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mraY Is an Essential Gene for Cell Growth in *Escherichia coli*

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The synthesis of the murein precursor lipid I is performed by MraY. We have shown that *mraY* is an essential gene for cell growth. Cells depleted of MraY first swell and then lyse. The expression of *mraY* DNA in vitro produces a 40-kDa polypeptide detectable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

In *Escherichia coli*, the *mra* (murein region a [14]) cluster is located at 2 min on the genetic map. The region contains 16 open reading frames (ORFs), the functions of 14 of which have been identified (Fig. 1). The genes are involved in either cell division or murein synthesis or both (for reviews, see references 3, 10, and 19). The *mraY* and *murG* genes encode enzymes which together catalyze the formation of the lipid-linked disaccharide pentapeptide, lipid II [undecaprenyl-pyrophosphoryl-*N*-acetyl muramyl-(pentapeptide)-*N*-acetyl glucosamine], the precursor required for murein biosynthesis (8, 13, 15, 19). The first step of this two-stage process involves MraY, a membrane-bound translocase, which catalyzes the binding of UDP-MurNAc-(pentapeptide) to bactoprenol, a membrane-bound C_{55} isoprenoid lipid, to produce lipid I [undecaprenyl-pyrophosphoryl (PP)-MurNAc-pentapeptide] (8, 15, 20). The *mraY* mutant phenotype is not known. In this study, the phe-

notype of an *mraY*-null mutant is described. The proposed *mraY* ORF of 1,080 bp, identified by Ikeda et al., (7), is shown to complement an *mraY*-null allele. This ORF also produced a polypeptide of ~40 kDa when it was expressed in an in vitro transcription and translation system.

Insertional inactivation of *mraY*. An 8.4-kb *Kpn*I fragment from Kohara phage λ6F3 (110) was cloned into pUC18 to create pDEG1 (Fig. 1) (1, 9). The *mraY* locus in pDEG1 was inactivated by the insertion of the chloramphenicol resistance gene (*cat*) into the *Xmn*I site of *mraY* to make pDYC1 (Fig. 1). The disrupted *mraY*::*cat* construct was used to replace a wild-type *mraY* allele in the *recD* strain DSB1 [*rodA*(Am) recD::*minitet* Tet^r Kan^r Sup^0], which has a duplication of the 2-min region (1). Chloramphenicol-resistant (Cmp^r) colonies were isolated from cells transformed with a linear 9.3-kb *Kpn*I restriction fragment from pDYC1. Southern blot analysis of
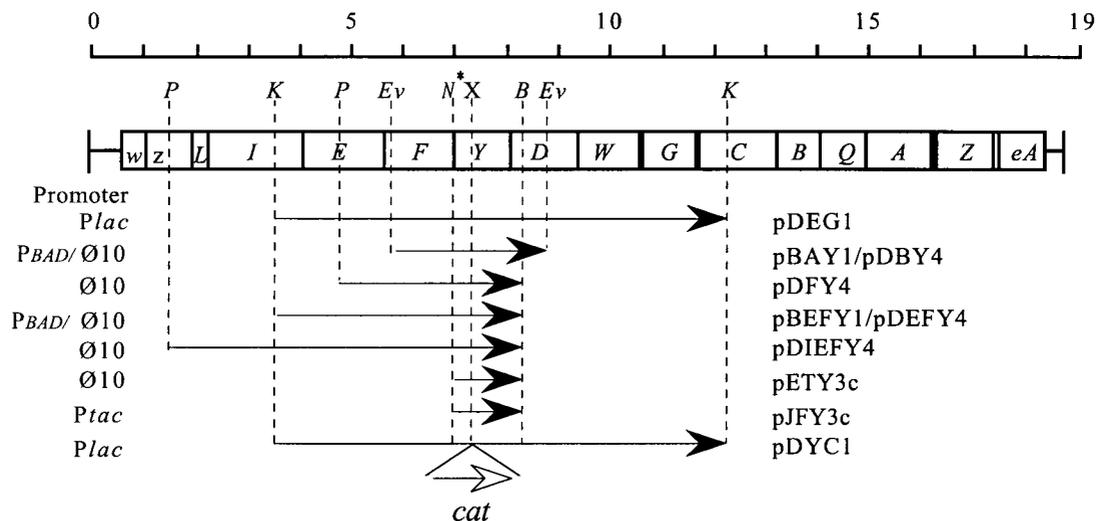

FIG. 1. Organization of the *mra* region and the plasmids constructed for the purpose of this study. The upper part shows the ORFs in the *mra* region to scale in kilobase pairs. The boxed letters refer to the genes within this region: *w*, mraW; *z*, mraZ; *L*, ftsL; *I*, ftsI; *E*, murE; *F*, murF; *Y*, mraY; *D*, murD; *W*, ftsW; *G*, murG; *C*, murC; *B*, ddlB; *Q*, ftsQ; *A*, ftsA; *Z*, ftsZ; and *eA*, envA. Also shown are the cloned fragments with direction of expression as indicated by arrowheads. The types of promoter used to express these ORFs are listed at the left. *P*, *Pvu*II; *K*, *Kpn*I; *Ev*, *Eco*RV; *N**, *Nde*I; *X*, *Xmn*I; and *B*, *Bgl*II. The *Nde*I site was introduced by PCR-directed mutagenesis.

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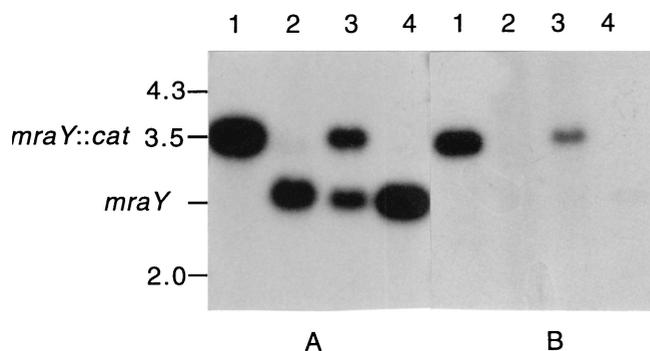
FIG. 2. Southern blot analysis of the genomic DNA from the partial diploid strains DSB1 (*mraY* wild type, lane 2) and DBYC1 (*mraY*::*cat* mutant, lane 3). Lanes 1 and 4 contain control DNAs *mraY*::*cat* (pDYC1) and *mraY* wild type (pDEG1), respectively. All of the DNAs were restricted with *Eco*RV, electrophoresed through agarose, and Southern blotted onto a nylon membrane. The blot was first probed with *mraY* (A) to show *mraY*::*cat* at 3.5 kb and *mraY* at 2.7 kb (lanes 1 and 4, respectively). Lane 2 shows strain DSB1 to have only wild-type *mraY* at 2.7 kb. Lane 3 shows both the wild-type and disrupted forms of *mraY* from DBYC1 DNA. Probing the same blot with *cat* (B) shows that only pDYC1 (lane 1) and DBYC1 (lane 3) contain the *mraY*::*cat* fragment at 3.5 kb.

the genomic DNA from one Cmp^r clone showed that both the wild-type and the disrupted copy of *mraY* were present (Fig. 2). This strain was designated DBYC1. P1 lysates were grown on DBYC1 and used to transduce C600T (*galK leu*::Tn*10* Tet^r) to Cmp^r. No Cmp^r transductants were recovered. When C600T carrying pUC18 or pDEG1 was then transduced with the same P1 lysate, Cmp^r transductants were recovered only from cells carrying pDEG1. The inability to transduce C600T (with or without pUC18) suggested that *mraY* may be an essential gene. The genetic linkage between *leu*::Tn*10* and *mraY*::*cat* was determined by measuring the cotransduction frequency of the two markers. Of the Cmp^r isolates screened, 78% were Tet^s Leu^+, consistent with the proximity of *leu* and *mraY*.

Complementation analysis of *mraY*::*cat*. The region required for the independent expression of *mraY* was identified by using several clones of *mraY* in the vector pT7-4 (17, 18), a multicopy plasmid lacking exogenous promoters. These plasmids were pDBY4, pDFY4, pDEFY4, and pDIEFY4 (Fig. 1). The expression of cloned DNA in these constructs is dependent on the presence of T7 RNA polymerase, usually supplied by an inducible (*lacUV5*) λ bacteriophage (λDE3, absent in the host strain, C600T). Using P1 transduction, we were able to introduce the *mraY*::*cat* allele into C600T carrying either pDEFY4 or pDIEFY4 but not when it was carrying the other pT7-4-based *mraY* clones, pDBY4 or pDFY4. Hara et al. (6) recently demonstrated that transcription of the first seven genes in the *mra* cluster is dependent on P_*mra*, a promoter located at the start of the *mra* region. Because complementation of *mraY*::*cat* was achieved only in multicopy number plasmids, it is possible that a weak promoter-like sequence which exists in the upstream DNA is sufficient to complement the null allele when present at a high copy number.

The *mraY*::*cat* phenotype. To study the effects of MraY depletion in growing cells, a 2.7-kb *Eco*RV fragment containing *mraY* was cloned into the vector pBAD18 (5) to create pBAY1 (Fig. 1). Expression from P_BAD can be induced or repressed with arabinose or glucose, respectively. A second clone, pBEFY1, was made by subcloning a 4.5-kb *Eco*RI/*Hin*dIII fragment from pDEFY4 into pBAD18 (Fig. 1). C600T containing either pBAD18 or the other plasmids was transduced with P1 lysates prepared from DBYC1. The only Cmp^r transductants

recovered contained either pBAY1 or pBEFY1. Cmp^r isolates carrying pBAY1 were arabinose dependent for growth as expected, whereas Cmp^r isolates carrying pBEFY1 were able to grow in the presence of either glucose or arabinose, as expected, since *mraY* can be expressed from P_*mraY* independently of P_BAD.

To achieve more-controlled regulation of expression of *mraY* from pBAY1 in strain DBYC2 (*galK mraY*::*cat* Cmp^r), the plasmid copy number was reduced by introducing the *pcnB*::*kan* allele (11) by P1 transduction to give strain DBYC3 (as DBYC2 *pcnB*::*Kan* Kan^r). DBYC3/pBAY1 was initially grown in Luria broth (LB) plus chloramphenicol with 0.2% arabinose at 37°C until the cells reached exponential phase. The method for culturing DBYC3/pBAY1 in either LB plus 0.2% arabinose (wt/vol) or LB plus 0.2% glucose (wt/vol) was as described earlier (1). After 80 min, the growth rate of the glucose-containing culture started to decrease, and growth stopped after 120 min (Fig. 3). The arabinose-containing culture continued to grow unaffected (Fig. 3). Cells from the arabinose-containing culture at 0 and 180 min were mainly short rods (Fig. 4). But, after 180 min in glucose, the cells were misshapen or had lysed (Fig. 4). Cells of C600T/pBAY1 cultured under the same conditions were indistinguishable from DBYC3/pBAY1 grown in 0.2% arabinose with respect to growth rate and cell morphology (data not shown).

The phenotype of the *mraY*::*cat* strain is very similar to that of mutants of peptidoglycan precursor genes (19). This suggests that synthesis of peptidoglycan is interrupted, presumably by a depletion in the pool of lipid I, causing cell death by autolysis. We conclude that *mraY* is an essential gene required for cell wall growth in *E. coli*.

Identification of MraY. The MraY polypeptide has not been detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis even when overproduced in vivo (12), although increased levels of enzyme activity have been observed (2, 8). We failed to detect radiolabelled MraY
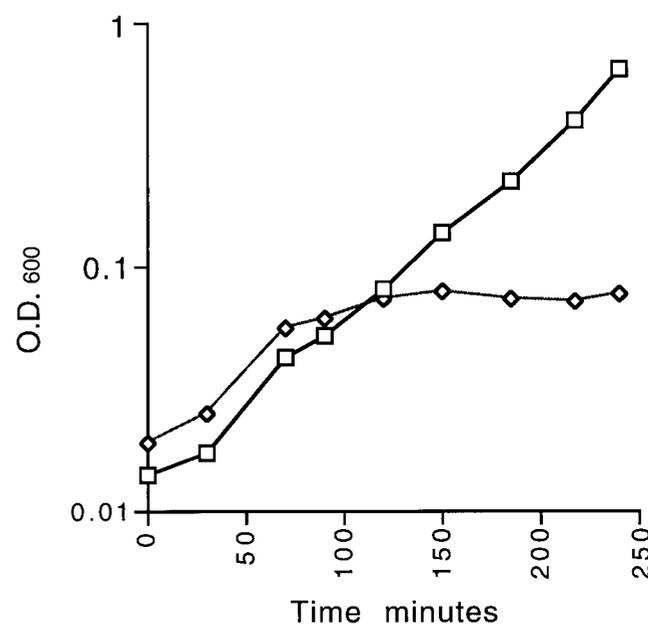

FIG. 3. Induction and repression of P_BAD::*mraY* in the *mraY*-null mutant strain DBYC3/pBAY1 cultured in LB plus kanamycin supplemented with either 0.2% (vol/vol) arabinose (□) or 0.2% (vol/vol) glucose (◇) at 37°C. O.D., optical density.
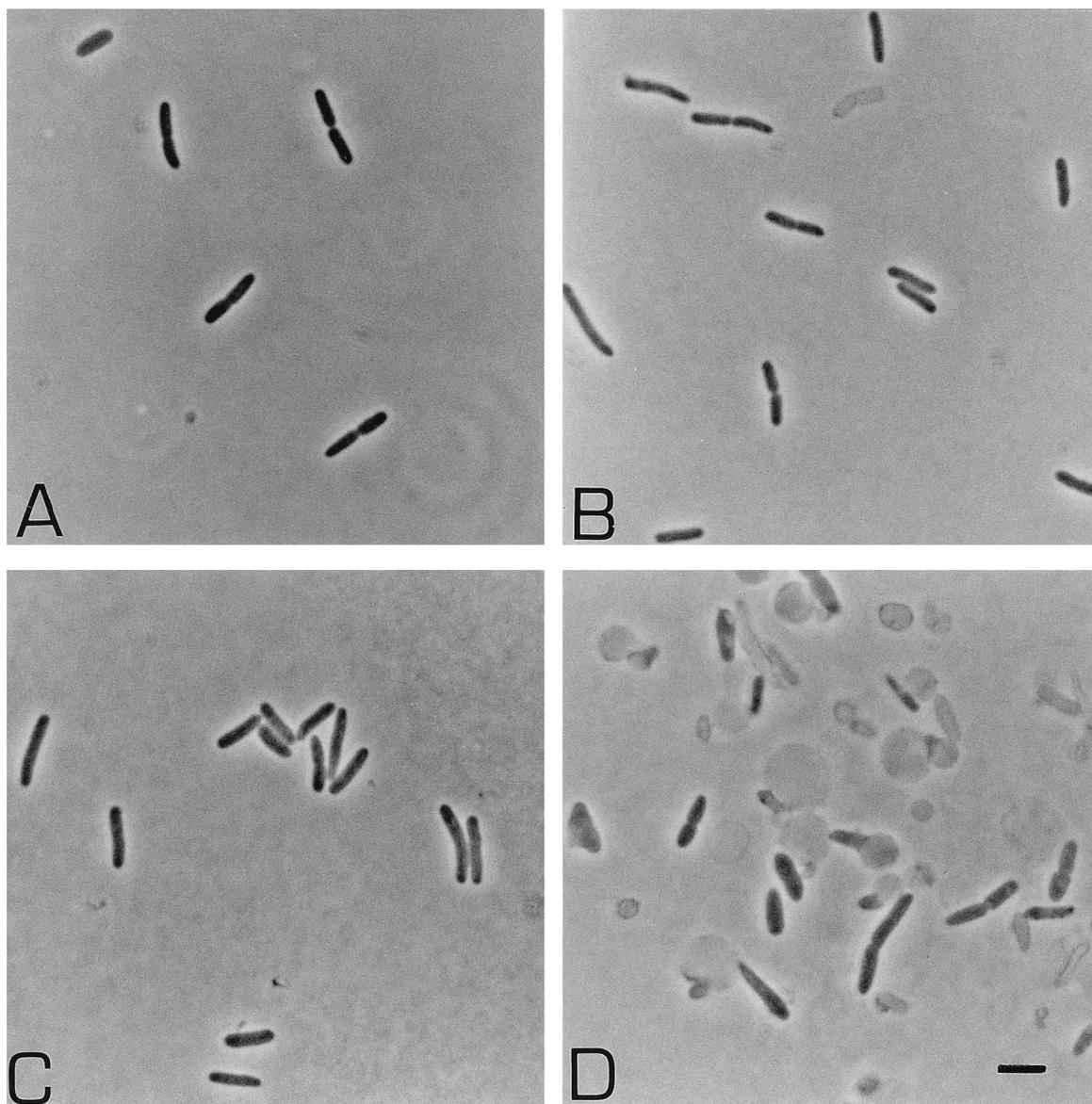
FIG. 4. Micrographs of DBYC3/pBAY1 grown in LB supplemented with either arabinose or glucose. (A and C) Cells from the arabinose-containing culture sampled at 0 and 180, respectively. (B and D) Cells from the glucose-containing culture sampled at 0 and 180 min, respectively. Bar, 5 μm.

from the T7 clones listed in Fig. 1 by SDS-PAGE. These included pETY3c, in which *mraY* was cloned in frame to the T7 gene 10 Shine-Dalgarno sequence by PCR-directed mutagenesis with primers DAV2 (5′ GGAGAATGGCATATGTTAG TTTGG 3′) and DAV3 (5′ CAATCAGATCTGCCGCCA 3′) (16). *mraY* was subcloned from pETY3c into pJF118EH (4) to create pJFY3c (Fig. 1). pJF118EH and pJFY3c DNA were used as the templates for in vitro translation. Analysis (SDS–10% PAGE) revealed that pJFY3c produced a peptide of ~40 kDa (Fig. 5) which was absent in the pJF118EH control (Fig. 5). In agreement with this result, pJFY3c complemented the null allele (introduced by P1 transduction to C600T/pJFY3c), whereas pJF118EH did not.
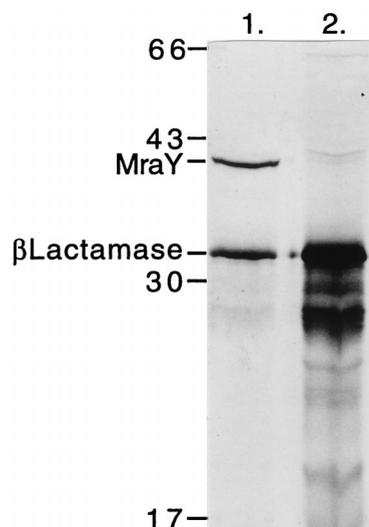

FIG. 5. SDS–10% PAGE analysis of the in vitro translation products (radiolabelled) produced by pJFY3c (lane 1) and pJF118EH (lane 2) after induction with 0.5 M isopropyl-β-D-thiogalactopyranoside. In lane 1 a unique polypeptide migrates as a 40-kDa peptide. This band is absent from the pJF118EH sample and is therefore presumed to be MraY.

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