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Localization of the *kdsA* Gene with the Aid of the Physical Map of the *Escherichia coli* Chromosome

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The isolation and analysis of two recombinant plasmids containing the *kdsA* gene from *Escherichia coli* chromosomal gene libraries is reported. The subfragments obtained from the inserts correspond to the fragment pattern around coordinate 1,282 kilobases of the physical map of the *E. coli* chromosome (Kohara et al. Cell 50:495–508, 1987). The *kdsA* gene has been located at coordinates 1,282 through 1,283 kilobases, corresponding to min 26.7 in the classical map coordinates. The *kdsA* gene is transcribed from this position toward the nearby *nar* gene.

The *kdsA* gene codes for 3-deoxy-D-*manno*-octulosonic acid 8-phosphate synthetase, a key enzyme in the lipopolysaccharide biosynthesis of gram-negative bacteria. The enzyme is responsible for the formation of the eight-carbon skeleton of 3-deoxy-D-*manno*-octulosonic acid from D-arabinose 5-phosphate and phosphoenol pyruvate (3). 3-Deoxy-D-*manno*-octulosonic acid functions as a linker between the hydrophobic portion of lipopolysaccharide, termed lipid A, and the hydrophilic polysaccharide chain. The *kdsA* gene has been cloned from *Escherichia coli* chromosomal DNA (6). It has been shown to be the last gene of an operon (7). The map position of the *kdsA* gene in *E. coli* is not known. It has been mapped in *Salmonella typhimurium* only approximately around min 39 (4). By using a 760-base-pair *Sau*3A fragment from the recombinant plasmid pMW100, which was described earlier (6) and is included here in Fig. 1, we prepared a radioactive probe by nick translation to screen an *E. coli* chromosomal *Eco*RI library linked with plasmid pUC119 by using colony hybridization as a selection technique (1). Two colonies were detected which gave signals above background with the probe. The background hybridization presumably originated from the complementary sequences in the chromosomal *E. coli* DNA. Positive colonies could be clearly discerned against the background signals even without amplification of the plasmid DNA in the recombinant cells. One of the two positive colonies was

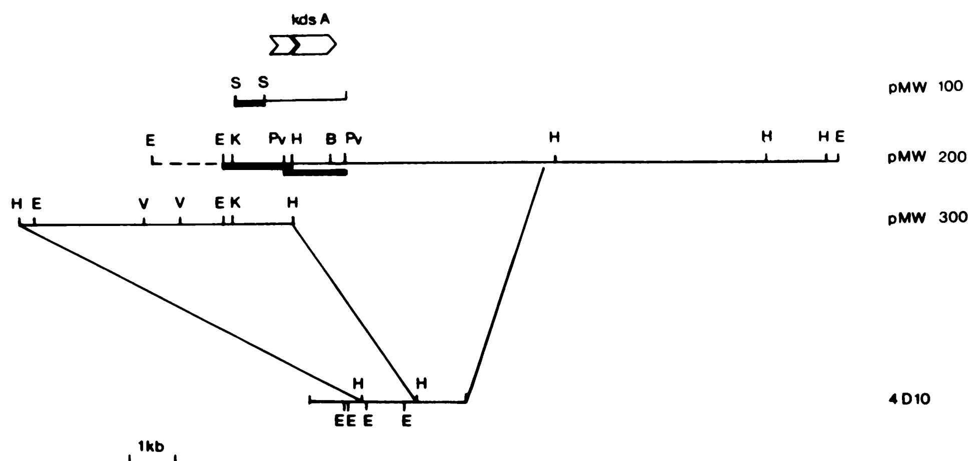

FIG. 1. Physical map of the inserts of the recombinant plasmids pMW100, pMW200, and pMW300 and the recombinant λ phage 4D10. The *kdsA* gene is shown above the linear maps as an open arrow pointing into the direction of transcription. The smaller arrow to the left represents the terminal part of an upstream open reading frame contained in pMW101 (7). Cleavage sites: E, *Eco*RI; K, *Kpn*I; H, *Hin*dIII; V, *Eco*RV; Pv, *Pvu*II; S, *Sau*3A. The *Kpn*I, *Pvu*II, and *Sau*3A sites were derived from nucleotide sequences (7; unpublished data). Thick lines indicate DNA probes for hybridization experiments. The *Eco*RI fragment on the left border of pMW200 (---) originated from another part of the *E. coli* chromosome and is unrelated to the *kdsA* operon.

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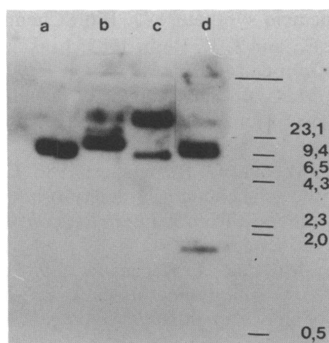

FIG. 2. Autoradiogram of a Southern hybridization experiment with fragmented DNA from phage 4D10 blotted to nitrocellulose after electrophoretic separation. Restriction enzymes (lanes): a, *Eco*RI; b, *Kpn*I; c, *Hin*dIII; d, *Hin*dIII-*Kpn*I. The lines on the right margin mark the positions of *Hin*dIII-cleaved λ DNA fragments.

isolated and used for the preparation of plasmid DNA, which was then characterized by cleavage with restriction enzymes. The recombinant plasmid was designated pMW200. It contained two *Eco*RI fragments of 14.1 and 1.8 kilobases (kb). The 1.8-kb fragment is unrelated to the *kdsA* operon; it was probably accidentally incorporated into pMW200 during the preparation of the gene library. The 14.1-kb *Eco*RI fragment represents a contiguous DNA fragment containing *kdsA*.

Since Southern hybridization assays with a probe from the *kdsA* region indicated that the latter was close to the edge of the 14.1-kb *Eco*RI fragment and preliminary sequence data showed that the beginning of the transcription unit was located beyond the *Eco*RI site, we searched for clones that included neighboring DNA regions. A *Hin*dIII library from *E. coli* chromosomal DNA in pUC119 was assayed with the colony hybridization technique by using the nick-translated 1.5-kb *Hin*dIII-*Eco*RI fragment from pMW200. One colony showing a positive signal was isolated. It contained a recombinant plasmid with an approximately 6.3-kb insert which showed a strong signal after cleavage with *Hin*dIII and electrophoretic separation in a Southern hybridization assay with the same probe as in the colony assay. The recombinant plasmid was designated pMW300. It was *Eco*RI-*Hin*dIII and *Eco*RV-*Hin*dIII digested, and the fragments were hybridized with the radioactive 1.5-kb *Eco*RI-*Hin*dIII probe. A 1.5-kb *Eco*RI-*Hin*dIII fragment and a 2.6-kb *Eco*RV-*Hin*dIII fragment showed positive signals. The 4.4-kb *Eco*RI fragment was isolated and cleaved with *Eco*RV. From these results we arranged the *Eco*RI and *Eco*RV cleavage sites as indicated in Fig. 1.

When we screened visually the physical map of the *E. coli* chromosome (2), starting with a search for the 14.1-kb *Eco*RI fragment that was overlapped by a 6.3-kb *Hin*dIII fragment, we found that the fragment pattern around the map coordinate 1,290 kb fit our data. We found one additional *Eco*RV site in the 6.3-kb *Hin*dIII fragment which is absent in the map of Kohara et al. (2). This site is shown in Fig. 1. Hence, the DNA from phage 4D10 described by Kohara et al. (2) was prepared (5) and cleaved with *Hin*dIII, *Eco*RI, and *Kpn*I and double digested with *Hin*dIII-*Kpn*I. The electrophoretically separated fragments were probed with nick-translated pMW101 DNA, the *Pvu*II fragment indicated in Fig. 1, which contains the entire *kdsA* gene (7) (Fig. 2). The following fragments showed clear signals: 6-kb *Eco*RI fragment, >20- and 5.5-kb *Hin*dIII fragments, 8-kb *Kpn*I fragment, and 6- and 1.5-kb *Hin*dIII-*Kpn*I fragments. Thus, the *kdsA* gene of *E. coli* is located at the left border of the large *Hin*dIII fragment between coordinates 1,282 and 1,288 kb. The neighboring *Hin*dIII fragment from 1,276 to 1,282 kb also shows a positive signal because the probe pMW101 contains upstream of *kdsA* part of another open reading frame that includes the *Hin*dIII site with the coordinate position 1,282 kb. On the basis of this analysis we assign the *kdsA* gene the map coordinates 1,282 through 1,283 kb. When expressed in the classical coordinates of the *E. coli* chromosomal map it has to be located at min 26.7. From these data we also conclude that transcription of the *kdsA* operon is directed toward the *nar* gene located at min 27.1 (Fig. 3).

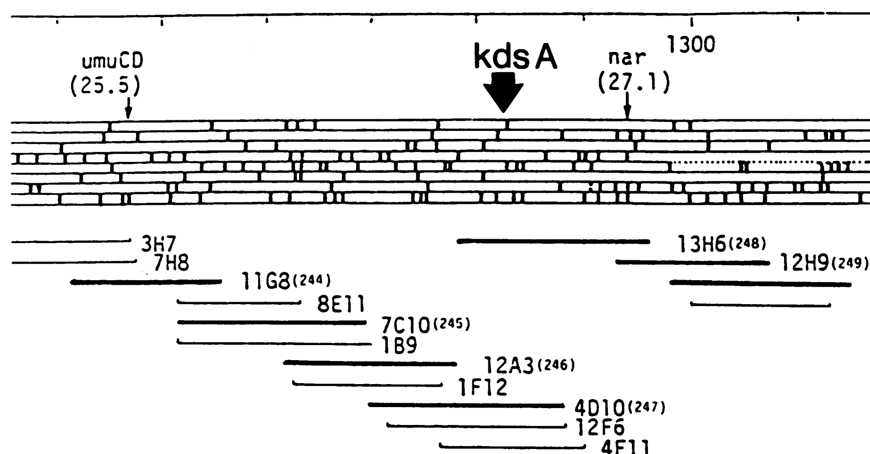

FIG. 3. Section of the physical map of the *E. coli* chromosome as described by Kohara et al. (2) around map position 27; the location of the *kdsA* gene is indicated. The lines containing the various restriction sites correspond (from top to bottom) to *Bam*HI, *Hin*dIII, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I, and *Pvu*II. The inserts contained in various λ phages are shown at the bottom. Reproduced from reference 2 with permission.

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