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Attenuation control of *pyrBI* operon expression in *Escherichia coli* K-12

(UTP-regulated gene expression/coupled transcription-translation/DNA sequence/*in vitro* transcription)

CHARLES L. TURNBOUGH, JR., KAROL L. HICKS, AND JOHN P. DONAHUE

Department of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294

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ABSTRACT **The *pyrBI* operon of *Escherichia coli* K-12 encodes the subunits of the pyrimidine biosynthetic enzyme aspartate transcarbamylase (carbamoylphosphate:L-aspartate carbamoyltransferase, EC 2.1.3.2). Expression of this operon apparently is negatively regulated by the intracellular levels of UTP. To elucidate the regulatory mechanism in which UTP functions, the nucleotide sequence of the promoter-regulatory region of the *pyrBI* operon was determined and DNA fragments containing this region were transcribed *in vitro*. These experiments revealed a ρ-independent transcriptional terminator (attenuator) located only 23 base pairs before the promoter-proximal end of the structural genes. Transcription initiated upstream at either of two potential *pyrBI* promoters was efficiently (≈98%) terminated at this site, indicating that the regulation of *pyrBI* expression involves attenuation control. Additional features identified suggest a model for regulation in which the relative rates of UTP-dependent transcription within the *pyrBI* leader region and coupled translation of the leader transcript control transcriptional termination at the attenuator.**

In *Escherichia coli* K-12 and closely related bacteria, *de novo* synthesis of UMP is catalyzed by six enzymes encoded by six unlinked pyrimidine genes and operons (1–3). The expression of these genes and operons appears to be noncoordinately regulated by pyrimidine nucleotides (4), but little is known about the regulatory mechanisms involved. The *pyrBI* operon encodes the catalytic (*pyrB*) and regulatory (*pyrI*) subunits of the pyrimidine biosynthetic enzyme aspartate transcarbamylase (ATCase; carbamoylphosphate:L-aspartate carbamoyltransferase, EC 2.1.3.2) (3). Previous *in vivo* studies have indicated that *pyrBI* expression is negatively regulated over a several hundredfold range by the levels of a uridine nucleotide (4). Recent experiments using an *in vitro* coupled transcription-translation system have identified UTP as the principal pyrimidine regulatory effector of this operon (5). This result and the observation that ATCase synthesis is preferentially stimulated by sublethal concentrations of inhibitors of transcriptional elongation in *Salmonella typhimurium* (unpublished data) suggest that the rate of UTP-dependent transcription is involved in the regulation of *pyrBI* expression. In addition, ATCase synthesis was shown to be selectively inhibited in a *hisT* strain of *S. typhimurium* (6), in which the rate of translational elongation is slowed (ref. 7; D. Palmer and S. Artz, personal communication). This result suggests that the rates of both transcription and translation, perhaps of a leader sequence preceding the *pyrBI* structural genes, are involved in regulation. The regulatory mechanism could be similar to the attenuation control mechanisms of amino acid biosynthetic operons (8).

In this study we determined the nucleotide sequence of the promoter-regulatory region of the *pyrBI* operon of *E. coli* K-12 and characterized *in vitro* transcription of DNA fragments containing this region. The results indicate that *pyrBI* expression is regulated by an attenuation control mechanism (8) that could be sensitive to the relative rates of UTP-dependent transcription within the *pyrBI* leader region and translation of the leader transcript.

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MATERIALS AND METHODS

DNA Preparations. The λ specialized transducing phage *λd valS argI pyrB* (yk14m5) was prepared from the *E. coli* K-12 lysogenic strain $AD_{11}m5$ (9) provided by Akihiko Kikuchi, and phage DNA was extracted (10). Plasmid DNA was isolated by the procedure of Birnboim and Doly (11) and purified further by CsCl/ethidium bromide density gradient centrifugation (12). DNA restriction fragments were separated by agarose gel electrophoresis (13) and extracted from the gel by electroelution (14). Fragments used as templates in *in vitro* transcription reactions were phenol extracted. The sizes of plasmids and restriction fragments were measured by agarose gel electrophoresis with appropriate standards.

Restriction Digests, Ligations, and Transformations. Restriction endonucleases were obtained from New England BioLabs and used as recommended by the supplier. Conditions for ligation of restriction fragments with T4 DNA ligase (New England BioLabs) were essentially as described (12). The protocol used for transformations was that of Morrison (15).

DNA Sequence Analyses. DNA sequences were determined by the method of Maxam and Gilbert (16).

***In Vitro* Transcription.** RNA polymerase was purified from *E. coli* strain MRE 600 (17, 18) and was a gift from David Wood and Jack Lebowitz. *In vitro* transcription assay conditions were as follows unless indicated otherwise in the text. Reaction mixtures (0.05 ml) contained: 20 mM Tris·HCl (pH 7.9); 10 mM $MgCl_2$; 50 mM KCl; 0.1 mM Na_2EDTA; 0.1 mM dithiothreitol; 0.2 mM GTP; 0.2 mM CTP; 0.2 mM UTP; 0.2 mM [α-^{32}P]ATP (2.5 Ci/mmol; 1 Ci = 3.7×10^{10} Bq; ICN); DNA at 21 pmol/ml; and RNA polymerase at 256 pmol/ml. Reaction mixtures lacking ribonucleoside triphosphates were preincubated for 5 min at 37°C. Reactions were initiated by the addition of the triphosphates; 30 sec later, rifampicin was added to a final concentration of 4 μM. Incubation was continued for 30 min at 37°C, and reactions were terminated by freezing in dry ice. Yeast carrier RNA (final concentration, 1 mg/ml) and 0.25 ml of extraction buffer (10 mM Tris·HCl, pH 7.9/10 mM Na_2EDTA/0.1% $NaDodSO_4$) were then added to the reaction mixtures and each was extracted with an equal volume of phenol. The RNA was precipitated with ethanol, dried, and dissolved in 99% formamide. Samples were placed in boiling water for 3 min, quick chilled in ice-water, and analyzed by electrophoresis on

Abbreviations: ATCase, aspartate transcarbamylase; bp, base pair(s).

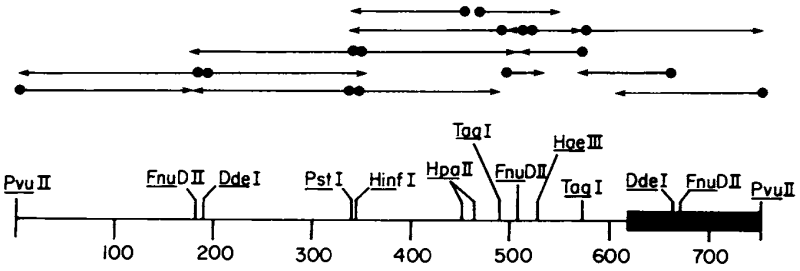

FIG. 1. Strategy and restriction sites used for sequence analysis of the 758-bp *Pvu* II fragment containing the promoter-regulatory region of the *pyrBI* operon. All restriction fragments were 5'-end labeled (16). Arrows indicate direction and extent of each sequence determination. The heavy bar represents DNA encoding the NH_2 terminus of the ATCase catalytic subunit.

7% polyacrylamide/Tris/borate/EDTA, pH 8.3, gels containing 7 M urea (19). Autoradiograms of gels were scanned with a densitometer to quantitate transcripts.

RESULTS

Subcloning of the *pyrB* Gene and Identification of the *pyrBI* Promoter-Regulatory Region. The *pyrB* gene of *E. coli* K-12, which is the first structural gene in the *pyrBI* operon (3), was subcloned from the λ specialized transducing phage *λd valS argI pyrB* (yk14m5) by ligating *Pvu* II or *Hinc*II restriction fragments of transducing phage and plasmid pBR322 (20) DNA. Recombinant plasmids were isolated from the ligation mixtures by transforming an *E. coli* K-12 *pyrB* auxotroph to prototrophy. Restriction maps of these plasmids (unpublished data) were used to localize the *pyrB*-complementing sequence within a 758-base pair (bp) *Pvu* II fragment and an adjacent 1,080-bp *Pvu* II/*Hinc*II fragment.

The 758-bp *Pvu* II fragment was prepared from one of the recombinant plasmids, and the sequence of the DNA adjacent to the 1,080-bp *Pvu* II/*Hinc*II fragment was determined. The first 140 bp of this end of the fragment (Fig. 1) were found to encode the NH_2-terminal sequence of the ATCase catalytic subunit (21), indicating that the promoter-regulatory region is included in the remaining 618 bp of this fragment. This conclusion was supported by experiments in which the 758-bp *Pvu* II fragment was inserted into the promoter-cloning plasmid pKO-1 (22), resulting in the genetic fusion of the inserted promoter and the *galK* structural gene. In strains containing this recombinant plasmid, the expression of the fused *galK* gene was regulated by the availability of pyrimidines in the same manner as that of the wild-type *pyrBI* operon (unpublished data).

DNA Sequence of the *pyrBI* Promoter-Regulatory Region. The nucleotide sequence of the entire 758-bp *Pvu* II fragment was determined as summarized in Fig. 1 and is presented in Fig. 2. The sequence encoding the NH_2 terminus of the ATCase catalytic subunit is included in nucleotides 619–758 and is preceded by a typical ribosome binding site (23). Computer-assisted inspection (24) of the entire nucleotide sequence revealed a number of features relevant to regulation of *pyrBI* expression. Perhaps the most striking was a putative ρ-independent transcription termination sequence (569–595) located only 23 bp from the start of the ATCase catalytic subunit sequence. This termination sequence was identified by the G+C-rich region of dyad symmetry followed by eight thymidine residues in the antisense strand (25). Previous studies indicated that this sequence is sufficient to cause transcriptional termination (26). The RNA hairpin encoded by the region of dyad symmetry, and which apparently is essential for transcriptional termination (26), has a calculated free energy of formation of −19.1 kcal/mol (1 cal = 4.184 J) (27). The close proximity of the transcription termination sequence to the start of the *pyrB* structural gene indicates that it functions as an attenuator (8). This se-

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                    20                  40                  60                  80                 100
CTGCGGGATG CACTTTTGAC GATATCATTG ATGTTACGAG CTTCCATACC GATCCAGAAA ACCAATTTGA AGACATCATG ACGGTGAAAA ATGAAATATT

                   120                 140                 160                 180                 200
TAGCGCCCCA CCTTATCCAA ACTGGACGGC GGTGGGTGTT ACATGGCTGG CAGGCTTTGA TTTTGAAATT AAAGTGATAG CGCGCATCCC TGAGCAGTAA

                   220                 240                 P1                  280                 300
GCAATAGTGT TAGCCGTTCG CTTTCAGAGT CCGCCCTATA AGTCGGATGA ATGGAATAAA ATGCATATCT GATTGCGTGA AAGTGAAAAA GGAAAAAGCA

                   320                 340                 360                 380                 400
GGGAATGTCT GCAATTATTG ATACCGAAGG ACAGTTCCCC TGCAGAATCA CATCAAATAA AAATGCATAT ACCTTGACTT TTAATTCAAA TAAACCGTTT

                   420                 440          P2     460                 480
GCGCTGACAA AATATTGCAT CAAATGCTTG CGCCGCTTCT GACGATGAGT ATAATGCCGG ACAATTTGCC GGCAGGATGT ATG GTT CAG TGT GTT CGA
                                                                                  MET Val Gln Cys Val Arg

500                 520                 540                 560                 580
 CAT TTT GTC TTA CCG CGT CTG AAA AAA GAC GCT GGC CTG CCG TTT TTC TTC CCG TTG ATC ACC CAT TCC CAG CCC CTC AAT CGA GGG
 His Phe Val Leu Pro Arg Leu Lys Lys Asp Ala Gly Leu Pro Phe Phe Phe Pro Leu Ile Thr His Ser Gln Pro Leu Asn Arg Gly

                   600                 620                 640                 660
GCT TTT TTT TGC CCA GGC GTC AGG AGA TAA AAG ATG GCT AAT CCG CTA TAT CAG AAA CAT ATC ATT TCC ATA AAC GAC CTT AGT CGC
Ala Phe Phe Cys Pro Gly Val Arg Arg         MET Ala Asn Pro Leu Tyr Gln Lys His Ile Ile Ser Ile Asn Asp Leu Ser Arg

        680                 700                 720                 740                 758
GAT GAC CTT AAT CTG GTG CTG GCG ACA GCG GCG AAA CTG AAA GCA AAC CCG CAA CCA GAG CTG TTG AAG CAC AAA GTC ATT GCC AG
Asp Asp Leu Asn Leu Val Leu Ala Thr Ala Ala Lys Leu Lys Ala Asn Pro Gln Pro Glu Leu Leu Lys His Lys Val Ile Ala Ser
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FIG. 2. Nucleotide sequence and encoded polypeptides of the 758-bp *Pvu* II fragment containing the *pyrBI* promoter-regulatory region. Only the sequence of the antisense strand is shown; numbering is from the 5' end. Dyad symmetries are indicated by the arrows with the center of symmetry shown by the dots. Pribnow box and −35 region sequences of promoters P_1 and P_2 are indicated by brackets. Further details are in the text.

quence is nearly identical to that of the tryptophan attenuator of *E. coli* K-12 (8).

Two potential *pyrBI* promoters showing homology with the consensus sequence for prokaryotic promoters (25) were located (designated P_1 and P_2 in Fig. 2). Transcription appears to be initiated at both of these promoters *in vitro*. In promoter P_1, the Pribnow box (257–263) is similar to the consensus sequence, but the −35 region is not. In promoter P_2, the Pribnow box (450–456) is identical to the consensus sequence and the highly conserved TTG sequence is present in the −35 region (428–430). The transcripts initiated at either promoter apparently could encode small polypeptides in addition to ATCase. Within the leader region of the shorter P_2 transcript is an open reading frame (481–612), preceded by an apparent ribosome binding site, that could encode a 44-amino acid polypeptide (Fig. 2). The termination codon for this polypeptide is only three nucleotides before the start of the ATCase sequence. The leader region of the P_1 transcript could encode two polypeptides—a 45-amino acid polypeptide and the 44-amino acid polypeptide included in the P_2 transcript. The 45-amino acid polypeptide (not shown in Fig. 2) would be encoded by nucleotides 305–439. The ribosome binding site for the synthesis of this polypeptide apparently would be included in a stable RNA hairpin (calculated free energy, −18.2 kcal/mol), which may inhibit translation (23).

One other region of dyad symmetry (511–533) capable of encoding a stable RNA hairpin (calculated free energy, −12.2 kcal/mol) was identified. This region and the adjacent thymidine-rich sequence included in nucleotides 541–548 appear to comprise a strong transcription pause site when the rate of transcription is limited by low levels of UTP (see below).

***In Vitro* Transcription of DNA Fragments from the *pyrBI* Promoter-Regulatory Region.** To localize sites of transcriptional initiation and termination, restriction fragments from the *pyrBI* promoter-regulatory region were transcribed *in vitro* and the resulting transcripts were analyzed by polyacrylamide gel electrophoresis. Transcription of the 758-bp *Pvu* II fragment produced two major transcripts approximately 328 and 135 nucleotides long (Fig. 3, lane 1). Transcripts of the same size also were synthesized from the 474-bp *Dde* I fragment (lane 3), which is an internal segment of the *Pvu* II fragment (Fig. 3 *Lower*). These results suggested that the two transcripts were initiated at the previously identified promoters P_1 and P_2 with termination of both transcripts occurring at the putative attenuator preceding the *pyrB* structural gene. To examine this possibility, the 758-bp *Pvu* II fragment was transcribed in a reaction in which GTP was replaced by the analog ITP. Substituting ITP for GTP has been shown to substantially decrease transcription termination at attenuators (28). If the above assignments are correct, this substitution should result in the synthesis of run-off transcripts approximately 491 and 298 nucleotides long. Transcripts of the predicted size were detected, although the amount of the 298-nucleotide transcript was less than expected (Fig. 3, lane 2). No 135-nucleotide transcript was detected, and only a small amount of a 328-nucleotide transcript was synthesized. There appeared to be only slight differences in the electrophoretic mobilities of transcripts containing either ITP or GTP. To confirm the location of the apparent *pyrBI* promoters, the 304-bp *Dde* I/*Taq* I fragment (Fig. 3 *Lower*) was transcribed. Transcription initiated at promoters P_1 and P_2 should produce run-off transcripts approximately 230 and 37 nucleotides in length, respectively, and transcripts of this size were detected (Fig. 3, lane 4). From the data presented, it is presumed that transcription is initiated at promoters P_1 and P_2.

The relative frequency of transcription from promoters P_1 and P_2 was estimated by measuring the radioactivity in the 328- and 135-nucleotide transcripts synthesized from the *Pvu* II fragment (Fig. 3, lane 1). After correcting for the differences in the specific activities of the transcripts, it was determined that approximately the same number of transcripts were initiated at each promoter.

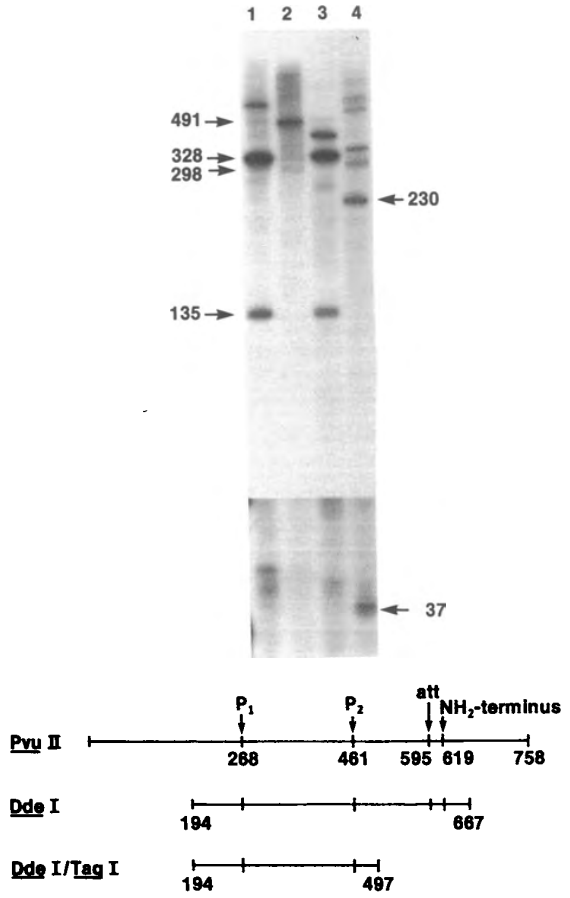

FIG. 3. *In vitro* transcription of the *pyrBI* promoter-regulatory region. (*Upper*) Autoradiogram of a polyacrylamide gel used to separate transcripts from: lane 1, 758-bp *Pvu* II fragment; lane 2, 758-bp *Pvu* II fragment with ITP substituted for GTP; lane 3, 474-bp *Dde* I fragment; and lane 4, 304-bp *Dde* I/*Taq* I fragment. Restriction fragment sizes represent the lengths of the sense strand. The lower part of the autoradiogram was enhanced using an intensifying screen to show the 37-nucleotide transcript, which has a relatively low specific activity. The major transcript above the 328-nucleotide transcript in lanes 1 and 3 and those above the 230-nucleotide transcript in lane 4 are approximately the same length as or larger than the respective templates and are presumed to result from nonspecific transcription. (*Lower*) DNA templates with probable transcriptional initiation sites for promoters P_1 and P_2, probable termination site at the attenuator, and NH_2 terminus of the ATCase catalytic subunit indicated. Transcript lengths were estimated from the gel by using single-stranded DNA standards.

The efficiency of transcriptional termination at the putative *pyrBI* attenuator was determined by comparing the levels of attenuated and run-off transcripts synthesized from the *Pvu* II fragment (Fig. 3, lane 1). Approximately 98% of the transcripts initiated at promoters P_1 and P_2 apparently were terminated at the attenuator.

To examine UTP-specific effects on transcription of the *pyrBI* promoter-regulatory region, the 758-bp *Pvu* II fragment was transcribed in a series of reactions in which one ribonucleoside triphosphate at a time was varied from the near optimal concentration of 200 μM (29) to 5 μM. The three other ribonu-

cleoside triphosphates were maintained at 200 μM. Reaction mixtures were incubated for 5 min after the addition of rifampicin and the transcripts were analyzed as above (data not shown). Reducing the concentration of UTP or any other ribonucleoside triphosphate did not increase the levels of the run-off transcripts. The most striking effect observed was that the reduction in the level of the 328-nucleotide transcript was much greater with a decrease in UTP level than with a decrease in the concentration of any other ribonucleoside triphosphate. Decreases in the level of this transcript caused by lowering the UTP concentration were accompanied by increases in the level of an approximately 280-nucleotide transcript, suggesting transcriptional pausing or termination before the putative attenuator.

UTP-sensitive transcriptional pausing was examined by transcribing the *Pvu* II fragment in four reactions in which the concentration of one ribonucleoside triphosphate was reduced to 20 μM, a suboptimal concentration (30), while the others were maintained at 200 μM. Reactions were initiated by the simultaneous addition of the ribonucleoside triphosphates and rifampicin (10 μM), which permits only a single round of transcription. Aliquots were removed at different times and transcripts were analyzed (Fig. 4). Only at suboptimal UTP, substantial levels of transcripts approximately 81 and 280 nucleotides long were detected at early time points. The pattern of disappearance of these two transcripts with the accumulation of the 135- and 328-nucleotide transcripts indicated that transcription initiated at promoters P_1 and P_2 paused before the putative attenuator at sites within the thymidine-rich sequence included in nucleotides 541–548 (Fig. 2). Additional transcripts approximately 160 and 169 nucleotides long also were detected at early time points and indicated pausing near promoter P_2 of transcription initiated at promoter P_1. This pausing was not ribonucleoside triphosphate-specific. Two other effects on transcription observed only at suboptimal UTP were a severalfold increase in the level of the 135-nucleotide transcript and a lag in the appearance of transcripts initiated at P_1. The cause and regulatory significance of these effects are not known.

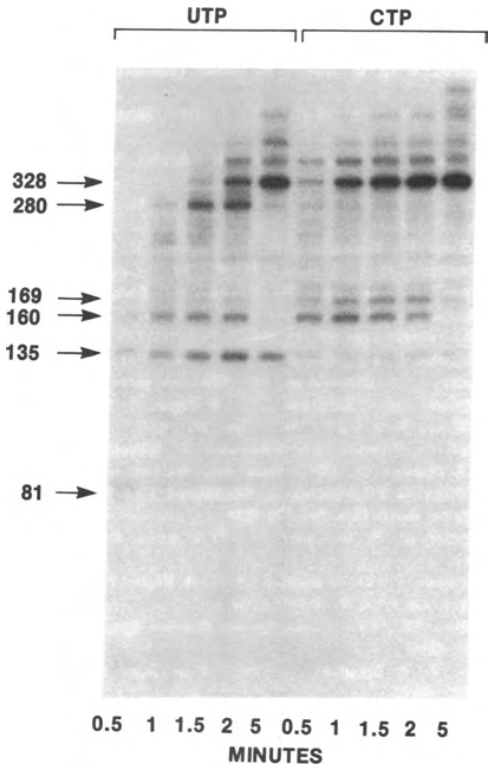

FIG. 4. *In vitro* transcription of the 758-bp *Pvu* II fragment at suboptimal ribonucleoside triphosphate concentrations. The figure shows an autoradiogram of a polyacrylamide gel used to separate transcripts synthesized during a single round of transcription at suboptimal concentrations (20 μM) of UTP or CTP. Transcription at suboptimal ATP or GTP was similar to that at suboptimal CTP. Aliquots for analysis were removed from the reaction mixtures at the indicated times. The RNA polymerase concentration was 84 pmol/ml in this experiment and other changes in the transcription protocol described in *Materials and Methods* are indicated in the text. Transcript lengths were estimated as in Fig. 3.

DISCUSSION

The presence of a strong ρ-independent transcriptional terminator immediately preceding the *pyrBI* structural genes indicates that the expression of this operon is regulated by an attenuation control mechanism (8). The apparent involvement of transcription and translation in the regulation of *pyrBI* expression suggests that this mechanism may be similar to the attenuation control mechanisms described for many amino acid biosynthetic operons in enteric bacteria (8). Unlike these operons, however, the *pyrBI* operon does not contain sequences preceding the G+C-rich region of dyad symmetry in the attenuator that permit the formation of alternative stem-loop structures in the leader transcript as a means of regulating transcriptional termination. A mechanism that could function in regulating transcriptional termination at the *pyrBI* attenuator is suggested from studies on attenuation control of histidine operon expression in *S. typhimurium* (7). These studies indicate that if a translating ribosome is allowed to follow closely behind RNA polymerase during the transcription of the attenuator region, then it can disrupt or prevent the formation of the attenuator-encoded RNA hairpin necessary for termination and thereby permit transcription to continue into the structural genes. Because the *pyrBI* attenuator is included in a leader polypeptide-encoding sequence (Fig. 2), regulating the relative rates of transcription and translation of this sequence could control transcriptional termination and the expression of the structural genes.

Based on the results presented in this study and other data, we propose the following model for regulation of the *pyrBI* operon. Transcription is initiated at promoter P_1 or P_2. When the intracellular level of UTP is low, RNA polymerase slows or stops temporarily within the run of thymidine residues in the UTP-sensitive pause site located approximately 20 bp before the attenuator. This pause provides enough time for a ribosome to initiate translation of the 44-amino acid leader polypeptide and translate up to the stalled RNA polymerase. When the polymerase eventually passes this pause site and transcribes the attenuator, the formation of the attenuator-encoded RNA hairpin is disrupted or precluded by the adjacent translating ribosome. In the absence of the termination hairpin, RNA polymerase continues transcribing into the *pyrBI* structural genes. Translation of the leader polypeptide is terminated within the ribosome binding site preceding the ATCase catalytic subunit cistron, which should ensure the immediate initiation of translation of this protein. When the intracellular level of UTP is high, RNA polymerase does not pause during the transcription of the leader region. This does not provide enough time for a ribosome to bind to the transcript and catch up to RNA polymerase before the formation of the attenuator-encoded hairpin and subsequent termination of transcription before the structural genes.

In this attenuation control mechanism there is no requirement for a purely regulatory protein. Several attempts to isolate

mutants with altered pyrimidine regulatory protein activity have been unsuccessful (31–33). Recently, a mutant of *S. typhimurium* containing increased levels of ATCase and several other pyrimidine biosynthetic enzymes was isolated (34). The mutation responsible for these effects was mapped within the gene cluster encoding the β and β' subunits of RNA polymerase, indicating a regulatory role for this enzyme in the control of pyrimidine gene expression. This observation is entirely consistent with the proposed model in which an alteration in RNA polymerase reducing the rate of transcriptional elongation or the efficiency of attenuation should result in increased expression of the *pyrBI* operon. The observation that other pyrimidine genes are affected by this mutation suggests that they may be regulated by similar attenuation mechanisms.

An important feature of the proposed model is the UTP-sensitive transcription pause site preceding the attenuator. Because regions of dyad symmetry are frequently associated with pausing (35), it is likely that this site includes the sequence of dyad symmetry preceding the thymidine-rich region within which pausing occurs (Fig. 2). We presume that these sequences constitute a uniquely strong pause site when the rate of transcription through the thymidine-rich region is limited by the availability of UTP. It has been shown that substantial derepression of *pyrBI* expression occurs only when the concentration of UTP is below an apparent threshold of approximately 0.2 mM (5, 36), which is the UTP concentration below which the rate of transcriptional elongation is reduced *in vitro* (29, 30). Although UTP appears to be the principal nucleoside triphosphate effector of *pyrBI* expression, it has been suggested that GTP also is involved in regulation, based on limited stimulation of expression that occurs upon guanine starvation (33). It is possible that low levels of GTP cause limited transcriptional pausing within the leader region. The physiological significance of this regulation is unclear.

The proposed model can accommodate transcriptional initiation at either promoter P_1 or P_2, but there is no requirement that both function. The only significant difference between the leader transcripts initiated at the two promoters appears to be that the P_1 transcript encodes a second leader polypeptide as described in *Results*. We have not assigned a regulatory function for this polypeptide, and it is possible that its translation would be precluded by secondary structure in the transcript. If, in fact, there is only one *pyrBI* promoter *in vivo*, the better candidate is promoter P_2. It is closer to the structural genes and shows greater homology with the consensus sequence for prokaryotic promoters. In addition, *in vitro* transcription initiated at this promoter is selectively inhibited by guanosine tetraphosphate (unpublished data), which has been shown to be a negative effector of ATCase synthesis *in vivo* and *in vitro* in a coupled transcription-translation system (5). Promoters negatively affected by guanosine tetraphosphate typically contain a G+C-rich sequence between the Pribnow box and the site of transcriptional initiation (37), and such a sequence is present only in promoter P_2. In formulating the model, we have assumed that regulation involving guanosine tetraphosphate occurs at the level of transcriptional initiation and is independent of the attenuation control mechanism.

Although the proposed attenuation control mechanism is consistent with the known features of *pyrBI* expression, additional experiments are clearly required to confirm the model. We also think that similar mechanisms of regulation may function in the expression of other pyrimidine genes and similar studies should provide useful information.

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