JOURNAL OF BACTERIOLOGY, Feb. 1992, p. 758–764
0021-9193/92/030758-07$02.00/0
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Vol. 174, No. 3

Influence of Cyclic AMP, Agmatine, and a Novel Protein Encoded by a Flanking Gene on *speB* (Agmatine Ureohydrolase) in *Escherichia coli*

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The *speB* gene of *Escherichia coli* encodes agmatine ureohydrolase (AUH), a putrescine biosynthetic enzyme. The *speB* gene is transcribed either from its own promoter or as a polycistronic message from the promoter of the *speA* gene encoding arginine decarboxylase. Two open reading frames (ORF1 and ORF2) are present on the strand complementary to *speB*; approximately 90% of ORF2 overlaps the *speB* coding region. Analysis of transcriptional and translational fusions of ORF1 or ORF2 to *lacZ* revealed that ORF1 encoded a novel protein while ORF2 was not transcribed. Deletion of ORF1 from a plasmid containing ORF1, ORF2, and *speB* reduced the activity of AUH by 83%. In contrast, the presence of plasmid-encoded ORF1 caused an 86% increase in chromosomally encoded AUH activity. ORF1 did not stimulate alkaline phosphatase expressed from a Φ(*speB-phoA*) transcriptional fusion encoded on the same plasmid. Western analysis (immunoblot) of a Φ(ORF1-*lacZ*) translational fusion revealed that ORF1 encodes a 25.3-kDa protein. Agmatine induced transcription of Φ(*speB-phoA*) but not Φ(*speA-phoA*) fusions. Consequently, agmatine affects selection between the monocistronic and the polycistronic modes of *speB* transcription. In contrast, cyclic AMP (cAMP) repressed AUH activity of chromosomally encoded AUH but had no effect on plasmid-borne *speB* nor Φ(*speB-phoA*). It is concluded that ORF1 encodes a protein which is a posttranscriptional regulator of *speB*, agmatine induces *speB* independent of *speA*, and cAMP regulates *speB* indirectly.

In *Escherichia coli* putrescine can be synthesized from arginine by decarboxylation of arginine to agmatine, catalyzed by arginine decarboxylase; agmatine is hydrolyzed to putrescine, catalyzed by agmatine ureohydrolase (AUH) (13). Arginine decarboxylase and AUH are encoded by the *speA* and *speB* genes, respectively, located at approximately minute 63.5 on the *E. coli* chromosome (1). We have sequenced the *speB* gene and characterized its pattern of transcription (12). The *speB* gene is encoded by one open reading frame (ORF); however, it can be transcribed as either a monocistronic or a polycistronic message. The *speB* promoter, initiating the monocistronic transcript, is unusual in that it does not require any sequence specificity upstream from the −10 Pribnow box for its activity. The polycistronic message is initiated from the promoter of *speA* residing immediately upstream from *speB* (12).

The source of the *speB* gene was plasmid pKA5, which contains a 7.5-kb *Eco*RI fragment of *E. coli* chromosome subcloned into pBR322 (2). It had been previously established that this *Eco*RI fragment contains the *speB*, *speA*, and *metK* genes, encoding AUH, arginine decarboxylase, and methionine adenosyltransferase, respectively. In the initial search for the *speB* gene on pKA5, a 3.0-kb fragment subcloned into pBR322 resulted in overproduction of AUH activity (12). A small deletion at one end of this fragment caused a significant decrease in AUH activity. After establishing the location of the *speB* gene within the 3.0-kb fragment, it became apparent that the deletion did not map within the *speB* gene. Rather, the deletion included the 5′ region of ORF1, the first of the two ORFs (ORF1 and ORF2) located on the strand complimentary to *speB*. These observations prompted us to investigate the nature of the relationship between ORF1, ORF2, and expression of *speB*. The data reported here indicate that ORF1 encodes a protein which stimulates expression of AUH at a posttranscriptional level.

Agmatine is a substrate of the reaction catalyzed by AUH. A number of *E. coli* strains either bearing a chromosomal copy of *speB* or transformed with pKA5 show increased AUH activity following agmatine supplementation (9). Our results show that agmatine is an inducer of the *speB*, but not of the *speA*, promoter.

The involvement of cyclic AMP (cAMP) in regulation of AUH in *E. coli* has been the subject of controversy. Halpern and Metzer (4) reported that cAMP supplementation of strain CS101B of *E. coli* K-12 induced the expression of AUH activity. In contrast, Satishchandran and Boyle (9) showed that cAMP supplementation of other *E. coli* K-12 strains reduced the activity of AUH. The later inhibition by cAMP required the cAMP receptor protein (CRP), as *crp* strains supplemented with cAMP did not exhibit reduced AUH activity. Satishchandran and Boyle (9) concluded that cAMP was either directly or indirectly involved in the transcriptional regulation of the *speB* gene. Our study was designed to distinguish between these two possibilities. In this work, we demonstrate that cAMP supplementation of wild-type *E. coli* results in a significant decrease in AUH activity. However, neither the activity of AUH encoded on a plasmid, either monocistronically or polycistronically, nor the activities of the *speB*, *speA*, or ORF1 promoters showed any response to cAMP. Thus, the effect of cAMP on *speB* expression is indirect.

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MATERIALS AND METHODS

Bacterial hosts, media, and growth conditions. In all experiments involving vector pCB267 and its derivatives, the host was *E. coli* CB806 (*lacZ galK phoA8 rpsL thi recA56*) (9). The fusion protein encoded on plasmid pX28ILΔP was expressed in *E. coli* JM109 (DE3) (*endA1 recA1 gyrA96 thi hsdR17 relA1 supE44 Δlac-proAB*) [F' *traD36 proAB lacI*^{q} ZΔM15] λ(DE3) (Promega Corp., Madison, Wis.). *E. coli* DH5α (F$^-$ *endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1* γ80 *lacZ* ΔM15) (Bethesda Research Laboratories) was used as a host for other plasmids. Wild-type *E. coli* K-12 was obtained from H. Yamasaki, Carleton University, Ottawa, Canada.

The bacteria were grown at 37°C with shaking; MOPS (morpholinepropanesulfonic acid) minimal medium (8) with 0.2% glucose was used when the effect of either agmatine or cAMP was measured. In other experiments the bacteria were grown in Luria-Bertani (LB) broth (7). When *E. coli* DH5α and its derivatives were grown in MOPS minimal medium, 25 μg of amino acids (except arginine) per ml and 1 mM thiamine were included. For all *lacZ* promoter assays, IPTG (isopropyl-β-D-thiogalactoside) was added to the media to a final concentration of 1 mM. Where indicated, cAMP or agmatine sulfate (Sigma Chemical Co.) were used at 5 and 10 mM final concentrations, respectively.

Vectors. Plasmid pBR322 was purchased from Bethesda Research Laboratories, Inc., plasmid pCB267 (11) was obtained from T. Larson, Virginia Polytechnical Institute and State University, Blacksburg. Plasmids pGEM-3Z and pGEMEX-2 were purchased from Promega Corp.

AUH assay. Urea production was measured by using Sigma Diagnostics Urea Nitrogen Determination Procedure no. 640 as described previously (12). Crude extract of the wild-type *E. coli* K-12 was prepared from a 200-ml culture grown to 90 Klett units; the final volume of the crude extract was 1.0 ml. The activity of chromosomally encoded AUH in cells transformed with plasmids was measured in crude extracts prepared from cells disrupted in a French pressure cell (15,000 lb/in^2, 4°C).

Alkaline phosphatase and β-galactosidase assays. Colormetric assays utilizing *p*-nitrophenylphosphate or *o*-nitrophenyl-β-D-galactoside were performed as described previously (11).

Construction of the recombinant plasmids. Construction of the deletion plasmids pKB1, pKB2, pKB2S, pKB2H, pBB15N, and pCO3B15P was described previously (12). The transcriptional fusion plasmids pCO1L, pCO2O1L, pCO2L, pCO12L, pCO3P, pCO3PO1, pCO4P, pCLL, and pCLP are derivatives of the vector pCB267. The promoterless *lacZ* and *phoA* indicator genes in pCB267 flank the multiple cloning site. A promoter fragment inserted into the multiple cloning site will thus activate one of the indicator genes in a transcriptional fusion (11).

The DNA inserts in all these plasmids except pCLL and pCLP are derived from pKA5; their original location is represented in Fig. 1 and 6. Generation of plasmid pCO3PO1 is shown schematically in Fig. 2. This plasmid was generated by substituting the 1.93-kb *SacI-BglII* fragment of *lacZ* in plasmid pCO3P [containing Φ(*speB-phoA*)] with the *SmaI-BglII* fragment (containing ORF1) derived from pKA5 (see Fig. 2).

A fragment containing the promoter of ORF4 (*speA*) (see Fig. 6) was ligated into pCB267 to produce pCO4P. In this plasmid the *speA* promoter activates the expression of the *phoA* gene.

A 0.39-kb *PvuII* fragment of plasmid pGEM-3Z containing the promoter, operator, and CRP-binding site of *lacZ*, as well as the first 120 bp of the structural *lacZ* sequence, was ligated into pCB267 in both orientations, creating the plasmids pCLL and pCLP.

The names of these plasmids reflect the orientation of their inserts with respect to the orientation of either the *phoA* or the *lacZ* promoterless structural gene: "O" in the name designates the ORF in the insert, while "L" or "P" designates the indicator gene (*lacZ* or *phoA*, respectively) codirectional with the ORF. Thus, O1L in the name indicates that ORF1 is in the same orientation as the *lacZ* structural gene.

Plasmid pKB01 was created by subcloning of the *SmaI-BamHI* fragment, containing ORF1 (see Fig. 6), into pBR322. Generation of pBORF1LΔP was initiated by insertion of a 1.09-kb *EcoRI-PvuI* fragment, derived from plasmid pKG1 (12), between the *EcoRI* and *PvuI* restriction sites of the vector pMC1403 (3). The 1.09-kb *EcoRI-PvuI* fragment contains the region upstream from ORF1, starting with the *SmaI* site pictured in Fig. 1 and 6 (the *EcoRI* end is derived from multiple cloning site of the source plasmid, pKG1, and maps immediately upstream from the *SmaI* site) down to the *PvuI* site, residing within the ninth codon upstream from the C terminus of ORF1. The pMC1403 vector contains, downstream from the *EcoRI* site, a 6.2-kb fragment of the *lac* operon; the *PvuI* site maps 425 codons upstream from the C terminus of *lacZ*. Thus, the insertion produced an in-frame fusion between ORF1 and the 425 C-terminal codons of *lacZ*. The *EcoRI-SmaI* fragment containing this fusion was subcloned between the *EcoRI* and *PstI* site of pBR322, producing plasmid pBORF1LΔP.

Plasmids pBWLΔP(T) and pX28ILΔP (a gift from T. Caceci, Virginia Polytechnical Institute and State University) contain the same fragment of *lacZ* as pBORF1LΔP with an additional sequence (codons 8 through 45 of *lacZ*) present upstream from the 425-codon C terminus. In pBWLΔP(T) a promoterless synthetic ORF, encoding 103 amino acids, is fused in frame to the *lacZ* sequences. The location of this fusion within the pBR322 is the same as in pBORF1LΔP. In construction of plasmid pX28ILΔP the synthetic ORF::*lacZ* fusion from plasmid pBWLΔP(T) was inserted downstream from a promoter and 25 N-terminal codons of the T7 bacteriophage gene 10 in the vector pGEMEX-2. The resulting fusion (T7 gene 10:synthetic ORF::*lacZ*) gene encodes 591 amino acids.

DNA sequencing and analysis. The strategies and procedures used in sequencing of the *SmaI-BamHI* insert of the plasmid pKB2S (see Fig. 5) have been described previously (12). Sequence analysis was performed utilizing the University of Wisconsin Genetics Computer Group software; the Wordsearch program was used to search both the GenBank and NFBR data bases for sequences homologous to ORF1 and ORF2.

Expression, electrotransfer, and immunodetection of fusion proteins. *E. coli* DH5α(pBORF1LΔP) and DH5α[pBWLΔP(T)] were grown at 37°C to a density of 90 Klett units in LB media containing 25 μg of tetracycline per ml. The cells were pelleted at 15,000 × *g* for 5 min at 4°C. *E. coli* JM109(DE3)(pX28ILΔP) was grown as described above (except in LB containing 100 μg of ampicillin per ml) to a density of 100 Klett units. The cells were pelleted (2,500 × *g*, 15 min, room temperature), resuspended in fresh LB media containing 200 μg of ampicillin per ml and 0.5 mM IPTG, grown for an additional 2 h a 37°C, and harvested as described above.

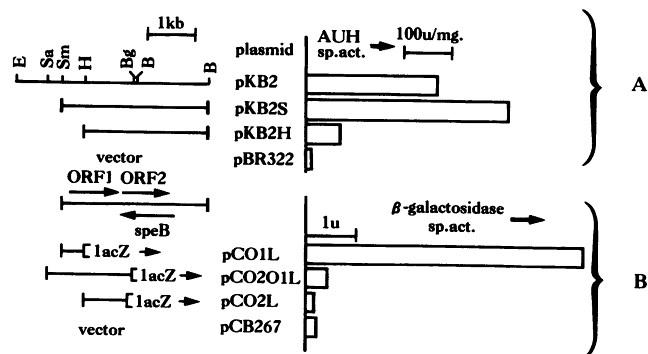

FIG. 1. Promoter activity and influence of deletions in sequences flanking *speB*. The horizontal lines at the left specify the inserts in the named plasmids. Projection of these lines on the restriction map (top of A) or on the structural map (between A and B) specifies their sizes and origins relative to ORFs. (A) Ability of strains containing the specified deletion plasmids to express AUH activity; 1 U of AUH activity = 1 nmol of urea per min. (B) Ability of DNA fragments derived from pKB2 to activate transcription (in the direction indicated by the arrows) of the promoterless *lacZ* gene in the vector pCB267. All cultures were grown in LB medium. 1 U of β-galactosidase activity = optical density at 410 nm × 10^{-2}/min/Klett unit/ml. E, *Eco*RI; H, *Hin*dIII; Sa, *Sal*I; Sm, *Sma*I; Bg, *Bgl*I; B, *Bam*HI.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out in 7.5% acrylamide as described by Laemmli (5). After electrophoresis the proteins were electrotransferred to a nitrocellulose membrane (0.22 μm; MSI, Westboro, Mass.) for 2 h at 100 V in 25 mM Tris–192 mM glycine–20% (vol/vol) methanol, pH 8.3. Immunodetection was performed with rabbit anti-β-galactosidase- and anti-rabbit horseradish peroxidase-conjugated antibodies and 4-chloro-1-naphthol.

Nucleotide sequence accession number. The sequence data reported in this paper are located in the GenBank data base, accession number M32363.

RESULTS

Influence of neighboring sequences on expression of the *speB* gene. Our initial search for the *speB* gene within pKA5 showed that deletion of a 460-bp *Sma*I-*Hin*dIII fragment 3′ to *speB* reduced AUH expression 83% (Fig. 1A). The deleted fragment included the upstream sequences and the 5′ end of ORF1. Our initial hypothesis was that either ORF1 or ORF2 (as the result of read-through from ORF1) encoded a protein necessary for activation of the *speB* transcription.

To establish whether either of the two ORFs represented an actively transcribed gene, we searched for the presence of a functional promoter upstream from ORF1. We constructed a transcriptional fusion between the 460-bp *Sma*I-*Hin*dIII fragment and the promoterless *lacZ* gene in the vector pCB267. Strains bearing this fusion (pCO1L) exhibited a 29-fold increase in β-galactosidase activity (Fig. 1B). Plasmids were constructed to establish the presence of an independent promoter for ORF2 (pCO2L) or a read-through transcription from ORF1 into ORF2 (pCO201L), respectively. The results indicated that ORF2 does not have its own promoter and that a transcriptional terminator must be present upstream from the *Bgl*II site (Fig. 1B), preventing a read-through from the ORF1 promoter into the ORF2. To establish whether ORF1 encodes a transcriptional regulator

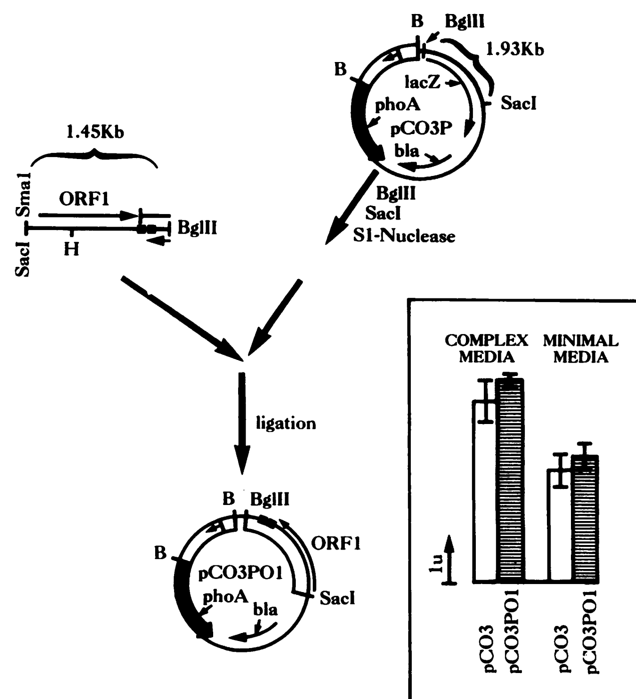

FIG. 2. **Construction of plasmid pCO3PO1.** The two black boxes downstream from ORF1 in the *Sac*I-*Bgl*II insert depict the position of the two long tandem repeats (see Fig. 5) containing the *speB* terminator and three other palindromes. B, *Bam*HI; H, *Hin*dIII. (Insert) Alkaline phosphatase activity of extracts from *E. coli* CB806 transformed with either pCO3P or pCO3PO1 and cultured in complex or minimal medium. 1 U of activity = optical density at 410 nm = 10^{-3}/min/Klett unit/ml.

of the *speB* expression, we inserted the *Sma*I-*Bgl*II fragment of pKA5 (Fig. 2), containing ORF1 and flanking sequences, into pCO3P, which contained a Φ(*speB-phoA′*) fusion, creating plasmid pCO3PO1 (Fig. 2). If ORF1 encoded an inducer of the *speB* promoter, alkaline phosphatase activity in the cells transformed with pCO3PO1 should be significantly higher than in cells transformed with pCO3P. As shown in Fig. 2, alkaline phosphatase activity did not change in presence of the ORF1 gene in *E. coli* cultured either in enriched or in defined medium. To assess whether ORF1 encodes a *trans*-acting factor, we compared AUH activity in extracts derived from *E. coli* transformed with either pBR322 or pKBO1 (ORF1 only). The activity of chromosomally encoded AUH increased 86% in extracts of *E. coli*

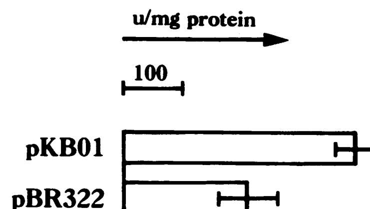

FIG. 3. Activity of chromosomally encoded AUH as a function of the presence (pKB01) or absence (pBR322) of plasmid-encoded ORF1. The bars indicate specific activities of chromosomally encoded AUH measured in the extracts of *E. coli* DH5α(pKB01) or *E. coli* DH5α(pBR322). One unit of AUH activity is defined as in the legend to Fig. 1A.

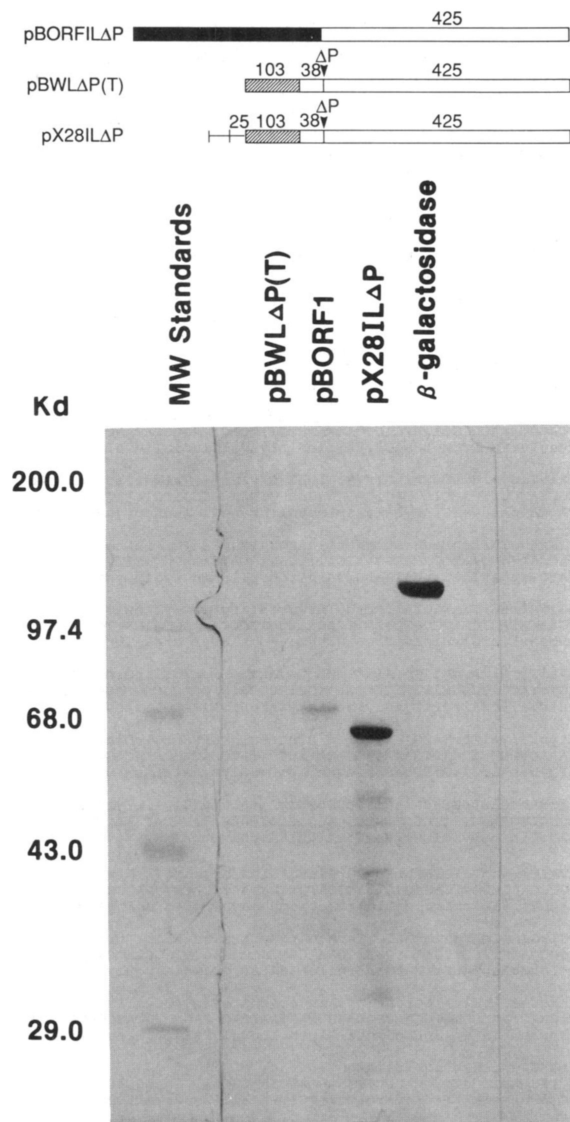

FIG. 4. Western blot of the extracts of *E. coli* DH5α[pBWLΔP (T)], *E. coli* DH5α(pBORF1LΔP), and *E. coli* JM109(DES) (pX28ILΔP). The extracts in each lane represent the equivalent of 30 Klett units/ml of culture. The top diagram (drawn to scale) depicts the relevant inserts present in the plasmids. The empty boxed region represents the fusion fragment derived from *lacZ'*; the black box represents the fragment between nucleotides 1 and 1092 of the sequence shown in Fig. 5 (containing the ORF1 upstream region); the hatched box represents a synthetic ORF; the thin line represents the promoter and upstream coding sequence of bacteriophage T7's gene 10. The arrowheads (ΔP) depict an internal in-frame deletion in the *lacZ* portion of the fusions. The numbers specify the number of codons present in the fragments beneath them.

(pKBO1) (Fig. 3), suggesting that a factor was encoded by ORF1 which acts in *trans* to stimulate AUH activity.

To establish whether ORF1 encodes a protein, we constructed a translational fusion in plasmid pBORF1LΔP. In this plasmid, the 9th codon upstream from the C terminus of ORF1 is fused in frame to the 425 C-terminal codons of *lacZ*. When extracts of *E. coli* transformed with the pBORF1LΔP were resolved by electrophoresis and analyzed by Western blotting (immunoblotting) (Fig. 4), a 68.5-kDa protein appeared. The fusion protein encoded by the control plasmid pX28ILΔP appeared at position corresponding to 63 kDa. This protein encodes 166 amino acids more than the *lacZ* portion of the ORF1::*lacZ* fusion encoded by pBORF1LΔP. Assuming 114 Da per amino acid (the average mass per amino acid in β-galactosidase), we calculated that ORF1 is translated into a protein about 222 amino acids long. This places the tentative start of translation of the ORF1-encoded protein at approximately position 451 of the sequence shown in Fig. 5, which maps 7 nucleotides upstream from the *Hin*dIII site (shown in Fig. 1).

Influence of agmatine and cAMP on promoter activities of *speB*, *speA*, and ORF1. The activities of alkaline phosphatase or β-galactosidase encoded by transcriptional fusions were assayed in extracts from *E. coli* grown in defined medium supplemented with cAMP or agmatine sulfate (Fig. 6). All three *E. coli* strains bearing the *speB* promoter (plasmids pCO3B15P, pCO3P, and pCO3PO1) exhibited greater than 100% induction of the reporter genes by agmatine. Plasmid pCO3B15P, which contains only the minimal upstream sequence essential for the *speB* promoter activity (deleted for all sequences upstream from position −11 [12]) was fully induced with agmatine. Thus, the sequences necessary for induction of *speB* by agmatine must be located downstream of position −11 in the *speB* gene.

The presence of ORF1 did not enhance the induction of alkaline phosphatase by agmatine in *E. coli* transformed with pCO3PO1 carrying both ORF1 and Φ(*speB-phoA*); nor was transcription from the ORF1 promoter itself (pCO1L and pCO12L) affected by either agmatine or cAMP. Neither the *speB* nor the *speA* (pCO4P) fusions were affected by cAMP; in contrast, the control transcriptional fusions of the *lacZ* promoter (pCLL and pCLP) were induced by cAMP (Fig. 6). Thus, cAMP-CRP is not the factor limiting the response of the assayed promoters.

Influence of agmatine and cAMP on AUH expression. AUH activities were measured in extracts from *E. coli* transformed with either pKA5 or pBB15N or from wild-type *E. coli* K-12 grown with or without cAMP or agmatine (Fig. 7). The AUH activity encoded by pBB15N (*speB*) was induced 100% by agmatine. cAMP drastically reduced the expression of chromosomally encoded AUH in wild-type *E. coli*. However, cAMP did not cause any decrease in the activity of plasmid-encoded AUH whether expressed monocistronically (pBB15N) or as part of the *speAB* operon (pKA5). *E. coli* DH5α transformed with pGEM-3Z was used as a positive control for cAMP response. The chromosomal copy of the *lacZ* operon in *E. coli* DH5α is deleted for the codons 11 to 41 of the *lacZ* gene. It produces an inactive β-galactosidase, whose activity can be restored by complementation with the α peptide of β-galactosidase encoded by plasmid pGEM-3Z. As shown in Fig. 7, the β-galactosidase level in this control strain increased by 575% as result of cAMP supplementation of the growth media.

DISCUSSION

We previously reported the presence of two ORFs, ORF1 and ORF2, on the strand opposite the *speB* coding strand (12). In the present study a promoter activity was identified in sequences upstream from ORF1. Although ORF2 is separated from ORF1 by only 31 nucleotides, transcription initiated at the ORF1 promoter did not read through ORF2. Since no promoter activity was detected immediately upstream from ORF2, it is either a pseudogene or it represents a gene that is not transcribed under the conditions used. A search of the GenBank and NBRF data bases revealed that

FIG. 5. Nucleotide sequence encoding ORF1, ORF2, *speB*, and flanking regions in pKB2S. The amino acid sequences of ORF1 (130 to 1116) and ORF2 (1151 to 2185) are given above the nucleotide sequence; the amino acid sequence of AUH as well as the last codons of *speA* is given below the sequence. Long thin arrows above the sequences indicate the palindromes present within the underlined sequence of the two long tandem repeats. The palindrome under the thick arrow represents the *speB* terminator; the *speB* promoter and Shine-Dalgarno sequence are boxed. Asterisks designate the centers of symmetry in the palindromes.

the 5′ end of ORF2 has significant regions of homology (about 150 of 170 bp) to the 5′ ends of the *gltA* and *sucB* genes encoding citrate synthase and dihydrolipoyltranssuccinase components of α-ketoglutarate dehydrogenase, respectively (1). The significance of this homology is not clear.

We established that ORF1 is translated in vivo, producing a protein of approximately 222 amino acids. A computer search of the GenBank and NBRF data bases revealed that ORF1 contained no significant regions of homology to any reported nucleotide or amino acid sequences. The N terminus of the ORF1 protein, as tentatively deduced from the size of the ORF1::*lacZ* fusion, maps at position 451 of the sequenced DNA (Fig. 5) and is located in close proximity to two Shine-Dalgarno-like sequences, AGGAG, at positions 450 and 521. Neither of these consensus sequences is followed at a plausible distance (e.g., 4 to 9 bp) by an ATG codon.

Two observations indicate that the ORF1 gene product is involved in positive regulation of the AUH expression: deletion of the ORF1 promoter region from a plasmid encoding both ORF1 and AUH resulted in an 83% loss of AUH activity, and the presence of plasmid-encoded ORF1 caused an 86% increase in the activity of chromosomally encoded AUH. This effect is not due to direct transcriptional control, as alkaline phosphatase activity was unaffected when both the Φ(*speB-phoA*) fusion and ORF1 were present on the same plasmid. It is not certain at this point whether the ORF1-encoded protein represents the regulatory factor; our experiments do not exclude the possibility of a small regulatory RNA, coencoded on ORF1, being responsible for its effect on the AUH activity. It is thus of interest to establish the exact nature of the regulatory gene product as well as the mechanisms involved in the regulation. It was found that the ORF1 promoter is strongly induced by high temperatures; the promoter activity ratio at 42 versus 28°C was approximately 25 (6). In light of this and our own

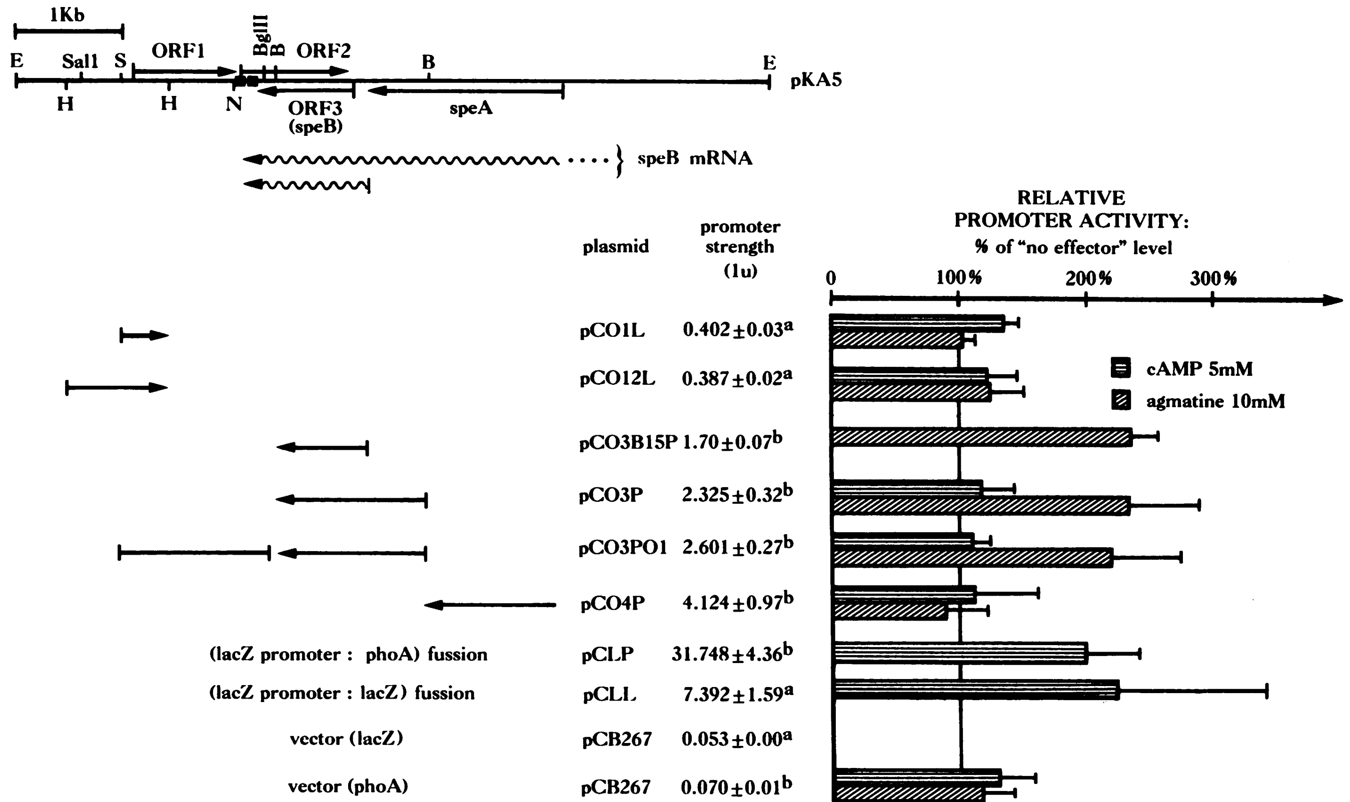

FIG. 6. Influence of cAMP or agmatine on the promoter activities of *speB*, *speA*, and ORF1. (Top) Structural map (with relevant restriction sites indicated) of the 7.5-kb pKA5 insert and the locations of sequences included in the *speB* transcripts (wavy lines). E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; N, *Nru*I; S, *Sma*I. (Bottom) The horizontal lines specify the inserts in the named plasmids and the direction (arrows) in which promoter activity was measured. Projection of the lines on the pKA5 map specifies the fragments' original locations. The promoter strength column lists the specific activities resulting from the indicator gene expression (*phoA* or *lacZ*) when no effectors were included in the growth medium. One unit is defined as in the legend to Fig. 2. Superscript indicate data from three or more β-galactosidase (a) or alkaline phosphatase (b) assays.

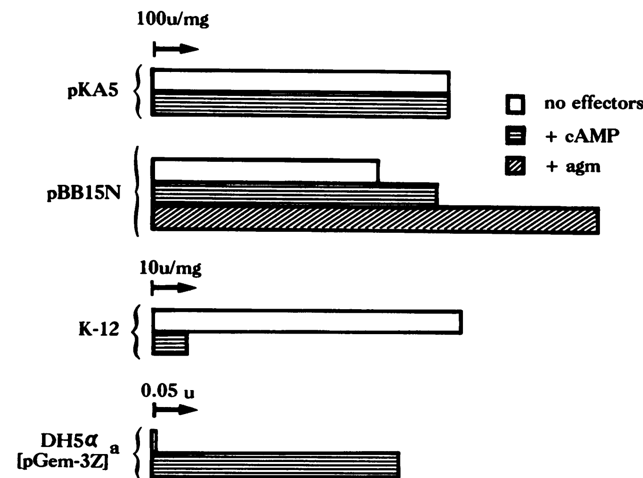

FIG. 7. Specific activities of AUH in crude extracts of *E. coli* supplemented with cAMP or agmatine. The strains are *E. coli* DH5α(pKA5), *E. coli* DH5α(pBB15N), *E. coli* HY1, and *E. coli* DH5α(pGEM-3Z). Data are from three experiments. One unit of AUH activity is defined as in the legend to Fig. 1A. One unit of β-galactosidase-specific activity is defined as in the legend to Fig. 2.

results, it appears that the stimulatory effect of the ORF1 gene product on AUH activity represents a novel aspect of *speB* regulation.

Satishchandran and Boyle (9) showed that cAMP supplementation inhibited the expression of AUH activity in *E. coli* strains able to produce CRP. They also demonstrated that agmatine stimulated expression of either chromosomally or plasmid-encoded AUH. Our earlier observation that *speB* can be expressed polycistronically from the promoter of *speA* (12) combined with the data presented here demonstrates that both ORF1 and *speA* (12) influence expression of *speB*. Thus, it was of interest to learn whether the promoter of *speB*, ORF1, or *speA* was responding directly to cAMP or agmatine. Our results showed that agmatine induced the activity of the *speB* promoter but did not affect the promoter of either *speA* or ORF1. Consequently, agmatine selectively induced the monocistronic transcription of *speB* and must be part of a switch mechanism involved in selection of the polycistronic versus monocistronic transcription of *speB* (12).

Although the chromosomally encoded AUH was significantly inhibited by cAMP, neither the activities of the *speB*, *speA*, and ORF1 promoters nor the AUH activity encoded by pKA5 (*speB*, ORF1, *speA*, and *metK*) or pBB15N (*speB*) was affected. These results demonstrate that the expression of *speB* is not regulated through direct interaction between the cAMP-CRP complex and the promoters of either *speB* or

speA. However, the effect of cAMP on the chromosomally encoded AUH in wild-type *E. coli* K12 indicates that a cAMP-regulated factor must be involved. This factor is either strain specific or easily titrated out when the *speB* gene is encoded on a multicopy plasmid. Both assumptions require that the regulatory factor be encoded by a region of the chromosome not present in pKA5 and that its expression in the cell or its affinity for the target (DNA, RNA, or protein) be low. A possible inhibitory protein was identified during the purification of AUH by Satishchandran and Boyle (9). They demonstrated that a chromatofocused protein fraction with a pI of approximately 5.0 caused inhibition of AUH. This inhibitory activity could be eliminated by heat or proteinase K treatment. It would be of interest to determine whether a chromatofocused extract from a *cya crp E. coli* strain still produces this inhibitory effect. In another study, Satishchandran and Boyle showed that maximum repression of AUH was observed 2.5 generations following the addition of cAMP to *E. coli* (10). This would be consistent with the cAMP regulatory effect involving synthesis of a small amount of an inhibitory protein.

ACKNOWLEDGMENTS

We thank Timothy Larson for helpful discussion, Bob Moore for the promoter of the *speA* gene, Sandra Peters for performing the Western blot, and T. Caceci for the plasmids pBWLΔP(T) and pX28ILΔP.

This work was supported by a grant (DMB-8508917) from the National Science Foundation (to S.M.B.) and a graduate fellowship from the Department of Biochemistry and Nutrition (to M.B.W.S.).

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