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Construction of a *tyrP-lac* Operon Fusion Strain and Its Use in the Isolation and Analysis of Mutants Derepressed for *tyrP* Expression

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The gene *tyrP*, which codes for a component of the tyrosine-specific transport system, has been localized on the *Escherichia coli* K-12 chromosome at min 42. A *tyrP-lac* operon fusion was constructed and used to isolate mutants that have altered expression from the *tyrP* promoter. All putative *tyrP* operator mutations were transferred onto a plasmid vector by recombination in vivo. Restriction enzyme analysis of the resultant plasmids suggests that some of these mutants arose from either an insertion or a deletion of DNA occurring within the region of DNA that contains the *tyrP* promoter.

In *Escherichia coli* K-12 there is a transport system specific for tyrosine (6). The only gene so far identified that codes for a component of this system is the *tyrP* gene (45). Because of the inability to readily screen large numbers of colonies for their $TyrP^+$ or TyrP phenotype, previous results have only established an approximate map position for this gene (45).

The expression of the tyrosine-specific transport system is enhanced in the presence of phenylalanine and repressed in the presence of tyrosine. Both the enhancement and repression of this system are dependent on the presence of the regulatory protein coded by the gene *tyrR* (46). This gene has also been shown to be essential for regulation of the common aromatic amino acid transport system (46) and of a number of enzymes involved in aromatic biosynthesis (7, 10, 21, 25, 44). In previous studies, the regulation of the tyrosine-specific transport system was measured by studying the transport of tyrosine into cells. No direct measurement of the *tyrP* gene product was undertaken.

In this paper, we report on a more accurate mapping of *tyrP* by using the transposon Tn*10*, the creation of *tyrP-lac* operon fusion strains in which transcriptional regulation from the *tyrP* promoter can be readily studied, and the isolation and preliminary characterization of a number of derepressed mutants.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophage. The bacterial strains used in this work are all derivatives of *E. coli* K-12 and are described in Table 1. The plasmids also are listed in Table 1.

The bacteriophage λNK370 (*b221 cI857 cI171*::Tn*10 O261*) was obtained from N. Kleckner. λp1(209) and Mu d1(*lac* Ap^r) were obtained from M. Casadaban (11, 12). λ p*tyrP-lac*-1 is described in this paper.

Chemicals and media. The chemicals used were all obtained commercially and were not further purified. L-[U-^{14}C]tyrosine (504 mCi/mmol) was purchased from the Radiochemical Centre, Amersham, England. The preparation of D-erythrose-4-phosphate and phosphoenolpyruvate was as described previously (10).

Luria agar and Lennox broth were routinely used as nutrient media, and minimal media were prepared from the 56/2 buffer of Monod et al. (39).

Drugs were used at final concentrations in minimal and nutrient media, respectively, of (micrograms per milliliter): tetracycline, 5 and 15; streptomycin, 400 and 200; ampicillin, 25 and 25; trimethoprim, 10 and 10; and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 40 and 40.

Transport and enzyme assays. Cells were prepared and assayed for their ability to transport tyrosine by a previously described method (46). Transport activities are expressed in milliunits per milligram (dry weight) of cells, where 1 U equals 1 μmol of substrate transported per min.

3-Deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) synthetase assays were performed as previously described (9). Enzyme activities are expressed in international units, and specific activities are expressed as milliunits per milligram of protein in the cell extracts. β-Galactosidase was assayed with toluene-treated cells as described by Miller (38). Activities are expressed as units, defined as follows. Units = $1{,}000 \times \{[OD_{420} - (1.75 \times OD_{550})]/[t \times v \times OD_{600}]\}$, where t is the time (in minutes) of the assay, v is the volume (in milliliters) of cell culture used in the assay, and OD_{420} is the optical density at 420 nm of the cell culture used.

Protein estimations in cell extracts were determined by the method of Lowry et al. (35).

Tyrosine cross-feeding. The method used to detect cross feeding was based on that described by Gibson and Jones (22), except that 0.01% peptone was omitted from the medium. The tyrosine auxotroph AT2471 was used as the indicator strain in these experiments.

Genetic methods. P1 *kc* transduction and conjugation were performed by methods described previously (40, 41).

λ lysates were prepared by UV induction of lysogens (38) or by lytic propagation in Luria broth. λ lysogens were obtained as described previously (23). Lysate of Mu d1(*lac* Ap^r) was prepared and used in mutagenesis by a previously described method (12).

Random transposition of Tn*10* into the chromosome was achieved by the method of Kleckner et al. (31). Tetracycline-sensitive derivatives of strains carrying Tn*10* were selected by methods previously described (5, 36).

Isolation of mutants defective in tyrosine-specific transport. Cells were mutagenized with either Tn*10* or Mu d1(*lac* Ap^r) as described above. Before the mutagenized cultures were plated onto the various selective media (see below), they were grown in minimal media containing the appropriate

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TABLE 1. *E. coli* K-12 strains and plasmids used in this work

Strain	Plasmid	Characteristics[a]	Source or reference
AT2471		*tyrA4*	A. L. Taylor
DFF1/JC1553		F150/*leuB6 hisG1 argG6 metB1 recA1 rpsL104*	(34)
JP2405		*aroG aroH aroP tyrP474 argE proA*	45
JP2867		*his-29 ilv-1 ΔlacU169*	23
JP2879		*aroG aroH aroP his*	P1-sensitive derivative of strain JP2864 (45)
JP2881		JP2879 *tyrP476*::Tn*10*	This work
JP3061		*aroG aroH aroP argE his ΔlacU169*	From strain KB1366 (45) through several intermediate strains
JP3067		JP3061 *tyrP492*::Mu d1(*lac* Ap^r)	This work
JP3073		JP3067 *tyrR366 zci-2*::Tn*10*	By P1 transduction
JP3502		*tyrR366 zci-2*::Tn*10 ΔlacU169* (λ p*tyrP-lac*-1)	From strain JP3311 (23) through several intermediate strains
JP3508		*ΔlacU169 recA56 Δ(srl-1300*::Tn*10)605* (λ p*tyrP-lac*-1)	From strain JP3311 (23) through several intermediate strains
JP3509		JP3508(pMU400)	By transformation
JP3516		JP3508 *tyrR497*	This work
JP3544		F637 *srl-1300*::Tn*10 thi-1 tyrA2 pyrD34 his-68 trp-45 thyA33 recA1 str-118*	From strain KLF43(KL259) (34) by P1 transduction
JP3923		*thr leu Δ(lacZM15 gyrA379 rpsL743 recA56 srl-1300*::Tn*10 Δ(aroL478*::Tn*10)606*	From strain JFM43 (43) through several intermediate strains
JP4052		F637/JP3508 *tyrPo501*	This work
JP4054		F637/JP3508 *tyrPo503*	This work
JP4055		F637/JP3508 *tyrPo506*	This work
JP4057		F637/JP3508 *tyrPo508*	This work
JP4061		F637/JP3508 *tyrPo512*	This work
JP4066		F637/JP3508 *tyrPo516*	This work
JP4070		F637/JP3508 *tyrPo520*	This work
KLF48(KL158)		F148 *arg aroD5 his proA2 recA1 rpsL*	(34)
MAL103		[Mu cts d1(*lac* Ap^r)] [Mu cts] Δ(*proAB-lacIPOZYA*)XIII *rpsL*	11
MC1022		*ΔlacZM15 rpsL*	13
W3110		*thi-1*	C. Yanofsky
	pBR322	Ap^r Tc^r	42
	pRBD13	Tc^r Gal$^+$	(R. B. Davey, P. I. Bird, S. M. Nikoletti, J. Praskier, and J. Pittard, Plasmid, in press)
	pMU400	Ap^r *tyrR*$^+$	18
	pMU510	Tp^r Lac$^+$	This work
	pMU517	Ap^r Gal$^+$ *rpsL*$^+$	This work
	pMU518	Tp^r *rpsL*$^+$	This work
	pNO1523	Ap^r *rpsL*$^+$	20
	pREG151	Tp^r	S. Falkow

[a] The nomenclature for genetic symbols follows that described by Bachmann (2) and for transpositional insertions follows that described by Kleckner et al. (32). Allele numbers are indicated where they are known. Fermentation markers are not described. Ap^r, Ampicillin resistance; Tc^r, tetracycline resistance; Tp^r, trimethoprim resistance. Mutants that had derepressed β-galactosidase levels but exhibited wild-type tyrosine-specific transport were provisionally designated as *tyrP* operator mutants.

antibiotic for four generations to express the TyrP$^-$ phenotype.

Isolation of λ strains carrying *tyrP-lac* operon fusions. λ phages that carry the *tyrP-lac* operon fusion were isolated by a method previously described (23). First, strains containing *tyrP*::Mu d1(*lac* Ap^r) were lysogenized with λp1(209). The *tyrP476*::Tn*10* allele was introduced into the lysogens by P1 transduction. Only those lysogens which upon introduction of the tetracycline resistance determinant become λ sensitive were kept. Each lysogen was induced by UV irradiation, and the corresponding λ lysate obtained was then used to transduce strain JP2867 to Lac$^+$. The Lac$^+$ transductants were purified, tested for λ lysogeny, and then assayed for their tyrosine-specific transport and β-galactosidase activities under various growth conditions. The strains in which the expression of the *tyrP* and lactose structural genes exhibited identical regulation to that observed for the wild-type *tyrP* gene were then induced by UV irradiation. In all cases this resulted in the production of high-frequency transducing Lac$^+$ λ lysates. These lysates were found to produce blue plaques on a lawn of strain JP2867 (Δlac) on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside indicator plates.

Isolation of mutants with increased β-galactosidase activity. Lysogens of λ p*tyrP-lac*-1 were streaked for single colonies on nutrient agar plates. These colonies were then singly used to obtain individual overnight cultures of the lysogens grown in minimal media containing 10^{-3} M tyrosine. These cultures

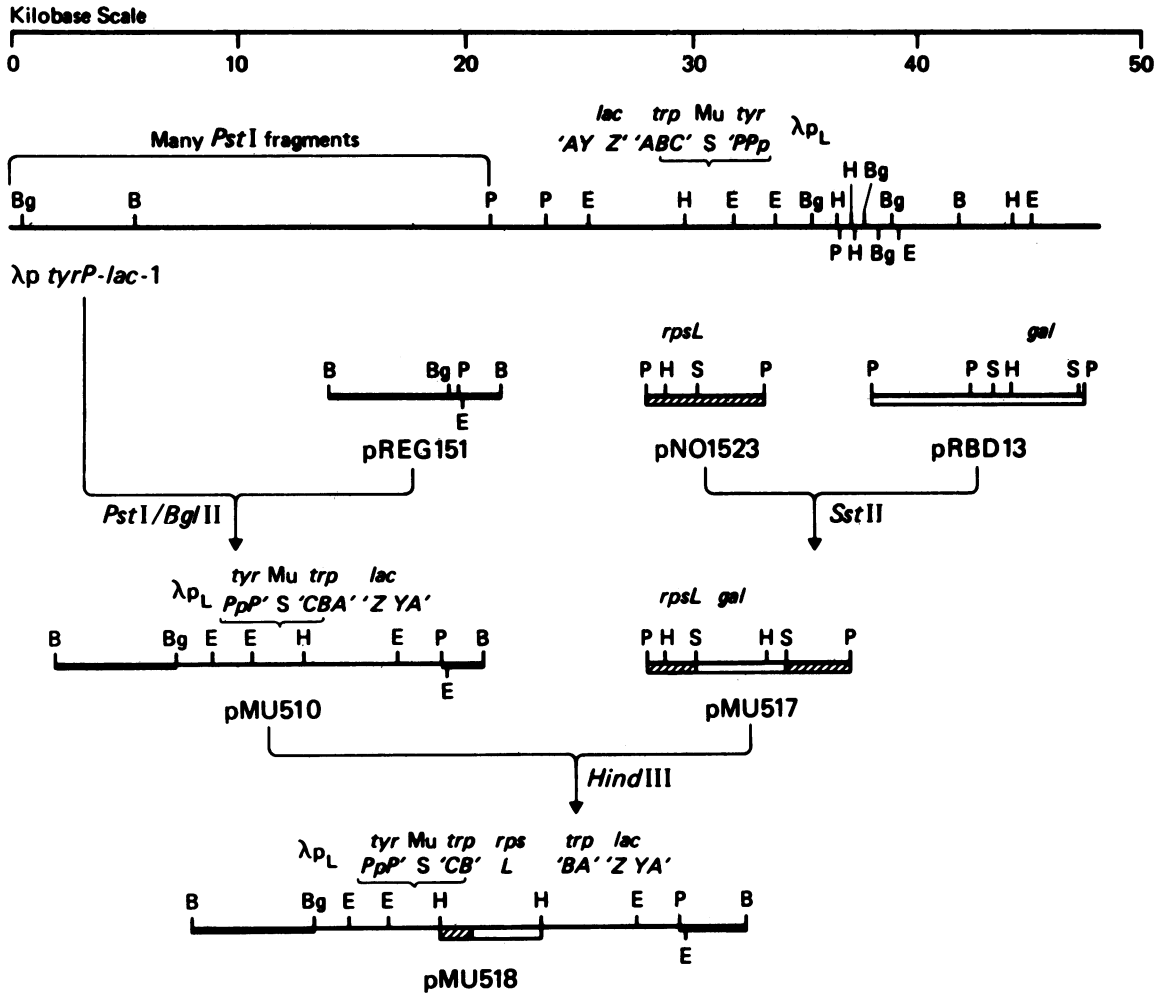

FIG. 1. Physical map of λ p*tyrP-lac*-1 and the construction of various plasmids carrying the *tyrP-lac* operon fusion. Plasmids were constructed as outlined in the text. P, *Pst*I; H, *Hin*dIII; S, *Sst*II; E, *Eco*RI; B, *Bam*HI; *Bgl*II.

were then separately plated onto media containing lactose as the sole carbon source and 10^{-3} M tyrosine. After incubation periods of between 40 and 64 h at 37°C, the plates were scored for Lac$^+$ colonies. These strains were then characterized and grouped into different classes (see below), in which each member of a particular class arose from a separate overnight culture of the parent strain.

Recombinant DNA technique. Plasmid DNA was prepared either by the method of Birnboin and Doly (4) or by centrifuging cleared lysates on cesium chloride-ethidium bromide gradients (15, 24). λ DNA was prepared by a previously described method (37). Transformation were performed by the method of Kushner (33). Restriction endonucleases and T4 DNA ligase were purchased from commerical sources and used as recommended. Agarose gel electrophoresis and visualization of DNA restriction fragments were by standard procedures (19).

Plasmid constructions. Figure 1 outlines the steps involved in the construction of plasmid pMU518, which was subsequently used in the transfer of putative *tyrP* operator mutations from the chromosomal *tyrP-lac* operon fusion by recombination in vivo (see below). The first step involved cloning the 11.7-kilobase (kb) *Bgl*II-*Pst*I generated fragment (which contains the *tyrP-lac* fusion) from λp*tyrP-lac*-1 into plasmid pREG151. The resulting plasmid pMU510 confers a Lac$^+$ phenotype. The second step involved cloning the *rpsL*$^+$ gene into the *tyrP-lac* operon fusion of pMU510, as this provided a positive selection for in vivo recombinants (see below). A *Hin*dIII generated fragment containing the *rpsL*$^+$ gene was constructed by cloning the 3.9-kb *Sst*II generated fragment from pRBD13 into plasmid pNO1523 (*rpsL*$^+$). The resultant plasmid pMU517 contains the *rpsL*$^+$ gene on a 4.4-kb *Hin*dII generated fragment. This fragment was subsequently cloned into the *Hin*dIII site of pMU510, resulting in plasmid pMU518.

During the course of the plasmid constructions, it was observed that the Mu region of *tyrP-lac* operon fusion lacked *Kpn*I and *Hin*dIII cut sites. This contradicts the mapping data of Casadaban and Cohen (13), which shows a *Kpn*I and a *Hin*dII site located in the Mu region of *lac*-operon fusions. This discrepancy is unlikely to be due to a deletion occurring in the Mu region when Mu dl(*lac* Ap^r) inserted into the *tyrP* gene, as other fusions isolated in this laboratory also fail to show these sites in the Mu region (data not shown).

TABLE 2. Effect of tyrosine and phenylalanine on β-galactosidase activities

Strain	β-Galactosidase activity in [a]:		
	MMP	MM	MMT
JP3067	421	45	4[b]
JP3073 (*tyrR366*)	113	114	110

[a] Growth in minimal media without aromatic amino acid supplement (MM), supplemented with 10^{-3} M phenylalanine (MMP), and supplemented with 10^{-3} M tyrosine (MMT).

[b] Grown in the presence of 0.005% shikimic acid.

Transfer of *tyrP* operator mutations onto plasmid pMU518. The putative *tyrP* operator mutations were transferred onto pMU518 by recombination in vivo by a modification of the strategy used by Cobbett et al. (16). The strains containing these mutations (see below) were made $RecA^+$ by transferring the F-prime F637 into them. These $RecA^+$ derivatives were then transformed to trimethoprim resistance with pMU518. To obtain derivatives of pMU518 which had recombined with the chromosomal *tyrP-lac* operon fusion, plasmid DNA from each trimethoprim-resistant, $RecA^+$ strain was isolated and used to transform strain JP3923 (*rpsL743*) to trimethoprim resistance in the presence of streptomycin. Since streptomycin resistance is recessive (20), only those recombinants of pMU518 which had lost the wild-type *rpsL* gene were selected. These recombinant plasmids occurred at a frequency of 10^{-2} to 10^{-3}, and the strains containing them were screened by using β-galactosidase assays for those plasmids that carried a putative *tyrP* operator mutation.

RESULTS

Mapping of *tyrP*. Strains (*aroG aroH aroP*) which contain only the tyrosine-repressible and -inhibitable DAHP synthetase and are defective in the general aromatic transport systems are unable to grow on media supplemented with tyrosine or various tyrosine analogs. However, *tyrP* derivatives of such strains show no such inhibition (45). By using this observation, a P1 lysate, prepared on cells of strain W3110 in which the transposon Tn*10* had been randomly inserted into the chromosome, was used to infect strain JP2879 (*aroG aroH aroP*), and transductants were selected on minimal media containing tetracycline and tyrosine (10^{-5} M), 3-fluorotyrosine (10^{-5} M) or 4-fluorophenylalanine (10^{-4} M). Transductants that were able to grow due to the introduction of wild-type $aroG^+$ or $aroH^+$ genes were identified by growth inhibition tests (44) and discarded. The remaining strains were then assayed for their ability to transport tyrosine, and 19 were found to have a defective tyrosine-specific transport system. Cross-feeding studies revealed that all of these mutants excrete tyrosine into their growth media as does the original *tyrP* mutant JP2405 (45). When P1 was grown on one of the Tn*10*-induced mutants, JP2881, and used to transduce the gene for tetracycline resistance into JP2879, 99% of the transductants were found to inherit the mutant *tyrP* allele. On the basis of these results, we assume that Tn*10* is inserted in *tyrP* in strain JP2881.

Conjugational mapping of the gene for tetracycline resistance in strain JP2881 showed that it was located ca. 2 min counterclockwise from *his* (data not shown). By using phage P1, it was transduced with *shiA* at a frequency of 3% and with *uvrC* at a frequency of 93%. Two F-primes, F148 and F150, which cover the *E. coli* K-12 chromosome counterclockwise from *his* (34), failed to complement the *tyrP* mutations of strains JP2405 and JP2881. However both F-primes have been found not to carry the *uvrC* locus (34; B. Bachmann, personal communication). The $tyrP^+$ gene has recently been cloned onto a plasmid vector, and this has been found to complement both *tyrP* alleles (P. Wookey, personal communication).

Isolation of a strain with a *tyrP-lac* operon fusion. Strain JP3061 was lysogenized with Mu d1(*lac* Ap^r) as described above, and ampicillin-resistant colonies were selected on minimal media containing 3-fluorotyrosine (5×10^{-5} M). Putative *tyrP*::Mu d1(*lac* Ap^r) clones were purified and screened for the *tyrP* phenotype as described above. They were also screened for Lac^+, immunity to Mu, and temperature sensitivity.

The β-galactosidase levels of one such strain, JP3067, were found to be repressed in the presence of tyrosine and enhanced in the presence of phenylalanine (Table 2). The degree of repression and enhancement was similar to that previously reported for the tyrosine-specific transport system (46). When the *tyrR366* allele was introduced into strain JP3067, the β-galactosidase levels of the resultant strain

TABLE 3. β-Galactosidase activities of various *tyrP-lac* fusion mutants

Strain	Parent strain	Mutant class	No. in class	β-Galactosidase activity in[a]:					
				MMT (10^{-3} M)	MMT (10^{-4} M)	MMPT (10^{-3} M)	MMPT (10^{-4} M)	MM	MMP
JP3502				109	102	112	119	118	113
JP3508				4	5	5	32	53	392
JP4054	JP3508	A	7	245	257	265	250	272	263
JP4066	JP3509	A		285	271	268	261	253	307
JP4057	JP3508	B	1	69	97	93	165	233	1,683
JP4055	JP3508	C	5	67	61	50	71	90	854
JP4061	JP3509	C		37	34	36	73	92	1,070
JP4052	JP3508	D	7	114	88	308	603	120	911
JP4070	JP3509	D		205	133	388	596	116	666

[a] Growth in minimal media without aromatic amino acid supplement (MM) or supplemented with 10^{-3} M phenylalanine (MMP), 10^{-3} M tyrosine [MMT(10^{-3} M)], 10^{-4} M tyrosine [MMT(10^{-4} M)], 10^{-3} M tyrosine and 10^{-3} M phenylalanine [MMPT(10^{-3} M)], or 10^{-4} M tyrosine and 10^{-3} M phenylalanine [MMPT(10^{-4} M)].

TABLE 4. Specific activities of β-galactosidase and DAHP synthetase in strains JP3508 and JP3516

Strain	Sp act of[a]: β-galactosidase			DAHP synthetase Tyr (mU/mg of protein)			DAHP synthetase Phe (mU/mg of protein)		
	MMP	MM	MMT	MMP	MM	MMT	MMP	MM	MMT
JP3508	423	51	5	28	30	22	22	61	62
JP3516	707	78	498	405	381	348	30	115	41

[a] For definitions of abbreviations, see Table 2, footnote *a*.

JP3073 were no longer repressed or enhanced in the presence of tyrosine or phenylalanine (Table 2).

A P1 lysate prepared on strain JP2881 was used to transduce the *tyrP* region into strain JP3067 by selecting for tetracycline resistance. All the tetracycline-resistant transductants were found to be temperature resistant, sensitive to Mu infection, and Lac$^-$. These results lead us to conclude that in strain JP3067, Mu dl(*lac* Ap^r) has inserted into the chromosome at the *tyrP* locus, resulting in the lactose structural genes being under the control of the *tyrP* promoter.

Isolation and characterization of λ p*tyrP-lac* and plasmids carrying *tyrP-lac*. A λ phage that carries the *tyrP-lac* operon fusion from strain JP3067 was isolated (see above) and called λ p*tyrP-lac*-1. The β-galactosidase levels exhibited by the two strains JP3508 and JP3502 (*tyrP*) (Table 3), which carry λ p*tyrP-lac*-1, reveal that *lacZ* expression is similar to that observed in strain JP3067.

A restriction endonuclease cleavage map of λ p*tyrP-lac*-1 is shown in Fig. 1. The site where Mu dl(*lac* Ap^r) has inserted into *tyrP* is expected to be within the 2.2-kb *Eco*RI-*Hin*dIII fragment. This is because the *Hin*dIII site previously has been shown to be located at the MuS end of Mu dl(*lac* Ap^r) within the *trp*B gene (13). Since no *Eco*RI sites are found in the MuS region, it is thought that the observed *Eco*RI site is from the *tyrP* region of the chromosome. This *Eco*RI-*Hin*dIII fragment is also expected to contain the *tyrP* promoter (*tyrPp*) as the entire *tyrP* gene has been shown to be contained within a 2.8-kb *Eco*RI fragment (47). According to this argument, the 6.4-kb *Eco*RI fragment from *tyrP-lac*-1 should contain the *tyrP* promoter and part of the *lacZ* structural gene. Expression of LacZ′ can be detected by complementation in strain MC1022 (13) and should be subject to the same controls as *tyrP*. This was confirmed by cloning the 6.4-kb insert in both orientations into pBR322 and demonstrating that β-galactosidase synthesis was repressed by tyrosine (data not shown).

Strains that carried these plasmids were unable to grow in the presence of phenylalanine (10^{-3} M) when tyrosine was absent. This phenotype is also observed in strains that contain the entire *tyrP*$^+$ gene cloned into pBR322 (P. Wookey, personal communication). Although we do not know the reason for this growth inhibition, it should be noted that under these conditions the cell is making greatly amplified amounts of the *tyrP* protein or a peptide containing its amino-terminal end. We assume that growth inhibition is, in some way, caused by the very high levels of the amino end of the *tyrP* protein. The finding that another *tyrP-lac* fusion in which Mu dl(*lac* Ap^r) has inserted 0.3 kb closer to the *tyrP* promoter does not show the phenylalanine-sensitive phenotype when cloned into pBR322, coupled with the failure to observe the phenylalanine-sensitive phenotype when the first *tyrP-lac* fusion is cloned in the low-copy-number vector pREG151, supports this general hypothesis. To avoid conditions that might inadvertantly select for alterations to *tyrP* expression, all subsequent cloning work involved pREG151. The strategy used to construct the various plasmids (Fig. 1) is described above.

Selection for mutants with increased β-galactosidase activity. Although lysogens of λ p*tyrP-lac*-1 were stable, they tended, at a frequency of ca. 10^{-5}, to give rise to colonies containing more than one copy of λ p*tyrP-lac* phage integrated in the chromosome. To avoid this problem, *recA* lysogens were used when selecting for mutants with increased levels of β-galactosidase activity. An overnight culture of strain JP3508, grown in the presence of tyrosine, was plated onto media containing lactose as the sole carbon source and 10^{-3} M tyrosine (see above). Mutants were obtained at a frequency between 10^{-6} and 10^{-7}/CFU plated. One class of mutants that would be expected to be found among the strains growing on the lactose-tyrosine media would be mutants altered in the regulator gene *tyrR*. In such *tyrR* mutants, repression of transcription from the *tyrP* promoter by tyrosine should be abolished. Many *tyrR* mutants confer resistance to the tyrosine analog 3-fluorotyrosine because of the derepression of other genes in the *tyrR* regulon affecting tyrosine biosynthesis (10). To identify this class of mutant, strains were screened for growth on minimal medium containing 2×10^{-4} M 3-fluorotyrosine. Of 120 strains tested, 86 were found to be resistant to 3-fluorotyrosine and were considered to be putative *tyrR* mutants. The 34 remaining strains were then analyzed to determine whether their regulatory mutations were *cis*- or *trans*-acting. Among the latter, we expected to find other *tyrR* mutants altered in *tyrR* function but not conferring resistance to levels of 3-fluorotyrosine as high as 2×10^{-4} M. In addition, if other cytoplasmid molecules are involved in the control of *tyrP* expression, some mutants might be found with alteration to these functions. *Cis*-acting mutations were expected to affect the putative *tyrP* operator locus associated with the *tyrP-lac* fusion and to be located in the promoter-operator region of the *tyrP-lac*. As the parent strains contained two copies of the *tyrP* promoter, one linked to *lacZ* and the other to *tyrP*, discrimination between *cis*- and *trans*-acting mutations could be made by studying repression of the *tyrP*-coded tyrosine-specific transport systems in addition to β-galactosidase. Measurement of tyrosine uptake in these 34 mutants revealed that 22 had mutations in a *trans*-acting gene as evidenced by derepressed transport and 12 had mutations affecting only *tyrP-lac* (data not shown). Genetic mapping showed that all of the 22 mutants had mutations that mapped in the *tyrR* region (data not shown). To circumvent the selection of *tyrR* mutants, the multicopy *tyrR*$^+$ plasmid pMU400 was introduced into strain JP3508 to produce strain JP3509. This was used for subsequent mutant isolations, and the only additional mutants isolated were those with *cis*-acting mutations linked to *tyrP-lac*.

Analysis of *cis*-acting mutants. To simplify comparisons between all of the *cis*-acting mutations, mutants derived from strain JP3509 were grown in the absence of ampicillin,

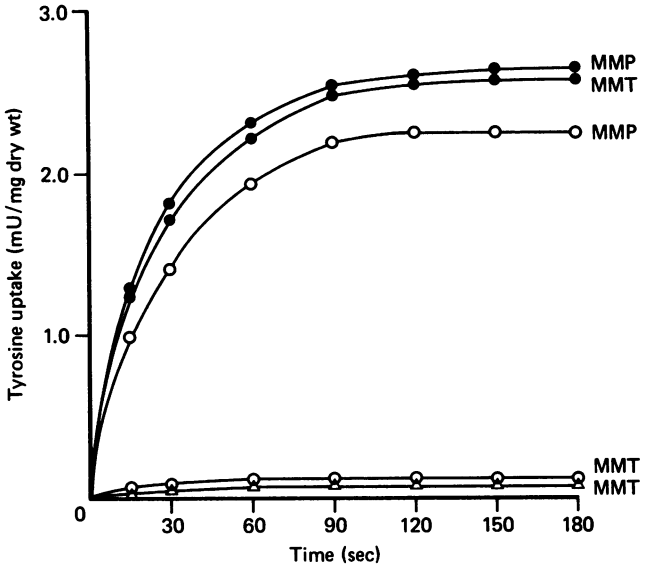

FIG. 2. Uptake of tyrosine by the tyrosine-specific transport system in strains JP3508 (○), JP3516 (●), and JP3516 MU400 (△). Growth was in minimal media supplemented with either 10^{-3} M tyrosine (MMT) or 10^{-3} M phenylalanine (MMP).

and derivatives were isolates that had lost pMU400. These derivatives were used in subsequent experiments.

The β-galactosidase activities of all these putative operator mutants were determined under a variety of growth conditions, and these results were used to group the mutants into a number of arbitrary classes (Table 3). Although the significance of these various mutants will ultimately rely on an analysis of the nucleotide sequences in their *tyrP* promoter regions, some preliminary observations can be made by considering the variation in enzyme levels and by examining changes in their restriction maps.

The β-galactosidase activities exhibited by the class A mutants, which remained unaffected by the presence of either tyrosine or phenylalanine in the growth media, do not correspond to any of those observed for the wild-type or *tyrR* strains. The simplest explanation is that in these strains the lactose structural genes are now being expressed from another promoter. If this is in fact correct, then the region of DNA upstream from the lactose structural genes would be expected to show some alteration. To test this, the mutations from the seven class A strains were transferred onto plasmid pMU518 by recombination in vivo (see above). The resultant plasmids were purified, digested with *Eco*RI and *Hin*dIII, and compared with an identical digest of pMU510. In all cases, a 3.6-kb *Eco*RI-*Hin*dIII fragment was found to have replaced the original 2.2-kb *Eco*RI-*Hin*dIII fragment of pMU510 which carries the *tyrP* promoter. It is postulated that this extra 1.4 kb of DNA contains a promoter which now controls *lac* expression (see below).

When the class B mutation was transferred onto pMU518, it was found that the *tyrP* promoter was now contained within a 1.95-kb *Eco*RI-*Hin*dIII generated fragment. This indicates that a deletion of DNA from the original 2.2-kb fragment has resulted in increased expression of the *lacZ* gene in all growth conditions (Table 3). The β-galactosidase levels in this mutant are repressed 2- to 4-fold in the presence of tyrosine, whereas there is a greater than 10-fold repression in the parent strain JP3508. In the case of growth in the presence of phenylalanine, the relative increase in β-galactosidase activity is the same in both strains. This deletion thus appears also to have partially abolished repression by tyrosine.

In class C mutants, expression of *lacZ* is still partially repressed in the presence of tyrosine, whereas in the class D mutants, there is no such repression (Table 3). Both classes also have a higher level of *lacZ* expression in the presence of 10^{-3} M phenylalanine than that observed in the original *tyrP-lac* operon fusion. In the class D mutants, some induction by phenylalanine occurs even in the presence of tyrosine. When these mutations were transferred onto pMU518, it was found that all class C mutants now contained the *tyrP* promoter on a 2.1-kb *Eco*RI-*Hin*dIII fragment, indicating that a deletion of 0.1 kb had occurred. In the case of the class D mutants, the *tyrP* promoter was still contained on a 2.2-kb *Eco*RI-*Hin*dIII fragment.

Analysis of *trans*-acting mutants. (i) Fluorotyrosine-resistant mutants. The 86 3-fluorotyrosine-resistant strains isolated were presumed to be *tyrR* mutants. To confirm this, 10 strains were chosen for further study. Assays for β-galactosidase activity and ability to transport tyrosine, under various growth conditions (data not shown), revealed that these strains possessed the same phenotype as the *tyrR* strain JP3502. To verify that the phenotype observed was indeed due to a lesion in *tyrR*, the strains were transduced to tetracycline resistance, using a P1 lysate prepared on a strain having Tn*10* inserted near *tyrR*$^+$. In all cases, the inheritance of fluorotyrosine sensitivity showed identical linkage to the tetracycline resistance gene as that previously observed between Tn*10* and *tyrR*$^+$ (17).

(ii) Fluorotyrosine-sensitive mutants. β-Galactosidase assays and tyrosine-specific transport studies on the 22 3-fluorotyrosine-sensitive mutants revealed that in these strains expression from the *tyrP* promoter is enhanced, instead of repressed, in the presence of tyrosine. The β-galactosidase activities (Table 4) and the levels of tyrosine-specific transport (Fig. 2) are shown for one of the mutants, JP3516. P1 transduction studies, as described for the fluorotyrosine-resistant mutants, revealed that the mutation causing this phenotype in strain JP3516 was located at the *tyrR* locus. The introduction of the multicopy *tyrR*$^+$ plasmid pMU400 into strain JP3516 and the 21 other mutants resulted in *tyrP* being subject once again to tyrosine repression (Fig. 2). The mutation in strain JP3516 was given the allele number *tyrR497*.

The two isoenzymes DAHP synthetase Tyr and DAHP synthetase Phe are subject to *tyrR*-mediated repression (7, 25). Results obtained on the expression of these enzymes in strain JP3516 and the parent strain JP3508 (Table 4), along with those obtained on the expression of *tyrP*, indicate that when the *tyrR497* gene product interacts with tyrosine the

TABLE 5. Effect of different growth conditions on β-galactosidase activities

Growth medium[a]	β-galactosidase activity of strain:	
	JP3502 (*tyr366*)	JP3508
MM	126	30
MM + 0.2% Casamino Acids	532	109
Luria broth	381	94

[a] MM, Minimal media without aromatic amino acid supplement.

resulting phenotype is similar to that observed when the wild-type TyrR protein interacts with phenylalanine.

Effect of growth in enriched media on *tyrP* expression. When strains JP3508 and JP3502 (*tyrR*) are grown in enriched media, they exhibit a higher β-galactosidase activity than when grown in minimal media (Table 5). A similar increase in tyrosine uptake via the tyrosine-specific transport system was also observed in these enriched media (data not shown). Since this increase in *tyrP* expression occurs in either *tyrR*$^+$ or *tyrR* strains, it is not mediated by the TyrR protein. The common aromatic amino acid transport system, which also transports tyrosine (6), shows no increase in expression in these enriched media (P. Wookey and M. L. Chye, unpublished data). The mechanism and components involved in the increase of *tyrP* expression is now being investigated in this laboratory.

DISCUSSION

Earlier studies on the regulation of the tyrosine-specific transport system (46) suggested that expression of *tyrP* is enhanced in the presence of phenylalanine and repressed in the presence of tyrosine, with both enhancement and repression being dependent on the presence of a functional TyrR protein. Studies on the *tyrP-lac* operon fusion, generated by Mu dl(*lac* Ap^r) insertions, have confirmed this. However, these studies also revealed an increase in *tyrP* expression when strains were grown in enriched media. This increase in expression is independent of the TyrR protein and thus represents another mechanism by which *tyrP* expression is regulated.

By using the *tyrP-lac* operon fusion, putative operator mutants were isolated in which tyrosine repression of expression from the *tyrP* promoter is either partially or totally abolished. In the cases of the class B, C, and D mutants, the expression from the *tyrP* promoter was still enhanced in the presence of phenylalanine. These results suggest that there are two distinct sites associated with the *tyrP* promoter in which the TyrR protein exerts its repressing and enhancing effects on *tyrP* expression. For the sake of simplicity, we are assuming a single *tyrP* promoter at this stage.

In all studies on strains that have a wild-type *tyrP* promoter, the repressing effects of tyrosine have been observed to be dominant over the phenylalanine-induced enhancement of expression (46; this paper). For example, expression from the *tyrP* promoter is fully repressed in media containing 10^{-3} M tyrosine and 10^{-3} M phenylalanine. In the case of the class D mutants, the repressing effects of tyrosine appear to be completely abolished (Table 3). These mutants now show enhancement of *tyrP* expression by phenylalanine even in the presence of tyrosine. A simple model to explain these observations would involve separate target sites in the DNA for the tyrosine- and phenylalanine-mediated interactions. Binding of the TyrR protein-tyrosine complex to its target would prevent *tyrP* transcription. The effect would be dominant if its binding either rendered the TyrR protein-phenylalanine target inaccessible or negated the effect of the interaction, between the TyrR protein-phenylalanine and its target, on *tyrP* expression. In class D mutants, we assume that the TyrR protein-tyrosine target has been altered so that the repressor no longer binds to it. Although tyrosine by itself has no repressing effect on the expression from the *tyrP* promoters in class D mutants, it is interesting that in these mutants the presence of tyrosine still causes reduction in the level of phenylalanine enhancement. This does not seem to reflect residual activity at the TyrR protein-tyrosine target site as tyrosine alone causes no repression. A possible explanation for this is that tyrosine also exerts a dominant effect over phenylalanine at another level, e.g., at the level of interaction with the TyrR protein. In this case, high concentrations of tyrosine would either inhibit interactions between the TyrR protein and phenylalanine or affect the activity of the TyrR protein-phenylalanine complex, perhaps by binding to it as well.

The class B mutant that was isolated is unusual in that *tyrP* expression was elevated in all growth conditions tested. This increase in expression appears to be due to a 0.25-kb deletion. Possible explanations are that this deletion either has resulted in an increase in promoter activity or has removed a region of DNA that causes a reduction in transcription. It has been well established in other systems such as *trp* and *his* that deletions which remove attenuator loci can result in increased expression of adjacent structural genes (3, 26, 28, 29). Another example of deletions in *lac* operon fusion strains resulting in increased levels of β-galactosidase has, however, been reported (16) for a system in which DNA sequence analysis of wild-type genes has clearly established the absence of any attenuator locus adjacent to the specific promoter from which *lacZ* is transcribed (G. Hudson and B. E. Davidson, personal communication). It is possible that in this case and perhaps in the case of *tyrP-lac* the deletions may have removed transcription termination signals which do not form part of the regulation of the wild-type operon but which result from the insertion of the Mu dl(*lac* Ap^r) or Mu and λp1(209) into the chromosome. Another possibility is that the observed increase in β-galactosidase activity is due to an alteration in the efficiency with which the *lacZ* gene is translated. Aksoy et al. (1) have recently shown that in Mu dl(*lac* Ap^r), part of the *trpA* gene is fused to the *lacZ* gene. The fusion gene has the translational start properties of *trpA*, and the resultant β-galactosidase protein is joined between amino acid 59 of *trpA* and amino acid 3 of *lacZ*. Translation of this fused gene was shown to be coupled to the translation of *trpB*. When a frameshift mutation was introduced into *trpB*, causing premature translation termination in that gene, there was a significant reduction in the synthesis of β-galactosidase. In the class B mutant, a deletion was found to occur in the *Eco*RI-*Hin*dIII fragment that also contains the *trpB* translational start signal. It is possible that the deletion has resulted in an increase in efficiency with which *trpB* is translated, which in turn could result in an increase in the synthesis of β-galactosidase.

In the class A mutants that were isolated, *lacZ* appears to be expressed from another promoter. In these strains, the original 2.2-kb *Eco*RI-*Hin*dIII fragment that carries the *tyrP* promoter is replaced by a 3.6-kb *Eco*RI-*Hin*dIII fragment. We believe this increase in fragment size is due to an insertion element as there are many uncharacterized IS elements reported that correspond to this size (8). It has also been shown that some IS elements can provide promoter functions (14, 27, 30).

The fluorotyrosine-resistant *tyrR* mutants that were isolated closely resemble *tyrR* mutants that have been previously reported (10). The fluorotyrosine-sensitive *tyrR* mutants, however, appear to be a new class. Our data suggest that in these mutants the structure of the TyrR protein has been altered so that when it binds tyrosine it assumes a configuration more closely resembling that of the wild-type TyrR protein which has bound phenylalanine.

It is difficult to see why these mutants remain sensitive to fluorotyrosine as tyrosine synthesis should be derepressed in

these strains. The most likely explanation is that the enhanced level of the *tyrP* system in these strains results in higher intracellular pools of 3-fluorotyrosine. A *tyrR* mutant has been previously described in which tyrosine-mediated repression is abolished and phenylalanine-mediated effects are enhanced (25, 46), but this is the first example of a mutant in which the mutation has resulted in the tyrosine-mediated effects being similar to those produced by phenylalanine.

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