Up-Promoter Mutations in the *trpBA* Operon of *Pseudomonas aeruginosa*

CHUN-YA HAN, IRVING P. CRAWFORD,[†] AND CAROLINE S. HARWOOD*

Department of Microbiology, University of Iowa, Iowa City, Iowa 52242

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In *Pseudomonas aeruginosa*, the operon encoding tryptophan synthase (*trpBA*) is positively regulated by the TrpI protein and an intermediate in tryptophan biosynthesis, indoleglycerol phosphate (InGP). A gene fusion in which the *trpBA* promoter directs expression of the *Pseudomonas putida xylE* gene was constructed. By using a *P. putida* F1 *todE* mutant carrying this fusion on a plasmid, three *cis*-acting mutations that increased *xylE* expression enough to allow the *todE* strain to grow on toluene were isolated. The level of *xylE* transcript from the *trpBA* promoter was increased in all three mutants. All three mutations are base substitutions located in the -10 region of the *trpBA* promoter; two of these mutations make the promoter sequence more like the *Escherichia coli* RNA polymerase σ^{70} promoter consensus sequence. The activities of the wild-type and mutant *trpBA* promoters, as monitored by *xylE* expression, were assayed in *P. putida* PpG1 and in *E. coli*. The up-regulatory phenotypes of the mutants were maintained in the heterologous backgrounds, as was *trpI* and InGP dependence. These results indicate that the *P. aeruginosa trpBA* promoter has the key characteristics of a typical *E. coli* positively regulated promoter. The results also show that the *P. aeruginosa* and *P. putida trpI* activator gene products are functionally interchangeable.

Bacteria of the genus *Pseudomonas* are important human and plant pathogens. The pseudomonads also have beneficial attributes, the most notable of which is their extreme metabolic versatility. The ability of this group of bacteria to catalyze commercially valuable bioconversions is being intensively investigated, and the pseudomonads also show great promise as agents of bioremediation (45). An understanding of gene expression signals typically used by these gram-negative bacteria is important if their biological potential is to be fully appreciated and would also be required for practical applications in which the expression of cloned *Pseudomonas* genes in heterologous bacteria, such as *Escherichia coli*, is mandated.

Often, *Pseudomonas* genes are not very well expressed in *E. coli* (27, 29, 34, 47). Most of the genes that have been studied in detail are positively regulated, and the requirement for specific activator proteins and effector molecules probably, at least partially, explains the observed poor expression (25, 30, 38–40, 44, 52). This explanation cannot wholly account for the effects seen however, because constitutive genes are often also poorly expressed in *E. coli* (27). Thus it would seem likely that inefficient recognition of *Pseudomonas* sequences by particular components of the *E. coli* transcription-translation machinery represents an additional barrier to effective expression.

To date, studies of *Pseudomonas* gene expression have focused almost exclusively on transcription initiation. Comparisons of nucleotide sequences upstream from transcriptional start sites of *Pseudomonas* genes have allowed the compilation of promoter sequences. Some of these sequences show some similarity to the σ^{70} and/or σ^{54} consensus sequences of *E. coli* (1, 13, 47). There are also *Pseudomonas* promoter sequences for which no *E. coli* consensus similarity can be discerned (47). Because only a very small

number of promoters are available for sequence comparison and because most of these are positively regulated promoters, which would be expected to show significant deviation from *E. coli* consensus sequences in any case (41), it is difficult to know whether promoter sequences unique to *Pseudomonas* species exist or whether similar sequences define functional promoters in both *Pseudomonas* species and *E. coli*.

Here, we have addressed this question by isolating and characterizing up-promoter mutations of the *Pseudomonas aeruginosa trpBA* operon. In *P. aeruginosa* and *Pseudomonas putida*, expression of the *trpBA* genes, encoding the two subunits of tryptophan synthase, is positively regulated by the substrate, indoleglycerol phosphate (InGP), and by the product of the *trpI* gene (8, 10, 11, 30). This is in contrast to the situation in *E. coli*, in which the synthesis of the *trpA* and *trpB* products is not inducible but instead is directly repressed by tryptophan (51). *trpI* and the *trpBA* operon are divergently transcribed in both *Pseudomonas* species; the translational start sites are separated by 103 bp in *P. aeruginosa* and by 110 bp in *P. putida*. The *P. aeruginosa* TrpI activator binding site and the effect of InGP on the interaction of *P. aeruginosa* TrpI with DNA have been examined by gel retardation, DNA footprinting, and in vitro transcription (6–8, 20).

We fused the *trpBA* control region from *P. aeruginosa* to the promoterless *xylE* gene carried on the expression vector pVDX18 (28). Plasmid mutations leading to increased *xylE* expression were isolated by selecting for complementation of a *P. putida* mutant unable to grow on toluene. The locations of the mutations, the specific base substitutions involved, and the levels of *xylE* expression that the mutations conferred in an *E. coli* background all indicate that the *trpBA* promoter has the characteristics of a typical *E. coli* σ^{70} -specific promoter. Our results also show that the *P. aeruginosa* and *P. putida* TrpI activator proteins are functionally interchangeable.

* Corresponding author.

[†] Deceased 8 October 1989.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Source or reference
Strains		
<i>P. putida</i>		
F107	<i>todE</i> ; derived from wild-type strain F1	17
IC1651	<i>trpE278 trpA506</i> ; derived from wild-type strain PpG1	Crawford laboratory
<i>E. coli</i>		
JM109	<i>recA1 endA1 gyrA96 thi hsdR17 supE44 relA1</i> λ^- Δ (<i>lac-proAB</i>) (F' <i>traD36 proAB lacI^qZ</i> Δ M15)	50
HB101	F ⁻ <i>leuB6 proA2 recA13 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL20</i> λ^- <i>supE44 hsdS20</i>	3
IC361	<i>r_B⁻ m_B⁻ trpE3B248A2</i>	12
Plasmids		
pRK2013	Km ^r ; ColE1 replicon with IncP Tra functions	16
pVDX18	IncQ Amp ^r (Cb ^r) <i>mob⁺ xylE⁺</i> (promoterless)	28
pMIB85	Cm ^r ; <i>trpI</i> from strain PAC174 cloned into pACYC184; <i>trpI</i> expressed from its own promoter	8
pMI603	Amp ^r ; <i>KpnI</i> fragment of <i>P. aeruginosa</i> PAO1 carrying <i>trpI</i> and the <i>trpI-trpBA</i> intragenic region, cloned into pUC18	6
pHH1	Amp ^r ; <i>P. aeruginosa</i> PAO1 <i>trpBA</i> control region, 176-bp <i>Bss</i> II- <i>Eco</i> O109 fragment from pMI603 inserted into <i>Xba</i> I-digested pVDX18 by blunt-end ligation to generate a <i>xylE</i> transcriptional fusion	This study
pHHB	A 1,044-bp <i>Ava</i> I fragment of p1395 (15) containing the <i>P. aeruginosa</i> PAO1 <i>trpG</i> promoter inserted into the <i>Xba</i> I site of pVDX18 in the reverse orientation relative to <i>xylE</i> transcription	This study
pHH2	Amp ^r ; pHH1 with mutation X9 (−8 T → C) in <i>trpP_B</i>	This study
pHH3	Amp ^r ; pHH1 with mutation X56 (−10 G → T) in <i>trpP_B</i>	This study
pHH4	Amp ^r ; pHH1 with mutation X57 (−8 T → A) in <i>trpP_B</i>	This study

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used are described in Table 1.

Media and antibiotics. *E. coli* and *P. putida* were grown at 37 and 30°C, respectively. Luria broth (LB) (35) was the complete medium. Growth on toluene was tested with cells grown on plates of a defined mineral salts medium (46). Toluene was supplied as a vapor (23). Vogel-Bonner (V-B) minimal salts medium E (48) supplemented with 0.2% glucose, 0.05% acid-hydrolyzed casein, and 5 μ g of tryptophan per ml was the defined medium used for all other experiments. Media were solidified with 15 g of agar (Difco Laboratories, Detroit, Mich.) per liter. *Pseudomonas* isolation agar (PIA), obtained from Difco, was used to select against *E. coli* donors in bacterial conjugations with *P. putida* strains.

Antibiotics were used in the following concentrations (in micrograms per milliliter): for *E. coli*, ampicillin, 100; chloramphenicol, 25; kanamycin, 50; for *P. putida*, ampicillin, 100; chloramphenicol, 500; kanamycin, 250; carbenicillin (in PIA), 1,000.

Plasmid manipulation. Procedures used for plasmid isolation and purification, restriction enzyme digestion, DNA ligation, and agarose electrophoresis were as previously described by Maniatis et al. (31). *E. coli* cells were made competent for transformation by the CaCl₂ method (9). Plasmids were introduced into *P. putida* strains in triparental matings with plasmid pRK2013 providing mobilization functions (14).

Selection of *cis*-acting mutations in pHH1. Plasmid pHH1, which carries the *trpBA* control region directing expression of the promoterless *xylE* gene (encoding catechol 2,3-dioxygenase) was conjugated into *P. putida* F107, a *todE* (3-

methylcatechol dioxygenase) mutant. *P. putida* F107(pHH1) cells were then grown overnight in LB, harvested, washed twice, and resuspended in an equal volume of 0.85% (wt/vol) NaCl. Samples (0.1 ml) of the washed cell suspensions were spread onto toluene minimal plates and incubated at 30°C. After 2 days, each plate had about 50 dark brown colonies. Seventy colonies were picked and restreaked on toluene minimal plates to verify the revertant phenotype. Plasmid DNA from each mutant was then purified and used to transform *E. coli* JM109, with selection on Luria (L)-agar plates containing ampicillin. The plasmid in each transformant was mobilized for transfer to *P. putida* F107 in a triparental mating with *E. coli* HB101(pRK2013). Of 59 exconjugants, 4 were able to grow on toluene plates and were assumed to carry a mutant pHH1 plasmid. The *trpBA* control region (176 bp) from each mutant plasmid was then sequenced.

Localization of plasmid mutations. To be certain that the observed mutant phenotypes were due to the identified base changes in the sequenced *trpBA* control region and not to other unidentified changes elsewhere in the plasmids, we digested the DNA with *Hind*III and *Eco*RI and recloned the *P. aeruginosa* DNA inserts into pVDX18 to generate plasmids pHH2, pHH3, and pHH4. The catechol 2,3-dioxygenase assays reported in this paper were carried out with these constructs. Assays with strains harboring the original mutant plasmids gave very similar results.

DNA sequencing. DNA fragments were labeled by filling in protruding 5' ends, using the Klenow fragment of *E. coli* DNA polymerase I and the appropriate α -³²P-labeled deoxynucleotide triphosphate (31). Sequencing of the *trpBA* control region upstream (between the *Hind*III and *Bam*HI sites of the multiple cloning site of pVDX18) from the *xylE*

transcriptional fusions was performed by the procedure of Maxam and Gilbert (33). In primer extension experiments, nucleotide sequencing was performed by the dideoxy-chain termination method (43) with the *Taq* DNA polymerase sequencing kit from United States Biochemical Corp.

Cell extracts and catechol 2,3-dioxygenase assays. Bacterial cells were grown in V-B minimal medium plus 0.2% glucose and any other supplements or antibiotics indicated. Preparation of cell extracts by sonication and assays for catechol 2,3-dioxygenase activity were performed as described by Konyecsni and Deretic (28). One unit of catechol 2,3-dioxygenase is defined as the amount of enzyme which converts catechol to 1 nmol of 2-hydroxymuconic semialdehyde (a yellow product with a molar extinction coefficient of 4.4×10^4 at 375 nm) per min. The catechol 2,3-dioxygenase data presented in each of the tables are from the single trial (of at least three trials) that gave the highest activities. Activities varied by as much as a factor of 4, depending on the trial. However, the relative activities between strains assayed in a given trial were always the same. The protein concentrations of cell extracts were determined by the method of Bradford (4).

Preparation of RNA. Total cellular RNA was isolated from 100-ml cultures of cells harvested by centrifugation at an A_{550} of 0.15 to 0.25, as described previously (2).

Primer extension mapping. A 31-residue oligonucleotide (5'-GAATTCGAGCTCGGTACCCGGGGATCCTCTA-3') complementary to a region of the pVDX18 multiple cloning site 59 bases from the presumed *trpBA* mRNA start site (6), was 5' end labeled with [γ^{32} P]ATP and T4 polynucleotide kinase and used in primer extension mapping of mRNA. The primer extension mapping was performed by the procedure of Sambrook et al. (42). The synthetic oligonucleotide was obtained from the Biotechnology Research Service at Northwestern University.

Northern (RNA) blot hybridization. The procedures used in Northern blot hybridization were those described by Sambrook et al. (42). Total cellular RNA was fractionated by electrophoresis through 1.2% agarose gels containing formaldehyde and then transferred to nylon membranes. The RNA of interest was detected by hybridization with the same radiolabeled synthetic oligonucleotide as was used for primer extension mapping.

RESULTS

Selection of up-regulatory mutants. Plasmid pHH1 was constructed by insertion of a 176-bp *Bss*II-*Eco*O109 fragment containing the *P. aeruginosa trpI-trpBA* control region (6) into the promoter probe vector pVDX18. The pVDX18 reporter gene, *xylE*, originates from the transmissible plasmid TOL, which carries a series of genes whose products are required for the degradation of toluene (18). *xylE* encodes a broad-substrate-specificity catechol 2,3-dioxygenase which is able to catalyze the oxidation of 3-methylcatechol as well as unsubstituted catechol (37, 49). *P. putida* F1 dissimilates toluene by a pathway (distinct from the TOL plasmid pathway) in which the oxidation of 3-methylcatechol by the product of the *todE* gene is a required step (17). Since the *xylE* and *todE* gene products have overlapping substrate specificities, one would predict that *xylE* would complement a *todE* defect. Colonies of the *todE* mutant *P. putida* F107 carrying pHH1 turned yellow on glucose minimal plates when sprayed with catechol, indicating that the plasmid expressed significant background (constitutive) catechol 2,3-dioxygenase activity. Nevertheless, the *trpBA* control re-

gion-*xylE* fusion plasmid did not contribute sufficient catechol 2,3-dioxygenase activity to complement the *todE* mutation, because cells carrying pHH1 were unable to form colonies on toluene minimal plates. Plating of *P. putida* F107(pHH1) cells on toluene minimal plates therefore imposed a strong selection for mutations resulting in higher levels of *xylE* expression. As outlined above (Materials and Methods), 59 mutant strains were isolated, 4 of which had plasmid-associated mutations. The nucleotide sequence of the *trpI-trpBA* control region from each mutant plasmid was determined in order to locate the mutation. As shown in Fig. 1, the mutations are located in the -10 region of the *trpBA* operon. Two of the plasmids had identical mutations [-8 T $\rightarrow$ C]).

To measure the effect of each mutation on *xylE* expression, we assayed catechol 2,3-dioxygenase levels in *P. putida* F107 carrying the three different mutant plasmids and compared them with those expressed from the parent plasmid (Table 2). As expected, all three mutations increased the levels of *xylE* expression.

Regulation of the mutant fusions by tryptophan and InGP. In *P. aeruginosa* and *P. putida* expression of several of the genes required for the synthesis of InGP are repressible by tryptophan (5, 32). The addition of exogenous tryptophan to growth medium will therefore decrease the intracellular concentration of the inducer (InGP) of the *trpBA* operon. As shown in Table 2, tryptophan, when present at a concentration that had a twofold effect on expression from the wild-type fusion, failed to repress *xylE* expression from the mutant fusion plasmids, indicating that the plasmid mutations provide at least partial relief from InGP dependence. To determine whether InGP had an activating effect on *xylE* expression from the mutant plasmids, the fusions were transferred into *P. putida* IC1651, a *trpE trpA* mutant. The *trpE* mutation results in loss of activity of the first specific tryptophan biosynthetic enzyme, anthranilate synthase, while the *trpA* mutation inactivates tryptophan synthase. Intact *trpD* and *trpC* genes catalyze the conversion of anthranilate, an intermediate of tryptophan synthesis, to InGP. As shown in Table 3, in the absence of anthranilate the mutations produce higher levels of *xylE* product than does the wild-type fusion. In addition, high intracellular InGP produced in the presence of anthranilate induced extremely high levels of catechol 2,3-dioxygenase activity in strains bearing each of the four *xylE* fusion plasmids.

Assignment of the mutations to *trpP_B*. The locations of the mutations in the *trpBA* control region and the resulting phenotypes indicate that the plasmid mutations are up mutations in the *trpBA* promoter (*trpP_B* mutations). Wild-type and mutant *xylE* fusions had identical transcriptional start sites, as determined by primer extension analysis (Fig. 2). Thus the mutations do not create a new promoter. The location of the *trpB* transcriptional start site (Fig. 1) confirms the placement of the mutations at -8 and -10 in *trpP_B*. By analogy with *E. coli*, these mutations define the nucleotide sequence of the *P. aeruginosa trpP_B* -10 region as 5'-TAGATT-3', which agrees with the *E. coli* -10 consensus sequence (5'-TATAAT-3') at four positions (24).

Effect of the mutations on *trpP_B-xylE* transcription. To test directly whether the plasmid mutations increased transcription from the *trpBA* promoter, we used Northern blot analysis to measure the relative amounts of *trpP_B-xylE* mRNA produced in *P. putida* IC1651 carrying the wild-type and mutant plasmids. For each of the mutants, we found that increased catechol 2,3-dioxygenase activity correlated with higher levels of specific transcript (Fig. 3). Comparison of

FIG. 1. Locations of the *cis*-acting mutations in the *trpBA* control region. At the top is the entire *trpI-trpBA* control region. The lightly stippled region represents the vector, pVDX18. The *Hind*III (H) and *Eco*RI (E) sites in the vector are indicated. The DNA sequence of the *trpBA* promoter region is given at the bottom of the figure. The transcriptional start site, direction of transcription, ribosome binding site, and the -10 and -35 regions of the *trpBA* promoter are indicated, as are the up-promoter mutations isolated in this study. The start site was determined in this study by primer extension (see Fig. 2). It is shifted by two bases from the tentative assignment made previously (6).

the amount of *trpP_B-xylE* mRNA synthesized by cells grown in the presence and absence of anthranilate provides the first direct evidence that InGP regulates the *trpBA* operon at the transcriptional level. The mutations increased the level of uninduced gene expression and also increased induced levels at least twofold. These data as well as the data from Tables 2 and 3 also confirm that the *P. putida* and *P. aeruginosa* TrpI proteins are functionally interchangeable.

Expression of *trpP_B-xylE* fusions in *E. coli*. The wild-type and mutant fusions were transferred to an *E. coli trpABE* strain expressing *trpI* from plasmid pMIB85 in order to monitor *xylE* expression in the presence and absence of InGP generated from exogenous anthranilate. As shown in Table 4, the up-regulatory phenotypes of the mutants were

retained in the *E. coli* background. However, the levels of activity observed were much lower than those seen in *P. putida* genetic backgrounds. Induction by InGP was also seen, and the mutations again increased levels of enzyme produced in the presence of InGP. No measurable catechol 2,3-dioxygenase activity was seen in the absence of *trpI* provided in *trans* (data not shown), indicating that transcription from the mutant *trpBA* control regions requires TrpI protein.

DISCUSSION

The *P. putida todE* mutant, F107, carrying a *trpBA* control region-*xylE* fusion plasmid (pHH1) was unable to grow with toluene as its sole source of carbon and energy. By selection for growth on minimal toluene plates, mutants expressing higher levels of catechol 2,3-dioxygenase were isolated. In

TABLE 2. Regulation of the *trpP_B-xylE* fusions by tryptophan in *P. putida* F107

Plasmid	Trp fusion	Catechol 2,3-dioxygenase activity ^a	
		Trp limitation ^b	Trp excess ^c
None		<1	ND ^d
pVDX18	None	318	323
pHHB	Wild type (<i>trpG</i>), inverted	15	ND
pHH1	Wild type	234	127
pHH2	X9 (-8 C)	1,585	1,695
pHH3	X56 (-10 T)	1,487	1,238
pHH4	X57 (-8 A)	2,398	2,015

^a Units of specific activity are nanomoles of 2-hydroxymuconic semialdehyde formed minute⁻¹ milligram of protein⁻¹ at 28°C.

^b Tryptophan was present in V-B medium at a concentration of 5 µg/ml (see Materials and Methods).

^c Tryptophan was present in V-B medium at a final concentration of 20 µg/ml.

^d ND, not determined.

TABLE 3. Enzymatic activity of *trpP_B-xylE* fusions in *P. putida* IC1651 (*trpAE*)

Plasmid	Trp fusion	Catechol 2,3-dioxygenase activity ^a	
		Without anthranilate	With anthranilate ^b
None		<1	<1
pVDX18	None	687	3,277
pHHB	Wild type (<i>trpG</i>), inverted	10	317
pHH1	Wild type	259	46,863
pHH2	X9 (-8 C)	4,948	86,480
pHH3	X56 (-10 T)	4,117	87,044
pHH4	X57 (-8 A)	7,803	97,786

^a Units of specific activity are nanomoles of 2-hydroxymuconic semialdehyde formed minute⁻¹ milligram of protein⁻¹ at 28°C.

^b Anthranilate, added to growth medium at a concentration of 20 µg/ml, is converted intracellularly to InGP in *trpAE* strains.

FIG. 2. Primer extension analysis of the *trpP_B-xylE* fusion transcripts. RNA (10 μ g) was isolated from *P. putida* IC1651 carrying pHH1 to pHH4. The cells were grown in LB plus 100 μ g of ampicillin per ml. The RNA was primed with a 31-base oligonucleotide complementary to the multiple cloning site of pVDX18. After extension with reverse transcriptase, products were separated by electrophoresis; a dideoxy sequencing ladder is shown for comparison. Lanes: 1, RNA prepared from IC1651(pHH1); 2, RNA prepared from IC1651(pHH2); 3, RNA prepared from IC1651(pHH3); 4, RNA prepared from IC1651(pHH4).

this paper, we have focused on several plasmid mutations that were found to be located in the -10 region of the *trpBA* promoter. In the original selection, 55 additional mutant strains were isolated, 10 of which appear to be *trpI* mutants, as determined on the basis of the ability of a wild-type copy of *P. aeruginosa trpI* to restore the wild-type phenotype (21a). Thus, the selection procedure described here should be generally useful for isolating mutations in activator genes encoded in the *P. putida* F107 chromosome, as well as for the isolation of up-promoter mutations.

Footprinting and gel retardation studies have indicated that InGP stimulates the expression of the *trpBA* operon by increasing the binding of the activator protein, TrpI, to specific sites in the *trpBA* control region (6, 7). *xylE* expression from the three *trpP_B-xylE* fusion mutants described herein was partially independent of InGP (Tables 2 and 3), and levels of specific mRNA were higher in each of the mutants than in cells containing the parental plasmid. Runoff transcription experiments using *trpP_B* DNA as the template also show that each of the mutations increases the activity of *trpP_B* (20). Both in vitro and in vivo results indicate,

FIG. 3. Effect of growth with anthranilate on the relative levels of *trpP_B-xylE*-specific mRNA in *P. putida* IC1651 cells carrying wild-type or mutant fusion plasmids. Cells were grown in V-B medium supplemented with 0.2% glucose, 0.05% acid-hydrolyzed casein, 5 μ g of tryptophan per ml, and 100 μ g of ampicillin per ml. Anthranilate (added at a concentration of 20 μ g/ml) is converted intracellularly to InGP by strain IC1651. RNA (15 μ g [total] per lane) was separated by electrophoresis, transferred to nylon membranes, and probed with a 32 P-end-labeled synthetic oligonucleotide as described in Materials and Methods. Lanes: wt, RNA prepared from IC1651(pHH1); 2, RNA prepared from IC1651(pHH2); 3, RNA prepared from IC1651(pHH3); 4, RNA prepared from IC1651(pHH4).

therefore, that the plasmid mutations increase *xylE* expression by increasing transcription initiation.

Two of the three mutations examined (-10 G $\rightarrow$ T and -8 T $\rightarrow$ A) have an up-promoter phenotype in *E. coli* and also bring the sequence of the -10 region of the *P. aeruginosa trpBA* operon promoter (5'-TAGATT-3') into closer similarity with the -10 consensus sequence 5'-TATAAT-3' for *E. coli* σ^{70} -specific promoters (24). On the basis of the frequencies of nucleotides seen at -8 in *E. coli* promoters, the up-promoter phenotype of the third plasmid mutation (-8 T $\rightarrow$ C) would not have been predicted (22). However an up phenotype for this nucleotide change has been observed in the *ant* promoter of *Salmonella* phage P22 in the context of a down mutation in the -35 region (36). We did not isolate any mutants with base substitutions in the -35 region of *trpP_B*. This region shows an extremely poor match to the *E. coli* -35 consensus (6, 24) and may in fact be so unlike the consensus that any single mutation is unlikely to have any observable effect on promoter strength. In summary, the *trpBA* promoter has the features of a typical *E. coli* positively regulated, σ^{70} -specific promoter. Such a promoter is characteristically weak, since transcription of positively regulated promoters, by definition, does not proceed very well in the presence of RNA polymerase alone (41).

TABLE 4. Enzymatic activity of *trpP_B-xylE* fusions in *E. coli* IC361(pMIB85)

Plasmid	Trp fusion	Catechol 2,3-dioxygenase activity ^a	
		Without anthranilate	With anthranilate ^b
pVDX18	None	2.8	3.7
pHHB	Wild type (<i>trpG</i>), inverted	<1.0	<1.0
pHH1	Wild type	2.2	104.0
pHH2	X9 (-8 C)	6.7	437.0
pHH3	X56 (-10 T)	5.9	686.0
pHH4	X57 (-8 A)	10.0	738.0

^a Units of specific activity are nanomoles of 2-hydroxy-muconic semialdehyde formed minute⁻¹ milligram of protein⁻¹.

^b Anthranilate, added to growth medium at a concentration of 20 μ g/ml, is converted intracellularly to InGP in strain IC361 (*trpABE*).

The observation that the *trpP_B* mutations had up-regulatory phenotypes in two *P. putida* backgrounds, as well as in the *E. coli* background, suggests that there is a form of *Pseudomonas* RNA polymerase with promoter recognition properties similar to the σ^{70} holoenzyme of *E. coli*. *E. coli* and *P. putida* RNA polymerases have been shown to display identical patterns of DNA binding with promoters (21). Recent studies (19) have shown that *E. coli*, *P. aeruginosa*, and *Pseudomonas syringae* RNA polymerases all initiate transcription from four bacteriophage λ promoters and also from the *P. aeruginosa trpBA* and *trpI* promoters. Moreover, the amounts of transcript formed in vitro from each of the six promoters ranked in the same order for each of the three RNA polymerases. The *Pseudomonas* polymerases were, however, more active with the weaker promoters than the *E. coli* RNA polymerase, indicating that the *Pseudomonas* enzymes may be more efficient than their *E. coli* counterpart. The reasons for this difference in efficiency are not known but could reflect a more stringent requirement of the *E. coli* enzyme for recognizable sequences in the -35 region.

Although the *P. aeruginosa trpBA* promoter is likely to be read by a *Pseudomonas* RNA polymerase that is similar to the *E. coli* σ^{70} enzyme, levels of expression of the *trpP_B-xylE* fusions are still very low in *E. coli* in comparison with the levels expressed in *P. putida* backgrounds (Tables 2, 3, and 4). *xylE* expression in *E. coli* requires transcription from the *trpI* promoter carried on plasmid pMIB85, because TrpI is needed to activate *trpP_B*. A 10-fold lower level of transcription from both the *trpI* and the *trpBA* promoters in *E. coli* could account for levels of catechol 2,3-dioxygenase activity that are 100-fold lower than those measured in *P. putida*. The more-stringent promoter recognition properties of the *E. coli* RNA polymerase (19) are likely to be at least partially responsible for reduced levels of transcription from the two *trp* promoters. Numerous other factors, such as upstream DNA sequence elements, specific *Pseudomonas* host proteins, or a particular intracellular salt concentration characteristic of *Pseudomonas* species, could also be important for optimal promoter recognition and may either be missing or not configured properly for optimal gene expression in *E. coli*.

The observed low levels of catechol 2,3-dioxygenase are probably not due to enzyme instability because *xylE*, when cloned into *E. coli* expression vectors, is expressed in *E. coli* at levels comparable with those seen in *P. putida* (26).

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