JOURNAL OF BACTERIOLOGY, Oct. 1987, p. 4710–4715
0021-9193/87/104710-06$02.00/0

Vol. 169, No. 10

Molecular Analysis of the Regulatory Region of the *Escherichia coli* K-12 *tyrB* Gene

JI YANG AND JAMES PITTARD*

Department of Microbiology, The University of Melbourne, Parkville, Victoria 3052, Australia

Received 6 May 1987/Accepted 17 July 1987

The *tyrB* gene from *Escherichia coli* K-12 was cloned and sequenced, and the transcriptional start point of *tyrB* was determined by primer extension. By using a fusion plasmid in which the *lacZ* structural gene is transcribed from the *tyrB* promoter, it was shown that the expression of *tyrB* is controlled at the transcriptional level by the TyrR protein, with tyrosine as corepressor. The fusion plasmid was used to isolate mutants in which the repression of *tyrB* had been abolished. The *tyrB* promoter-operator region of these mutants was sequenced, and the *tyrB* operator was identified. A comparison between the *tyrB* operator and those of the other genes belonging to the *tyrR* regulon is presented.

The last reaction in the phenylalanine and tyrosine biosynthetic pathways involves the transamination of the keto acids phenylpyruvate and 4-hydroxyphenylpyruvate, respectively. The key enzyme for carrying out this reaction is an aminotransferase coded for by the *tyrB* gene (10). Another aminotransferase coded for by the very closely related *aspC* gene can also carry out these reactions, although it has a lower affinity for these substrates and seems to be primarily involved in the formation of aspartate (10). A third aminotransferase encoded by the unrelated *ilvE* gene has some limited ability to synthesize phenylalanine but is primarily involved in the synthesis of the branched-chain amino acids (10).

The expression of the *tyrB* gene has been shown to be repressed by tyrosine in cells with but not without a functional TyrR repressor (24, 26). The *tyrB* gene is, therefore, a part of the *tyrR* regulon. Eight separate transcription units are controlled by the TyrR protein, and the operator sequences controlling six of these have been described (3, 5–7, 13, 14).

Recently, two papers have been published reporting the nucleotide sequence of the *tyrB* gene (8, 17). In this study we identified the operator locus of the *tyrB* gene and determined the start point of transcription.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this study were all derivatives of *Escherichia coli* K-12. Their relevant genotypes are shown in Table 1. The plasmids used are shown in Table 2.

Media and chemicals. The minimal medium used was prepared from the 56/2 buffer described by Monod et al. (20). When tyrosine was added to the medium, it was added at a concentration of 10^{-3} M.

The chemicals used were all obtained commercially and not purified further. Trimethoprim and ampicillin were used in minimal medium at final concentrations of 10 and 50 μg/ml, respectively. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was used at a final concentration of 25 μg/ml. [α-^{35}S]dATP (1,200 Ci/mmol; 7.9 mCi/ml) used in DNA sequencing and primer extension was obtained from Amersham.

* Corresponding author.

β-Galactosidase assay. β-Galactosidase activity was assayed as described by Miller (19).

Aromatic amino acid aminotransferase assay. The aromatic amino acid aminotransferase was assayed by the method described previously by Wallace and Pittard (26), except that specific activities are expressed in international units (1 μmol of product formed per min per mg of protein at 37°C).

Recombinant DNA techniques. Standard recombinant DNA procedures were used essentially as described by Maniatis et al. (18). DNA fragments to be sequenced were cloned in both orientations in M13tg130 and M13tg131 vectors (15), and deletions were generated by using exonuclease III (12). Nucleotide sequences were determined by the chain termination method described by Sanger et al. (23).

Primer extension technique. The 5′ end of the in vivo transcripts was determined by the primer extension technique as described by Hudson and Davidson (13).

Sodium bisulfite mutagenesis. In vitro mutagenesis with sodium bisulfite was based on the method of Peden and Nathans (22). Treatment was with 0.9 M sodium bisulfite for 20 min.

RESULTS

Cloning the *tyrB* gene. Gelfand and Steinberg (10) have previously described a strain, DG30, which, as a result of mutations in *tyrB*, *aspC*, and *ilvE*, is defective in all aromatic aminotransferase activity. However, this strain is not readily transformed, and so a new strain (JP4287) was constructed which carries the same *tyrB*, *aspC*, and *ilvE* mutations but was readily transformed with plasmid DNA (data not shown).

A number of plasmids in the Clark and Carbon collection have been previously listed as carrying the *tyrB*$^+$ gene (21). We obtained three of these plasmids (pLC28-33, pLC33-42, and pLC44-14) from the *E. coli* Genetic Stock Center, Yale University, New Haven, Conn. Plasmid DNA was prepared and was used to transform JP4287. Of the three plasmids used, only pLC28-33 converted JP4287 to a TyrB$^+$ phenotype. The *tyrB* gene was then subcloned from pLC28-33 on a 1.7-kilobase (kb) *Hin*dIII-*Bam*HI fragment to form plasmid pMU1484.

This plasmid was introduced into three strains which differed in their *tyrR* genotype. JP4443 has the mutant *tyrR* allele *tyrR366*, JP4441 is a normal haploid *tyrR*$^+$, and JP4442 carries many copies of the *tyrR*$^+$ gene on the multicopy

TABLE 1. *E. coli* strains used in this study

Strain	Characteristic(s)[a]	Source or reference
DG30	*tyrB507 ilvE12 aspC13 thi-1 argE3 proA2 hisG4 hsdS14 hppT29 lacY1 galK2 ara-14 xyl-5 mtl-1 rpsL31 tsx-33* λ^- *supE44 recB21 recC22 sbcB15*	D. H. Gelfand (10)
JP4279	*thi-1 aroB351 thr-1 rpoB312 supE44 gal-351 lacY1 rpsL744*	This laboratory
JP4287	JP4279 *aroB*$^+$ *tyrB507 ilvE12 aspC12 argE3*	A series of steps involving DG30 and JP4279. This laboratory.
JP4431	JP4287 *tyrR366*	By P1 *kc* transduction and by the method of Bochner et al. (2)
JP3561	*thr-1 leu-1 ΔlacZM15 Δ(aroL478::*Tn*10)606 supE44 fhuA2 gyrA379*	P. Kasian (14)
JP4822	JP3561 *tyrR366*	By P1 *kc* transduction and by the method of Bochner et al. (2)
JP4441	JP4287(pMU1484)	By transformation
JP4442	JP4441(pMU400)	By transformation
JP4443	JP4431(pMU1484)	By transformation
JP4453	JP3561(pMU1490)	By transformation
JP4454	JP4453(pMU360)	By transformation
JP4455	JP4822(pMU1490)	By transformation
JP4456	JP3561(pMU360)	By transformation
JP6528	JP4456(pMU1491)	By transformation
JP6529	JP4456(pMU1492)	By transformation
JP6530	JP4456(pMU1493)	By transformation
JP6531	JP4456(pMU1494)	By transformation

[a] The genetic nomenclature is that described by Bachmann (1). *zci-2*::Tn*10* describes the position of the transposon insertion in accordance with the nomenclature of Kleckner et al. (16). Allele numbers are given when known.

TABLE 2. Plasmids used in this study

Plasmid	Characteristic(s)[a]	Source or reference
pMU530	Tp^r pREG151 replicon (pREG151 is a mini R388 replicon)	P. Kasian (14)
pMU1484	pMU530 with a 1.7-kb *Bam*HI-*Hin*dIII *tyrB*$^+$ fragment	This study
pMU400	Ap^r *tyrR*$^+$ ColE1 replicon	E. Cornish (5)
pMU575	Promoter cloning vector based on pMU530	This study
pMU1490	A pMU575 derivative with a 251-bp *Bam*HI-*Sma*I fragment carrying *tyrB* regulatory region	This study
mpMU27	M13tg131 with an insert comprising the *tyrB* regulatory region	This study
pMU1491	pMU1490 derivative carrying *tyrB* promoter mutation	This study
pMU1492	pMU1490 derivative carrying *tyrB* promoter mutation	This study
pMU1493	pMU1490 derivative carrying *tyrB* operator mutation	This study
pMU1494	pMU1490 derivative carrying *tyrB* operator mutation	This study
pLC28-33	*tyrB*$^+$ ColE1 replicon	F. Neidhardt et al. (21)
pMU360	Ap^r *tyrR*$^+$ ColE1 replicon	E. Cornish (5)

[a] Ap^r, Ampicillin resistance; Tp^r, trimethoprim resistance.

plasmid pMU400. These strains were grown in the presence and absence of tyrosine and assayed for their aminotransferase activity with tyrosine as the substrate.

The results (Table 3) confirm that pMU1484 contains a gene (*tyrB*) which codes for an aminotransferase active in the interconversion of 4-hydroxyphenylpyruvate and tyrosine whose synthesis is repressed by the TyrR protein and tyrosine. The results also show that increasing the number of *tyrR*$^+$ gene copies in the cell further increases the extent of *tyrB* repression. By using exonuclease *Bal* 31, a 1.4-kb fragment was obtained which showed the same regulated expression of *tyrB* (data not shown). The entire sequence of this 1.4-kb fragment was then determined by the dideoxy method of Sanger et al. as described in Materials and Methods.

While this work was being completed, the entire *tyrB* sequence was reported by Kuramitsu et al. (17). Since then, a second report of the *tyrB* sequence has also appeared (8). Our sequence, which is in agreement with the sequences in these reports, is not presented but was used to subclone the *tyrB* promoter and operator region.

Subcloning the *tyrB* promoter-operator region. Inspection of the sequence at the amino terminus and upstream of the *tyrB* structural gene revealed possible promoter-operator sites. These were present on a 251-base-pair (bp) fragment that had been prepared by exonuclease III digestion as part of the strategy of sequencing the gene. This fragment, the sequence of which is shown in Fig. 1, was cloned from mpMU27 into the *Bam*HI and *Sma*I sites of the promoter cloning vector pMU575.

Plasmid pMU575 (Fig. 2; A. Wright, unpublished data) was constructed by using the pREG151 replicon. It is a *galK'-lacZ'YA'* fusion vector in which the amino terminus of the *galK* gene, containing all the translational start signals but no promoters, is fused in phase with the eighth codon of the *lacZ* gene. This plasmid has termination codons present in all three reading frames between the polylinker region and the start of the *galK* gene, so that the translation of the *lacZ* message should be independent of any translation events initiated within the inserted fragment. In addition, a transcription termination signal has been inserted upstream of the polylinker site of pMU575 to eliminate readthrough transcription from upstream promoters into the *lacZ* gene.

The new plasmid (pMU1490) was transformed into the various *tyrR* and *tyrR*$^+$ strains as before, and after purification the transformants were grown in the presence and

TABLE 3. Aromatic amino acid aminotransferase activities in strains containing pMU1484 and different copy numbers of the *tyrR*$^+$ gene

Strain	Description	Copy no. of *tyrR*$^+$ gene	Sp act (mU) of aromatic amino acid aminotransferase[a] from cells grown in[b]:	
			MM	MM + Tyr
JP4443	JP4431(pMU1484)	0	38	34
JP4441	JP4287(pMU1484)	1	17	4
JP4442	JP4287(pMU1484, pMU400)	Multicopy	12	2.5

[a] The aromatic amino acid aminotransferase activity was measured by estimating the conversion of tyrosine to 4-hydroxyphenylpyruvate.

[b] MM, Minimal medium; MM + Tyr, minimal medium supplemented with 10^{-3} M tyrosine.

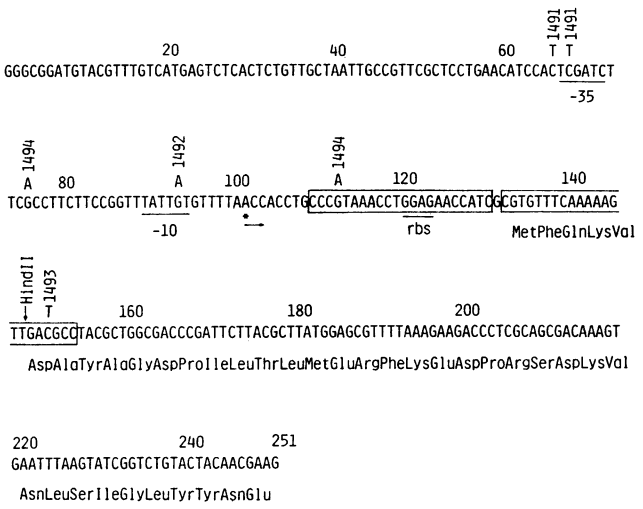

FIG. 1. Nucleotide sequence of antisense strand of the *tyrB* regulatory region. The Shine-Dalgarno box is labeled (rbs) and underlined (8). The *tyrB* transcription start site, determined by primer extension, is marked with an asterisk and an arrow, and the putative −35 and −10 regions are underlined. The *Hin*dII restriction site on the sequence used to prepare the probe for primer extension is indicated. The double TYR R boxes are boxed. The base changes in the mutant plasmids are as follows: pMU1491, C-to-T substitutions at positions 66 and 68; pMU1492, G-to-A substitution at position 94; pMU1493, C-to-T substitution at position 151; pMU1494, G-to-A substitutions at positions 76 and 113.

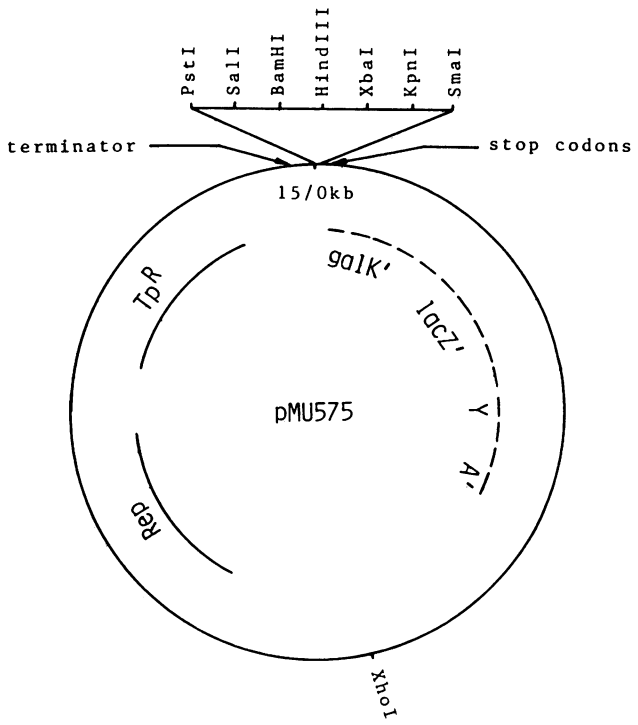

FIG. 2. Physical map of promoter cloning vector pMU575. Details are given in the text.

absence of tyrosine and assayed for β-galactosidase. Strains with pMU1490 synthesized β-galactosidase, and the synthesis was repressed when *tyrR*$^+$ cells were grown in the presence of tyrosine (Table 4). The failure to observe repression in *tyrR* cells and the enhanced repression in a multicopy *tyrR*$^+$ strain confirm that this 251-bp fragment contains the *tyrB* promoter-operator sequence.

Determination of the site of transcription initiation within the *tyrB* promoter region. The primer extension technique described by Hudson and Davidson (13) was used to determine the 5′ endpoint of the *tyrB* transcript, which presumably represents the transcription start point of the *tyrB* message. A 150-base *Hin*dII single-stranded DNA primer was generated from the M13tg131 derivative (mpMU27) which carries the 251-bp *tyrB* regulatory region. In this instance, single-stranded mpMU27 was first annealed with the conventional 17-base M13 sequencing primer. [α-^{35}S]dATP was used as a label, and after de novo synthesis, the enzyme *Hin*dII, which cuts the DNA at position 148 (Fig. 1), was used for digestion. The *Hin*dII site lies in the coding region of the gene and must therefore be downstream from the transcription start point. After digestion, the DNA was denatured at 100°C and the single strands were separated on a 6% sequencing gel. The single strand comprising the sequence from bases 148 to 251 and corresponding to the sense strand of the sequence represented in Fig. 1 with the addition of the M13 sequencing primer on the 5′ (base 251) end was prepared and used as a single-stranded primer. After hybridization to mRNA from a strain carrying pMU1490 grown under conditions which derepress *tyrB*, the primer was extended with reverse transcriptase. The product of extension was electrophoresed next to the sequencing reactions of the identical clone used to generate the primer. The results of the primer extension (Fig. 3) demonstrate that the start point of transcription is at position 102 of the sequence shown in Fig. 1. An examination of the sequence upstream from the start point revealed a possible promoter whose −10 and −35 regions are underlined in Fig. 1.

Localized mutagenesis of the *tyrB* promoter-operator region and characterization of the mutant plasmids. Plasmids pMU575 and pMU1490 differ only by the 251-bp insert present in pMU1490. Since this insert contains the promoter-operator region, these two plasmids can readily be used together to create the appropriate single-stranded target for sodium bisulfite mutagenesis (22).

Plasmid pMU575 was digested with *Sma*I and *Bam*HI, and pMU1490 was digested with *Xho*I. Both digests were then denatured, mixed, and reannealed. The resultant heteroduplexes contained the 251-base insert as a single-stranded region. The heteroduplexes were mutagenized with sodium bisulfite as described in Materials and Methods, and the gap was filled in in vitro with Klenow fragment and deoxynucleoside triphosphates. Plasmid DNA was then transformed into a strain (JP4456) which carries the multicopy *tyrR*$^+$ plasmid pMU360, and transformants were plated onto minimal medium supplemented with X-Gal and tyrosine. On this

TABLE 4. Repression of β-galactosidase in strains carrying the *tyrB-lacZ* fusion plasmid

Strain	Description	Copy no. of *tyrR*$^+$ gene	β-Galactosidase activity[a] of cells grown in[b]:	
			MM	MM + Tyr
JP4455	JP4822(pMU1490)	0	508	451
JP4453	JP3561(pMU1490)	1	338	131
JP4454	JP3561(pMU1490, pMU360)	Multicopy	156	34

[a] The units of β-galactosidase specific activity are those defined by Miller (19).

[b] Abbreviations are defined in Table 3, footnote *b*.

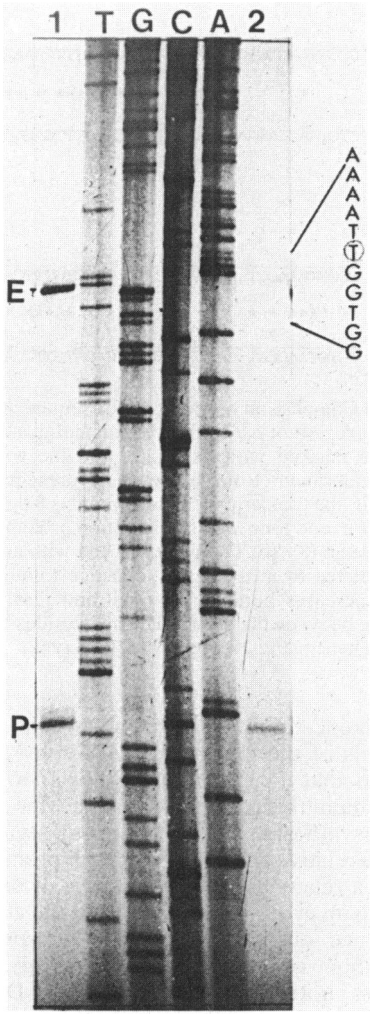

FIG. 3. Determination of the start site of *tyrB* transcription by primer extension. A 150-bp *Hin*dII ^{35}S-labeled probe was used as the primer (band P), and the product of extension (band E) using this primer corresponded to the circled T residue in the sequencing ladder of the sense strand of the *tyrB* regulatory region. Lane 1, Probe extended when annealed to RNA from a strain carrying pMU1490; lane 2, probe extended in the absence of RNA. The other lanes show the products of the dideoxynucleotide sequencing reactions obtained when the identical M13tg131 clone used to generate the primer was sequenced.

medium, derepressed mutants produce colonies that are a significantly deeper blue than the pale blue colonies of the wild-type strain. This difference is maximized by the presence of pMU360 in the recipient strain. A number of deep blue colonies were chosen for further study. To confirm that the altered phenotype was caused by mutation in the pMU1490 derivatives, these plasmids were, in each instance, separated from pMU360, transformed again into JP4456, and assayed for β-galactosidase. The β-galactosidase activities of four of these transformed strains are shown in Table 5. All four strains showed greatly enhanced β-galactosidase activity in both minimal medium and minimal medium supplemented with tyrosine. Strains carrying plasmid pMU1493 or pMU1494 were not repressed at all by tyrosine and synthesized β-galactosidase at levels slightly less than that observed in the *tyrR* mutant JP4455 (Table 4). Strains with plasmid pMU1491 or pMU1492, on the other hand, synthesized β-galactosidase at levels three times higher than those observed in JP4455, and the addition of tyrosine to the medium still caused repression, although significantly less than that observed with the wild type.

TABLE 5. Expression of β-galactosidase from wild-type and mutant *tyrB-lacZ* fusions

Strain	Description	β-Galactosidase activity of cells grown in[a]:	
		MM	MM + Tyr
JP4454[b]	JP3561(pMU1490, pMU360)	156	34
JP6528	JP3561(pMU1491, pMU360)	1,594	916
JP6529	JP3561(pMU1492, pMU360)	1,222	894
JP6530	JP3561(pMU1493, pMU360)	354	313
JP6531	JP3561(pMU1494, pMU360)	356	334

[a] Abbreviations are defined in Table 3, footnote *b*.
[b] Data for JP4454 are repeated from Table 4.

The DNA fragment carrying the *tyrB* promoter-operator region was cloned from each of the mutant plasmids into M13tg130 and M13tg131 and sequenced. The base changes in these four mutants are shown in Fig. 1. In Fig. 1 we boxed a sequence which resembles the double TYR R boxes previously reported for other genes of the *tyrR* regulon whose expression is controlled by tyrosine. Although the agreement with the TYR R box consensus sequence is not strong, the hypothesis is validated by the finding that both mutants in which repression was abolished (pMU1493 and pMU1494) have base changes affecting G · C pairs in these putative TYR R boxes. Plasmid pMU1494 has a second base change at position 76 whose effect, if any, is unknown. The double TYR R boxes in *aroF*, *aroL*, *aroP*, *tyrP*, and *tyrB* and the base changes that have been identified in operator constitutive mutants are shown in Fig. 4.

The base substitutions in the two mutant plasmids (pMU1491 and pMU1492) which showed levels of β-

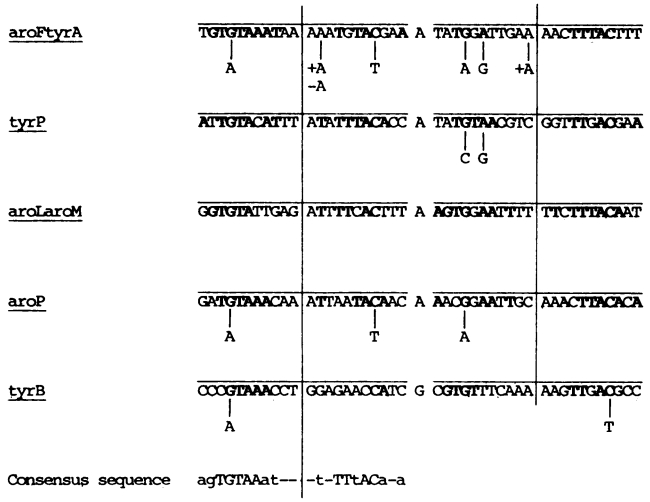

FIG. 4. Comparison of the double TYR R boxes of *aroF-tyrA*, *tyrP*, *aroL-aroM*, *aroP*, and *tyrB* (3, 4, 7, 9, 14). The consensus sequence for the TYR R box (3) is shown; capital letters indicate bases found in at least 9 of the 12 previously reported TYR R boxes, whereas lowercase letters denote those common to at least 6 boxes. Nucleotide changes in various operator constitutive mutants are marked. The nucleotides that match the consensus sequences are shown in boldface type. Both *aroF-tyrA* and *aroL-aroM* have an additional box at a distance of 30 and 31 bases, respectively, from the double boxes.

galactosidase in excess of those observed in a *tyrR* mutant are found in the −10 and −35 regions of the postulated *tyrB* promoter. In both instances the base changes increased homology with the promoter consensus sequence (11).

DISCUSSION

The identification of the operator site for the *tyrB* gene brings to seven the number of transcription units of the *tyrR* regulon whose operator loci have been sequenced (3–7, 9, 13, 14). Only *mtr* remains to be analyzed. Base changes resulting in a loss of operator function have been studied for *aroF* (4, 9), *tyrP* (14), *aroP* (3), *aroG* (N. Bassegio and B. E. Davidson, unpublished results), *aroL* (A. Wright and J. Pittard, unpublished results), and *tyrB* (this study). These results have allowed us to identify a family of sequences which are all closely related to the consensus sequence agTGTAAat---t-TTtACa-a. These sequences have been termed TYR R boxes. All of the operator mutations involving simple base substitutions fall within the TYR R boxes. Most of these affect the G · C and C · G pairs that are invariably found 14 bases apart in the left and right arms of the TYR R boxes. The two genes of the regulon whose expression is not controlled by tyrosine, *aroG* and *tyrR*, contain a single TYR R box adjacent or overlapping the −35 region of the promoter (5, 6). Both of these boxes are highly symmetrical and show a close agreement with the consensus sequence. On the other hand, the transcription units whose expression is controlled by tyrosine, *aroF*, *aroL*, *tyrP*, and *aroP*, have all been shown to contain at least two TYR R boxes and consistently exhibit double boxes separated by a single A · T base pair (3, 4, 7, 9, 14). In *tyrB* the same arrangement is conserved, although in this instance a G · C base pair intervenes between the two boxes (Fig. 4). In general, both the symmetry and the agreement with the consensus sequence are weaker in the TYR R boxes occurring in pairs. In *aroF* and *tyrP* the double boxes overlap the −35 regions, in *aroL* they overlap the −10 region, and in *aroP* they are situated 40 or so bases downstream from the −10 sequence. In *tyrB*, as reported above, the TYR R boxes are adjacent to the −10 region and overlap the ribosome-binding site sequence and the beginning of the structural gene.

The mutations which affect the TyrR-mediated repression of *tyrB* occur in one or the other of the palindromic G · C pairs which have been found to be altered in other operator mutants of the *tyrR* regulon (Fig. 4). Two of the mutants selected for their enhanced β-galactosidase expression but which still showed some repression have base changes that alter either the −10 or −35 sequence of the promoter. In both instances, the new sequences show a closer homology with the ideal consensus sequence (11). The diminished repression in these mutants would seem to result from an increased affinity between the RNA polymerase and the promoter rather than a change in the affinity between repressor and operator. This will have to be confirmed by in vitro studies.

In comparison with the *aroF-tyrA* operon, which codes for the tyrosine-repressible enzymes of the first reaction of the common pathway and of the terminal pathway, *tyrB* is relatively insensitive to repression. When cells are grown in minimal medium, the endogenous tyrosine pool results in about a 90% repression of *aroF-tyrA* (25) but 0 to 40% repression of *tyrB*. Overall, the TYR R boxes of *tyrB* show a weaker homology with the TYR R consensus sequence than do the *aroF* boxes (Fig. 4), and this may be the explanation

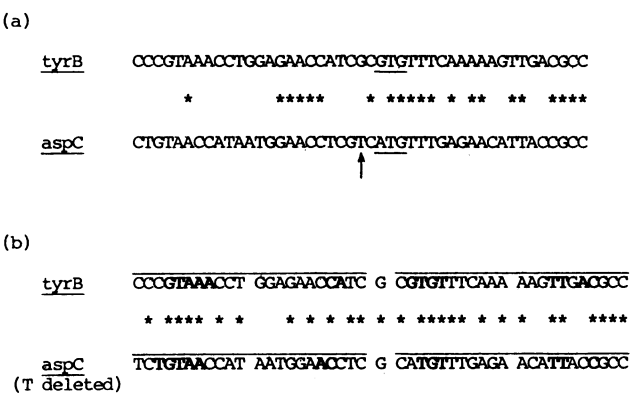

FIG. 5. (a) Comparison of the operator sequence of *tyrB* and the corresponding region of *aspC* (8). The nucleotides common in both sequences are marked with asterisks, and the translational start codons are underlined. Both sequences represent transcribed regions. For *tyrB*, the transcriptional start point is 8 bp upstream of the sequence shown, and for *aspC*, transcription probably commences 9 or 10 bp upstream (8). (b) T residue marked with an arrow in panel a has been deleted from the *aspC* sequence to align the left-hand boxes. This increases homology overall and reestablishes the required spacing between the boxes. The nucleotides which match the consensus sequence (Fig. 4) are in boldface type.

for the difference. Furthermore, a consideration of derepressed levels of the enzyme coded for by *aroF-tyrA* and *tyrB* suggests that if *tyrB* expression was 90% repressed in minimal medium, this reaction would become rate limiting in tyrosine biosynthesis. It is tempting to argue that in this instance, the relative strength of the *tyrB* promoter may have selected for a relatively inefficient operator or vice versa.

Fotheringham et al. (8) have reported the complete nucleotide sequence of both the *tyrB* and *aspC* genes. The sequences show an overall DNA homology of 51%. The investigators note, however, that the DNA encoded nonconserved regions of the proteins have undergone considerable evolutionary change. Since *tyrB*, but not *aspC*, is controlled by the TyrR protein, it was of interest to compare the nucleotide sequences of these two genes in the regions of the TYR R boxes.

In Fig. 5 the *tyrB* and *aspC* sequences have been aligned so that the translation start codons for the two enzymes coincide. The right-hand TYR R box of *tyrB* also codes for the first seven amino acids of the *tyrB*-encoded aminotransferase. Perhaps for this reason sequence homology between *tyrB* and *aspC* remains high (15 of 22 bases) in this region. On first inspection, however, sequence homology seems to fall to a much lower level (6 of 22 bases) in the region of the left-hand box. When the *aspC* sequence was examined for the presence of TYR R boxes, the following observations were made. The sequence corresponding to the right-hand TYR R box has 6 of 17 bases of the TYR R consensus sequence, two less than the corresponding region in *tyrB*. The upstream sequence also contains a recognizable TYR R box, but in *aspC* two bases rather than one separate the two boxes. By deleting a thymine nucleotide (Fig. 5), overall homology between the two sequences is restored and the double TYR R box appears in *aspC* with the same spacing as in other genes of the regulon.

The observation that *aspC* is not controlled by the TyrR protein suggests that neither the sequence of the single right-hand box nor that of the single left-hand box is sufficient for control. Similarly, *tyrB* mutants which have only one box

intact are no longer controlled by the TyrR protein. Whether a deletion of a single thymine nucleotide (Fig. 5) would result in *aspC* coming under TyrR-mediated control or whether it would require other base changes within the TYR R box region has yet to be tested. The marked similarity between the two genes does, however, pose the possibility that the TYR R boxes evolved to control *tyrB* before *aspC* was produced by duplication and that since that event an *aspC* control that was inappropriate was discarded.

Finally, recent studies of the regulation of *tyrP* have shown that if the number of bases between the TYR R boxes is changed from one to three, tyrosine repression is abolished (A. Wright, unpublished results).

ACKNOWLEDGMENTS

We thank L. Vizard and M. Wright for technical assistance. We thank our colleagues for helpful discussion and B. E. Davidson and A. P. Kyne for making the MELBDBSYS suite of computer programs available to us.

This study was supported by the Australian Research Grant Scheme. J.Y. holds a University of Melbourne Postgraduate Research Award.

LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Bochner, B. R., H.-C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143**:926–933.
3. **Chye, M.-L., and J. Pittard.** 1987. Transcription control of the *aroP* gene in *Escherichia coli* K-12: analysis of operator mutants. J. Bacteriol. **169**:386–393.
4. **Cobbett, C. S., and M. L. Delbridge.** 1987. Regulatory mutants of the *aroF-tyrA* operon of *Escherichia coli* K-12. J. Bacteriol. **169**:2500–2506.
5. **Cornish, E. C., V. P. Argyropoulos, J. Pittard, and B. E. Davidson.** 1986. Structure of the *Escherichia coli* K-12 regulatory gene *tyrR*: nucleotide sequence and sites of initiation of transcription and translation. J. Biol. Chem. **261**:403–412.
6. **Davies, W. D., and B. E. Davidson.** 1982. The nucleotide sequence of *aroG*, the gene for 3-deoxy-D-arabinoheptulosonate-7-phosphate synthetase (phe) in *Escherichia coli* K-12. Nucleic Acids Res. **10**:4045–4058.
7. **DeFeyter, R. C., B. E. Davidson, and J. Pittard.** 1986. Nucleotide sequence of the transcription unit containing the *aroL* and *aroM* genes from *Escherichia coli* K-12. J. Bacteriol. **165**:233–239.
8. **Fotheringham, I. G., S. A. Dacey, P. P. Taylor, T. J. Smith, M. G. Hunter, M. E. Finlay, S. B. Primrose, D. M. Parker, and R. M. Edwards.** 1986. The cloning and sequence analysis of the *aspC* and *tyrB* genes from *Escherichia coli* K-12. Biochem. J. **234**:593–604.
9. **Garner, C. G., and K. M. Herrmann.** 1985. Operator mutants of the *Escherichia coli aroF* gene. J. Biol. Chem. **260**:3820–3825.
10. **Gelfand, D. H., and R. A. Steinberg.** 1977. *Escherichia coli* mutants deficient in the aspartate and aromatic amino acid aminotransferases. J. Bacteriol. **130**:429–440.
11. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11**:2237–2255.
12. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
13. **Hudson, G. S., and B. E. Davidson.** 1984. Nucleotide sequence and transcription of the phenylalanine and tyrosine operons of *Escherichia coli* K-12. J. Mol. Biol. **180**:1023–1051.
14. **Kasian, P. A., B. E. Davidson, and J. Pittard.** 1986. Molecular analysis of the promoter operator region of the *Escherichia coli* K-12 *tyrP* gene. J. Bacteriol. **167**:556–561.
15. **Kieny, M. P., R. Lathe, and J. P. Lecocq.** 1983. New versatile cloning and sequencing vectors based on the bacteriophage M13. Gene **26**:91–99.
16. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. J. Mol. Biol. **116**:125–159.
17. **Kuramitsu, S., K. Inoue, T. Ogawa, H. Ogawa, and H. Kagamiyama.** 1985. Aromatic amino acid aminotransferase of *Escherichia coli*: nucleotide sequence of the *tyrB* gene. Biochem. Biophys. Res. Commun. **133**:134–139.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Monod, J., G. Cohen-Bazire, and M. Cohn.** 1951. Sur la biosynthese de la β-galactosidase (lactase) chez, *Escherichia coli*. La specificite de l'induction. Biochim. Biophys. Acta **7**:585–599.
21. **Neidhardt, F. C., V. Vaughn, T. A. Phillips, and P. L. Bloch.** 1983. Gene-protein index of *Escherichia coli* K-12. Microbiol. Rev. **47**:231–284.
22. **Peden, K. W. C., and D. Nathans.** 1982. Local mutagenesis within deletion loops of DNA heteroduplexes. Proc. Natl. Acad. Sci. USA **79**:7214–7217.
23. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
24. **Silbert, D. F., S. E. Jorgenson, and E. C. C. Lin.** 1963. Repression of transaminase A by tyrosine in *Escherichia coli*. Biochim. Biophys. Acta **73**:232–240.
25. **Tribe, D. E., H. Camakaris, and J. Pittard.** 1976. Constitutive and repressible enzymes of the common pathway of aromatic biosynthesis in *Escherichia coli* K-12: regulation of enzyme synthesis at different growth rates. J. Bacteriol. **127**:1085–1097.
26. **Wallace, B. J., and J. Pittard.** 1969. Regulator gene controlling enzymes concerned in tyrosine biosynthesis in *Escherichia coli*. J. Bacteriol. **97**:1234–1241.