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 β-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 pTQA-A and pTQA-B, respectively, were constructed. pTQA-A was able to complement a rodA mutant, strain SJC21 (3), but pTQA-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 hydrophathy 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 hydrophathy 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 1110 to 1113), of the pbpA gene, encoding PBP 2 (2), with a 2-nucleotide spacing. Two possible Shine-Dalgarno sequences, GGAGG (nucleotides 115 to 117) and AGGA (nucleotides 119 to 120), 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, Avall, NruI, MutI, Apal, BssHI, DdeI, EcoT22I, HaeII, BclI, Dde1, HaeII, and AvaII are indicated. TRM, Termination codon.

codon, ATG (nucleotides 1 to 3), of the rodA gene is preceded by the termination codon, TAA (nucleotides 1110 to 1113), 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 MutI DNA fragment carried by pH520 (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 β-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.

β-Galactosidase activities were determined according to the method of Miller (18) in strain MC1061 recA56 srcC300::Tn10, a conjugal derivative of strain MC1061, carrying ΔlacX74 and rpsL (5), and strain JC10240, carrying HfrP045, recA56, and srcC300::Tn10 (6), harboring pKAM411 and pKAM813. pKAM411 gave 330 U of β-galactosidase activity per mg of cellular protein, whereas pKAM813 gave
2.0 U of activity, which indicated that the \textit{ppbA} promoter functions at more than 100 times the rate of the internal \textit{rodA} promoter. On the basis of the nucleotide sequence (Fig. 1) and the effect of the \textit{ppbA} promoter on expression of the gene fusion, we concluded that the \textit{ppbA} and \textit{rodA} genes constitute a single transcriptional unit, which we call the \textit{rodA} operon, whereas the \textit{rodA} gene has its own promoter within the \textit{ppbA} gene.

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

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**LITERATURE CITED**


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