than the corresponding event for a plaque forming phage and as such, the lysate contains a large number of defective phage. The titer of the defective phage was lowered by making a second lysate from the original UV-induced phage using a low MOI (*i.e.*, MOI = <0.1). This decreased the number of defective phage by 100-fold.

Identification and characterization of λpTC101: The secondary lysates, prepared as described above, were used to transduce NT1060 (*lamBs60*) to Kan^r on indicator agar that allowed simultaneous scoring of the Dex phenotype. Lysates from PC110, PC111, PC113, and PC115 gave 0.03, 1.5, 1.6 and 0.8% Dex$^+$ Kan^r colonies, respectively. One Dex$^+$ Kan^r lysogen from each lysate was purified for further testing. Specialized transducing phage from each lysogen were obtained from the supernatants of broth cultures

TABLE 2

Complementation tests on excised phage

	No. of Kan^r transductants			
	NT1060		NT116	
Phage	Dex$^+$	Dex$^-$	Dex$^+$	Dex$^-$
λPC110	120 (44%)	150 (56%)	2 (1%)	155 (99%)
λPC111	498 (99%)	3 (1%)	167 (97%)	5 (3%)
λPC113	4 (2%)	201 (98%)	6 (1%)	656 (99%)
λPC115	31 (31%)	68 (69%)	0 (0%)	78 (100%)

and, following plaque purification, high titer lysates were prepared.

The *prlC1* transducing phages may be of types (a), (c) or (e) (Figure 1). As described above, transducing phage of type (a) are not desired, therefore it is important to demonstrate that the phage are missing one of the Mu ends. Since both ends are genetically marked, this can be done by two simple tests. If the *s* end of Mu is present, the *lacZ* gene must also be present and the phage will form light blue plaques on the indicator XG. If the *c* end of Mu is present, the phage will express Mu immunity in a lysogen. All four of the phage checked form white plaques on L + XG plates thus indicating they had lost the *s* end of Mu and the *lacZ* gene and, as expected, they express Mu immunity as lysogens. Therefore, these phage are all of excision type (c).

Due to the fact that the *prlC1* phage were identified by transduction, we can conclude only that they must carry the *prlC1* mutation and not necessarily the entire *prlC1* gene. λp*lac*Mu9H contains a deletion of *attP* and accordingly, the transducing phage must integrate into the chromosome by homology. It is quite possible that these phage carry only that part of the *prlC* gene in which the *prlC1* mutation is located. In this case, a *prlC1* gene would be formed in the transductants by recombination. To identify those phage which *trans* complement and thus carry the entire gene, we used the high titer lysate for transduction into two slightly different strains. In the first strain, NT1060 (*lamBs60*), the phage must integrate at *prlC* because this is the only homology available. The second strain, NT116, is identical to NT1060 except that it carries a λ prophage at *tsx* and as such, has two regions of homology for integration of the transducing phage. If any of the phage can *trans* complement, they should yield only Dex$^+$ lysogens in both strains. However, if recombination is necessary to reconstitute *prlC1*, then many of the lysogens of strain NT116 will be Dex$^-$ because of the additional homology available for integration. Table 2 shows that of the four phage tested, only the one derived from PC111 is able to *trans* complement. The 1–3% Dex$^-$ transductants observed in this test for the phage from PC111 are a result of the instability of the phage carrying *prlC1*, as described below. The *trans* complementing phage, λpTC101, was used for further characterization and subcloning of *prlC*.

Since λpTC101 carries the *prlC1* gene, it should be possible to use this phage to exchange alleles of *prlC* with the chromosome by recombination and thus clone the wild-type *prlC* gene. When λpTC101 is lysogenized by homology over *prlC*$^+$, the result is a λ phage flanked by two directly repeated copies of *prlC*, one being *prlC*$^+$ and the other *prlC1*. If the phage is allowed to excise spontaneously from the chromosome, it should produce two different transducing phage. The first is from a recombination similar to that resulting in integration. In this case, *prlC*$^+$ will remain on the chromosome and *prlC1* on the phage. The second results from a recombination on the opposite side of the *prlC1* mutation. Here the phage will carry *prlC*$^+$ and *prlC1* will remain in the chromosome. Allele exchange was carried out with λpTC101 lysogenized into NT1060 by screening for Kan^s colonies and counterscoring their Dex phenotype. The chromosomal allele (*prlC*$^+$) was changed to *prlC1* at a frequency of 40%.

This chromosomal allele exchange is a RecA-dependent event (data not shown) and therefore, should be reciprocal. Since the *prlC1* allele can be left in the chromosome 40% of the time, we would expect to find *prlC*$^+$ on the phage 40% of the time. Twenty spontaneously released phage from the λpTC101 lysogen were checked for the presence of the wild-type gene. We found that all twenty phage still carry the *prlC1* gene. This suggests that *prlC*$^+$ cannot be carried on λ. To further test this idea, we isolated Dex$^-$ homogenotes (*prlC*$^+$/*prlC*$^+$) of NT1060 (λpTC101) and measured the frequency of spontaneously released phage. With this lysogen, the frequency of release is three logs lower (10^3 pfu/ml) than the starting heterogenote (10^6 pfu/ml). Moreover, it appears that many, if not all, of these phage are mutants (see below). We therefore conclude that it is not possible to isolate *prlC*$^+$ on λpTC101. This could indicate that the wild-type gene is larger than *prlC1* resulting in a phage with too much DNA to be packaged, or more likely in light of results described below, that the wild-type gene interferes in some manner with the λ life cycle.

The fact that λ cannot carry *prlC*$^+$ was not totally unexpected. The *prlC1* transducing phage, λpTC101, forms small plaques which have 10–100-fold fewer phage than the parent phage, λp*lac*Mu9H (10^4–10^5 pfu/plaque for λpTC101 and 10^6 pfu/plaque for λp*lac*Mu9H). In the course of growing λpTC101, large plaques appear in the lysates at a frequency of 1–2%. The occurrence of these large plaques is RecA-

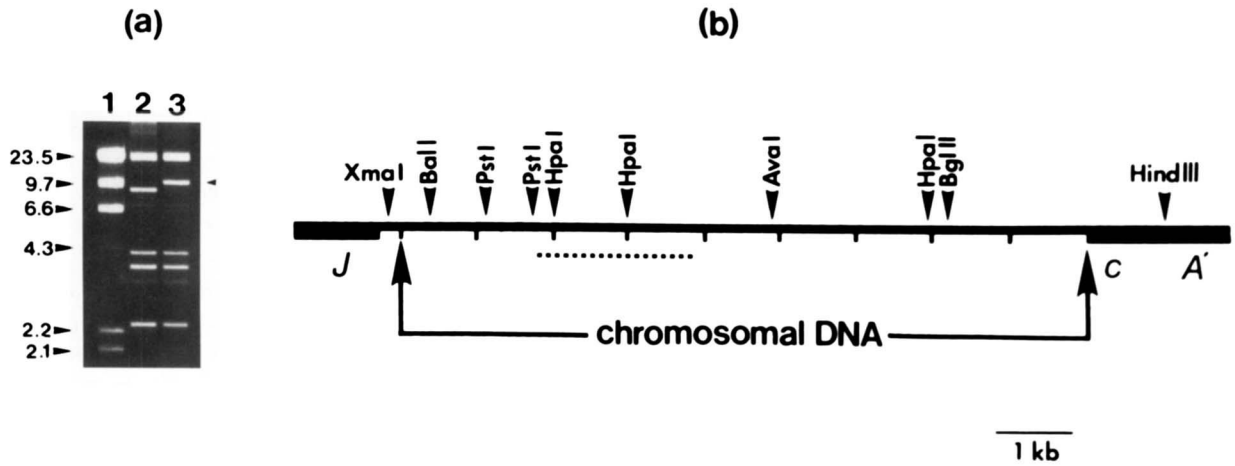

FIGURE 2.—Size determination and restriction site analysis of λpTC101. Panel (a) shows an *Xma*I-*Hin*dIII digest of λp*lac*Mu9H (lane 2) and λpTC101 (lane 3). Lane 1 contains molecular weight standards with their corresponding molecular weight shown on the left. The arrow to the right indicates the chromosomal DNA carried in λpTC101. This 10.5 kb band is composed of 9 kb of chromosomal DNA, 1 kb of the Mu *c* end and 0.5 kb of λ DNA from the *J* region. Samples were electrophoresed on a 0.8% agarose gel. Panel (b) shows a restriction map of the chromosomal DNA located between the *Xma*I and *Hin*dIII sites in λpTC101. There are no restriction sites in this DNA for *Bam*HI, *Bcl*I, *Bgl*I, *Cla*I, *Eco*RI, *Hin*dIII, *Kpn*I, *Nco*I, *Sac*I, *Stu*I, *Xba*I and *Xma*I. The *dotted line* indicates the approximate position of the *prlC* gene as determined from linkage data.

independent and the number of phage per large plaque is now equivalent to λp*lac*Mu9H. These large plaques contain phage which have lost the ability to either *trans* complement or to recombine to generate a functional *prlC1* allele. We believe the instability of λpTC101 when it is grown lytically is caused by a detrimental effect of the *prlC1* gene product on λ lytic growth. If the *prlC1* gene is removed by mutation, normal growth, as seen in the large plaque mutants, is restored. Apparently *prlC*$^+$ causes even more severe effects on λ growth.

Restriction mapping and subcloning of *prlC1*: Phage DNA was isolated from λp*lac*Mu9H and λpTC101 and physical mapping was performed using restriction endonucleases. These results (Figure 2) show that λpTC101 carries approximately 9 kb of chromosomal DNA. Because it has very few 6 bp restriction enzyme recognition sequences, we decided to subclone the entire insert by taking advantage of restriction sites in the flanking phage DNA. There is an *Xma*I site located in the λ DNA on the left side of the insert. On the right side of the insert is a *Hin*dIII site located in the Mu *c* end. This *Xma*I-*Hin*dIII fragment, which carries *prlC1*, was subcloned into pTA108 (Figure 3) by screening the transformants for insertional inactivation of *lacZ*. The plasmids that contain an insert were subsequently checked for their ability to confer a Dex$^+$ phenotype in NT1060 (*lamBs60*). Of 12 complementing plasmids identified, all contain the same 9-kb fragment which is identical to that present in λpTC101. One of the plasmids, pTC101, was chosen for further analysis.

Transductional allele exchange: We have found

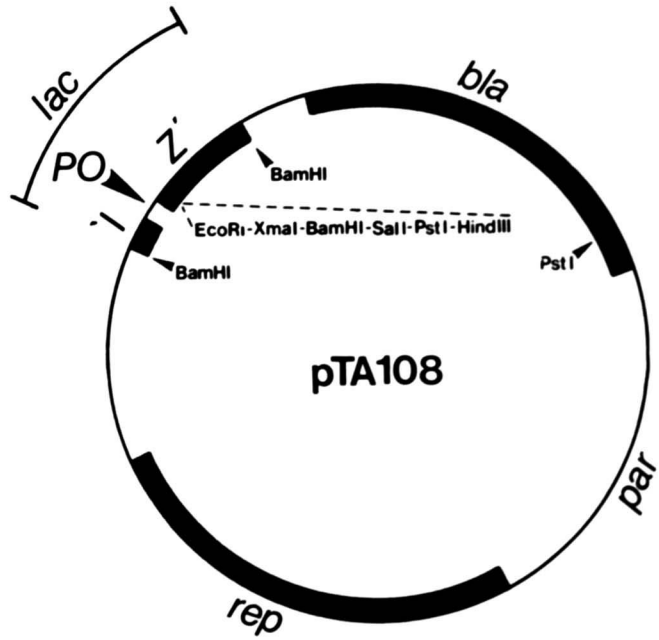

FIGURE 3.—Low copy number vector pTA108. The construction of pTA108 is described in MATERIALS AND METHODS. It is a derivative of pSC101 which contains the *lac* DNA from pUC8. As such, it can be used for insertional inactivation cloning. The *Xma*I-*Hin*dIII fragment from λpTC101 was inserted into the *Xma*I-*Hin*dIII sites of pTA108 to form pTC101.

that pTA108 cannot be moved between strains by P1 transduction (Table 3). However, the chromosomal insert in pTC101 allows mobilization between strains in a P1-mediated fashion. The frequency of this mobilization is similar to the frequency of transducing a chromosomal marker. This implies that pTC101 must have a crossover with the chromosome before P1 can package it. We, therefore, tested if during the cross-

TABLE 3

Transductional allele exchange

Chromosomal allele	Plasmid allele	No. of Amp^r transductants[a] Dex$^+$	Dex$^-$	Percent exchange of plasmid and chromosomal alleles
prlC$^+$	pTA108	0	0	NA
prlC1	*prlC1*	118	0	NA
prlC$^+$	*prlC1*	145	9	5.8[b]
prlC1	*prlC*$^+$	9	62	12.7[b]
prlC2	*prlC*$^+$	5	40	11.1
prlC5	*prlC*$^+$	7	424	1.6
prlC7	*prlC*$^+$	15	438	3.3
prlC8	*prlC*$^+$	46	273	14.4
prlC11	*prlC*$^+$	15	661	2.2
prlC12	*prlC*$^+$	14	177	7.3
prlC18	*prlC*$^+$	15	327	4.3

NA, not applicable.

[a] P1 was grown on the strains containing the indicated plasmid and chromosomal alleles. This lysate was used to transduce NT1060 to Amp^r and the Dex phenotype was scored. All of the suppressor mutations are dominant.

[b] The discrepancy between the two frequencies in the *prlC*$^+$ by *prlC1* cross can be explained by the fact that *prlC1* is dominant to *prlC*$^+$. In the cross used to move *prlC*$^+$ onto the plasmid, the recessive allele is being scored. The transducing fragment that carries the *prlC*$^+$ allele from the chromosome will also contain the *prlC1* allele from the plasmid. Therefore, the frequency of identifying the recessive allele is lower than the frequency of identifying the dominant allele (cross with *prlC*$^+$ on the plasmid).

over event, the *prlC* allele in the plasmid could be exchanged with the *prlC* allele present in the chromosome. These results are shown in Table 3. If the *prlC1* allele is on the chromosome and the plasmid, the only allele that should be found in the transductants is *prlC1*. When this cross was performed, all the transductants had a plasmid which conferred a Dex$^+$ (*prlC1*) phenotype. This result indicates that the plasmid is not being rearranged or altered. We next transformed pTC101 into NT1060 (*prlC*$^+$) and mobilized the plasmid by transduction. Approximately 6% of the transductants now carry a plasmid which confers a Dex$^-$ (*prlC*$^+$) phenotype in NT1060 (*lamBs60*). To confirm that the plasmid is not being rearranged during the transduction, ten of the plasmids presumed to carry *prlC*$^+$ were checked by physical mapping. The plasmids were digested with restriction enzymes that recognize both 4 base pair (bp) and 6 bp sequences. In all cases, the digest patterns were the same for ten *prlC*$^+$ plasmids as well as for pTC101 (data not shown). Therefore, we believe that the plasmids which confer a Dex$^-$ phenotype do indeed carry *prlC*$^+$. One of the *prlC*$^+$ containing plasmids, pTC100, was chosen for further study.

pTC100 was transformed into all of the *prlC* mutations and transductional allele exchange was carried out. This allowed both isolation of plasmids containing the new *prlC* mutations and measurement of the frequency with which they will recombine with pTC100

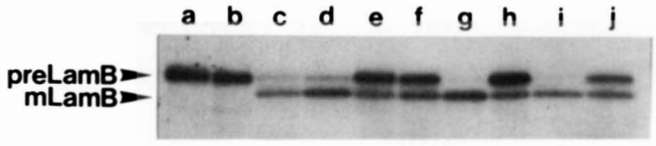

FIGURE 4.—The strength of the *prlC* suppressor mutations as determined by processing of LamBs78. LamBs78 was immunoprecipitated from [^{35}S]methionine labeled whole cell extracts of maltose induced cells. Extracts were from JS1013 containing the following plasmids: (a) pTA108; (b) pTC100 (*prlC*$^+$); (c) pTC101 (*prlC1*); (d) pTC102 (*prlC2*); (e) pTC105 (*prlC5*); (f) pTC107 (*prlC7*); (g) pTC108 (*prlC8*); (h) pTC111 (*prlC11*); (i) pTC112 (*prlC12*); (j) pTC118 (*prlC18*). LamBs78 was used because it is not possible to detect processing with LamBs60.

(Table 3). Based on these frequencies, the suppressor mutations map at several different positions. After the plasmids carrying the new *prlC* alleles were purified, the mutations were crossed, in a pairwise fashion, against each other. The appearance of *prlC*$^+$ recombinants in these crosses proves that the new alleles represent different mutations (data not shown).

Mapping the *prlC1* gene in pTC101: The position of *prlC1* in pTC101 is inferred from the transductional allele exchange frequencies and the linkage between *prlC1* and the original λp*lac*Mu9H insertion. If the gene was located in the middle of the cloned chromosomal DNA, we would expect the transductional allele exchange frequencies to approach 50%. Because these frequencies are so low, the gene should be located near one end of the cloned fragment. This fragment was subcloned from λpTC101 using sites in the λ DNA and Mu *c* end, therefore the position of the original λp*lac*Mu9H insertion is marked by the DNA present from the Mu *c* end. Because this insertion shows only 85% linkage to *prlC1*, the *prlC1* gene should be located near the end of the cloned fragment that is not adjacent to the Mu *c* end. This position is shown in Figure 2. Preliminary deletion mapping results of pTC101 have shown this placement of the gene to be correct (data not shown).

Biochemical analysis of the *prlC* mutations: The plasmids containing *prlC*$^+$, *prlC1*, *prlC2*, *prlC5*, *prlC7*, *prlC8*, *prlC11*, *prlC12* and *prlC18*, respectively, were transformed into JS1013 (*lamBs78*). *lamBs78* is an in-frame deletion that removes the bases corresponding to amino acids 10–13 of the *lamB* signal sequence (EMR and SILHAVY 1980). Mid-logarithmic cultures of these strains were labeled with [^{35}S]methionine and the LamB immunoprecipitated. An SDS-polyacrylamide gel of these samples is shown in Figure 4. As can be seen in lanes 1 and 2, the *lamBs78* mutation causes LamB to accumulate in the precursor form (signal sequence uncleaved). However, in the presence of the *prlC* suppressor mutations (lanes 3–10) different amounts of LamB are found in the mature form. Due to the fact that leader peptidase resides on the periplasmic side of the inner membrane (ZWIZINSKI

TABLE 4

Processing of *lamBs78* in the presence of *prlC* suppressors

Plasmid in JS1013	*prlC* allele	Percent precursor LamB	Percent mature LamB	Mutation class
pTA108	NA	95	5	NA
pTC100	*prlC*$^+$	95	5	NA
pTC101	*prlC1*	28	72	2
pTC102	*prlC2*	23	77	2
pTC105	*prlC5*	64	35	1
pTC107	*prlC7*	61	39	1
pTC108	*prlC8*	7	93	3
pTC111	*prlC11*	66	34	1
pTC112	*prlC12*	11	89	3
pTC118	*prlC18*	55	45	1

NA, not applicable.

and WICKNER 1980), the mature form of LamB indicates that LamB has been translocated across the inner membrane and thus represents a measure of the amount of defective LamB that can be exported in the presence of the suppressor mutations. A quantitation of the percentage precursor and mature LamB in each suppressor mutation is shown in Table 4.

DISCUSSION

Localized spontaneous mutagenesis allows for rapid screening of mutations which confer a selectable phenotype and map to a specific region on the chromosome. In the case of *prlC*, this technique has yielded six new alleles that suppress signal sequence mutations in *lamB*. These six alleles, as well as the two previously isolated alleles, fall into three classes based on their suppression of *lamBs78*. Class 1 (*prlC5*, *prlC7*, *prlC11*, *prlC18*) shows 35–45% of the total LamB in the mature form, Class 2 (*prlC1*, *prlC2*), 75% mature and Class 3 (*prlC8*, *prlC12*), 90% mature. In addition, Class 1 mutations show a similar frequency of recombination in the transductional allele exchange (1.6–4.3%). This apparent clustering suggests that these mutations are altering the same region of the *prlC* gene. Class 2 mutations are also clustered (11.1% and 12.7%), whereas the Class 3 mutations have very different recombination frequencies (7.3 and 14.4%). These data, along with the fact that the *prlC* suppressor mutations can recombine to generate a *prlC*$^+$ gene, indicate that several mutations within *prlC* will enable it to suppress signal sequence defects in exported proteins. Based on the strength of suppression by Class 3 mutations, we believe that the mutant LamB protein is being exported via the normal pathway. Whether or not PrlC is affecting export in a direct or indirect manner, awaits the isolation and characterization of defined *prlC* null mutations.

As a first step in the characterization of *prlC*, we have cloned the gene. To this end, an *in vivo* cloning procedure has been developed. This procedure has the obvious advantage that it allows cloning of any gene of interest for which there is a phenotype, regardless of how much additional information is available about the gene. The logic behind λp*lac*Mu9H cloning is the same as that for the *in vivo* cloning method of SHIMADA, WEISBERG and GOTTESMAN (1972) which uses 2° λ *att* sites to position λ at different places in the chromosome. One improvement λp*lac*Mu9H cloning offers, is the phage can transpose randomly into the bacterial chromosome. Therefore, a gene does not have to map near a 2° *att* site in order for *in vivo* cloning to be successful. Another advantage of λp*lac*Mu9H cloning is that all of the excision events produced by UV treatment are the result of illegitimate recombination. Thus, many illegitimate excision events can be screened to find those which generate the desired specialized transducing phage.

The instability of λpTC101 and its inability to carry the *prlC*$^+$ gene can be attributed to the unusual nature of the *prlC* gene product and does not detract from the generality of λp*lac*Mu9H cloning. Two results which strengthen this conclusion are that the *prlC1* gene, when present on a pBR322 derivative is also unstable (data not shown) and the λpTC101 instability can be alleviated by mutations in *prlC1* (*i.e.*, the large plaque mutants).

Due to the lack of restriction sites near *prlC*, the gene was subcloned from λpTC101 using sites in the flanking λ and Mu DNA. Both the *Xma*I and the *Hin*dIII sites used are in DNA that does not have to be present in all transducing phage. However, if these restriction sites are missing, others within the λ *J* gene and λ *exo* region can be used for subcloning. Thus, regardless of the restriction map of the chromosomal insert, it can be subcloned from the transducing phage.

The subcloning of *prlC1* into pTA108 has offered many advantages. Because pTA108 is present in only four to five copies per cell, the detrimental effects seen when *prlC1* is present on pBR322 have been alleviated. We have also found that transduction of pTA108 by P1 is dependent on chromosomal homology. By taking advantage of this observation, *prlC*$^+$ and all of the *prlC* suppressor mutations have been recombined onto the plasmid. Restriction digest analysis of the plasmids containing *prlC*$^+$, *prlC1* and *prlC2* has indicated that the suppressor mutations are not the result of any large insertion or deletion in the *prlC* gene (data not shown). Using the new alleles of *prlC* and the plasmids that carry them, we can now begin to delineate the role of PrlC in the export of proteins in *E. coli*.

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