JOURNAL OF BACTERIOLOGY, Nov. 1997, p. 6665–6673 Vol. 179, No. 21
0021-9193/97/$04.00+0

Regulation of *upp* Expression in *Escherichia coli* by UTP-Sensitive Selection of Transcriptional Start Sites Coupled with UTP-Dependent Reiterative Transcription

ANH-HUE THI TU AND CHARLES L. TURNBOUGH, JR.*

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

Received 24 April 1997/Accepted 18 August 1997

Expression of the *upp* gene of *Escherichia coli*, which encodes the pyrimidine salvage enzyme uracil phosphoribosyltransferase, is negatively regulated by pyrimidine availability. In this study, we demonstrate that this regulation occurs mainly by UTP-sensitive selection of alternative transcriptional start sites, which produces transcripts that differ in the ability to be productively elongated. The *upp* initially transcribed region contains the sequence GATTTTTTTTG (nontemplate strand). Transcription is initiated primarily at the first two bases in this sequence, designated G6 and A7 (counting from the promoter −10 region). High intracellular levels of UTP favor initiation at position A7; however, the resulting transcripts are subject to reiterative transcription (i.e., repetitive nucleotide addition) within the run of T residues in the initially transcribed region. The resulting AUUUU$_n$ (where $n = 1$ to >50) transcripts are not extended to include downstream *upp* sequences. In contrast, low intracellular levels of UTP strongly favor initiation at position G6, which results in transcripts that generally do not engage in reiterative transcription and thus can be normally elongated. This mechanism ensures that high levels of uracil phosphoribosyltransferase are produced only under conditions of pyrimidine limitation. The mechanisms that account for UTP-sensitive start site selection and different fates of *upp* transcripts, as well as the general use of UTP-dependent reiterative transcription in gene regulation, are discussed in detail.

Reiterative transcription, which is also known as pseudo-templated transcription, transcriptional slippage, and RNA polymerase stuttering, is a reaction catalyzed by a number of RNA polymerases, including bacterial, phage, viral, and eukaryotic enzymes (14, 15, 20). In this reaction, nucleotides are repetitively added to the 3′ end of a nascent transcript due to slippage between the transcript and template (either DNA or RNA). Typically, slippage occurs between a homopolymeric sequence in the transcript and at least three complementary bases in the template. The mechanism apparently involves one or more rounds of a one-base upstream shift of the transcript so that the same nucleotide in the template specifies multiple residues in the transcript (11). Reiterative transcription can occur during initiation or elongation, resulting in transcripts that can be immediately released from the transcription complex or (in some cases) extended by normal elongation after a switch to nonreiterative nucleotide addition. Although reiterative transcription can involve the addition of any nucleotide, at least under certain conditions, addition of U or A residues appears most efficient (42). This increased efficiency presumably reflects a requirement in the reaction for disruption of a hybrid between transcript and template, which should be easiest with U:A or A:T base pairing.

Recent studies indicate that reiterative transcription plays an important role in the expression and regulation of a number of bacterial and viral genes by a variety of mechanisms (14, 21, 34). In *Escherichia coli*, for example, reiterative transcription is involved in pyrimidine-mediated regulation of *pyrBI* and *codBA* operon expression. In the case of the *pyrBI* operon, which encodes the two subunits of the pyrimidine biosynthetic enzyme aspartate transcarbamylase, high intracellular levels of UTP induce reiterative transcription within the initially transcribed region of the operon (Fig. 1), resulting in the synthesis of transcripts with the sequence AAUUUU$_n$. These transcripts are not extended downstream to include additional leader region and structural gene sequences, and their synthesis reduces the level of productive initiation and full-length *pyrBI* transcript synthesis. This mechanism accounts for regulation over an approximately sevenfold range (21).

In the case of the *codBA* operon, which encodes the pyrimidine salvage enzymes cytosine permease and cytosine deaminase, reiterative transcription plays a somewhat different regulatory role. UTP-dependent reiterative transcription within a run of six T residues (nontemplate strand) in the initially transcribed region prevents productive extension of all transcripts initiated at the A8 start site (Fig. 1), which is the primary start site when the intracellular level of UTP is high. In contrast, reiterative transcription is avoided for the most part when initiation occurs at the more upstream start site G7, apparently because the transcript forms a stable hybrid with the template. Low intracellular levels of UTP strongly favor transcriptional initiation at position G7 by inhibiting initiation at position A8, which relies on a high concentration of UTP to form the critical first internucleotide bond of the transcript. Thus, regulation occurs over an approximately 30-fold range by UTP-sensitive selection of transcriptional start sites with different potentials for nonproductive reiterative transcription (34).

The modest requirements for regulation of *pyrBI* and *codBA* expression through UTP-dependent reiterative transcription suggest that similar mechanisms exist in *E. coli* (and in other species). Consistent with this prediction is the fact that 10% of the approximately 300 well-characterized *E. coli* promoters contain a run of three to eight T residues (nontemplate strand) within two nucleotides of the transcriptional start site, regions

* Corresponding author. Mailing address: Department of Microbiology, 409 BBRB/Box 6, University of Alabama at Birmingham, Birmingham, AL 35294. Phone: (205) 934-6289. Fax: (205) 975-5479. E-mail: chuckt@uab.edu.

FIG. 1. Sequences of the initially transcribed regions of the *upp*, *pyrBI*, and *codBA* promoters. The nontemplate strand sequence is shown. The −10 region of each promoter is underlined, and the major transcriptional start sites are indicated by asterisks. Start sites are identified in the text according to their positions downstream from the −10 region; e.g., the major *upp* start sites are designated G6 and A7.

within which reiterative transcription may occur. In addition, transcriptional initiation at many of the promoters in this group are suspected to be regulated by pyrimidine availability. One of these is the promoter for the *upp* gene (Fig. 1), which encodes uracil phosphoribosyltransferase. This pyrimidine salvage enzyme catalyzes the formation of UMP from uracil and phosphoribosylpyrophosphate. In this study, we investigated the mechanism of pyrimidine-mediated regulation of *upp* expression. Our results show that this regulation occurs by a mechanism analogous to that described for the *codBA* operon with some interesting differences.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains are *E. coli* K-12 derivatives. Strain MC4100 [F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 thiA1 relA1 deoC1 ptsF25 flbB5301 rbsR*] (6) served as a source of chromosomal DNA. Strain CLT42 (MC4100 *araD*$^+$ *car-94*) (38) was used as the parent in the construction of λ lysogens. Strain CLT240 (CLT42 *pcnB80 zad*::Tn*10*) was constructed by transducing CLT42 with phage P1 *vira* grown on strain KE94 (HB101 *pcnB80 zad*::Tn*10*) (35). Strain CLT240 was used as the recipient in transformations that introduced multicopy plasmids containing wild-type and mutant *upp*::*lacZ* gene fusions. The *pcnB80* mutation of strain CLT240 reduces plasmid copy number, which was found to be essential in maintaining mutant plasmids in the absence of secondary mutations that reduce *upp*::*lacZ* expression.

The multicopy plasmid used to construct the *upp*::*lacZ* gene fusions was pMLB1034, which contains the *lacZ* gene without a promoter, a ribosomal binding site, and the first eight codons for β-galactosidase (39). This plasmid contains an *Eco*RI/*Sma*I/*Bam*HI multiple cloning site immediately preceding the *lacZ* gene. Gene fusions were made by first digesting plasmid pMLB1034 with *Eco*RI and *Bam*HI and then ligating the linear plasmid to a 247-bp *Eco*RI-*Bam*HI restriction fragment containing the wild-type *upp* promoter region or an equivalent fragment containing a mutant promoter region. The downstream sequence in these promoter-region fragments extends through *upp* codon 30. All fusion constructions were confirmed by DNA sequence analysis using a Sequenase kit (U.S. Biochemical).

Restriction digests, ligations, transformations, and PCR. Conditions for restriction digests, ligations, and transformations were as previously described (38). PCR amplification of DNA was performed with *Pfu* DNA polymerase (Stratagene), using the reaction mixture recommended by the supplier. PCR conditions were 94°C/2 min, 42°C/1 min, and 72°C/1 min for 30 cycles.

DNA preparations and site-directed mutagenesis. Plasmid DNA was isolated by use of Qiagen plasmid kits. Chromosomal DNA was prepared as previously described (26). The wild-type *upp* promoter region, which contains nucleotides −100 to +127 counting from the G6 transcriptional start site (Fig. 1), was amplified by PCR for use in plasmid constructions. The template in the PCR was chromosomal DNA from strain MC4100. The forward and reverse DNA primers were 5′*CCGGAATTCAGCTG*CCATCTGAATATAAAATAAC and 5′*CGCG GATCCAGCTG*TTCGCGAAAGCGCTTGG, respectively (italicized sequences indicate added *Eco*RI, *Bam*HI, and *Pvu*II restriction sites). The resulting PCR product (after digestion with *Eco*RI and *Bam*HI to trim the fragment ends) was used in the construction of the plasmid pMLB1034 derivative carrying the wild-type *upp*::*lacZ* gene fusion described above.

Site-directed mutations were introduced into the *upp* promoter region by using a PCR-based procedure similar to that described by Barettino et al. (4). The products of this procedure are essentially the same as the PCR product described above except with a specific change in the *upp* promoter. These products served as a source of mutant *upp* promoter regions (again after *Eco*RI and *Bam*HI digestion) in the construction of gene fusion plasmids.

The PCR products containing the wild-type and mutant *upp* promoter regions were also digested with *Pvu*II (which cuts just inside the *Eco*RI and *Bam*HI sites). Each *Pvu*II promoter fragment was ligated to the 2,364-bp *Pvu*II fragment of plasmid pUC18 to produce a recombinant plasmid. These plasmids were introduced into *E. coli* DH5α by transformation, amplified, and then used (after *Pvu*II digestion) as a source of blunt-ended templates for in vitro transcription. Correct *upp* promoter region sequences in the pUC18 derivatives were verified by DNA sequence analysis.

In vitro transcription. Purified RNA polymerase holoenzyme containing σ^{70} was prepared as previously described (5, 10). DNA templates were either a 233-bp *Pvu*II restriction fragment containing the wild-type *upp* promoter region or an equivalent fragment containing a mutant promoter region. The templates were prepared, and their concentrations and purity were determined as previously described (32). In the standard assay, transcription reaction mixtures (10 μl) contained 10 nM DNA template, 100 nM RNA polymerase, 20 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 100 mM potassium glutamate, 0.2 mM Na_2EDTA, 0.1 mM dithiothreitol, 800 μM each ATP, CTP, and GTP, and from 50 to 1,000 μM UTP, depending on the experiment. In the reaction mixture, one of the nucleoside triphosphates was ^{32}P labeled. The specific activities of the label (purchased from NEN) were 0.625 Ci/mmol for [γ-^{32}P]ATP and [γ-^{32}P]GTP and 0.25 Ci/mmol for [α-^{32}P]UTP. Reactions were initiated by addition of RNA polymerase, and the reaction mixtures were incubated at 37°C for 15 min. Heparin (1 μl of a 1-mg/ml solution) was then added to the mixture, and incubation was continued for an additional 10 min. Reactions were terminated by adding 10 μl of stop solution (7 M urea, 2 mM Na_2EDTA, 0.25% [wt/vol] each bromophenol blue and xylene cyanol) and placing the samples on ice. The samples were heated at 100°C for 3 min, and typically an equal volume of each sample was removed and run on a 25 or 6% polyacrylamide (29:1 acrylamide: bisacrylamide)–50 mM Tris-borate (pH 8.3)–1 mM Na_2EDTA sequencing gel containing 7 M urea (32). Transcripts were visualized by autoradiography and quantitated by scanning gels with a Molecular Dynamics PhosphorImager.

Transcripts used for primer extension mapping were synthesized in 50-μl reaction mixtures containing the same ingredients as in the standard assay except that 200 μM each ATP, CTP, and GTP was used and the UTP concentration was varied as indicated in the text. None of the nucleoside triphosphates were radiolabeled in this case. Reaction mixtures were incubated as in the standard assay. After incubating with heparin, reactions were stopped and RNA was precipitated and dried as described by Wilson et al. (43). RNA was dissolved in 7 μl of diethyl pyrocarbonate-treated water (32), and the entire sample was used for primer extension analysis.

Transfer of *upp*::*lacZ* gene fusions from plasmids to the *E. coli* chromosome. Wild-type and mutant *upp*::*lacZ* gene fusions carried on derivatives of plasmid pMLB1034 were individually transferred to the chromosome of strain CLT42 by using phage λRZ5 (37). The presence of a single prophage at the λ attachment site was confirmed by PCR analysis (31). In this procedure, the concentration of each primer was 500 nM.

Media and culture methods. Cells used for enzyme assays and RNA isolations were grown to an optical density at 650 nm of 0.5 (mid-log) at 37°C with shaking in N$^-$C$^-$ medium (1) supplemented with 10 mM NH_4Cl, 0.4% (wt/vol) glucose, 0.015 mM thiamine hydrochloride, 1 mM arginine, and either 1 mM uracil or 0.25 mM UMP. Culture densities were measured with a Gilford model 260 spectrophotometer, and doubling times were determined between optical densities at 650 nm of 0.1 and 0.2.

Enzyme assays. Cell extracts were prepared by sonic oscillation (37). β-Galactosidase activity (26) and protein concentration (23) were determined as previously described.

Isolation of cellular RNA and primer extension mapping. Cellular RNA was isolated quantitatively as described by Wilson et al. (43). Primer extension mapping of the 5′ ends of *upp*::*lacZ* transcripts was performed as described by Liu and Turnbough (22) except that 50 μg of RNA from uracil-grown cells and 38 μg of RNA from UMP-grown cells were used for analysis. The difference in amounts of RNA isolated from the same mass of cells reflects the different levels of stable RNA in cells growing at different rates. The primer used in these experiments was 5′TTTTCCCAGTCACGACGTTG, which was labeled with ^{32}P at the 5′ end. This primer hybridizes to *lacZ* sequence just downstream from the fusion junction in the *upp*::*lacZ* transcript. Primer extension mapping of in vitro transcripts was performed essentially as described above except that the primer was 5′CCCAGCTTGTGTTTGACGAG. This primer hybridizes to the *upp* sequence from +84 to +65 (using start site G6 as +1) and was designed to detect runoff (actually any long productively extended) transcripts. In all primer extension mapping experiments, the primer was in large molar excess.

RESULTS

Identification of transcriptional start sites and UTP-dependent reiterative transcription at the *upp* promoter. To identify *upp* transcriptional start sites and determine whether reiterative transcription occurs within the initially transcribed region of the *upp* promoter, we transcribed a DNA template containing this promoter in a reaction mixture containing 800 μM each ATP, CTP, GTP, and [α-^{32}P]UTP. [α-^{32}P]UTP was used as the radiolabel to allow detection of all transcripts initiated at

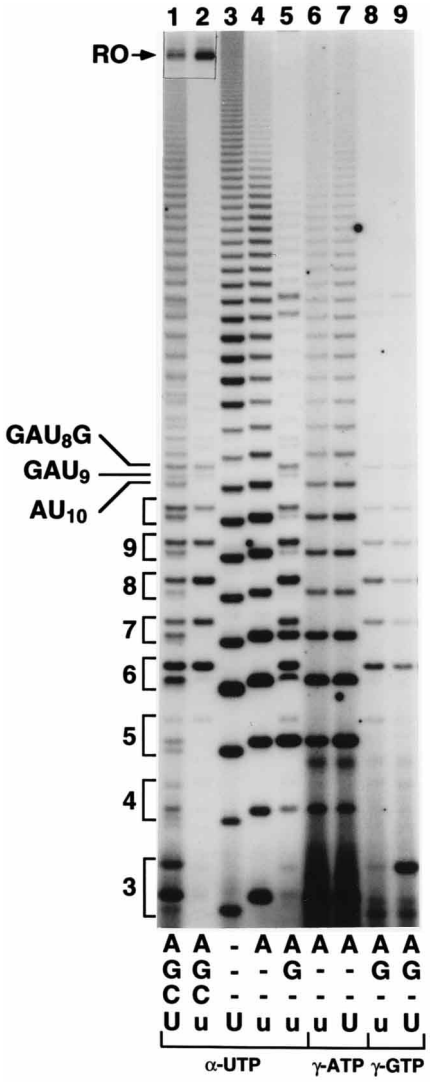

FIG. 2. Analysis of transcripts initiated at the *upp* promoter. A DNA template containing the wild-type *upp* promoter region was transcribed in vitro in reaction mixtures containing selected nucleoside triphosphates. The nucleotides included in each reaction mixture and their concentrations are indicated at the bottom as follows: A, 800 μM ATP; C, 800 μM CTP; G, 800 μM GTP; U, 800 μM UTP; and u, 50 μM UTP. The ^{32}P-labeled nucleotide (i.e., α-UTP, γ-ATP, or γ-GTP) in each reaction mixture also is indicated at the bottom. The autoradiograph is of the 25% polyacrylamide gel used to separate the transcripts. Brackets enclose triplet bands corresponding to transcripts of the same length but initiated at a different start site. The number next to each bracket indicates the length (in nucleotides) of the transcripts in the enclosed triplet. In addition, the sequences of the primary 11-nucleotide-long transcripts are shown. The same volume of reaction mixture was loaded in each lane except lane 3, where only half as much sample was loaded to compensate for a higher level of transcript synthesis. The inset at the top of lanes 1 and 2 shows *upp* runoff transcript bands (RO). Runoff transcripts were analyzed after separation in a 6% polyacrylamide gel.

previously identified (3) and other potential (22) transcriptional start sites located six, seven, and eight nucleotides downstream from the promoter −10 region (designated G6, A7, and T8, respectively) (Fig. 1). Transcripts synthesized in the reaction were separated on a 25% polyacrylamide sequencing gel and visualized by autoradiography.

The pattern of transcripts was extremely complicated (Fig. 2, lane 1). There were triplet bands for transcripts up to 10 nucleotides long, with the middle band of each triplet comigrating with marker transcripts containing the sequence AAU_n that were produced by reiterative transcription at the *pyrBI* promoter (data not shown). (For triplets containing transcripts longer than five nucleotides, note that the lower band, which is light, migrates very close to the middle band and is difficult to see in the figure.) The triplet bands indicate that transcription was initiated at three sites. Based on a comparison of the gel mobilities of the *upp* transcripts and short *codBA* transcripts (data not shown), the known effects of transcript sequence on gel mobility (34), and the sequence of the *upp* transcription initiation region, we tentatively identified the top, middle, and lower bands of each triplet as transcripts initiated at start sites G6, A7, and T8, respectively. Based on the relative band intensities in the triplets, it appears that initiation occurs inefficiently at position T8.

In addition to the triplet bands, transcription products included a ladder of longer transcripts from 11 to more than 50 nucleotides in length (Fig. 2, lane 1). This ladder is a clear indication of reiterative transcription at the *upp* promoter. To determine if synthesis of ladder transcripts is sensitive to UTP concentration, as previously observed with the *pyrBI* and *codBA* promoters, we performed another transcription reaction in which the reaction mixture contained 800 μM each ATP, CTP, and GTP and 50 μM [α-^{32}P]UTP. The results show that synthesis of the long ladder transcripts was greatly reduced (Fig. 2, lane 2). Also, synthesis of all putative A7- and T8-initiated transcripts, including very short transcripts that must have been produced by simple abortive initiation, was severely restricted. In contrast, synthesis of a number of the putative G6-initiated transcripts was stimulated at the lower UTP concentration. These results indicate that a low UTP concentration inhibits transcriptional initiation at positions A7 and T8 and that most reiterative transcription requires initiation at these sites, particularly A7.

Another effect of the low UTP concentration was a 2.1-fold increase in the level of *upp* runoff transcripts produced by transcription to the end of the DNA template (Fig. 2, inset). The levels of these 130-nucleotide-long transcripts were measured after separating transcripts on a 6% polyacrylamide gel.

Further characterization of the *upp* transcripts and reiterative transcription at the *upp* promoter. To establish the identities of the *upp* transcripts and transcriptional start sites and more thoroughly examine reiterative transcription, we transcribed the *upp* promoter in reaction mixtures containing selected nucleoside triphosphates and different radiolabels. With a reaction mixture containing only 800 μM [α-^{32}P]UTP, a long ladder of poly(U) transcripts was produced (Fig. 2, lane 3). This ladder grew one nucleotide at a time and clearly exceeded 50 nucleotides in length. The transcripts in this ladder comigrated with the bottom band of the triplets produced by transcription in the presence of 800 μM each nucleoside triphosphate (compare lanes 1 and 3 in Fig. 2). These results support our tentative identification of the bottom-band transcripts as T8-initiated transcripts and also indicate that reiterative transcription readily occurs with transcripts initiated at position T8.

With a reaction mixture containing 800 μM ATP and 50 μM [α-^{32}P]UTP, another long and simple ladder of transcripts was produced (Fig. 2, lane 4). These transcripts comigrated with the middle band of the triplets produced with all four nucleoside triphosphates. These bands were not detected (nor were any others) with a reaction mixture containing only 800 μM [α-^{32}P]ATP, indicating that they contained both A and U residues (data not shown). With another set of reaction mixtures containing 800 μM [γ-^{32}P]ATP and either 50 or 800 μM UTP, apparently identical ladders of transcripts were detected (Fig. 2, lanes 6 and 7). This result indicates that all of the transcripts

in lanes 4, 6, and 7 start with ATP. Together, these results provide convincing evidence that the middle bands of each triplet are indeed initiated at position A7, that these transcripts contain the sequence AU_n, and that reiterative transcription occurs efficiently with A7-initiated transcripts. Additionally, the results with the reaction mixtures containing 800 μM [γ-^{32}P]ATP and either 50 or 800 μM UTP indicate that a low UTP concentration does not substantially inhibit initiation at position A7 (or reiterative transcription involving A7-initiated transcripts) in the absence of GTP and CTP (compare lanes 2 and 6 in Fig. 2). Apparently, such inhibition requires another nucleotide (i.e., GTP) that can serve as a competing initiating nucleotide.

When 800 μM GTP was included in the reaction mixture along with 800 μM ATP and 50 μM [α-^{32}P]UTP, doublet bands were detected (Fig. 2, lane 5). The bottom bands migrated as A7-initiated AU_n transcripts. The top bands of each doublet comigrated with the top bands of the triplets produced with all four nucleoside triphosphates (compare lanes 1 and 5 in Fig. 2). These bands clearly require GTP for their synthesis and in fact are not synthesized if either GTP, ATP, or UTP is omitted from the reaction mixture (complete data not shown). When [γ-^{32}P]GTP was used as the radiolabel instead of [α-^{32}P]UTP in reaction mixtures containing ATP, GTP, and UTP, bands corresponding to the top bands of the doublets (and triplets) were synthesized (Fig. 2, lanes 8 and 9). These results show that the top-band transcripts are initiated with GTP and contain G, A, and U residues, which provides strong support for the identification of these transcripts as G6-initiated transcripts.

The results of the transcription assays using [γ-^{32}P]GTP, ATP, and UTP also show that reiterative transcription does not occur efficiently with transcripts initiated at position G6, as indicated by the absence of a ladder of long transcripts. Presumably, the ladder of short G6-initiated transcripts (up to 11 nucleotides long) is produced primarily by simple abortive initiation. Evidence in support of this conclusion was provided by identifying the two 11-nucleotide long G6-initiated transcripts observed in lanes 8 and 9 (and also in lanes 1, 2, and 5) of Fig. 2. Identification was made by differential radiolabeled-nucleotide incorporation essentially as described for *codBA* transcripts (34). The major species (which was about 2.5 times more abundant) was identified as GAU_8G, a predicted product of abortive initiation. The minor 11-mer was determined to be GAU_9, apparently a product of low-level reiterative transcription. The effects of UTP concentration on the levels of G6-initiated transcripts synthesized with only GTP, ATP, and UTP (lanes 8 and 9) were similar to those observed in reactions with all four nucleoside triphosphates (lanes 1 and 2); these effects will be described in detail below.

Effects of UTP concentration on transcription from the *upp* promoter. To examine in more detail the effects of UTP concentration on transcription from the *upp* promoter, we transcribed the promoter region in reaction mixtures containing 800 μM each ATP, CTP, and GTP and concentrations of [α-^{32}P]UTP ranging from 50 to 1,000 μM. The lowest and highest UTP concentrations used here are similar to those found in *E. coli* grown under conditions of severe pyrimidine limitation and pyrimidine excess, respectively (2, 27). The results show that the level of each A7-initiated transcript increased linearly over an approximately 20-fold range with increasing UTP concentration (Fig. 3A). The pattern for one of these transcripts, AU_{10}, is shown in Fig. 3B. As mentioned above, it appears that the effect of UTP concentration on the synthesis of A7-initiated transcripts occurs at the level of transcriptional initiation (as opposed to reiterative transcription),

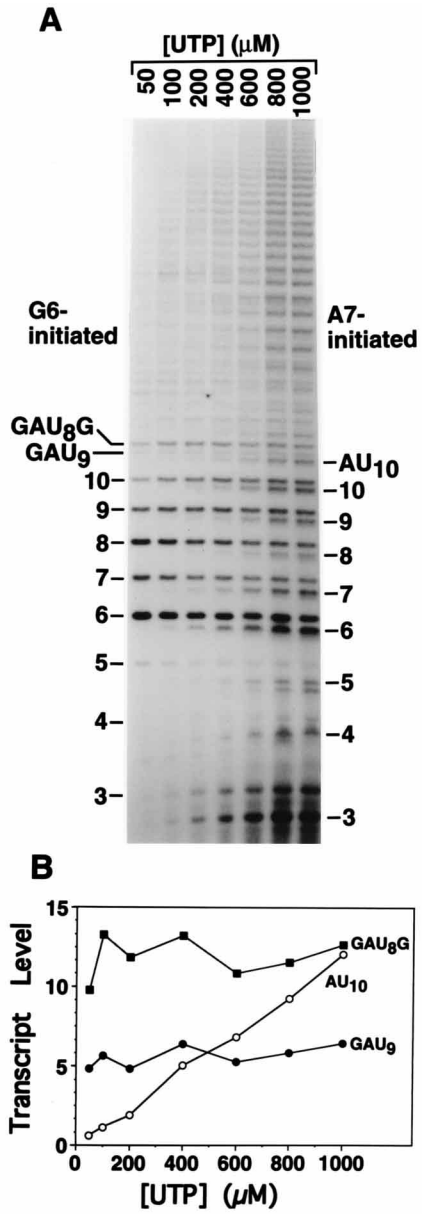

FIG. 3. Effects of UTP concentration on *upp* transcription. Standard in vitro transcription reactions were performed in which the UTP concentration was varied as indicated and the radiolabel was [α-^{32}P]UTP. Transcripts were separated on a 25% polyacrylamide sequencing gel, visualized by autoradiography, and quantitated. (A) Autoradiograph of the gel, with nucleotide lengths or sequences of selected G6-initiated and A7-initiated transcripts indicated on the left or right, respectively. (B) Levels of the major 11-nucleotide-long *upp* transcripts AU_{10}, GAU_8G, and GAU_9 plotted as a function of UTP concentration. The units for transcript levels are arbitrary. Transcript levels have been corrected for the different number of radiolabeled U residues in each transcript.

because the same pattern of synthesis is observed for transcripts produced by both reiterative transcription (e.g., AU_{10}) and abortive initiation (e.g., AU_2 and AU_3). The pattern of synthesis of the minor T8-initiated transcripts (not labeled in Fig. 3A) appeared identical to that of the A7-initiated transcripts. We assume that UTP concentration affects initiation at the T8 start site in the same way that it does at A7; i.e., initiation is directly proportional to the UTP concentration.

The synthesis of G6-initiated transcripts produced a pattern

strikingly different from that described above. The effects of UTP concentration were much smaller, and they varied with different transcripts (Fig. 3A). The synthesis of G6-initiated transcripts containing four or fewer nucleotides increased nearly linearly over a severalfold range with increasing UTP concentration. The synthesis of transcripts containing five to eight nucleotides decreased nearly linearly over an approximately twofold range with increasing UTP concentration. Finally, the synthesis of transcripts containing more than eight nucleotides was not significantly affected by the UTP concentration. The latter response was observed with transcripts produced by either abortive initiation (e.g., GAU_8G) or low-level reiterative transcription (e.g., GAU_9) (Fig. 3B). The reason for the complex pattern of G6-initiated transcript synthesis is not obvious. However, the overall effect of increasing the UTP concentration from 50 to 1,000 μM UTP was a nearly twofold increase in the total level of G6-initiated transcripts.

Transcriptional fates of *upp* transcripts. In our previous characterization of transcription from the *codBA* promoter, we found that transcripts equivalent to the *upp* transcripts initiated at positions A7 and T8 were never extended past the long run of T residues in the initially transcribed region to include additional leader region and structural gene sequences. To determine if the *upp* A7 and T8 transcripts were in fact subject to the same fate, we mapped by primer extension the 5′ ends of *upp* runoff transcripts synthesized in reaction mixtures containing 200 μM each ATP, CTP, and GTP and either 50 or 1,000 μM UTP. We used lower than standard concentrations of ATP, CTP, and GTP in this case because doing so greatly enhances initiation at positions A7 and T8 (primarily A7), at least in the presence of high UTP (compare lane 1 of Fig. 2 with lane 2 of Fig. 4A). The primer extension data indicate, however, that no A7- or T8-initiated transcripts are extended to produce runoff transcripts. All runoff transcripts produced at 50 and 1,000 μM UTP appear to be initiated at position G6 (Fig. 4B). The set of minor bands migrating below the G6 band in the gel are most likely due to a small amount of degradation of the primer extension product or impurities (i.e., shorter oligonucleotides) in the primer.

The primer extension data also show that the level of runoff transcript synthesis was severalfold higher at 50 μM UTP than at 1,000 μM UTP (Fig. 4B). This difference is similar to that observed when the levels of short G6-initiated transcripts synthesized at 50 and 1,000 μM UTP are compared (Fig. 4A). These data, along with the results presented above, indicate that increased synthesis of runoff transcripts is due to stimulation of initiation at position G6.

Regulatory effects of *upp* promoter mutations that inhibit reiterative transcription. To assess the role of reiterative transcription in pyrimidine-mediated regulation of *upp* expression in vivo, we first constructed two site-directed mutations that alter the run of eight T residues in the *upp* initially transcribed region. One mutation changes the third T to a G and is designated the TTG mutation. The other mutation deletes six T residues and is called the ΔT_6 mutation. These mutations were predicted to severely inhibit or abolish reiterative transcription at the *upp* promoter. To confirm such an effect, DNA templates containing the promoter mutations plus a wild-type template as a control were transcribed in reaction mixtures containing 800 μM each ATP, CTP, and GTP and either 800 or 50 μM [α-^{32}P]UTP. Analysis of the transcript products indicated that reiterative transcription was in fact eliminated by each mutation, as judged by the absence of a very long, regularly spaced ladder of transcripts in the presence of 800 μM UTP (Fig. 5). However, transcription of the mutant templates did generate an unusual, nonuniform ladder of transcripts up to approximately 20 nucleotides in length. These ladders were produced even at low UTP, where initiation appears to occur almost exclusively at position G6 just as observed with the wild-type promoter. Thus, we assume that the unusual ladders of transcripts are G6-initiated transcripts produced by atypical abortive initiation not involving UTP-dependent reiterative transcription.

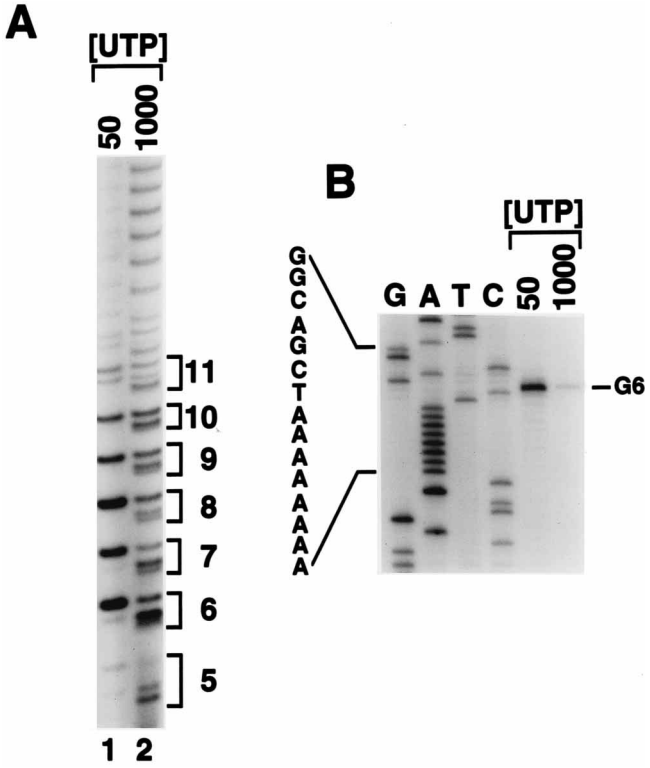

FIG. 4. Characterization of short and runoff *upp* transcripts synthesized in reaction mixtures containing 200 μM each ATP, CTP, and GTP and either 50 or 1,000 μM UTP. (A) Short transcripts were analyzed as described in the legend to Fig. 2. Transcripts less than five nucleotides long are not shown because only very low levels were synthesized with either 50 or 1,000 μM UTP. Transcripts for this analysis were labeled with [α-^{32}P]UTP. (B) Runoff transcripts were synthesized in scaled-up in vitro reaction mixtures that did not contain a radiolabeled nucleotide. The 5′ ends of runoff transcripts were determined by primer extension mapping as described in Materials and Methods. The autoradiograph is of the 10% polyacrylamide sequencing gel used to separate primer extension products. UTP concentrations (micromolar) are indicated at the top. The band corresponding to G6-initiated transcripts is marked on the right. The dideoxy sequencing ladder (G, A, T, and C) of the *upp* promoter region was generated with the primer used for primer extension.

The mutant and wild-type promoter regions were then used to construct *upp*::*lacZ* gene fusion strains. The promoter regions were inserted separately into plasmid pMLB1034 to create *lacZ* gene fusions, which were recombined onto phage λRZ5. The recombinant phage were used to infect strain CLT42 (*car-94* Δ*lacZYA*), and lysogens carrying a single prophage at the chromosomal λ attachment site were isolated and designated CLT5178 (wild-type fusion), CLT5214 (TTG mutant), and CLT5218 (ΔT_6 mutant). These strains are pyrimidine auxotrophs because the *car-94* mutation inactivates the first enzyme of the pyrimidine biosynthesis pathway. Wild-type and mutant strains were grown in glucose-minimal salts medium containing either uracil or UMP as the pyrimidine source, which provides a condition of pyrimidine excess or limitation, respectively. The levels of β-galactosidase in these strains were assayed as an indicator of *upp* expression (Table

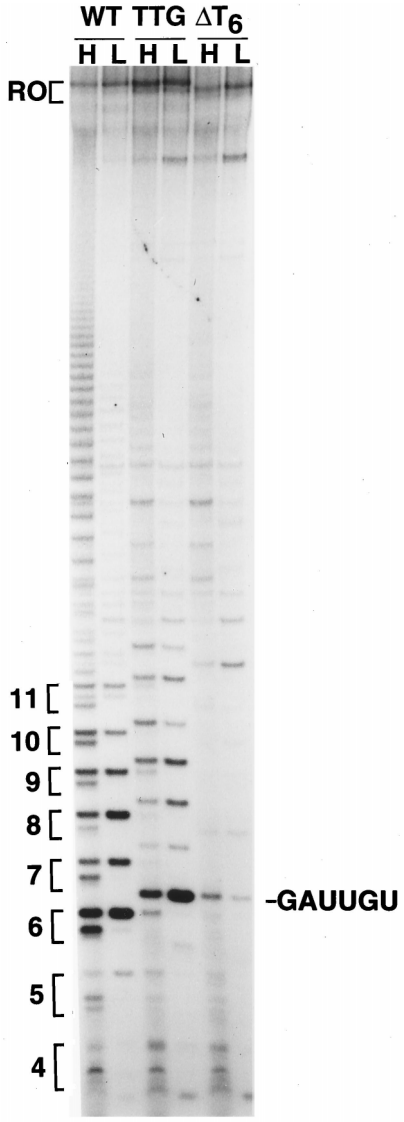

FIG. 5. Effects of *upp* promoter mutations on reiterative transcription. Standard in vitro transcription reactions were performed with either wild-type (WT) or mutant (TTG and ΔT_6) DNA templates. Two reactions were run with each template: one with 800 μM (high [H]) and the other with 50 μM (low [L]) [α-^{32}P]UTP. Transcripts were separated on a 25% polyacrylamide sequencing gel and visualized by autoradiography. Wild-type transcript lengths and runoff (RO) transcripts are labeled on the left. The position of the G6-initiated 6-mer, GAUUGU, which is produced with both mutant templates, is indicated on the right for reference. Note that for transcripts longer than five residues, mutant transcripts migrate more slowly than corresponding (i.e., same length and start site) wild-type transcripts. This difference is observed because the mutant transcripts contain a higher fraction of G residues, which slow migration more than any other nucleotide. As an example, the G6-initiated 6-mer (GAUUGU) produced with the TTG template migrates above the G6-initiated 6-mer (GAUUUU) produced with the wild-type template. However, the latter transcript (GAUUUU) comigrates with the A7-initiated 6-mer (AUUGUU) produced with the TTG template because they have the same nucleotide content.

1). The results show that wild-type fusion expression is regulated over a nearly sixfold range by pyrimidine availability, similar to that previously reported for the intact wild-type *upp* gene (3, 36). In contrast, expression of the mutant fusions was regulated only 1.7- and 1.6-fold in strains CLT5214 (TTG) and CLT5218 (ΔT_6), respectively. The reduced regulation was due primarily to increased expression under conditions of pyrimidine excess. This result indicates that UTP-dependent reiterative transcription accounts for nearly all pyrimidine-mediated negative regulation of *upp* expression. The lower level of expression in strain CLT5218 than in strain CLT5214 apparently reflects a general negative effect of the ΔT_6 mutation on fusion expression.

TABLE 1. Effects of promoter mutations on regulation of *upp*::*lacZ* expression[a]

Strain (promoter genotype)	β-Galactosidase activity (nmol/min/mg)[b]		Fold regulation
	Uracil	UMP	
CLT5178 (wild type)	3,020	17,500	5.8
CLT5214 (TTG)	10,800	18,800	1.7
CLT5218 (ΔT_6)	5,600	8,900	1.6

[a] Doubling times were 46 ± 2 min for cells grown on uracil and 69 ± 3 min for cells grown on UMP.
[b] Mean of five experiments with variation of ≤8%.

Quantitative primer extension mapping of *upp*::*lacZ* transcripts synthesized in vivo from wild-type and mutant promoters. Quantitative primer extension mapping was used to measure the levels and determine the start sites of *upp*::*lacZ* transcripts synthesized in strains CLT5178 (wild type), CLT5214 (TTG), and CLT5218 (ΔT_6) grown on uracil or UMP (same cultures as described in Table 1). The primer used in these experiments hybridizes to *lacZ* sequence contained in the fusions and therefore detects only *upp*::*lacZ* transcripts in the Δ*lacZYA* strains. The results for the wild-type and TTG transcripts are shown in Fig. 6. The results for the ΔT_6 transcripts are not shown because they were essentially the same as those for the TTG transcripts. In the case of wild-type transcripts, only G6-initiated transcripts were detected. The level of these transcripts from UMP-grown cells was sevenfold higher than the level from uracil-grown cells, roughly reflecting cellular β-galactosidase activities (Table 1). In the case of TTG transcripts, both G6- and A7-initiated transcripts were detected with cells grown on either pyrimidine source. However, the pyrimidine source dramatically affected the relative amounts of the two transcripts. For uracil-grown cells, about 40% of the transcripts were initiated at position G6 and about 60% were initiated at position A7. For UMP-grown cells, about 90% of the transcripts were initiated at position G6 and only about 10% were initiated at position A7. The total level of *upp* transcripts for UMP-grown cells was 40% higher than the total level for uracil-grown cells, again roughly reflecting the β-galactosidase activities measured in these cells.

The primer extension mapping experiments also revealed a number of transcripts that are shorter than the G6- and A7-initiated transcripts (Fig. 6A). These transcripts appear to be products of in vivo *upp* transcript degradation. Such bands were not detected in primer extension mapping experiments using *upp* transcripts synthesized in vitro (Fig. 4B). From the pattern of the shorter transcripts shown in Fig. 6A, it appears that G6- and A7-initiated transcripts were degraded similarly. This is indicated by the fact that the level of shorter transcripts reflects the total *upp* transcript level and not the level of one particular species.

DISCUSSION

Our in vitro transcription studies identified a number of features of transcriptional initiation at the *upp* promoter that are clearly important for pyrimidine-mediated regulation of *upp* expression. The results show that transcriptional initiation at the *upp* promoter can occur at multiple sites designated G6, A7, and T8 and that the selection of these sites is sensitive to

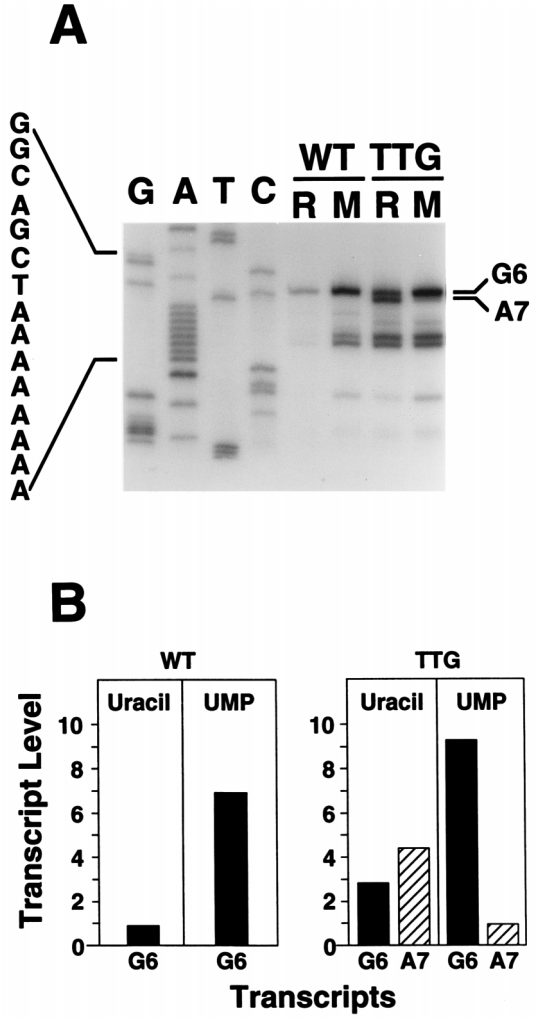

FIG. 6. Levels of *upp*::*lacZ* transcripts initiated at the wild-type (WT) and TTG mutant (TTG) promoters in strains CLT5178 and CLT5214, respectively. Cellular RNA was quantitatively isolated from cells grown on either uracil (R) or UMP (M). Transcript levels were measured by primer extension mapping as described in Materials and Methods. (A) Autoradiograph of the 10% polyacrylamide sequencing gel that was used to separate the primer extension products. The bands corresponding to G6- and A7-initiated transcripts are shown on the right. The dideoxy sequencing ladder of the wild-type *upp* promoter region, which was used to identify transcripts, was generated with the primer used for primer extension. Note that the spaces between bands in sequencing ladders of the wild-type and TTG mutant promoter regions are identical, and thus only one ladder is shown. (B) Transcript levels quantitated with a PhosphorImager and plotted in arbitrary units.

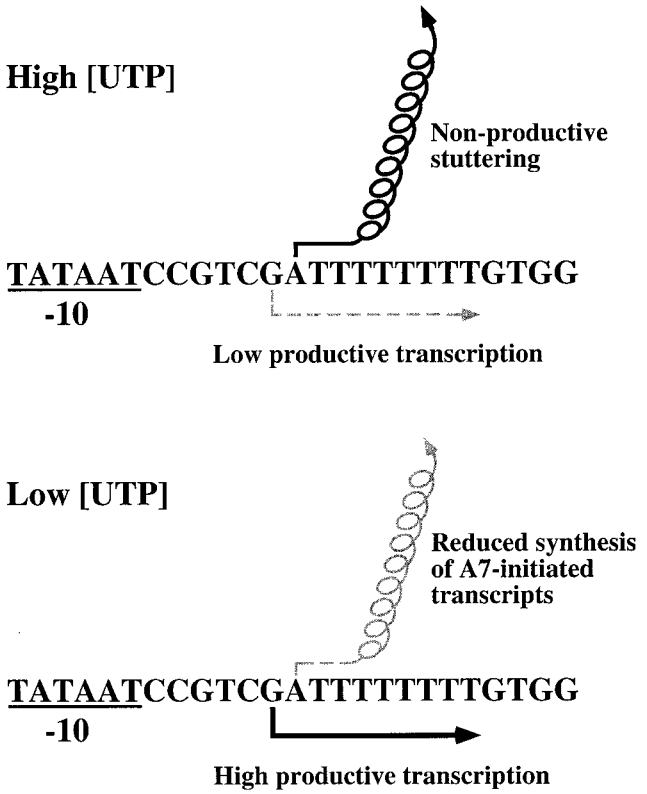

FIG. 7. Model for pyrimidine-mediated regulation of *upp* expression, showing the effects of UTP concentration on the selection of transcriptional start sites and the fates of transcripts initiated at alternative sites.

the concentration of UTP. High concentrations of UTP support efficient initiation at positions G6 and A7 and a low level of initiation at position T8. Low concentrations of UTP stimulate initiation at position G6 while nearly abolishing initiation at the other sites. In addition, low levels of UTP enhance synthesis of *upp* runoff transcripts. The results also show that UTP-dependent reiterative transcription occurs efficiently with A7- and T8-initiated transcripts and that these transcripts are not extended past the initially transcribed region. In contrast, most G6-initiated transcripts avoid reiterative transcription and are either terminated by simple abortive initiation or productively extended to include structural gene sequence.

The characterization of in vivo transcription of *upp*::*lacZ* gene fusions containing wild-type and mutant promoter regions provided additional information necessary to understand the regulatory mechanism. The results demonstrate that UTP-dependent reiterative transcription within the *upp* initially transcribed region is required for nearly all pyrimidine-mediated regulation of *upp* expression. The data also show that transcriptional initiation in vivo can occur efficiently at start sites G6 and A7; however, there was no clear indication of initiation at position T8. Detection of A7-initiated transcripts by primer extension required the use of a mutant promoter that prevented reiterative transcription, because otherwise these transcripts are not extended downstream to include sequences that hybridize to the primer. Quantitation of mutant *upp*::*lacZ* transcripts revealed the same dramatic UTP-sensitive transcriptional start site switching observed in vitro. Finally, the levels of full-length *upp*::*lacZ* transcripts (wild type or mutant) paralleled the levels of β-galactosidase activity in the cell.

A model for *upp* regulation. On the basis of these observations, we propose the following model for pyrimidine-mediated regulation of *upp* expression (Fig. 7). When intracellular levels of UTP are high, RNA polymerase preferentially initiates transcription at position A7. The resulting nascent transcript is extended until it contains three or perhaps four U residues (i.e., AU_3 or AU_4). At this point, weak base pairing between the transcript and DNA template permits the transcript to slip one base upstream. RNA polymerase then adds a U residue to the 3′ end of the transcript, and from this point on the transcript is irreversibly directed into a nonproductive transcription pathway. The transcript can be released from the initiation complex or another round of slippage, and U addition can occur. This process can be repeated many times with a certain probability of transcript release after every U addition. Synthesis of the resulting AU_n transcripts in effect occludes the

promoter, thereby reducing the opportunity for initiation at position G6 and the production of full-length *upp* transcripts. In contrast, when intracellular levels of UTP are low, RNA polymerase initiates transcription almost exclusively at position G6. The resulting transcripts avoid reiterative transcription presumably due to the formation of a more stable hybrid between the transcript and DNA template. Thus, these transcripts are elongated normally and can be extended downstream to generate translatable *upp* transcripts. As a consequence of this regulatory mechanism, high levels of the enzyme required for uracil salvage are produced only under conditions of pyrimidine limitation. In all of its key features, this model is analogous to the proposed mechanism for pyrimidine-mediated regulation of *codBA* expression (34).

In the model, we did not include a role for T8-initiated transcripts because it is not clear that they are synthesized in vivo. If in fact T8-initiated transcripts are produced under physiological conditions, they would presumably function in the proposed regulatory mechanism just like A7-initiated transcripts.

Selection of transcriptional start sites. UTP-sensitive selection of alternative transcriptional start sites is a key feature in the proposed model. As argued previously (34), this process is probably based on a requirement for rapid formation of a covalent bond between the first and second nucleotides of a transcript to achieve efficient transcriptional initiation. Rapid formation of the first internucleotide bond requires ample concentrations of both nucleotides. A low concentration of the second nucleotide can dramatically reduce initiation efficiency (25, 28), or it can cause the selection of an alternative start site (at the same promoter) that does not use the limiting nucleotide in the formation of the first internucleotide bond (40). Accordingly, position A7 is the primary start site at the *upp* promoter when the concentration of UTP, the second nucleotide, is high. This selection apparently is based on a preference for initiating transcription seven bases downstream from the −10 region and for the use of ATP as the initiating nucleotide (22). However, when the UTP concentration is low, resulting in inefficient initiation at position A7, RNA polymerase readily selects the secondary start site G6. Transcription from this site uses GTP and ATP to form the first internucleotide bond and thus is not inhibited by a low concentration of UTP.

In the foregoing scenario, we assume that both ATP and GTP are present in ample amounts in all cases: ATP and GTP concentrations are greater than 1 mM in cells grown under conditions of pyrimidine excess or limitation (27, 30). However, ATP and GTP pools do increase substantially (two- to threefold) upon pyrimidine limitation (30), and it is possible that this effect favors initiation at position G6 and contributes to the observed transcriptional start site switching. Such a mechanism requires that GTP levels are not saturating for transcriptional initiation at the *upp* promoter, while ATP levels are above or close to the level required for maximal initiation (34).

Different fates of *upp* transcripts and the role of an extended RNA-DNA hybrid. We assume in our model that the different fates of G6- and A7-initiated transcripts are related to their ability to form a stable hybrid with the DNA template during the initiation phase of transcription. Specifically, nascent A7-initiated transcripts cannot form a hybrid that is stable enough to prevent transcript slippage and realignment, which results in reiterative transcription. On the other hand, nascent G6-initiated transcripts can form a hybrid that is stable enough to preclude slippage (in most instances) and thus avoid reiterative transcription. The difference in the two cases is that G6-initiated transcripts form a hybrid that includes an extra rG:dC base pair at the beginning of the heteroduplex.

For this explanation to be correct, it appears necessary that the RNA-DNA hybrid contains at least 10 base pairs. This length is based on the distance between the first G residue of the G6-initiated transcript and the end of the run of eight U residues specified by the initially transcribed region. The existence of such an extended hybrid has been proposed in one popular model for transcriptional elongation (8, 44) and is consistent with a number of different types of experimental observations (9, 13, 17–19, 24, 29, 42, 45). However, an alternative model that advocates a short hybrid containing three or fewer base pairs has been proposed (7, 41). If the latter model is correct, then some features other than the strength of the RNA-DNA hybrid must control reiterative transcription (otherwise slippage would occur after synthesis of the nascent 5-mer with the sequence GAUUU). The most likely possibility is a sequence-specific interaction between the 5′ end of the transcript and RNA polymerase. However, recent studies in this lab have shown that the extent of reiterative transcription during initiation is clearly not due to such a transcript-RNA polymerase interaction and correlates directly with the apparent strength of the RNA-DNA hybrid (33). Thus, we think that G6-initiated transcripts (but not A7-initiated transcripts) escape reiterative transcription because the sequence at their 5′ ends allows the formation of a stable hybrid between the nascent transcript and DNA template, which persists long enough for the transcript to be extended downstream of the run of eight U residues that induce slippage.

An assumption in our estimation of the length of the extended hybrid described above is that slippage of G6-initiated transcripts is prevented only by base pairing at the 5′ end of the transcript. Another possible explanation is that after the transcript contains four or more U residues, it encounters a (presently unknown) barrier to slippage. If such a barrier exists, then we may be overestimating the length of the hybrid. However, based on the studies of Wagner et al., which indicate that slippage occurs upon the addition of 10 or 11 U residues to the 3′ end of an elongating transcript (42), we do not favor the latter explanation.

Difference between the *upp* and *codBA* regulatory mechanisms. Although there are many similarities between the mechanisms of pyrimidine-mediated regulation of *upp* and *codBA* expression, there is one striking difference. The range of regulation observed with the *upp* gene is less than 6-fold, whereas that for the *codBA* operon is approximately 30-fold. This difference could be due to the different locations of the transcriptional start sites in the two promoters (relative to the −10 region) and to preferences in selecting these sites (22). The G and A start sites are located at positions 6 and 7 in the case of *upp* and at positions 7 and 8 in the case of *codBA*. Pyrimidine-mediated start site switching between these two sites at the *upp* promoter is not as extensive as that seen at the *codBA* promoter. Switching is much closer to an all-or-none situation in the case of the *codBA* promoter. More extensive start site switching should result in a wider range of regulation.

Overview. The regulatory mechanism described in this report increases to three the number of clear examples of regulation involving reiterative transcription in *E. coli* (or any bacterial species). Another possible example not yet discussed involves UTP-mediated regulation of transcriptional initiation at the *galP2* promoter of the *galETK* operon, although the reported range of regulation is only 1.5-fold (16). This small range of regulation may be due to the use of apparently inadequate conditions for pyrimidine limitation. We have recently demonstrated that pyrimidine-mediated regulation of *carAB* expression occurs in part through a mechanism similar to that described for regulating initiation at the *pyrBI* promoter (12).

The *carAB* operon encodes the first enzyme in the pyrimidine biosynthetic pathway.

Thus, all of the examples of regulation by reiterative transcription in *E. coli* involve genes with an obvious connection to pyrimidine metabolism (*galP2* through the synthesis of UDP-galactose). However, many of the more than 30 *E. coli* promoters containing a run of three or more T residues in the nontemplate strand of the initially transcribed region are for genes and operons that do not appear to be directly associated with pyrimidine metabolism. It seems reasonable to suspect that many of these genes are in fact regulated by mechanisms involving UTP-dependent reiterative transcription, although the details of these mechanisms may be quite different from those characterized to date. Discovery of many new examples would indicate that the cell uses UTP much like a global regulatory effector, similar to cyclic AMP and guanosine tetraphosphate. It is known that large changes in UTP levels are tolerated by the cell, and such changes could be used as an indicator of cellular fitness.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant GM29466 to C.L.T. During part of this work, A.-H. T. T. was supported by training grant AI07041 from the National Institutes of Health.

REFERENCES

1. **Alper, M. D., and B. N. Ames.** 1978. Transport of antibiotics and metabolite analogs by systems under cyclic AMP control: positive selection of *Salmonella typhimurium cya* and *crp* mutants. J. Bacteriol. **133:**149–157.
2. **Andersen, J. T., K. F. Jensen, and P. Poulsen.** 1991. Role of transcription pausing in the control of the *pyrE* attenuator in *Escherichia coli*. Mol. Microbiol. **5:**327–333.
3. **Andersen, P. S., J. M. Smith, and B. Mygind.** 1992. Characterization of the *upp* gene encoding uracil phosphoribosyltransferase of *Escherichia coli* K12. Eur. J. Biochem. **204:**51–56.
4. **Barettino, D., M. Feigenbutz, R. Valcárcel, and H. G. Stunnenberg.** 1993. Improved method for PCR-mediated site-directed mutagenesis. Nucleic Acids Res. **22:**541–542.
5. **Burgess, R. R., and J. J. Jendrisak.** 1975. A procedure for the rapid, large-scale purification of *Escherichia coli* DNA-dependent RNA polymerase involving polymin P precipitation and DNA-cellulose chromatography. Biochemistry **14:**4634–4638.
6. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104:**541–555.
7. **Chamberlin, M. J.** 1995. New models for the mechanism of transcription elongation and its regulation. Harvey Lect. **88:**1–21.
8. **Chan, C. L., and R. Landick.** 1994. New perspectives on RNA chain elongation and termination by *E. coli* RNA polymerase, p. 297–321. *In* R. C. Conaway and J. W. Conaway (ed.), Transcription: mechanisms and regulation. Raven Press, New York, N.Y.
9. **Daube, S. S., and P. H. von Hippel.** 1992. Functional transcription elongation complexes from synthetic RNA-DNA bubble duplexes. Science **258:**1320–1324.
10. **Gonzalez, N., J. Wiggs, and M. J. Chamberlin.** 1977. A simple procedure for resolution of *Escherichia coli* RNA polymerase holoenzyme from core polymerase. Arch. Biochem. Biophys. **182:**404–408.
11. **Guo, H.-C., and J. W. Roberts.** 1990. Heterogeneous initiation due to slippage at the bacteriophage 82 late gene promoter in vitro. Biochemistry **29:**10702–10709.
12. **Han, X., and C. L. Turnbough, Jr.** Unpublished data.
13. **Hanna, M. M., and C. F. Meares.** 1983. Topography of transcription: path of the leading end of nascent RNA through the *Escherichia coli* transcription complex. Proc. Natl. Acad. Sci. USA **80:**4238–4242.
14. **Jacques, J. P., and D. Kolakofsky.** 1991. Pseudo-templated transcription in prokaryotic and eukaryotic organisms. Genes Dev. **5:**707–713.
15. **Jeong, S. W., W. H. Lang, and R. H. Reeder.** 1996. The yeast transcription terminator for RNA polymerase I is designed to prevent polymerase slippage. J. Biol. Chem. **271:**16104–16110.
16. **Jin, D. J.** 1994. Slippage synthesis at the *galP2* promoter of *Escherichia coli* and its regulation by UTP concentration and cAMP-cAMP receptor protein. J. Biol. Chem. **269:**17221–17227.
17. **Kainz, M., and J. Roberts.** 1992. Structure of transcription elongation complexes in vivo. Science **255:**838–841.
18. **Komissarova, N., and M. Kashlev.** 1997. Transcriptional arrest: *Escherichia coli* RNA polymerase translocates backward, leaving the 3′ end of the RNA intact and extruded. Proc. Natl. Acad. Sci. USA **94:**1755–1760.
19. **Lee, D. N., and R. Landick.** 1992. Structure of RNA and DNA chains in paused transcription complexes containing *Escherichia coli* RNA polymerase. J. Mol. Biol. **228:**759–777.
20. **Linton, M. F., M. Raabe, V. Pierotti, and S. G. Young.** 1997. Reading-frame restoration by transcriptional slippage at long stretches of adenine residues in mammalian cells. J. Biol. Chem. **272:**14127–14132.
21. **Liu, C., L. S. Heath, and C. L. Turnbough, Jr.** 1994. Regulation of *pyrBI* operon expression in *Escherichia coli* by UTP-sensitive reiterative RNA synthesis during transcriptional initiation. Genes Dev. **8:**2904–2912.
22. **Liu, J., and C. L. Turnbough, Jr.** 1994. Effects of transcriptional start site sequence and position on nucleotide-sensitive selection of alternative start sites at the *pyrC* promoter in *Escherichia coli*. J. Bacteriol. **176:**2938–2945.
23. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
24. **Macdonald, L. E., Y. Zhou, and W. T. McAllister.** 1993. Termination and slippage by bacteriophage T7 RNA polymerase. J. Mol. Biol. **232:**1030–1047.
25. **McClure, W. R., C. L. Cech, and D. E. Johnston.** 1978. A steady state assay for the RNA polymerase initiation reaction. J. Biol. Chem. **253:**8941–8948.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Neuhard, J., and P. Nygaard.** 1987. Purines and pyrimidines, p. 445–473. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
28. **Nierman, W. C., and M. J. Chamberlin.** 1979. Studies of RNA chain initiation by *Escherichia coli* RNA polymerase bound to T7 DNA. Direct analysis of the kinetics and extent of RNA chain initiation at T7 promoter A_1. J. Biol. Chem. **254:**7921–7926.
29. **Nudler, E., A. Mustaev, E. Lukhtanov, and A. Goldfarb.** 1997. The RNA-DNA hybrid maintains the register of transcription by preventing backtracking of RNA polymerase. Cell **89:**33–41.
30. **Poulsen, P., and K. F. Jensen.** 1987. Effect of UTP and GTP pools on attenuation at the *pyrE* gene of *Escherichia coli*. Mol. Gen. Genet. **208:**152–158.
31. **Powell, B. S., D. L. Court, Y. Nakamura, M. P. Rivas, and C. L. Turnbough, Jr.** 1994. Rapid confirmation of single copy lambda prophage integration by PCR. Nucleic Acids Res. **22:**5765–5766.
32. **Qi, F., C. Liu, L. S. Heath, and C. L. Turnbough, Jr.** 1996. *In vitro* assay for reiterative transcription during transcriptional initiation by *Escherichia coli* RNA polymerase. Methods Enzymol. **273:**71–85.
33. **Qi, F., and C. L. Turnbough, Jr.** Unpublished data.
34. **Qi, F., and C. L. Turnbough, Jr.** 1995. Regulation of *codBA* operon expression in *Escherichia coli* by UTP-dependent reiterative transcription and UTP-sensitive transcriptional start site switching. J. Mol. Biol. **254:**552–565.
35. **Quinn, C. L., B. T. Stephenson, and R. L. Switzer.** 1991. Functional organization and nucleotide sequence of the *Bacillus subtilis* pyrimidine biosynthetic operon. J. Biol. Chem. **266:**9113–9127.
36. **Rasmussen, U. B., B. Mygind, and P. Nygaard.** 1986. Purification and some properties of uracil phosphoribosyltransferase from *Escherichia coli* K12. Biochim. Biophys. Acta **881:**268–275.
37. **Roland, K. L., C. Liu, and C. L. Turnbough, Jr.** 1988. Role of the ribosome in suppressing transcriptional termination at the *pyrBI* attenuator of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **85:**7149–7153.
38. **Roland, K. L., F. E. Powell, and C. L. Turnbough, Jr.** 1985. Role of translation and attenuation in the control of *pyrBI* operon expression in *Escherichia coli* K-12. J. Bacteriol. **163:**991–999.
39. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **Sørensen, K. I., K. E. Baker, R. A. Kelln, and J. Neuhard.** 1993. Nucleotide pool-sensitive selection of the transcriptional start site in vivo at the *Salmonella typhimurium pyrC* and *pyrD* promoters. J. Bacteriol. **175:**4137–4144.
41. **Surratt, C. K., S. C. Milan, and M. J. Chamberlin.** 1991. Spontaneous cleavage of RNA in ternary complexes of *Escherichia coli* RNA polymerase and its significance for the mechanism of transcription. Proc. Natl. Acad. Sci. USA **88:**7983–7987.
42. **Wagner, L. A., R. B. Weiss, R. Driscoll, D. S. Dunn, and R. F. Gesteland.** 1990. Transcriptional slippage occurs during elongation at runs of adenine or thymine in *Escherichia coli*. Nucleic Acids Res. **18:**3529–3535.
43. **Wilson, H. R., C. D. Archer, J. Liu, and C. L. Turnbough, Jr.** 1992. Translational control of *pyrC* expression mediated by nucleotide-sensitive selection of transcriptional start sites in *Escherichia coli*. J. Bacteriol. **174:**514–524.
44. **Yager, T. D., and P. H. von Hippel.** 1991. A thermodynamic analysis of RNA transcript elongation and termination in *Escherichia coli*. Biochemistry **30:**1097–1118.
45. **Zaychikov, E., L. Denissova, and H. Heumann.** 1995. Translocation of the *Escherichia coli* transcription complex observed in the registers 11 to 20: "jumping" of RNA polymerase and asymmetric expansion and contraction of the "transcription bubble." Proc. Natl. Acad. Sci. USA **92:**1739–1743.