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Physical Map Location of the *rpoN* Gene of *Escherichia coli*

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The *Escherichia coli rpoN* gene, encoding an RNA polymerase sigma factor, σ^{54}, has been genetically mapped to 70 min on the *E. coli* K-12 chromosome (3), cloned (8), and sequenced (19). In this study, we cloned the *rpoN* region from phage DNAs 7E3 (522) and 3G10 (523) of the Kohara miniset library (11) and determined its nucleotide sequence. The restriction map made from the nucleotide sequence was laid on Rudd's physical map (17), the unique *Bam*HI site in this region being matched with the same site on the physical map. The results defined the position for the *rpoN* gene at around 3364 kb (Fig. 1). There were four open reading frames (ORFs) with the same transcription direction in this region: ECRNORF1 (241 amino acids), ECRNORF2 (= *rpoN*; 477 amino acids), ECRNORF3 (95 amino acids), and ECRNORF4 (163 amino acids). Similar ORFs were arranged in the same order in chromosomes of *Rhizobium meliloti* (2), *Pseudomonas putida* (9), *Klebsiella pneumoniae* (13, 14), *Thiobacillus ferrooxidans* (4), and *Azotobacter vinelandii* (15), suggesting that this region has a common physiological significance in these bacteria.

The putative protein from ECRNORF1, which is on direct matching 59.2 and 57.5% identical to the *T. ferrooxidans* (4) and *R. meliloti* (2) counterparts, respectively, resembles a family of membrane-binding transport proteins having two consensus nucleotide-binding motifs, such as *E. coli* CysA (20) and LivF (1). *E. coli* RpoN shows sequence identities of 88.7, 51.4, 48.8, 48.5, and 38.7% to those of *K. pneumoniae* (13), *A. vinelandii* (14), *P. putida* (9), *T. ferrooxidans* (4), and *R. meliloti* (16), respectively. The putative protein from ECRNORF3 has 87.4, 53.8, 53.8, and 33.3% identities to those from *K. pneumoniae* (13), *A. vinelandii* (15), *P. putida* (9), and *T. ferrooxidans* (4), respectively. The protein has 36.5% matching amino acids in 95 residues, 52.0% in 25 residues, and 35.1% in 37 residues compared with a putative protein from an ORF upstream from the *E. coli pheA* operon (7), another putative protein from an ORF upstream from the *Bacillus subtilis div*$^+$ gene (18), and spinach chloroplast ribosomal protein (22), respectively. The putative protein from ECRNORF4 matches 88.8% compared with that of *K. pneumoniae* (13). Interestingly, it has 25.2% matching amino acids in 131 residues, 24.1% in 112 residues, and 24.3% in 107 residues compared with *E. coli* mannitol enzyme II (12), *Salmonella typhimurium* fructose enzyme III (6), and *Staphylococcus carnosus* mannitol enzyme III (5) of the bacterial phosphotransferase system. The protein also has 36.1% matching amino acids in 36 residues and 35.1% in 37 residues compared with *Caulobacter crescentus* FlaY (10) and *R. meliloti* nodulation protein C (21), respectively.

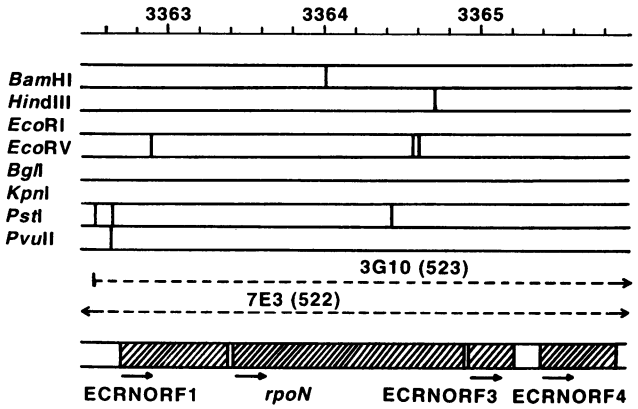

FIG. 1. Location of the *rpoN* gene on the physical map of *E. coli*. This region is covered by the Kohara phages 3G10 (523) and 7E3 (522). The latter clone extends to both flanking regions. The *Eco*RV site near the C-terminal coding sequence of *rpoN* contained two sites. The *Pst*I site near the 5′ end of this region also contained two sites. A *Pvu*II site was newly found at the 5′ end of this region. The hatched areas depict ORFs, which are transcribed as indicated by arrows.

Nucleotide sequence accession number. The nucleotide sequence data obtained here will appear in the DDBJ, EMBL, and GenBank data bases under accession no. D12938.

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