Physical Map Location of the rffC and rffA Genes of Escherichia coli

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Enterobacterial common antigen (ECA) is an outer-membrane glycolipid synthesized by all members of the Enterobacteriaceae (3, 7, 9). The carbohydrate portion of ECA consists of a linear heteropolysaccharide chain comprising the amino sugars N-acetyl-D-glucosamine (GlcNAc), N-acetyl-D-mannosaminuronic acid (ManNAcA), and 4-acetamido-4,6-dideoxy-D-galactose (Fuc4NAc) (6, 8). These sugars are linked to one another to form the trisaccharide repeat unit \( \text{GlcNAc-(1→4)}\)\(\text{β-D-ManNAcA-(1→4)}\)\(\text{α-D-GlcNAc-(1→6)}\). Individual heteropolysaccharide chains are linked to phosphatidic acid through phosphodiester linkage (4). The genetic determinants of ECA synthesis include the rfe-rff gene cluster located at approximately 85 min on the Escherichia coli chromosome (5, 10). The rfe-rff gene cluster was recently cloned in the cosmid pHC79, and restriction endonuclease mapping and complementation studies utilizing the original cosmid clone and six subclones established the partial gene order rfe-rffDirffE-rffA/rffC-rffT-rffM (11).

The entire DNA sequence of the E. coli chromosome from 84.5 to 86.5 min, including the rfe-rff gene cluster, was recently reported by Daniels et al. (1). Three open reading frames, o181, o299, and o416, were designated as possible coding sequences for the rffA and rffC genes (Fig. 1). The rffA gene codes for the transaminase that catalyzes the conversion of thymidine diphosphate (TDP)-4-keto-6-deoxy-D-glucose to TDP-4-amino-4,6-dideoxy-D-galactose, the immediate precursor of TDP-Fuc4NAc, whereas the rffC gene appears to be involved in ECA chain elongation (12). Exper-

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**FIG. 1.** Physical map locations of the rffC and rffA genes within the rfe-rff gene cluster of the E. coli chromosome. (A) The sequence of open reading frames and genes within the rfe-rff gene cluster is presented as determined by Daniels et al. (1). The locations of these open reading frames and genes on the revised physical map of Rudd (14) from 3994 to 4008 kb is indicated. Plasmid pRL106 was constructed by subcloning the 2.8-kb XhoI-SmaI fragment containing o181 and o299 from pCA32 (11) into the multicloning site of pBluescript KS. Plasmids pRL115 and pRL113 were derived from pRL106 by deletion of the indicated XhoI-Bpu1102 and EcoRV-EcoRV fragments (cross-hatched areas), respectively. (B) The physical map of Daniels et al. (1). (C) The physical map of Rudd (14). Abbreviations: B, BamHI; H, HindIII; R, EcoRI; V, EcoRV; G, BglII; K, KpnI; S, FspI; P, PvuII.

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iments were carried out to determine which of these putative open reading frames encodes the rffA and rffC gene products. A 2.8-kb XhoI-SmaI fragment containing o181 and o299 as the only complete open reading frames was sub-cloned into the multicloning site of pBluescript KS to give plasmid pRL106 (Fig. 1). Passive hemagglutination assays using mouse anti-ECA polyclonal antibodies (13) revealed that the defects in ECA synthesis in mutants possessing Tn10 insertions in either rffA or rffC were complemented by pRL106. No promoter sequences were identified in the 2.8-kb insert fragment, and expression of the rffA and rffC genes was dependent on the lac promoter of the vector. These data preclude the possibility that o116 is the coding sequence for either rffA or rffC. Deletion of the 1.8-kb EcoRV fragment from pRL106 by digestion with EcoRV followed by ligation gave rise to the 4.0-kb plasmid pRL113, which contained o181 as the only complete open reading frame. Passive hemagglutination assays established that ECA synthesis was restored in ECA-negative mutants possessing Tn10 insertions in rffC following transformation with pRL113. In contrast, pRL113 was unable to complement the defect in ECA synthesis in mutants possessing Tn10 insertions in rffA. These data support the conclusion that o181 encodes the rffC gene product; they also provide indirect evidence that o299 is the structural gene for the transaminate. Direct evidence that o299 encodes the rffA gene product was obtained by digestion of pRL106 with Bpu1102 and XhoI followed by end filling and blunt-end ligation (Fig. 1). The resulting 5.1-kb plasmid, pRL115, contained o299 as the only complete open reading frame in the cloned fragment. Plasmid pRL115 was used to transform ECA-negative mutants possessing Tn10 insertions in either the rffA or the rffC gene. Passive hemagglutination assays revealed that pRL115 was able to complement the defect in ECA synthesis only in rffA::Tn10-insertion mutants. Accordingly, these studies establish o181 and o299 as the rffC and rffA genes, respectively. The nucleotide sequences of rffC and rffA predict proteins of 19.6 and 33.2 kDa, respectively (1). In addition, restriction map data in the region of rffC and rffA correspond to the region between 4002 and 4004 kb of the revised E. coli genomic restriction map of Rudd (14) and to the region between 3733 and 3730 kb of the restriction map of Kohara et al. (which is reversed in this region with respect to the Rudd map) (2).

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REFERENCES


