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New *mre* Genes *mreC* and *mreD*, Responsible for Formation of the Rod Shape of *Escherichia coli* Cells

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New shape-determining genes in the *mre* cluster at 71 min on the *Escherichia coli* chromosome map, named *mreC* and *mreD*, were identified by complementation experiments using Δ*mre-678* mutant cells, which have a 5-kilobase-pair deletion encompassing the *mre* region, and by DNA sequencing. The Δ*mre-678* mutant cells required three genes, the previously reported *mreB* gene and the two new genes, to restore the normal rod shape of the cells and normal sensitivity of growth to mecillinam. The *mreC* gene is preceded by the *mreB* gene and by a 65-base-pair spacing sequence containing a palindrome sequence and a possible Shine-Dalgarno sequence. The deduced amino acid sequence of the MreC protein consists of 367 amino acid residues with a molecular weight of 39,530. The initiation codon of the *mreD* gene overlaps the termination codon of the *mreC* gene by one nucleotide residue. The deduced amino acid sequence of the MreD protein consists of 162 amino acid residues with a molecular weight of 18,755. In vitro, the coding frames of *mreC* and *mreD* produced proteins with M_rs of 40,000 and 15,000, respectively, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Two regions on the *Escherichia coli* chromosome, which we named *mrd* (14) and *mre* (15), are responsible for determining cell shape and sensitivity to an amidinopenicillin, mecillinam. The *mrd* region, located at 15 min on the *E. coli* chromosome map, involves two genes, *mrdA* (also called *pbpA*), coding for the peptidoglycan synthetase penicillin-binding protein 2, and *mrdB* (also called *rodA*), coding for the RodA protein. These genes are thought to function together in an early step of cell elongation (8). The *mre* region at 71 min on the chromosome map includes *mreB* and several other genes that also function in forming normal rod-shaped cells (4). The *mreB* gene, which encodes a 37-kilodalton (kDa) protein, is thought to regulate cell division negatively (16). Previous maxicell experiments showed that the 6.5-kilobase-pair (kb) *Sal*I fragment of the chromosome encompassing the *mre* region encodes at least four proteins, including the MreB protein (4), but precise analysis of the genes other than *mreB* has not yet been carried out.

Here we report the physical mapping of two genes in the *mre* region, *mreC* and *mreD*, involved in formation of the rod shape of the cell, their DNA sequences, and preliminary identification of their encoded proteins.

MATERIALS AND METHODS

Bacterial strains and media. The properties of *E. coli* K-12 PA340 (F⁻ *argH1 thr-1 leuB6 gdh-1 hisG1 gltB31 thi-1 lacY1 gal-6 xyl-7 ara-14 mtl-2 malA1 rpsL9 tonA2*) and its *mre* derivatives PA340-129 (the same as PA340 but *gltB*⁺ *mreB-129*) and PA340-678 (the same as PA340 but *gltB*⁺ Δ*mre-678*) were described previously (15). Strain JM109 [Δ(*lac-proAB*) *recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* λ⁻ F′ *traD36 proAB lacI*ᵠ Δ*lacZM15*] was used for plasmid construction and DNA sequencing experiments. Modified Lennox broth supplemented with 20 mg of thymine and 100 μg of lipoic acid per liter (L'lip broth) was used for growing cells. Appropriate antibiotics were added to the broth for growing cells carrying plasmids.

Plasmid construction. Low-copy-number plasmid pLG339 (11) was obtained from B. G. Spratt, University of Sussex, Sussex, England. High-copy-number plasmids pHGS398 and pHSG399 (13) were purchased from Takara Shuzo Co., Kyoto, Japan. Plasmids pMEL1C, pMEL1Ba, pMEL1S, and pMEL1Bg (Fig. 1) were constructed as follows. Plasmid pMEL1 was digested by appropriate restriction endonucleases to form one-cut fragments, and these fragments were purified electrophoretically on a 0.7% agarose gel. The

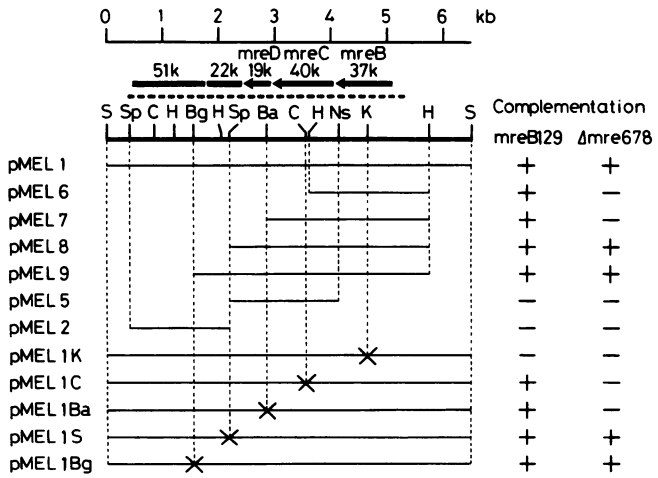

FIG. 1. Complementation of the Δ*mre-678* mutation by chromosomal fragments of various lengths and those with frame-shift mutations (×). Alignment of *mre* genes and flanking genes is shown by bold lines. Symbols: ◂, direction of transcription; – – –, chromosomal deletion in the Δ*mre-678* mutation. Abbreviations for restriction endonucleases: Ba, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; H, *Hin*cII; K, *Kpn*I; Ns, *Nsp*7524I; S, *Sal*I; Sp, *Sph*I. *Nsp*7524I also recognizes *Sph*I sites.

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TABLE 1. Plasmids used

Plasmid	Source and construction
pMEL1	Constructed by ligation of a 6.5-kb *Sal*I fragment containing the *mreB*, *mreC*, and *mreD* genes and two other coding frames with *Sal*I-digested pLG339 (15)
pMEL2	1.8-kb *Sph*I fragment of pMEL1 and *Sph*I-digested pLG339
pMEL5	2.0-kb *Sph*I-*Nsp*7524I fragment of pMEL1 and *Sph*I-digested pLG339
pMEL6	2.1-kb *Hin*cII fragment containing the *mreB* gene and *Hin*cII-digested pLG339 (16)
pMEL6′	Same as pMEL6 but in the reverse direction
pMEL7	1.8-kb *Bam*HI-*Kpn*I fragment of pMEL1 and 7.0-kb *Bam*HI-*Kpn*I fragment of pMEL6′
pMEL8	2.4-kb *Sph*I-*Kpn*I fragment of pMEL1 and 7.2-kb *Sph*I-*Kpn*I fragment of pMEL6′
pMEL9	3.0-kb *Bgl*II-*Kpn*I fragment of pMEL1 and 7.0-kb *Bam*HI-*Kpn*I fragment of pMEL6′
pMEG1[a]	2.0-kb *Sph*I-*Nsp*7524I fragment of pMEL1 and *Sph*I-digested pHSG398
pMEG2[a]	1.1-kb *Sph*I-*Pst*I fragment of pMEG1 and *Sph*I-*Pst*I-digested pHSG398
pMEG3[a]	1.3-kb *Bam*HI-*Nsp*7524I fragment of pMEG1 and *Bam*HI-*Sph*I-digested pHSG399
pMEL1K	Same as pMEL1 but a frameshift at the *Kpn*I site in the *mreB* gene (15)
pMEL1C[b]	Same as pMEL1 but a frameshift at the *Cla*I site in the *mreC* gene
pMEL1Ba[b]	Same as pMEL1 but a frameshift at the *Bam*HI site in the *mreD* gene
pMEL1S[b]	Same as pMEL1 but a frameshift at the *Sph*I site in the coding frame of the 22-kDa protein
pMEL1Bg[b]	Same as pMEL1 but a frameshift at the *Bgl*II site in the coding frame of the 51-kDa protein

[a] Constructed so that the *mreC* and/or *mreD* genes are located under the *lacUV5* promoter of the vector plasmids in the same direction.
[b] Details of construction are described briefly in Materials and Methods.

isolated fragments were then filled in at the cohesive ends with nucleotides by treatment with the Klenow fragment of *E. coli* DNA polymerase I (for 5′ protruding fragments) or with the Klenow fragment after mung bean nuclease treatment (for 3′ protruding fragments). The fragments were then ligated, and *E. coli* JM109 was transformed with the ligation mixture. Correct construction of plasmids was confirmed by restriction endonuclease digestion. Constructions of plasmids are summarized in Table 1.

DNA sequencing. The dideoxynucleotide method (10) with [α-^{32}P]dCTP was applied directly to the denatured double-stranded DNA plasmids carrying the *E. coli* chromosome fragments (6).

In vitro protein synthesis. In vitro protein synthesis according to the system of DeVries and Zubay (3) was carried out by using a procaryotic protein expression kit (Amersham International plc, Buckinghamshire, England) and L-[^{3}H]leucine.

Radioactive materials and reagents. [α-^{32}P]dCTP (410 Ci/mmol) and L-[4,5-^{3}H]leucine (145 Ci/mmol) were purchased from Amersham. Mecillinam was a gift of Leo Pharmaceutical Products, Copenhagen, Denmark. Other reagents and enzymes used for DNA recombination experiments were commercial products.

RESULTS

Complementation experiments on Δ*mre678* mutants. As reported previously, the Δ*mre-678* mutation caused by a 5-kb deletion in the *mre* region was complemented by plasmid pMEL1, carrying a 6.5-kb *Sal*I fragment containing the whole *mre* region, but not by plasmid pMEL4, carrying a 3.6-kb *Bam*HI-*Sal*I fragment containing *mreB* and its short flanking regions (15). To determine the minimum region required for complementation of the Δ*mre-678* mutation, we constructed plasmids that carried various lengths of the 6.5-kb *Sal*I fragment (Fig. 1). We also constructed plasmids with frameshift mutations at several sites in plasmid pMEL1 (Fig. 1). The results of the complementation tests are shown in Fig. 2 (cell shape) and Table 2 (MIC of mecillinam) and are summarized in Fig. 1.

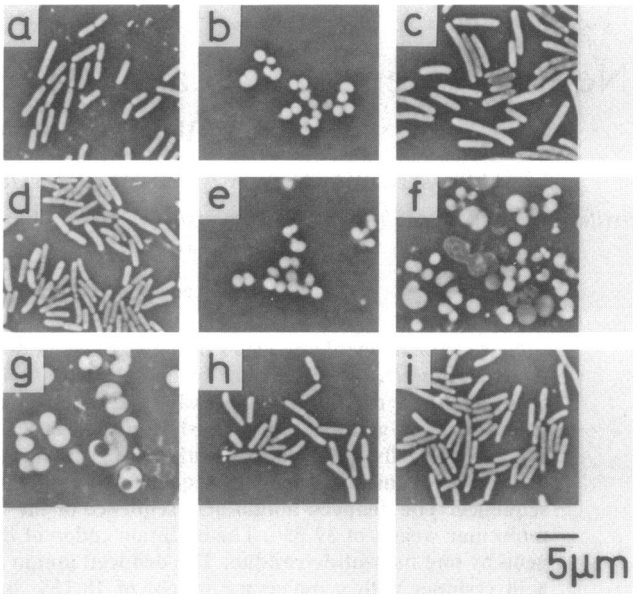

FIG. 2. Recovery of cell shape of Δ*mre-678* mutants. Cells were grown on L'lip-agar plates containing 25 mg of kanamycin per liter at 30°C overnight; the cells were then suspended in the same broth, and their shape was examined by dark-field phase-contrast microscopy. a, PA340(pLG339); b, PA340-678(pLG339); c, PA340-678(pMEL1); d, PA340-678(pMEL8); e, PA340-678(pMEL1K); f, PA340-678(pMEL1C); g, PA340-678(pMEL1Ba); h, PA340-678(pMEL1S); i, PA340-678(pMEL1Bg).

The plasmid carrying a 3.5-kb *Sph*I-*Hin*cII fragment (pMEL8) could complement the Δ*mre-678* mutation. Plasmids carrying a longer fragment than pMEL8 (pMEL1 and pMEL9) could also complement this mutation, but those

TABLE 2. Complementation of MIC for mecillinam of the Δ*mre-678* mutant

Strain	MIC for mecillinam (μg/ml)[a]	
	30°C	42°C
PA340(pLG339)	0.10	0.03
PA340-678(pLG339)	74	25
PA340-678(pMEL1)	0.03	0.10
PA340-678(pMEL8)	0.03	0.10
PA340-678(pMEL1K)	74	8.2
PA340-678(pMEL1C)	25	8.2
PA340-678(pMEL1Ba)	74	25
PA340-678(pMEL1S)	0.03	0.10
PA340-678(pMEL1Bg)	0.03	0.10

[a] Determined in threefold serial dilution tests on L'lip-agar plates containing 25 mg of kanamycin per liter with an inoculum of 10^4 cells.

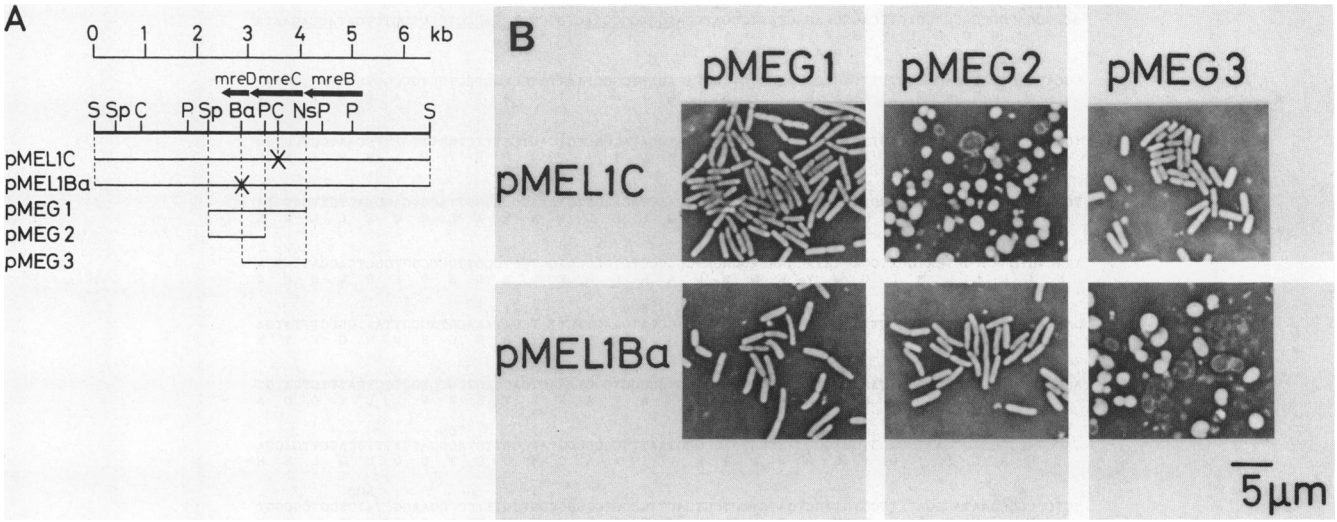

FIG. 3. Locations of *mreC* and *mreD* by cotransformation of the Δ*mre-678* mutant. (A) Plasmids carrying the 6.5-kb chromosomal fragment with frameshifts (×) at the *Cla*I and *Bam*HI sites and those carrying smaller fragments complementing the frameshift mutations; (B) recovery of cell shape of the Δ*mre-678* mutant.

carrying smaller fragments (pMEL5, pMEL6, and pMEL7) could not. Plasmid pMEL2, which contains a 1.9-kb *Sph*I fragment flanking the 3.5-kb *Sph*I-*Hin*cII fragment, also could not complement the mutation. Plasmid pMEL1K, which was derived from pMEL1 and has a frameshift at the *Kpn*I site in the *mreB* gene, also did not complement the Δ*mre-678* mutation, as reported previously (15). Likewise, plasmids with a frameshift at either the *Cla*I site or the *Bam*HI site, both located downstream of the *mreB* gene (pMEL1C and pMEL1Ba, respectively), could not complement the mutation. On the other hand, plasmids pMEL1S and pMEL1Bg, which have frameshifts at the *Sph*I and *Bgl*II sites, respectively, farther downstream of the *mreB* gene, could complement the mutation. These results indicated that at least one other gene containing the *Cla*I and *Bam*HI sites in addition to the *mreB* gene was required by the Δ*mre-678* mutant cells for restoration of the normal rod shape and normal mecillinam sensitivity.

Mecillinam sensitivity of the mutant cells was restored by plasmid pMEL1, pMEL8, pMEL1S, or pMEL1Bg to a level similar to that of wild-type cells but was not restored substantially by pMEL1K, pMEL1C, and pMEL1Ba (Table 2). Plasmids pMEL1K and pMEL1C caused some reduction in resistance of the mutant cells to mecillinam. The round shape of the mutant cells was restored to the normal rod by transformation with pMEL1, pMEL8, pMEL1S, or pMEL1Bg but not with pMEL1K, pMEL1C, or pMEL1Ba (Fig. 2). The shape of the cells transformed with pMEL1C or pMEL1Ba was, however, different from that of cells transformed with pMEL1K: pMEL1K transformants were nearly the same round shape as the mutant cells, but transformants with pMEL1C or pMEL1Ba appeared as swollen spheres, and some of them underwent lysis. Probably a defect in the genes containing the *Cla*I or *Bam*HI site or amplification of the defective genes is more harmful to the cell than similar alterations of the *mreB* gene.

To determine the precise location of the gene(s) including the *Cla*I and *Bam*HI sites, the Δ*mre-678* mutant was cotransformed with either pMEL1C or pMEL1Ba and plasmids carrying smaller wild-type fragments in the region closely downstream of the *mreB* gene. Strain PA340-678(pMEL1C) was complemented by plasmids pMEG1 and pMEG3, which contained regions (1.8 and 1.2 kb, respectively) contiguous to *mreB*, but not by pMEG2, which contained a 1.1-kb region 1 kb downstream of *mreB* (Fig. 3). On the other hand, strain PA340-678(pMEL1Ba) was complemented by plasmids pMEG1 and pMEG2 but not by pMEG3. These results indicated that two genes in addition to *mreB* are necessary for complementation of the Δ*mre-678* mutation; one of these genes is located in the 1.3-kb *Bam*HI-*Nsp*7524I fragment, which contains the *Cla*I site, and the other is located in the 1.0-kb *Sph*I-*Pst*I fragment, which contains the *Bam*HI site. These genes could be transcribed by *lacUV5* promoter of the vector plasmids.

Determination of the DNA sequences of the *mreC* and *mreD* genes. The 2.0-kb *Sph*I-*Nsp*7524I fragment, which complemented the two mutations caused by the frameshift at the *Cla*I and *Bam*HI sites, was sequenced (Fig. 4). Two open reading frames were found in this fragment, as expected from the complementation study; one involved the *Cla*I site, and the other involved the *Bam*HI site. The former, which was named *mreC*, extends from ATG (nucleotides 101 to 103) to TAG (nucleotides 1202 to 1204), whereas the latter, which was named *mreD*, extends from GTG (nucleotides 1204 to 1206) to TAA (nucleotides 1690 to 1692).

The *mreC* gene is preceded by *mreB* (only 32 nucleotides of *mreB* are shown in Fig. 4). The termination codon of *mreB* and the start codon of *mreC* are separated by a 65-base-pair (bp) spacing sequence. A possible Shine-Dalgarno sequence (ACGAG; positions 82 to 86) and palindrome sequence (positions 43 to 74) that could form a 12-bp stem with one mismatch and an 8-bp loop are seen in this region. However, no promoterlike sequence could be found in the close upstream region of the *mreC* gene. The deduced amino acid sequence of the MreC protein consists of 367 amino acid residues with a molecular weight of 39,530. A hydrophobic domain in the N-terminal region, which might anchor the protein to the membrane, is seen in the MreC protein. The MreC protein has a proline-rich domain at the C-terminal region (20 proline residues from amino acid residues 279 to 367).

The initiation codon of the *mreD* gene overlaps the termi-

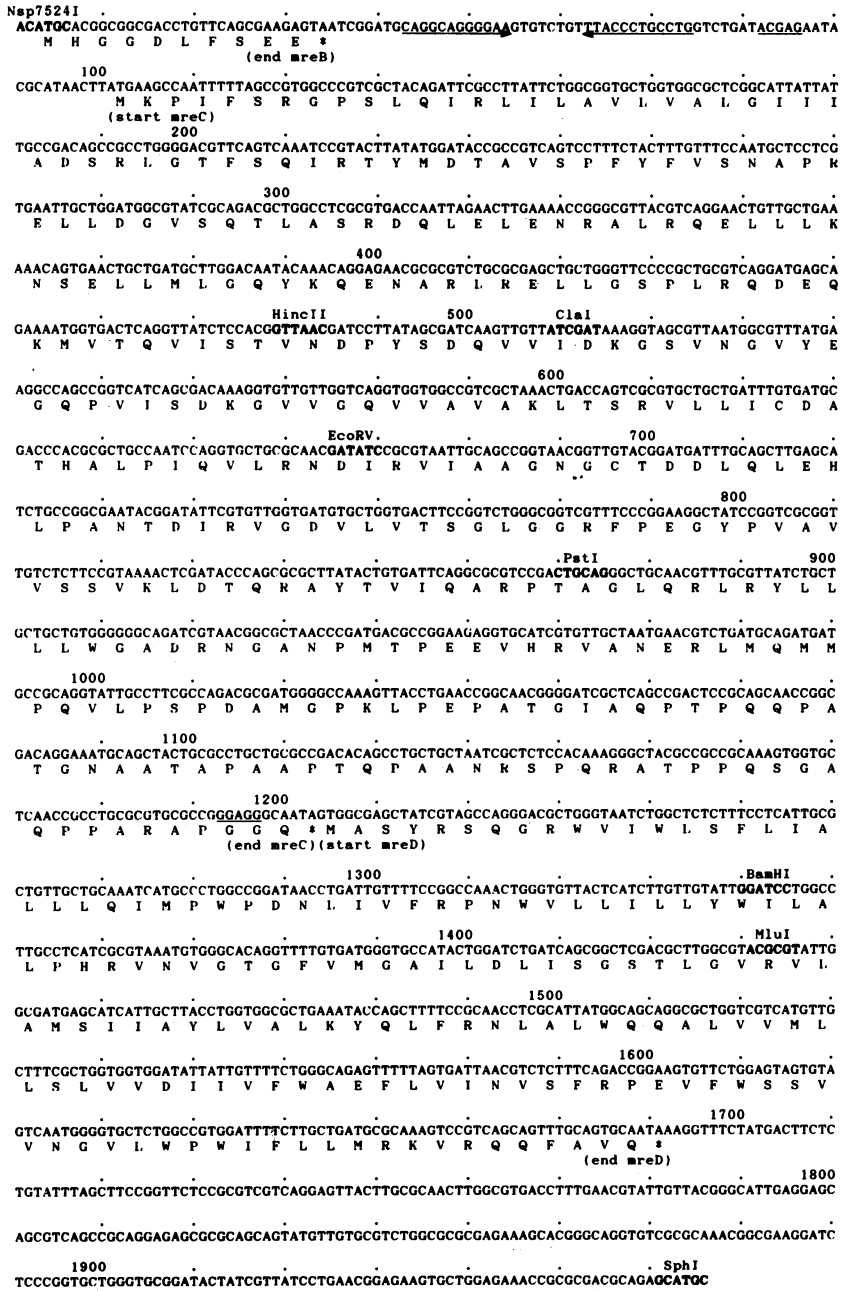

FIG. 4. Base sequence of the 2.0-kb *Nsp*7524I-*Sph*I fragment encompassing the *mreC* and *mreD* genes and deduced amino acid sequences. Possible Shine-Dalgarno sequences are underlined. Arrows indicate palindrome sequences. *, Termination codons.

nation codon of the *mreC* gene by one nucleotide, G (1204). A Shine-Dalgarno-like sequence (GGAGG; positions 1193 to 1197) is seen in the coding frame of *mreC*, but no promoter-like sequence could be found in the close upstream region. The deduced amino acid sequence of the MreD protein consists of 162 amino acid residues with a molecular weight of 18,755. The hydropathy profile revealed that the MreD protein was extremely hydrophobic, 71% of its amino acid residues being hydrophobic.

Identification of the *mreC* and *mreD* gene products in an in vitro protein synthesis system. Proteins encoded by genes located on the 2.0-kb *Sph*I-*Nsp*7524I fragment were detected in an in vitro protein synthesis system. A fluorogram of the gel after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the proteins labeled by L-[^{3}H]leucine is shown in Fig. 5. Plasmid pMEG1, which carried the 2.0-kb *Sph*I-*Nsp*7524I fragment containing both the *mreC* and *mreD* genes, formed two proteins, with M_rs of about 40,000 and 15,000, as judged by SDS-PAGE, in addition to the product of the chloramphenicol resistance gene on the vector plasmid. The 40-kDa protein was also formed by plasmid pMEG3, which carried the 1.3-kb *Bam*HI-*Nsp*7524I fragment containing the *mreC* gene, but not by pMEG2, which carried the 1.0-kb *Sph*I-*Pst*I fragment containing the *mreD* gene. On the other hand, the 15-kDa protein was formed by plasmid pMEG2 but not by pMEG3. These results indicate that the 40-kDa protein was formed by the *mreC* gene and that the 15-kDa protein was formed by

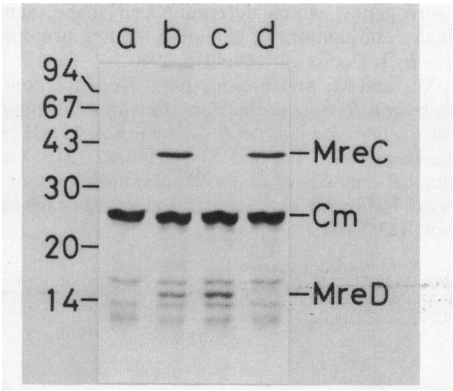

FIG. 5. Proteins synthesized in vitro from plasmids carrying either or both of the *mreC* and *mreD* genes. A fluorogram of SDS-PAGE (15% gel) is shown. Lanes: a, pHSG398; b, pMEG1; c, pMEG2; d, pMEG3. Numbers at the left indicate M_r values in thousands. Cm, Chloramphenicol acetyltransferase.

the *mreD* gene. Interestingly, the molecular weight of the MreD protein estimated by SDS-PAGE was rather smaller than its calculated molecular weight. This was probably because of its extremely high hydrophobicity, since similar discrepancies have been observed with the RodA (12) and LacY (5) proteins.

DISCUSSION

In this work, we have demonstrated the alignment in the *mre* region of genes that are involved in formation of the rod shape of *E. coli* cells and determined their DNA sequences. Downstream of *mreB*, we found two genes, *mreC* and *mreD*, that could code for proteins of 40 and 19 kDa, respectively. The Δ*mre-678* mutant cells, which have a 5-kb deletion encompassing the *mre* region, required three genes, *mreB*, *mreC* and *mreD*, for growth as normal rod-shaped cells with normal sensitivity to mecillinam. We previously reported that four proteins, of 37 (MreB) 40, 22, and 51 kDa, are formed from the 6.5-kb *Sal*I fragment in a maxicell system (4). MreC corresponds to the 40-kDa protein found in the maxicell system, but MreD, which was found to be a 15-kDa protein on SDS-PAGE of proteins synthesized in an in vitro synthesis system, was not detected in the previous maxicell experiments. Probably the high hydrophobicity of MreD prevented its detection in the membrane fraction on SDS-PAGE.

The precise functions of MreC and MreD are unknown. Judging from the results of DNA sequencing, the three *mre* genes, *mreB*, *mreC*, and *mreD*, could be transcribed as an operon and function together, but this possibility requires further investigation. The amino acid sequence of the C-terminal half of MreB shows strong similarity with the C-terminal half of the cell division gene product, FtsA (4, 9). Moreover, the total area of MreB shows some similarities with those of proteins in the heat shock protein 70 family, which were suggested to have protein kinase activity (1, 2, 7; data not shown). These facts suggest that the function of the MreB protein is regulatory rather than structural or biosynthetic. This protein may function in switch-on/off of cell division, as previously proposed from physiological experiments (16). We previously reported that the *mreB* mutation caused overproduction of the septum-peptidoglycan synthetase penicillin-binding protein 3. Probably the MreB protein negatively regulates the formation of penicillin-binding protein 3, and this may contribute to switching of the processes of cell division and cell elongation. As the Δ*mre-678* mutant showed even higher overproduction of penicillin-binding protein 3 and some increase of penicillin-binding protein 1B, the *mreC* and *mreD* genes may also contribute to regulation of formation of penicillin-binding proteins. We are now investigating this point.

The *mreB-129* mutant is supersensitive to mecillinam, but the Δ*mre-678* mutant carrying a 5-kb chromosomal deletion encompassing the *mreB*, *mreC*, and *mreD* genes is resistant to this antibiotic (15). However, the *mreB* mutant constructed by introduction of plasmid pMEL1K into the Δ*mre-678* mutant was resistant to mecillinam (15; Table 2). Westling-Häggström and Normark (17) also isolated a mutant, named *envB*, that was spherical and mecillinam supersensitive like *mreB129* and that became mecillinam resistant by a double mutation of *envB* and *sloB*. The *envB* mutation was probably located in the *mreB* gene, but the *sloB* mutation has not yet been precisely located (15, 17). The mechanism of mecillinam sensitivity is still a puzzling problem.

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LITERATURE CITED

1. **Bardwell, J. C. A., and E. A. Craig.** 1984. Major heat shock gene of *Drosophila* and the *Escherichia coli* heat-inducible *dnaK* gene are homologous. Proc. Natl. Acad. Sci. USA **81**:848–852.
2. **Bienz, M.** 1984. *Xenopus hsp* 70 genes are constitutively expressed in injected oocytes. EMBO J. **3**:2477–2483.
3. **DeVries, J. K., and G. Zubay.** 1967. DNA-directed peptide synthesis. II. The synthesis of the α-fragment of the enzyme β-galactosidase. Proc. Natl. Acad. Sci. USA **57**:1010–1012.
4. **Doi, M., M. Wachi, F. Ishino, S. Tomioka, M. Ito, Y. Sakagami, A. Suzuki, and M. Matsuhashi.** 1988. Determinations of the DNA sequence of the *mreB* gene and of the gene products of the *mre* region that function in formation of the rod shape of *Escherichia coli* cells. J. Bacteriol. **170**:4619–4624.
5. **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.
6. **Hattori, M., and Y. Sakaki.** 1986. Dideoxy sequencing method using denatured plasmid templates. Anal. Biochem. **152**:232–238.
7. **Ingolia, T. D., E. A. Craig, and B. J. McCarthy.** 1980. Sequence of three copies of the gene for the major Drosophila heat shock induced protein and their flanking regions. Cell **21**:669–679.
8. **Ishino, F., W. Park, S. Tomioka, S. Tamaki, I. Takase, K. Kunugita, H. Matsuzawa, S. Asoh, T. Ohta, B. G. Spratt, and M. Matsuhashi.** 1986. Peptidoglycan synthetic activities in membranes of *Escherichia coli* caused by overproduction of penicillin-binding protein 2 and RodA protein. J. Biol. Chem. **261**: 7024–7031.
9. **Robinson, A. C., D. J. Kenan, G. F. Hatfull, N. F. Sullivan, R. Spiegelberg, and W. D. Donachie.** 1984. DNA sequence and transcriptional organization of essential cell division genes *ftsQ* and *ftsA* of *Escherichia coli*: evidence for overlapping transcriptional units. J. Bacteriol. **160**:546–555.
10. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
11. **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.
12. **Stoker, N. G., J. M. Pratt, and B. G. Spratt.** 1983. Identification of the *rodA* gene product of *Escherichia coli*. J. Bacteriol. **155**:854–859.

13. **Takeshita, S., M. Sato, M. Toba, W. Masahashi, and T. Hashimoto-Gotoh.** 1987. High-copy-number and low-copy-number plasmid vectors for *lacZ* α-complementation and chloramphenicol- or kanamycin-resistance selection. Gene **61**:63–74.
14. **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.
15. **Wachi, M., M. Doi, S. Tamaki, W. Park, S. Nakajima-Iijima, and M. Matsuhashi.** 1987. Mutant isolation and molecular cloning of *mre* genes, which determine cell shape, sensitivity to mecillinam, and amount of penicillin-binding proteins in *Escherichia coli*. J. Bacteriol. **169**:4935–4940.
16. **Wachi, M., and M. Matsuhashi.** 1989. Negative control of cell division by *mreB*, a gene that functions in determining the rod shape of *Escherichia coli* cells. J. Bacteriol. **171**:3123–3127.
17. **Westling-Häggström, B., and S. Normark.** 1975. Genetic and physiological analysis of an *envB* spherelike mutant of *Escherichia coli* K-12 and characterization of its transductants. J. Bacteriol. **123**:75–82.