The Tyrosine Repressor Negatively Regulates aroH Expression in Escherichia coli†

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The levels of the tryptophan-sensitive isoenzyme of 3-deoxy-o-arabino-heptulosonate 7-phosphate synthase of Escherichia coli, encoded by the aroH gene, were elevated in tyrR and/or trpR mutants. The effect of tyrR and trpR lesions on aroH expression was confirmed by using a lacZ reporter system. The mutational elimination of either repressor led to a threefold increase in β-galactosidase.

In bacteria and plants, aromatic amino acid biosynthesis proceeds by the common aromatic, or shikimate, pathway. This metabolic sequence delivers chorismate to several terminal pathways, including the three metabolic routes that generate phenylalanine, tyrosine, and tryptophan (16, 22). In Escherichia coli, carbon flow through the shikimate pathway is controlled by modulation of the catalytic activity of the first enzyme, 3-deoxy-o-arabino-heptulosonate 7-phosphate (DAHP) synthase (EC 4.1.2.15) (21). E. coli and Salmonella typhimurium have three DAHP synthase isoenzymes encoded by the unlinked genes aroF, aroG, and aroH.

The aroF and aroG genes are repressed by the Tyr repressor, the tyrR gene product. Repression is mediated by tyrosine and phenylalanine, respectively (7). cis-acting regulatory sites specific for aroF have been identified mutational-ly (9, 12). The presumptive operator sequences reside within three boxes, designated aroFol (12), aroFo2 (12), and Ty box 1 (9). The aroG gene has a single operator box similar in sequence to the aroF operator boxes (1, 12). The aroG operator has been further defined through the characterization of constitutive mutants and through tyrosine repressor footprinting studies (1).

Several other genes of aromatic amino acid biosynthesis or transport, including aroL, aroF, tyrB, tyrP, and mtr, are regulated by the Tyr repressor (8, 15, 17, 27, 28). Operator mutations have been isolated for most of these. All of the known or presumptive Tyr repressor targets consist of 22-bp imperfect palindromes that share sequence similarity with aroFol and aroFo2 (12). A proposed consensus sequence (1, 11) contains a G-N14-C motif with the palindromic G and C as the only invariant bases. Many but not all of the structurally characterized operator mutations are single-base-pair changes of this G or C (1, 12, 28).

The expression of aroH is controlled by the Trp repressor (13, 29), a protein that also regulates the expression of the trp operon (3), trpR itself (5, 6), and mtr (15). The regulatory regions of these four operons contain similar target sequences whose role as Trp repressor binding sites rests in part upon analogies in structure to positions of known operator-constitutive mutations within the primary trp operon regulatory region (3). The trp operator has been further delineated by saturation mutagenesis (2). The interaction of the Trp repressor with the four known targets has been examined in detail both in vitro and in vivo (5, 15, 18). We now demonstrate not only that the aroH gene is regulated by the tryptophan repressor but also that aroH expression is elevated in tyrR strains and that the aroH regulatory region contains a G-N14-C motif.

DAHP synthase. We measured the activities of the three DAHP synthase isoenzymes (24) in extracts of a series of related E. coli strains. The levels of the tyrosine- and phenylalanine-sensitive isoenzymes were determined by assays in the presence of the corresponding feedback inhibitors; the levels of the tryptophan-sensitive isoenzyme were calculated to be the amounts of enzyme activity not subject to inhibition by the other two aromatic amino acids. In a tyrR tyrR strain the phenylalanine-sensitive isoenzyme was the predominant species, followed by the tyrosine-sensitive isoenzyme. The tryptophan-sensitive isoenzyme contributed only about 5% to the total activity. In a tyrR strain, aroF, aroG, and, unexpectedly, also aroH were derepressed. While aroF and aroG expression was unchanged in a trpR strain, the aroH product was elevated. In a tyrR trpR double mutant, the aroH product constituted about 15% of the total DAHP synthase activity. Thus, our preliminary studies suggested that aroH might be regulated not only by trpR but also by tyrR. Since the tryptophan-sensitive DAHP synthase is the minority isoenzyme, it was difficult to precisely assess the effect of the two repressors on aroH expression by direct assays of the primary gene products. Therefore, the regulation of aroH was examined by assaying β-galactosidase in extracts of cells harboring aroH-lacZ fusions.

β-Galactosidase levels in strains containing aroF-lacZ and aroH-lacZ fusions. In the form of prophages, fusions of aroF or aroH to lacZ were introduced in single copy into the chromosomes of appropriate tyrR and trpR strains. Strain SP564 [Δ(lac-pro) zci-223::Tn10] is a derivative of CHS63 [Δ(lac-pro)] obtained by transduction with phage P1 grown on PLK 1336 (zci-223::Tn10 near min 28; obtained from P. L. Kuempel). Strain SP564 has a Tn10 insertion near tyrR but is Tet+ and TyrR+ (3-fluorotyrosine sensitive). Strain SP564-1 [Δ(lac-pro) Δ tyrR] was derived from strain SP564 by the imprecise excision of Tn10 (4). Strain SP564-1 is Tet+ and TyrR+ (3-fluorotyrosine resistant). Strain SP1238 [Δ(lac-pro) (serB-trpR)]37-1 is a CHS63 derivative obtained by transduction with P1 grown on SP516 [Δ(lac-pro) Δ tyrR]37-1.

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TABLE 1. Effects of mutations in tyrR and trpR on expression from aroH- and aroF-lac fusions

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>β-Galactosidase sp act a in strain carrying:</th>
<th>(aroH-lacZ)</th>
<th>(aroF-lacZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP564</td>
<td>tyrR+ trpR+</td>
<td>65 ± 5</td>
<td>881 ± 44</td>
<td></td>
</tr>
<tr>
<td>Sp564-1</td>
<td>tyrR trpR+</td>
<td>163 ± 10</td>
<td>9,893 ± 482</td>
<td></td>
</tr>
<tr>
<td>SP1238</td>
<td>tyrR+ trpR</td>
<td>168 ± 4</td>
<td>659 ± 58</td>
<td></td>
</tr>
<tr>
<td>Sp1239</td>
<td>tyrR trpR</td>
<td>521 ± 28</td>
<td>8,585 ± 812</td>
<td></td>
</tr>
</tbody>
</table>

a Each value is the average ± standard deviation of four independently selected lysogens assayed in quadruplicate; β-galactosidase activities are in Miller units (19).

FIG. 1. Putative repressor binding sites in the aroH regulatory region. Upper line, part of the sequence for the aroH regulatory region. Brackets indicate the 22-bp imperfect palindromes, the potential targets for the trpR gene product around −35 and the tyrR gene product around +20; numbers indicate nucleotides, numbered relative to the transcription start (18). Lower line left, trp operator consensus sequences. Capital letters indicate invariant base pairs, and lowercase letters indicate base pairs that are found in three of the four targets (15, 18). Lower right line, Tyr box consensus. Capital letters indicate invariant base pairs, and lowercase letters indicate base pairs that are found in more than half of the 14 published targets (1); considering all known Tyr boxes (1), the G and C of the G-N14-C motif are the only invariant bases.

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