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Mutant Isolation and Molecular Cloning of *mre* Genes, Which Determine Cell Shape, Sensitivity to Mecillinam, and Amount of Penicillin-Binding Proteins in *Escherichia coli*

MASAAKI WACHI, MASAKI DOI,† SHIGEO TAMAKI,‡ WAN PARK, SADAYO NAKAJIMA-IIJIMA, AND MICHIO MATSUHASHI*

Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo, Japan

Received 11 May 1987/Accepted 24 August 1987

A chromosomal region of *Escherichia coli* contiguous to the *fabE* gene at 71 min on the chromosomal map contains multiple genes that are responsible for determination of the rod shape and sensitivity to the amidinopenicillin mecillinam. The so-called *mre* region was cloned and analyzed by complementation of two closely related but distinct *E. coli* mutants characterized, respectively, by the mutations *mre-129* and *mre-678*, that showed a rounded to irregular cell shape and altered sensitivities to mecillinam; the *mre-129* mutant was supersensitive to mecillinam at 30°C, but the *mre-678* mutant was resistant. The *mre-678* mutation also caused simultaneous overproduction of penicillin-binding proteins 1Bs and 3. A chromosomal region of the wild-type DNA containing the total *mre* region and the *fabE* gene was first cloned on a lambda phage; a 7-kilobase (kb) fragment containing the whole *mre* region, but not the *fabE* gene, was then recloned on a mini F plasmid, pLG339; and finally, a 2.8-kb fragment complementing only *mre-129* was also cloned on this low-copy-number plasmid. The whole 7-kb fragment was required for complementing the *mre-678* mutant phenotypes. Fragments containing *fabE* but not the *mre-129* region could be cloned on a high-copy-number plasmid. Southern blot hybridization indicated that the *mre-678* mutant had a large deletion of 5.25 kb in its DNA, covering at least part of the *mre-129* gene.

One of the most fundamental questions in cell biology is how the characteristic shape of a cell is determined. A clue to the mechanism of determining the rod shape of bacteria has been sought by isolating mutants that show different cell shapes and then analyzing them genetically and biochemically. Many mutants of *Escherichia coli* K-12 that could not form rod-shaped cells have been isolated. Among them, mutants with an osmotically stable, spherical shape were thought to have a defect in one of the steps involved in determination or formation of the rod shape of the cell. These *rod* mutations could be classified into three main groups on the basis of their positions in the gene map. In the first group, the mutations were located at 14.5 min (referred to as the *mrd* region) on the *E. coli* chromosome map. This group included the *rodA* (9), *rodX* (4), *pbpA* (16), and *mrdAB* (19) mutations. In the second group, the mutations were located at 71 min (referred to as the *mre* region), and this group included the *rodY* (4), *envB* (11), and *mreBC* (8) mutations. The third group consisted of mutations affecting formation of cyclic AMP (5) and the mechanism in which cyclic AMP is involved (5, 21), and their gene map positions were diverse. Most of these mutations showed altered sensitivities to an amidinopenicillin, mecillinam (MPC) (8, 16, 19, 21, 23). These results indicated that formation of the rod shape of the cell in *E. coli* involves a step that is sensitive to amidinopenicillin, and the mutation that causes a defect in this step results in a spherical shape of cell with altered sensitivity to MPC. Thus, this antibiotic was used for selecting Rod$^-$ mutants and cloning genes involved in formation of the rod shape.

Here we report the isolation and genetic analysis of *mre* mutations and cloning of the *mre* region of DNA on a low-copy-number plasmid. During the study, the neighboring *fabE* gene coding for acetyl-CoA carboxylase (14) was also cloned, as described briefly in this paper.

MATERIALS AND METHODS

Bacterial strains and growth media. The properties of the *E. coli* K-12 strains used are summarized in Table 1. Modified Lennox broth (6), called L′-broth (19), supplemented with 50 μg of lipoic acid per liter, was usually used for growing cells. Tryptone medium (1% [wt/vol] tryptone [Difco], 0.25% [wt/vol] NaCl, adjusted to pH 7.0 with NaOH), supplemented with 0.2% maltose and 10 mM $MgSO_4$, was used for preparation and titration of λ phage lysates. The antibiotics used for selection of the strains were chloramphenicol (25 mg/liter), tetracycline (25 mg/liter), and kanamycin (25 mg/liter). The broth was solidified with agar at 1.5% (wt/vol) for plates and 0.5% (wt/vol) for the top agar. Mutants AT1325-129 (*mre-129* mutant) and AT1325-678 (*mre-678* mutant) were obtained from *E. coli* K-12 AT1325 as described below.

Isolation of the λ transducing phage carrying the *fabE-mre* region of the chromosome. The procedure for isolation of the specialized λ transducing phage was essentially as described by Schrenk and Weisberg (12). A mixed low-frequency transducing phage lysate was prepared from *E. coli* KS302 [Δ(*gal-att*λ-*bio*), lysogenized with λ *c*I857 *S*am7] and was used to transduce *E. coli* LA2-22, which had been lysogenized with wild-type λ phage, to *fabE*$^+$. The *fabE*$^+$ transductants were selected for growth at 42°C. A high-frequency transducing phage lysate was prepared by inducing a *fabE*$^+$ transductant with mitomycin C (1 mg/liter). Then

* Corresponding author.
† Present address: Yamanouchi Pharmaceutical Co., Ltd., Itabashi-ku, Tokyo, 174 Japan.
‡ Present address: Research Center, Taisho Pharmaceutical Co., Ltd., Ohmiya-shi, Saitama-ken, 330 Japan.

TABLE 1. Strains used

Strain	Genotype	Source
AT1325	F⁻ *lip-9 thi-1 his-4 purB15 proA2 mtl-1*	B. J. Bachmann
AT1325-129	As AT1325 but *mre-129*	This work
AT1325-678	As AT1325 but *mre-678*	This work
AT2472	Hfr *aroE24 thi-1 relA1*	A. L. Taylor
AT2472-129	As AT2472 by *aroE*⁺ *mre-129*	*aroE*⁺ *mre-129* transduction of AT2742 (donor: AT1325-129)
AT2472-678	As AT2472 but *aroE*⁺ *mre-678*	*aroE*⁺ *mre-678* transduction of AT2742 (donor: AT1325-678)
PA340	F⁻ *argH1 thr-1 leuB6 ghd-1 gltB31 thi-1 lacY1 gal-6 xyl-7 ara-14 mtl-2 malA1 rpsL9 tonA2*	M. A. Berkerich
PA340-129	As PA340 but *hisG1 mre-129*	Sexual cross: AT2472-129 × PA340
PA340-678	As PA340 but *hisG1 mre-678*	Sexual cross: AT2472-678 × PA340
KS302	F⁻Δ(*gal-attλ-bio*) *sup*⁰ *thi rpsL*	K. Shimada (13)
LA2-22	F⁻ *galA fabE22 lct thi-1 ara-14 lacY-1 galK12 xyl-5 mtl-1 strA20 tsx-57 tfr-5* λ	D. F. Silbert (2)

we obtained a defective transducing phage strain, λ d*fabE mre-678* c1857 *S*am7, which carried a chromosomal part that complemented all three mutations, *fabE*, *mre-129*, and *mre-678* (see also reference 8).

Preparation of DNA and recombinant DNA methods. Phage lysate was prepared from a double lysogen of PA340-678 (λ d*fabE mre-678* c1857 *S*am7 and the helper phage λ), and the transducing phage particles were purified by polyethyleneglycol 6000 sedimentation and CsCl gradient ultracentrifugation as described by Yamamoto et al. (22). Purified phage particles were digested with proteinase K (Sigma, 50 μg/ml), and DNA was prepared by the usual phenol extraction method.

Plasmid DNA was prepared by the alkaline lysis method (7), and chromosomal DNA was prepared by sucrose gradient ultracentrifugation of a sodium dodecyl sulfate-treated lysozyme lysate of *E. coli* cells (3). The methods of Maniatis et al. (7) were used for the reactions of the restriction endonucleases, T4 DNA ligase, and T4 DNA polymerase. Electrophoresis of DNA was carried out on 0.7% agarose (Seakem) gels. The electrophoresis buffer was 89 mM Tris-borate buffer (pH 8.3) containing 2.5 mM disodium EDTA. Low-copy-number plasmid pLG339 (18) was obtained from B. G. Spratt, University of Sussex, England. Transformation was carried out by the method of Norgard et al. (10). For construction of the plasmids, see the text and Figs. 1 and 2. For construction of plasmid pMEL1K, which contained a deletion of several nucleotides around the restriction endonuclease *Kpn*I site, pMEL1 was digested with *Kpn*I and the protruding 3′ end was digested with T4 DNA polymerase in the absence of deoxynucleotides. Subsequently, four kinds of deoxynucleotides were added to form blunt ends, which were joined with T4 DNA ligase.

Detection of PBPs. Penicillin-binding proteins (PBPs) were detected by the procedure of binding [^{3}H]benzylpenicillin followed by separation of the proteins on a sodium dodecyl sulfate-polyacrylamide gel and detection of the radioactivity by fluorography as described previously (17, 19).

Southern blot hybridization. Chromosomal and plasmid DNA fragments digested with appropriate restriction endonucleases were separated on 0.7% agarose gels and then transferred to a nitrocellulose filter as described by Southern (15). A radioactive probe was prepared by the multiprime labeling method of Feinberg and Vogelstein (1), using [α-^{32}P]dCTP. Hybridization was carried out at 65°C.

Radioactive materials and reagents. [*benzyl*-4-^{3}H]benzylpenicillin (*N*-ethylpiperidinium salt, 65.2 mCi/mg, dissolved in acetone) was a generous gift from P. Cassidy, Merck Sharp & Dohme, Rahway, N.J. [α-^{32}P]dCTP (410 Ci/mmol) was a product of Amersham. MPC was a gift of Leo Pharmaceutical Products, Copenhagen, Denmark. T4 DNA ligase and T4 DNA polymerase were purchased from Takara Biochemicals, Kyoto, Japan. Other reagents were commercial products of reagent grade.

RESULTS

Isolation and genetic analysis of the *mre-129* and *mre-678* morphology mutants and their properties. Mutants with a spherical shape and increased resistance to MPC were derived from *E. coli* K-12 AT1325 by spontaneous mutagenesis and selected on an agar plate of L′-broth at 42°C in the presence of 100 μg of MPC per ml (19). Many mutants obtained had a mutation linked to *leuS* and *lip* at 14.5 min on the *E. coli* chromosome map (*mrdAB* mutations, also called *pbpA* and *rodA*; see reference 19), but some had mutations that did not show this linkage. Two of the latter mutants were selected, strains AT1325-129 (*mre-129* mutation) and AT1325-678 (*mre-678* mutation). These strains differed from each other in sensitivity to MPC: mutant AT1325-129 was resistant to the drug at 25 μg/ml at 42°C but supersensitive at 37 and 30°C, whereas mutant AT1325-678 was highly resistant at any of the above temperatures (Table 2). Mutants AT1325-129 and −678 both showed higher sensitivities to most other β-lactam antibiotics (penicillin, cephalosporin, cefamycin, and nocardicin) than their parent strain. Moreover, the *mre-678* mutant showed increased levels of PBPs 1Bs and 3 simultaneously.

The two *mre* mutations were cotransducible with *aroE* or *rpsL* by transduction with phage P1. A three-point test showed that the most plausible order was *mre-aroE-rpsL*, with a frequency of cotransduction of about 12 to 15% with *aroE*. After the P1 transduction (Aro⁺ selection), transductants with *mre* mutations showed phenotypes similar to those of the original *mre* mutants, except that the *mre-129* transductants were supersensitive to MPC at both 30 to 37 and 42°C (data not shown).

The two *mre* mutations were recessive to the wild type. By introduction of an F′ plasmid, F′-20, which carried the chromosomal part from 68 to 96 min (*aroE*, *argH*, and several other mutant genes), both the rod shape and the wild-type sensitivities to β-lactam antibiotics of the cell could be recovered in both the *mre-129* and *mre-678* mutants (data not shown).

Cloning of the *mre* genes on a lambda phage. A lambda phage, λ d*fabE mre-678* c1857 *S*am7, which carried a large fragment of the *E. coli* chromosome covering the *mre* region and the closely linked *fabE* gene, was isolated (for details, see Materials and Methods). This phage could also complement both the *mre-129* and *mre-678* mutant phenotypes when used to transduce appropriate mutant cells.

Preparation of the restriction map and recloning of DNA fragments that complemented the *mre* and *fabE* mutations. A

TABLE 2. Cell shapes and sensitivities of *mre* mutants to β-lactam antibiotics

Temp (°C)	Strain	Cell shape	MIC[a] (μg/ml) of:				
			MPC	ABPC	CEX	CFX	NCA
30	AT1325	Rod	0.1[b]	6	10	6	100
	AT1325-129 (*mre-129*)	Sphere	0.03[b]	1	10	3	100
	AT1325-678 (*mre-678*)	Sphere	74[b]	0.3	3	3	100
42	AT1325	Rod	0.9	6	15	10	100
	AT1325-129 (*mre-129*)	Sphere	25	1	6	3	30
	AT1325-678 (*mre-678*)	Sphere	74	1	6	3	30

[a] Abbreviations: ABPC, aminobenzylpenicillin; CEX, cephalexin; CFX, cefoxitin; NCA, nocardicin A. Values represent MICs for growth on L'-agar plates.
[b] Same MIC as at 37°C.

31-kilobase (kb) *cosL Eco*RI fragment of λ d*fabE mre-678* c1857 *S*am7 was isolated, and a restriction map was prepared (the 13.6-kb *Sal*I-*Bgl*II region is shown in Fig. 1). Then, the 7-kb *Sal*I fragment was cloned on the low-copy-number plasmid pLG339 at the *Sal*I site. Plasmid pMEL1 was thus obtained. Plasmid pMEL10, which contained a larger 13.6-kb *Sal*I-*Bgl*II fragment, was obtained by ligation of the 7.5-kb *Bgl*II-*Bam*HI fragment of the plasmid pMEL1 (which contained the 1.6-kb *Bgl*II-*Sal*I fragment from the chromosome and the 5.9-kb *Sal*I-*Bam*HI fragment from the vector) with the 12-kb *Bgl*II fragment from the λ phage. Plasmid pMEL12 (see Fig. 2) was constructed by deleting a 4.3-kb *Bam*HI fragment from pMEL10. The complementation of the *mre-129* and *mre-678* mutations by the plasmids containing these DNA fragments is summarized in Fig. 2, and detailed data are shown in Table 3 and Fig. 3 and 4. All the

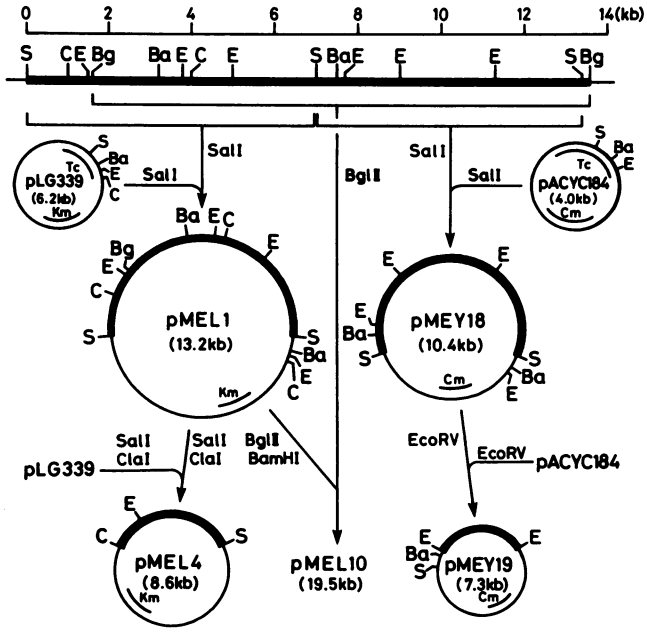

FIG. 1. Construction of plasmids. Abbreviations for restriction endonucleases: Ba, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RV; S, *Sal*I. Restriction sites for *Kpn*I and *Hin*cII are not shown.

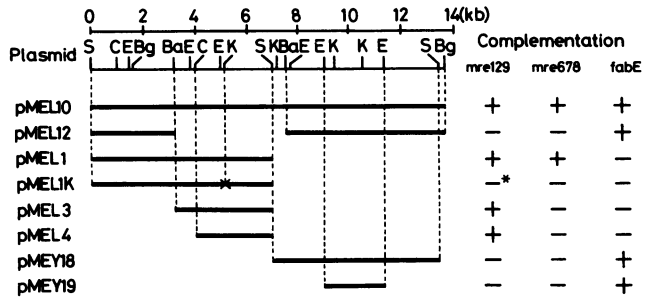

FIG. 2. Complementation of DNA fragments from the *mre-fabE* region. Abbreviations are as for Fig. 1; K, *Kpn*I. The asterisk indicates no recovery of MPC sensitivity but partial recovery of cell shape.

phenotypes of *mre* mutations, i.e., the spherical shape and altered sensitivities to MPC in both *mre-129* and *mre-678*, as well as the increased amounts of PBPs 1Bs and 3 in *mre-678*, could be recovered by the 13.6-kb *Sal*I-*Bam*HI fragment (pMEL10) and the 7-kb *Sal*I fragment (pMEL1). None of the *mre* mutant phenotypes could be complemented by pMEL12.

The 7-kb *Sal*I fragment contained a unique *Kpn*I site. Deletion of several nucleotides around this site (possibly also a frameshift) caused significant decrease of the potency in restoring the *mre* phenotypes (Table 3 and Fig. 2 through 4). The sensitivities to MPC of both *mre-129* and *mre-678* mutants could not be affected by transformation with the plasmid pMEL1K. With regard to cell shape, plasmid pMEL1K partially restored the rod shape of PA340-129 (*mre-129*) but not that of PA340-678 (*mre-678*), whereas pMEL1 almost completely restored the cell shapes of both *mre-129* and *mre-678* mutants (Fig. 3). Similar results were obtained when the original mutants AT1325-129 and AT1325-678 were used for complementation (data not shown).

The overproduction of PBPs 1Bs and 3 by the *mre-678* mutation could be decreased to normal levels by transduction with λ d*fabE mre-678* c1857 *S*am7 or by plasmids containing the 13.6-kb *Sal*I-*Bgl*II fragment (pMEL10) or 7-kb *Sal*I fragment (pMEL1), but not by the plasmid containing the modified 7-kb *Sal*I fragment (pMEL1K) or other smaller plasmids prepared from the 7-kb *Sal*I fragment (pMEL3 and pMEL4) (Fig. 4).

These results strongly suggested that a DNA sequence that involves this *Kpn*I site is responsible for formation of

TABLE 3. Complementation of sensitivities of *mre* mutants to MPC by plasmids carrying *E. coli* chromosome DNA fragments of the *mre* area

Strain	MPC MIC[a] (μg/ml) coded by plasmid:							
	pLG339		pMEL1		pMEL1K		pMEL4	
	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C
AT1325	0.10	0.91						
AT1325-129	0.03	25	0.10	2.7	0.03	25	0.10	0.91
AT1325-678	74	74	0.03	0.10	74	74	74	74
PA340	0.10	0.31						
PA340-129	0.01	0.004	0.03	0.10	0.01	0.004	0.03	0.10
PA340-678	74	25	0.03	0.10	74	25	74	25

[a] MIC for growth at the two temperatures on L'-agar plates in the presence of 25 μg of kanamycin per ml.

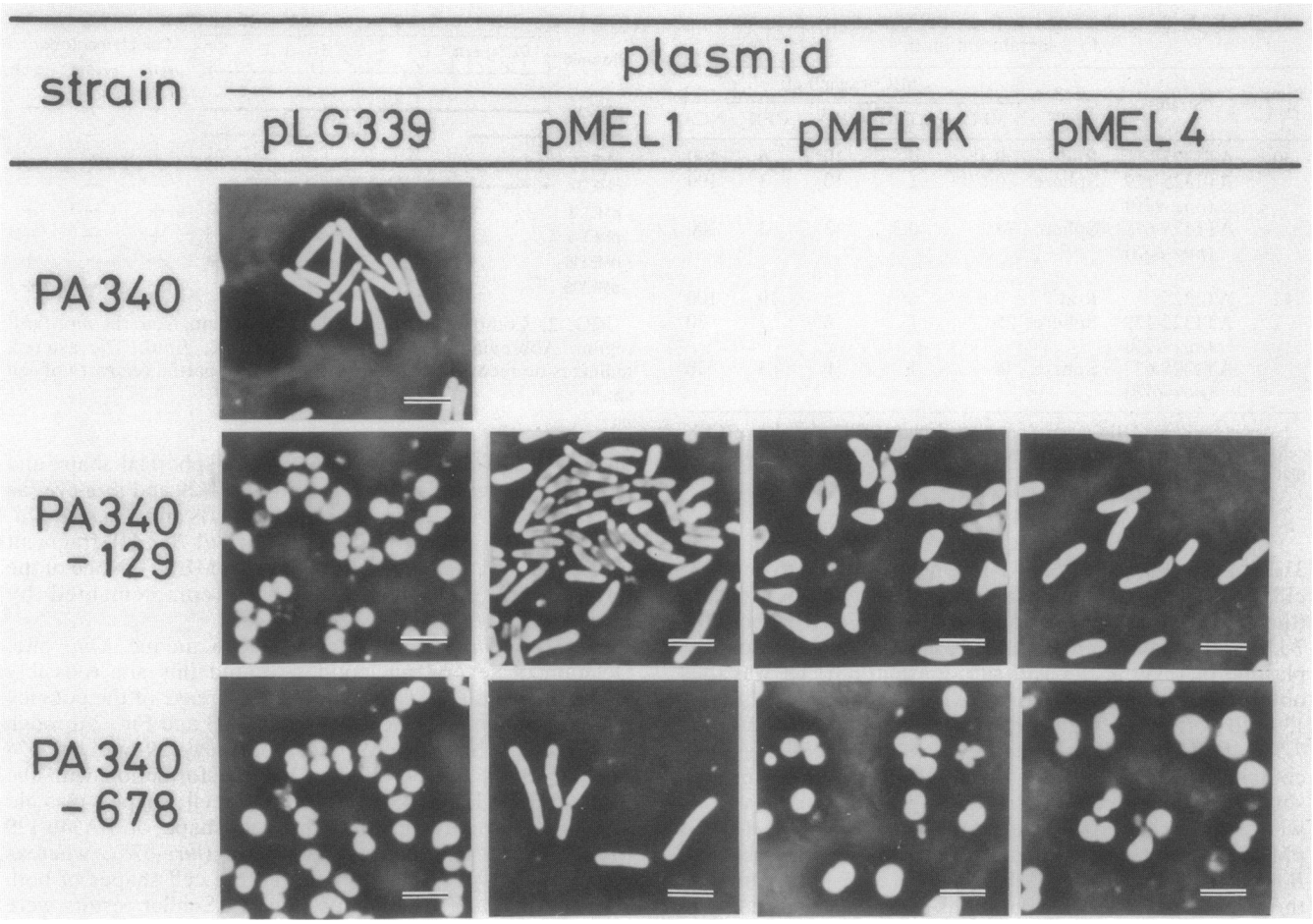

FIG. 3. Cell shapes of *mre* mutants and recovery by plasmids containing *mre* region DNA fragments. Cells of *E. coli* strain were cultured in L'-broth at 30°C to 10^8 cells per ml and observed by dark-field phase-contrast microscopy. Bar, 5 μm.

the rod shape of the cell, normal sensitivity of cell growth to MPC, and the normal amounts of PBPs 1Bs and 3.

To locate the *mre-129* mutation, we recloned smaller DNA fragments from the 7-kb *Sal*I fragment. Fragments that contained the 2.8-kb *Cla*I-*Sal*I region on which the *Kpn*I site was located could only be cloned on low-copy-number plasmids (such as pMEL3 or 4). Plasmids containing the 2.8-kb *Cla*I-*Sal*I fragment (pMEL3 and 4) could complement the total phenotypes of the *mre-129* mutant (Table 3; Fig. 2 through 4), indicating that the *mre-129* gene is in this 2.8-kb *Cla*I-*Sal*I region. This 2.8-kb fragment also restored the phenotypes of the original *mre-129* mutant and *mre-129* transductants as well as the recombinant. Unlike the phenotypes of the *mre-129* mutant, none of those of the *mre-678* mutant could be restored by DNA fragments shorter than 7-kb *Sal*I fragments. This finding suggested that some other part of the 7-kb *Sal*I fragment was required for complementing the phenotypes of the *mre-678* mutant. This possibility was investigated next.

For complementation of both *mre* and *fabE* mutations, a larger fragment in λ d*fabE mre-678* c1857 *S*am7 or the 13.6-kb *Sal*I-*Bgl*II fragment (pMEL10) was required. The *fabE* phenotype alone could be complemented by smaller fragments, the 6.4-kb *Sal*I fragment (pMEY18) and 2.3-kb *Eco*RV fragment (pMEY19; see Fig. 1 and 2). Moreover, these fragments could also be cloned on a high-copy-number plasmid (Fig. 1). Thus, the *mre* genes were located on the 7-kb *Sal*I region of the chromosome, whereas the *fabE* gene was located on the 2.3-kb *Eco*RV fragment which was at least 2 kb away from the *mre* genes.

DNA deletion of 5.25 kb in the *mre-678* mutant. The *mre-678* mutant showed much tighter phenotypical changes than the *mre-129* mutant, and a complementation experiment (summarized in Fig. 2) strongly indicated that the *mre-678* mutant had double or multiple mutations or a large chromosomal deletion. A Southern blot hybridization experiment showed that the *mre-678* mutant had a large chromosomal deletion of 5.25 kb involving the *Kpn*I site.

The 7-kb *Sal*I fragment from the plasmid pMEL1 was uniformly labeled with [α-^{32}P]dCTP and used as a probe for the hybridization (Fig. 5). The plasmid pMEL1 and chromosomes of the *mre*$^+$ strain AT1325 and *mre-129* strain AT1325-129 gave a 7-kb *Sal*I fragment that could hybridize with the 7-kb *Sal*I fragment of pMEL1, whereas the chromosome of the *mre-678* strain AT1325-678 gave a much smaller hybridizing fragment of 1.75 kb (Fig. 5A). When the restriction enzyme *Hin*cII was used, the plasmid pMEL1 and the chromosomes of the *mre*$^+$ and *mre*129 strains gave five smaller DNA fragments, whereas the chromosome of the *mre-678* strain gave two fragments (Fig. 5B). One of the two fragments from the *mre-678* strain was 0.7 kb and was common to all the DNAs examined, but the other, of 1.05

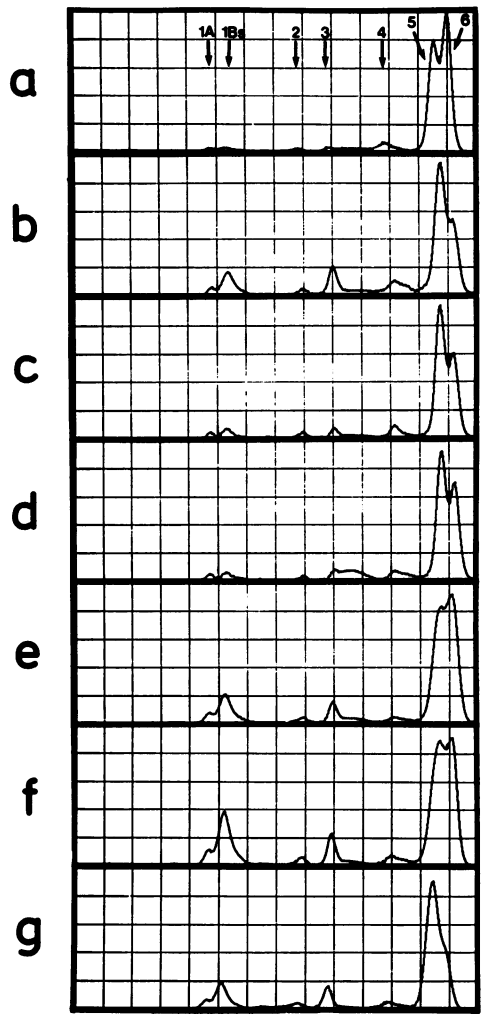

FIG. 4. Overproduction of PBPs 1Bs and 3 in the *mre-678* mutant and recovery of overproduction by plasmids carrying *mre* region DNA fragments. A densitogram of a fluorogram of the electrophoretic gel is shown. (a) PA340; (b) PA340-678; (c) PA340-678(λ d*fabE* *mre-678* *c*I857 *S*am7); (d) PA340-678 (pMEL1); (e) PA340-678(pMEL3); (f) PA340-678(pMEL4); (g) PA340-678(pMEL1K).

kb, was not obtained from the wild type or *mre-129* DNA. Thus we estimated that there was a deletion of a total of 5.25 kb in the *mre* mutant DNA.

DISCUSSION

The results obtained in this work elucidated the peculiar features of the *mre* (murein formation gene cluster E) region at 71 min on the *E. coli* chromosome map, which involves the genes responsible for formation of the rod shape and normal MPC sensitivity of the *E. coli* cell. This region is included in the 7-kb *Sal*I fragment very closely linked to the *fabE* gene. All fragments that contained the 2.8-kb *Cla*I-*Sal*I region could be cloned on a low-copy-number plasmid (mini F) but not on high-copy-number plasmids, suggesting that overproduction of the product(s) of this region is lethal. Two spherical cell mutants with mutations in the *mre* region (*mre-129* and *mre-678*, both recessive to the wild type) were isolated and analyzed. The *mre-129* mutation caused supersensitivity to MPC, while the *mre-678* mutation caused high resistance to MPC and overproduction of PBPs 1Bs and 3.

A peculiar feature of the *mre-129* mutation was that the original *mre-129* mutant isolated was supersensitive to MPC at 30°C but resistant at 42°C, whereas its *mre-129* transductants were supersensitive to this antibiotic at both 30 and 42°C. Plasmids that covered the 2.8-kb *Cla*I-*Sal*I region recovered both sensitivities. One possible explanation for this is that the original *mre-129* mutant might have two mutations, i.e., the *mre-129* mutation, which causes supersensitivity of the cell at both temperatures, and an additional mutation that causes resistance to MPC at higher temperatures only together with the *mre-129* mutation. Another possibility is that the *mre-129* mutation generally causes MPC supersensitivity at lower temperatures and MPC resistance at higher temperatures and that the threshold temperature for the change of sensitivity depends on the genetic background of the cell.

The *mre* region thus seems to contain two or more genes that are responsible for the expression of the normal rod shape and MPC sensitivity of the cell and the amount of PBPs. A defect in an *mre* gene (*mre-129* mutation) may cause rounding of the cell and MPC supersensitivity, and such a defect extending to two or more *mre* genes (*mre-678* mutation) may cause rounding of the cell, MPC resistance, and overproduction of PBPs 1Bs and 3. Products of these genes may function in some unknown manner in formation of a rod-shaped cell having normal sensitivities to MPC and other β-lactam antibiotics. It is also unknown whether these products react in some way with products of other rod shape-determining genes, such as PBP 2 and the RodA protein formed from the *mrd* (murein formation gene cluster D) region of the chromosome. Sequencing of the *mre* genes and characterization of their products are in progress.

Normark et al. (11) previously reported isolation of a

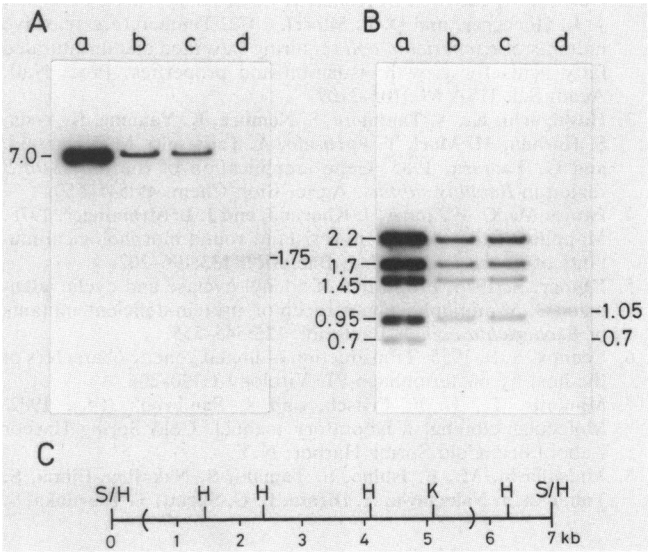

FIG. 5. Deletion in the *mre-678* mutant identified by Southern blot hybridization. Autoradiograms of (A) *Sal*I-digested DNA fragments and (B) *Hinc*II-digested DNA fragments. (C) Restriction map of the 7-kb *Sal*I fragment of pMEL1; the supposed deletion area in *mre-678* (5.25 kb) is shown approximately by parentheses. Source of DNA: lane a, pMEL1; lane b, AT1325; lane c, AT1325-129; lane d, AT1325-678. A uniformly labeled 7-kb *Sal*I fragment from pMEL1 was used as a probe. Abbreviations for the restriction enzymes: H, *Hinc*II; K, *Kpn*I; S, *Sal*I.

spherical, MPC-resistant mutant that could be separated by P1 transduction into two mutants, a rounded, MPC-supersensitive strain carrying *envB* and a rod-shaped, MPC-resistant strain carrying *sloB*. The *envB* mutation was located close to *aroE* (24), and complementation tests with plasmids pMEL1 and pMEL4 suggested that it was in the same complementation group as *mre-129* (data not shown). However, the *sloB* mutation that rendered the *envB* mutant highly MPC resistant was located on the opposite side of the *rpsL* gene (24) about 2 min away from the *envB* gene. In our case, a 5.25-kb deletion in the *mre* region caused resistance to MPC. It seems that the high MPC resistance in the *mre-678* mutant has a different mechanism from that of the *sloB* mutant.

Previously, a gene that can affect the amount of PBPs 1A and 2 simultaneously (20) was found and mapped close to the *mre* mutations (8), but this mutation was not investigated further.

Incidentally, during cloning of the *mre* region DNA, we also cloned the closely located *fabE* gene, which is reported to code for acetyl-CoA carboxylase (14). In contrast to the *mre* genes, the *fabE* gene could be cloned on a high-copy-number plasmid as well as on a low-copy-number plasmid.

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