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Pyrimidine Regulation of Tandem Promoters for *carAB* in *Salmonella typhimurium*

CHUNG-DAR LU,[1] MOGENS KILSTRUP,[2] JAN NEUHARD,[2] AND AHMED ABDELAL[1]*

Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia 30303,[1] and Institute of Biological Chemistry B, University of Copenhagen, Copenhagen, Denmark[2]

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The *carAB* operon of *Salmonella typhimurium* encodes the two subunits of the enzyme carbamoylphosphate synthetase. Transcription of the operon is initiated at tandem promoters that are subject to control by pyrimidines and arginine. Pyrimidine regulation was examined by quantitative primer extension experiments under conditions in which densitometric measurements of the transcripts were linear with the amount of RNA. RNA was obtained from mutant strains that permit manipulations of pyrimidine nucleotide pools. The data showed that a uridine nucleotide repressed the upstream promoter (P1), whereas arginine repressed the downstream promoter (P2). Exogenous cytidine, which increased the intracellular CTP pool in certain mutant strains, did not affect either promoter. However, CTP limitation resulted in derepression of the pyrimidine-specific promoter as well as the downstream arginine-specific promoter. The effect of pyrimidines on P2 was confirmed in a *carA::lacZ* transcriptional fusion in which the activity of the pyrimidine-specific promoter was abolished. Primer extension experiments with an *argR*::Tn*10* derivative showed that repression of P1 by uridine nucleotides did not require a functional arginine repressor and that repression of P2 by arginine did not interfere with elongation of transcripts initiated at the upstream P1 promoter.

Carbamoylphosphate synthetase (CPSase) in *Salmonella typhimurium* (1) and *Escherichia coli* (11) catalyzes the synthesis of carbamoylphosphate, a common precursor for arginine and pyrimidines (Fig. 1). The two subunits of the enzyme are encoded by the *carAB* operon, the transcription of which is cumulatively repressed by arginine and pyrimidines (11, 21). Recent studies (21) with *S. typhimurium* showed that transcription of the *carAB* operon is initiated at tandem promoters (Fig. 2A). As is the case with *E. coli* (5, 7, 11, 30), transcription from the upstream promoter (P1) is subject to pyrimidine control, and transcription from the downstream promoter (P2) is negatively controlled by arginine (21). The P2 region contains two 18-base-pair (bp) sequences homologous to the ARG box that characterizes operator sequences sensitive to the arginine repressor (11). The sequence of the P1 region does not show any features resembling the attenuators preceding the *pyrBI* (32, 36) or *pyrE* (4) operon. Both of these operons have been shown to be regulated through a UTP-sensitive attenuation control mechanism (4, 10, 32, 36). In the case of *pyrBI*, recent studies (25) indicate that an additional mechanism, the nature of which is unknown, contributes to pyrimidine regulation of this operon. Studies with *pyrC* (29, 39), *pyrD* (22), and *pyrF* (35, 37) indicate that regulation of expression of these genes does not involve attenuation control mechanisms. Thus, with the exception of *pyrBI* and *pyrE*, the mechanisms that control expression of genes of pyrimidine biosynthesis are yet to be elucidated.

Studies with mutant strains of *S. typhimurium* that permit manipulations of individual nucleotide pools (20, 34) led to the conclusion that expression of *pyrB*, *pyrE*, and *pyrF* is repressed by a uracil nucleotide different from UMP, whereas *pyrC* and *pyrD* expression is controlled primarily by a cytosine nucleotide other than CMP. It has recently been reported (18, 21) that both uracil and cytosine nucleotides control *carAB* expression in *S. typhimurium*.

The present article deals with the specific effects of cytidine and uridine nucleotides on expression of *carAB*. We report quantitative primer extension experiments in mutant strains that permit manipulations of pyrimidine nucleotide pools. The results show that exogenous uridine negatively controls the activity of the upstream promoter (P1), whereas exogenous cytidine does not affect *carAB* expression. Surprisingly, CTP limitation results in derepression not only of P1 but also of the downstream promoter (P2). The downstream promoter was previously believed to be controlled only by arginine. The effect of pyrimidines on P2 is supported by experiments with a *carA::lacZ* transcriptional fusion in which the activity of P1 is abolished. Primer extension experiments also showed that repression of the downstream promoter by arginine does not interfere with the elongation of transcripts initiated at the upstream promoter.

MATERIALS AND METHODS

Strains. All strains used were derivatives of *Salmonella typhimurium* LT2. Strain AA57 (*argR*::Tn*10*) was derived from KR62 (*proAB21 argR*::Tn*10*), provided by R. A. Kelln (12). Strain KD1109 (*cdd-4*) was also obtained from R. A Kelln (20). Strain JL1280 (*pyrG1611 cdd-7 udp-2 glpT*) was obtained from J. L. Ingraham (3). The *pyrG1611* mutation is leaky, and the CTP synthetase encoded by this allele is more temperature labile than the wild-type enzyme.

Enzyme assays. Cells were suspended in 100 mM potassium phosphate (pH 7.5) containing 0.5 mM EDTA, and cell extracts were prepared by sonic disruption or passage through an Aminco French pressure cell. CPSase activity was assayed by incorporation of [^{14}C]bicarbonate into carbamoylphosphate (1). Enzyme activities were computed from results obtained with reaction mixtures containing less than 100 μg of protein (reaction rates are not proportional to enzyme concentrations at higher protein levels). Dihydroorotase (39), β-galactosidase (27), and β-lactamase (15) were assayed as described previously.

Nucleotide pool determination. Cells were labeled for at

* Corresponding author.

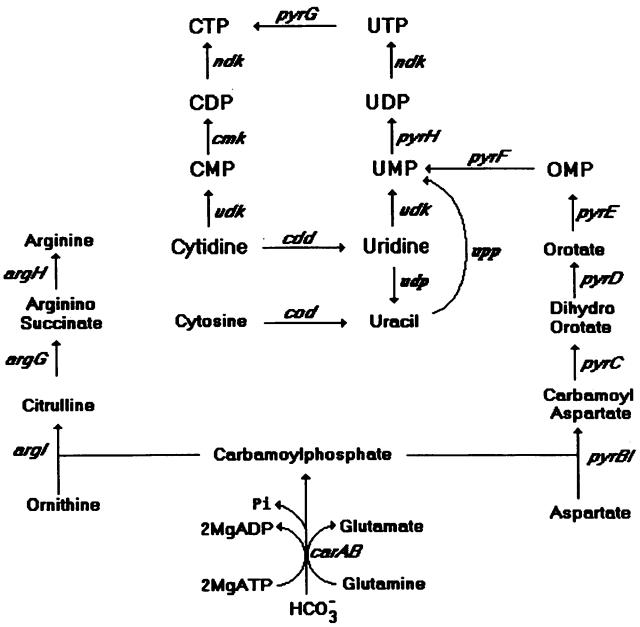


FIG. 1. Pathways of arginine and pyrimidine biosynthesis. The genes encoding the enzymes involved are indicated: *argI*, ornithine carbamoyltransferase; *argG*, argininosuccinate synthetase; *argH*, argininosuccinase; *carAB*, carbamoylphosphate synthetase; *cod*, cytosine deaminase; *cdd*, cytidine deaminase; *cmk*, cytidine monophosphate kinase; *ndk*, nucleoside diphosphate kinase; *pyrBI*, aspartate carbamoyltransferase; *pyrC*, dihydroorotase; *pyrD*, dihydroorotate oxidase; *pyrE*, orotate phosphoribosyltransferase; *pyrF*, orotidine 5'-phosphate decarboxylase; *pyrG*, CTP synthetase; *pyrH*, UMP kinase; *udk*, uridine kinase; *udp*, uridine phosphorylase; *upp*, uracil phosphoribosyltransferase.

least one generation of exponential growth in the Tris-buffered medium of Irr and Gallant (16) containing 0.3 mM $^{32}P_i$ with a specific activity of 33 Ci/mol. Extraction of cells and thin-layer chromatographic separation of nucleoside triphosphates were done as described by Jensen et al. (19).

RNA extraction. The cultures were grown in minimal medium (38) supplemented with 0.2% glucose and arginine (100 μg/ml), cytidine (1 mM), or uridine (1 mM) where indicated. A 10-ml sample of logarithmically growing culture (OD_{600}, 0.5) was added to the RNA extraction buffer containing 10% sodium dodecyl sulfate at 65°C, and RNA was extracted quantitatively by the procedure described by Hagen and Young (13). In one experiment (KD1109), RNA was extracted by the procedure described by Aiba and co-workers (2). All solutions were made with 0.2% diethylpyrocarbonate-treated water. The final RNA precipitate was dissolved in 100 μl of 10 mM Tris hydrochloride (Tris-HCl) (pH 8.0) containing 1 mM EDTA. The RNA concentration was determined by measuring the OD_{260}. The quality of the RNA was monitored by electrophoresis on a 1% agarose gel in sodium phosphate buffer (pH 7.0) after denaturation of the RNA with glyoxal as described by Lehrach and co-workers (23).

Primer extension experiments. A synthetic oligonucleotide primer complementary to codons 8 to 13 of the *carA* gene was prepared with an Applied Biosystems model 381 DNA synthesizer. The primer was applied to a 20% polyacrylamide gel and detected by UV shadowing as described by the manufacturer (Applied Biosystems). The purified primer was eluted from the gel with a C-18 cartridge (Waters Associates). Purification of the oligonucleotide primer significantly improved the quality of primer extension experiments. The primer was labeled at the 5' end by phosphorylation with polynucleotide kinase and [γ-^{32}P]ATP (6,000 Ci/mmol; New England Nuclear Corp.). The reaction mixture was extracted with phenol and chloroform, and the labeled primer was precipitated by adding 1/10 volume of 4 M ammonium acetate and 2 volumes of ethanol and incubating for 10 min in a dry ice-ethanol bath. The primer was collected by centrifugation (4°C), washed with 70% ethanol, dried under vacuum, and dissolved in water. The labeled primer (10 ng) was mixed with RNA (10 to 50 μg) in 0.02 M Tris-HCl, pH 8.0, containing 0.1 M NaCl and 0.1 mM EDTA in a 1.5-ml microfuge tube (final volume, 30 μl). The reaction mixture was incubated at 85°C for 5 min and then transferred to 55°C for 3 h. The reaction mix was cooled slowly to 37°C in a covered water bath, followed by centrifugation at 12,000 × *g* for 5 s. Then, 30 μl of 0.2 M Tris-HCl, pH 8.0, containing 20 mM $MgCl_2$, 0.1 M KCl, 10 mM dithiothreitol, 1 mM each dATP, dCTP, dGTP, and dTTP, and 200 U of cloned Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) was added. The reaction mix was incubated at 37°C for 1 h, and the reaction was terminated by heating at 65°C for 5 min. RNA was digested by the addition of 5 μg of pancreatic RNase A and incubation at 37°C for 10 min. The sample was extracted with phenol and chloroform, and the products of primer extension were precipitated by the addition of 0.1 volume of 4 M ammonium acetate and 2.5 volumes of ethanol and incubation in a dry ice-ethanol bath for 10 min. The primer extension products were collected by centrifugation (4°C) at 12,000 × *g* for 15 min, washed once with 200 μl of 70% ethanol, and dried in vacuo. The sample was dissolved in 7 μl of DNA-sequencing dye, and 3 μl was applied to a 0.2-mm sequencing 8% polyacrylamide gel with an LKB Macrophor electrophoresis unit. Following electrophoresis, the gel was soaked in 10% acetic acid for 15 min and then dried at 80°C for 1 h. Following exposure of an X-ray film for 15 h, the relative levels of transcripts were obtained by scanning the film with an LKB Ultrascan XL laser densitometer and using an LKB 2400 Gelscan XL software package. Figure 3A shows a photograph of an autoradiograph obtained with different concentrations of RNA from JL1280. The downstream promoter is represented by three bands, as previously shown in primer extension and S1 experiments (21). The basis for the multiple bands of P2 observed under all physiological conditions in *S. typhimurium* (21) as well as *E. coli* (5, 7) is not known. The peak areas obtained by densitometric measurements of the autoradiograph for P1 and P2 were plotted as a function of RNA concentrations. The resulting graph (Fig. 3B) shows the linear relationship between the densitometric measurements and the amount of RNA used in primer extension experiments.

DNA sequencing. DNA sequencing was performed by the method of Sanger et al. (33) with recombinant M13 template and the same primer that was used for primer extension.

RESULTS

Repression of P1 and P2 by uridine and arginine. In wild-type *S. typhimurium* and *E. coli*, exogenous cytidine is rapidly and quantitatively converted to uridine by cytidine deaminase, encoded by *cdd* (3, 28) (Fig. 1). In contrast, *cdd* mutants convert exogenous cytidine effectively to CTP (20, 34). In such *cdd* mutants, the addition of uracil to the growth medium increases the UTP pool and to a lesser extent the

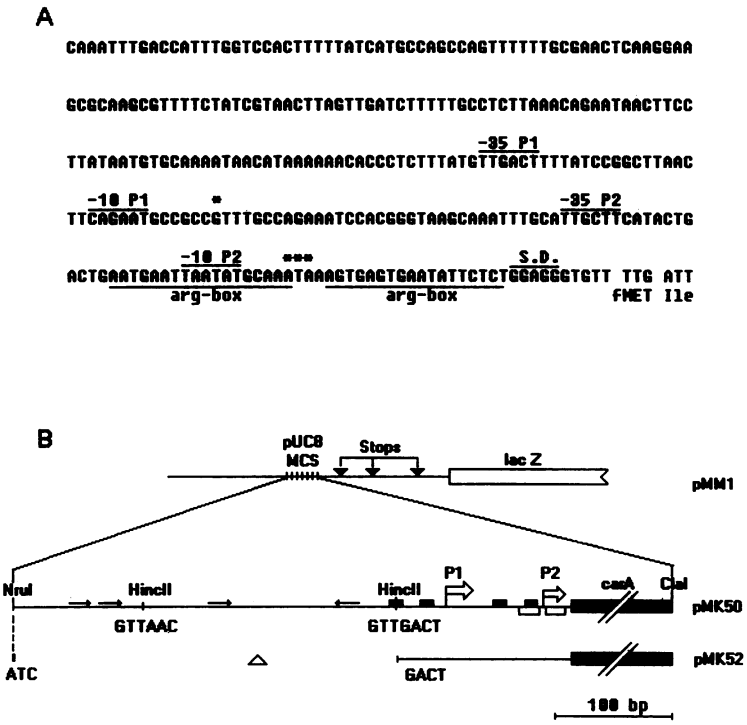


FIG. 2. (A) Nucleotide sequence of the *carAB* control region of *S. typhimurium* (21). The −10 and −35 regions for the pyrimidine-specific promoter (P1) and the arginine-specific promoter (P2) are overlined and labeled. Asterisks indicate the transcriptional initiation sites corresponding to the tandem promoters. The operator sites for the arginine repressor are underlined and labeled arg-box. The Shine-Dalgarno sequence is overlined and labeled S.D. (B) Structure of *carA*::*lacZ* operon fusion plasmids. The expression vector pMM1 (21) contains the multiple cloning sites (MCS) of pUC8 followed by a 96-bp leader with translational stops in all three reading frames. Translation of the *lacZ* gene is initiated at the *lacZ* ribosome-binding site. Open bars, *lacZ* structural gene; shaded bars, *carA* structural gene; open boxes, operator sites; solid boxes, promoter −35 and −10 regions; open arrows, mRNA start points; thin arrows, repeated sequences.

CTP pool, whereas the addition of cytidine increases the CTP pool about twofold without affecting the UTP pool significantly (20, 34).

Strain KD1109 was grown in the absence and presence of uridine, cytidine, or arginine, and quantitative primer extension experiments were carried out as described under Materials and Methods. The results (Table 1) show that exogenous uridine and exogenous arginine significantly repressed P1 and P2, respectively. In contrast, exogenous cytidine had no significant effect on either P1 or P2 expression. Dihydroorotase, assayed in the same cultures, was repressed by exogenous cytidine. Dihydroorotase has been shown previously to be primarily controlled by a cytidine nucleotide (20, 34) and can thus serve as an index of the intracellular CTP pool in the cultures used for primer extension and CPSase assay. Figure 4 shows the results of another experiment with KD1109 in which the X-ray film was exposed for 72 h rather than the 15 h required for the densitometric measurements. The photograph clearly shows that exogenous cytidine did not repress P1 activity.

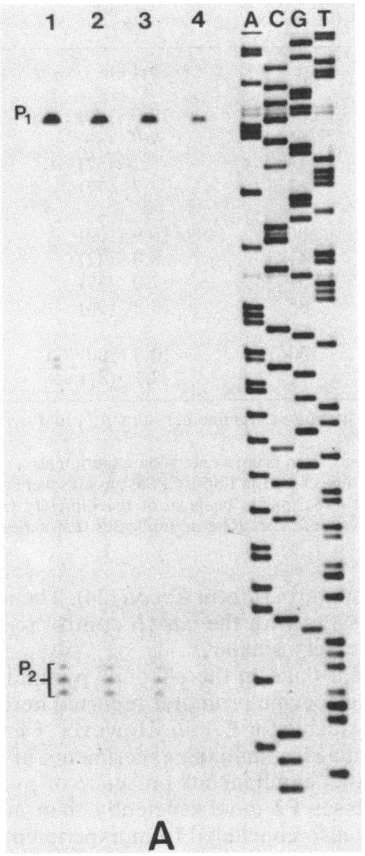
The absence of repression by exogenous cytidine in strain KD1109 (*cdd-4*) was reproducible. Similar results were obtained in primer extension experiments with strains KP1968 (*cdd-2*) and JL1278 (*cdd-7*).

Comparison of CPSase and dihydroorotase levels between LT2 and KD1109 (Table 1) showed higher levels for these enzymes in KD1109. A higher level of ornithine carbamoyltransferase in KD1109 relative to LT2 was also noted in an earlier report (20). The basis for the differences in the levels of the three enzymes between KD1109 and LT2 is not known. This variation in enzyme levels does not affect the conclusion about the lack of repression by exogenous cytidine, since this observation applies to a number of *cdd* derivatives regardless of the CPSase levels in cultures grown in glucose minimal medium.

Both P1 and P2 are derepressed upon cytidine limitation. The effect of cytidine limitation was examined in strain JL1280, which harbors a bradytrophic mutation in *pyrG* (CTP synthetase) as well as a *cdd* mutation (Fig. 1). When this strain was grown in the absence of exogenous cytidine, the CTP pool was reduced and the UTP pool was elevated (Table 2). The results of primer extension experiments are shown in Table 1 and Fig. 5 (the X-ray film was exposed for 15 h for densitometric measurements and 72 h for photography). Under conditions of cytidine limitation, in which derepressed synthesis of CPSase and dihydroorotase was observed, both P1 and P2 were derepressed, despite the elevated UTP pool (Table 1). Addition of exogenous uridine did not affect the low CTP pool but increased the UTP pool even further (Table 2). This growth condition, which resulted in repressed CPSase levels but high levels of dihydroorotase, caused repression of P1 and a slight lowering of the elevated P2 level (Table 1). These results were reproducible and establish that P2 is also controlled by pyrimidines. Consistent with this conclusion, cytidine starvation decreased arginine repression of P2 (Table 1).

Pyrimidine control of P1 is maintained in an *argR* background. The results of primer extension experiments with strain AA57 (*argR*::Tn*10*) are shown in Table 1. These results show that repression of P1 by pyrimidines does not require a functional *argR* product.

Transcriptional fusion experiments confirm the effect of



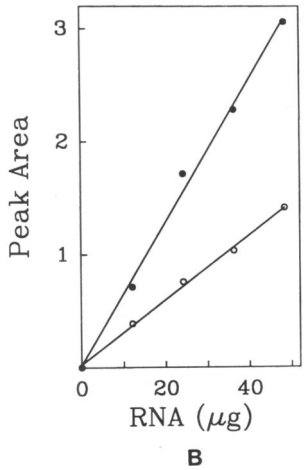


FIG. 3. Relationship between the amount of RNA used in primer extension experiments and the peak areas obtained from densitometric measurements. The RNA used was obtained from strain JL1280 grown in glucose minimal medium at 37°C. (A) Products of primer extension with different amounts of RNA. Lane 1, 48 μg; lane 2, 36 μg; lane 3, 24 μg; lane 4, 12 μg. The dideoxy ladder shown to the right of the autoradiogram was generated with the same primer used for extension. (B) Peak areas obtained from densitometric measurements of the autoradiogram plotted as a function of RNA concentration. The values indicated for P2 represent the sum of the peak areas for the three bands associated with this promoter. Symbols: ●, P1; ○, P2.

pyrimidine nucleotides on P2. We described earlier (21) the construction of *carA::lacZ* operon fusions on a low-copy-number plasmid. Figure 2B shows the structure of two *carA::lacZ* fusions used in this work. pMK50 contains a chromosomal fragment carrying the two promoters, the upstream region, and the first 340 bp of the structural gene encoding the small subunit. pMK52 carries a deletion of all sequences upstream of the P1 promoter, and the −35 region of P1 is changed from TTGACT to TCGACT, resulting in abolishment of P1 activity (21). The plasmid used is temperature sensitive for replication. Accordingly, expression of *carA* was studied by measuring the β-galactosidase levels in strain JL1280 at 30°C, at which the low copy number is maintained.

The results with pMK50 (Table 3) show that under conditions of cytidine limitation, transcription of *carAB* was significantly regulated with cytidine or uridine. In the case of pMK52, transcription from P2 was significantly regulated by cytidine and somewhat less by uridine. As expected, arginine resulted in strong repression of P2 activity in pMK52. However, fully repressed levels of P2 were only obtained in the presence of both arginine and cytidine.

The data in Table 3 also indicate that P1 was significantly more sensitive than P2 to the presence of both cytidine and uridine. Thus, in the case of pMK50 with arginine (i.e., P1), addition of cytidine reduced the specific activity by 3.6-fold (7,650 versus 2,120 U), whereas cytidine plus uridine reduced it 10-fold (7,650 versus 748 U). In contrast, with pMK52 (i.e., P2), cytidine reduced expression 2.5-fold (1,930 versus 782 U), and cytidine plus uridine reduced it only slightly more, i.e., 2.7-fold.

Comparison of transcript and CPSase levels. Examination of the data presented in Table 1 shows reasonable correspondence within each experiment between CPSase levels and the sum of P1 and P2 transcripts. These results indicate that the translational efficiencies of P1 and P2 mRNA do not differ significantly in *S. typhimurium*.

DISCUSSION

This article reports primer extension experiments with derivatives of *S. typhimurium* that are blocked in the conversion of exogenous cytidine to uridine (*cdd*; Fig. 1). In such strains, exogenous cytidine specifically increases the CTP pool without affecting the UTP pool, whereas exogenous uridine increases the UTP pool and to a lesser extent the CTP pool (20, 34). The results obtained with KD1109 (Table 1) establish that exogenous uridine and exogenous arginine repress P1 and P2, respectively. However, exogenous cytidine, which swells the intracellular CTP pool, has no effect on transcriptional initiation of *carAB* in the *cdd* derivative. Dihydroorotase, which has been shown to be controlled by a cytosine nucleotide (20, 34), was repressed in the same cultures under these conditions (Table 1).

The primer extension data with strain JL1280 (*pyrG cdd*) revealed that CTP limitation results in derepression not only of P1 but also of P2 (Table 1). The effect of pyrimidines on the activity of the two promoters was confirmed with the transcriptional fusions pMK50 and pMK52, of which pMK52 lacks P1 activity (Fig. 2B and Table 3). The effect of pyrimidines on P2 is surprising, since this promoter overlaps the two ARG boxes for *carAB* (Fig. 2A). The two ARG boxes are similar to the operator modules for the arginine regulon (11), and a recent report by Charlier et al. (7) has shown that the purified arginine repressor (24) protects the ARG boxes of the *E. coli carAB* operon against DNase I.

TABLE 1. Regulation of levels of P1 and P2 transcripts and CPSase

Expt no.	Strain (relevant genotype)	Addition(s) to medium[a]	Transcript level[b]		% of total transcripts (P1 + P2)	Sp act (nmol/min per mg of protein)	
			P1	P2		CPSase (%)[c]	Dihydroorotase
1	LT2Z (wild type)	None	1.8	0.5		5.1	50
2	KD1109 (*cdd*)	None	1.5	1.1	100	10.0 (100)	100
		CR	1.4	1.1	96	8.9 (89)	38
		UR	0.3	1.1	54	5.2 (52)	48
		Arg	1.3	0.3	61	7.7 (77)	100
3	JL1280 (*pyrG cdd*)	None	4.4	1.6	100	18.9 (100)	210
		CR	1.5	0.9	41	8.0 (42)	23
		UR	0.9	1.3	37	7.0 (37)	180
		Arg	4.4	0.7	85	18.2 (96)	220
4	AA57 (*argR*::Tn*10*)	Arg	1.8	3.24	100	10.8 (100)	37
		Arg, UR	0.4	3.20	71	8.7 (81)	17

[a] Cultures were grown at 37°C in glucose minimal medium (38) with the indicated additions: Arg, 100 μg of arginine per ml; CR, 1 mM cytidine; UR, 1 mM uridine.

[b] Transcript levels represent peak areas obtained by densitometric measurements of autoradiograms from primer extension experiments.

[c] Numbers in parentheses represent percent CPSase activity, with the level in the absence of pyrimidines taken as 100%. CPSase assays were done in duplicates that varied within 2%. However, it is less accurate to compare CPSase levels between different experiments because of the difficulty in making precise determination of the specific radioactivity of preparations of [^{14}C]bicarbonate. Dihydroorotase assays were also done in duplicates that varied less than 7%.

The nucleotide sequence of *argR* from *S. typhimurium* was recently determined (C. Lu and A. Abdelal, manuscript in preparation). The deduced amino acid sequence for the arginine repressor from *S. typhimurium* exhibits 95% homology with its counterpart from *E. coli* (24). The interactions of the two repressors with the *carAB* control regions are thus expected to be very similar.

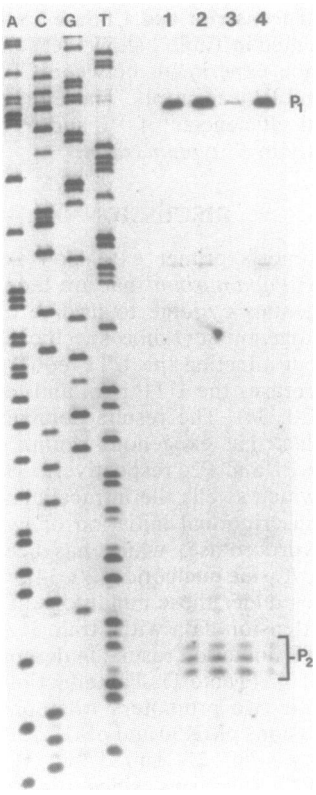


FIG. 4. Primer extension experiments with strain KD1109 (*cdd*). All reaction mixes contained 25 μg of RNA. Lane 1, No additions to glucose minimal medium; lane 2, 1 mM cytidine; lane 3, 1 mM uridine; lane 4, 100 μg of arginine per ml. The X-ray film was exposed for 72 h.

No equivalent data on the effect of pyrimidine limitation on the arginine-specific promoter reported here for *S. typhimurium* are available for *E. coli*. However, Charlier et al. (7) reported S1 nuclease mapping experiments in *E. coli* which indicate that the simultaneous presence of pyrimidines and arginine represses P2 more efficiently than arginine alone. These authors also concluded from experiments with *carA*::*lacZ* fusions that this intensification of arginine repression by pyrimidines of P2 expression does not occur when P1 is inactive (7). These results contrast with the data reported here (Table 3) that indicate the presence of pyrimidine control of P2 in a *carA*::*lacZ* fusion in which P1 is inactive. In fact, the results with this fusion also show intensification of arginine repression of P2 by pyrimidines. The differences between the results reported here and those reported for *E. coli* may reflect the use of a high-copy-number vector in the studies reported by Charlier et al. (7). We recently reported primer extension experiments with *S. typhimurium* (21), which indicate the absence of pyrimidine control of transcriptional initiation under high-copy conditions. Similarly, uracil repression was also reported to be absent in a high-copy-number *carA*::*lacZ* transcriptional fusion in *E. coli* by Bouvier et al. (5). The escape from pyrimidine control suggests the involvement of a titratable regulatory element in pyrimidine control of *carAB*. In such a case, the pyrimidine effect on P2 could reflect protein-protein interactions be-

TABLE 2. Nucleoside triphosphate pools for strain JL1280[a]

Addition to medium	Pool size (nmol/mg [dry wt] of cells)			
	ATP	GTP	CTP	UTP
None	8.9	4.2	0.4	8.3
Cytidine	5.1	2.4	2.3	1.5
Uridine	6.6	2.5	0.4	11.8

[a] Strain JL1280 (*pyrG cdd*) was grown at 37°C in low-phosphate minimal medium (16) with glucose as the carbon source. Cytidine or uridine was added at 1 mM as indicated.

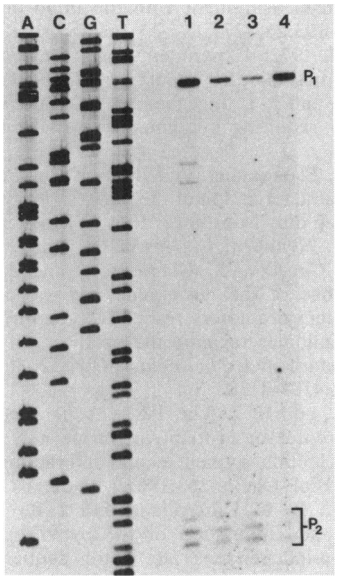


FIG. 5. Primer extension experiments with strain JL1280 (*pyrG cdd*). All reaction mixes contained 25 μg of RNA. Cultures were grown in glucose minimal medium at 37°C. Lane 1, No additions; lane 2, 1 mM cytidine; lane 3, 1 mM uridine; lane 4, 100 μg of arginine per ml. The X-ray film was exposed for 72 h.

tween the arginine repressor and a putative pyrimidine-regulatory protein. One candidate for a pyrimidine-regulatory protein is the product of the *use* gene (6). A mutation in this gene results in temperature-dependent sensitivity to uracil (6), an effect shown to be exerted at P1 of the *carAB* operon (21).

The results presented in Table 1 clearly show that repression of P2 by arginine does not interfere with elongation of transcripts initiated at P1, 69 nucleotides upstream of P2. These results contrast with those obtained with *lac* (14) and *aroP* (8), in which a repressor bound at a downstream site from the transcription initiation site was effective in preventing elongation by RNA polymerase.

Finally, it should be pointed out that nucleotide sequences resembling the consensus PUR box (31) are located in the *carAB* region; one (AAGCGCAAGCGTTTTCTA) is found approximately 100 bp upstream of P1, and the other is located 660 bp into the coding region of *carA* (L. M. Meng and M. Kilstrup, personal communication). The PUR box characterizes operator sequences for certain genes of purine biosynthesis that are regulated by the product of *purR* (31). The significance of the finding that sequences resembling the PUR box are present in the *carAB* operon is not clear. Purines have been previously shown to affect expression of the *pyr* genes in *S. typhimurium* (17, 18); however, their exact role in *carAB* regulation is not known.

TABLE 3. β-Galactosidase levels in JL1280 (*pyrG cdd*) harboring plasmid-borne *carA::lacZ* transcriptional fusion

Plasmid	Addition(s) to minimal medium[a]	β-Galactosidase sp act, nmol/min per mg of protein (%)[b]
pMK50	None	8,840 (100)
	CR	4,050 (46)
	UR	4,330 (49)
	CR, UR	1,560 (18)
	Arg	7,650 (87)
	Arg, CR	2,120 (24)
	Arg, UR	2,750 (31)
	Arg, CR, UR	748 (8)
pMK52	None	1,930 (100)
	CR	782 (41)
	UR	1,030 (54)
	CR, UR	720 (37)
	Arg	196 (10)
	Arg, CR	72 (4)
	Arg, UR	140 (7)
	Arg, CR, UR	80 (4)

[a] Cells were grown in AB medium (9) at 30°C. The following additions were made as indicated: Arg, arginine at 100 μg/ml; CR, 1 mM cytidine; UR, 1 mM uridine.

[b] Values were normalized relative to β-lactamase encoded by the plasmids. Protein was determined by the method of Lowry et al. (26). Numbers in parentheses indicate percentage of activity with no addition.

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