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Role of the Purine Repressor in the Regulation of Pyrimidine Gene Expression in *Escherichia coli* K-12

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The *pyrC* and *pyrD* genes of *Escherichia coli* K-12 encode the pyrimidine biosynthetic enzymes dihydroorotase and dihydroorotate dehydrogenase, respectively. A highly conserved sequence in the promoter regions of these two genes is similar to the *pur* operator, which is the binding site for the purine repressor (PurR). In this study, we examined the role of PurR in the regulation of *pyrC* and *pyrD* expression. Our results show that *pyrC* and *pyrD* expression was repressed approximately twofold in cells grown in the presence of through a mechanism requiring PurR. A mutation, designated *pyrCp926*, which alters a 6-base-pair region within the conserved sequence in the *pyrC* promoter eliminated PurR-mediated repression of *pyrC* expression. This result indicates that PurR binds to the *pyrC* (and presumably to the *pyrD*) conserved sequence and inhibits transcriptional initiation. We also demonstrated that the *pyrCp926* mutation had no effect on pyrimidine-mediated regulation of *pyrC* expression, indicating that pyrimidine and purine effectors act through independent mechanisms to control the expression of the *pyrC* and *pyrD* genes.

In *Escherichia coli* K-12, six unlinked genes and small operons designated *carAB*, *pyrBI*, *pyrC*, *pyrD*, *pyrE*, and *pyrF* encode the six enzymes required for the de novo synthesis of UMP, the precursor of all pyrimidine nucleotides (20). The expression of these genes and operons is negatively regulated in a noncoordinate manner by the intracellular levels of pyrimidine nucleotides (20, 30). The expression of the *pyrBI* operon and the *pyrE* and *pyrF* genes is repressed by a uridine nucleotide, whereas *pyrC* and *pyrD* gene expression appears to be repressed primarily by a cytidine nucleotide. The expression of the *carAB* operon, which is essential for arginine as well as pyrimidine biosynthesis, is cumulatively repressed by arginine and both uridine and cytidine nucleotides (16, 21). Over the last several years, a number of studies have provided detailed information about pyrimidine-mediated regulation of *pyrBI* and *pyrE* expression, which occurs, at least primarily, through similar UTP-sensitive attenuation control mechanisms (10, 12–14, 23, 25, 26, 31). In contrast, little is known at present about the mechanisms through which the expression of the other pyrimidine genes is controlled by pyrimidine nucleotide effectors.

In a previous study designed to characterize the *pyrC* gene and its expression, we identified a highly conserved sequence, which contains a region of dyad symmetry, in or near the *pyrC*, *pyrD*, and *carAB* promoters (33). We proposed that this operatorlike sequence might be the binding site for a repressor protein involved in cytidine nucleotide-mediated regulation. Subsequently, we discovered that this conserved sequence is similar to the binding site for the purine repressor (PurR), which is encoded by the *purR* gene (11, 28) (Fig. 1). The PurR-binding site, designated the *pur* operator, is found within or overlapping the promoters of the unlinked *pur* regulon genes, which encode the purine biosynthetic enzymes, and also downstream of the *purR* promoter (1, 28). It appears that PurR mediates the negative regulation of *pur* gene expression in cells grown under conditions of purine excess by binding to the *pur* operator and inhibiting transcription (28). Recently, it was shown that there is a sequence similar to the *pur* operator immediately upstream of the promoter of the *prsA* gene, which encodes phosphoribosylpyrophosphate synthetase (5) (Fig. 1). In addition, it has been found that insertional inactivation of the *purR* gene results in increased expression of the *codA* gene encoding cytosine deaminase (11). These results indicate that PurR is involved in regulating the expression of genes required for nucleotide metabolism other than purine biosynthesis. In the present study, we examined the possibility that purine-mediated repression requiring PurR is involved in the regulation of *pyrC* and *pyrD* gene expression. Our results indicate that PurR represses the expression of these genes under conditions of purine excess by interacting with the conserved sequences previously identified in the *pyrC* and *pyrD* promoter regions.

MATERIALS AND METHODS

Bacterial strains. All strains used are *E. coli* K-12 and are described in Table 1. Bacteriophage P1-mediated transductions used in strain constructions were performed essentially as described in reference 19.

Construction of plasmids and recombinant bacteriophage M13. Plasmid pBHM105, which carries the *pyrC* gene and flanking sequences, was constructed as described previously (33). This plasmid contains a 1.6-kilobase-pair (kb) *Nru*I-*Pvu*II fragment of *E. coli* K-12 chromosomal DNA, which had been modified with *Eco*RI linkers, inserted into the *Eco*RI site of plasmid pBR322. Plasmid pBHM108 was constructed by inserting the same linker-modified 1.6-kb *Nru*I-*Pvu*II chromosomal fragment present in plasmid pBHM105 into the *Eco*RI site of plasmid pUC18. Plasmid pBHM177 was constructed from plasmid pBHM105 by replacing the 727-base-pair (bp) *Eco*RI-*Bam*HI fragment containing the wild-type *pyrC* promoter with an equivalent 727-bp *Eco*RI-*Bam*HI fragment containing the *pyrCp926* mutation. Plasmid pBHM221 also was derived from plasmid pBHM105 by inserting a 1.3-kb kanamycin resistance cassette (Pharmacia, Inc., Piscataway, N.J.) into the *Bam*HI site at codon 85 of the *pyrC* gene. The *pyrC* and *kan* genes are transcribed in opposite directions in this plasmid. Recombinant bacteriophage M13mp9amC6 (amber muta-

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***pur* operator consensus**	**cGCAAaCGttT-c-tt**
purF	**CGCAAACGTTTTCTTT(N6)TAGAAT**
pyrC	**GGAAAACGTTTCCGCTTATCCT**
pyrD	**GGAAAACGTTTGCGTT(N41)CATAAT**
carAB	**GGAAAACGCTTGCGCA**
prsA	**AGAAAACGTTTTCGCG(N27)TAGAAT**

FIG. 1. Consensus sequence of the *pur* operator, the sequence of the *pur* operator in the *purF* promoter, and similar sequences in the *pyrC*, *pyrD*, *carAB*, and *prsA* promoter regions. The *pur* operator consensus sequence was derived from the sequences of the *purF*, *purHD*, *purL*, *purMN*, *purEK*, *purC*, and *purR* promoter regions (1); totally conserved and highly (≥75%) conserved nucleotides are indicated by capital and lowercase letters, respectively, and the dashes indicate positions at which the sequence is not highly conserved. The sequence of the −10 regions of the *purF*, *pyrC*, *pyrD*, and *prsA* promoters is underlined; the number of nucleotides (N) between the conserved sequences and the −10 regions is indicated in the parentheses. The *carAB* conserved sequence is found in the opposite orientation 109 nucleotides upstream of the −10 region of the pyrimidine-regulated promoter P_1 (4, 22).

tions in phage genes I and II) was made by inserting the 748-bp *Bam*HI fragment from plasmid pBHM108, which includes the promoter region of the *pyrC* gene, into the *Bam*HI site of the replicative form of bacteriophage M13mp9am.

DNA preparations, restriction digests, ligations, and transformations. DNA preparations, restriction digestions, ligations, and transformations were performed as previously described (25, 26). Restriction endonucleases and T4 polynucleotide kinase were purchased from either New England BioLabs, Inc. (Beverly, Mass.) or Boehringer Mannheim Biochemicals (Indianapolis, Ind.). T4 DNA ligase and the Klenow fragment of DNA polymerase I were obtained from New England BioLabs.

In vitro oligonucleotide-directed mutagenesis to construct the *pyrCp926* mutation. A mutation designed to inactivate the putative *pur* operator of the *pyrC* promoter was constructed by using the gapped heteroduplex method of oligonucleotide-directed mutagenesis (3). The protocol followed was the same as that previously described (14), except that the single-stranded template DNA was isolated from the recombinant bacteriophage M13mp9amC6 and the sequence of the mutagenic oligonucleotide was 5′-dGCGGAAACGAAAA GGTTTGCACGA. The resulting *pyrC* promoter mutation, which was designated *pyrCp926*, changed a 6-bp sequence within the putative *pur* operator from GGAAAA to CCTTTT (Fig. 1). The mutation was verified by determining the DNA sequence of the *pyrC* promoter region (29). The *pyrCp926* mutation was excised from replicative-form bacteriophage DNA as part of a 727-bp *Eco*RI-*Bam*HI fragment, which was then used to construct plasmid pBHM177.

TABLE 1. *E. coli* K-12 strains

Strain	Genotype	Source
JC7623	*recB21 recC22 sbcB15 arg ara his leu pro thr*	G. Walker (34)
MC4100	F⁻ *araD139* Δ(*argF-lac*) *U169 rpsL150 thiA1 relA1 deoC1 ptsF25 flbB5301 rbsR*	M. Casadaban (6)
R100	MC4100(λ *purF*::*lacZ*)[a]	H. Zalkin (27)
R300	R100 *purR300*	H. Zalkin (27)
CLT68	MC4100 *pyrB482*::*kan*	This laboratory (14)
CLT104	JC7623 *pyrC487*::*kan*	This study
CLT105	MC4100 *pyrC487*::*kan*	P1(CLT104) × MC4100
CLT106	R100 *pyrC487*::*kan*	P1(CLT104) × R100
CLT107	R300 *pyrC487*::*kan*	P1(CLT104) × R300
CLT108	JC7623 *pyrCp926*	This study
CLT109	MC4100 *pyrCp926*	P1(CLT108) × CLT105
CLT110	R100 *pyrCp926*	P1(CLT108) × CLT106
CLT111	R300 *pyrCp926*	P1(CLT108) × CLT107
CLT112	MC4100 *pyrB482*::*kan* *pyrCp926*	P1(CLT68) × CLT109

[a] λ *purF*::*lacZ* is a recombinant phage containing a *purF*::*lacZ* gene fusion and is integrated into the chromosome at the lambda attachment site (27).

Introduction of the *pyrCp926* mutation into the promoter of the chromosomal *pyrC* gene of selected *E. coli* K-12 strains. The *pyrCp926* mutation was introduced into the promoter of the chromosomal *pyrC* gene of strains MC4100, R100, and R300 in the following manner. First, we constructed a strain carrying a selectable *pyrC* mutation by transforming strain JC7623 with *Eco*RI-digested plasmid pBHM221 (*pyrC487*::*kan*). (Strain JC7623 and its derivatives were used as recipients in the transformations described here because they can be readily transformed with linear DNA [34].) A kanamycin-resistant, pyrimidine-auxotrophic transformant was isolated and designated strain CLT104. This strain was shown to be ampicillin and tetracycline sensitive, indicating that plasmid pBHM221 (*bla tet*) had not integrated into the chromosome. The *pyrC487*::*kan* mutation of strain CLT104 then was transferred to strains MC4100, R100, and R300 by P1-mediated transduction, generating strains CLT105, CLT106, and CLT107, respectively. Next, we replaced the insertionally inactivated *pyrC* gene and flanking sequences of strain CLT104 (JC7623 *pyrC487*::*kan*) with the *pyrC* region carried by plasmid pBHM177 (*pyrCp926*) by transforming this strain with the 1.6-kb *Eco*RI insert of plasmid pBHM177. A pyrimidine-prototrophic, kanamycin-sensitive transformant which, when compared with strain JC7623, exhibited increased *pyrC* expression in an adenine-supplemented medium was isolated and designated strain CLT108 (JC7623 *pyrCp926*). The *pyrCp926* mutation of strain CLT108 then was transferred to the chromosome of strains CLT105 (MC4100 *pyrC487*::*kan*), CLT106 (R100 *pyrC487*::*kan*), and CLT107 (R300 *pyrC487*::*kan*) by P1-mediated transduction. Pyrimidine-prototrophic, kanamycin-sensitive transductants were isolated and designated CLT109 (MC4100 *pyrCp926*), CLT110 (R100 *pyrCp926*), and CLT111 (R300 *pyrCp926*). A

pyrimidine-auxotrophic derivative of strain CLT109 was constructed by transducing this strain with phage P1 grown on strain CLT68 (*pyrB482*::*kan*). A kanamycin-resistant, pyrimidine-auxotrophic transductant was designated strain CLT112.

Confirmation of the presence of the chromosomal *pyrCp926* mutation. The presence of the *pyrCp926* mutation in the *pyrC* promoter of strains CLT110, CLT111, and CLT112 was confirmed by Southern hybridization. Chromosomal DNA was prepared from these three strains and also from strain MC4100 (*pyrC*$^+$) to serve as a control. For each Southern blot, a 100-μg sample of each DNA preparation was digested to completion with *Nru*I and *Bam*HI. The digested DNA was prepared for hybridization and probed as described by Maniatis et al. (17) with the following modifications. The DNA was transferred from a 1% agarose gel to nitrocellulose filter paper by vacuum blotting. One blot was probed with the 5′-^{32}P-labeled mutagenic oligonucleotide used in the construction of the *pyrCp926* mutation, and a second blot was probed with a 5′-^{32}P-labeled oligonucleotide with the sequence 5′-dGCGGAAACGTTTTCCTTTGCAC GA, which is complementary to the operatorlike sequence of the wild-type *pyrC* promoter shown in Fig. 1. The oligonucleotides were radiolabeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (5,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Hybridizations were done for 20 h at 52°C. The high-temperature wash was done for 30 min at 52°C. The data showed that the mutagenic oligonucleotide hydridized to a 721-bp *Nru*I-*Bam*HI fragment from strains CLT110, CLT111, and CLT112 but did not hybridize to DNA from strain MC4100. In contrast, the oligonucleotide complementary to the wild-type *pyrC* promoter sequence hybridized to a 721-bp *Nru*I-*Bam*HI fragment from strain MC4100 and not to DNA from the other three strains (data not shown).

To demonstrate that the introduction of the *pyrCp926* mutation was not accompanied by large insertions, deletions, or rearrangements, we compared the *pyrC* regions of strains CLT110, CLT111, CLT112, and MC4100 by Southern hybridization. Chromosomal DNA from each of these strains was digested to completion with *Hinc*II. Based on the restriction map of the *pyrC* region, digestion with *Hinc*II should yield a 2-kb fragment containing the *pyrC* promoter plus the first 308 codons of the *pyrC* gene and a 0.9-kb fragment containing the remaining 40 codons of the gene (33). Blots were prepared as described above. The ^{32}P-labeled DNA used as a probe was synthesized by random priming (8) and was complementary to codons 85 through 348 of the *pyrC* gene and also to additional downstream sequences. Hybridizations were done for 20 h at 68°C, and high-temperature washes were done for 2.5 h at 68°C. The data showed that DNA from all four strains contained two fragments of the expected size that hybridized to the probe (data not shown). This result indicates that the introduction of the *pyrCp926* mutation into strains CLT110, CLT111, and CLT112 occurred by homologous recombination.

Media and culture methods. All cultures (30 ml in a 125-ml flask) used for enzyme assays were grown in N$^-$C$^-$ medium (2) supplemented with 10 mM NH_4Cl, 0.4% glucose, and 0.015 mM thiamine hydrochloride. Strains MC4100, R100, R300, CLT110, and CLT111 were grown at 37°C with shaking in medium with an additional 0.74 mM adenine supplement when indicated. Strains CLT68 and CLT112 were grown at 30°C with shaking in medium containing an additional supplement of 1 mM arginine and either 1 mM uracil or 0.25 mM UMP. Strains CLT68 and CLT112 were grown at 30°C instead of 37°C because the level of pyrimidine-mediated regulation of *pyr* gene expression in these strains is higher at the lower temperature. The solid media used in strain constructions were LB medium (19) with ampicillin (25 μg/ml), kanamycin sulfate (50 μg/ml), or tetracycline hydrochloride (25 μg/ml) added when required and VBCG (minimal glucose) (32) supplemented with 0.3 mM amino acids and 0.015 mM thiamine hydrochloride when required. All solid media contained 1.5% agar (Difco Laboratories, Detroit, Mich.). Growth on solid media was at 37°C.

Enzyme assays. Cultures were grown to an A_{650} of 0.5 (as measured with a Gilford model 260 spectrophotometer), and 25-ml samples were taken. Cells were collected by centrifugation, washed, and disrupted by sonic oscillation as described previously (33). Each extract was divided into two portions. Cell debris was removed from one portion by centrifugation at 17,000 × *g* for 30 min at 4°C. This extract was used to assay dihydroorotase, β-galactosidase, and aspartate transcarbamylase activities. The unspun extract was used to assay dihydroorotate dehydrogenase activity. Dihydroorotase (33), dihydroorotate dehydrogenase (30), β-galactosidase (19), and aspartate transcarbamylase (25) activities were measured as described previously with the following modifications. Dilutions of cell extracts used to measure dihydroorotase activity were made in 50 mM sodium phosphate (pH 7.0) containing 50 μg of bovine serum albumin per ml. The dihydroorotate concentration in the reaction mixture used to assay dihydroorotate dehydrogenase activity was increased to 5 mM, and the assay was done at 30°C. The protein concentration in spun and unspun sonic extracts was determined by the method of Lowry et al. (15) with bovine serum albumin as the standard.

RESULTS

Role of PurR in the regulation of *pyrC* and *pyrD* expression. To determine the role of PurR in the control of *pyrC* and *pyrD* expression, we initially measured the levels of dihydroorotase and dihydroorotate dehydrogenase, which are encoded by the *pyrC* and *pyrD* genes, respectively, in strain R100 grown in a glucose-minimal salts medium with or without an adenine supplement. These conditions were chosen because growth in the presence of adenine results in a high level of PurR-mediated repression of *pur* gene expression (27, 28). Strain R100 was used in this experiment because it contains a *purF*::*lacZ* fusion carried as part of a recombinant lambda phage integrated into the chromosome at the lambda attachment site. This fusion permitted us to readily monitor *purF* expression as a control for PurR-mediated repression by measuring β-galactosidase activity. The results show that *pyrC* and *pyrD* expression was repressed approximately twofold in cells grown in the presence of adenine (Table 2). In comparison, *purF*::*lacZ* expression was repressed 15-fold under the same conditions. As an additional control, the level of aspartate transcarbamylase, which is encoded by the *pyrBI* operon, was measured in cells grown under the two conditions. The results show that *pyrBI* expression was not repressed in the presence of adenine; in fact, expression was approximately 25% higher under this condition (data not shown).

To determine whether PurR is required for the observed repression of *pyrC* and *pyrD* expression, we measured the levels of dihydroorotase and dihydroorotate dehydrogenase in strain R300 (*purR300*) grown in the presence or absence of adenine. This strain is identical to strain R100 except for a mutation in the *purR* gene that inactivates the repressor (27).

TABLE 2. Purine-mediated regulation of *pyrC*, *pyrD*, and *purF*::*lacZ* expression[a]

Strain (genotype)	Adenine	Enzyme activity (nmol/min per mg)[b]					
		Dihydroorotase (*pyrC*)		Dihydrooroatate dehydrogenase (*pyrD*)		β-Galactosidase (*purF*::*lacZ*)	
R100 (*purR*$^+$)	−	194	(2.1)	69.7	(1.8)	77.0	(15)
	+	93.2		39.4		5.15	
R300 (*purR300*)	−	202	(1.1)	76.8	(1.0)	130	(1.0)
	+	186		73.9		124	

[a] Doubling times were 50 and 47 min in the absence and presence of adenine, respectively.
[b] Activities are the mean of two experiments. Numbers in parentheses indicate fold regulation.

TABLE 3. Effect of the *pyrCp926* mutation on purine-mediated regulation of *pyrC* expression[a]

Strain (genotype)	Adenine	Enzyme activity (nmol/min per mg)[b]			
		Dihydroorotase (*pyrC*)		β-Galactosidase (*purF*::*lacZ*)	
CLT110 (*purR*$^+$ *pyrCp926*)	−	274	(1.2)	80.6	(16)
	+	228		5.02	
CLT111 (*purR300 pyrCp926*)	−	276	(1.2)	130	(1.0)
	+	226		126	

[a] Doubling times were 49 and 47 min in the absence and presence of adenine, respectively.
[b] Activities are the mean of two experiments. Numbers in parentheses indicate fold regulation.

The results show that nearly all purine-mediated repression of *pyrC* and *pyrD* expression was eliminated in the absence of a functional PurR, indicating the important role of this protein in the regulation (Table 2). Essentially all purine-mediated repression of *purF*::*lacZ* expression was abolished, confirming that the *purR300* mutation completely destroys PurR activity. The level of *purF*::*lacZ* expression in strain R300 was 70% higher than that measured in strain R100 grown without an adenine supplement. This result indicates that limited PurR-mediated repression of *purF*::*lacZ* expression occurred in strain R100 grown in the absence of adenine. It appears that this limited PurR-mediated repression also had a slight effect on the level of *pyrC* and *pyrD* expression in strain R100. Aspartate transcarbamylase levels also were measured in stain R300 and were found to be the same as those in strain R100.

Effect of the *pyrCp926* mutation on PurR-mediated repression of *pyrC* expression. The presence of a sequence similar to the *pur* operator in or near the *pyrC* and *pyrD* promoters (Fig. 1) suggests that PurR binds to these sites to cause repression of gene expression. To demonstrate that PurR interacts with the putative *pur* operator in the *pyrC* promoter, we constructed a mutation which changes this sequence from 5′-GGAAAACGTTTCCGCT to 5′-CCTTT TCGTTTCCGCT. This 6-bp mutation, designated *pyrCp926*, destroys the dyad symmetry in this region. This mutation was introduced into the *pyrC* promoters of strains R100 (*purR*$^+$) and R300 (*purR300*) by homologous recombination to generate strains CLT110 (*purR*$^+$ *pyrCp926*) and CLT111 (*purR300 pyrCp926*). The effect of the *pyrCp926* mutation on purine-mediated repression of *pyrC* expression was determined by measuring the level of dihydroorotase in strains CLT110 and CLT111 grown in the presence or absence of adenine. The results show that dihydroorotase levels were the same in the two strains, with the levels in cells grown in the presence of adenine nearly as high as those in cells grown without the adenine supplement (Table 3). These data indicate that the *pyrCp926* mutation eliminated PurR-mediated regulation, apparently by abolishing its binding site. It appears, however, that there is a low level of residual purine-mediated repression that occurs in a PurR-independent manner.

In this experiment, we also measured the levels of β-galactosidase encoded by the *purF*::*lacZ* fusion as a control for PurR-mediated repression. The data show that *purF*::*lacZ* expression was repressed 16-fold in strain CLT110 (*purR*$^+$ *pyrCp926*), indicating that the expression of PurR-repressible genes other than *pyrC* was still subject to normal regulation (Table 3). No purine-mediated repression of *purF*::*lacZ* expression was observed in strain CLT111 (*purR300 pyrCp926*). The results presented in Tables 2 and 3 show that the derepressed level of *pyrC* expression was higher in strains containing the *pyrCp926* mutation than in those with the wild-type *pyrC* promoter. This difference apparently reflects an enhancement of transcriptional initiation at the mutant promoter.

Effect of the *pyrCp926* mutation on pyrimidine-mediated regulation of *pyrC* expression. To examine the role of the *pur* operatorlike sequence within the *pyrC* promoter in pyrimidine-mediated regulation of *pyrC* expression, we measured dihydroorotase levels in strains CLT68 (*pyrC*$^+$ *pyrB482*::*kan*) and CLT112 (*pyrCp926 pyrB482*::*kan*). Both strains are pyrimidine auxotrophs and were grown in a glucose-minimal salts medium containing either uracil or UMP as the pyrimidine source. Growth on uracil causes repressed *pyr* gene expression, whereas growth on UMP, which is slowly utilized by cells grown under the present conditions, causes derepressed *pyr* gene expression. The results show that the level of pyrimidine-mediated regulation of *pyrC* expression was the same in both strains, indicating that the *pyrCp926* mutation had no effect on this regulation (Table 4). The mutation did have an effect, however, on the level of *pyrC* expression. Expression was increased approximately 25% in strain CLT112 grown on either pyrimidine source. We presume that this increase is due to the stimulatory effect of the *pyrCp926* mutation on *pyrC* transcription that was observed in strains CLT110 and CLT111 (Table 3), although in the present experiment we cannot exclude the possibility that the increase in *pyrC* expression in strain CLT112 is due to a loss of low-level PurR-mediated repression.

DISCUSSION

The experiments described in this paper demonstrate that the expression of the *pyrC* and *pyrD* genes is repressed in

TABLE 4. Effect of the *pyrCp926* mutation on pyrimidine-mediated regulation of *pyrC* expression[a]

Strain (genotype)	Pyrimidine source	Dihydroorotase (nmol/min per mg)[b]	
CLT68 (*pyrC*$^+$ *pyrB482*::*kan*)	Uracil	160	(12)
	UMP	1,910	
CLT112 (*pyrCp926 pyrB482*::*kan*)	Uracil	196	(12)
	UMP	2,400	

[a] Doubling times were 68 and 111 min on uracil and UMP, respectively.
[b] Activities are the mean of five experiments. Numbers in parentheses indicate fold regulation.

cells grown under conditions of purine excess. Nearly all the observed purine-mediated repression is eliminated in the absence of a functional PurR. The *pyrC* and *pyrD* promoter regions contain a sequence similar to the *pur* operator. We showed that the *pyrCp926* mutation, which alters the sequence of the putative operator in the *pyrC* promoter, eliminates PurR-mediated repression of *pyrC* expression. These results indicate that under conditions of purine excess, PurR binds to an operator in the *pyrC* and *pyrD* promoter regions and inhibits transcriptional initiation. We also showed that the expression of the *pyrBI* operon, which does not contain a *pur* operatorlike sequence in its promoter region, is not repressed under conditions of purine excess. This result indicates that PurR-mediated repression is not involved in regulating the expression of all *pyr* genes.

The level of PurR-mediated repression of *pyrC* and *pyrD* expression is approximately twofold. In contrast, *purF* expression is repressed 15-fold. The lower level of repression observed for the *pyrC* gene appears to be due to the sequence of the *pyrC* operator, which differs from that of the *purF* operator, as well as the consensus *pur* operator, at positions 1, 3, and 15 (Fig. 1). Presumably, the efficiency of PurR binding at the *pyrC* operator is reduced because of these differences. It is unlikely that the position of the *pyrC* operator contributes to the lower level of repression, because both the *pyrC* and *purF* operators are located at similar positions entirely within the promoter (Fig. 1). The lower level of repression observed for the *pyrD* gene may be due to both the sequence of the *pyrD* operator and its location. The sequence of the *pyrD* operator differs from that of the *purF* and consensus *pur* operators at positions 1 and 3 (Fig. 1). In addition, the *pyrD* operator is located 18 bp upstream of the −35 region of the *pyrD* promoter. Based on previously published footprinting experiments (18, 28), it appears that the binding sites for PurR and RNA polymerase overlap by only a few base pairs in the *pyrD* promoter region. This limited overlap may not be sufficient for PurR to inhibit RNA polymerase binding efficiently at this site.

In this study, we also examined the effect of the *pyrCp926* mutation on pyrimidine-mediated regulation of *pyrC* expression. The experiments presented show that the mutation has no effect on this regulation, although the mutation apparently enhances transcriptional initiation at the *pyrC* promoter. This result indicates that the *pyrC* and presumably the *pyrD* operators are not involved in pyrimidine-mediated regulation of gene expression.

In support of the proposed role of PurR in purine-mediated repression of *pyrC* expression, it recently was demonstrated by using a gel retardation assay and also by footprinting that PurR binds to the *pyrC* operator (7). Binding of PurR to the *pyrC* operator is relatively weak. Approximately seven times more PurR is required for half-maximal binding to the *pyrC* operator than to the *purF* operator. The lower affinity of PurR for the *pyrC* operator is consistent with the lower level of PurR-mediated repression of *pyrC* expression. In addition, it has been demonstrated that the *pyrCp926* mutation, which abolishes PurR-mediated repression, eliminates PurR binding to the *pyrC* promoter region (K. Y. Choi and H. Zalkin, personal communication).

In a recent paper by Jensen (9), data were presented which suggest that the expression of the *pyrC* and *pyrD* genes is stimulated by a guanine nucleotide in *Salmonella typhimurium*. In the present study, we observed only purine-mediated repression of *pyrC* and *pyrD* expression. These results may be explained in one of two ways. First, the stimulation of *pyrC* and *pyrD* expression observed in *S. typhimurium* occurs because of an increase in the intracellular concentration of a guanine nucleotide effector which binds to and inactivates PurR. In cells grown in adenine, the concentration of this effector is reduced, thereby permitting PurR-mediated repression. Second, there are two counteracting purine-mediated control mechanisms that modulate *pyrC* and *pyrD* expression, perhaps under different conditions. One mechanism uses a guanine nucleotide effector to stimulate gene expression in a PurR-independent manner, and the other mechanism uses a different purine compound, for example hypoxanthine or guanine as suggested by Houlberg and Jensen (8a), as the corepressor of PurR. Clearly, additional experiments are required to establish the identity of the purine effector of PurR-mediated repression and to determine the relationship between the regulatory mechanism proposed by Jensen (9) and that described in this report.

More than 95% of the pyrimidine and purine nucleotides synthesized in the cell are used as precursors of nucleic acids (20). To ensure efficient nucleic acid biosynthesis, the cell needs to maintain a proper balance between pyrimidine and purine nucleotide pools. There appear to be a number of regulatory mechanisms by which this occurs, acting at the level of enzyme activity and gene expression. For example, the enzymes carbamyl phosphate synthetase and aspartate transcarbamylase, which catalyze the first two reactions in the pyrimidine nucleotide biosynthetic pathway, are activated by IMP and ATP, respectively (20). In addition, GTP appears to be a negative effector of *pyrBI* and *pyrE* expression, most likely acting through the attenuation control mechanisms (9, 24). The PurR-mediated repression of *pyrC* and *pyrD* expression described in this study provides yet another mechanism by which purine levels affect pyrimidine nucleotide biosynthesis. Acting in concert, these mechanisms should provide the cell with the ability to appropriately adjust the intracellular levels of pyrimidine and purine nucleotides under a variety of physiological conditions.

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