Physical Map Location and Transcriptional Orientation of the *tyrR* Gene of *Escherichia coli* K-12†

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The *tyrR* gene of *Escherichia coli* encodes a regulatory protein of 513 amino acids that controls the rates of expression of eight genes (6). The genes of the *tyrR* regulon specify proteins important in the transport and synthesis of aromatic amino acids (6). The approximate map position of *tyrR* was determined by classical mating methods (8) and by P1 transduction (1). The *tyrR* gene was cloned and sequenced (2) a number of years ago. We have confirmed the structure of the *tyrR* gene and flanking sequences (7). The *tyrR* gene has now been precisely located on the physical map of Kohara et al. (4) through the use of Southern blotting procedures (5) and polymerase chain reaction techniques.

In preliminary studies, four phage DNAs from the Kohara miniset library, namely, E14F6 (256), 1C2 (257), 2D3 (258), and 3H8 (259), were used as polymerase chain reaction templates. Sequences specific for *tyrR* were detected only in phage 259, when primers specific to the two ends of the *tyrR* structural gene were used (data not shown). Further Southern blotting studies with the ordered Kohara miniset library (3) (*E. coli* Gene Mapping Membrane; Takara Biochemical, Inc., Berkeley, Calif.) showed that only phages 3H8 (259) and 6B4 (260) contained DNA that hybridized with the *tyrR* structural gene (data not shown). Thus, the *tyrR* gene must lie within that segment of the chromosome common to phages 259 and 260 (Fig. 1).

Among the eight restriction endonucleases used by Kohara et al. to generate their physical map (4), only one (*EcoRV*) cleaves the known (2) sequence of *tyrR*. From this information one could not deduce the transcriptional orientation of *tyrR* with respect to the rest of the map even if the position of the critical *EcoRV* site were known. To rigorously locate *tyrR* and determine its transcriptional orientation, two ³²P-labeled probes were used in Southern blotting experiments. One was a 5'-end-labeled 19-mer (5'-TTGGTG CAGGAATTCTGAC3') that complements (with one mismatch) the sequence in the *tyrR* promoter region situated upstream of the *EcoRV* site. The second probe was specific for the *tyrR* structural gene (7), situated downstream of the *EcoRV* site. In the region of DNA that is common to phages 259 and 260, there is only one reported *EcoRV* site, near coordinate 1408 (Fig. 1). If the physical map of this region is correct, the *tyrR* gene would be predicted to lie near this *EcoRV* site. However, when Southern blotting analysis was performed with phage 259 DNA, an *EcoRV* fragment of 7.5 kb hybridized with the labeled 19-mer (Fig. 2B, lane 5), while an *EcoRV* fragment of 5.3 kb hybridized with the labeled structural gene probe (Fig. 2A, lane 5). This result is not compatible with the published physical map of this region, which predicts that *EcoRV* fragments of 13 and 1 kb should

hybridize to the 19-mer and the *tyrR* structural gene, respectively (Fig. 1). Our result implies either that another *EcoRV* site exists or that the *EcoRV* site at coordinate 1408 had been mismapped. Additional *EcoRV* digests of a *Bam*HI-*Eco*RI fragment preparatively isolated from phage 259 (Fig. 1) showed that this segment of the chromosome actually contains three *EcoRV* sites (data not shown). Two *EcoRV* sites, at coordinates 1396 and 1408, correspond to established (4) map positions. The third *EcoRV* site, at coordinate 1403, has not previously been reported. When a *Bam*HI-*Hind*III fragment derived from phage 259 (Fig. 1) was digested with *EcoRV*, three cleavage products were observed. The intact *Bam*HI-*Hind*III fragment contained DNA segments that hybridized with both the labeled 19-mer and the labeled *tyrR* structural gene probes (Fig. 2A and B, lane 2). There is thus an additional *EcoRV* site in the *Bam*HI-*Hind*III interval common to the inserts of phages 259 and 260. The *tyrR* gene is located near the newly identified *EcoRV* site. The presence of an additional *EcoRV* site is in perfect accord with the sizes of the 7.5- and 5.3-kb *EcoRV* fragments that were found to hybridize with the labeled 19-mer and the *tyrR* structural gene probes.

The transcriptional orientation of *tyrR* was deduced by additional Southern blotting experiments. When the labeled 19-mer was used as probe, an *EcoRV* fragment of 7.5 kb was detected by hybridization in both the full-length insert and the *Bam*HI-*Hind*III fragment of phage 259 (Fig. 2B, lanes 1 and 5). However, the *tyrR* structural gene probe hybridized

FIG. 1. Location of the *tyrR* gene on the physical map of *E. coli* (4). The figure shows the insert of Kohara phage 3H8 (259), drawn approximately to scale. The newly identified *EcoRV* site at coordinate 1403 is drawn as a bold vertical line. The horizontal arrow indicates the transcriptional orientation of *tyrR*. The hatched area depicts the sequenced (2) *tyrR* region, which lies predominantly downstream of the *EcoRV* site that was identified in the course of the present study. There are flanking *Eco*RI sites that originate from the polylinker regions of the vector (4).

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FIG. 2. Southern blotting analysis of Kohara phage 3H8 (259), using as probe either the *tyrR* structural gene (7) (A) or a 5'-³²P-labeled 19-mer (5'TGGTGCAGGAATTCTGAC3') (B) specific for sequences upstream of the *EcoRV* site in the published sequence of *tyrR* (2). DNA from phage 259 was digested with *EcoRI*-*Bam*HI (lane 7), *EcoRI*-*Bam*HI-*EcoRV* (lane 6), *EcoRV* (lane 5), *EcoRI*-*Hind*III (lane 4), or *Hind*III-*EcoRV* (lane 3). A gel-purified *Bam*HI-*Hind*III fragment of the 259 insert was run in lane 2. The same *Bam*HI-*Hind*III fragment was cleaved with *EcoRV*, and the digestion products were applied to lane 1. After electrophoretic separation on 1% agarose, the DNA fragments were transferred to a nitrocellulose membrane. Hybridization was first performed in 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaPO₄, and 1 mM EDTA [pH 7.7]) at 42°C (overnight), using the 19-mer probe. After autoradiography, the same membrane was washed free of radioactivity and then reprobbed in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C, using nick-translated [³²P]DNA specific for the *tyrR* structural gene. In panel A, the approximate sizes of the hybridizing fragments are 1.9 kb (lanes 1 and 3), 9.4 kb (lane 2), 14 kb (lane 4), 5.3 kb (lanes 5 and 6), and 16 kb (lane 7). In panel B, the approximate sizes of the hybridizing fragments are 7.5 kb (lanes 1, 3, 5, and 6), 9.3 kb (lane 2), 14 kb (lane 4), and 16 kb (lane 7). Lanes marked M show the migration positions of molecular weight markers (λ -*Hind*III).

to a 5.3-kb *EcoRV* fragment derived from the full-length insert (Fig. 2A, lane 5). The structural gene probe hybridized to a fragment of 1.9 kb when the isolated *Bam*HI-*Hind*III fragment was digested with *EcoRV* (Fig. 2A, lane 1). The newly identified *EcoRV* site must therefore lie 1.9 kb to the left of the unique *Hind*III site in clone 259 (Fig. 1). The *tyrR* structural gene must be located to the right of the newly identified *EcoRV* site (Fig. 1). Thus, the transcriptional orientation of the *tyrR* gene is clockwise (Fig. 1).

The assignment of the newly identified *EcoRV* site to coordinate 29.27 min (1403 kb) is reasonable. None of the seven other restriction enzyme sites of Kohara are present within 1.9 kb of DNA downstream of this *EcoRV* site, in accord with the published *tyrR* sequence (2). The Southern hybridization results (Fig. 2) confirm that the location of the *tyrR* gene is consistent with the map of this region (Fig. 2A and B, lanes 3, 4, 6, and 7). Except for the previously undetected *EcoRV* site, the other tested restriction endonuclease cleavage sites match the published (4) physical map (Fig. 2A and B, lanes 3, 4, 6, and 7).

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