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Physical Map Locations of the *trxB*, *htrD*, *cydC*, and *cydD* Genes of *Escherichia coli*

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The *Escherichia coli* thioredoxin reductase enzyme is a flavoprotein which catalyzes the transfer of reducing potential from NADPH to thioredoxin (4, 9). Thioredoxin participates in the reduction of ribonucleotides during DNA biosynthesis in *E. coli* (9) and is essential for sulfate reduction during cysteine biosynthesis (8, 9). In addition, thioredoxin functions in vitro in the reduction of methionine sulfoxide and protein disulfides (9). An *E. coli* mutant deficient in thioredoxin reductase activity has been isolated, defining the *trxB* gene (4). The *trxB* gene has been genetically mapped to a position between 20 and 21 min on the *E. coli* K-12 chromosome (6), cloned (14), and sequenced (15).

We have recently reported the isolation of a new *E. coli* gene, designated *htrD*, which is required for growth at high temperature (3). The *htrD* gene has been mapped by using the Kohara library of overlapping λ phage clones (7). First, cosmid clones of *htrD*$^+$ were shown to hybridize specifically to these phages. Second, a mini-Tn*10* (Tet^r) insertion within the *htrD* gene was shown to specifically recombine with Kohara bacteriophages λ213 and λ214 (1H1 and 1F10 respectively). Third, these same phages were shown to complement the HtrD Ts$^-$ phenotype. Further analysis of subclones precisely localized the *htrD* gene to a position centered around kb 942.5 on the *E. coli* physical map (Fig. 1) (7). Sequence analysis of the *htrD* gene (to be published elsewhere) revealed that it lies immediately adjacent to the 3′ end of the *trxB* gene. To confirm this new *trxB* position, we obtained a *trxB* null mutant (A326), *trxB*::Kan^r, for further analysis (15). We then transduced this *trxB* mutation into strain W3110 (wild type) by using the generalized transducing bacteriophage P1. The *trxB*::Kan^r allele was found to recombine with Kohara phages λ213 and λ214 exclusively. This result supports the data obtained from the sequence analysis and defines a new position for the *trxB* gene at 943 to 944 kb on the *E. coli* physical map (Fig. 1). The restriction map of *htrD* and *trxB* subclones is also in agreement with that of phages λ213 and λ214.

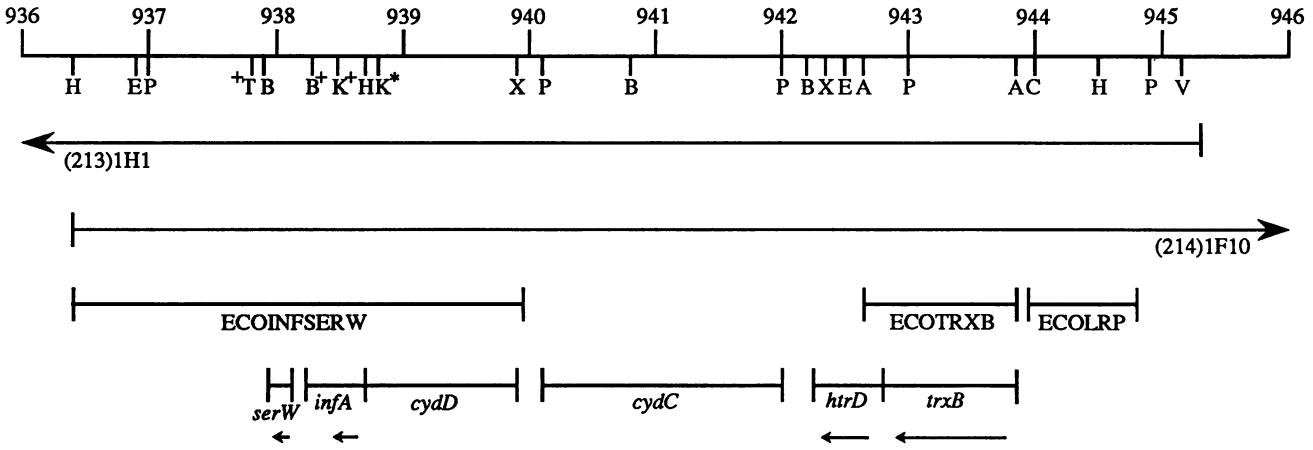

FIG. 1. Physical and genetic map of the 19.3-min region of the *E. coli* chromosome (936 to 946 kb) (7, 13). Numbering is according to reference 12, with all coordinates approximately 2 kb lower than in reference 7. Miniset clones (7), GenBank DNA sequences (ECOINFSERW, ECOTRXB, and ECOLRP), and newly positioned or unsequenced genes are shown. Restriction enzyme cleavage sites are indicated as follows: A, *Hpa*I; B, *Bgl*I; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pvu*II; T, *Pst*I; V, *Eco*RV; and X, *Xho*I. All restriction sites are as described by Kohara et al. (7), except *Cla*I, *Hpa*I, and *Xho*I, which were positioned in this study. An asterisk designates a restriction site present in neither the corresponding GenBank sequence nor the plasmid subclones covering the region. A plus designates a restriction site whose position is derived from a GenBank sequence. The *lrp* sequence position (ECOLRP) is from references 10 and 16. Where known, the open reading frame and direction of transcription for each gene are indicated by a small arrow. GenBank accession numbers for published sequences are as follows: ECOINFSERW, M63145; ECOTRXB, JO3762; and ECOLRP, M35869.

E. coli contains at least two separate terminal oxidase complexes which catalyze the oxidation of ubiquinol and reduce molecular oxygen to water (1). These two membrane-bound enzymes are the cytochrome *o* and cytochrome *d* complexes. The cytochrome *o* complex is encoded by the *cyoABCDE* operon (10.2 min), while the cytochrome *d* complex is encoded by the *cydAB* operon (16.6 min) (2). Two genes other than *cydAB* which are involved in cytochrome *d*

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function have been isolated. These are the *cydD* and *cydC* genes (2, 5, 11).

The *cydD* gene was isolated on the basis of the absence of cytochrome *d* activity resulting from mutations mapping in genes unlinked to *cydAB* (11). The *cydD* mutant thus isolated was also shown to be characteristically zinc sensitive (11). By using this mutant, the *cydD* gene was genetically mapped near the 19.3-min region of the *E. coli* chromosome. A subset of overlapping phages from the Kohara library spanning this region was tested for complementation of the zinc-sensitive phenotype of a *cydD* mutant. Only phages λ213 and λ214 were able to restore *cydD* growth in the presence of zinc. Further complementation analysis using plasmid subclones of the region was used to precisely position the *cydD* gene (Fig. 1). Sequence analysis of *cydD*-complementing plasmid subclones further established the location of this gene within a previously sequenced and positioned region, ECOINFSERW (GenBank accession number M63145) (12), containing the *infA* and *serW* genes (Fig. 1).

The *cydC* gene was defined in a search for genes other than *cydAB* that are required for expression of the functional form of cytochrome *d* (5). Initial characterization of the *cydC*::λ*plac*Mu53 mutant thus isolated indicates that this gene is required for synthesis of the heme *d* prosthetic group found associated with the cytochrome *d* complex (5). The *cydC* gene was genetically mapped to the 19.2-min region of the *E. coli* chromosome and later was more precisely mapped to the overlap region of Kohara phages λ213 and λ214 (2, 5). In order to map the *cydC* gene more accurately in relation to *htrD* and *cydD*, Southern blot analysis was performed with chromosomal DNA from wild-type and *cydC*::λ*plac*Mu53 bacterial strains. This DNA was digested and probed with randomly primed ^{32}P-labelled DNA restriction fragments from the overlap region of phages λ213 and λ214. The results of this analysis (data not shown) indicate that the *cydC*::λ*plac*Mu53 insertion lies within a 1,900-bp *Pvu*II fragment positioned between the *cydD* and *htrD* genes (Fig. 1).

The results reported here establish the relative order of genes in the 19.3-min region of the *E. coli* chromosome to be *serW*, *infA*, *cydD*, *cydC*, *htrD*, and *trxB*, reading in a clockwise direction. Furthermore, these results establish a new position for the *trxB* gene. It is interesting that two genes essential for proper cytochrome *d* function lie in such close proximity to one another. Perhaps *htrD* or other neighboring genes are also involved in this pathway.

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