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Locations of the *lip*, *poxB*, and *ilvBN* Genes on the Physical Map of *Escherichia coli*

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The *lip* locus, located at ca. 14.5 min on the *Escherichia coli* chromosome (6), is thought to encode an enzyme (or enzymes) involved in the terminal step(s) of lipoic acid biosynthesis (1, 14). We have used a representative mutant (JRG26) from the single genetic class of lipoic acid-auxotrophic strains described by Herbert and Guest (7) to identify the Kohara λ phage which carries the complementing *lip* gene (Table 1). This phage also carries a DNA segment corresponding to a second genetic class of lipoic acid-auxotrophic mutants recently isolated in our laboratory (to be published elsewhere). Our restriction data from the *lip* locus are in general agreement with the data of Kohara et al. (9). However, we have not attempted to exhaustively verify the Kohara physical map of phage λ168.

Our restriction data (4, 5) for *Eco*RV, *Pvu*II, *Bam*HI, *Hin*dIII, *Eco*RI, *Kpn*I, and *Pst*I sites around the *poxB* gene are in agreement with the Kohara restriction map (9). The restriction data of Wek et al. (15) for the *ilvBN* gene agrees with the Kohara map except for an *Eco*RV site not present in the Kohara map.

It should be noted that our result (*ilvBN* at 3860 kbp) is essentially identical to those of Kohara (8) and Brewer (2), who systematically compared the directly determined restriction map (9) with that predicted from data base DNA sequences. In contrast, Médigue et al (10) and Rudd et al. (11) report *ilvBN* at 3919 and 3923 kbp, respectively. The apparent disagreement is due to computer-simulated reversal of the *rrnD-rrnE* inversion of strain W3110 mapped by Kohara et al. (9). When converted to the W3110 coordinates, the locations of *ilvBN* are 3860.5 kbp (11) and 3864.5 kbp (10), respectively. However, it should be noted that Médigue et al. (10) based their location on matching only 6 of the 12 predicted restriction sites.

TABLE 1. Physical locations of the *poxB*, *ilvBN*, and *lip* genes

Gene	Genetic map location (min)	Physical map location (kbp)	Phage(s)[a]		Comment
			Tested	Positive on test	
poxB	19	920–930	101–676	211[b]	Further localized to ~925 kbp by comparison of the published restriction map (5,6) with the physical map of Kohara et al. (9)
ilvBN	82	3855–3865	101–676	568, 569[b]	Further localized to ~3860 kbp[c] by comparison of the published restriction map (15) with the physical map of Kohara et al. (9)
lip	14.5	664–682	165–170	168[d]	Further localized to 675–680 kbp by analysis of plasmid subclones (14)

[a] Numbers refer to bacteriophage clones from the miniset of Kohara (8).
[b] DNA fragments encoding either the *poxB* or *ilvBN* gene were labeled with ^{32}P and used to probe the entire miniset of Kohara λ phages (101–676).
[c] The location given is that on the original map of Kohara et al. (9). The computer-generated maps (10, 11) give different values because of correction of the *rrnD-rrnE* inversion of strain W3110 (9).
[d] Spot complementation/recombination assays were performed by spotting ~2×10^7 PFU of each Kohara phage alone or with ~2×10^7 PFU of λ cI857 onto a lawn of JRG26 on minimal medium lacking lipoic acid. The appearance of colonies within the test spot following incubation at 30°C was interpreted as a positive result. Plasmid subclones derived from Kohara phage 168 that also complemented the *lip-2* mutation in strain JRG26 were subsequently obtained, thus confirming this assignment (14). Our complementation results are consistent with the data of Spratt et al. (12).

Pyruvate oxidase (pyruvate:ubiquinone-8 oxidoreductase; EC 1.2.2.2) of *E. coli*, encoded by the *poxB* locus, is a lipid-activated flavoprotein that catalyzes the oxidation of pyruvate to acetate and CO_2 (3). The acetohydroxy acid synthase isozymes (EC 4.1.3.18) encoded by the *ilvBN* locus catalyze a common step in the biosynthesis of the branched-chain amino acids, valine, leucine, and isoleucine (13). Evidence from our laboratory has suggested that pyruvate oxidase is an evolutionary ancestor of the acetohydroxy acid synthase isozymes (3). We have used plasmid clones of these genes as hybridization probes to identify the corresponding Kohara λ phages harboring these DNA fragments (Table 1).

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