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Organization and Subcloning of the *dacA-rodA-pbpA* Cluster of Cell Shape Genes in *Escherichia coli*

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The transducing bacteriophage λpBS10 carries a small cluster of *Escherichia coli* penicillin-binding protein/cell shape genes, including *pbpA*, *rodA*, and *dacA*. Deletion mapping and subcloning showed that these genes, and the gene for a cytoplasmic membrane protein of molecular weight 54,000, are located within a 5.6-kilobase region and are probably contiguous. The *dacA* gene, which codes for penicillin-binding protein 5, was cloned on a 1.5-kilobase fragment into a low-copy-number plasmid vector, but insertion into high-copy-number plasmids produced deleterious effects on bacterial growth, and the plasmids could not be stably maintained. The direction of transcription of *dacA* was determined. The *rodA* gene was cloned on a 1.6-kilobase fragment into both low- and high-copy-number plasmids, and the identification of its gene product is described in the accompanying paper (Stoker et al., J. Bacteriol. **155**:854–859). The *pbpA* gene, which codes for penicillin-binding protein 2, was cloned on a 3.7-kilobase fragment in low-copy-number plasmids, but insertion of the fragment into high-copy-number plasmids resulted in deleterious effects on bacterial growth, and the plasmids could not be stably maintained.

Bacterial cells derive their strength and shape from the rigidity of the peptidoglycan layer of the cell wall (13). Rod-shaped bacteria synthesize cylindrical peptidoglycan during cell elongation and hemispherical peptidoglycan during cross wall formation at cell division. Different enzyme systems appear to be involved in the synthesis of peptidoglycan of different shapes (14), but little is known about the mechanism by which cell shape is determined. We described a small cluster of genes involved in cell shape determination and peptidoglycan synthesis, including *pbpA*, the structural gene for penicillin-binding protein (PBP) 2, and *rodA* (1, 17). Both of these genes appear to be essential for the synthesis of cylindrical peptidoglycan, as inactivation of either gene causes cells to grow as spheres (17, 20). *dacA*, the structural gene for PBP5, also maps within the cluster. Although inactivation of PBP5 does not result in serious growth defects (8, 9, 16), overproduction of PBP5 in *Escherichia coli* results in spherical cells (7).

The genes of this cluster were cloned into bacteriophage λ to produce the plaque-forming specialized transducing phage λpBS10, which contains a 15-kilobase (kb) *Hin*dIII fragment carrying the region *leuS-pbpA-rodA-dacA* (17). The proteins synthesized by λpBS10 and related transducing phage were analyzed, and the gene products of the *leuS*, *pbpA*, and *dacA* genes were identified, but no protein corresponding to the *rodA* gene product was found (17).

We describe here the further characterization of this gene cluster and the separate subcloning of *pbpA*, *rodA*, and *dacA* into low-copy-number plasmid vectors.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, and plasmids. The bacterial strains used in this work are listed in Table 1. The bacteriophage used were P1 *vir*, λ *imm*^{434}, λpBS10, and λ d*lip5* (17). The low-copy-number plasmid cloning vectors used were pSC105 (3), pLG318, and pLG338 (18). The media and conditions of growth for bacteria and phage were described previously (17).

DNA preparation, manipulation, and analysis. The preparation of plasmid and bacteriophage DNAs and techniques for the manipulation and transformation of DNA were described previously (17, 18).

Genetic techniques. Generalized transduction with phage P1 *vir* was performed as described previously (17). *E. coli* W3110 *pbpA6* was constructed from strain W3110 by cotransduction of *pbpA6* with an adjacent Tn*10* insertion by using P1 phage grown on strain SP60. *E. coli* NGS34 *leuS*(Ts) *rodA*(Ts) was constructed from strain TMRL12 *leuS*(Ts) by cotransduction of *rodA52*(Ts) with Tn*10* by using P1 phage grown on strain SP5212.

The presence of the *leuS* and *rodA* genes on λ transducing phage was tested with strains TMRL12(λ *imm*^{434}) and SP5211(λ *imm*^{434}), respectively, as de-

TABLE 1. *E. coli* strains

Strain no.	Relevant properties	Reference or source
C600	*thr leu thi tonA supE*	Our collection
159	*gal uvrA*	17
SP45	*trp tyr ilv supD*(Ts) *pbpA45*(Ts)	17
SP60	*trp tyr ilv supD*(Ts) *pbpA6 ponA6 zbe*::Tn*10*	17
W3110	sup^0	Our collection
W3110 *pbpA6*	sup^0 *zbe*::Tn*10 pbpA6*	This paper
SP5211	*his purB proA thi lacY rpsL rodA52*(Ts)	17
SP5212	SP5211 *zbe*::Tn*10*	This paper
TMRL12	*arg pro purB his thi lacY leuS*(Ts)	M. Matsuhashi (8)
NGS34	TMRL12 *zbe*::Tn*10 rodA52*(Ts)	This paper
SP5003	*his supF srl*::Tn*10 recA56* Δ(*rodA-dacA-lip*) (λ d*rodA*1)	16

scribed previously (17). A cleaner test for the presence of *rodA* was used when the phage carried an intact *leuS* gene. Strain NGS34(λ imm^{434}) was transduced to $leuS^+$ at 42°C, and the cell morphology was examined to determine whether *rodA* was present. Transduction of *pbpA* was measured by selecting for growth of strain W3110 *pbpA6*(λ imm^{434}) on Penassay agar (antibiotic medium; Difco Laboratories, Detroit, Mich.) at 37°C. This selection is based on the fact that fast-growing spherical strains (e.g., strain W3110 *pbpA6*) survive on minimal media but not on Penassay medium.

Isolation of deletion derivatives of λpBS10. Deletion derivatives of λpBS10 were isolated by selecting for resistance to EDTA (10); 0.1-ml dilutions of λpBS10 were adsorbed to 0.1 ml of an overnight culture of strain C600 for 10 min at room temperature and then plated in 2.5 ml of Trypticase soft agar (BBL Microbiology Systems, Cockeysville, Md.) on Trypticase agar plates containing between 0.8 and 1.4 mM EDTA. Large plaques that appeared on a background lawn of pinprick plaques were picked and purified.

Analysis of proteins synthesized by bacteriophage and plasmids. Proteins synthesized by λpBS10 and its derivatives were analyzed in heavily UV-irradiated cells of *E. coli* 159(pGY101) as described previously (17). pGY101 is a plasmid carrying the phage 434 repressor gene (6).

Proteins synthesized by plasmids were analyzed in minicells prepared and labeled with [^{35}S]methionine as described by Reeve (12).

The assay of PBPs in whole cells with [^{3}H]benzylpenicillin (30 Ci/mmol; a gift from Patrick Cassidy, Merck Laboratories, Rahway, N.J.) was described previously (2).

RESULTS

Isolation of deletion derivatives of λpBS10. We reported the mapping of genes carried by the transducing phage λpBS10 and an analysis of the gene products, but ambiguity remained about the precise location and relative order of some of the genes (17). To remove some of this ambiguity, deletion derivatives of λpBS10 were isolated and tested for the presence of the *leuS*, *pbpA*, and *rodA* genes. Six phage that still transduced *leuS* but failed to transduce *rodA* were chosen for analysis in detail (Table 2). The approximate size and location of the deletions were determined by digestion with restriction endonucleases (Fig. 1).

The proteins synthesized after infection of UV-irradiated cells with the deletion derivatives of λpBS10 were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis followed by fluorography (Fig. 2 and Table 3). Eight proteins were synthesized from the chromosomal genes of λpBS10; these are numbered by decreasing molecular weight as described previously (17). Comparison of the proteins no longer synthesized by deletion derivatives of λpBS10 with the extent and location of their deletions allowed more precise mapping of the genes carried by the phage.

The gene encoding protein 8 (M_r, 11,000) was shown to map very close to the *pbpA* gene, but the order was not determined (17). λpBS10Δ2 still transduced *leuS*, but was deleted of *pbpA*, *rodA*, and *dacA*; yet it still synthesized protein 8. The gene encoding protein 8 must therefore map on the *leuS* side of *pbpA*.

Similarly, the gene encoding protein 3 (M_r, 54,000 [54K protein]) was shown to map very close to *dacA*, but the order was not determined (17). λpBS10Δ8 and λpBS10Δ13 were $rodA^-$ but $dacA^+$ and failed to synthesize protein 3 (the 54K protein). Therefore the 54K protein must be encoded either by *rodA* or by a gene that maps on the *pbpA* side of *dacA*.

TABLE 2. Genes transduced by λpBS10 and its deletion derivatives

Gene	Transduction by:						
	λpBS10	λpBS10Δ2	λpBS10Δ6	λpBS10Δ8	λpBS10Δ13	λpBS10Δ34	λpBS10Δ51
leuS	+	+	+	+	+	+	+
pbpA	+	−	+	+	+	−	−
rodA	+	−	−	−	−	−	−

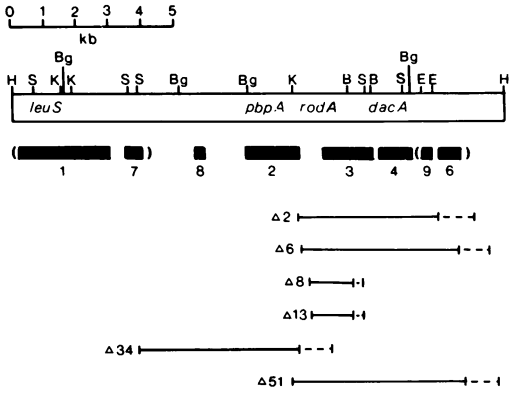

FIG. 1. Map of the chromosomal DNA of λpBS10. Lines at the bottom indicate the extent of deletions in derivatives of λpBS10. Dotted regions show the ambiguous end points of the deletions. Solid blocks represent the map location and relative size of the genes encoding the proteins synthesized by the 15-kb *Hin*dIII fragment, assuming that 1 kb can code for a protein of molecular weight 37,000. Details of the location of the *pbpA*, *rodA* and *dacA* genes are given in the text. The gene products are numbered in order of decreasing molecular weight (17) (Fig. 2). The assigned genes and gene products are: *leu*S, protein 1 (leucyl tRNA synthetase); *pbpA*, protein 2 (PBP2); *dacA*, protein 4 (PBP5) (17). Abbreviations for restriction endonuclease cleavage sites: H, *Hin*dIII; S, *Sal*I, K, *Kpn*I; Bg, *Bgl*II; B, *Bam*HI: E, *Eco*RI. The restriction map differs slightly from an earlier one (17) due to the detection of several small DNA fragments that were previously overlooked.

The *rodA* gene maps between *pbpA* and *dacA* (17), and the only protein that we showed was synthesized from this region is the 54K protein. λpBS10Δ34, which failed to transduce *pbpA* or *rodA*, was found to synthesize the 54K protein. The 54K protein cannot therefore be the *rodA* gene product, and the gene encoding the 54K protein (the 54K gene) must map between *rodA* and *dacA* (Fig. 1). The identification of the *rodA* gene product is described elsewhere (19).

The *pbpA*, *rodA*, 54K, and *dacA* genes map together in a small region of the *E. coli* chromosome, and all code for cytoplasmic membrane proteins (5, 11, 17, 19; unpublished data). Nothing is known of the function of the 54K protein, but the others are implicated in peptidoglycan synthesis and cell shape. To allow a more detailed investigation of the genes in this region, we subcloned the *dacA*, *rodA*, and *pbpA* genes separately into plasmid vectors.

Subcloning the *dacA* gene. The *dacA* gene was cloned on a 3-kb fragment from a partial *Sau*IIIa digest of λ d*lip5* into the *Bam*HI site within the tetracycline resistance region of the low-copy-number plasmid pSC105 to produce pBS25 (7). *E. coli* strains carrying pBS25 grow as spherical cells due to the disturbing effect of elevated levels of PBP5 on bacterial growth (7). The *dacA* gene was further localized to a 1.5-kb *Bam*HI-*Eco*RI fragment (7), and this fragment was purified from λ d*lip5* and inserted between the *Bam*HI and *Eco*RI sites within the tetracycline resistance region of pLG338 to produce pBS59. The level of *dacA* expression in pBS59 was lower than in pBS25, as some expression occurs from the *tet* promoter in the latter plasmid, and strain C600(pBS59) grew as swollen cells rather than as the completely spherical cells produced by strain C600(pBS25). Attempts to clone the *dacA* gene into high-copy-number plasmid vectors produced transformants with bizarre morphology, and these were too unstable to be studied further.

The direction of transcription of *dacA* was determined by inserting the gene in both orientations in front of the powerful phage λ leftward promoter (p_L) in the low-copy-number expression vector pBS41 (Fig. 3). pBS41 was constructed by inserting a 4.0-kb *Bam*HI-*Eco*RI fragment of phage λ, carrying the *N* p_L *c*I857 Δ (*cro*) region (map coordinates, 35,269 to 40,000; deleted between the *Bgl*II sites at 38,935 and 39,646 to remove *cro* [4]), into pLG318. The *cro*-deleted λ fragment was prepared from the expression vector pGWp_L1, kindly provided by Geoff Wilson. DNA inserted into the unique *Bam*HI site of pBS41 is transcribed from λ p_L at 42°C due to inactivation of the thermolabile *c*I857 repressor. The *dacA* gene was obtained as a *Bam*HI fragment as follows. λ d*lip5* DNA was partially digested with *Sau*IIIA, and 1- to 5-kb fragments were cloned into the *Bam*HI site of pSC105. Plasmids carrying *dacA* were detected by the reappearance of PBP5 in *E. coli* SP5003, which is deleted of the chromosomal *dacA* gene (16). The resulting *dacA*$^+$ plasmids were screened for those that produced two fragments after digestion with *Bam*HI due to the generation of *Bam*HI sites at both ends of the inserted fragment. A plasmid, pBS30, which carried *dacA* on a 3.1-kb *Bam*HI fragment was obtained, and the fragment was purified and inserted into the *Bam*HI site of pBS41. Transformants were obtained at 30°C, and those carrying recombinant plasmids were detected by their increased size on plasmid screening gels (18). Two plasmids (pBS43 and pBS48) that had the 3.1-kb *Bam*HI fragment inserted in opposite orientations were studied. Strain C600(pBS43) grew as swollen cells at 30°C, and the cells became spherical and produced high levels of PBP5 when the temperature was shifted to 42°C. Cells of strain C600(pBS48) did not alter their shape or overproduce PBP5 when shifted from 30 to 42°C (Fig. 4). The *dacA* gene in pBS43 was therefore in the correct orientation for transcrip-

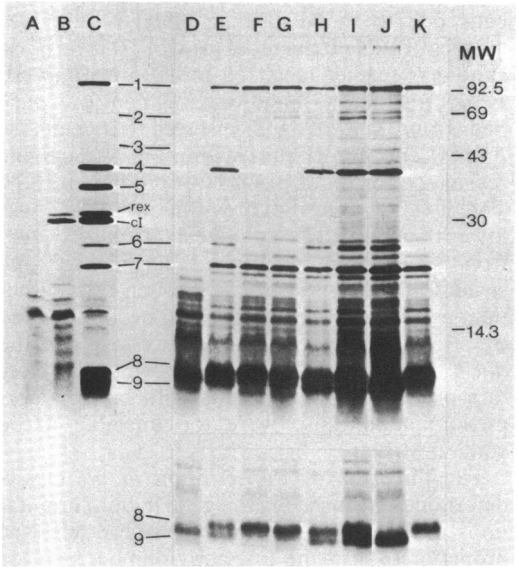

FIG. 2. Proteins synthesized by λ d*lip5*, λpBS10, and deletion derivatives. Lanes show [^{35}S]methionine-labeled proteins synthesized in UV-irradiated cells of strain 159(λ$^+$) infected with (A) no phage, (B) λ$^+$, or (C) λ d*lip5* or in UV-irradiated cells of strain 159(pGY101) infected with (D) no phage, (E) λpBS10, (F) λpBS10Δ2, (G) λpBS10Δ6, (H) λpBS10Δ8, (I) λpBS10Δ13, (J) λpBS10Δ34, or (K) λpBS10Δ51. Cells were fractionated on a 15% SDS-polyacrylamide gel and detected by autoradiography. The minor proteins 2 and 3 were difficult to show clearly without overexposing the X-ray film and are not visible in this figure. The lower part of the figure shows the bottom of the same gel exposed to X-ray film for a short period to show the resolution of proteins 8 and 9. Molecular weights (MW) ($\times 10^3$) are shown to the right.

tion from λ p_L, and the gene is therefore transcribed from the *Bam*HI toward the *Eco*RI site (Fig. 1 and 3).

Subcloning the *rodA* gene. The 5.0-kb *Bgl*II fragment from λpBS10 was purified and ligated with a *Bam*HI digest of pSC105 (Fig. 5). This was transformed into the temperature-sensitive *rodA* mutant strain SP5211, and kanamycin-resistant colonies were selected on nutrient agar at 42°C. A mixture of large and small colonies appeared. The smaller colonies consisted of spherical cells, whereas the larger colonies contained rod-shaped cells, indicating the presence of a functional *rodA* gene. Plasmids pLG340 and pLG341 were shown to carry the *Bgl*II fragment cloned in opposite orientations.

pLG340 and pLG341 did not complement the *pbpA45* mutation, indicating that the 5.0-kb *Bgl*II fragment does not contain the entire *pbpA* gene; neither did they carry an intact *dacA* gene, as *Bgl*II cuts within the *dacA* gene (7). The 0.7-kb *Bam*HI fragment, which lies within the 5.0-kb *Bgl*II fragment, was removed by digesting the plasmids with *Bam*HI, followed by religation at a low DNA concentration (Fig. 5). The resulting plasmids (pLG342 and pLG344) were still *rodA*$^+$. Since the *pbpA* gene extends across the *Kpn*I site within the 5.0-kb *Bgl*II fragment (see below), it was anticipated that *rodA* lay within the 1.6-kb *Kpn*I-*Bam*HI fragment. This fragment was therefore cloned into pLG318, a derivative of pSC105 with a single *Kpn*I site (18). The resultant plasmid, pLG346, was *rodA*$^+$ (Fig. 5). The 1.6-kb *Kpn*I-*Bam*HI fragment carrying *rodA* could readily be inserted into high-copy-number plasmid vectors (19), and these plasmids had no obviously deleterious effect on cell growth or morphology.

The proteins synthesized by plasmids carrying *rodA* were analyzed in minicells with both [^{35}S]methionine and a ^{3}H-amino acid mixture (data not shown). No candidate for the *rodA* gene product was found in these experiments, and the identification of the *rodA* gene product is described elsewhere (19).

Subcloning the *pbpA* gene. The *pbpA* and *rodA* genes are located on a 7-kb *Sal*I fragment (17) (Fig. 1). To subclone *pbpA* separately from *rodA*, we used λpBS10Δ13, which lacks *rodA*

TABLE 3. Proteins synthesized by λpBS10 and its deletion derivatives[a]

Protein no.	Mol wt	Gene	Production by phage:							Cellular location[b]
			λpBS10	λpBS10Δ2	λpBS10Δ6	λpBS10Δ8	λpBS10Δ13	λpBS10Δ34	λpBS10Δ51	
1	105,000	*leuS*	+	+	+	+	+	+	+	C
2	66,000	*pbpA*	+	−	+	+	+	−	−	CM
3	54,000	?	+	−	−	−	−	+	−	CM
4	42,000	*dacA*	+	−	−	+	+	+	−	CM
6	22,000	?	+	−	−	+	+	+	−	C
7	19,000	?	+	+	+	+	+	+	+	CM
8	11,000	?	+	+	+	+	+	−	+	C
9	10,000	?	+	−	−	+	+	+	−	C

[a] Proteins synthesized by λpBS10 and its deletion derivatives in UV-irradiated cells of *E. coli* 159(pGY101). Proteins are numbered by the method of Spratt et al. (17).

[b] Taken from references 15 and 17 and unpublished data. C, Cytoplasm; CM, cytoplasmic membrane.

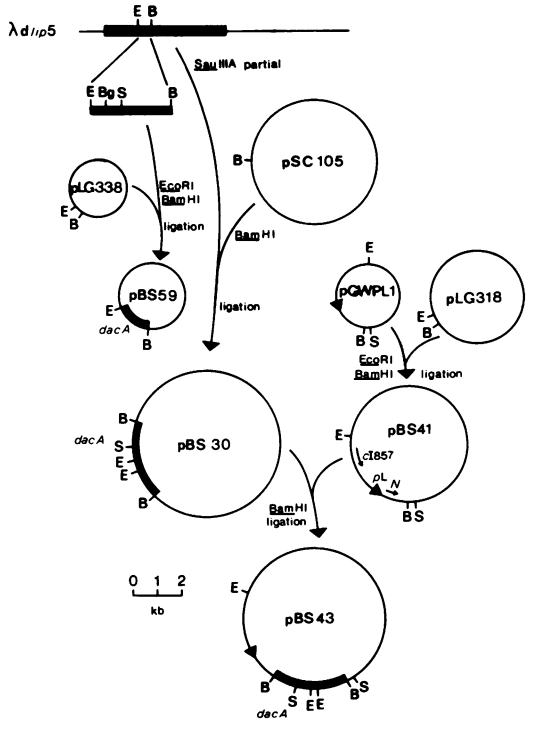

FIG. 3. Subcloning of the *dacA* gene. *dacA* was subcloned from the defective transducing phage λ d*lip5* (17) as described in the text. Solid blocks indicate chromosomal DNA. Abbreviations are described in the legend to Fig. 1. The plasmids (but not λ d*lip5*) are drawn to scale.

due to a 1.2-kb deletion within the *Sal*I fragment (Fig. 1). A *Sal*I digest of λpBS10Δ13 DNA was ligated with pLG318 cut with *Sal*I, and the mixture was used to transform the temperature-

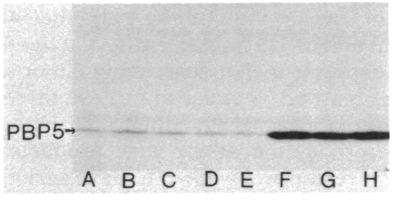

FIG. 4. Direction of transcription of *dacA*. *E. coli* C600 carrying pBS48 (lanes A through D) or pBS43 (lanes E through H) was grown at 30°C in Luria broth containing 0.1% glucose to an absorbancy at 550 nm of 0.2, shifted to 42°C for 15 min, and then grown at 37°C. Samples were taken at the time of the shift to 42°C (A and E) and at 30 (B and F), 60 (C and G), and 90 (D and H) min after the shift. The absorbancy of the samples was adjusted to 0.2 with Luria broth, and 250-μl samples were assayed for PBPs with high-specific-activity [^{3}H]benzylpenicillin (40 μg/ml; 30 Ci/mmol) as previously described (2). The fluorograph was exposed to show PBP5, and the other PBPs are not visible.

sensitive *pbpA* mutant strain SP45 to kanamycin resistance at 42°C. A mixture of large and small colonies were obtained. The small colonies consisted of spherical cells, whereas the large colonies contained rod-shaped cells, indicating the presence of the cloned *pbpA* gene. pBS46 and pBS47 contained the deleted 5.8-kb *Sal*I fragment in opposite orientations (Fig. 6).

The deleted 5.8-kb *Sal*I fragment was inserted into the *Xho*I site of pLG318 to produce the *pbpA*$^+$ plasmid pBS57 (Fig. 6). This plasmid was digested with *Kpn*I, religated at low DNA concentration, and used to transform strain SP45 to tetracycline resistance at 42°C. The resulting transformants formed small colonies of spherical cells, indicating that removal of the DNA between the *Kpn*I sites of pBS57 resulted in the inactivation of *pbpA*. Similarly, confirmation that the *pbpA* gene extended across a *Bgl*II site was obtained by showing that removal of the DNA between the two *Bgl*II sites in pBS47 resulted in a plasmid, pBS49, which could no longer complement the *pbpA* mutation (Fig. 6). The gene therefore extended across the 1.4-kb *Bgl*II-*Kpn*I fragment in pBS47 (Fig. 6), and it was further subcloned by isolating the *Sma*I-*Sal*I fragment from pBS47 and inserting it be-

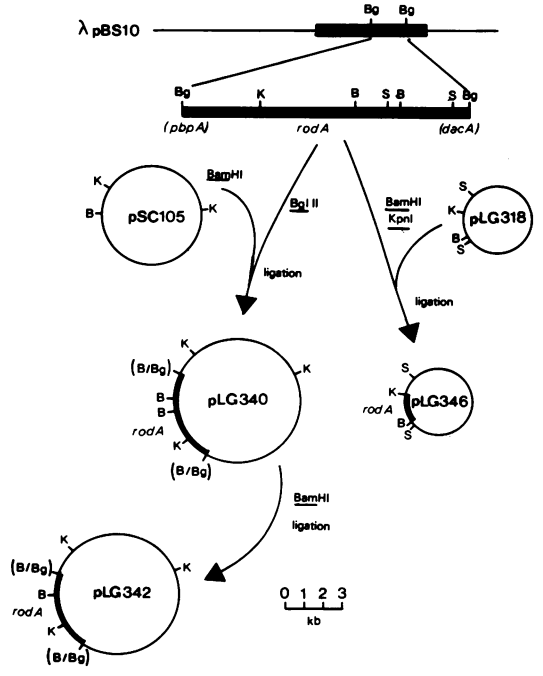

FIG. 5. Subcloning of the *rodA* gene. *rodA* was subcloned from λpBS10 as described in the text. Solid blocks indicate chromosomal DNA. Abbreviations are described in the legend to Fig. 1. B/Bg is a hybrid *Bam*HI-*Bgl*II site. The plasmids (but not λpBS10) are drawn to scale.

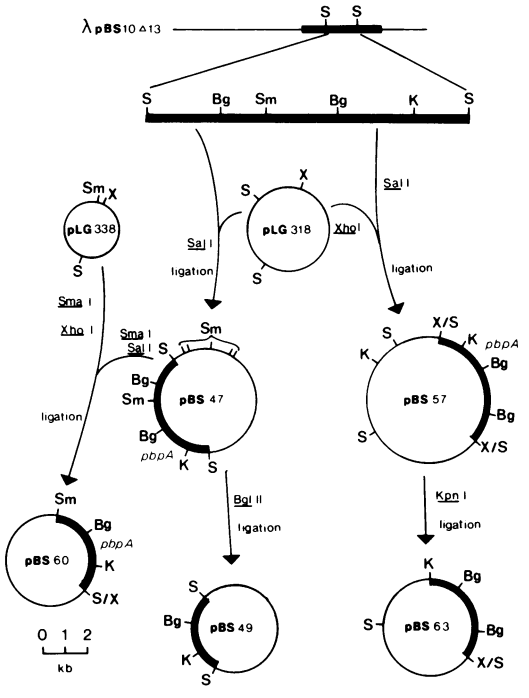

FIG. 6. Subcloning of the *pbpA* gene. *pbpA* was subcloned from λpBS10Δ13, which has a 1.2-kb deletion that removes *rodA* (Fig. 1). Solid blocks indicate chromosomal DNA. Abbreviations are given in the legend to Fig. 1. Other abbreviations: Sm, *Sma*I; X, *Xho*I; S/X and X/S, hybrid *Sal*I-*Xho*I sites. The plasmids (but not λpBS10Δ13) are drawn to scale.

tween the *Sma*I and *Xho*I sites of pLG338 to produce the *pbpA*$^+$ plasmid pBS60 (Fig. 6).

Strain C600 carrying pBS47, pBS57, or pBS60 had approximately six times the normal level of PBP2 and grew as normal rod-shaped cells. The deleted 5.8-kb *Sal*I fragment could be inserted into pBR322, but cells carrying the resultant plasmid grew poorly, with grossly distorted morphology, suggesting that the presence of many copies of the *pbpA* gene and high levels of PBP2 were deleterious to the cell. Attempts to determine the direction of transcription of *pbpA* by positioning the gene in front of the p_L promoter were inconclusive.

DISCUSSION

The analysis of deletion derivatives of λpBS10 allowed the construction of a genetic and physical map of the genes carried by the transducing phage. Four of the genes (*pbpA*, *rodA*, 54K, and *dacA*) form a cluster; three of these are known to be involved in peptidoglycan synthesis and cell shape, but the function of the 54K gene remains unknown. Earlier experiments suggested that the 54K protein might be the *rodA* gene product, but the isolation of λpBS10Δ34, which failed to transduce *rodA* but synthesized the 54K protein, established that the 54K gene and *rodA* are distinct genes. However, no candidate for the *rodA* gene product was found. Recently, *rodA* was shown to encode a cytoplasmic membrane protein (M_r, 31,000) that is not detected by the standard method of SDS-polyacrylamide gel electrophoresis due to protein aggregation after boiling in SDS buffer (19).

The products of the *pbpA*, *rodA*, 54K, and *dacA* genes are therefore all components of the cytoplasmic membrane (15, 17, 19; unpublished data). The size of the region containing these four genes can be accurately determined. The *pbpA* gene extends across the 1.4-kb *Bgl*II-*Kpn*I fragment (Fig. 1 and 6). As the estimated size of the gene is 1.8 kb, it could only extend about 400 base pairs on the *leuS* side of the *Bgl*II site. The other end of the gene cluster is known from the DNA sequence of the *dacA* gene (J. K. Broome-Smith, A. Edelman, and B. G. Spratt, manuscript in preparation), which ends 138 base pairs before the *Eco*RI site (Fig. 1). The four genes *pbpA*, *rodA*, 54K, and *dacA* therefore map within a 5.6-kb region, and the molecular weights of their gene products (66,000, 31,000, 54,000, and 42,000, respectively) indicate a coding region of at least 5.3-kb. As no other gene products were detected from this region, it seems likely that these four genes are contiguous.

The direction of transcription of *dacA* was shown to be from left to right (Fig. 1) by analysis of the level of PBP5 produced by plasmids in which *dacA* was inserted in each orientation downstream from the p_L promoter. The direction of transcription was confirmed from the DNA sequence of *dacA* (Broome-Smith et al., in preparation). The *rodA* gene is also transcribed in the left to right direction (19), but the directions of transcription of *pbpA* and the 54K gene are unknown. The *pbpA*, *rodA*, and *dacA* genes were expressed when inserted in either orientation in plasmid vectors, and these three genes therefore appear to have their own promoters.

Overproduction of the gene products of the *leuS-lip* region was shown to have serious morphological effects on *E. coli* cells and prevented cloning of the region into high-copy-number plasmids (7, 17). We have now subcloned the *pbpA*, *rodA*, and *dacA* genes into low-copy-number plasmid vectors and showed that neither the *pbpA* or *dacA* gene can be stably cloned in high-copy-number plasmids. The *rodA* gene can, however, be cloned at high copy number without significant disturbance of cell growth. Overproduction of PBP5 (the *dacA* gene product) is extremely toxic to *E. coli* cells. Low levels of overproduction (e.g., 10-fold) cause the organism to grow as spherical cells, whereas higher

levels are lethal, presumably because of excessive removal of terminal D-alanine residues from peptidoglycan precursors, which results in depletion of the substrate for peptidoglycan transpeptidation (7). The reason for the deleterious effect of PBP2 (the *pbpA* gene product) overproduction is not known.

The precise location of these genes and the construction of plasmids that carry each gene separate from the others will facilitate studies of the physiology and biochemistry of the gene products and their role in cell wall synthesis and morphogenesis.

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