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Escherichia coli mraR Gene Involved in Cell Growth and Division

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The *mraR* gene, which has a coding frame of 363 bp and lies close to and upstream of the *ftsI* gene of *Escherichia coli*, is involved in both cell division and cell lysis. It is thought to function in regulating the two distinct steps of the cell cycle, as two different one-base mutations in this unique gene caused different phenotypical changes in the cell. Comparison of nucleotide sequences of the mutant type *mraR* DNAs with the wild type suggested that filamentation of the cell was caused by a mutation in the putative start codon, whereas lysis of the cell was caused by a mutation which led to a change of one internal glutamate residue to lysine.

Many genes involved in the growth and division of *Escherichia coli* cells are located on the chromosome, forming several gene clusters (8). Among them, the largest cluster yet found is located at 2 min on the chromosome map. This area, which we call the *mra* (murein synthesis gene cluster A) area, encompasses at least 13 cell growth and division genes (*ftsI*, *murE*, *murF*, *mraY*, *murD*, *ftsW*, *murG*, *murC*, *ddl*, *ftsQ*, *ftsA*, *ftsZ*, and *envA*) which closely flank each other. The base sequences of the total area are known, and the functions of proteins encoded by most genes have also been determined. We previously isolated two mutants defective in cell growth (*lts-33* mutant) and division (*fts-36* mutant) and located the mutations inside the 600-bp *Pvu*II-*Mlu*I fragment (3). This fragment contained the 5′-terminal 125-base sequence of *ftsI* (9), which codes for septum peptidoglycan synthetase penicillin-binding protein 3 (4, 7, 10), and its upstream sequence, encompassing a short open reading frame which was first reported to contain 330 bp (9). In this report, we show that this open reading frame contains 363 bp and codes for a 14.5-kDa protein and that the *lts-33* and *fts-36* mutants have mutations in this unique open reading frame. As this gene is supposed to function in regulating cell growth and division, we refer to it as *mraR*, the *mraR* mutant allele from the *lts-33* mutant strain being *mraR33* and that from the *fts-36* mutant strain being *mraR36*.

The parental-type 363-bp reading frame was shown to form a protein in in vitro protein synthesis (Fig. 1). Plasmids used in this experiment were pTR1′ ΔPstI, pJOB701 (3), and pJM4, which was prepared in this work by inserting the 0.8-kb *Cla*I fragment from pJD16 (3) into the *Cla*I site of pBR322. Among the three plasmids, each containing different portions of the 1.9-kb *Cla*I-*Pst*I fragment, only pJOB701 and pTR1′ΔPstI, which contained the full-length 363-bp open reading frame, could complement the two mutations and, moreover, form a 14.5-kDa protein. Plasmid pJM4, which contained only a short 5′-terminal sequence of this open reading frame, was incapable of both complementation and protein synthesis.

DNA sequences of the fragments from the two mutants and their parent strain *E. coli* JE1011 (3) are shown in Fig. 2. As it was not possible to clone the mutant *mraR* genes directly, the polymerase chain reaction (PCR) method was used. The PCR was carried out by using a TR-100 thermoprocessor (TAITEC, Saitama-ken, Japan) with two pairs each of primers a and c and b and d (Fig. 2) to amplify the parent and two mutant *mraR* genes. DNA sequencing was done with an ABI 370A DNA sequencer with fluorescent primers in two directions each. The results showed that the two mutant *mraR* genes contained replacements of one base each in the coding frame of the wild type. Replacement of one guanine residue by adenine, causing replacement of Glu (amino acid residue 88 from the N-terminal methionine residue in the wild-type protein) by Lys, was the only replacement in the *mraR33* DNA, and that of guanine by adenine in putative ATG start codon was the only replacement in *mraR36* DNA. Since these mutations were induced by NH_2OH treatment (3), which causes G:C to A:T conversion, substitutions in both *mraR33* and *mraR36* mutant DNAs are consistent. The latter replacement may cause a shift of the start codon for 12 bp downstream to a second start codon sequence, GTG, which might produce a protein shorter by four amino acids. No other possible start codon was found upstream of the first, ATG start codon. As a Shine-Dalgarno sequence-like sequence does not occur upstream of the putative second start codon, GTG, the efficiency of translation of the *mraR36* gene from this start codon could be lower than that of wild-type *mraR*, in which the ATG start codon is preceded by a normal Shine-Dalgarno sequence-like sequence. Production of the MraR36 protein was not, however, tested. The base sequences of the entire coding frame of wild-type *mraR* were the same as those reported previously by Nakamura et al. (9), except for one adenine residue not found by us between G-570 and C-571 (Fig. 2). Deletion of this one adenine residue may result in termination of the frame for 33 bp downstream and, consequently, production of a protein longer by 11 amino acid residues, as shown in Fig. 2. This adenine residue was also not found in DNAs of the two mutant *mraR* genes prepared by PCR.

The MraR protein may play an important role in the

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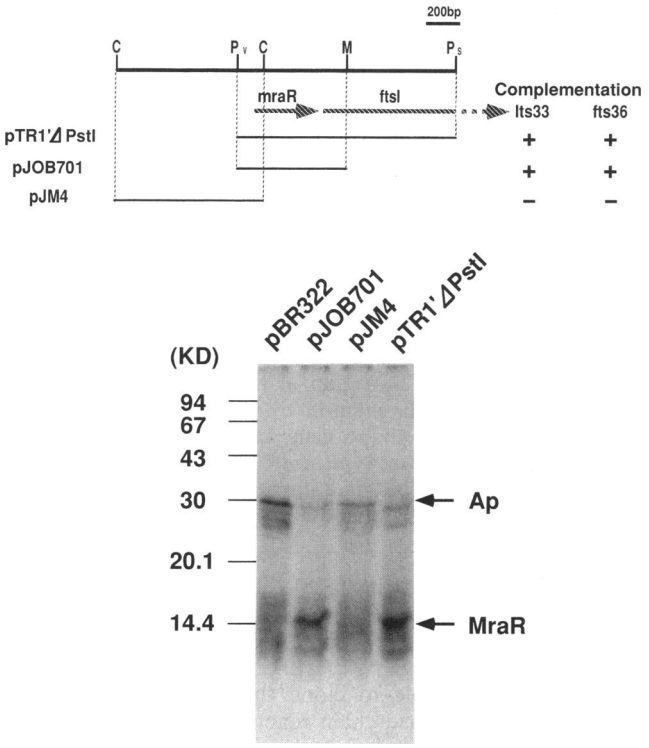

FIG. 1. Complementation of the *mraR33* and *mraR36* mutations with DNA fragments encompassing *mraR* and identification of the *mraR* gene products. For complementation experiments, *E. coli* mutant strains JLB33 and JLB36 (3) were used as host strains, and for in vitro protein synthesis, the system of De Vries and Zubay (1) was used. The product protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a 15% acrylamide gel (5). Abbreviations for restriction endonucleases: C, *Cla*I; M, *Mlu*I; Ps, *Pst*I; Pv, *Pvu*II. kD, kilodaltons. Ap, β-lactamase.

processes of cell growth and division. If this protein is altered by changing Glu-88 into the basic residue Lys, the cells lyse at the nonpermissive temperature of 42°C, growing as short rods. However, if the start codon is changed the frame may be either translated from a less efficient second start codon to form a protein truncated by four amino acid residues or not translated practically. It is unknown whether truncation of the protein or the decrease in its formation disturbs cell division. The two distinct mutations in the single gene *mraR*, as described above, which cause lysis of growing rod cells in the one case (*mraR33*) and defective cell division in the other (*mraR36*), suggest the regulatory function of this gene. Recently, it was found in the laboratory of J. Beckwith (2a) that null mutations in this gene affect cell division.

Judging from its hydropathy profile, the MraR protein could have a transmembrane domain in the N-terminal region. Possibly, this protein functions in the cell membrane to regulate the activity of the cell growth and division machinery. Alternatively, however, the MraR protein could also be an enzyme essential for cell division, deletion of which may cause inhibition of cell division but certain mutation of which may cause expression of cell-lytic activity. The precise mechanism of action of the MraR protein requires further study.

The *mraR* gene is located upstream of 13 closely located genes of the *mra* region with only 18 bp of spacing between

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CCCGGGTGGGCGGCTTTCGATCATCAGCTTCCACTCGCTGGAAGACCGTATTGTGAAACG  60

TTTTATGCGTGAAAACAGCCGCGGTCCGCAAGTTCCGGCAGGGTTACCGATGACTGAAGA 120

GCAGCTCAAAAAACTGGGTGGCCGTCAGCTGCGAGCACTAGGCAAGTTAATGCCGGGCGA 180

AGAAGAGGTGGCTGAGAACCCTCGTGCCCGTAGTTCAGTTCTGCGTATTGCAGAGAGGAC 240

GAATGCATGATCAGCAGAGTGACAGAAGCTCTAAGCAAAGTTAAAGGATCGATGGGAAGC 300
mraRwildMetIleSerArgValThrGluAlaLeuSerLysValLysGlySerMetGlySer
  mraR36--A       (Met)
CACGAGCGCCATGCATTGCCTGGTGTTATCGGTGACGATCTTTTGCGATTTGGGAAGCTG 360
HisGluArgHisAlaLeuProGlyValIleGlyAspAspLeuLeuArgPheGlyLysLeu

CCACTCTGCCTGTTCATTTGCATTATTTTGACGGCGGTGACTGTGGTAACCACGGCGCAC 420
ProLeuCysLeuPheIleCysIleIleLeuThrAlaValThrValValThrThrAlaHis

CATACCCGTTTACTGACCGCTCAGCGCGAACAACTGGTGCTGGAGCGAGATGCTTTAGAC 480
HisThrArgLeuLeuThrAlaGlnArgGluGlnLeuValLeuGluArgAspAlaLeuAsp

ATTGAATGGCGCAACCTGATCCTTGAAGAGAATGCGCTCGGCGACCATAGCCGGGTGGAA 540
IleGluTrpArgAsnLeuIleLeuGluGluAsnAlaLeuGlyAspHisSerArgValGlu
                  mraR33A--
                        Lys
AGGATCGCCACGGAAAAGCTGCAAATGCAGCATGTTGATCCGTCACAAGAAAATATCGTA 600
ArgIleAlaThrGluLysLeuGlnMetGlnHisValAspProSerGlnGluAsnIleVal

GTGCAAAAATAAGGATAAACGCGACGCATGAAAGCAGCGGCGAAAACGCAGAAACCAAAA 660
ValGlnLys***          ftsI MetLysAlaAlaAlaLysThrGlnLysProLys

CGTCAGGAAGAACATGCCAACTTTATCAGTTGGCGTTTTGCGTTGTTATGCGGCTGTATT 720
ArgGlnGluGluHisAlaAsnPheIleSerTrpArgPheAlaLeuLeuCysGlyCysIle

CTCCTGGCGCTGGCTTTTCTGCTCGGACGCGTAGCGTGGTTACAAGTTATCTCCCCGGAT 780
LeuLeuAlaLeuAlaPheLeuLeuGlyArgValAlaTrpLeuGlnValIleSerProAsp

ATGCTGGTGAAAGAGGGCGACATGCGTTCTCTTCGCGTTCAGCAAGTTTCCACCTCCCGC 840
MetLeuValLysGluGlyAspMetArgSerLeuArgValGlnGlnValSerThrSerArg

(a) 5' tgtaaaacgacggccagtCCGGCAGGGTTACCGATGA 3'

(b) 5' tgtaaaacgacggccagtTTATTTTGACGGCGGTGAC 3'

(c) 5' caggaaacagctatgaccTATGGTCGCCGAGCGCATT 3'

(d) 5' caggaaacagctatgaccCACCAGCATATCCGGGGAG 3'
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FIG. 2. Sequence of DNA fragments encompassing the wild-type and two mutant *mraR* genes. The entire sequence of the wild type and substitutions in the mutants are shown. In parentheses is the methionine residue encoded by the putative second start codon. The Symbol *** shows a termination codon, and underlining shows a putative Shine-Dalgarno sequence. Sequences a to d show the primers used for PCRs. Sequences with lowercase letters are complementary to fluorescent sequencing primers, and those with capital letters are complementary to *mraR* sequences. The arrows indicate PCR primer-binding sites.

the coding frames of *mraR* and *ftsI*. The *mraR* gene might also be involved in expression of this series of cell wall- and septum peptidoglycan-synthetic enzyme genes. Moreover, an SOS box-like sequence was found close to and upstream of the *mraR* gene (3). It would be interesting to know whether *mraR* is part of the SOS regulon, but neither the *mraR33* nor the *mraR36* mutant was supersensitive to UV irradiation.

Further upstream of *mraR*, one open reading frame, *orfB* (2), has been reported with one base overlapping with the start codon of *mraR*. The function of *orfB* is unknown. Our DNA sequencing results showed no change in 133 nucleotide residues upstream of the reading frames of the two mutant *mraR* genes. There is another gene, *shl* (6), further upstream of *orfB*, separated by a 1-kb spacing sequence. The similarity of the deduced amino acid sequence of the Shl protein to those of the GalR and LacI proteins suggests that the Shl protein functions as a transcription regulator.

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