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The Tyrosine Repressor Negatively Regulates *aroH* Expression in *Escherichia coli*†

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The levels of the tryptophan-sensitive isoenzyme of 3-deoxy-D-*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-D-*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 mutationally (9, 12). The presumptive operator lesions reside within three boxes, designated *aroFo1* (12), *aroFo2* (12), and Tyr 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*, *aroP*, *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 *aroFo1* and *aroFo2* (12). A proposed consensus sequence (1, 11) contains a G-N_{14}-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-N_{14}-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 $tyrR^+$ $trpR^+$ 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*::Tn*10*] is a derivative of CSH63 [Δ(*lac-pro*)] obtained by transduction with phage P1 grown on PLK 1336 (*zci-223*::Tn*10* near min 28; obtained from P. L. Kuempel). Strain SP564 has a Tn*10* insertion near *tyrR* but is Tet^r and $TyrR^+$ (3-fluorotyrosine sensitive). Strain SP564-1 [Δ(*lac-pro*) Δ*tyrR*] was derived from strain SP564 by the imprecise excision of Tn*10* (4). Strain SP564-1 is Tet^s and $TyrR^-$ (3-fluorotyrosine resistant). Strain SP1238 [Δ(*lac-pro*) $(serB\text{-}trpR)^{\blacktriangledown 37\text{-}1}$ *zjj*::Tn*10*] is a CSH63 derivative obtained by transduction with P1 grown on SP516 [$(serB\text{-}trpR)^{\blacktriangledown 37\text{-}1}$

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

Strain	Genotype	β-Galactosidase sp act[a] in strain carrying:	
		aroH-lacZ	*aroF-lacZ*
SP564	*tyrR*$^+$ *trpR*$^+$	65 ± 5	881 ± 44
Sp564-1	*tyrR trpR*$^+$	163 ± 10	9,893 ± 482
SP1238	*tyrR*$^+$ *trpR*	168 ± 4	659 ± 58
Sp1239	*tyrR trpR*	521 ± 28	8,585 ± 812

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

zjj::Tn*10*]. Strain SP1239 was derived from SP564-1 by transduction with P1 grown on SP516. Strain SP1239 is TrpR$^-$ TyrR$^-$.

Phage λCLG3, obtained by homologous recombination between λRZ5 and pCLG3 (13), carries a transcription-translation fusion (26) wherein codon 10 of *aroH* is fused to codon 6 of *lacZ*. In phage λGME77, obtained by homologous recombination between λRZ5 and pGME77, codon 77 of *aroF* is fused to codon 6 of *lacZ*. Plasmid pGME77 is a pMLB1034 (26) derivative that carries the 565-bp *Eco*RI-*Dra*I fragment of M13GME (20).

To obtain single lysogens, phages were adsorbed to host cells of fresh overnight cultures. Lac$^+$ lysogens were selected on tryptone agar supplemented with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and ampicillin. Six lysogens from each λRZ5 derivative were purified on minimal salts medium.

Table 1 shows the β-galactosidase levels in extracts of these lysogens. As judged by the β-galactosidase levels in strains with *aroF-lacZ* fusions, the Trp repressor had no effect on *aroF* expression. Transcription from the *aroF* promoter was about 10-fold derepressed in the *tyrR* strain, in good agreement with the DAHP synthase data.

The data from the set of strains harboring the *aroH-lacZ* fusion confirmed that *aroH* expression was governed not only by the Trp repressor but also by the Tyr repressor. The amplitude of regulation for the two repressors was the same. The level of β-galactosidase in the *tyrR trpR* strain indicated at least an additive effect of the two repressors on *aroH* expression.

The Tyr repressor controls a number of genes, most of which have been sequenced (1, 10, 14, 23, 25, 28). On the basis of structural homology and the nature of operator mutations, a consensus target sequence for the Tyr repressor was proposed (1, 11). The unit of recognition is a G-N_{14}-C motif-containing 22-bp palindrome that is positioned at highly variable locations within the regulatory region of Tyr repressor-responsive genes. In four cases, one of the Tyr boxes overlaps the −35 region of the promoter; in several cases, boxes are upstream or downstream from the promoter within the leader region of the gene. One Tyr box is even found within a protein-coding region.

The *aroH* regulatory region contains a Tyr box with a G-N_{14}-C motif within a 117-bp leader region (Fig. 1). The similarity to the consensus sequence and the involvement of the Tyr repressor in *aroH* expression suggest that this *aroH* G-N_{14}-C motif may be a good candidate for the Tyr repressor binding site. This presumptive operator is 38 bp away from the known Trp repressor binding site that is situated astride the −35 hexamer of the *aroH* promoter. Thus, *aroH* expression appears to be jointly controlled through the action of two different repressors acting at separate target sites in the *aroH* regulatory region.

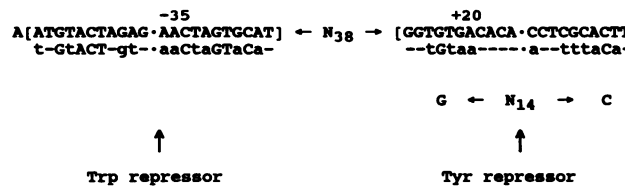

FIG. 1. Putative repressor binding sites in the *aroH* regulatory region. Upper line, part of the sequence for the *aroH* regulatory region. Brackets indicate the 22-bp imperfect palindromes, the potential targets for the *trpR* gene product around −35 and the *tyrR* gene product around +20; numbers 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 line right, 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-N_{14}-C motif are the only invariant bases.

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