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Localization of the Ferredoxin (*fdx*) Gene on the Physical Map of the *Escherichia coli* Chromosome

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Escherichia coli contains a [2Fe-2S] ferredoxin first described by Knappe and colleagues (1, 5). The physiological function of the protein is not known, but its properties are similar to those of ferredoxins from animal mitochondria and *Pseudomonas putida*, which mediate electron transfer to cytochrome P-450 enzymes. We have recently cloned, sequenced, and overexpressed the gene (designated *fdx*) encoding this ferredoxin (4). Two overlapping clones, totaling ~5 kb of DNA, were obtained from two different genomic libraries. Clone 6 was isolated from a library derived from *E. coli* K-12 strain W1485 (lot 1331; Clontech, Palo Alto, Calif.; ATCC 12435) and included most of the coding sequence and ~2 kb of 3′ noncoding DNA. Clone 10 was isolated from a library derived from *E. coli* B (Clontech lot 1131, ATCC 11303) and included the complete coding sequence and ~2 kb of 5′ upstream DNA. Nucleotide sequence data obtained for the overlapping region of the two clones were identical. Herein we report the use of probes derived from these clones together with restriction digest analysis to determine the physical map location of the *fdx* gene.

The position of the *fdx* gene was initially determined with a λ phage miniset membrane prepared with the clones isolated by Kohara et al. (Takara Biochemical, Berkeley, Calif.) (2, 3). An *Ava*II-*Ava*II fragment of *fdx* which included the coding sequence (4) hybridized to two λ phage clones, 429 (7F8) and 430 (5E10), localizing the coding region to the area of overlap (~3 kb) of these clones. *Eco*RI-*Hin*dII fragments from the 3′ terminus of clone 6 (600 bp) and the 5′ terminus of clone 10 (260 bp) were then separately hybridized to each of these clones. The 5′ probe hybridized to both λ phage clones 7F8 and 5E10, whereas the 3′ probe hybridized only to clone 7F8. This establishes that the gene is oriented counterclockwise on the *E. coli* chromosome and localizes its approximate position relative to the miniset clones (Fig. 1).

Alignment of clones 6 and 10 with the restriction map reported by Kohara et al. (2) for *E. coli* K-12 strain W3110 predicts the occurrence of a *Hin*dIII site near the 5′ terminus, a *Pvu*II site near the 3′ terminus, and a *Bgl*I site near the middle of these clones. The partial restriction map previously determined contained a *Hin*dIII site ~0.5 kb from the 5′ end of clone 10 and a *Pvu*II site ~1 kb from the 3′ end of clone 6 (4), consistent with the alignment shown in Fig. 1. However, neither clone 6 nor clone 10 was cut by *Bgl*I. The failure to digest with *Bgl*I could be due to sequence differences between the *E. coli* strains used for cloning *fdx* and the strain used by Kohara et al. for establishing the physical map. We therefore prepared Southern blots of restriction digests of genomic DNAs from K-12 strain W3110 (ATCC 27325) used by Kohara et al. and from strain B (ATCC 11303) used by Clontech for preparation of the genomic library used for isolation of clone 10. Figure 2 shows the results obtained with DNAs from both strains with six of the restriction enzymes tested by Kohara et al. K-12 strain W3110 yielded the expected pattern, including two *Bgl*I fragments of 2.9 and 4.2 kb. Strain B, on the other hand, yielded only a single *Bgl*I fragment, ~7 kb, suggesting the absence of the *Bgl*I site near kb 2658.5 on the physical map.

Analysis of the nucleotide sequence determined for the *fdx* gene revealed several undecanucleotide sequences differing from a *Bgl*I site by only a single base. To determine whether one of these corresponds to the *Bgl*I site present in K-12 strain W3110, we used the polymerase chain reaction to amplify DNA in the region of the *fdx* gene of genomic DNAs from strain W3110 and from strain B. The sequence of the amplified DNA was determined, and the *Bgl*I site present in strain W3110 was found to occur in the sequence GCCct gatGGC (uppercase letters represent the recognition site), corresponding to bases 38 to 48 of the *fdx* coding sequence. For strain B, base 39 was found to be T instead of C, thereby leading to loss of the *Bgl*I site and explaining the different restriction pattern of this strain. The C→T transition occurs in the third position of the codon for Cys-13 (residue 12 of the mature protein) and does not result in a change in the amino acid sequence of the ferredoxin. The alignment shown in Fig. 1, based initially on hybridization results, is therefore supported by restriction and sequence analysis. The *fdx* gene is thus localized between kb 2658 and 2659 (~54 min) on the *E. coli* chromosome.

Nucleotide sequence accession number. The nucleotide sequence of *fdx* has been assigned GenBank accession number M88654.

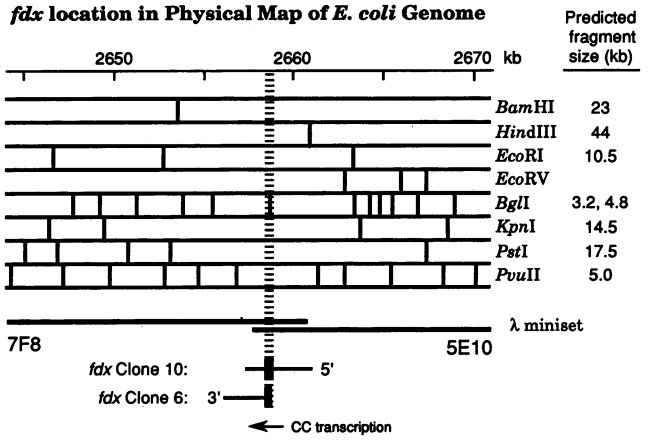

FIG. 1. Restriction map, adapted from that described by Kohara et al. (2), showing the locations of miniset λ phage clones 7F8 and 5E10 and *fdx* clones 6 and 10. The coding sequence of *fdx* is indicated by the filled boxes. The restriction fragment sizes predicted to hybridize to probes of *fdx* are listed on the right.

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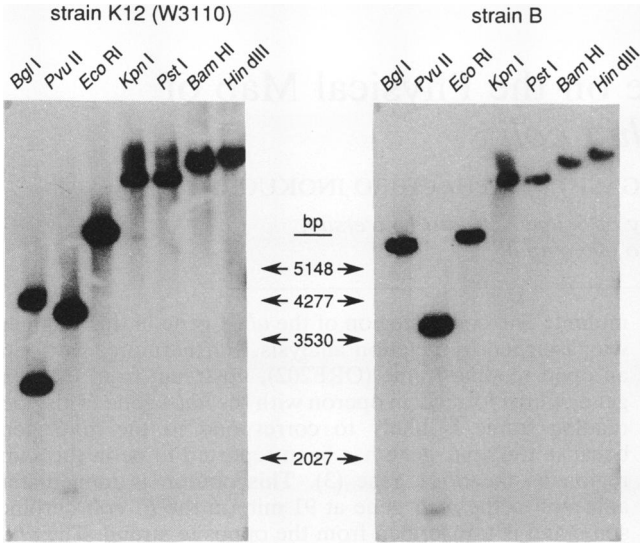

FIG. 2. Southern blots of restriction enzyme digests of genomic DNAs from *E. coli* K-12 strain W3110 and *E. coli* B probed with ^{32}P-labeled *fdx*.

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