

## NOTES

# Nucleotide Sequence of the *rodA* Gene, Responsible for the Rod Shape of *Escherichia coli*: *rodA* and the *pbpA* Gene, Encoding Penicillin-Binding Protein 2, Constitute the *rodA* Operon

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The *rodA* gene, which is responsible for the rod shape of *Escherichia coli*, was located 5 nucleotides downstream of another rod-shape-determining gene, *pbpA*, encoding penicillin-binding protein 2. The coding region for the RodA protein was 1,110 base pairs in length. Two plasmids, carrying a *rodA-lacZ* gene fusion with and without the *pbpA* promoter upstream of the gene fusion, were constructed. On the basis of the difference between the expression levels of the  $\beta$ -galactosidase activity dependent on and independent of the *pbpA* promoter, we concluded that the *pbpA* and *rodA* genes constitute a single transcriptional unit called the *rodA* operon.

*Escherichia coli* mutants with a spherical shape have been isolated and studied genetically and biochemically; *rodA* (also called *mrdB*) (12, 16, 30), *pbpA* (also called *mrdA*) (12, 23, 30), *envB* (12, 21), *mre* (32), and *cya* (13) mutants have been reported on. Both *pbpA* (23) and *rodA* (15, 16) mutants grow as spheres and are resistant to mecillinam. The *pbpA* mutants are defective in penicillin-binding protein (PBP) 2 (23), but PBP 2 of the *rodA* mutant is normal (15). The *pbpA* and *rodA* genes are located on the *E. coli* chromosome at about 15 min in the *leuS-lip* region (3, 16, 23, 24, 30). Both genes were cloned and shown to be contiguously located on the chromosome (3, 24, 25), each gene being expressed through its respective promoter (3). The *rodA* gene product (RodA protein) is a cytoplasmic membrane protein with a molecular weight of 31,000 and is not made as a preprotein (26). The RodA protein is essential for catalysis by PBP 2 (the *pbpA* gene product) of the peptidoglycan transglycosylase and transpeptidase reactions in membrane preparations (10, 11).

**Nucleotide sequence of the *rodA* gene.** The *rodA* gene is located in the 1.6-kilobase *KpnI-BamHI* fragment carried by pMA106 (3). The DNA fragment was completely sequenced on both strands by the method of Maxam and Gilbert (17). Figure 1 shows the nucleotide sequence of the *rodA* gene, including the sequences of the 3'-terminal part of the *pbpA* gene (2) and the 5'-terminal part of the *rlpA* gene for a 36,000-molecular-weight lipoprotein (36K lipoprotein) (29). The DNA fragments from the *TaqI*-A site (nucleotide -81) and *TaqI*-B site (nucleotide 48) (Fig. 1) to the *BamHI* site (nucleotide 1339; position not shown in Fig. 1; see reference 29) were inserted in the *HindIII* site of pBR322 downstream of the *bla* promoter, P1 (27), and plasmids pTAQ-A and pTAQ-B, respectively, were constructed. pTAQ-A was able to complement a *rodA* mutant, strain SJC21 (3), but pTAQ-B was not (data not shown). On this basis, the *rodA* gene was considered to be located in the region of nucleotides 1 to

1110 (Fig. 1). The deduced amino acid sequence of the RodA protein comprised 370 amino acid residues (Fig. 1), and its molecular weight was calculated to be 40,093.

Two possible Shine-Dalgarno sequences (7, 22), GGAGG (nucleotides -15 to -11) and AGGA (nucleotides -13 to -10), precede the initiation codon, ATG (nucleotides 1 through 3), for the coding sequence (Fig. 1). The *rodA* gene is expressed independently of the *pbpA* gene (3) and was located close to and downstream of (5-nucleotide spacing) the *pbpA* gene, which indicated that the promoter for the *rodA* gene is present in the region of the *pbpA* gene.

The hydropathy profile of the RodA protein (data not shown), obtained according to the method of Kyte and Doolittle (14), revealed that the RodA protein is extremely hydrophobic. The average hydropathy of each 19-residue segment of the sequence suggested that the RodA protein has at least nine membrane-spanning segments and is located in the cytoplasmic membrane, as previously reported (26).

The enzymatic function of the RodA protein remains unknown. It was shown that PBPs 1A (9) and 1B (19, 20, 28, 31), responsible for cell elongation, and PBP 3 (8), responsible for septum formation, are bifunctional enzymes having transglycosylase and transpeptidase activities. However, PBP 2 shows the two activities only in the presence of the RodA protein as measured in membrane preparations (10, 11). Since the penicillin-binding site of PBP 2 is considered to be the active site of transpeptidase and a water-soluble form of PBP 2 (PBP 2\*) exhibits penicillin-binding activity in the absence of the RodA protein (1), PBP 2 seems to catalyze the transpeptidase reaction by itself. In that case, the RodA protein would regulate the transglycosylase activity of PBP 2 or catalyze the transglycosylase reaction by itself. However, it is also possible that the RodA protein regulates the transpeptidase activity of PBP 2. In any case, the RodA protein seems to form a complex with PBP 2 in the cytoplasmic membrane.

**Expression of the *rodA* gene.** The nucleotide sequence shown in Fig. 1 indicates that the directions of transcription of the *pbpA* and *rodA* genes are identical, and the initiation

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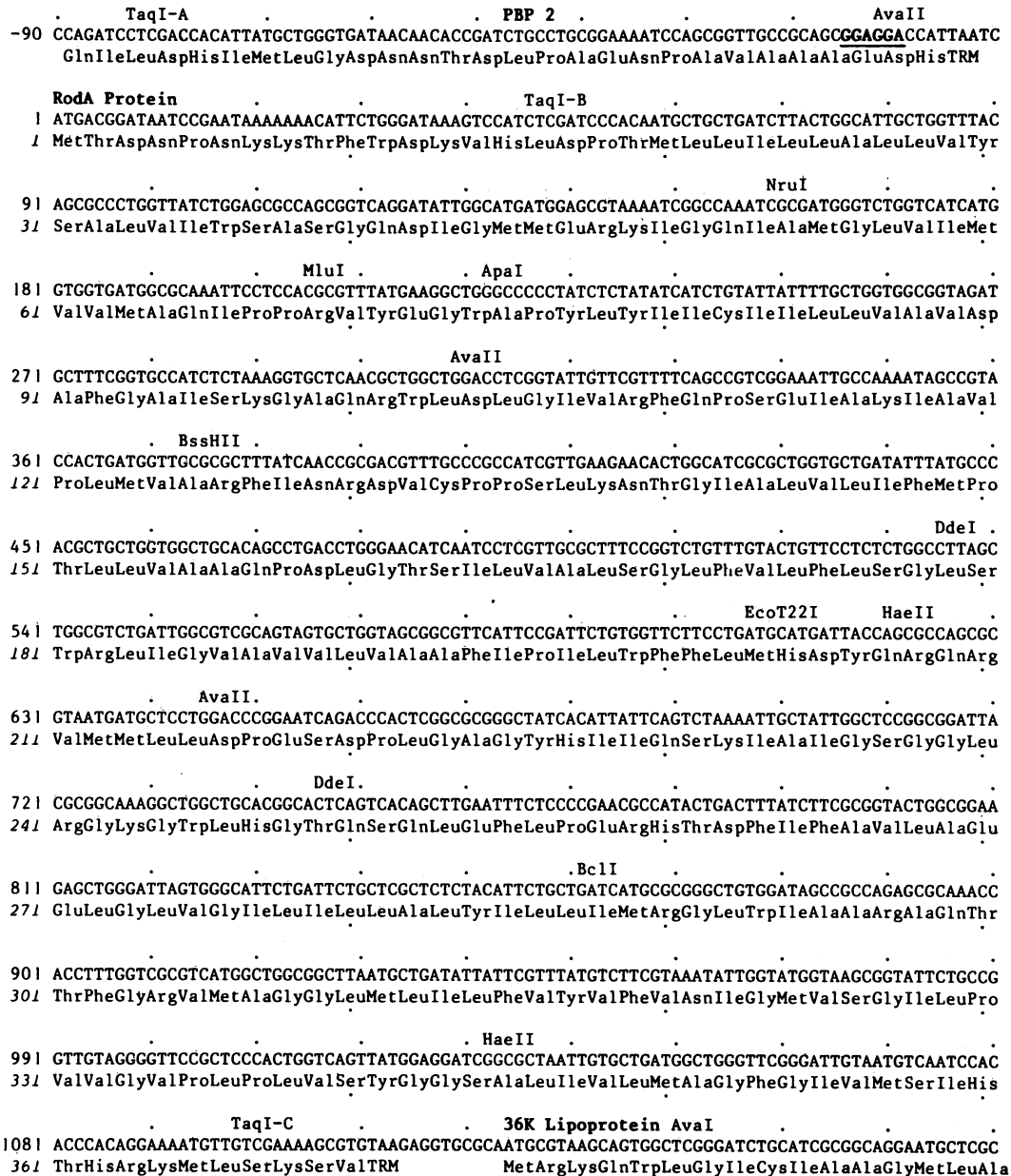


FIG. 1. Nucleotide sequence of the *rodA* gene and its 5'- and 3'-flanking regions and deduced amino acid sequence of the RodA protein. The coding region for the *rodA* gene (nucleotides 1 to 1110) follows the termination codon, TAA (nucleotides -5 to -3), of the *pbpA* gene, encoding BBP 2 (2), with a 2-nucleotide spacing. Two possible Shine-Dalgarno sequences, GGAGG (nucleotides -15 to -11) and AGGA (nucleotides -13 to -10), preceding the coding sequence for the *rodA* gene, are underlined. The ribosome-binding sequences are located in the coding region for the *pbpA* gene. The amino acid residues of the RodA protein (residues 1 to 370) are numbered from the N terminus in italics. The *rplA* gene, encoding the 36K lipoprotein (29), starts with a 13-nucleotide spacing downstream of the *rodA* gene. The restriction sites for *TaqI*, *AvaII*, *NruI*, *MluI*, *ApaI*, *BssHII*, *DdeI*, *EcoT22I*, *HaeII*, *BclI* and *AvaI* are indicated. TRM, Termination codon.

codon, ATG (nucleotides 1 to 3), of the *rodA* gene is preceded by the termination codon, TAA (nucleotides -5 to -3), of the *pbpA* gene with a 2-nucleotide spacing. These findings suggested that the *pbpA* and *rodA* genes constitute a single transcriptional unit. Therefore, we constructed the *rodA-lacZ* gene fusion with or without the *pbpA* promoter upstream of the gene fusion. The 3.4-kilobase *MluI* DNA fragment carried by pHS202 (3) contains the *pbpA* promoter, the *pbpA* gene, and the 5'-terminal part of the *rodA* gene, including the *rodA* promoter (Fig. 1). The DNA fragment was inserted in *BamHI*-digested pMC1403 (4) so as to be in the correct translational frame in the fusion protein between

RodA and  $\beta$ -galactosidase, and pKAM411 was obtained. Then the 1.5-kilobase *EcoRI-BglII* fragment containing the promoter for and the 5'-coding region of the *pbpA* gene was deleted from pKAM411, resulting in pKAM813 carrying the *rodA* promoter and the gene fusion.

$\beta$ -Galactosidase activities were determined according to the method of Miller (18) in strain MC1061 *recA56 srlC300::Tn10*, a conjugational derivative of strain MC1061, carrying  $\Delta lacX74$  and *rpsL* (5), and strain JC10240, carrying HfrPO45, *recA56*, and *srlC300::Tn10* (6), harboring pKAM 411 and pKAM813. pKAM411 gave 330 U of  $\beta$ -galactosidase activity per mg of cellular protein, whereas pKAM813 gave

2.0 U of activity, which indicated that the *pbpA* promoter functions at more than 100 times the rate of the internal *rodA* promoter. On the basis of the nucleotide sequence (Fig. 1) and the effect of the *pbpA* promoter on expression of the gene fusion, we concluded that the *pbpA* and *rodA* genes constitute a single transcriptional unit, which we call the *rodA* operon, whereas the *rodA* gene has its own promoter within the *pbpA* gene.

There are two genes, encoding a 7,700-dalton and a 17,000-dalton protein, between the *pbpA* promoter and the *pbpA* gene (2, 29). Moreover, the *rtpA* gene, encoding the 36K lipoprotein (previously called the 54K protein), starts with a 13-nucleotide spacing downstream of the *rodA* gene (29) (Fig. 1). Therefore, it is very likely that the *rodA* operon contains these three genes in addition to the *rodA* and *pbpA* genes.

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