

E. COLI MAP†

Physical Locations of Genes in the *rne (ams)-rpmF-plsX-fab* Region of the *Escherichia coli* K-12 Chromosome

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The *plsX* gene of *Escherichia coli* is involved in membrane lipid synthesis in a biochemically undefined manner. A defect in *plsX* (*plsX50*) together with *plsB26* (encoding a K_m -defective *sn*-glycerol 3-phosphate acyltransferase) is required for conferral of a glycerol-3-phosphate-auxotrophic phenotype (5). The *plsX* gene maps near min 24 on the linkage map of *E. coli*, while *plsB* maps near min 92 (1, 5). Recently, several genes from the 24-min region of the linkage map have been cloned and sequenced, including *rne (ams)*, *rpmF*, *fabHDG*, and *acpP* (2, 3, 6, 8-11). Each of these genes is located on lambda phage 14C1 (235 of the miniset) of the Kohara library (4). We have found that the same phage complements a *plsB26 plsX50* mutant strain. The *plsX* gene was located by subcloning of restriction fragments from phage 235 together with complementation and recombination tests. Approximately 4,900 bp of DNA surrounding the *plsX* gene was sequenced (from coordinates 1163.3 to 1168.2) (Fig. 1). The DNA sequence obtained contained information from three nonoverlapping neighboring sequences in GenBank and thus closed two gaps in the sequence of the chromosome. A map of the region based on the merged nucleotide sequences is shown in Fig. 1A. The physical map of Kohara et al. (4) (Fig. 1B) is in relatively

good agreement with the restriction map predicted by the nucleotide sequence, except the *HindIII* and *EcoRV* sites were missing in the *rne (ams)* region.

The first gap that was closed contained the DNA from the *PstI* site within *orfX* (3) to the *SalI* site located just upstream of the *g30k-rpmF* operon (9). Knowledge of the complete nucleotide sequence allowed translation of *orfX* to a termination codon which would result in a protein of 320 amino acids with a predicted molecular weight of 36,008. This protein may be the 31-kDa protein identified by Claverie-Martin et al. (2, 3). Another open reading frame, labeled *orfY* (Fig. 1), was found within the first gap. The open reading frame is comprised of 207 codons and encodes a protein with a predicted molecular weight of 23,226. Plasmids containing this DNA region directed the synthesis of a protein of 23 kDa in a maxicell system, and a similarly sized protein was overexpressed when an appropriate restriction fragment was cloned into an expression vector (7). The functions of the OrfX and OrfY proteins are not known. The amino acid sequence of OrfY exhibited no apparent significant similarity with the translated sequences in GenBank.

The second gap contained the DNA from the leftmost *PstI* site located near coordinate 1165 (Fig. 1) to the *NruI* site

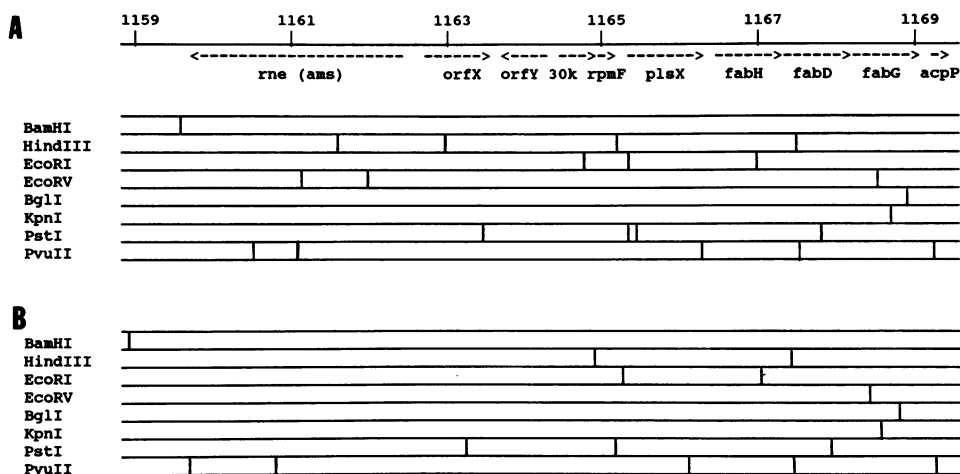


FIG. 1. Physical map of the *rne (ams)-rpmF-plsX-fab* region of the *E. coli* chromosome. (A) The scale of Kohara et al. (4) is given at the top in kilobase pairs. The restriction map is based on the nucleotide sequence, with the positions of the genes indicated above the map. GenBank accession numbers for the sequenced regions are as follows: *rne (ams)-orfX*, M62747 and M36288 (3); *orfX-orfY*, M96791 (this work); *g30k-rpmF*, M29698 (9); *plsX*, M96793 (this work); *fabH*, M77744 (10); *fabD*, Z11565 and M87040 (6, 11); and *fabG-acpP*, M84991 (8). (B) The physical map of Kohara et al. (4) is shown.

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upstream of *fabH* (10). The *plsX* gene was located in this region between *rpmF* and *fabH* and is transcribed in the same direction. The *plsX* gene comprises 346 codons and encodes a protein with a predicted molecular weight of 37,100. A protein of 36 kDa was identified as the *plsX* gene product by using a maxicell system (7).

With the closure of the two gaps in the nucleotide sequence, a continuous sequence of 10,133 bp of DNA in this region is now complete. The positions of all of the genes are known, and all of the predicted gene products have been identified.

Nucleotide sequence accession numbers. The GenBank accession numbers for the sequences discussed here are M96791 (*orfX-orfY*) and M96793 (*plsX*).

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