JOURNAL OF BACTERIOLOGY, Mar. 1992, p. 1707–1708
0021-9193/92/051707-02$02.00/0

Vol. 174, No. 5

Location of the Transketolase (*tkt*) Gene on the *Escherichia coli* Physical Map

GEORG A. SPRENGER

Institut für Biotechnologie 1 des Forschungszentrums Jülich GmbH, P.O. Box 1913, D-W-5170 Jülich, Germany

Transketolase (EC 2.2.1.1) catalyzes the interconversion of sugar phosphates in the pentose phosphate cycle. *Escherichia coli* K-12 mutants with a defect in transketolase (*tkt* mutants) have been isolated (5), and a map position of 62 min was assigned. However, as P1-mediated cotransduction attempts with the markers *argA*, *lysA*, and *serA* had failed, this location remained unverified (2, 5).

Recently, the *tkt* gene was cloned as a *Bam*HI DNA fragment of about 6 kb from *E. coli*, but no chromosomal location was given (4). Independently, the gene was cloned on an RP4miniMu prime vector (8). Subcloning from this vector allowed us to locate the *tkt* gene on a 2.5-kb DNA (*Sau*3A partial plus *Kpn*I) fragment present in plasmid pGSJ425 (9). The sequence of the insert DNA was determined, and an open reading frame (ORF) of 1,992 bp was found. This could encode a protein subunit with a molecular weight of 73,000, which was in good agreement with the subunit size determined for purified transketolase. The ORF was verified by demonstrating that the predicted N-terminal amino acid sequence matched the N residues of the purified enzyme (9).

For physical mapping of the *tkt* gene, a collection of lambda clones from the Kohara miniset (6) spanning the 61- to 64-min region was used. An internal 0.35-kb *Hin*dIII fragment of the cloned *tkt* gene served as the probe for DNA-DNA hybridizations with restricted phage DNAs. The two clones 472 (6C5) and 473 (1H10) gave positive results in hybridization and also were able to complement the *tkt* defect of strain BJ 502 (*tkt-2*; 5) as judged by acid production on MacConkey 1% xylose plates. A comparison of the *tkt* restriction map with the region from kb 3085 to 3105 of the Kohara map (6) is given in Fig. 1; the map location of *tkt* was

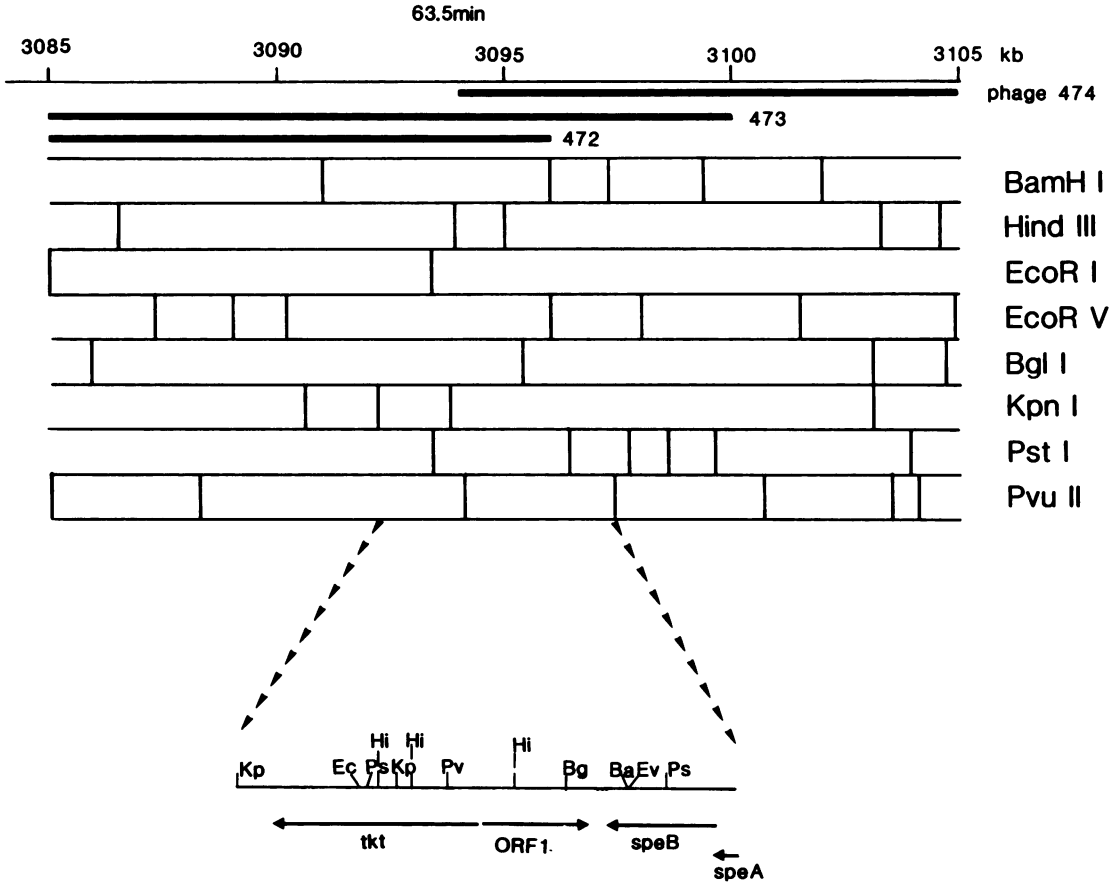

FIG. 1. Alignment of the physical map in the 3085- to 3105-kb region of the *E. coli* chromosome (6) with the data from this and previous work (7). Recombinant phages overlapping the region are displayed as solid bars. Arrows above the genes show transcriptional direction. The additional 0.35-kb *Hin*dIII DNA fragment present in the *tkt* gene is adjacent to the 1.5-kb *Hin*dIII fragment at around 3094 kb.

determined to be at 63.5 min or 3092 to 3094 kb. The direction of transcription is counterclockwise. The restriction map of *tkt* overlapped with that of the adjacent genes (7, 10) involved with putrescine synthesis or its regulation (*speA*, *speB*, and ORF1). The *tkt* ORF is separated by only 50 bp of DNA from an ORF (called ORF1 and read in the direction opposite to that of *tkt*). ORF1 encodes a protein that has a positive regulatory effect on the activity of agmatine ureohydrolase encoded by *speB* (2). Downstream of the *tkt* gene (at a distance of 4 kb) follows a cluster of glycolysis genes (1). The fine-restriction map together with data from the DNA sequence revealed that the Kohara map (6) is correct in the 63.5-min region with the one exception that a small (0.35-kb) additional *Hin*dIII fragment (9) is present.

I thank Y. Kohara for providing the miniset and additional data. My special thanks to S. M. Boyle for sharing sequence information prior to publication and for critically reading the manuscript.

REFERENCES

1. **Alefounder, P. R., and R. N. Perham.** 1989. Identification, molecular cloning and sequence analysis of a gene cluster encoding the Class II fructose 1,6-bisphosphate aldolase, 3-phospho-glycerate kinase and a putative second glyceraldehyde 3-phosphate dehydrogenase of *Escherichia coli*. Mol. Microbiol. **3**:723–732.
2. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54**:130–197.
3. **Boyle, S. M.** Unpublished data.
4. **Draths, K. M., and J. W. Frost.** 1990. Synthesis using plasmid-based biocatalysis:plasmid assembly and 3-deoxy-D-*arabino*-heptulosonate production. J. Am. Chem. Soc. **112**:1657–1659.
5. **Josephson, B. L., and D. G. Fraenkel.** 1969. Transketolase mutants of *Escherichia coli*. J. Bacteriol. **100**:1289–1295.
6. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:495–508.
7. **Satishchandran, C., G. D. Markham, R. C. Moore, and S. M. Boyle.** 1990. Locations of the *speA*, *speB*, *speC*, and *metK* genes on the physical map of *Escherichia coli*. J. Bacteriol. **172**:4748.
8. **Sprenger, G. A.** 1991. Cloning and preliminary characterization of the transketolase gene from *Escherichia coli* K-12, p. 322–326. *In* H. Bisswanger and J. Ullrich (ed.), Biochemistry and physiology of thiamin diphosphate enzymes. VCH Verlagsgesellschaft, Weinheim, Germany.
9. **Sprenger, G. A.** Unpublished data.
10. **Szumanski, M. B. W., and S. M. Boyle.** 1990. Analysis and sequence of the *speB* gene encoding agmatine ureohydrolase, a putrescine biosynthetic enzyme in *Escherichia coli*. J. Bacteriol. **172**:538–547.