JOURNAL OF BACTERIOLOGY, Aug. 1988, p. 3786–3788
0021-9193/88/083786-03$02.00/0

Vol. 170, No. 8

Determination of Gene Products and Coding Regions from the *murE-murF* Region of *Escherichia coli*

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We report the cloning of *murE* and *murF* genes and the identification of their gene products. The *murE* and *murF* genes encode diaminopimelic acid- and D-alanyl-D-alanine-adding enzymes, respectively, and both genes are involved in cell wall peptidoglycan biosynthesis in *Escherichia coli*.

In *Escherichia coli* K-12 there is a cluster of genes in the 2-min region of the genetic map that are all required for cell wall synthesis and cell division. The cluster consists of seven genes for cell wall peptidoglycan synthesis, *mraA*, *mraB*, *murE*, *murF*, *murG*, *murC*, and *ddl*; four cell division genes, *ftsI* (*sep* or *pbpB*), *ftsQ*, *ftsA*, and *ftsZ* (*sulB* or *sfiB*); a cell permeability gene, *envA*; and *secA*, which is involved in the secretion of cell envelope protein (1). To understand how cell wall peptidoglycan synthesis and septum formation are organized during cell growth in *E. coli*, a structural analysis of this gene cluster might be important. For this purpose, a number of clones encompassing part of the cluster have been isolated (5, 10, 13). In this report, we describe the localization of the *murE* and *murF* genes on the restriction map and the identification of their gene products.

One of the peptidoglycan precursors in the cytoplasmic steps of cell wall biosynthesis is UDP-*N*-acetylmuramyl-L-Ala-D-Glu-*m*-diaminopimelic acid-D-Ala-D-Ala (UDP-Mur NAc pentapeptide) (6). The amino acids are sequentially added to UDP-MurNAc by specific adding enzymes. Among these, diaminopimelic acid- and D-alanyl-D-alanine-adding enzymes are encoded by the *murE* and *murF* genes, respectively (8, 9). Mutants with temperature-sensitive mutations in these genes lyse at high temperatures, due to the impaired peptidoglycan synthesis. Genetic analysis shows that the *murE* and *murF* genes are adjacent to the *ftsI* gene, and Takeda et al. (18) showed that one of the chromosomal clones, pLC26-6, includes the segment from *leuA* to *murF*, as judged by genetic complementation tests with cosmid DNAs derived from an *E. coli* genomic library (4). Using this cosmid clone as starting material, we have subcloned DNA segments into plasmid vector pACYC184 (3) or pBR322 (2) in order to determine the precise positions of the *murE* and *murF* genes on the restriction map.

Figure 1 shows the restriction map of the *ftsI-murF* chromosomal region and plasmids harboring various segments. Plasmids pIM301 and pMS316 were constructed by subcloning of 6.1-kilobase (kb) *Sma*I-*Eco*RI and 2.6-kb *Pvu*II-*Pvu*II fragments, respectively, into the *Bam*HI site of the tetracycline resistance (Tc^r) gene in a pACYC184 vector after purification of the fragments followed by blunt-end ligation. Plasmids pAY7 and pAY10 are deletion derivatives of pIM301. pAY4 and pIM28 are clones harboring 4.4-kb *Kpn*I-*Hpa*I and 1.8-kb *Nar*I-*Mlu*I fragments, respectively, at the *Bam*HI site of the Tc^r gene in a pBR322 vector. pAY6 was made from pAY4 by deletion. pMS201 was constructed by cloning a 4-kb *Eco*RV fragment into pBR322 at the *Eco*RV site of the Tc^r gene.

To test whether a set of clones could genetically complement the thermosensitive mutations, we prepared $CaCl_2$-treated competent cells (7) of *E. coli* JE6602 [Hfr P4X *thi ftsI730*(Ts) *leuA thr*] for *ftsI*, JE6604 [F^- *murE*(Ts) *thr leu thi pyrF codA thyA argG ilvA his lacY tonA tsx phx supE ths dra sus uvrB str*] for *murE*, and JE6606 [same as for JE6604 but *murF*(Ts) *murE*$^+$] for *murF* and transformed them with a series of plasmid DNAs. The cell suspension mixed with DNA was separated into two parts after allowing time for expression of plasmid-coded genes and was plated onto two plates containing an appropriate antibiotic. These two plates were separately incubated at 30 or 40°C. The thermosensitivity of individual colonies of transformants on the plate incubated at 30°C was confirmed by streaking onto a new plate followed by incubation at 42°C. The results of the complementation tests are shown in Table 1. These results enabled us to identify the *murE* and *murF* gene products coded on plasmid clones. We employed the maxicell method

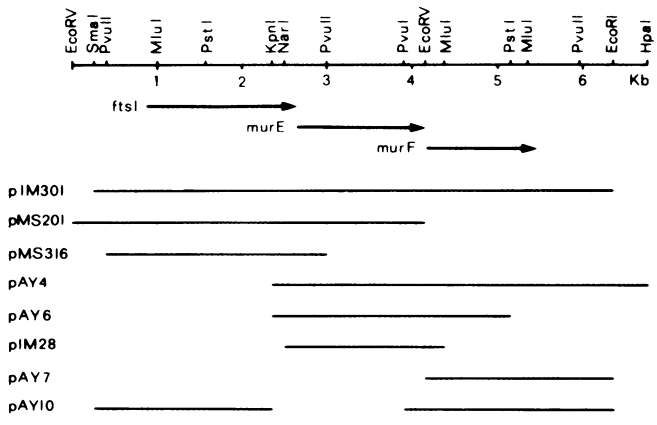

FIG. 1. Restriction map of the *ftsI-murF* chromosomal region and the fragments cloned in plasmids. The horizontal bars with plasmid names show the chromosomal regions which were cloned into plasmid vectors (see text). Arrows underneath the restriction map indicate proposed polypeptide-coding regions and their orientations of *murE* and *murF*, based on the molecular masses of native and truncated products determined in this work; the *ftsI* gene position has been precisely determined by DNA sequencing (12).

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TABLE 1. Genetic complementation of *ftsI*, *murE*, and *murF* mutations with plasmid clones

Plasmid	Complementation[a] with:		
	ftsI	*murE*	*murF*
pIM301	+	+	+
pMS201	+	+	−
pMS316	+	−	−
pAY4	−	+	+
pAY6	−	+	−
pIM28	−	+	−
pAY7	−	−	−
pAY10	−	−	+

[a] +, Complementation; −, no complementation.

developed by Sancar et al. (14). *E. coli* CSR 603 (*uvrA6 recA1 phr thr leu pro his thi arg lac gal ara xyl mtl str*) was transformed with purified plasmid DNA, and the plasmid-coded proteins were subsequently labeled with [^{35}S]methionine (Amersham Corp.) in UV-irradiated whole cells. The labeled proteins were analyzed on a sodium dodecyl sulfate-polyacrylamide gel (10%) (Fig. 2).

Plasmid pIM301 complemented all three mutations, *ftsI*, *murE*, and *murF*. This is consistent with the finding that the plasmid encodes three proteins in addition to chloramphenicol acetyltransferase (15) on the vector pACYC184 (Fig. 2). Of the three, the highest-molecular-mass protein, that of 60 kilodaltons (kDa), is the *ftsI* gene product (penicillin-binding protein 3) (12). Therefore, the other two proteins, those of 56 and 52 kDa, should be the *murE* and *murF* gene products. Genetic complementation of *murE* by using a set of clones showed that the functional *murE* gene is encoded in a 1.6-kb *Nar*I-*Eco*RV segment which corresponds to the overlapping region of pMS201 and pIM28 (Table 1 and Fig. 1). The pAY6 clone complemented only a *murE* mutant and produced a 56-kDa protein, indicating that the *murE* gene encodes the 56 kDa protein. This is further supported by evidence that the 56-kDa protein is produced in pAY4, which shows successful complementation of the *murE* mutation, but not in pMS316, which does not complement the defect. It thus appears that the 56-kDa protein is the *murE* gene product and is coded within the 1.6-kb region downstream of the *ftsI* gene. The truncated *murE* product of 29 kDa is produced by pMS316, suggesting that the transcriptional direction of the *murE* gene would be clockwise on the *E. coli* genetic map. The molecular weight of the truncated protein is higher than that of the predicted coding region, which could be due to transcriptional readthrough into the vector. Moreover, it seems that *murE* has its own promoter, because the transcriptional direction of *murE* in the insert is opposite to that of the Tc^r gene in pAY4 and pAY6, and *murE* is nevertheless expressed on these plasmids.

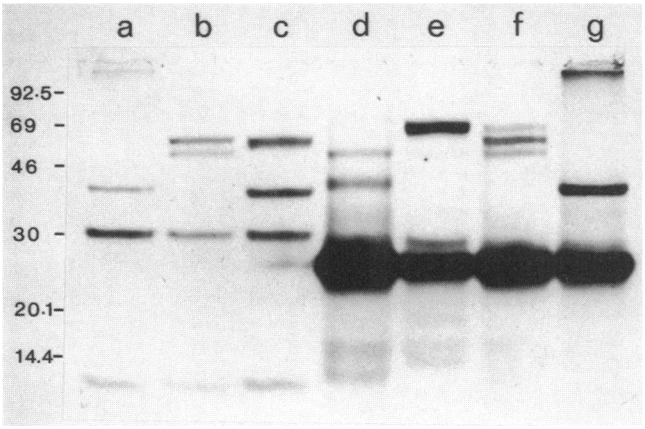

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of plasmid-coded proteins. The ampicillin resistance gene product (30 kDa) in lanes a through c and chloramphenicol acetyltransferase (27 kDa) in lanes d through g are coded by plasmid vectors of pBR322 and pACYC184, respectively (15, 16). Due to insertion of a chromosomal fragment, the tetracycline resistance gene product (34 kDa) (17) in lanes a and g, which are reference lanes for pBR322 and pACYC184 vectors, is missing in lanes b through f. Molecular mass standards are indicated in kilodaltons on the left. Lanes: a, CSR 306(pBR322); b, CSR 306(pAY4); c, CSR 306(pAY6); d, CSR 306(pAY10); e, CSR 306(pMS316); f, CSR 306(pIM301); g, CSR 306(pACYC184).

Since pIM301, pAY4, and pAY10 complemented the *murF* mutation and produced the 52-kDa protein in the maxicell experiment, it is clear that the *murF* gene encodes the 52-kDa protein on the 2.5-kb *Pvu*I-*Eco*RI fragment. Recently, van Heijenoort and his colleagues have partially purified D-alanyl-D-alanine-adding enzyme from *E. coli* and have shown that its molecular mass is 51 ± 2 kDa (11). This is quite consistent with our estimate. Furthermore, it seems likely that the *murF* gene is transcribed in the same direction as *ftsI* and *murE*, because pAY6 produces truncated *murF* product in addition to a native *murE* protein. These results are summarized in Fig. 1.

We are grateful to Y. Onishi for help in preparing the manuscript and to M. Soma for technical assistance.

This work was supported by a grant from the Ministry of Education, Science and Culture of Japan to Y.H. I.N.M. was funded by a JSPS postdoctoral fellowship.

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