JOURNAL OF BACTERIOLOGY, May 2003, p. 3139–3146 Vol. 185, No. 10
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.10.3139–3146.2003
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Transcription Regulation Coupling of the Divergent *argG* and *metY* Promoters in *Escherichia coli* K-12

Evelyne Krin,[1]* Christine Laurent-Winter,[2] Philippe N. Bertin,[1]† Antoine Danchin,[1] and Annie Kolb[3]

Unité de Génétique des Génomes Bactériens,[1] Génopole-Plateau Protéomique,[2] and Laboratoire des Régulations Transcriptionnelles,[3] Institut Pasteur, 75724 Paris Cedex 15, France

Received 17 December 2002/Accepted 3 March 2003

The cAMP-catabolite activator protein (CAP) complex is a pleiotropic regulator that regulates a vast number of *Escherichia coli* genes, including those involved in carbon metabolism. We identified two new targets of this complex: *argG*, which encodes the arginosuccinate synthase involved in the arginine biosynthetic pathway, and *metY*, which encodes one of the two methionine tRNA initiators, $tRNA^{Met}_{f2}$. The cAMP-CAP complex activates *argG* transcription and inhibits *metY* transcription from the same DNA position. We also show that ArgR, the specific repressor of the arginine biosynthetic pathway, together with its arginine cofactor, acts on the regulation of *metY* mediated by CAP. The regulation of the two divergent promoters is thus simultaneously controlled not only by the cAMP-CAP complex, a global regulator, but also by a specific regulator of arginine metabolism, suggesting a previously unsuspected link between carbon metabolism and translation initiation.

All forms of life degrade carbon-containing molecules. The cAMP-catabolite activator protein (CAP) complex is a global regulator involved in the regulation, repression as well as activation, of a vast number of *Escherichia coli* genes. Initially, its role was solely thought to control of the use of alternative carbon sources when glucose was lacking. Indeed, the largest group of targets controls the catabolism of carbohydrates, amino acids, and nucleosides. However, it is now clear that CAP also controls the expression of genes involved in many noncatabolic functions, including genes encoding membrane proteins, involved in metabolic transport (e.g., *proP* [39]), in carbon starvation, and in resistance to stress (e.g., *gadA* [8]). The absence of an obvious link between these various targets suggests that CAP controls the expression of genes involved in adaptation to growth conditions under limited nutrient supply. The promoters of these genes are usually regulated by multiple factors, and CAP tends either to be involved in coactivation together with a second activator or to act in tandem with a repressor (for example, at the *lac* promoter [33]). CAP seems therefore to sense a global signal (e.g., glucose starvation as reflected by the intracellular cAMP concentration), whereas the specific regulator monitors the level of a specific metabolite that may or may not be present.

In the present work, the two-dimensional protein pattern of a CAP-deficient strain revealed an alteration in the level of ArgG, the arginosuccinate synthase involved in the arginine biosynthetic pathway. The study of the DNA promoter region of this gene showed the presence of the divergent promoter of *metY*, which encodes one of the two methionine tRNAs required for the initiation of protein synthesis. Since *argG* is known to be regulated by a specific repressor, ArgR, we studied the direct effect of CAP and ArgR, in vivo and in vitro, on the transcription of *argG* and *metY*. Finally, we demonstrated the coupling between the arginine biosynthetic pathway and a gene responsible for the initiation of protein synthesis.

* Corresponding author. Mailing address: Institut Pasteur, Unité de Génétique des Génomes Bactériens, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: (33) 01 40 61 35 56. Fax: (33) 01 45 68 89 48. E-mail: ekrin@pasteur.fr.

† Present address: Laboratoire de Dynamique, Evolution et Expression de Génomes de Microorganismes, FRE 2326 ULP/CNRS, 67083 Strasbourg Cedex, France.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The *E. coli* K-12 derivatives and plasmids used in this work are listed in Table 1. The *crp* gene was mutated by inserting an Sm^r/Spc^r cassette into the *Bcl*I site of the *crp* gene, yielding *crp*::Sm. This mutation was introduced into strain MG1655 by allelic replacement using the M13mp8 phage, giving rise to strain to BE1815 (5). The *metZΔ2*::*cat* mutation carried by strain IBPC 6411 (transduced from strain TK2 [21] and kindly provided by M. Springer) was introduced into MG1655 by transduction, giving rise to strain BE1623. Plasmid pDIA530 was constructed as follows: the fragment containing positions −417 to +90 of the *argG* promoter according to reference 9 was PCR amplified from genomic DNA using the Expand high-fidelity PCR system (Roche) and synthetic oligonucleotides 5′-CA GAAGGATCCTTTCAAATCCC-3′, containing a *Bam*HI site, and 5′-GGAGA AGCTTGAGAATCGTCGTC-3′, containing a *Hin*dIII site. After purification, the fragment was cloned into the *Bam*HI and *Hin*dIII restriction sites of PKK232-8 (Pharmacia). Plasmid pDIA560 was similar to pDIA530 except that the CAP binding site was mutated by PCR amplification with the synthetic oligonucleotide 5′AATCTGCAGGCATTATAGTAATCCACGCTCGATTTT GTCAACGTTTATTGC-3′. Plasmid pDIA539 contained the 268-bp *argG* promoter (see below), cloned into the *Hin*cII blunted site of pJCD01 (24). Strains were grown at 37°C in M9 minimal medium (26) supplemented with mannose (0.4%), thiamine (5 mg/ml), a mixture of all amino acids with or without arginine (0.005% of each), and when necessary 100 μg of ampicillin/ml. All experiments were performed in accordance with the European regulation requirements concerning the contained use of genetically modified organisms of group I (agreement no. 2735).

Two-dimensional gel electrophoresis. Ten micrograms of total proteins, extracted from a culture at an optical density at 600 nm (OD_{600}) of 0.4 to 0.5, was resolved on a two-dimensional (2D) gel (23). Polypeptides were detected by silver staining (29). Proteins were quantified as previously described (4). Spots of interest were excised from multiple gels and subjected to internal amino acid (37). The amino acid sequence homology search was carried out using the BLASTP software (2).

Quantitative analysis of mRNA. RNA was prepared, subjected to a slot blot, and quantified as previously described (36). The experiment was performed using two independent cultures with a probe corresponding to a 700-bp fragment of *argG*, generated by PCR amplification using the PCR DIG probe synthesis kit (Roche). Quantitation was made with the Bio-Rad Multi-Analyst system.

Gel mobility shift DNA binding assay. A 268-bp fragment corresponding to the promoter region of *argG* (−212 to +56) according to reference 9 was

TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Genotype	Source or reference
Strains		
MG1655	Prototrophic *E. coli* K-12	CGSC 6300[a]
BE1815	MG1655 *crp*::Sm	This study
IBPC 6411	*thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44 rpsL pps metZ*Δ*2*::*cat*	M. Springer
BE1723	MG1655 *metZ*Δ*2*::*catZ*	This study
Plasmids		
pJCD01	PUC19 derivative containing a polylinker flanked by the divergent terminators *rpoCt* and *rrnBT1T2*	24
pKK232-8	Cloning vector for promoter analysis	Pharmacia
pDIA530	pKK232-8 derivative carrying the −417 to +90 *argG* promoter region	This study
pDIA539	pJCD01 derivative carrying the −212 to +56 *argG* promoter region	This study
pDIA560	As pDIA530 but with a mutated CAP site: AGTGAtccacgCCACA→ AGTAAtccacgCTCGA[b]	This study

[a] CGSC, *E. coli* Genetic Stock Center, Yale University.
[b] Underlining indicates mutated residues.

amplified from genomic DNA by PCR using *Pfu* DNA polymerase (Stratagene) and synthetic oligonucleotides 5′-GTGTACCGAGACGGGACG-3′ and 5′-TTAACTGATGATGAGCCTGG-3′ (one of which was labeled with [γ-^{32}P]ATP by use of T4 polynucleotide kinase). The PCR product was purified with the High pure PCR product purification kit (Roche). Wild-type and mutated H159L CAP proteins were purified according to the method of Ghosaini et al. (13). Gel retardation experiments were performed as previously described (10), with some modifications: cAMP-CAP was bound to the labeled DNA fragment (0.2 nM) in a HEPES-Mg-K glutamate reaction buffer (40 mM HEPES [pH 8], 10 mM $MgCl_2$, 100 mM K-glutamate) in the presence of 160 μM cAMP, at room temperature for 20 min.

DNase I footprinting experiments. The binding of cAMP-CAP and/or ArgR (kindly provided by D. Charlier) to the 268-bp DNA fragment encompassing the *argG* promoter was performed in HEPES-Mg-K glutamate buffer in the presence of 160 μM cAMP as previously described (10). After 20 min at room temperature, DNase I was added at a final concentration of 0.1 μg/ml. Reaction mixtures without any regulator were incubated at 37°C for 15 s and those containing CAP or/and ArgR were incubated at 37°C for 25 s. Reactions were stopped and were subjected to electrophoresis after heating at 90°C. Protected bands were identified by comparison with the same fragment treated for A+G sequencing reactions (25).

Chloramphenicol acetyltransferase assay. Bacteria carrying pDIA530 or pDIA560 were grown to log phase. Three 1.5-ml samples from two independent cultures were centrifuged for 5 min at 13,000 × *g*. Pellets were resuspended in 500 μl of 100 mM Tris-HCl (pH 7.8) and treated as described previously (35, 36).

In vitro transcription assays. In vitro transcription experiments were performed at least two times with pDIA539 (1.2 nM) in buffer containing 40 mM Tris-HCl (pH 8), 10 mM $MgCl_2$, 100 mM KCl, 0.5-mg/ml bovine serum albumin, 160 μM cAMP, and 1.4 mM dithiothreitol. After 20 min at room temperature, 14 μl of the mixture containing CAP or H159L CAP and/or ArgR at different concentrations was incubated at 30°C for 3 min. Then, 3.5 μl of a mixture containing nucleoside triphosphates, [α-^{32}P]UTP, and RNA polymerase (15 nM) was added to perform polymerization at 30°C for 10 min. The reaction was stopped by adding 1% sodium dodecyl sulfate and xylene blue formamide. After heating at 70°C and electrophoresis gel, the data were quantified with a PhosphorImager (Molecular Dynamics).

β-galactosidase assay. Overnight cultures were freshly diluted to an OD_{600} of 0.1 and were incubated 30 and 60 min at 30°C in M63 medium (26) supplemented with isopropyl-β-D-thiogalactopyranoside (IPTG) (1 mM) and glycerol (0.4%) ± arginine (10 mM). β-galactosidase activity was then determined by the method of Miller (27). Each assay was performed with two independent cultures.

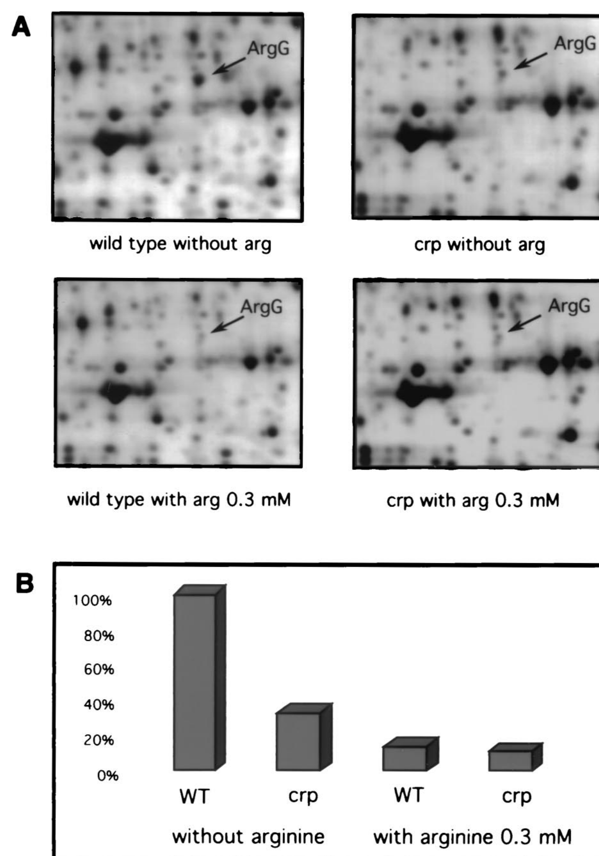

FIG. 1. (A) 2D gels of the wild-type strain (MG1655) and the *crp* mutant strain (BE1815) grown in minimal medium with or without 0.3 mM arginine. (B) Relative amounts of the ArgG protein in the four conditions.

RESULTS

The expression of *argG* is reduced in a *crp* mutant. Preliminary two-dimensional experiments with *argH* mutants (which are unable to synthesize arginine) showed that the level of several polypeptides was altered in a *crp* background (data not shown). Among them, arginosuccinate synthase was identified by microsequencing and a BLASTP search: the internal sequence of the protein was TFSDDVEMMLEANRI, which is 100% identical to an ArgG peptide (Swissprot accession number p22767). To rule out any effect of the *argH* mutation, a second set of experiments was performed in a wild-type background. Figure 1A shows representative 2D patterns of silver-stained proteins isolated from wild-type MG1655 and its *crp* derivative, BE1815, grown in M9 minimum medium supplemented with mannose and with or without arginine. The ArgG accumulation level was quantified (Fig. 1B): in the *crp* strain, BE1815, it corresponded to 33% of the wild-type level. In the presence of 0.3 mM arginine, both strains contained around 10% of the level found in the wild type in the absence of

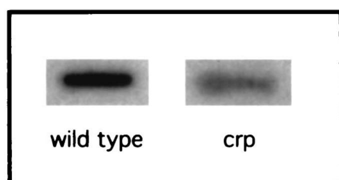

FIG. 2. The amount of *argG* mRNA in the wild-type and *crp* mutant strains was analyzed by slot blot hybridization with a specific 700-bp probe. Cultures were grown in minimal medium without arginine.

arginine. This was expected since arginine is a repressor of arginine biosynthesis genes (9).

To determine whether the variations in protein levels observed correlated with a difference in the level of mRNA, a slot blot experiment with a chemiluminescent *argG* probe was performed with total RNA extracted from an exponential-phase culture grown in minimal medium without arginine (Fig. 2). A quantitative analysis showed that the mutant contained just 27% of the amount of *argG* mRNA contained by the wild type. This demonstrated that the regulation of ArgG synthesis by the cAMP-CAP complex is directly correlated with the amount of its mRNA.

Identification of a CAP-binding site in the *argG* promoter region. A putative cAMP-CAP-binding site close to the known consensus binding site (7) was identified, centered at −166.5 bp with respect to the transcriptional start site. To confirm this, a gel mobility shift DNA binding assay was performed with the purified cAMP-CAP complex. Figure 3 shows that the presence of cAMP-CAP, at a low CAP concentration (1.5 nM), led to a significant retardation of the DNA fragment carrying the *argG* promoter region between −212 and +56, suggesting that the cAMP-CAP complex binds to the *argG* promoter region.

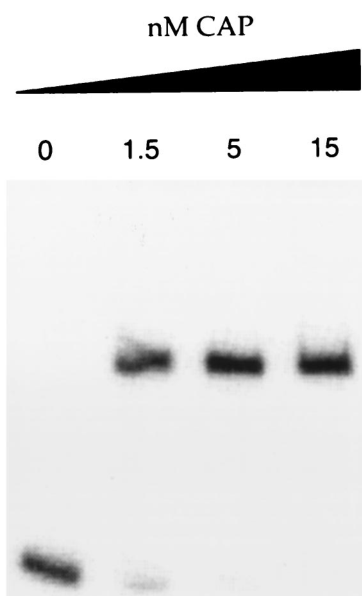

FIG. 3. Competitive gel retardation assay with CAP and the *argG* promoter region (positions −212 and +56 with respect to the transcriptional start site). DNA fragments were incubated with the indicated concentrations of CAP.

The precise location of the binding site was determined by DNase I footprinting. A footprint characteristic of the cAMP-CAP complex marked by protected bands with two hypersensitive bands 10 bp apart was detected in the presence of cAMP and 10 nM CAP (Fig. 4A). The site was fully occupied, and some hypersensitive bands were observed up- and downstream of the site in the presence of 100 nM CAP (Fig. 4A). Our data show that the cAMP-CAP complex binds specifically between positions 35 and 61, according to the numbering of the sequence in Fig. 5, which corresponds to the predicted cAMP-CAP binding site from which CAP may activate *argG* transcription. Moreover, the CAP-binding site overlaps both the −10 box and the upstream sequence of the *metY-yhbC-nusA-infB* operon P_{-1} promoter (15). This operon encodes successively the $tRNA^{Met}_{f2}$, a protein of unknown function, the transcription-translation coupling factor NusA, and the translation initiation factor IF2. This suggests that the binding of the cAMP-CAP complex at a position centered on −19.5 upstream of the *metY* transcription start site may repress the transcription of the *metY-nusA-infB* operon from the P_{-} promoter.

The cAMP-CAP complex activates *argG* transcription in vivo. The cAMP-CAP-binding site is distant from the *argG* transcription start site as it is centered at position −166.5. To determine whether the cAMP-CAP complex acts directly on *argG* transcription, an *argG-cat* transcriptional fusion was constructed on plasmid PKK232.8 with an intact (pDIA530) or an altered (pDIA560) CAP binding site. The altered CAP site was designed to prevent cAMP-CAP fixation, as previously described (6), i.e., tatAGTGAtccacgCCACAttt changed to tatA GTAAtccacgCTCGAttt. A gel mobility shift DNA binding assay confirmed that the cAMP-CAP complex was unable to bind to the altered site even at a high CAP concentration (40 nM). CAT activity during log phase was measured in *argG* transcriptional fusions with both wild-type and mutated promoters. Three to four colonies were picked from two independent cultures (Fig. 6). For the *crp*+ strain, the mutated CAP-binding site on pDIA560 caused a reduction to 63% in beta-galactosidase activity of the fusion compared to the nonmutated binding site in pDIA530. However, for the *crp* mutant (BE1815), both the mutated and the nonmutated CAP binding sites caused the same reduction in beta-galactosidase activity, to 36% of the activity seen in the wild-type reference strain. These results are consistent with the amounts of the ArgG protein and *argG* mRNA (Fig. 1 and 2). The CAT activity in the wild-type strain carrying the mutated CAP binding site (pDIA560) was between that obtained with the wild-type fusion (pDIA530) in the wild-type strain and that obtained for the *crp* mutant strain. This suggested that the cAMP-CAP complex exerts an additional and indirect effect on *argG* transcription.

It is known that *argG* is regulated by the hexameric ArgR repressor. In fact, ArgR, in conjunction with its corepressor arginine, represses *argG* transcription by binding to three ARG boxes: first to tandem ARG boxes, constituted of 18-bp boxes separated by 3 bp, located around the +1 site of *argG*, and then to a third single ARG box, located 101 bases upstream (Fig. 5) (9). This prompted us to study the interaction between the binding of the cAMP-CAP complex and the hexameric ArgR repressor in *arg* regulation.

Binding of ArgR to the *argG* promoter region in the pres-

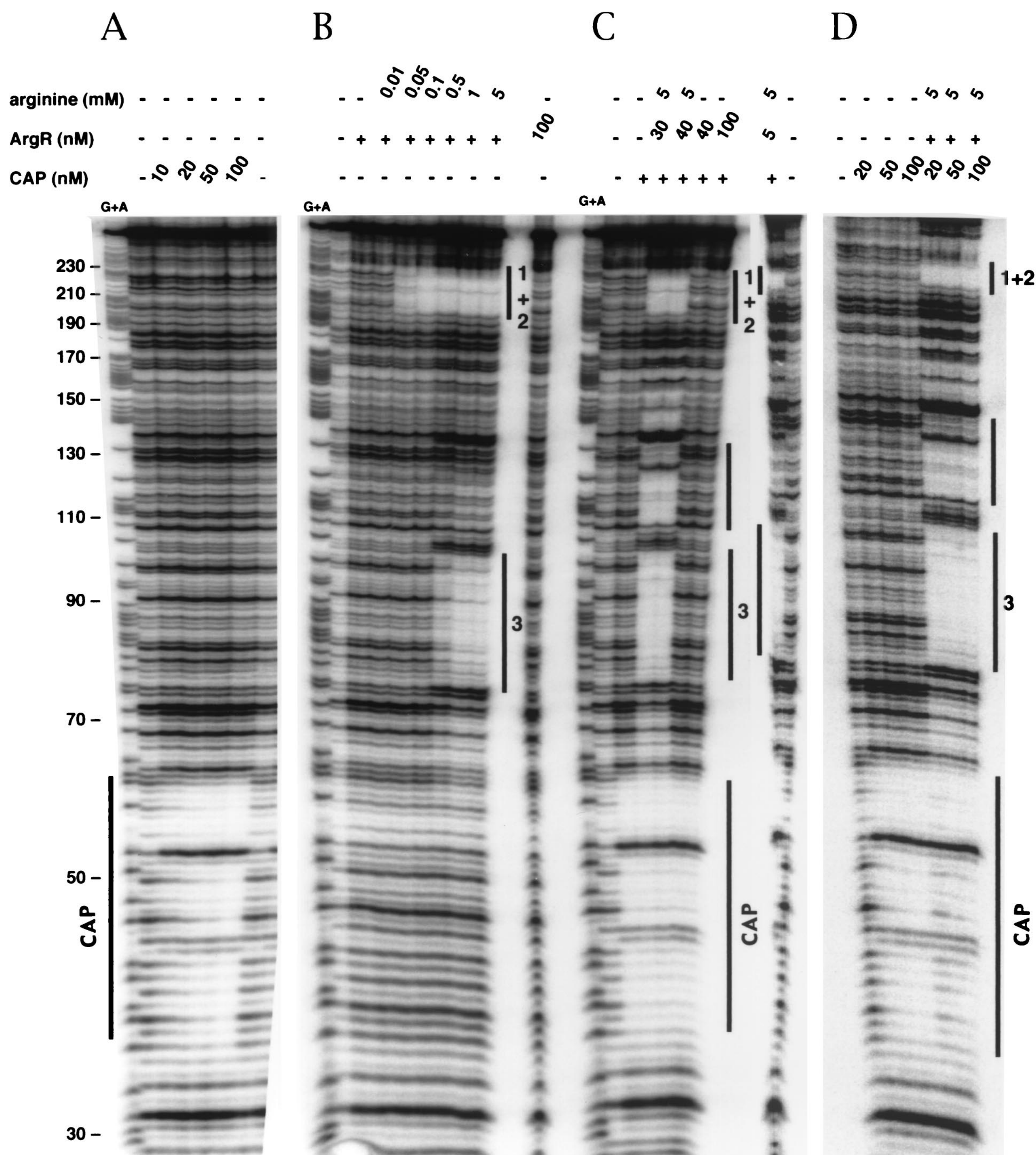

FIG. 4. Analysis of CAP- and ArgR-binding sites in the *argG* and *metY* promoter region by DNase I footprinting assays. The labeled DNA fragment represents the coding strand of *argG* with cAMP-CAP complex alone (A), with 5 nM ArgR alone (+) (numbers correspond to ARG boxes) (B), with 100 nM CAP (+) and then with ArgR (C), and with 20 nM ArgR (+) and then with CAP (D). Protected regions are marked by solid lines. The coordinates were determined with G+A and correspond to the numbering used in Fig. 5. −, no protein was present.

ence and absence of CAP. DNase I footprint experiments were first performed with ArgR (5 nM) at the *argG* promoter region (0.6 nM) and various arginine concentrations. It has been suggested that the degree of repression mainly depends on the concentration of arginine (14). No binding to the DNA fragment was observed in the absence of arginine, even at a high ArgR concentration (100 nM) (Fig. 4B). In the presence of 0.05 mM arginine, ArgR (7.5 nM) bound to the tandem ARG boxes around the *argG* +1 start site, whereas with 0.5 mM arginine, ArgR also bound to the third single ARG box (Fig. 4B). In the presence of 5 mM arginine and at a higher ArgR concentration (30 nM), a protected region was observed between the tandem ARG boxes and the single ARG box (positions 110 to 132) (data not shown; also Fig. 4C and D). This is

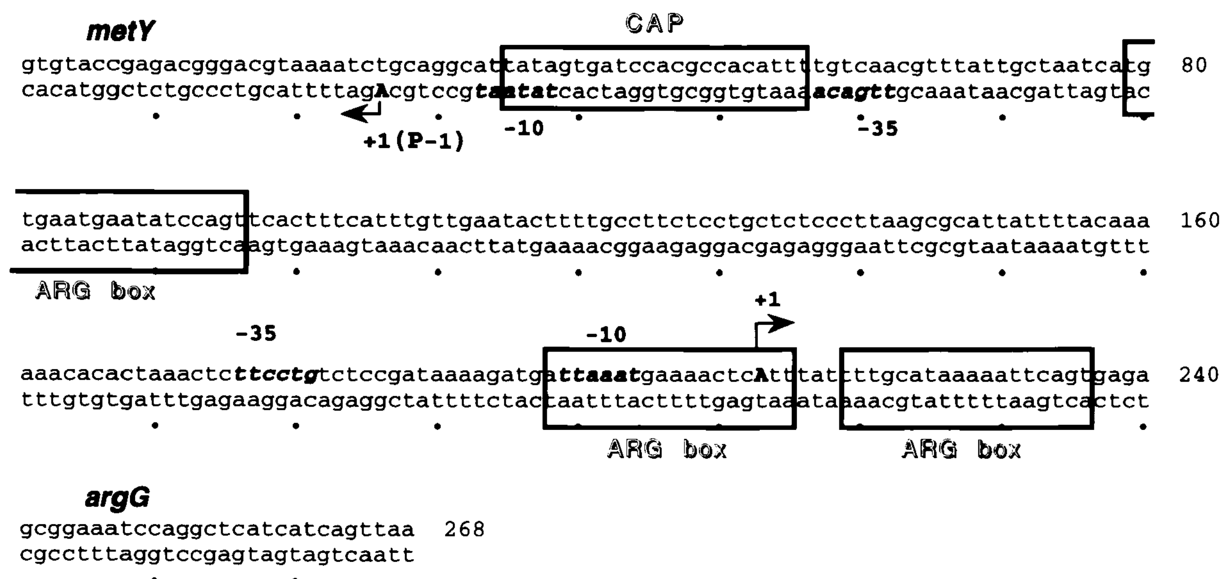

FIG. 5. Regulatory region of *argG* and *metY*. The nucleotide sequence corresponds to the fragment used in in vitro experiments. cAMP-CAP and ArgR-binding sites are indicated by boxes. The transcriptional start sites (+1) of *argG* and *metY* (promoter P−1) are indicated by arrows. The −10 and −35 boxes are indicated in bold.

thought to form a DNA loop, resulting from the binding to the three ARG boxes (9). DNase I footprint experiments were then performed with various concentrations of both regulators and in the presence and absence of 5 mM arginine (Fig. 4C and 4D). Again, in the absence of arginine, the ArgR regulator did not bind and had no effect on the binding of the cAMP-CAP complex to the operator. In the presence of arginine, ArgR bound to the three boxes, even at a high concentration of CAP (100 nM) (Fig. 4C and 4D). In contrast, the order with which the two regulators were added affected CAP binding. Indeed, the cAMP-CAP complex remained stably bound to DNA when ArgR was subsequently added in the presence of arginine (Fig. 4C). In contrast, when ArgR with arginine was already bound to DNA, the binding of CAP to DNA was less efficient, even at a high concentration (100 nM). However, its binding site was partially protected (Fig. 4D). Moreover, the hypersensitive bands observed between the CAP-binding site and the third ARG box with CAP alone were no longer visible in the presence of ArgR and arginine. These results suggest that the binding of each of the two regulators interferes with the binding of the other and that ArgR binding overrides CAP binding.

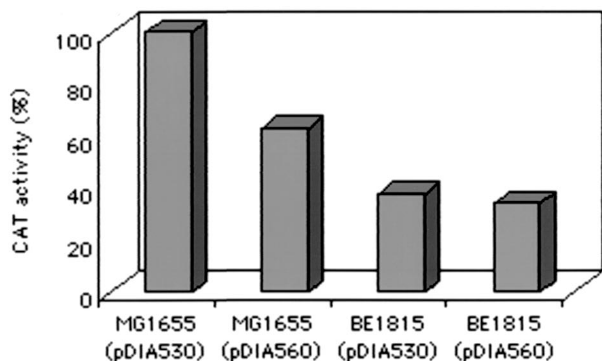

FIG. 6. Effect of a *crp* mutation (BE1815) on *argG-cat* transcriptional fusion activity. pDIA530 carries the intact promoter, whereas pDIA560 carries a promoter with an altered CAP site.

The cAMP-CAP complex activates *argG* and represses *metY*, whereas ArgR also represses *metY* but only in the presence of CAP. In the different conditions of regulator binding, we performed in vitro transcription assays on plasmid pDIA539, which contained the *metY-argG* promoter region used in DNase I footprint experiments (Fig. 5). Again, a range of regulator concentrations as well as of the cofactor arginine was used. The effect of a mutated CAP protein carrying an H159L amino acid substitution in activating region I, which prevents interactions with the C-terminal domain of the α subunit of RNA polymerase (12), was also investigated. At 250 nM, CAP increased *argG* transcription twofold, whereas it decreased *metY* transcription 16-fold. The mutated protein had the same effect, although the inhibition of *metY* was at a slightly lower level (10-fold) (Fig. 7). This further supports the hypothesis that the binding of RNA polymerase to the *metY* promoter P_{-1} is inhibited in the presence of the cAMP-CAP complex. These results also suggest that the cAMP-CAP complex does not interact directly with RNA polymerase bound to the *argG* promoter: CAP could only act on DNA conformation, hence facilitating the binding of RNA polymerase to the −10 and −35 boxes of the promoter. In the absence of its corepressor arginine, the ArgR regulator had no effect on the transcription of either *metY* or *argG*, whether in the presence or in the absence of CAP (Fig. 7), in agreement with the DNase I footprinting results. With 0.05 mM arginine, when bound to the tandem ARG boxes, ArgR (9 nM) repressed *argG* transcription 10-fold (Fig. 7). In the presence of CAP, the inhibitory effect of ArgR plus arginine remained predominant and of the same magnitude as that on *argG* (Fig. 7). This result is

FIG. 7. In vitro transcription assay by RNA polymerase from *metY* P_{-1} and *argG* promoters. pDIA539 was incubated with RNA polymerase in the presence or the absence (-) of regulators. Asterisks correspond to the H159L mutated CAP protein. Different transcripts are indicated by arrows.

consistent with the difference in the levels of ArgG protein measured in two-dimensional gel experiments (Fig. 1). At a higher arginine concentration (0.5 mM), when ArgR also bound to the third isolated ARG box, no further inhibition was observed at the *argG* promoter. In contrast, ArgR repressed the transcription of *metY* twofold in the presence of CAP, although ArgR had no detectable effect without CAP. This suggests that both regulators can bind to their own sites together in vitro, as shown by footprinting experiments, and that the binding of ArgR to the third ARG box, which occurs at a high concentration of arginine, reinforces the repressor effect of CAP on the binding of RNA polymerase to the *metY* P_{-1} promoter.

At 37°C, *metY-infB* transcription is mainly terminated after *metY* (3, 32). Therefore, the regulation of the *metY* P_{-1} promoter in vivo was studied by monitoring the level of $tRNA^{Met}_{f2}$.

Regulation of *metY* by cAMP-CAP complex and ArgR in vivo. It was previously shown that the level of β-galactosidase in the presence of IPTG reflects the efficiency of translation from the AUG start codon at the initiation step, independently of transcription, and thus the amount of $tRNA^{Met}_{f}$ available (21). For a *metZ* mutant strain, in which $tRNA^{Met}_{f}$ is synthesized only by *metY*, the level of β-galactosidase translation is similar to that measured for a wild-type strain grown in minimal medium with glucose and Casamino Acids in the presence of IPTG (21). With these carbon sources, the level of cAMP is low. In contrast, in the presence of glycerol, the cAMP level is 10-fold higher (18). Therefore, the activity of β-galactosidase was measured in cells that had been freshly diluted in minimal medium supplemented with 0.4% glycerol and with and without 10 mM arginine. After 30 and 60 min with glycerol alone, the β-galactosidase level for the *metZ* strain BE1723 was 70% of that obtained for the wild type (Table 2). This further supports the repressive effect of the cAMP-CAP complex observed in vitro, although the effect was less marked in vivo. This could be explained by the presence of a second *metY* promoter, P_0, downstream of P_{-1} (17). Surprisingly, with glycerol and arginine, no difference was observed between wild-type and *metZ* strains (Table 2), suggesting that CAP had no more effect on the *metY* level. In this assay, a high arginine concentration (10 mM) was used to allow ArgR to bind to the third ARG box. The result obtained suggests that the binding of ArgR to the third single ARG box in the chromosome prevents the binding of the cAMP-CAP complex in vivo.

In summary, the regulation of *argG* and *metY* expression is coupled by the cAMP-CAP complex but in an opposite way and in an even more complex manner with ArgR and arginine.

DISCUSSION

For *E. coli* K-12, arginine biosynthesis starts from glutamate and carbamoylphosphate. The enzymes responsible are coded by the arginine regulon. This regulon is composed of 12 genes,

TABLE 2. β-Galactosidase activity[a]

Carbon source	Activity for strain: MG1655 (Wild-type)		BEI723 (*metZ* mutant)	
	30 min	60 min	30 min	60 min
Glycerol	2,100	3,920	1,420	2,770
Glycerol + arginine	1,460	3,650	1,700	3,400

[a] β-Galactosidase activity is expressed in Miller units/milligram of protein. Values are the means of two independent determinations. Activities were measured after 30 and 60 min in cultures freshly diluted to an OD_{600} of 0.1 in M63 medium.

among which only a few are organized in an operon. However, all the genes in the arginine regulon are regulated by a master transcriptional regulator and repressor, ArgR, which functions as a direct sensor of arginine availability. The binding of six L-arginine molecules at the trimer-trimer interface of ArgR activates the regulator, allowing it to bind to the ARG boxes in DNA (11). The repressor binds symmetrically to four consecutive helical turns (corresponding to the two palindromic ARG boxes separated by three nucleotides, a span of nearly 40 bp) on one face of the DNA (14). The number of ArgR molecules per cell appears to be relatively high, around 600 (300 in the presence of excess arginine). Since its affinity for arginine is quite low (K_d of around 10^{-4} M) and the K_d of the active repressor for its operator is between 10^{-9} and 10^{-10} M, the degree of repression will mainly depend on the arginine concentration (14). *argG*, which is a member of the arginine regulon, encodes arginosuccinate synthase. Its promoter contains two 18-bp ARG boxes, separated by 3 bp, extending from the −10 box to the +24 residue, with respect to the +1 start site, and a third single box located 101 bp upstream of the tandem boxes (Fig. 5). The binding of the repressor to the upstream single ARG box requires much more repressor than binding to the tandem boxes. It has been suggested that binding to the three ARG boxes leads to the formation of a DNA loop and that the third site may become occupied if a loop is formed (9, 14).

In our study, we showed that *argG* is also regulated at a transcriptional level by the cAMP-CAP complex. This complex binds at position −166.5, with respect to the +1 start site, to the tatAGTGAtccacgCCACAttt sequence, which is very close to the consensus binding site 5′aaaTGTGAtntanaTCACAttt3′ (7). At such a distant location, CAP would have no effect on transcription. However, we demonstrated that its binding resulted in a twofold increase in *argG* transcription. A CAP protein that had mutated in its activating region I (H159L), such that it was unable to interact with the C-terminal domain of the α subunit of RNA polymerase (12), had the same activating effect. This suggests that in the present case, the cAMP-CAP complex does not interact directly with RNA polymerase but only facilitates its binding to −10 and −35 boxes by the stabilization of the bending of this promoter region, making it more accessible for transcription initiation. The generation of a DNA loop upon ArgR binding supports the hypothesis that the region is particularly formed (9, 14). The binding of ArgR to the tandem ARG boxes around the +1 start site, in the presence of arginine (0.05 mM), repressed the transcription of *argG* 10-fold, even in the presence of the cAMP-CAP complex. Thus, a gene-specific regulation, i.e., mediated by ArgR, is here clearly predominant, as observed in the case of numerous targets of CAP (e.g., *lac* or *mal*). As a consequence, the cAMP-CAP complex may increase the rate of arginine synthesis under arginine starvation conditions. The *argG* gene should therefore be added to the list of genes induced by CAP and involved in the biosynthesis of amino acids such as isoleucine and valine, which are derived from glutamate (7). On the other hand, CAP is known to repress *gadA*, coding for a glutamate decarboxylase that participates in acid resistance and synthesizes GABA from glutamate (8). This suggests that CAP may favor the consumption of glutamate for amino acid biosynthesis instead of its use in other metabolic processes.

It is interesting that from the same position, the cAMP-CAP complex is both an activator of *argG* and a strong repressor of the *metY-yhbC-nusA-infB* operon, since its binding site overlaps the sequence between the −10 and −35 boxes of the P_{-1} promoter of the latter (15), which encodes $tRNA_{f2}^{Met}$, the unknown protein YhbC, NusA, and the translation initiation factor IF2, respectively. However, an internal promoter, P_2 (which directs the transcription of the protein coding part), and two intercistronic terminators, t1 and t2, are present downstream of *metY*. At 37°C, only a *metY* transcript is mainly observed, whereas under cold shock, distal genes are expressed through a transcription antitermination mechanism that is mediated by cold shock-induced Csp proteins, such as CspA (3, 32).

Two isoacceptor species of $tRNA_{f}^{Met}$ are present in *E. coli* K-12: $tRNA_{f1}^{Met}$ coded by a three-tandem-repeated gene *metZ* (20), which represents the major fraction of the initiator tRNA pool, and $tRNA_{f2}^{Met}$ coded by *metY*, which represents the minor fraction (16). However, start codons AUG and GUG are recognized by both initiator tRNAs, and a *metZ* mutant strain is able to grow, although at a slower rate than the wild type or a *metY*-deficient strain (19, 21). The transcription of *metZ* is sensitive to ppGpp, the chemical mediator of stringent control, whereas this is not the case for *metY* (30). Before the present work, no regulation of the transcription of *metY* from its two promoters, P_{-1} and P_0 (15, 17), had been described, although the relative fraction of $tRNA_{f2}^{Met}$ in the tRNA pool seemed to be dependent on the growth rate and on the Fis activator (31, 38). In this article, we show that a regulation mechanism for *metY* transcription exists, mediated by the cAMP-CAP complex and by the repressor of *argG*, ArgR. In fact, the cAMP-CAP complex repressed the transcription of *metY* from its promoter P_{-1} 16-fold in vitro, and a significant inhibitory effect was also observed in vivo. By binding to the −10 and −35 boxes of the promoter, the cAMP-CAP complex blocked the access of RNA polymerase to promoter elements, as described for *osmY* (22) and *cya* P2 (1, 28), a mechanism that is very rarely used by CAP. ArgR only exerted its effect in the presence of CAP and a high concentration of arginine, i.e., when the third ARG box, which is centered at position −60 with respect to the +1 position of *metY*, was occupied by ArgR. However, the effects observed in vitro and in vivo seemed to contradict each other: ArgR repressed *metY* in vitro by increasing the cAMP-CAP complex repression effect, whereas in vivo it seemed to prevent the inhibitory action of CAP. It should be noted that the distance between the CAP binding site and the distal ArgR box is 42 bp, i.e., exactly four full turns of the DNA double helix, assuming a DNA pitch of 10.5 bp. This may mean that CAP and ArgR are located on the same side of the DNA helix, especially when the concentration of CAP is high, thus allowing them to act synergistically to repress the *metY* promoter in vitro. However, in footprinting experiments, ArgR with arginine, when bound first, partially prevented the binding of CAP, especially when CAP was added at the same concentration as ArgR. The intracellular concentration of cAMP is known to remain relatively low, even when cells are grown on glycerol, and in contrast to ArgR, the cAMP-CAP complex is a pleiotropic regulator that possesses many more available targets on the chromosome than the specific regulator ArgR. This suggests that more molecules of ArgR than of CAP are

available to bind to the *metY* promoter. This could explain why ArgR prevents CAP binding in vivo.

Finally, our results confirm the hypothesis that the different promoters of Met-tRNA initiators respond to different signals under changing physiological conditions (15). Indeed, we showed that the synthesis of one of the two tRNA initiators is coregulated with one of the arginine biosynthetic genes, *argG*, by both the cAMP-CAP complex and the ArgR-specific repressor of arginine biosynthesis. The function of these gene products indicates that they are coupled through their direct involvement in protein biosynthesis. However, the direct relationship between arginine and one of the two methionine tRNA initiators remains unexplained, although it was recently found that methionine seems to act on the level of RNA messenger of genes involved in arginine biosynthesis for *Bacillus subtilis* (34). This further supports the existence of a strong link between methionine and arginine biosynthesis, namely polyamine biosynthesis, for the synthesis of spermidine.

ACKNOWLEDGMENTS

We are grateful to W. Maas for helpful advice and to E. Tate for critical reading. We thank M. Springer for providing us with the *metZ*-deficient strain and D. Charlier for providing the purified ArgR protein.

Financial support came from the Institut Pasteur and the Centre National de la Recherche Scientifique (URA 1129 and URA 2171 and FRE2364).

REFERENCES

1. **Aiba, H.** 1985. Transcription of the *Escherichia coli* adenylate cyclase gene is negatively regulated by cAMP-cAMP receptor protein. J. Biol. Chem. **260:** 3063–3070.
2. **Altschul, W., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Bae, W., B. Xia, M. Inouye, and K. Severinov.** 2000. *Escherichia coli* CspA-family RNA chaperones are transcription antiterminators. Proc. Natl. Acad. Sci. USA **97:**7784–7789.
4. **Bertin, P., N. Benhabiles, E. Krin, C. Laurent-Winter, C. Tendeng, E. Turlin, A. Thomas, A. Danchin, and R. Brasseur.** 1999. The structural and functional organization of H-NS-like proteins is evolutionarily conserved in Gram-negative bacteria. Mol. Microbiol. **31:**319–329.
5. **Blum, P., D. Holzchu, H. S. Kwan, D. Riggs, and S. Artz.** 1989. Gene replacement and retrieval with recombinant M13mp bacteriophages. J. Bacteriol. **171:**538–546.
6. **Buchet, A., K. Eichler, and M.-A. Mandrand-Berthelot.** 1998. Regulation of the carnithine pathway in *Escherichia coli*: investigation of the *cai-fix* divergent promoter region. J. Bacteriol. **180:**2599–2608.
7. **Busby, S., and A. Kolb.** 1996. The CAP modulon, p. 255–279. *In* E. C. C. Lyn and A. S. Lynch (ed.), Regulation of gene expression in *Escherichia coli*. R.G. Landes Company, Georgetown, Tex.
8. **Castanie-Cornet, M.-P., and J. W. Foster.** 2001. *Escherichia coli* acid resistance: cAMP receptor protein and a 20 bp cis-acting sequence control pH and stationary phase expression of the *gadA* and *gadBC* glutamate decarboxylase genes. Microbiology **147:**709–715.
9. **Charlier, D., M. Roovers, F. V. Vliet, A. Boyen, R. Cunin, Y. Nakamura, N. Glansdorff, and A. Piérard.** 1992. Arginine regulon of *Escherichia coli* K-12: a study of repressor-operator interactions and of *in vitro* binding affinities *versus in vivo* repression. J. Mol. Biol. **226:**367–386.
10. **De Reuse, H., A. Kolb, and A. Danchin.** 1992. Positive regulation of the expression of the *Escherichia coli pts* operon. Identification of the regulatory regions. J. Mol. Biol. **226:**623–635.
11. **Duyne, G. D. V., G. Ghosh, W. K. Maas, and P. B. Sigler.** 1996. Structure of the oligomerization and L-arginine binding domain of the arginine repressor of *Escherichia coli*. J. Mol. Biol. **256:**377–391.
12. **Ebright, R. H.** 1993. Transcription activation at class I CAP-dependent promoters. Mol. Microbiol. **8:**797–802.
13. **Ghosaini, L., A. Brown, and J. Sturtevant.** 1988. Scanning calorimetric study of the thermal unfolding of catabolic activator protein from *Escherichia coli* in the absence and presence of cyclic mononucleotides. Biochemistry **27:** 5257–5261.
14. **Glansdorff, N.** 1996. Biosynthesis of arginine and polyamines, p. 408–433. *In* F. C. Neidhardt, R. Curtis III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
15. **Granston, A. E., D. L. Thompson, and D. I. Friedman.** 1990. Identification of a second promoter for the *metY-nusA-infB* operon of *Escherichia coli*. J. Bacteriol. **172:**2336–2342.
16. **Ikemura, T., and H. Ozeki.** 1977. Gross map location of *Escherichia coli* transfer RNA genes. J. Mol. Biol. **117:**419–446.
17. **Ishii, S., K. Kuroki, and F. Imamoto.** 1984. $tRNA_{f2}^{Met}$ gene in the leader region of the *nusA* operon in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81:**409–413.
18. **Joseph, E., C. Bernsley, N. Guiso, and A. Ullmann.** 1982. Multiple regulation of the activity of adenylate cyclase in Escherichia coli. Mol. Gen. Genet. **185:**262–268.
19. **Kenri, T., F. Imamoto, and Y. Kano.** 1992. Construction and characterization of an *Escherichia coli* mutant deficient in the *metY* gene encoding $tRNA_{f2}^{Met}$: either $tRNA_{f1}^{Met}$ or $tRNA_{f2}^{Met}$ is required for cell growth. Gene **114:**109–114.
20. **Kenri, T., F. Imamoto, and Y. Kano.** 1994. Three tandemly repeated structural genes encoding tRNA(f1Met) in the *metZ* operon of *Escherichia coli* K-12. Gene **138:**261–262.
21. **Kenri, T., K. Kohno, N. Goshima, F. Imamoto, and Y. Kano.** 1991. Construction and characterization of an *Escherichia coli* mutant with a deletion of the *metZ* gene encoding $tRNA_{f1}^{Met}$. Gene **103:**31–36.
22. **Lange, R., M. Barth, and R. Hengge-Aronis.** 1993. Complex transcriptional control of the σ^s-dependent stationary-phase-induced and osmotically regulated *osmY* (*csi-5*) gene suggests novel roles for Lrp, cyclic AMP (cAMP) receptor protein-cAMP complex, and integration host factor in the stationary-phase response of *Escherichia coli*. J. Bacteriol. **175:**7910–7917.
23. **Laurent-Winter, C., S. Ngo, A. Danchin, and P. Bertin.** 1997. Role of *Escherichia coli* histone-like nucleoid structuring protein in bacterial metabolism and stress response: identification of targets by two-dimensional electrophoresis. Eur. J. Biochem. **244:**767–773.
24. **Marschall, C., V. Labrousse, M. Kreimer, D. Weichart, A. Kolb, and R. Hengge-Aronis.** 1998. Molecular analysis of the regulation of *csiD*, a carbon starvation-inducible gene in *Escherichia coli* that is exclusively dependent on σ^s and requires activation by cAMP-CRP. J. Mol. Biol. **276:**339–353.
25. **Maxam, A., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Nat. Acad. Sci. USA **74:**560–564.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
27. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **Mori, K., and H. Aiba.** 1985. Evidence for negative control of *cya* transcription by cAMP and cAMP receptor protein in intact *Escherichia coli* cells. J. Biol. Chem. **260:**14838–14842.
29. **Morrissey, J. H.** 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. Anal. Biochem. **117:** 307–310.
30. **Nagase, T., S. Ishii, and F. Imamoto.** 1988. Differential transcriptional control of the two $tRNA_f^{Met}$ genes of *Escherichia coli* K-12. Gene **67:**49–57.
31. **Nilsson, L., and V. Emilsson.** 1994. Factor for inversion stimulation-dependent growth rate regulation of individual tRNA species in *Escherichia coli*. J. Biol. Chem. **269:**9460–9465.
32. **Regnier, P., and M. Grunberg-Manago.** 1989. Cleavage by RNaseIII in the transcripts of the *metY-nusA-infB* operon of *Escherichia coli* releases the tRNA and initiates the decay of the downstream mRNA. J. Mol. Biol. **210:**293–302.
33. **Reznikoff, W. S.** 1992. The lactose operon-controlling elements: a complex paradigm. Mol. Microbiol. **6:**2419–2422.
34. **Sekowska, A., S. Robin, J.-J. Daudin, A. Henaut, and A. Danchin.** 2001. Extracting biological information from DNA arrays: an unexpected link between arginine and methionine metabolism in *Bacillus subtilis*. Genome Biol. **2:**0019.1–0019.12.
35. **Shaw, W. V.** 1975. Chloramphenicol acetyltransferase from chloramphenicol resistant bacteria. Methods Enzymol. **43:** 742–743.
36. **Soutourina, O., A. Kolb, E. Krin, C. Laurent-Winter, S. Rimsky, A. Danchin, and P. Bertin.** 1999. Multiple control of flagellum biosynthesis in *Escherichia coli*: Role of H-NS protein and the cyclic AMP-catabolic activator protein complex in transcription of the *flhDC* master operon. J. Bacteriol. **181:**7500–7508.
37. **Touati, E., C. Laurent-Winter, P. Quillardet, and M. Hoffnung.** 1996. Global response of *Escherichia coli* cells to a treatment with 7-methoxy-2-nitronaphtho(2,1-*b*)furan (R7000), an extremely potent mutagen. Mutat. Res. **349:**193–200.
38. **Verbeek, H., L. Nilsson, G. Baliko, and L. Bosch.** 1990. Potential binding sites of the *trans*-activator FIS are present upstream of all rRNA operons and of many but not all tRNA operons. Biochim. Biophys. Acta **1050:**302–306.
39. **Xu, J., and R. C. Johnson.** 1997. Cyclic AMP receptor protein functions as a repressor of the osmotically inducible promoter *proP* P1 in *Escherichia coli*. J. Bacteriol. **182:**4180–4187.