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Location of the *hemE* Gene on the Physical Map of *Escherichia coli*

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In *Escherichia coli*, heme, siroheme, and vitamin B_{12} are produced via the common tetrapyrrole pathway. The genes involved in the early part of the pathway, from aminolevulinate to uroporphyrinogen III formation, have been cloned (6, 7, 10). In this study, we isolated a strain with a mutation in the *hemE* gene (which encodes uroporphyrinogen decarboxylase), cloned the *hemE* gene, and determined its location on the *E. coli* physical map.

Isolation and characterization of an *E. coli hemE* mutant. Strain W3110 was infected with λ *b*221 *c*I857 Tn*5* (2) at 37°C and plated on L agar (3) containing 10 μg of kanamycin per ml. Under these conditions, the phage, which lacks the attachment site, cannot lysogenize. The resulting kanamycin-resistant colonies are therefore due to transposition of the Tn*5*. Mutants with insertions in *hemE*, *hemF*, *hemG*, or *hemH* should accumulate porphyrins; this would cause the colonies to fluoresce under long-wave-length UV light. Additionally, *hem* mutants require a fermentable carbon source. Therefore, small fluorescent colonies were scored for glucose dependence. Those colonies that were glucose dependent were then scored for the absence of heme-associated peroxidase activity with 3,3′,5,5′-tetramethylbenzidine (9). All of the strains tested lacked peroxidase activity. Tetrapyrrole intermediates were extracted from these strains with ethyl acetate-acetic acid (4). The esterified tetrapyrroles were identified by thin-layer chromatography (4). Among the mutants was one that overproduced uroporphyrinogen III. This strain was designated AJB550.

Determination of Tn*5*-containing restriction fragment sizes. AJB550 genomic DNA was digested with *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I, and *Pvu*II and blotted to nitrocellulose. A Tn*5*-containing plasmid, pSUP2021 (8), was used to probe the DNA. The probe hybridized to the following fragments (corrected for the Tn*5*): *Bam*HI, 2.2 kb; *Eco*RI, 15 kb; *Eco*RV, 1.4 kb; *Hin*dIII, 20 kb; *Pst*I, 10.6 kb; and *Pvu*II, 5.0 kb. Fragments of these sizes, except for *Eco*RV, can be found centered at approximately 3508 kb on the map of Kohara et al. (5). This location agrees with the location of *hemE* on the *E. coli* genetic map (1).

Complementation of AJB550. A 3.7-kb *Eco*RI-*Pvu*II fragment and a 2.2-kb *Bam*HI fragment were subcloned from the Kohara phage 7B7 (kindly provided by F. Blattner) into pBR328. This phage contains a portion of the *E. coli* chromosome from 3502 to 3520 kb (5) (Fig. 1). The plasmids were transformed into AJB550 by electroporation. The plasmid containing the *Eco*RI-*Pvu*II fragment (pCAP101) complemented AJB550, while the plasmid containing the *Bam*HI fragment (pCAP99) did not complement the mutant. Restriction mapping of 7B7 demonstrated that there are two additional unmapped *Eco*RV sites in the insert. One is within the 2.2-kb *Bam*HI fragment, and the other is within the 4.0-kb *Bam*HI fragment. Restriction mapping of pCAP99 indicated that the first *Eco*RV site is 0.6 kb from the left end of the 2.2-kb *Bam*HI fragment. The *Eco*RV site within the 4.0-kb *Bam*HI fragment has not been precisely mapped. Because of this, and because we have not confirmed the existence of several other *Eco*RV sites located in this region of the Kohara map, the *Eco*RV restriction map is not depicted in Fig. 1.

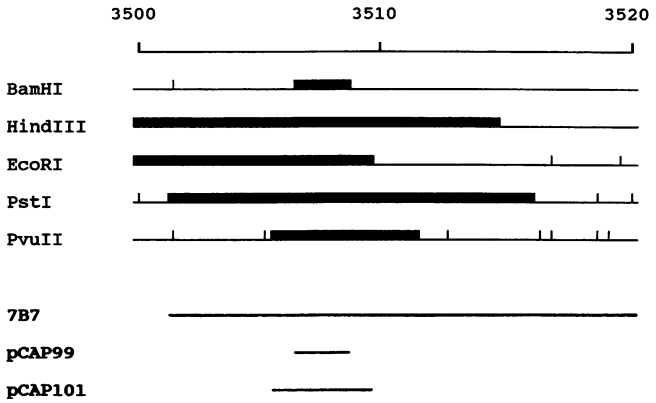

FIG. 1. Restriction map of the region surrounding *hemE*. The heavy lines indicate the restriction fragments that contained the Tn*5* insertion in AJB550.

These results demonstrate that the *hemE* gene is contained within a 3.7-kb *Eco*RI-*Pvu*II fragment located at 3506 to 3510 kb on the map of Kohara et al. (5). While the Southern blots indicate that the Tn*5* insertion in AJB550 is located between 3,505 and 3,507 kb, the inability of pCAP99 to complement AJB550 suggests that the *hemE* gene spans the *Bam*HI site between the 4-kb *Bam*HI fragment and the 2.2-kb *Bam*HI fragment.

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