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Identification of the *rodA* Gene Product of *Escherichia coli*

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Plasmids that carry the *Escherichia coli* cell shape gene *rodA* directed the synthesis of a cytoplasmic membrane protein (M_r, 31,000 [31K protein]) in minicells, maxicells, and an in vitro-coupled transcription-translation system. The 31K protein was identified as the *rodA* gene product, because it was not synthesized from the vector plasmids or from a plasmid in which the *rodA* gene was inactivated by insertion of Tn*1000*. Furthermore, a purified 1.6-kilobase *Kpn*I-*Bam*HI DNA fragment that contained the intact *rodA* gene directed the synthesis of only the 31K protein in an in vitro system. The apparent molecular weight of the protein was identical whether synthesized in vivo or in vitro, indicating that the *rodA* gene product is not made as a preprotein. The direction of transcription of *rodA* was from the *Kpn*I site towards the *Bam*HI site. The 31K protein was unusual in that it could only be detected when cell membranes were solubilized at low temperature (e.g., 37°C) before sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Apparently the *rodA* gene product aggregates after being boiled in sodium dodecyl sulfate and fails to enter a polyacrylamide gel.

The growth of rod-shaped bacteria requires the insertion of wall materials to produce cylindrical cell surface during cell elongation and hemispherical surface during cross wall formation at cell division. Strains that produce filaments or grow as spherical cells should include mutants defective in gene products required for the synthesis of the cross wall and the cylindrical wall, respectively. Identification of the gene products that are defective in spherical mutants should therefore define the components involved in the initiation and growth of the cylindrical portion of the bacterial cell wall.

Mutations that result in the growth of *Escherichia coli* as spherical cells map in either *pbpA* or *rodA* (also called *mrdA* and *mrdB*, respectively [24]) at 14 min on the *E. coli* chromosome (1, 9, 13, 18, 21) or in *envB* at 70 min (26). *pbpA* is the structural gene for penicillin-binding protein 2 (PBP2), which is the killing target for mecillinam, a β-lactam antibiotic that causes spherical growth of *E. coli* cells (18). Nothing is known about the *envB* gene product. The *rodA* gene maps next to *pbpA* in a small cluster of cell shape and peptidoglycan biosynthesis genes (20, 21, 24). This cluster also includes the gene (54K gene) for a cytoplasmic membrane protein (M_r, 54,000 [54K protein]) of unknown function, and the *dacA* gene that codes for PBP5, a major D-alanine carboxypeptidase (20). Although inactivation of PBP5 has no serious effect on cell morphology, overproduction of PBP5 results in the growth of *E. coli* as spherical cells (12).

All four genes are located within a 5.6-kilobase (kb) region in the order *pbpA-rodA*-54K gene-*dacA* (21). The *rodA* and *pbpA* genes are probably contiguous, but they are clearly distinct as shown by complementation analysis (20, 24), deletion mapping, and subcloning (21). They are also distinguishable by biochemical criteria, since *pbpA* mutants have detects in PBP2 production, whereas *rodA* mutants do not (20, 24).

The *rodA* gene was cloned on both bacteriophage λ and plasmid vectors, but attempts to identify the gene product by phage infection of UV-irradiated bacteria, minicells, or maxicells and an in vitro transcription-translation system were unsuccessful (20, 21; unpublished data). We show here that the *rodA* gene product is a cytoplasmic membrane protein (M_r, 31,000) that had previously escaped detection because it aggregates irreversibly when boiled in sodium dodecyl sulfate (SDS) buffer before gel electrophoresis.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strains used were: SP5211 *rodA52*(Ts) *his purB proA thi lacY rpsL* (20); SP5211 *recA* = SP5211 *srl*::Tn*10 recA56*; DS410 *minA minB*; CSR603 *phr uvrA6 recA1 thr leuB proA argE thi ara lacY galK mtl tsx supE gyrA rpsL* (17); and RB308 F^+ *deoC thyA lacY* (from Roger Buxton). Plasmids used were pACYC184 (2), pLG346, pLG355, and pGWp_L1 (21).

Media and growth conditions were described previously (20).

Preparation and manipulation of DNA. The preparation of plasmid DNA and techniques for the manipulation of DNA and transformation were described previously (20, 22).

Identification of plasmid-encoded gene products. Minicells were prepared from strain DS410 carrying appropriate plasmids and labeled with [^{35}S]methionine (1,500 Ci/mmol; Amersham International) as described by Reeve (16). The maxicell technique was done as described by Sancar et al. (17) with strain CSR603. The synthesis of proteins directed by DNA templates was performed with an in vitro coupled transcription-translation system prepared and used as described previously (14).

Fractionation of cells and analysis of proteins. Cell envelopes were prepared by sonicating the bacteria, followed by differential centrifugation, and separated into cytoplasmic and outer membrane fractions by extraction with sodium *N*-lauroyl sarcosinate as described previously (19). PBPs were assayed by using cell envelopes as previously described (19) with [^{3}H]benzylpenicillin (30 Ci/mmol), which was a gift from Patrick Cassidy, Merck Laboratories, Rahway, N.J. SDS-polyacrylamide gel electrophoresis and fluorography were carried out as described previously (19), except that, where stated, the proteins were solubilized in SDS sample buffer for 1 h at 37°C rather than for 5 min at 100°C.

RESULTS

Estimation of *rodA* gene size. The *rodA* gene was cloned on a 1.6-kb *Kpn*I-*Bam*HI fragment into the low-copy-number plasmid vector pLG318 to produce pLG346 (21), but no candidate for the gene product was found. One possible reason for this was that the *rodA* gene product was very small and difficult to detect with minicells or maxicells. The size of the *rodA* gene was therefore estimated by mapping TN*1000* insertions (5) that inactivate *rodA*.

First, a high-copy-number plasmid carrying *rodA* was constructed. The 2.6-kb *Bam*HI-*Sal*I fragment containing the *rodA* gene was cloned from pLG346 (21) into the high-copy-number plasmid vector pACYC184 to produce pLG355 (Fig. 1). Cells carrying pLG355 grew with normal morphology. *E. coli* RB308(pLG355) was then mated with strain SP5211, and transconjugants were selected on nutrient agar containing chloramphenicol (25 μg/ml) and streptomycin (200 μg/ml) at 42°C. Most of the colonies that appeared were large and consisted of rod-shaped cells owing to complementation of the cell shape defect of strain SP5211 by the transferred pLG355:Tn*1000* plasmids, but 5.5% were small colonies consisting of spherical cells produced by TN*1000* insertions that inactivated the *rodA* gene of pLG355. Thirty-one isolates that contained plasmids with insertions of Tn*1000* into the *rodA* gene were purified, and the plasmid DNA was prepared. Tn*1000* has single cleavage sites for *Bam*HI and *Kpn*I close to the δ end (5). Since the *rodA* gene lies within a 1.6-kb *Bam*HI-*Kpn*I fragment, insertions of Tn*1000* into *rodA* in either orientation could be mapped accurately.

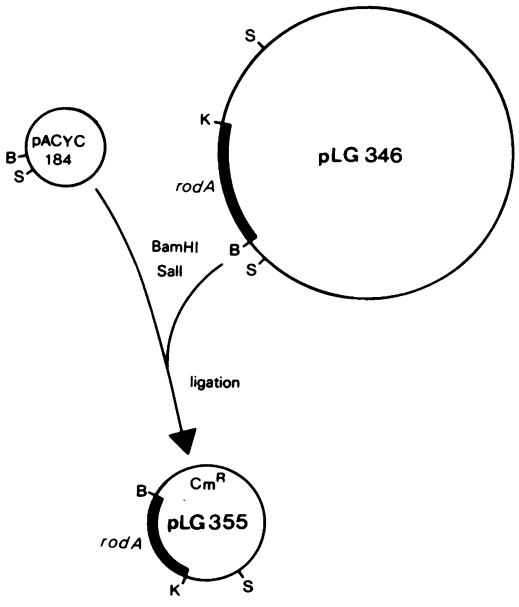

FIG. 1. Construction of pLG355. Abbreviations: S, *Sal*I; K, *Kpn*I; B, *Bam*HI; Cm^r, chloramphenicol resistance.

The location of the 31 Tn*1000* insertions that inactivated *rodA* are shown in Fig. 2. Although some error may be introduced by combining the two insertion maps, insertions in the same orientation could be compared accurately. Insertions in orientations 1 and 2 were spread over 1.0 and 0.9 kb, respectively. This provides an estimate of the size of the *rodA* gene and indicates that it could code for a protein with a molecular weight of up to about 37,000.

Identification of the *rodA* gene product. As the *rodA* gene product was unlikely to be unusually small, we considered the possibility that it behaved abnormally in the SDS-polyacrylamide

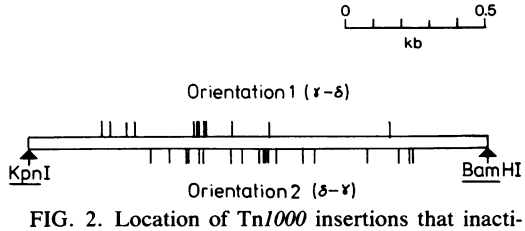

FIG. 2. Location of Tn*1000* insertions that inactivate *rodA*. Each vertical line shows the site of a Tn*1000* insertion that inactivated *rodA* in the 1.6-kb *Kpn*I-*Bam*HI fragment of pLG355. Orientations 1 and 2 are the two possible orientations of Tn*1000* insertion.

gel system. Teather et al. (25) reported that the lactose permease of *E. coli* could not be detected on SDS-polyacrylamide gels when proteins were solubilized in SDS sample buffer at 100°C because this treatment irreversibly aggregated the protein so that it failed to enter the gel. However, solubilizing the proteins at 37°C prevented aggregation, and the permease was identified. To see whether the *rodA* gene product also aggregated after being boiled, we prepared minicells from strain DS410 containing pACYC184, pLG355 (which carries *rodA*), or pLG355 *rodA*::Tn*1000*. The proteins synthesized in the minicells were labeled with [^{35}S]methionine, and SDS sample buffer was added. Half of each sample was boiled for 5 min (the standard procedure), and the other half was incubated at 37°C for 1 h, and the proteins synthesized were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. A minor protein (M_r, 31,000 [31K protein]) was synthesized by minicells containing pLG355 and detected in samples solubilized at 37°C, but not in samples that had been solubilized at 100°C (Fig. 3). The 31K protein was not synthesized in minicells carrying pACYC184 or pLG355 *rodA*::TN*1000*, strongly suggesting that it was the *rodA* gene product.

It is interesting that the tetracycline resistance protein (M_r, 34,000 [23]) encoded by pACYC184 was visible in greater amounts in samples solubilized at 37°C than in those solubilized at 100°C.

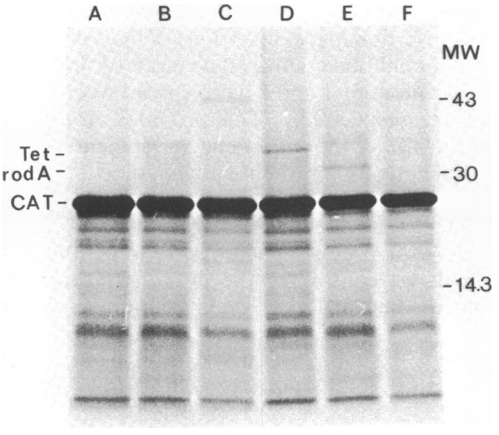

FIG. 3. Identification of the *rodA* gene product in minicells. Minicells were prepared from *E. coli* DS410 carrying pACYC184 (A and D), pLG355 (B and E), or pLG344 *rodA*::Tn*1000* (C and F). The plasmid-specified proteins were labeled with [^{35}S]methionine, separated on a 13% SDS-polyacrylamide gel, and detected by autoradiography. Proteins were solubilized in SDS sample buffer at 100°C for 5 min (A, B, C) or at 37°C for 1 h (D, E, F). Tet, Tetracycline resistance protein; CAT, chloramphenicol acetyltransferase. Molecular weights (MW) ($\times 10^3$) are shown to the right.

This protein may also aggregate to some extent on boiling.

Further evidence that the 31K protein was the *rodA* gene product was obtained by purifying the 1.6-kb *Kpn*I-*Bam*HI fragment that contained *rodA* and using this as a template for an in vitro transcription-translation system. A 31K protein was synthesized and detected in samples solubilized at 37°C, but not in those solubilized at 100°C (Fig. 4).

Cellular location of the *rodA* gene product. PBP2, PBP5, and the 54K protein, which are synthesized from the gene cluster that includes *rodA*, are all located in the cytoplasmic membrane (19, 20). It was therefore of interest to determine the location of the *rodA* gene product. As fractionation of minicells is troublesome, the plasmid-specified proteins were labeled by the maxicell system (17). The 31K protein was found exclusively in the cell envelope fraction and, after extraction of the cell envelopes with 1% sodium lauroyl sarcosinate, was recovered in the solubilized material, indicating that the *rodA* gene product was probably a cytoplasmic membrane protein (Fig. 5). As expected, the tetracycline resistance protein, a component of the cytoplasmic membrane, was also found exclusively in the solubilized material (Fig. 5).

***rodA* gene product not made as a preprotein.** Both PBP5 and the 54K protein synthesized from the gene cluster are made as preproteins and processed to their mature forms during insertion into the cytoplasmic membrane (6, 15; M. Jackson, personal communication). Since the 31K protein was also a cytoplasmic membrane protein, we examined whether it was made as a preprotein. The size of the *rodA* gene product synthesized in vivo was therefore compared with that of the primary translation product synthesized in vitro.

Proteins encoded by pLG355 were labeled with [^{35}S]methionine in minicells and in an in vitro transcription-translation system. Cyclic AMP was omitted from the in vitro system (14) to decrease transcription from the powerful promoter of the chloramphenicol acetyltransferase gene present on the plasmids (3). The proteins were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography (Fig. 6). No significant difference could be seen in the apparent molecular weight of the 31K protein synthesized in vivo or in vitro, and the *rodA* gene product appeared to enter the cytoplasmic membrane without processing.

***rodA* gene product not a PBP.** The *rodA* gene maps between the genes encoding PBP2 and PBP5 (20). Since the standard assay for PBPs involves boiling samples in SDS sample buffer before electrophoresis (19), it was possible that the *rodA* gene product was also a PBP that had

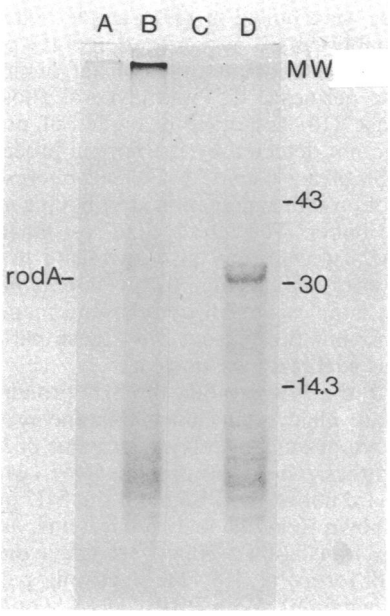

FIG. 4. Synthesis of the *rodA* gene product in vitro from a 1.6-kb *Kpn*I-*Bam*HI fragment. pLG355 was cleaved with *Kpn*I and *Bam*HI, and the 1.6-kb fragment that carries *rodA* was purified as described previously (19). A 2-μg sample of the DNA fragment was added to an in vitro transcription-translation system (14), and the proteins synthesized were labeled with [^{35}S]methionine, separated on a 15% SDS-polyacrylamide gel, and detected by autoradiography. Samples were solubilized in SDS sample buffer at 100 (lanes A and B) or 37°C (lanes C and D). Lanes A and C, Controls (no DNA added); lanes B and D, *rodA* DNA fragment. Molecular weights (MW) ($\times 10^3$) are shown to the right.

not been detected previously because of aggregation after boiling. We therefore assayed PBPs in cell envelopes prepared from several *E. coli* K-12 strains with [^{3}H]benzylpenicillin (30 μg/ml) as described previously (19), except that half of each sample was not boiled in SDS sample buffer before electrophoresis on a 12% SDS-polyacrylamide gel. No new PBP appeared in the unboiled samples, and we concluded that the *rodA* gene product is not a PBP (data not shown).

Direction of transcription of the *rodA* gene. The direction of transcription of *rodA* was determined by inserting the gene in both orientations in front of the powerful phage λ leftward promoter (p_L) in the temperature-sensitive expression vector pGWp_L1. This plasmid (kindly provided by Geoff Wilson) is a derivative of pBR322 that carries the *N* p_L *c*I857 region from phage λ (21). At 30°C the *c*I protein shuts off transcription, but at 42°C the repressor is inactivated and transcription occurs from p_L.

The *rodA* gene was cloned on a 2.9-kb *Sal*I fragment from pLG346 into the unique *Sal*I site in pGWp_L1. The ligation mixture was used to transform the temperature-sensitive *rodA* mutant SP5211 *rec*A(λ$^+$) to ampicillin resistance and rod shape at 42°C. Plasmid DNA was isolated from rod-shaped transformants, and restriction mapping showed that the transformants all contained the 2.9-kb *Sal*I fragment from pLG346 cloned in the same orientation. This plasmid was named pLG351. Recombinant plasmids containing the 2.9-kb *Sal*I fragment in the opposite orientation would possess a 275-base-pair inverted repeat and were presumably not recoverable (11). The *rodA* gene was therefore cloned in the opposite orientation by inserting the 2.6-kb *Bam*HI-*Sal*I fragment from pLG346 between the unique *Bam*HI and *Sal*I sites of pGWp_L1. The resulting plasmid was named pLG352.

The level of the *rodA* gene product synthesized in minicells carrying pLG351 and pLG352 was measured at 30 and 42°C. At 30°C both plasmids directed the synthesis of the 31K protein. At 42°C expression from p_L resulted in increased levels of the *rodA* gene product in minicells containing pLG351, whereas the level in minicells containing pLG352 became undetectable. The increased expression from pLG351

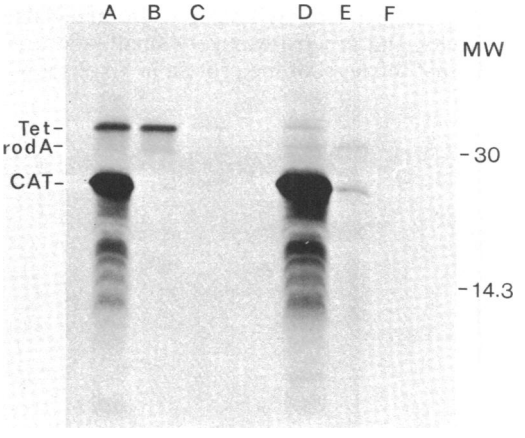

FIG. 5. Location of the *rodA* gene product in the cytoplasmic membrane. Proteins encoded by pACYC194 (lanes A through C) or pLG355 (lanes D through F) were labeled with [^{35}S]methionine by the maxicell system (17). A portion of labeled maxicells was solubilized (lanes A and D), and the remainder was used to prepare cytoplasmic (lanes B and E) and outer membrane (lanes C and F) fractions as previously described (19). The labeled proteins (solubilized at 37°C for 1 h) were analyzed on a 13% SDS-polyacrylamide gel, followed by fluorography. A protein with a mobility slightly faster than the *rodA* gene product is visible in lanes A and B. The protein was not always detected, and it may be a degradation product of the tetracycline resistance protein.

at 42°C indicated that the *rodA* gene was inserted in the correct orientation for transcription from the strong p_L promoter in this plasmid, and the *rodA* gene is therefore transcribed from the *Kpn*I site towards the *Bam*HI site. In the opposite orientation (pLG352), the level of 31K protein was decreased, presumably owing to antagonistic transcription of *rodA* from the more powerful p_L promoter.

DISCUSSION

We previously failed to detect the product of the *rodA* gene (20). In this paper we identified a protein (31K protein) encoded by plasmids that carry the *rodA* gene by solubilizing proteins at 37 rather than 100°C before electrophoresis. This protein is believed to be the *rodA* gene product, because all of the plasmids that complemented *rodA* in vivo produced the 31K protein, but it was not produced by the plasmid vectors or by a plasmid in which the integrity of the *rodA* gene had been destroyed by insertion of Tn*1000*. The size of the protein was consistent with the size of the gene estimated by mapping Tn*1000* insertions. Furthermore, the 31K protein was encoded by a 1.6-kb *Kpn*I-*Bam*HI fragment that was shown genetically to carry *rodA* (21), and this purified DNA fragment directed the synthesis of the 31K protein in an in vitro transcription-translation system. The amount of *rodA* gene product synthesized in minicells, maxicells, and in vitro was very small, and it is likely that the level of the protein in vivo is low.

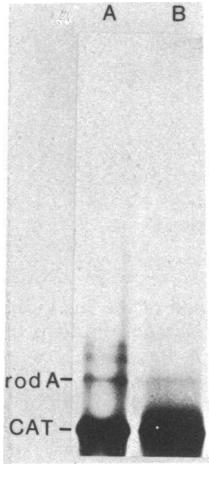

FIG. 6. Synthesis of the *rodA* gene product in vitro and in vivo. The proteins encoded by pLG355 were labeled with [^{35}S]methionine in minicells (A) or an in vitro transcription-translation system (B) and fractionated on a 13% SDS-polyacrylamide gel, followed by fluorography. CAT, Chloramphenicol acetyltransferase.

The failure to observe the 31K protein when samples were boiled in SDS sample buffer was presumably caused by aggregation of the protein so that it could not enter the polyacrylamide gel. Lactose permease (25) and glycerol phosphate permease (10) are other examples of proteins that are not detected by the normal procedures of SDS-polyacrylamide gel electrophoresis because of protein aggregation after boiling in SDS sample buffer. The tetracycline resistance protein (M_r, 34,000) may also aggregate to some extent after boiling (Fig. 3). It is interesting that these examples are all cytoplasmic membrane proteins, but the reasons why these particular proteins aggregate are unclear.

PBP2, the 54K protein, the 31K protein, and PBP5 are all cytoplasmic membrane proteins. Some cytoplasmic membrane proteins of *E. coli* are synthesized as preproteins (15), whereas others are not (4, 7). PBP5 and the 54K protein were shown to be made as preproteins, as their primary translation products are larger than the in vivo forms (6, 15; M. Jackson, personal communication). With PBP5, DNA sequencing confirmed the presence of an amino-terminal signal peptide of 29 residues that is removed during insertion of the protein into the cytoplasmic membrane (J. K. Broome-Smith, A. E. Edelman, and B. G. Spratt, unpublished data). The *rodA* gene product does not appear to be synthesized as a preprotein, because the size of the protein made in vitro and in vivo was indistinguishable. It is not yet known whether PBP2 is synthesized as a preprotein.

The *rodA* gene product is required for the growth of *E. coli* as rod-shaped cells, and it presumably plays an essential role in the synthesis of the cylindrical wall during cell elongation. The *rodA* gene maps within a 5.6-kb region that includes the genes for PBP2, the 54K protein, and PBP5 (21). The involvement of the *rodA* gene product in cell shape, its location in the cytoplasmic membrane, and the position of the gene between two genes (*pbpA* and *dacA*) that are known to code for enzymes of peptidoglycan synthesis make it very likely that the 31K protein is an enzyme active in the final stages of peptidoglycan synthesis. At present, however, nothing is known of its enzymatic activity.

Recently, a peptidoglycan transglycosylase and a mecillinam-sensitive transpeptidase activity were detected in cell envelopes prepared from *E. coli* cells that produced elevated levels of the products of the *leuS-pbpA-rodA-dacA-lip* region of the chromosome (8). The latter activity was attributed to PBP2, the only protein known to interact with mecillinam (18), but the former activity could be the result of one of the other products (e.g., the *rodA* gene product) of the *leuS-lip* region. The identification of the *rodA*

gene product and the construction of overproducing strains should allow it to be purified and its enzymatic activity in peptidoglycan synthesis investigated.

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LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
2. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
3. **de Combrugghe, B., I. Pastan, W. V. Shaw, and J. L. Rosner.** 1973. Stimulation by cyclic AMP and ppGpp of chloramphenicol acetyl transferase synthesis. Nat. New Biol. **241**:237–239.
4. **Ehring, R., K. Beyreuther, J. K. Wright, and P. Overath.** 1980. In vitro and in vivo products of *E. coli* lactose permease gene are identical. Nature (London) **283**:537–540.
5. **Guyer, M. S.** 1978. The γδ sequence of F is an insertion sequence. J. Mol. Biol. **126**:346–365.
6. **Herrero, E., M. Jackson, P. J. Bassford, D. Sinden, and I. B. Holland.** 1982. Insertion of a *malE*-β-galactosidase fusion protein into the envelope of *Escherichia coli* disrupts biogenesis of outer membrane proteins and processing of inner membrane proteins. J. Bacteriol. **152**:133–139.
7. **Higgins, C. F., P. D. Haag, K. Nikaido, F. Ardeshir, G. Garcia, and G. Ferro-Luzzi Ames.** 1982. Complete nucleotide sequence and identification of membrane components of the histidine transport operon of *S. typhimurium*. Nature (London) **298**:723–727.
8. **Ishino, F., S. Tamaki, B. G. Spratt, and M. Matsuhashi.** 1982. A mecillinam-sensitive peptidoglycan crosslinking reaction in *Escherichia coli*. Biochem. Biophys. Res. Commun. **109**:689–696.
9. **Iwaya, M., C. W. Jones, J. Khorana, and J. L. Strominger.** 1978. Mapping of the mecillinam-resistant round mutants of *Escherichia coli*. J. Bacteriol. **133**:196–202.
10. **Larson, T. J., G. Schumacher, and W. Boos.** 1982. Identification of the *glpT*-encoded *sn*-glycerol-3-phosphate permease of *Escherichia coli*, an oligomeric integral membrane protein. J. Bacteriol. **152**:1008–1021.
11. **Lilley, D. M. J.** 1981. In vivo consequences of plasmid topology. Nature (London) **292**:380–382.
12. **Markiewicz, Z., J. K. Broome-Smith, U. Schwarz, and B. G. Spratt.** 1982. Spherical *E. coli* due to elevated levels of D-alanine carboxypeptidase. Nature (London) **297**:702–704.
13. **Matsuzawa, H., K. Hayakawa, T. Sato, and K. Imahori.** 1973. Characterization and genetic analysis of a mutant of *Escherichia coli* K-12 with rounded morphology. J. Bacteriol. **115**:436–442.
14. **Pratt, J. M., G. J. Boulnois, V. Darby, E. Orr, E. Wahle, and I. B. Holland.** 1981. Identification of gene products programmed by restriction endonuclease DNA fragments using an *E. coli* in vitro system. Nucleic Acids Res. **9**:4459–4474.
15. **Pratt, J. M., I. B. Holland, and B. G. Spratt.** 1981. Precursor forms of penicillin-binding proteins 5 and 6 of *E. coli* cytoplasmic membrane. Nature (London) **293**:307–309.
16. **Reeve, J. N.** 1979. Use of minicells for bacteriophage directed polypeptide synthesis. Methods Enzymol. **68**:493–503.
17. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for identification of plasmid-coded proteins. J. Bacteriol. **137**:692–693.
18. **Spratt, B. G.** 1975. Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S.A. **72**:2999–3003.
19. **Spratt, B. G.** 1977. Properties of the penicillin-binding proteins of *Escherichia coli* K12. Eur. J. Biochem. **72**:341–352.
20. **Spratt, B. G., A. Boyd, and N. Stoker.** 1980. Defective and plaque-forming lambda transducing bacteriophage carrying penicillin-binding protein-cell shape genes: genetic and physical mapping and identification of gene products from the *lip-dacA-rodA-pbpA-leuS* region of the *Escherichia coli* chromosome. J. Bacteriol. **143**:569–581.
21. **Stoker, N. G., J. K. Broome-Smith, A. Edelman, and B. G. Spratt.** 1983. Organization and subcloning of the *dacA-rodA-pbpA* cluster of cell shape genes in *Escherichia coli*. J. Bacteriol. **155**:847–853.
22. **Stoker, N. G., N. F. Fairweather, and B. G. Spratt.** 1982. Versatile low copy number plasmid vectors for cloning in *Escherichia coli*. Gene **18**:335–341.
23. **Tait, R. C., and H. W. Boyer.** 1978. On the nature of tetracycline resistance controlled by the plasmid pSC101. Cell **13**:73–81.
24. **Tamaki, S., H. Matsuzawa, and M. Matsuhashi.** 1980. Cluster of *mrdA* and *mrdB* genes responsible for the rod shape and mecillinam sensitivity of *Escherichia coli*. J. Bacteriol. **141**:52–57.
25. **Teather, R. M., B. Muller-Hill, U. Abrutsch, G. Aichele, and P. Overath.** 1978. Amplification of the lactose carrier protein in *Escherichia coli* using a plasmid vector. Mol. Gen. Genet. **159**:239–248.
26. **Westling-Haggstrom, B., and S. Normark.** 1975. Genetic and physiological analysis of an *envB* spherelike mutant of *Escherichia coli* K-12 with rounded morphology. J. Bacteriol. **123**:75–82.